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Safety Information

INSTRUMENT CERTIFICATION

In accordance with Thermo Separation Products' commitment to customer service and safety, this autosampler and its accompanying documentation have satisfied the requirements for, and have received certification from T1DV/GS, CSA, and the FCC†. VDE certification is pending. This product complies with the following CSA Standard 022.2 regulations:

- O-M1982 – General Requirements (Canadian Electrical Code, Part II)
- 0.4–M1982 – Bonding and Grounding of Electrical Equipment (Protective Grounding)
- 150-M1986 – Laboratory Equipment

Designed and tested according to DIN 57411 Pt. 1/VDE 0411 Pt. 1, Safety Requirements for Electronic Measuring Apparatus, this autosampler has been shipped to you from our manufacturing facility in a safe condition.

IDENTIFYING SAFETY INFORMATION

This reference manual contains warnings and precautionary statements that can prevent personal injury, instrument damage, and loss of data if properly followed. All statements of this nature are called to your attention through the use of bold type and the following icons:


CAUTION!


WARNING!


HIGH VOLTAGE!

SPECIFIC HAZARD

Every instrument has specific hazards, so be sure to read and comply with the following precautions. They will help ensure the safe, long term use of your autosampler.

1. Only use fuses of the type and current rating specified. Do not use repaired fuses and do not short-circuit the fuse holder.

2. The supplied power cord must be inserted into a power outlet with a protective earth contact (ground). When using an extension cord, make sure that the cord also has an earth contact.

3. Do not change the external or internal grounding connections. Tampering with or disconnecting these connections could endanger you and/or damage the autosampler.



***NOTE**: The instrument is properly grounded in accordance with these regulations when shipped. You do not need to make any changes to the electrical connections or to the instrument's chassis to ensure safe operation.*

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4. Never run the autosampler without the top cover on. Permanent damage can occur. (The door may be opened during operation).

5. Do not turn the instrument on if you suspect that it has incurred any kind of electrical damage. Instead, disconnect the power cord and contact a LabAlliance Products Service Representative for a product evaluation. Do not attempt to use the instrument until it has been evaluated. (Electrical damage may have occurred if the autosampler shows visible signs of damage, or has been transported under severe stress.)

6. Damage can also result if the instrument is stored for prolonged periods under unfavorable conditions (*e.g.*, subjected to heat, water, etc.

7. Always disconnect the power cord before attempting any type of maintenance.

8. Capacitors inside the instrument may still be charged even if the instrument is turned off.

9. Never try to repair or replace any component of the instrument that is not described in this manual without the assistance of LabAlliance.

10. The autosampler is shipped with an aqueous methanol solution in the lines. Be sure to prime the autosampler with a miscible solvent as described in Appendix A prior to use.

GOOD LABORATORY PRACTICES

Always follow good laboratory practices whenever you operate any high-performance liquid chromatography.

Keep Good Records

To help identify and isolate problems with either your equipment or your chromatography, we recommend that you keep good records of all system conditions (*e.g.*, %RSDs on retention times and peak areas, peak shape and resolution, column pressure, and autosampler reproducibility). At a minimum, keep a chromatogram of a typical sample and standard mixture, well-documented with system conditions, for future reference. Careful comparison of retention times, peak shapes, column pressure, efficiency, and baseline noise can provide valuable clues to identifying and *solving* future problems.

Chemical Toxicity

Although the large volume of toxic and flammable solvents used and stored in laboratories can be quite dangerous, don't ignore the potential hazards posed by your samples. Take special care to read and follow all precautions that ensure proper ventilation, storage, handling, and disposal of both solvents and samples. Become familiar with the toxicity data and potential hazards associated with all chemicals by referring to the manufacturers' Material Safety Data Sheet (MSDS).

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Sample Preparation

Always consider the solubility of your sample in the mobile phase. Sample precipitation can plug the system by obstructing the flow through the injector and/or the column. This obstruction may result in irreparable damage to parts of the system. Particulate matter can be avoided by filtering the samples through 0.45- or 0.2-micron (or less) filters (see above).

Solvent Requirements

Many chemical manufacturers provide a line of high-purity or HPLC-grade reagents. Routine filtration of all solvents or eluants through a 0.45- or 0.2-micron (or less) fluorocarbon filter before placing them in the solvent reservoir will prolong the life and effectiveness of the inlet filters, check valves, seals, injectors, and columns.

Choose a mobile phase that is compatible with the sample and column you have selected for your separation. Remember that some solvents are corrosive to stainless steel. Inert/biocompatible instrument versions are also available from LabAlliance.

Degas the Eluants

Degas your LC system eluants by vacuum degassing or the helium sparging. Complete information for using LabAlliance equipment to perform either of these techniques is found in separate documentation provided with degas accessories.

Solvent Disposal

Make sure you have a solvent waste container or other kind of drain system available at or below the benchtop level. Most solvents have special disposal requirements and should not be disposed of directly down a drain. Follow all governmental regulations when disposing of any chemical.

High-pressure Systems and Leaks

LC systems operate at high pressure, but since liquids are not highly compressible, they do not store much energy. Thus, little immediate danger arises from the high pressure in an LC system unless a dangerous mobile phase is used, such as THF, CH_3CN, DMSO, H_2SO4, etc. However, if a leak occurs, it should be corrected as soon as possible. Finally, we recommend that you always wear eye and skin protection when working on an LC system and that you always shut down the system and return it to atmospheric pressure before attempting any maintenance.



***NOTE**: If, for any reason, you need to return your autosampler to LabAlliance, flush the lines with methanol.*

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Start-up Checklist

This list is a brief summary of tasks that should be completed to install your autosampler. Complete installation information is contained in Appendix A.



***NOTE**: Item marked with an asterisk (*) pertain to instruments with the sample preparation option installed. Item marked with two asterisks (**) are optional advanced installation procedures that should be performed only by very experienced users with specialized applications.*

Basic Installation

UNPACKING

- ❑ Unpack and inspect your instrument
- ❑ Remove cardboard/foam packing insert from sample tub
- ❑ Check for parts shortages
- ❑ Read the Safety Card

REAR PANEL

Place the autosampler with rear panel accessible

- ❑ Install flush bottle/bracket
- ❑ Install filter/solvent line and flush bottle
- ❑ Connect the external solvent tubing to tubing extensions (sample preparation only)*
- ❑ Connect heater/mixer to an air supply*
- ❑ Connect the power cord
- ❑ Hardwire external events, make connection to other SpectraSYSTEM instruments**

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FRONT PANEL

- ❑ Install solvent tray/waste container
- ❑ Connect autosampler to pump
  On instruments without the column oven or inert/biocompatible version, connect pump outlet line to part 6 on injector valve.

  Instruments with column oven installed, connect pump outlet line to solvent preheat tube
- ❑ Mount column On instruments without the column oven, connect inlet end to injector valve port 5

  On instruments with the column oven installed, install the column between the column mounts, resecure column mounts to oven, and connect inlet end to injector valve port 5.
- ❑ Connect autosampler to detector
  (Connect 12-inch 0.010 ID tubing to inlet side of flowcell)

STARTUP/PRIMING

- ❑ Turn on, check initial response to power-on
- ❑ Using the 2.5 µL syringe, flush lines with 5000 µL from the Btl reservoir.*

PERFORMANCE VERIFICATION

- ❑ Using the 250 µL syringe, flush lines with 500 µL
- ❑ Run a standard sample (a test mix is shipped with the instrument)
- ❑ Date and retain run results with instrument documentation
- ❑ Customize READY participation"
- ❑ Run an unretained sample to determine gradient delay time**
- ❑ Optimize needle height**

Installed by:_____ Date: _____

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List of Spare Parts, Consumable, Kits

Shown below is a list of spare parts, consumables, and kits available from LabAlliance for use with your SpectraSYSTEM® autosampler.

All Autosamplers

TS-A4214-010

Autosampler Vial Kit
(Kit consists of 144,1.5-mL glass vials, with crimp caps and silicone/PTFE septa.)

TS-A4216-010

Autosampler Vial Kit
(Kit consists of 144,750-mL polypropylene vials, with crimp caps and silicone/PTFE septa.)

TS-A4218-010

TS-A2203-010

Autosampler Vial Kit
(Kit consists of 144, 100-mL polypropylene vials, with crimp caps and silicone/PTFE septa.)

TS-3115-0400

Autosampler Vial Kit
(Kit includes 2-mL vials, caps, Teflon- liners and septa.) Vials are available in 1-, 2-,3-,4-, and 5-gross quantities.

TS-A2337-010
TS-2508-0260

Shell Vials

Autosampler MicroVial Kit
(Kit includes twelve 100-mL polypropylene vials, caps, and septa.)

TS-A4946-010
TS-A4947-010
TS-A4948-010
TS-A4950-010

Teflon Septa (I gross)
Teflon faced silicone septa 0 gross)

Clear Vials and Vial Kits

Standard Vials

Shell Vials, 100/package
Shell Caps, 100/package
Shell Vial Inserts, 300 mL, 100/package
Shell Vial Kit with Inserts, 100 /package

TS-A4951-010
TS-A4952-010
TS-A4953-010
TS-A4954-010

Crimp Vials/G

Standard Vials, 100/package
Standard Vial Septa, 100/package
Standard Vial Caps, 100/package
Standard Vial Kit, 100/package

TS-A4955-010
TS-A4956-010
TS-A4957-010

TS-A4962-010
TS-A4963-010

Crimp Caps, 100/package
Crimp Vials, 100/package, 1.8 mL
Crimp Vial Kit, 1 00/package

Poly Kits

Snap Crimp Kit, 100/package
Snap Caps,100/package

TS-A4958-010
TS-A4959-010

Inserts

Polypropylene Kits, 600 mL, 100/package
Polypropylene Kits, 100 mL, 1 00/package

TS-A4981-010
TS-A4960-010
TS-A4961-010

Spring for Insert, 100/package
Insert, 50 mL, 100/package
Insert, 100 mL, 100/package

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Amber Vials and Vial Kits

Crimp Vials	
TS-A4964-010	Amber Crimp Vials, 100/package
TS-A4965-010	Amber Vial Crimp Kit, 100/package
Standard Vials	
TS-A4966-010	Standard Amber Vials, 100/package
TS-A4967-010	Standard Amber Vial Kit, 100/package
PFTE Liners/Se	
TS-A4968-010	PTFE Liners, 100/package
TS-A4969-010	PTFE Vial Caps/Septa, 100/package
TS-A4970-010	PTFE-Silicone Septa, 100/package
Sample Trays	
TS-A3695-010	Sample Trays (3),40-vial capacity Sample Trays (3), 35-vial capacity
TS-A3696-010	(For autosamplers vAth Tray Temperature Control.)
Sample Trays	
TS-A3695-010	Sample Trays (3), 40-vial capacity Sample Trays (3), 35-vial capacity
TS-A3696-010	(For autosamplers with the Peltier cooling option/ upgrade.)
Cables and Adapters	
TS-A3981-010	**Autosampler-to-integrator Cable/Adapter**
TS-A3663-010	**BCD Communications Option** (This option requires field installation by LabAlliance unless purchased simultaneously with a SpectraSYSTEM autosampler.)

Standard Autosamplers

TS- A3566-010	**Injector Valve Assembly for the AS1000 autosamplers, 20 mL, SS** (Assembly includes the complete autosampler valve assembly.)
TS-A3566-030	**Injector Valve Assembly for the AS3000, 100mL, SS** (Assembly includes the complete autosampler valve assembly.)
TS-A4101-010	**Standard Maintenance Kit** (Kit includes everything necessary to maintain the instrument for one year: inlet filter, needle replacement kit, a valve rotor seal, syringe, lubricant, cleaner and an LC test sample.)
TS-A4051-010	**Standard Fittings Kit** (Kit includes stainless steel fittings and tubing used in a SpectraSYSTEM LC system.)
TS-A4719-010	**Autosampler Needle Replacement Kit** (Kit includes the standard, stainless-steel, deflected-point replacement needle.)
TS-A4719-020	**Autosampler Needle Replacement Kit** (Kit includes the standard, inert, deflected-point replacement needle.)

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Standard Autosamplers, con't.

Standard Sample Loops and Syringes

TS-3302-0230	Rheodyne Sample Loop, 20 µL, SS
TS-3302-0010	Rheodyne Sample Loop, 50 µL, SS
TS-3302-0020	Rheodyne Sample Loop, 100 µL, SS
TS-3302-0220	Rheodyne Sample Loop, 200 µL, SS
TS-3302-0030	Rheodyne Sample Loop, 500 µL, SS
TS-3302-0040	Rheodyne Sample Loop, 1 mL, SS
TS-A3588-020	Screw-tip Syringe, 250 µL, (standard and prep #1 drive)
TS-A3588-010	Screw-tip Syringe, 500 µL
TS-A3587-020	Screw-tip Syringe, 2.5 mL, (prep #2 drive)
TS-A4094-010	Solvent Inlet Filter Cartridge (pkg. of four)

Inert/Biocompatible Autosamplers

TS-A3566-020	**Injector Valve Assembly** (Assembly includes the complete autosampler valve assembly.)
TS-A4102-010	**Inert/Biocompatible Maintenance Kit** (Kit includes the inert/ biocompatible versions of everything in the Standard Maintenance Kit)
TS-A4061-010	**Inert/Biocompatible Fittings Kit** (Kit includes PEEK fittings and tubing used in an inert/biocompatible LC system.)
TS-A3564-020	**Inert/Biocompatible Autosampler Needle Replacement Kit**

Inert/Biocompatible Sample Loops and Syringes

TS-A4169-010	Rheodyne Sample Loop, 20 µL
TS-A4169-020	Rheodyne Sample Loop, 50 µL
TS-A4169-030	Rheodyne Sample Loop, 100 µL
TS-A4169-040	Rheodyne Sample Loop, 200 µL
TS-A4169-050	Rheodyne Sample Loop, 500 µL
TS-A4169-060	Rheodyne Sample Loop, 1 mL
TS-4169-070	Rheodyne Sample Loop, 2 mL
TS-A3588-020	Screw-tip Syringe, 250 µL
TS-A3588-010	Screw-tip Syringe, 500 µL
TS-A3587-020	Screw-tip Syringe, 2.5 mL, (prep #2 drive)
TS-A4094-010	Solvent Inlet Filter Cartridge (pkg. of 4)

Upgrade Kits

Upgrade kits are available for SpectraSYSTEM stainless-steel and inert/biocompatible autosamplers. Contact your local LabAlliance Representative for details.

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1

Getting Started

Introduction

This chapter provides you with the three basic rules you'll need for using your AS 1000, AS3000, or AS3500 SpectraSYSTEM® autosampler. It also introduces you to the instruments command center and describes the conventions well use in this manual.

Before you start this chapter, be sure that you read the Safety Information section beginning on page iii and install your autosampler as described in Appendix A.



***NOTE**: Throughout our explanations, we encourage you to explore the general architecture o your instrument's menus and screens. Use the Menu Tree in Appendix B or the menu card in the front pocket of this manual as your guide if you wish.*

Learning Your Way Around

AS EASY AS 1-2-3!

It's easy to learn your way around a SpectraSYSTEM autosampler. Just remember these three rules:

1. The arrow keys ([∧], [∨], [<], [>]) move the cursor in the direction printed on the key.



HINT: Press [MENU] to jump quickly to the top of the menu structure.

2. The shape of the cursor determines how you make a selection.
   - If a triangular cursor appears, press [ENTER].
   - If a blinking square cursor (©) appears, press the [+] or [-] keys to scroll up or down through preset choices, or to increase or decrease alphanumeric entries.

3. There are four ways to accept (and automatically save) an entry. Just move the cursor out of the field by any of the following methods:
   - Pressing [ENTER]
   - Using the arrow keys
   - Pressing [MENU]
   - Pressing [STATUS]

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NOTE: *You won't be able to leave a menu if errors are present or if you haven't filled in all the necessary entries.*

VISUAL CLUES

The following conventions are used on the autosampler's display:

1. Top-level menu choices are displayed in all-capital letters.

2. A field's square cursor changes to an underscore cursor when you're scrolling through preset choices or entering numerical values and characters.

3. A solid down-arrow (▼) on the right side of some displays indicates that the current menu continues on additional screens. To access additional menu lines, press the down-arrow key, [∨].

4. The last line of a longer menu is frequently a blank display line (without a solid down-arrow).

Instrument Control

Take a look at the keypad and two-line display located on the front panel (Fig. 1.1). This is the command center from which you'll access menus and control the instrument's operations. A brief explanation of the keys and the main menus and screens follows.

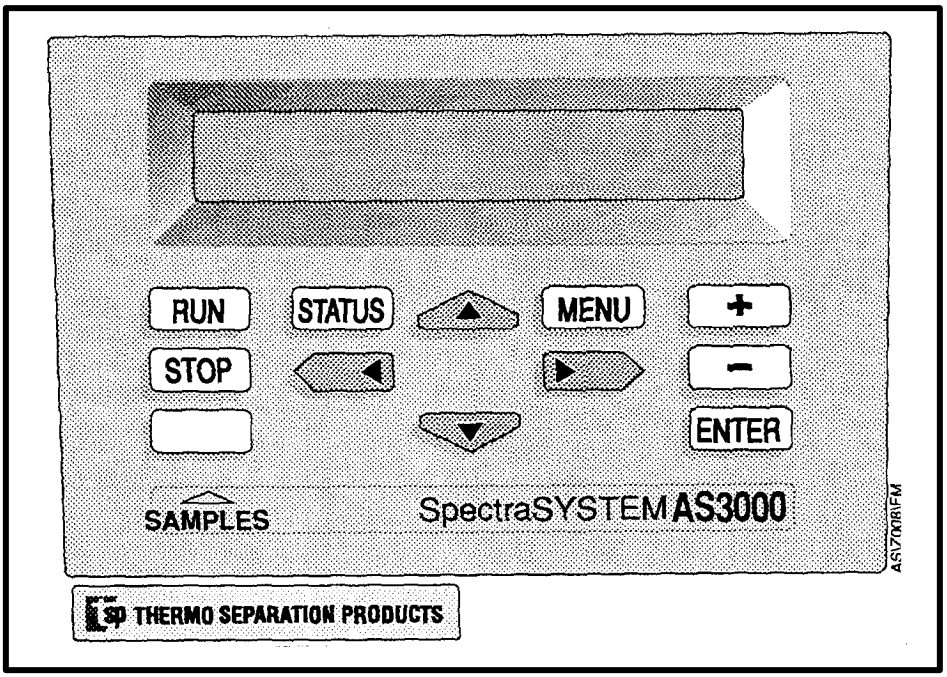


Figure 1.1 The autosampler's front panel
(With the exception of the model number, the front panel is identical for all SpectraSYSTEM autosamplers.)

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The keypad of each SpectraSYSTEM instrument consists of twelve keys. Four keys directly control the instrument's operation: [RUN], [STOP], [STATUS], and, on the autosampler, a blank key called [SAMPLES]. The remaining keys either access commands ([MENU] and [ENTER]), or are used to set parameters and move around the display ([A], [V], The function of each is explained below.

[RUN]

Pressing [RUN] starts the run.

The specific [RUN] operation depends on the contents of the sample queue:

1. If there are sample sets (groups of sample and associated calibration vials) in the sample queue, pressing [RUN] begins processing the first vial in the sample set.

2. If the sample queue is empty, pressing [RUN] prompts you to press [SAMPLES] to display the Samples Menu so that you can add sample sets to the sample queue.

[STOP]

Pressing [STOP] pauses the sample set currently running, aborts subsequent sample sets, and returns the autosampler to the initial run conditions. Refer to Chapter 3 for more information on the [STOP] key's function.

[STATUS]

Pressing [STATUS] displays the Status Screen. From the Status Screen you can monitor the run in progress



NOTE: Unlike the other SpectraSYSTEM instruments, on the autosamplers, you can make changes to the run in progress from the [SAMPLES] key rather than from the [STATUS] key (see below).

[SAMPLES]

The unlabeled key is the only variable key in the SpectraSYSTEM family. (It has a different function on each of the SpectraSYSTEM modules.) On the autosampler, the blank key is the [SAMPLES] key. The key's name appears on the nameplate below the key.
The [SAMPLES] key displays the Samples Menu that lets you define the resources (samples, reagents, and standards) and assign a file to each sample set. From the Samples Menu you also add sample sets to the sample queue.
Because you manage resources and files from the Samples Menu, the [SAMPLES] key gives you the real-time editing capability provided by the [STATUS] key on the other SpectraSYSTEM instruments. That is, from the Samples Menu you can make changes to the file that is currently running. Pressing the [SAMPLES] key during a ran lets you obtain information about, or edit the locations and injection volumes of, the sample and calibration vials. Chapter 3 and Appendix B contain more information on the [SAMPLES] key.

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[MENU]

Pressing [MENU] displays the Main Menu (Fig. 1.2). See below for more information.

[ENTER]

Pressing [ENTER] accepts a selected choice or menu entry. The [ENTER] key also advances the cursor to a new field, either on the same line of the display or in the line below.

[Ù], [Ú], [<], and [>]

Pressing any arrow key (up, down, left, or right) moves the cursor in the direction indicated on the key. The up- and down-arrow keys also move the cursor between menus and displays.

[+] and [-]

Pressing [+] and [-] keys scrolls you through a field's available choices or changes the value of alphanumeric entries. Holding down either key will continuously scroll the list of choices forward or backward until you release the key.

In fields that require alphabetical or numerical entries, the value of each digit is increased or decreased by one unit each time you press the [+] or [-] key. In fields that accept *either* numeric or character entries, such as the File Name field, the [+] and [-] keys scroll through the alphabet from A to Z, then through the numbers 0 to 9, and finally to a slash, hyphen, and blank space.

In other fields, the [+] key advances you through a present list of choices while the [-] key takes you back through the list.

MENUS, AND SCREENS

Your autosampler has two kinds of display: menus and screens. Menus require you to make selections or enter specific values. Screens display information that cannot be edited. The Menu Tree in Appendix B illustrates the structure and content of the autosampler's menus and screens.

Main Menu

The Main Menu (Fig. 1.2) is the top level of the menu structure. It gives you access to five other menus: FILES, COMMANDS, QUEUE, OPTIONS, and TESTS. To see the Main Menu, press the [MENU] key at any time.

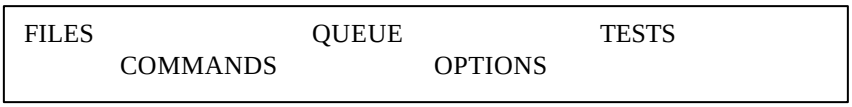


Figure 1.2 The Main Menu

From the Files Menu you can edit, load, or delete files. From the Queue Menu you can edit or change the order of sample sets in the sample Queue. The Tests Menu gives you access to the instrument's built-in diagnostics.

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The Commands Menu lets you run built-in instrument routines. In the Options Menu, you can set up or change your instrument's configuration. Refer to Chapters 3, 4, 5, and Appendices B, C, and D for more information on any of the instrument's menus.

Status Screen

The Status Screen automatically appears whenever you turn on the instrument or press the [STATUS] key. The Status Screen (Fig. 1.3a) displays the status of the sample queue, the current sample set, the current sample vial, and the total nuraber of samples in the set. Pressing the down-arrow once displays the Inject Status Screen (Figure 1.3b). Pressing the down-arrow again displays the Oven/Tray Temperature Status Screen (1.3c), if these options are present. Chapter 3 contains more information on the Status Screen.

QSTATUS	Set	#Samples
IDLE	1	1 / 1

Figure 1.3a The Queue Status Screen

Status	Vial	Vol	#Inj
READY	A01-S	1000	1 / 1

Figure 1.3b The Injector Status Screen

Oven	TrayTemp
OFF 23	OFF 25

Figure 1.3c The Oven/Tray Temperature Status Screen

MESSAGES

There are three different kinds of messages that can appear on your autosampler's display: user messages, confirmation messages, and error messages.

User messages

User messages tell you about an existing instrument condition or ask for further actions. Some of these will only appear on the display for three seconds. An example of a message requiring further action is shown in Figure 1.4.

* File Protection *
No Editing Allowed

Figure 1.4 An example of a user message

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Confirmation messages

Confirmation messages (Fig. 1.5), indicated on the display by asterisks, appear for one second after an operation has been carried out successfully.

```
* *   File Loaded   * *
```

Figure 1.5 An example of a confirmation message

Error messages

Error messages (Fig. 1.6), indicated on the display by exclamation points, are shown whenever an undesirable condition exists that prevents the instrument from carrying out an operation. Error messages remain on the display until you press a key.

```
! !   Unexpected vial in Hook   ! !
```

Figure 1.6 An example of an error message

Manual Conventions

This manual uses several conventions. Among them are menu displays, text conventions (brackets, slashes, etc.), standard words, and several different icons.

DISPLAYS

Figure 1.7 shows how we will depict the two-line display. Note that, in menu illustrations, the triangular cursor location is indicated by a caret (>).

```
> FILES          QUEUE          TESTS
    COMMANDS          OPTIONS
```

Figure 1.7 A two-line menu display

Frequently, the two lines shown on the display are only part of a longer menu. In this manual, menus having more than two lines are represented as in Figure 1.8.

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Injection Volume	1.0 *(AS3000)*	
Injection/Sample	1	
Cycle Time	2.5	
Col Oven: OFF__	Temp 18	
TrayTemp: OFF__	Temp 20	

Figure 1.8 A menu longer than two lines

COMMENTS

Fields that appear only on some SpectraSYSTEM autosampler **models or** only when certain options are installed are documented with a comment to the right of the field. For example, in Figure 1.8, the Injection Volume field appears only on the variable-injection volume (VIV) AS3000 autosamplers.

TEXT

Three typographic conventions are used to differentiate between keys, menus, and fields.

Brackets

Brackets, [ ], indicate instrument keys. For example: Press [MENU].

Slashes

Slashes, / /, are used around menu choices. For example: From the Main Menu, select /FILES/.

Capitalization

Capitalization is used to make field and menu names appear just as they do on the display. Generally the first letters of field names are capitalized. For example: Select /FILES/, /Edit/, /Injection/.

STANDARD WORDS

We have also standardized the meaning of two words: "select" and "enter."

select

The word "select" is used when you need to choose from among available options. For example, to "select" a particular menu choice, you would move the cursor to the appropriate choice and press [ENTER]. To "select" a field entry, move the cursor to the appropriate field and use the [+] and [-] keys to scroll to the desired preset value.

enter

The word "enter" is used when you need to specify individual alphanumeric digits. To "enter" a particular value, move the cursor to the desired field and use the [+] and [-] keys to increment or decrement each digit in the field until the desired value or letter appears.

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ICONS

The icons located in the left margin of this manual will alert you to the following situations.



WARNING! These statements alert you to situations that could result in personal injury. They also tell you how to avoid them. In addition to general warnings, this icon is used to call out chemical hazard warnings. All warnings appear in bold type.



WARNING!—Chemical Hazard! This icon alerts you to potential chemical hazards and tells you how to avoid them. All chemical hazard warnings appear in bold type.



WARNING!—High Voltage! This icon alerts you to the presence of high voltage and to the potential injury that could occur from electrical shock were you to come in contact with a specific instrument area or component. It also tells you how to avoid contact with the high—voltage areas in your instrument. All high—voltage warnings appear in bold type.



CAUTION! This icon alerts you to the correct operating or maintenance procedures needed to prevent equipment or data damage. All cautions appear in bold type.



HINT! This icon calls out general rules or shortcuts. They specify ways to obtain the best performance and results from your instrument. All hints appear in italics.



NOTE! This icon alerts you to important exceptions, side effects, or unexpected occurrences that may result form certain action(s). All notes appear in italics.

What's Next?

Now you're ready to try the practice example in Chapter 2, A *Quick Example.*

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2 A Quick Example

Introduction

In Chapter 1, you read about the three easy rules for using your autosampler's command center and some of its menus and screens. In this chapter you'll work through two examples. In the first example, you'll enter your run parameters from the autosampler's Samples Menu without first creating a file. In the second example, you'll create a file using the Edit Menu, load, and run that file. Each of these examples injects a 10 µL sample (distilled water) from the vial in position A01. In these examples you won't be changing any menu values. Instead, you'll use the default values already entered into the autosampler's menus as your run parameters. Use this practice run to make yourself comfortable with your autosampler's menus and file entry procedures, and to become familiar with the seven steps for setting up a run.

The steps for setting up a ran are as follows:

- Prepare your sample, solvents, mobile phase, vial(s), and sample tray(s)
- Enter your parameters into the Samples Menu or create an autosampler file
- Load your autosampler file
- Build your sample queue
- Enter your pump, detector, and data-output device parameters
- Install your sample tray(s)
- Start your run

The following practice examples use only a fraction of the features available on your autosampler, so we've included a more complete description of your autosampler s features and benefits first.

Before you start this chapter, be sure that you review the Safety Information section and complete the Start-up Checklist. This chapter assumes that you have installed your instrument as described in Appendix A and that your sample trays are installed.



NOTE: Your instrument is shipped with the sample trays installed. 1ffor some reason your trays are not installed, refer to Chapter 3 or to your Quick Reference Card for more information on installing your trays.

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About the SpectraSYSTEM Autosamplers

The SpectraSYSTEM autosamplers have been designed for ease of use and unsurpassed performance. Two different SpectraSYSTEM autosamplers are available each with a customized set of options and capabilities to fit a wide variety of HPLC analytical applications. SpectraSYSTEM autosamplers may be purchased with or without the built-in column oven and tray temperature control options. These options are available at the time of purchase or as upgrades. Instruments purchased without the tray temperature control option hold 120 vials; those purchased with the tray temperature control option hold 105 vials.

AS1 000

The AS 1000 fixed-loop autosampler can function as a stand-alone HPLC module or through the COMM. port as part of Thermo Separation Products' SpectraSYSTEM. In this configuration, all HPLC modules are controlled by a system controller via SpectraNET™ communication. This instrument offers additional optimization capability: it holds four user-programmable built-in files and the sample queue can process up to 39 sample sets plus one priority sample set.

AS3000

The AS3000 variable-injection volume (VIV) autosampler offers three injection methods: 1) full-loop, 2) traditional pun-loop where sample conservation is of primary concern, and 3) Thermo Separation Products' patented PushLoop® injection. Similar to the AS1000, it holds 39 user-programmable built-in files and the sample queue can process up to 39 sample sets plus one priority sample set. In addition to the built-in tray temperature control and column oven options, an automated sample-preparation option with accompanying heater/mixer is also available.

AS3500

The AS3500 autosampler has the same capabilities and available options as the AS3000. However, all "wetted" parts are made of corrosion-resistant, biocompatible materials.

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Practice Examples

Use these examples to get acquainted with your autosampler's menus. To keep these examples simple, yet still introduce you to as many menus as possible, we'll use the instrument's default parameters.

HINT: You may wish to refer to the Menu Tree in Appendix B as you work through this example. If at any time you lose your place, you can do one of the following:

- *Press [Ù] to return to a previous screen,*
  OR
- *Press [MENU] to return to the Main Menu (the top of the menu structure) and retrace your steps.*

PREPARE YOUR

Prepare your sample, solvents, mobile phase, vial, and sample tray as follows:

1. Fill a vial from your accessory kit with distilled water. Cap it securely, and place it in position A01, in the front left corner of tray A (Fig. 2.1).

   NOTE: Be sure to use either: 1) a 40=60 mil (1.0-1.5 mm) rubber or silicone septum with a 5 mil (0.1mm) Teflon™ liner on one side OR 2) a single 10 mil (0.25 mm) Teflon liner by itself.

2. Fill the flush-solvent bottle with a 70:30 MeOH:$H_2$0 solution. Refer to Appendix A for flush-bottle installation procedure.

ENTER YOUR PARAMETER

There are two ways to enter your run parameters into your autosampler: from the Samples Menu and from the Edit Menu. If you have an AS1000, you will use the Sample Menu method most often. Most likely you will want to enter into the Edit Menu those parameters that are least likely to change and those that change with each run into the Samples Menu. The Samples Menu allows you to vary individual parameters with successive runs. Should you decide to enter your parameters into the Edit Menu, there is no need to reenter them into the Samples Menu. They are automatically copied into the Samples Menu. Refer to Chapter 3 for more information on entering your run parameters. For now, you may wish to familiarize yourself with both approaches for entering your parameters.

From the Samples Menu

To enter your parameters from the Samples Menu:

1. Turn on your autosampler by pressing the on/off switch located in the lower right-hand comer of the front of the autosampler (Fig.2. 1).

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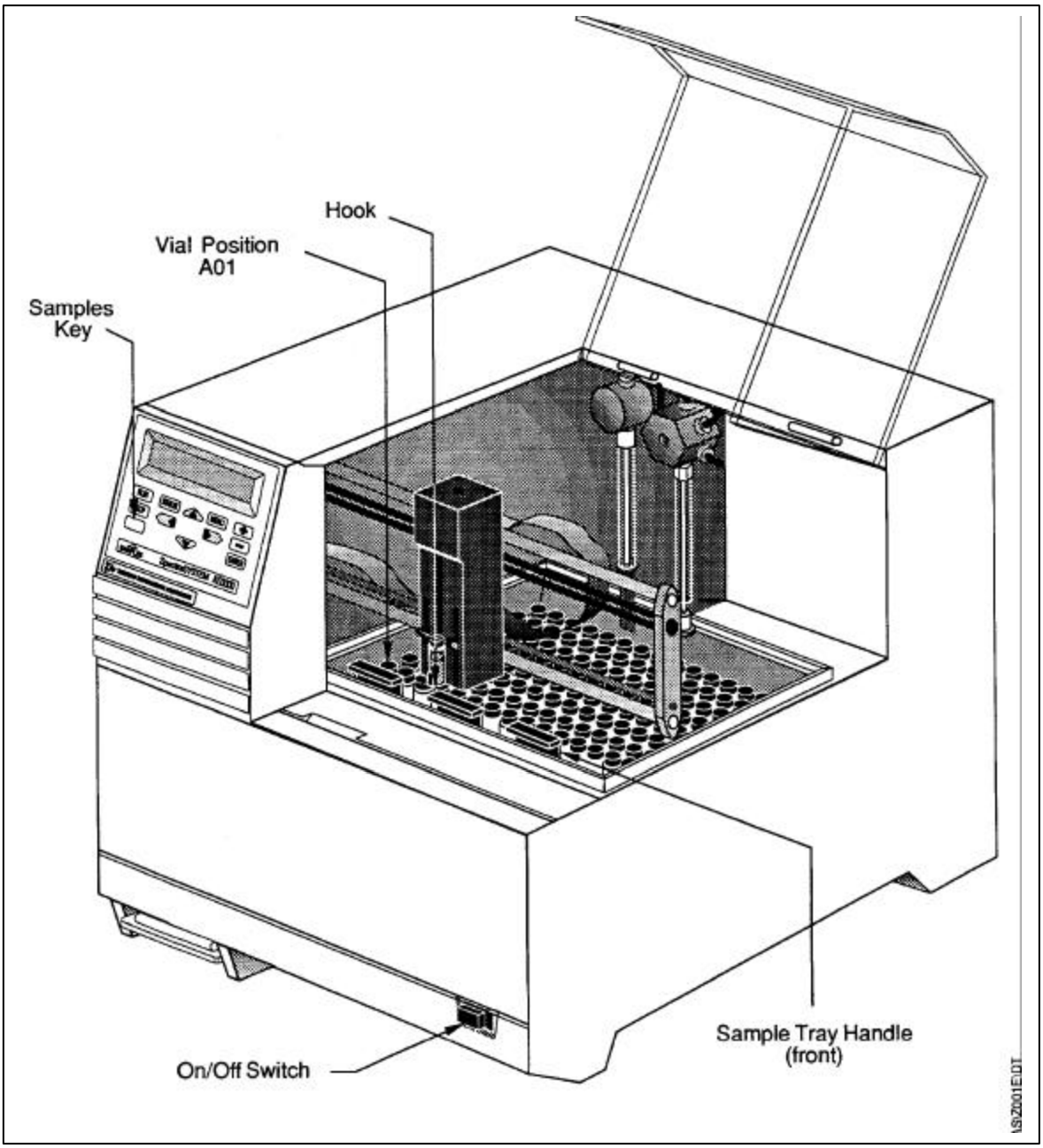


Figure 2.1 Front of the autosampler

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When the startup sequence is complete, the Status Screen appears (Fig. 2.2).

```
QStatus Set       #Samples
IDLE    0         0 / 0
```

Figure 2.2 The Status Screen

2. Press the [SAMPLES] key to display the Samples Menu (Fig. 2.3a). When the STATUS Menu first appears, the file name field is blank. Figure 2.3a shows how this field will appear when a file name is inserted.

```
Sample Set                          1
File 1:  FILENAME
-------------------------------------
Loop Size                          20
Injection/Sample                    1
Cycle Time                       10.0
First Sample Vial                A01
Number of Samples                   1
Add to Queue?                    Yes
```

Figure 2.3a The Samples Menu (AS1000)

```
Sample Set                          1
File 1:  FILENAME

Loop Size                          10
Injection/Sample                    1
Cycle Time                       10.0
First Sample Vial                A01
Number of Samples                   1
Add to Queue?                    Yes
```

Figure 2.3b The Samples Menu (AS3000)



3. Use the [∨], [<], [>], [+], and [-] keys to change the Cycle Time to 1.0.

4. Press the [∨] key to move down the Sample Menu and press [ENTER] to select “Yes” to add your sample set (1) to the queue.



NOTE: We’ll tell you more about building your queue later on in this chapter. For now, proceed to step 5.

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5. Press [RUN] to start your run. During the run, the autosampler hook picks up the vial, carries it to the sample tower, injects the sample, returns the vial to the sample tray, and flushes the lines. When the run is complete, the instrument' s status returns to "IDLE."

From the Edit Menu

In this example you'll use the Edit Menu to "create" and run the practice file. You'll use this method when you want to create more complex files and/or to create more than one file before starting a run. You will first specify a file name and then press [ENTER] in each menu field to accept its default value.

Use the following steps to create your autosampler file.

NOTE: If you worked through the last example, the autosampler returned you to the Status Screen at the completion of the run. Go on to step 1.

NOTE: If you skipped the last example, turn on your autosampler and wait for the Status Screen to appear. Then proceed to step 1.

1. Press [MENU] and select /FILES/ , /Edit/ to display the Edit Menu (Fig. 2.4). Notice that File 1 is already entered into the Edit File field. Press [ENTER] to edit File 1.

```
> Edit File                                    1_
File Name:  PRACTICE

Injection
Calibration
Timed Events
Sample Prep   (Appears only with Sample Preparation Option installed)
More
```

Figure 2.4 The Edit Menu

2. Use the [+], [-], and arrow keys to specify each character in the file name of your choice. We've chosen "PRACTICE" as our file name.

3. Move the cursor to each field in the Injection, Calibration, Timed Events, and More Menus and press [ENTER] to accept their default values. (If you were running a file other than this practice file, you would, of course, select other entries while moving through these menus.)

***NOTE**: For the practice example, press the [Ú] key to skip the Sample Prep Menu.*

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Notice that after the last field in each menu, the autosampler returns you to the next most probable choice in the menu structure. For example, after you accepted the Calibration Menu's default values, the autosampler automatically returned you to the Edit Menu with the cursor in the Timed Events field. Similarly, after you accepted the More Menu's default values, the autosampler places the cursor in the Load field of the Edit Menu. This is a pattern that occurs throughout the user interface. The instrument "anticipates" the menu choice you are mostly likely to need next. You can, of course, override that choice at any time by moving the cursor wherever you like.

LOAD YOUR FILE

Now that you've created your practice file, use the following steps to load your practice file manually.

1. Select /Load/ from the Edit Menu.
2. Verify that File I is entered, and press [ENTER] to load it into the autosampler's active memory. The message ""File Loaded"" appears briefly. Then the Status Screen is displayed (Fig. 2.5).

Loading the file manually activates any installed options (column oven or tray temperature control) that you set in the Injection Menu.

QStatus	Set	#Samples
IDLE	0	0 / 0

Figure 2.5 The Queue Status Screen with the queue idle

BUILD YOUR QUEUE

Build your sample queue by adding Sample Set I (that contains your practice sample) to the sample queue as follows:

1. Press [SAMPLES] to display the Samples Menu (Fig. 2.3).

2. Press [ENTER] in each field to accept the Samples Menu's default values.

If you were injecting an actual sample instead of water, you would enter your pump, detector, and integrator/recorder or data-system parameters now. For this example, however, simply load your trays, and close the autosampler's cover.

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START YOUR RUN

To start your run, press [RUN]. If everything's running properly, the Status Screen's QStatus field will read "RUN" (Fig. 2.6). The injection status field displays the time elapsed since the run was started.

QStatus	Set	#Samples
RUN	1	1 / 1

Figure 2.6 The Queue Status Screen with the queue running

Status	Vial	Vol	#Inj
0.05	A01-S	10	1 / 7

Figure 2.7 The Injector Status Screen with a run in progress

As you know from the first example, the autosampler injects vial A01. After the run is complete and the sample queue is empty, the vial is returned to the tray, and the instrument's queue status (QStatus) returns to "IDLE."

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3 Basic Operations

Introduction

This chapter describes the routine operation of the SpectraSYSTEM autosamplers. In this chapter you will learn the steps required for setting up and performing a run on your autosampler as follows:

- Prepare your chemicals
- Create a file that contains your autosampler run parameters
- Load your file
- Build your sample queue using the [SAMPLES] key and Samples Menu
- Start and stop a run
- Monitor the run status
- Change the parameters of a run in progress
- Insert a priority sample set into the queue

There are four options available for the AS3000 and AS3500:

- Column oven
- Tray temperature control
- Sample preparation
- BCD communication

The menus and fields used to control these options appear only if they are installed. This chapter includes the column oven and tray temperature control procedures. Chapter 4, *Advanced Operations*, describes the sample preparation menus (templates) and procedures. If you purchased the Sample Preparation Option, you'll first want to learn the procedures described in this chapter and then proceed to Chapter 4 to learn how to use the sample preparation templates and the heater/mixer. Contact your representative for information on the BCD option since this option requires specific installation procedures.

Before You Start

Before beginning this chapter, be sure that you've completed the Start-up Checklist found in the beginning of this manual and installed your instrument according to the procedures described in Appendix A.

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Run Preparation

The SpectraSYSTEM autosamplers allow you to process up to 39 non-priority sample sets (groups of sample vials and associated calibration vials) within a given run. To prepare for a run, use the following steps:

- Prepare your samples, solvents, and mobile phase
- Create your file by entering the parameters for each sample set into one of the four built-in files
- Load your file
- Build your sample queue
- Start your run

Once your run has started, you can:

- Monitor the progress of the run
- Modify the currently running file (the run file) to affect subsequent vials in the active sample set
- Change the run order of, add, or delete sample sets from the sample queue
- Add a priority sample set to the queue without disrupting the calibration routine of the active sample set

These procedures, along with the instrument's file-management features, are described in this section.

PREPARE YOUR CHEMICALS

Prepare your samples, solvents, and vials. Be sure that your sample(s) is (are) completely soluble in the mobile phase and that you have filtered your samples and solvents through a 0.5-micron filter. These techniques minimize sample precipitation in the lines and remove any particulate matter that could obstruct the flow through the autosamplers injector or column. Fill the solvent reservoir with flush solvent.



***NOTE**: If you are using the automated Sample Preparation Option, you can use up to four solvents. Refer to Chapter 4 for instructions on filling your external solvent reservoirs and for setting up solvent selection in your autosampler file.*

CREATE YOUR FILE

The group of autosampler parameters applied to a specific sample set is contained in a file. When your instrument is shipped, each of its four built-in files contains the default values. To create your own file, simply edit one of the existing files by entering your own parameters into the autosampler's Edit Menu. From the Edit Menu you can name your file and access additional menus to enter your injection, calibration, timed events, sample preparation, and other run parameters.

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***NOTE**: When you have entered all of your parameters in the Edit Menu, you7l have finished creating your edit file. You can't run your edit file by pressing the [RUN] key. Instead, you first have to load your edit file into the run file. For more information, see "Load your file" on page 30.*

To create your edit file:

1. Press the [MENU] key to display the Main Menu. From there, select /FIILES/, /Edit/ to display the Edit Menu (Fig. 3. 1).

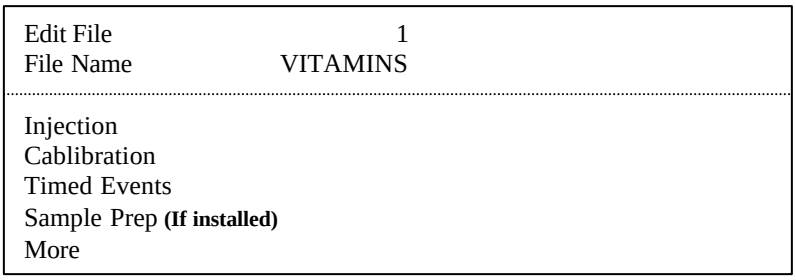


Figure 3.1 The Edit Menu

Edit File

An autosampler file is identified by its number (1 -4) and name, which you supply. (See "File Name," below.) The Edit File field indicates the number of the selected file. File I automatically appears in this field when the Edit Menu is first displayed. If you wish to edit a different file, press the [+] and [-] keys to select a different file number.

File Name

The File Name field allows you to name your file. When you first display the Edit Menu, the File Name field contains eight blank characters. Use the [+]/[-] and [<]/[>] keys to create a file name made up of the following characters: A-Z, 0-9, \ blank, dash, or hyphen.

Injection Menu

Selecting /Injection/ from the Edit Menu accesses the Injection Menu (Figs. 3.2a and b) from which you can enter your injection parameters. The next section describes the Injection Menu's fields.

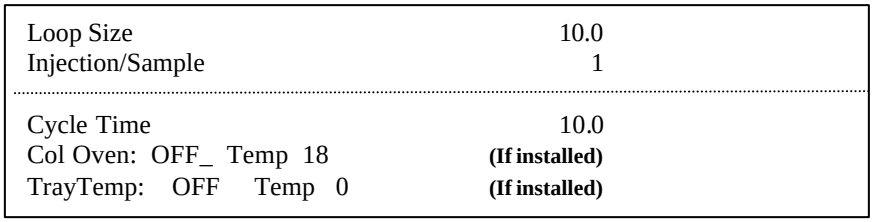


Figure 3.2a The AS1000 Injection Menu

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Loop Size			10.0
Injection/Sample			1
Cycle Time			10.0
Oven:	OFF	Temp 18	**(If installed)**
TrayTemp:	OFF	Temp 0	**(If installed)**

Figure 3.2b The AS3000 Injection Menu

Injection Volume (Loop Size)

Use the first field of the Injection Menu to specify the sample Loop Size (if you are using an AS 1000) or the sample injection volume (if you are using an AS3000). The injection volume value depends upon the injection method and range that you entered in the More Menu's Injection Type and Injection Range fields, respectively, (see page 28). To optimize injection volume, see page 233.

Full-loop injection: If you're using either full-loop injection on an AS3000 or an AS 1000, you can use the [+] and [-] keys to select one of the following values: 5, 10, 20, 50, 100, 200, 500, and 1000 PL.



***NOTE**: Be sure that the Loop Size field value reflects the size of the sample loop installed.*

Variable-Loop Injection: You can choose either Pull or Thermo Separation Products' patented PushLoop injection from the More Menu. These methods are described on page 29. If you use pull-loop injection, you can select a sample-injection volume from 1-1500 µL. If you use PushLoop injection, you can specify a sample-injection volume from either 0. 1 - 10.0 µL or 1-200, depending upon which Push type (Hi or Lo) you selected in the More Menu's Injection Type field. You should limit the injection volume to approximately one half the sample loop size.

Injection Sample

In this field, specify the number of injections to be made from each vial (1-99). If you have selected a sample preparation template, and want sample preparation without injections, enter 0 in this field.



***NOTE**: You can't enter a 0 unless you have first selected a sample preparation template.*

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Cycle Time

In the Cycle Time field enter the desired time (0-655.3 minutes) between the autosampler's injections (from the injection to the end of the ran). In calculating this value, be sure to allow enough time for the column to equilibrate (if you are running a gradient), all peaks to elute from the column, and for the integrator to print any post-run reports and perform any post-run calculations.

The autosampler is capable of making an injection approximately every 90 seconds, depending upon the injection and flush volumes selected. If your cycle time is controlled by a Pump Ready contact closure, you may set the Cycle Time to 0.0.



The default flush volume is 400 mL, so that the total injection cycle time (including the flush cycle) is approximately two minutes.

Column Oven

This field appears only if you purchased the Column Oven Option and set it to ON in your Configurations Menu. To use the column oven, move the cursor to the Col. Oven field and press the [+] and [-] keys to toggle the oven's state between on and off. In the Temp field in the same line, you can specify the oven temperature in one-degree increments from 15-800C.



***NOTE**: You can specify temperatures starting at 5'C above ambient.*



***NOTE**: Even if you set the oven's state to On, the oven isn't activated (turned on) until you load your file.*



***HINT**: The autosampler automatically delays the start of the run until the set temperatures have been reached. Once activated, the oven requires about 15 minutes to reach a temperature of 70'C. To save time, you "my wish to load your file so that the oven can be warming up while you're completing your run preparation and entering the file parameters for your run. See page 30for more information on loading your file.*

Tray Temperature Control

This option allows you to heat or cool the sample tray within a range of O'C to 60'C (in I' increments). This field appears only if you purchased the tray temperature control option and specified Yes in the Configurations Menu Tray Temp field. If you plan to use tray temperature control, use the [+] and [-] keys to turn on the tray heater/cooler in the Tray field. Then specify the temperature in its Temp field. If set to "On" in the Injection Menu, the heater/cooler will not be activated until you load the file manually (by pressing Load from the Files Menu), or automatically when you press [RUN] to start your queue.

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HINT.- *The autosampler automatically delays the start of the run until the set temperatures have been reached. Once activated, if empty, the tray requires about 30 minutes to reach a temperature of 0 'C and about 10 minutes to reach a temperature of 60 'C. To save time, you may wish to load your file so that the heater1cooler can be coming to temperature while you are completing your run preparation. See page 30for more information on loading your file. You can also save time by preheating or precooling your samples.*

The autosampler's Tray Temperature Control Option is primarily designed to maintain the temperature of the sample compartment within ± 2 'C. The actual temperature inside the autosampler vial is dependent upon the specified tray temperature, type of vial, sample buffer, etc. The following graph (see Fig. 3.2) indicates the approximate vial temperature given a specified tray set temperature.

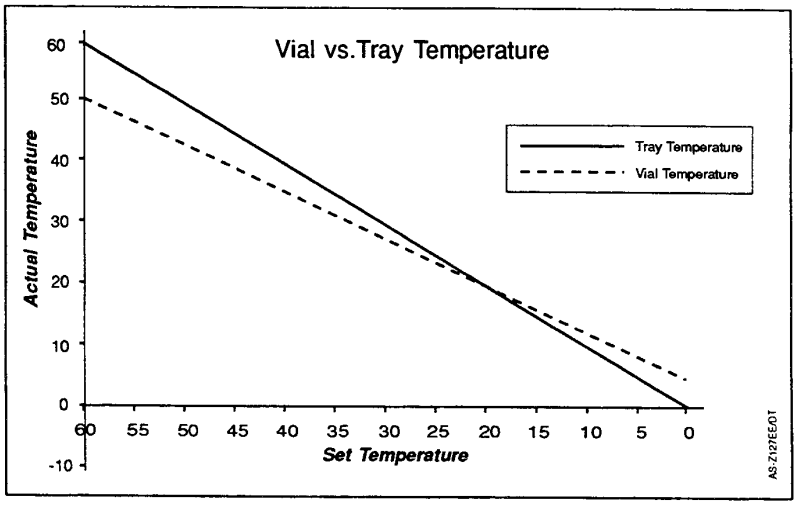


Figure 3.3 Plot showing the actual vial and tray temperature at a specified set temperature

The Tray Temperature Control Option can also be used to heat and cool samples. The time lag in heating and cooling is due to variations in ambient temperatures, indirect contact with temperature control units, and vial type. The accompanying graph (see Fig. 3.3) indicates the time required to heat (to 60'C) or cool (to O'C) the tray and vials from an ambient temperature of 23'C.

HINT: A 5'C change in ambient temperature results in approximately a PC change in vial temperature.

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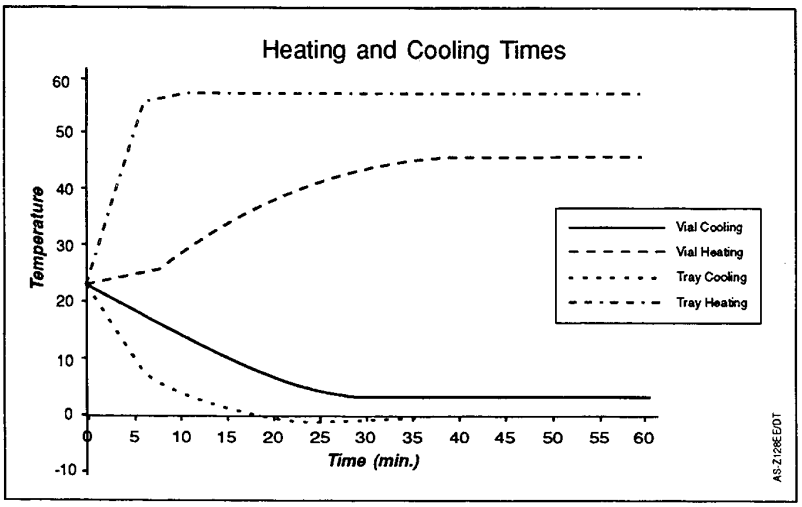


Figure 3.4 Plot of heating and cooling times of a standard glass vial and the autosampler tray. The starting ambient temperature was 230C.



HINT: You can save time by preheating or cooling your samples before placing them into the autosampler.

Calibration Menu

By selecting Calibration from the Edit Menu, you can call up the Calibration Menu (Fig. 3.5). This section tells you how to enter your calibration parameters and how each is used by other autosampler menus.

```
Number of Levels                    0
Samples/Calibration                 0
-------------------------------------------------------
Injections/Level                    1
Injection Volume                   10
Type                 Reuse same vials
Bracket samples                    No
```

Figure 3.5 The Calibration Menu

Number of Levels

Specify the number of calibration levels (0-9). The autosampler automatically copies this value to the Calib Vial 1 (of X) field in the Samples Menu, where X equals the number of levels. (Refer to page 32 for more information.) A value of zero in the Number of Levels field indicates that no calibration injections will be made for the specified sample set.

SamplesICalibration

From this field you can specify the calibration interval (the number of

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sample vials to be injected between each recalibration).

Injections/Levels

In this field you specify the number of injections (1-99) from each calibration level (0-9).

Injection Volume

The Injection Volume field in the Calibration Menu allows you to specify an injection volume for your calibration vials that is different from the sample injection volume you specified in the Injection Menu.

Similar to the Injection Volume field in the Injection Menu, this value depends upon the injection method you select in the More Menu's Injection Type field. As with the sample injection volume, you can choose between 0.1-10.0 µL and 1-200 µL if you are using PushLoop injection. (See page 28.)



***NOTE**: This field is not displayed if you have selected Full from the More Menu's Injection Type field or if you are using an AS1000. y*

Using variable-volume injection, you can choose either Pull or Thermo Separation Products' PushLoop injection from the More Menu. These methods are described on page 28. If you use pull-loop injection, you can select a calibration-vial injection volume from I200 RL. If you use PushLoop injection, you can specify a calibration vial injection volume from .1-10.0 µL or from 1-1500 RL, depending upon which Push type you selected in the Injection Type field. You should limit the injection volume to approximately one-half the sample loop size.

If you're using either full-loop injection on an AS3000, or an AS 1000, you can use the [+] and [-1 keys to select one of the following values 5, 10, 20, 50, 100, 200, 500, and 1000 µL. We recommend that you enter your sample-loop volume as your calibration-vial injection volume.



***NOTE**: If you want to determine optimum syringe and sample loop size, see page 233.*

Type

This field allows you to specify one of two types of patterns: 1) Reuse same vials or 2) Intersperse vials.

Reuse same vials programs the autosampler to use the same calibration vials each time a recalibration is made. Thus, each time a calibration is required, the autosampler goes to the first calibration vial position specified in the Sample Menu's Calib Vial 1 (of X) field and injects all the calibration vials. It then injects the number of samples indicated by the Calibration Menu's Samples/calibration value.

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***NOTE**: If you have selected the Reuse vial calibration type, do not place calibration vials between sample vials. Instead, place them before the sample vials in the same tray or in a different location.*

***NOTE**: When you reuse your calibration vials, you may place them in any positions in the sample tray(s). However, if you are doing multiple-level calibrations, your calibration vials must be placed sequentially in the tray.*

Intersperse vials programs the autosampler to use each calibration vial only once. You must manually intersperse your calibration vials between your sample vials within the sample set. The autosampler makes injections in tray-placement order. If you've specified a different injection volume for calibration vials versus sample vials, the autosampler uses the Samples/Calibration field value to determine whether the next vial is a calibration or a sample vial. It determines the location of the first calibration vial from the value entered in the Samples Menu's Calib l (of X) field. Figure 3.6 illustrates the vial order used by the two different calibration routines.

Injection Order	Reuse	Intersperse
C	A01	A01
C	A02	A02
S	A03	A03
S	A04	A04
C	A01	A05
C	A02	A06
S	A05	A07
S	A06	A08
Total vials used: 6		

Figure 3.6 The different injection orders used by the Reuse and Intersperse calibration routines

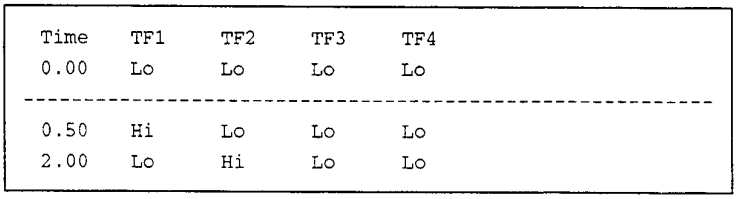
Bracket Samples

This field lets you specify whether or not you want to automatically end the sample set with a recalibration. If you select Yes, the autosampler processes the last sample and then repeats the calibration series specified for the sample set. If you select No, the autosampler simply stops and returns home after the last sample is injected.

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Timed Events Menu

Accessed from the Edit Menu, the Timed Events (external events) Menu (Fig. 3.7) allows you to create a time program to turn on/off external devices (such as column-switching valves and fraction collectors) at preset times during your nm. Pins 9-12 of the autosampler's 12-pin terminal are used for Timed Events. In this section, we'll show you how to create a time program. Refer to Appendices A and D for connecting external devices to your autosampler.



Time	TF1	TF2	TF3	TF4
0.00	Lo	Lo	Lo	Lo
0.50	Hi	Lo	Lo	Lo
2.00	Lo	Hi	Lo	Lo

Figure 3.7 The timed Events Menu

The Timed Events Menu holds a maximum of nine lines, each of which sets Hi/Lo one or more timed event outputs at the time specified. For example, in Figure 3.7, Time Function I (TFI) is set Hi (activated) at 0.5 minutes into the run; at 2 minutes into the run, Time Function 2 (TF2) is set Hi while Time Function I is set Lo (deactivated). By changing the Time value in each line, you can add, delete, or insert menu lines.

Adding a Line

To add a line to the end of your program:

1. Press [v] to move the cursor to the blank line after the last displayed line.

2. Press the [+] key to copy the last existing line with a new time incremented by one minute.

3. Use the arrow keys and the [+] and [-] keys to change the time and set the time functions' states (Hi/Lo).

Deleting a Line

To remove a line:

1. Move the cursor to the Time field in the line to be deleted.

2. Hold down the [-] key until the value is zero (0.00).

3. Press the [-] key once to erase the line.

If you are deleting a line from the middle of a timed events program, the blank line remains until you move the cursor to another line.



***NOTE**: If you delete both of the visible lines on the display, both lines disappear, but the cursor remains. Press* [A] *or [vj to move to the remaining lines in the program.*

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Inserting a Line

To insert a line between two existing lines:

1. Move the cursor to the blank line at the end of the program.
2. Enter a time that falls between two existing times.
3. Move the cursor off the new line. The autosampler automatically inserts the new line.
4. Then move the cursor to the newly inserted line(s) and set the Timed Events Outputs' states as desired.

Figures 3.8a-c illustrate this process.

Time	TF1	TF2	TF3	TF4
0.00	Lo	Lo	Lo	Lo
1.00	Lo	Lo	Hi	Lo
2.00	Lo	Hi	Lo	Lo

Figure 3.8a The original timed events program

Time	TF1	TF2	TF3	TF4
0.00	Lo	Lo	Lo	Lo
1.00	Lo	Lo	Hi	Lo
2.00	Lo	Hi	Lo	Lo
0.50	**Lo**	**Hi**	**Lo**	Lo

Figure 3.8b The original program with an added line -- unsorted

Time	TF1	TF2	TF3	TF4
0.00	Lo	Lo	Lo	Lo
0.50	**Lo**	**Hi**	**Lo**	Lo
1.00	Lo	Lo	Hi	Lo
2.00	Lo	Hi	Lo	Lo

Figure 3.8c The timed events program with an added line -- sorted



***NOTE**: The Run-time clock stops and resets at the completion of the Cycle Time. Time lines longer than the Cycle Time value will never be activated.*

Sample Prep

Once you've entered your external events parameters, the autosampler returns you to the Edit Menu with the cursor in the /Sample Prep/ field (if installed). If you did not purchase the Sample Preparation Option, this field won't appear in the Edit Menu. Instead, the autosampler will return you to the Edit Menu with the cursor in the /More/ field. See Chapter 4 for detailed information on the Sample Preparation Option.

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More Menu

The More Menu (Fig. 3.9) allows you to enter the remainder of your run parameters, each of which is discussed below.

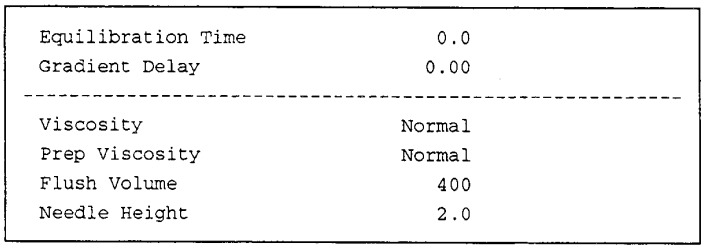


Figure 3.9a The AS1000 More Menu

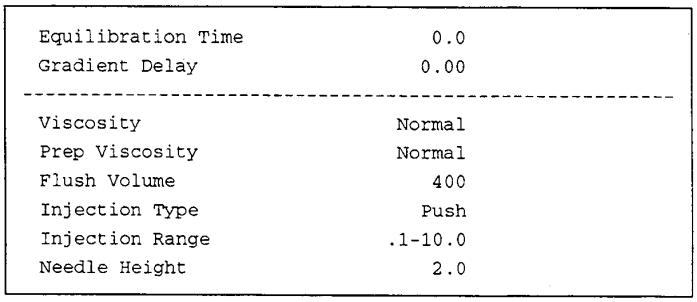


Figure 3.9b The AS3000 More Menu

Equilibration Time

In this field, specify the length of time (in minutes) required for the column or LC system to equilibrate. The equilibration time allows time for the column to equilibrate prior to the first injection of a new sample set. It is only in effect before the first injection. The default value of 0.0 is a good starting value for many applications.

Gradient Delay

The gradient delay field is used to specify the amount of time required to allow the mobile phase to travel from the pump's proportioning valve to the injection valve. This field is used only if you are running a gradient. To determine the gradient delay value, you'll need to calculate the precolumn volume, which is best done during installation. Refer to Appendix D for a procedure for calculating your precolumn volume and gradient delay value.

Viscosity

Specify the sample viscosity (Normal, Medium or Viscous). The viscosity setting affects the sample syringe draw-rate after the sample loop fails. A higher viscosity setting automatically decreases the syringe draw-rate to give a more viscous sample more time to be drawn into and to travel through the sample loop.



HINT: Use the Normal setting for running most applications.

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Prep Viscosity

Specify the viscosity (Normal, Medium or Viscous) of the sample preparation sample. The viscosity setting affects the preparation syringe (2.5 mL draw-rate. A higher viscosity setting automatically decreases the syringe draw-rate and increases the injection delay to give a more viscous sample more time to be drawn into and to travel through the sample lines. This also reduces cavitation caused by low boiling point solvents.



***NOTE**: This field appears only if the Sample Preparation Option is installed.*



***HINT**: When performing normal-phase chromatography, the viscous setting maximizes performance.*

Flush Volume

Specify the flush volume to be used for flushing between injections. The acceptable range is 200 - 5000 µL The default is 400 µL.

Injection Type (AS3000 and AS3500 only)

With the AS3000, you can choose from four different injection methods: 1) full-loop, 2) pull-loop 3) Thermo Separation Products' PushLoop injection using the high (Push Hi) injection volume range (1 -200 µL) and 4) PushLoop injection using the low (Push Lo) injection volume range (0.1-10 p-Q. The advantages of each type are j briefly described here. Refer to Appendix C for a description of how each method operates and for the excess sample volume required for injection.

The optimum injection method depends upon the amount of sample you have and the degree of precision desired.

Full: Full- or fixed-loop injection is useful when you want maximum precision and have unlimited sample. The maximum injection volume is 1000 µL and the range from 5-1000 µL.



***NOTE**: If you select full-loop injection, the Injection Menu's Injection Volume field changes to Loop Size and the Calibration Menu's Injection Volume field does not appear.*

Pull: Pull-loop injection (traditional variable-volume injection) is useful when sample conservation is important and when large injection volumes (>I 00 µL) are required.

Push: Thermo Separation Products' Flush Loop injection is a variable volume method that gives the precision of fixed-loop injection with limited amounts of sample. This method is most effective for smaller injection volumes (< 10 µL).

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Injection Range

The Injection Range field displays the sample- and calibration injection volume range, depending upon the Injection Type that you selected. For full-loop injection, the range is 5-1000 µL. For pull loop injection, the Injection Range field is automatically fined in with the 1-1500-microliter range. For PushLoop injection, the ranges are 1) 0. 1 - 10.0 microliters (Push Lo) or 2) 1-200 microliters (Push Hi), depending upon your sample-loop size.

Needle Height

The Needle Height field enables you to control the distance between the needle-tip and the bottom of the vial (0.0-20.0 mm). The default value is 2.0 mm.



***NOTE**: With this value, you are actually controlling the vial's height relative to the needle tip. The vial moves; the needle is stationary.*

SAVE YOUR FILE

Your file is saved automatically. Once you have filled in all of the Edit Menu's parameters, you have finished creating your edit file. Exiting the Edit Menu automatically saves your edit file but does not load it. For more about file management, see page 46.



***NOTE**: Pressing [RUN] after creating your file will not start your run. You must first add it to the sample queue. For more information, see "Build Your Sample Queue" below.*

LOAD YOUR FILE

The Load function is useful when you want to run a single sample set manually, for example to turn on your oven or tray heater/cooler.

To load your file, press /FILES/, /Load/. The Load Menu appears:

```
Load File 1:  FILENAME
```

If you want to load the last file you edited, use the [+) and [-] keys to select its file number and press [ENTER]. If, instead, you want to load a different file, select the correct file number and then press [ENTER]. The message **File Loaded" will be displayed. Once a file is loaded, it becomes your run file.

The Load function is used to run a single sample set; however, if you want to run a group of sample sets, this is done using the sample queue, by pressing [RUN]. Once [RUN] is pressed, the autosampler automatically loads each file into the queue before running it. You don’t need to manually load each file. For more information on running your samples from the sample queue, see "Build Your Sample Queue" below.

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BUILD YOUR SAMPLE QUEUE

The sample queue is the list of sample sets and associated files to be run. Building your sample queue requires adding each item to the queue from the Samples Menu and then arranging those items in the desired run order from the Queue Menu. If you run a group of files from the sample queue, each file is automatically loaded before it is run. These procedures are described below.

Adding Items to the Sample Queue

To add an item to the queue, press [SAMPLES] to access the Samples Menu. Then enter each of the values shown in the Samples Menu (Fig. 3. 10):



***HINT**: Since the method downloads most of the values automatically, the only values that require editing are those in the File Number and Vial location fields.*

Sample Set	1
File 1:	VITAMINS
Loop Size	100
Injections/Sample	1
Cycle Time	0.1
Calib Vial 1 (of 1)	A01 (*If calibration is used*)
First Sample Vial:	A02
Number of Samples	1
Add to Queue?	Yes

Figure 3.10a AS1000 Samples Menu

Sample Set	1
File 1:	VITAMINS
Injection Volume	100
Injections/Sample	1
Cycle Time	0.1
Calib Vial 1 (of 1)	A01 (*If calibration is used*)
First Sample Vial:	A02
Number of Samples	1
Add to Queue?	Yes

Figure 3.10b The AS3000 Samples Menu

Sample Set

Select the desired sample set you want to add to the queue. Each sample set can be used only one time in the sample queue. You can load a maximum of 39 sets into the sample queue, plus one priority set.

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File
Specify the number of the file to be applied to the specified sample set. The file's parameters will determine the sequence of Sample Set prompts.

Injection Volume, Injections/Sample, and Cycle Time
These values are copied from the Injection Menu of the associated file.

Calib Vial 1 (of X)
This value is copied from the Calibration Menu of the associated Me. Specify the location of the first vial in the calibration series. "X" is an indicator of the number of calibration levels specified in the file.



***NOTE**: The vial position automatically wraps. For example, if the first of three calibration vials is placed in C38, the first sample vial will be A01 for instruments without tray temperature control.*

First Sample Vial
Specify the first sample-vial location.

Number of Samples
Enter the number of sample vials in the sample set.

Add to Queue
Select Yes or No and press [ENTER] to add the selected sample set to the queue. Each set can be used only one time in the sample queue.

Once you have filled in the Samples Menu for the first sample set, pressing [SAMPLES] again returns you to the top of the menu so that you can add the next sample set.

Ordering Sets in the Sample Queue

You add items into the queue from the Samples Menu, which you access by pressing [Samples]. You can reorder them in the Queue Menu (Fig. 3.11). You can reorder any sets not currently running.

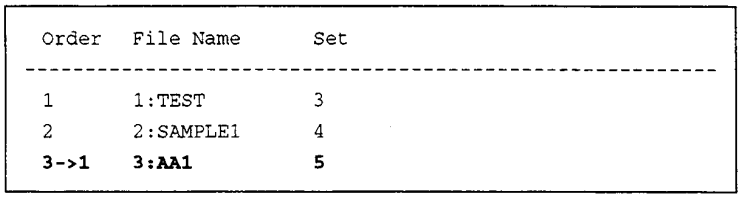
Order	File Name	Set
1	1:TEST	3
2	2:SAMPLE1	4
3	3:AA1	5

Figure 3.11 The Queue Menu with no priority-vial sample sets in the queue

Changing the Order of Sets Within the Queue

To change the order of items in the queue, move the cursor to the line where the change in order should occur and increment/decrement to the new Order value. Refer to the example in Figure 3.12. To run sample set 5 before sample set 3, move the cursor to the order column of the sample-set 5 line, and decrement the Order value to 1 (Fig. 3.12).

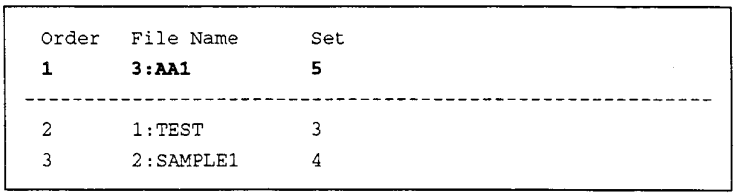
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Order	File Name	Set
1	1:TEST	3
2	2:SAMPLE1	4
3->1	**3:AA1**	**5**

Figure 3.12 Changing the order of sets in the queue

The lines rearrange themselves after you exit the field (Fig. 3.13).



Order	File Name	Set
1	**3:AA1**	**5**
2	1:TEST	3
3	2:SAMPLE1	4

Figure 3.13 The Queue Menu after the Order has been changed



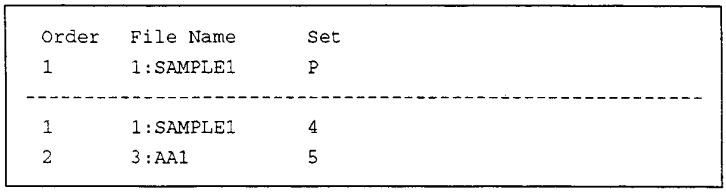
***NOTE**: Remember, Order 1 can only be changed if the sample set is not active. (When you press [Status] the QStatus column should read IDLE).*

Deleting Sets from the Queue

To delete an item from the sample queue, move the cursor to the line containing the item to be deleted, decrement the Order number to 0 and then blank. Moving the cursor to another line deletes the blank line from the display.

Managing a Priority Set Within the Queue

A priority set is inserted into the active set and its samples ran before other samples in the active set. It must be assigned to the active file. It appears in the Queue Menu with a P in the Set field (Fig. 3.14). You can't assign or change its priority status from the Queue Menu. A priority set will automatically be deleted from the queue upon completion of the active set or when the queue (QStatus) returns to its IDLE state (if you press [STOP]). For more information on running priority vials, see page 42.



Order	File Name	Set
1	1:SAMPLE1	P
1	1:SAMPLE1	4
2	3:AA1	5

Figure 3.14 A sample queue that contains a priority set

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ENTER YOUR OTHER LC PARAMETERS

Prepare your other LC modules for a run and enter the run parameters for your pump, detector and data-output device (recorder, integrator, or data system). Before you start your ran:

- Initialize your pump and degas your solvents as required for your application.
- Be sure your detector is stabilized. Don't start your analysis until you've achieved a stable baseline.
- Set up your data-output device.

For more information on connecting your autosampler to other LC modules, see Appendices A and D.

INSTALL SAMPLE TRAYS

Place your calibration and sample vials into the sample trays included in your Accessory Kit. Trays A, B, and C are arranged in the autosampler from left to right. Each tray contains 40 (35, with Tray Temperature Control) vials arranged in eight (seven) rows of five (Fig. 3.15). The first vial position is A01; the last is C40. The position labels are on the left of each position.



***NOTE**: If you purchased the Tray Temperature Control Option, each sample tray contains 35 vials.*

Your autosampler comes with a supply of vials, silicone and Teflon® septa, and screw-top vial caps. Additional vials may be ordered from LabAlliance. Be sure to use standard size vials to ensure smooth autosampler operation.



***NOTE**: Be sure to use either: 1) a 40-60 mil (1.0-1.5 mm) rubber or silicone septum with a 5 mil (0.1 mm) Teflon liner on one side or 2) a single 10 mil (0.25 mm) Teflon liner by itself.*

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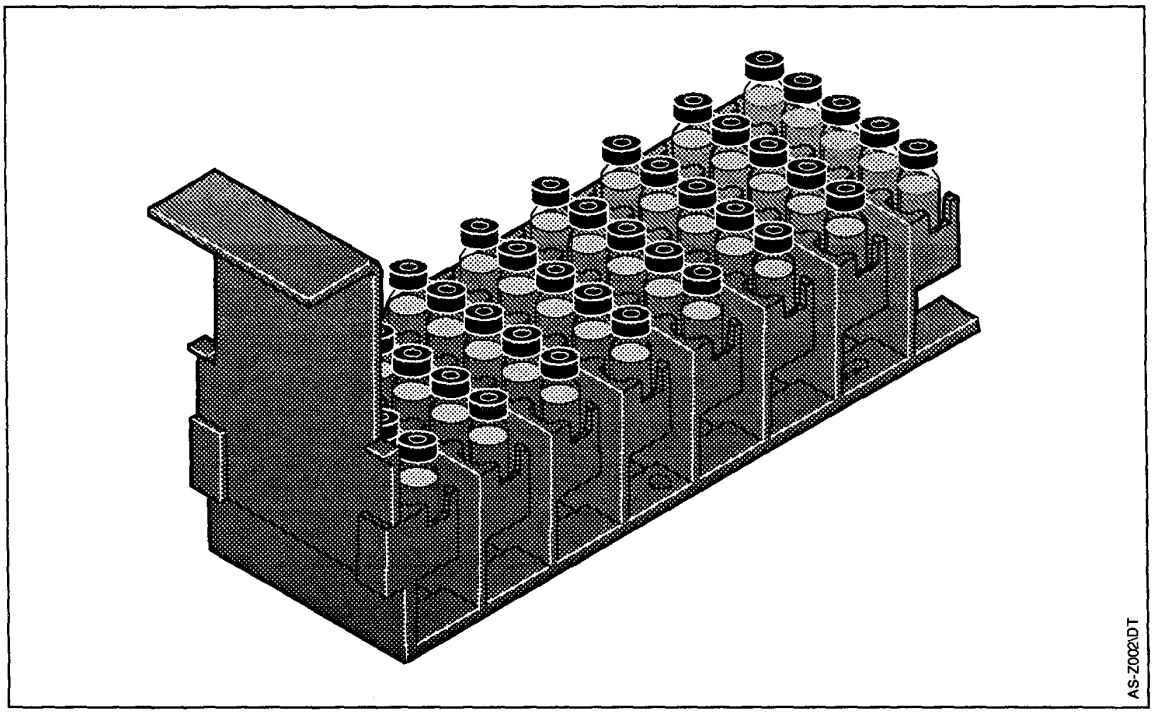


Figure 3.15 An autosampler tray

Place the calibration vials, and samples into the trays. After your vials are in place, install sample trays A, B, and C as follows:

1. Hold the tray handle, tilting the back end down (Fig. 3.16).

2. Insert the tray's "runners" into the slots in position A at the rear of the tub.

3. Lower the front of the tray into place and press down firmly to seat the tray.

***HINT**: Pull the handle back toward you until the tray snaps into place. Try moving each tray from side to side to be sure that it's seated completely.*

4. Insert the other two sample trays into positions B and C.

CAUTION! Be sure that each tray is properly seated. Each tray's upper front edge should fit under the upper lip of the tub. If a tray is not completely seated, the autosampler *will* not be able to correctly pick *up* or return vials to that sample tray.

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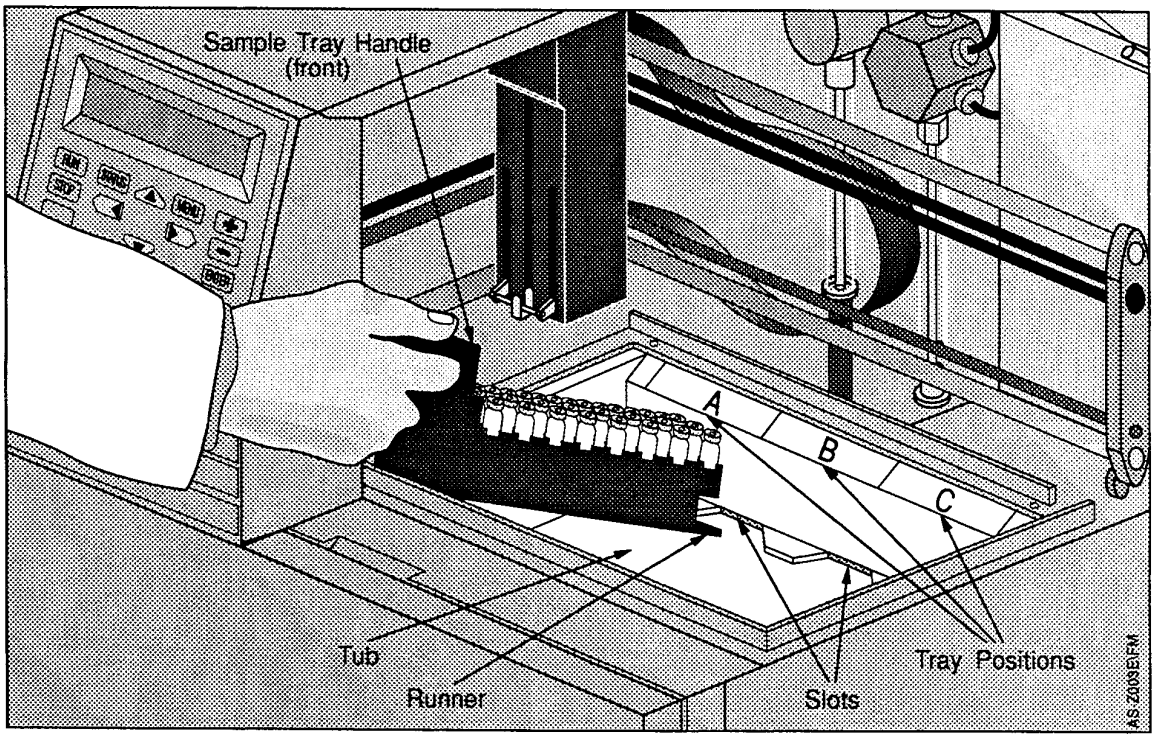


Figure 3.16 Installing a sample vial tray

Starting Your Run

Once you have created and loaded your file, added your sample set(s) to the sample queue, and installed your sample tray(s), you're ready to start your run.

Press [RUN] to start your run. If there are sample sets in the sample queue, the sample queue's "RUN" Status is displayed on the Status Screen (Fig. 3.17).

If the queue is empty, the autosampler prompts you to access the Samples Menu so that you can add sample sets to the queue. After you have added sets, press [RUN] to start your run.

MONITORING THE RUN STATUS

When [RUN] is pressed, the autosampler begins processing vials once the oven, heater/mixer and/or tray set temperatures have been reached, the equilibration delay time (entered in the More Menu) has elapsed, the door is closed, and the pump is ready. During the run, the Status Screen is displayed (Fig. 3.17).

***NOTE**: You can change the "Ready Requirements" from the Options, Ready Participation Menu. See "Ready Participation" on page 196 in Appendix D.*

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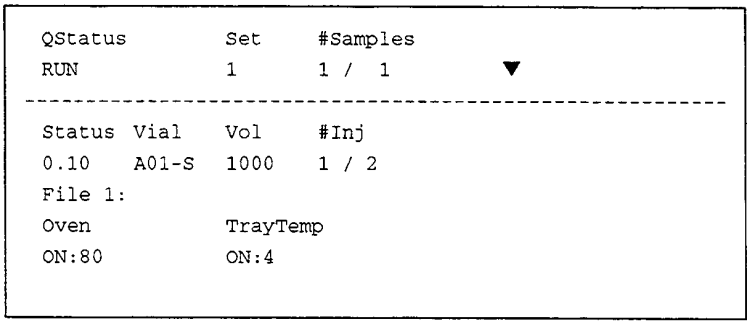


Figure 3.17 The Status Screen

The status screen gives you four kinds of information (described below): the autosampler queue status injection status oven and tray temperatures sample preparation status (if the Sample Preparation Option is installed)

***NOTE**: The sample preparation status screen is discussed in Chapter 4.*

You can't edit any information on the Status screen; however, editing during a run can be done from the Samples Menu (below).

Sample Queue information

Information on the sample queue is presented in the first two lines of the Status screen. The specific fields are described here.

QStatus

This field displays the status of the queue (IDLE, PAUSE, RUN, EQUIL, and ABORT). These conditions are described here.

IDLE indicates that no sample sets are currently being processed in the queue.

***NOTE**: The IDLE status doesn't tell you whether or not there are sets loaded into the queue. To check the contents of the sample queue, press [MENU] and select QUEUE.*

PAUSE indicates that a sample set is loaded, but that processing has been suspended. If the queue is paused (the [STOP] key has been pressed once), you can either resume processing with the current or subsequent vial, or abort processing of the current sample set. For more information, see "Stopping a Run" on page 44.

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RUN indicates that a sample set in the queue is being processed.

EOUIL indicates that the autosampler is waiting for the equilibration delay (temperature zone and initial conditions) to be reached before beginning the processing of the current sample set. This status will be displayed until the oven and tray reach the set temperatures entered in the Edit Menu and/or until the Equilibration Time entered in the More Menu has elapsed.

ABORT indicates that the processing of the current sample set has been stopped (the [STOP] key has been **pressed and ABORT** has been selected from the Abort/Continue Menu) or that a system error has occurred. Abort is displayed until the autosampler has returned the active vial to the sample tray and flushed all the lines. For more information about Abort, Continue, and Pause, see "Stopping a Run" on page 44.

Set

This field displays the number of the sample set currently being processed (1-39, P). If the queue is not running (the QStatus field reads other than RUN or PAUSE), the set field is blank.

#Samples

This field displays the number of the sample currently being processed and the total number of samples in the set: current sample number/total of samples. For example, if the autosampler is processing sample 50 in a set of 120 samples, the #Samples field will read 50 / 120.



***NOTE**: The total does not indicate the total number of vials or total number of injections in the set, -- it indicates the total number of sample vials in the set. Calibration vials are not included on this screen. This way, you'll have a more exact status of the run progress if you've chosen to reuse your calibration vials.*

Injection Status

Pressing [v] displays the next two status screens that contain information on the injection in progress.



***NOTE**: The Status Screen is updated every two seconds.*

Status

This field displays the progress of the current injection. A time in minutes in the Status field indicates that the queue is running (RUN) and that the current sample has been injected. This field displays the current run time (the time elapsed since the injection). If an injection has not occurred, the autosampler displays one of the following messages in the Status field:

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RDY indicates that the autosampler is ready for an injection (all temperature zones and gradient conditions have been reached), but that no injection is currently in progress.

NOTRDY indicates that the autosampler is not ready for an injection. NOTRDY may indicate that the temperature zones are not stabilized, the door is opened, or the Pump Ready contact closure is in the wrong state.

GRAD indicates that the autosampler is waiting for the set gradient conditions to be reached (for the gradient delay time set in the More Menu to elapse.)

HOLD indicates that an injection hold is in effect as determined by the Inject Hold input or as set in the Input Polarity Menu.

ABORT indicates that the current injection has been stopped in one of two ways: 1) [STOP] has been pressed to stop the Queue and ABORT selected from the Abort/Continue Menu, or 2) an error condition has occurred. During the ABORT sequence, the autosampler returns the current vial to the sample tray and flushes the lines.

DOOR indicates that the door is open. The autosampler automatically moves the arm to the home position to allow easy access to the sample tray. The arm automatically resumes where it left off when the door is closed.

Vial

This field indicates the position and type of vial (S for sample; C for calibration vial) currently being injected. If no injection is in progress (the Status is "NOTRDY"), this field is blank.

Vol

This field displays the injection volume for the current vial. If the current vial is a sample vial, this field indicates the Injection Volume value entered in the Injection Menu. If the current vial is a calibration vial, this field displays the Injection Volume value entered in the Calibration Menu.

#Inj

This field displays a fraction that indicates the current injection number over the total number of injections requested for the current vial: current injection number/injection per vial. For example, if the autosampler is performing the third of five injections the #Inj field will read 3 / 5.

File

Pressing [v I displays the number and name of the file assigned to the sample set currently being processed.

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Temperature Status

Pressing [v] accesses Oven/Tray Temperature Status screen which displays the actual-not the set-temperatures of the oven and tray, if the Column Oven and Tray Temperature Control Options are installed. The temperatures are displayed even if these options are turned off (not set). "OFF" indicates that an option is installed but not turned on in the current file's Injection Menu (Fig. 3.18).

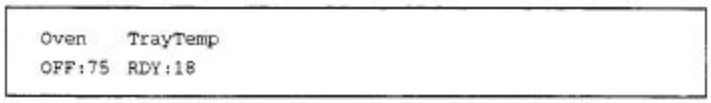


Figure 3.18 The Temperature Status screen with the oven turned off

The temperature field remains blank if one of these options is not present (Fig. 3.19). This screen does not appear if neither option is installed.



Figure 3.19 The Temperature status screen without the Tray Temperature Control Option installed



HINT: To check the set temperature for either the oven or the tray (or other parameters), you can return to the Edit Menu and view the information for the appropriate file as long as you have not loaded that file into the Queue. Therefore, you may wish to copy your file before loading it. That way you can edit the copy if you want to check any experimental parameters after your run file is loaded.

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Changing the Run in Progress

Once a run has started, you may wish to change the injection volume, cycle time, calibration interval, or set assignments. You make changes to the run currently in progress (the run file) from the Samples Menu. Figure 3.20 lists the changeable parameters in boldface type and indicates when those changes take effect. With the exception of the Cycle Time and Calib I (of X) values, changes made from the Samples Menu take effect on the next sample injection. Changes to the Cycle Time take effect on the current sample injection; changes to the Calib I (of X) field take effect on the next recalibration. Samples Menu changes remain in effect until the next sample set.

Sample Set	1	
File 1: VITAMINS		
Injections/Sample	**1**	*Next sample injection*
Cycle Time	**0.1**	*Current recalibration*
Calib 1 (of 3)	**A02**	*Reuse vials only*
First Sample Vial	A05	*Cannot change*
Number of Samples	**1**	*Next sample injection*
Set	2	*Next sample set*

Figure 3.20a The AS1000 Samples Menu

Sample Set	1	
File 1: VITAMINS		
Injection Volume	**5.0**	*Next sample injection*
Injections/Sample	**1**	*Next sample injection*
Cycle Time	**0.1**	*Current recalibration*
Calib 1 (of 3)	**A02**	*Reuse vials only*
First Sample Vial	A05	*Cannot change*
Number of Samples	**1**	*Next sample injection*
Set	2	*Next sample set*

Figure 3.20b The AS3000 Samples Menu



NOTE: Changing one vial location does not affect other vial locations.

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Priority sample sets

A priority sample set is one which can be inserted into the currently active sample set. It shares the same file and resources as specified for the currently active set. Priority sets can be added at any time.

Priority sample sets are like other sample sets with several important exceptions:

1. You assign their priority set number (P) in the Samples Menu (not in the Queue Menu).

2. Priority sets must be assigned to the currently active file.

3. You can't change the order of priority sample sets once they are loaded into the sample queue.

4. If you want to run priority sample sets, you must select Reuse same vials as your calibration routine type. (If you select Intersperse vials, the autosampler won't "know" whether a subsequent vial is a calibration vial or a sample vial and therefore won't be able to resume the calibration routine after the priority sample set is processed.)

5. Since a priority set is inserted into the currently active set, the file cannot be edited or changed between the currently active set and the priority set.

To insert a priority sample set into the sample queue, use the following steps (Fig. 3.21):

1. Press [SAMPLES].

2. Use the [+] and [-] keys to change the Set field value to P.

3. Fill in the other file parameters as you would for any other sample set.

4. Add the priority set to the queue.

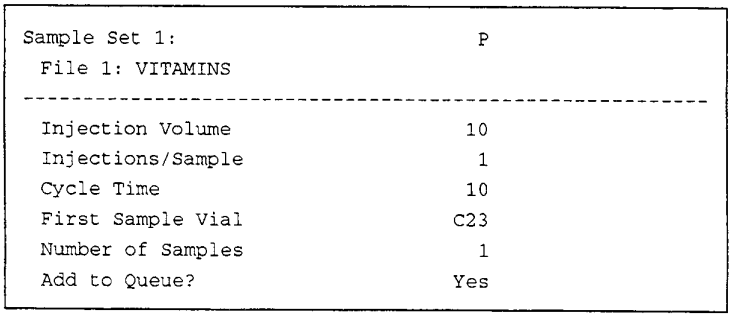


Figure 3.21 The Samples Menu with a priority sample set

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***NOTE**: The door is equipped with a safety interlock that automatically moves the autosampler's arm to the "home" position to allow you to add samples to the tray during a run. Once the door is closed, the arm returns to its position and resumes the operation in progress before the door was opened. However, you can disable the door interlock if you wish. Press [MENU], IOPTIONSI, Ready Participation, move to Door Interlock (page 197), and use the [+J and [-] keys to select DISABLE. For more information on the door interlock function, refer to Appendix D, page 196.*

The autosampler assigns a P (priority) order to the priority set as shown in the Queue Menu (Fig. 3.22):

```
 Order   File Name      Set
 1       1:VITAMINS     P
-------------------------------------------------------------
 1       1:VITAMINS     1
 2       2:BARBITUA     2
 3       3:EXPER 1      3
```

Figure 3.22 The Queue Menu with one priority sample set

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STOPPING A RUN

The [STOP] key has several functions depending upon the instrument status when the [STOP] key is pressed. Refer to Figure 3.23 as you read about the STOP/ABORT sequence.

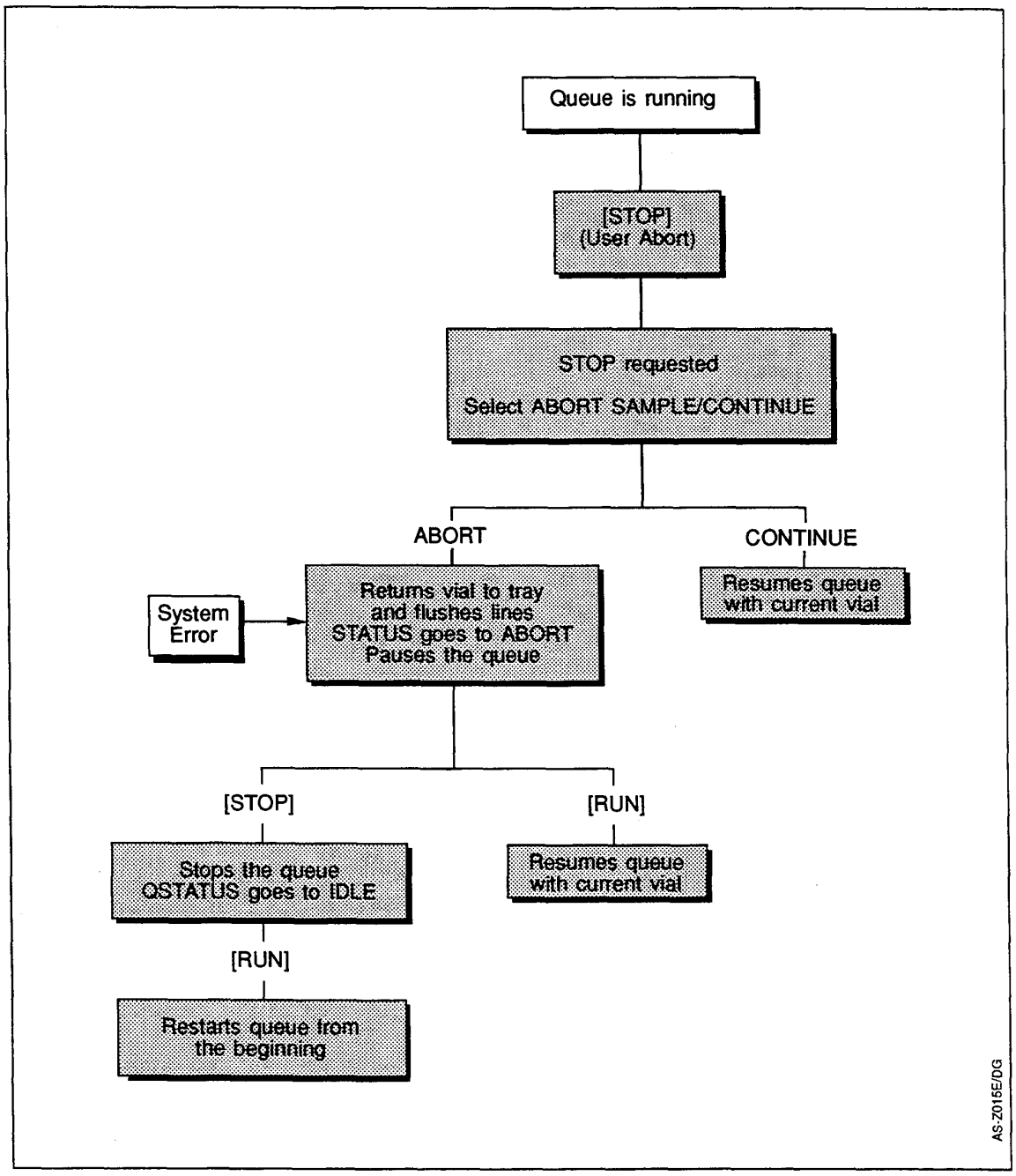


Figure 3.23 The Queue Stop/Abort Sequence

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If the queue is in RUN when you press the [STOP] key, the autosampler suspends its current operation and waits for you to make a selection to ABORT (Fig. 3.24a) or CONTINUE (Fig. 3.24b):

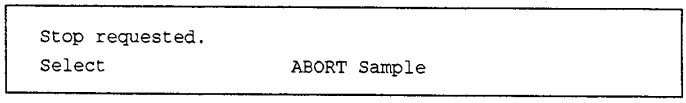


Figure 3.24a The Abort prompt

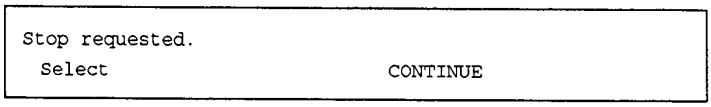


Figure 3.24b The Continue prompt

If you select Continue (by pressing the [+] key), the autosampler returns the queue to the ACTIVE state and resumes the motor activity (as if you had not pressed the [STOP] key).

If you select Abort or if a system error occurs, the autosampler:

- Aborts the processing of the current sample
- Returns the active vial to the vial tray
- Flushes the lines
- Puts the queue in the PAUSE state (Fig. 3.25)

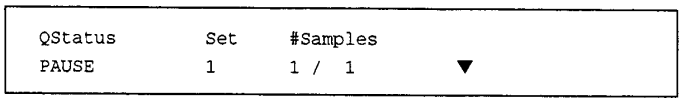


Figure 3.25 The Queue Status line with the autosampler in the PAUSE state

When the queue is PAUSED, you can either:

1. Press [RUN] to resume processing of the current vial or
2. Press [STOP] to stop the queue (the QStatus goes to IDLE) and then press [RUN] to restart processing from the beginning of the queue.

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This [STOP]/Abort sequence allows you several opportunities to stop and resume or restart the run as needed.

The ABORT/CONTINUE Menu and QPAUSE states let you remedy problems that could adversely influence the processing or data received from the current or subsequent vials (depleted solvent supply, etc) and resume the run without loss of previous data.

The ABORT, [STOP], [RUN] choice allows you to stop the current run and remedy problems that could have influenced the processing or data received from all prior injections (incorrect file assigned to the set, incorrect solvent concentration, etc.). This second option minimizes loss of resources (and time!).

File Management

You've learned how to edit and load files from the Files Menu. You can also copy, delete, protect files from editing, and lock files to prevent changes to the run file during a run.

SAVING FILES

As you know, the autosampler automatically saves your file when you exit the Edit Menu. If a power failure occurs while you are editing a file, all current changes will be lost. However, if a power failure occurs after you exit the Edit Menu, your changes will be saved.

COPYING FILES

To copy a file, press [MENU], and select /FILES/, /Copy/ to display the Copy Menu (Fig. 3.26).



Figure 3.26 The Copy Menu

Enter the number of the file to be copied in the Copy File field. Enter the number of the file you wish to copy to in the To field. When you press [ENTER], the message "File Copied" appears briefly and you are returned to the Files Menu.

DELETING FILES

Deleting a file restores all parameters to their default values. To delete a file, press [MENU], select /FILES/, and /Delete/. The Delete Menu appears (Fig. 3.27).



Figure 3.27 The Delete Menu

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Enter the number of the file you wish to delete and press [ENTER]. The message "File Deleted** appears briefly and you are returned to the Files Menu.

PROTECTING FILES

You can protect a file from being edited or deleted by using the file protection feature in the OPTIONS Menu. Because this feature enables you to control the changes to an original run file, you can easily incorporate your autosampler files into your standard good-laboratory-practice (GLP) protocols. To display the Configurations Menu (Fig. 3.28), press [MENU], select /OPTIONS/, /Configuration/.

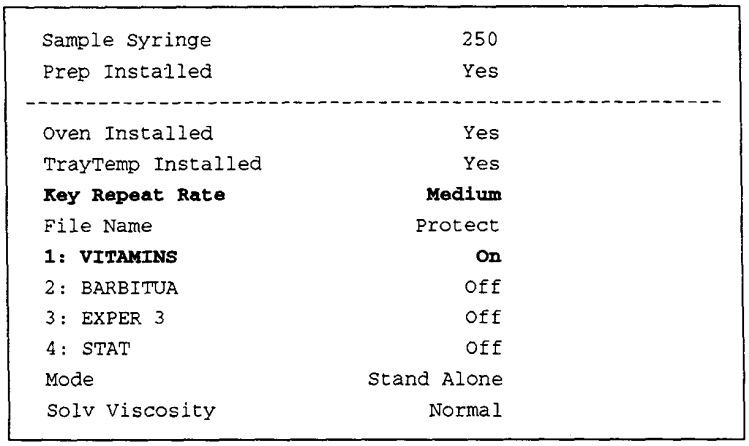


Figure 3.28 The Configuration Menu

To protect a Me, move the cursor down to the Protect field in the line that contains the file to be secured and press the [+]/[-] keys to turn ON the protect function. For example, File I VITAMINS in Figure 3.28 cannot be edited until the protect function is turned off. Attempting to edit a protected file displays the following message:

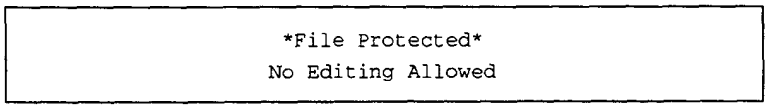


Figure 3.29 The confirmation message to prevent editing of a protected file.

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Changes to real-time editing

If a file is protected, you will not be able to change the injection volume, number of injections per vial, or cycle time for the ran in progress. These lines will not appear on the Samples Menu (Fig. 3.30).

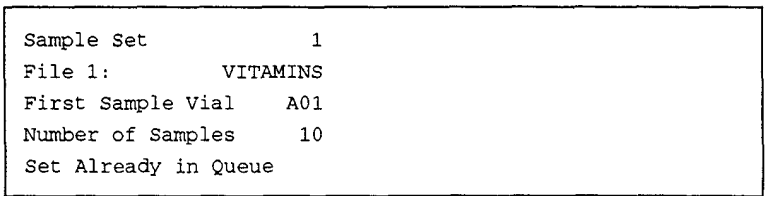


Figure 3.30 The Samples Menu for a protected file

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4 Advanced Operations

Introduction

This chapter tells you how to use the optional sample preparation system to automate sample treatment, heating, and mixing. In this chapter you will learn: The terminology used in the sample preparation templates How the sample preparation system works How to use the built-in templates How to create your own customized file How to use the Edit and Samples Menus and Status Screen with the Sample Preparation Option installed How to run an autosampler file that uses automated sample preparation

Maintenance and troubleshooting procedures for the sample preparation system are included in Chapter *5, Maintenance,* and Appendix *C, Troubleshooting,* respectively.

BEFORE YOU START

In presenting the material in this chapter, we assume that you have installed your sample preparation bottles and lines as described in Appendix A, that you have read and used the procedures described in Chapter 3, and that you are comfortable with the routine operations of your autosampler.

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Overview

Preparing to run a sample preparation file is similar to preparing to run any other autosampler file. First, you prepare your chemicals and fill the solvent bottle(s). Then you enter your file information into the Injection, Calibration, Timed Events, and More Menus as always. Next you enter your sample preparation parameters into one of the four built-in sample preparation menus (templates) accessible from the Edit Menu's Sample Prep field (Fig. 4. 1). Your sample preparation parameters are stored in the same file as your other run parameters. You load your file, enter your sample set information (build your sample queue) and start your run as we discussed in Chapter 3.

The Overlap Enable field at the end of each template allows you to program the autosampler to begin preparing the next sample vial while the current injection vial is still eluting.

The steps for running a sample preparation file are listed on the Sample Preparation Quickstart Card in the front of this manual.

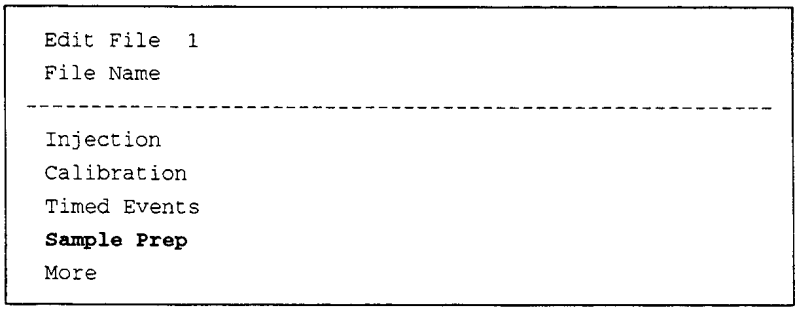


Figure 4.1 The Edit Menu

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Terminology

TEMPLATES

Each of the four sample preparation menus is a built-in procedure format called a template. Each template contains a different number o steps arranged in a preset and unchangeable order. Each step carries out a specific autosampler operation or group of operations. Some steps transfer liquid between vials or dispense solvents from any of the four external solvents. Others control the operation of the heater/mixer (included in the Sample Preparation Option) or the position of the hook to adjust the distance between the needle tip and the bottom of the vial.

By using the template default values, you can run one of the four built-in procedures:

1) Simple dilution

2) DABS-CL derivatization

3) Rainbow dilution

4) Linear dilution

Or, you can enter new values and create your own customized procedures. The autosampler automatically prepares your sample(s) according to the steps specified in the template.

DILUTION CYCLES

Within each template, the numbered steps are grouped into dilution cycles. For example, steps I - 6 in Figure 4.2 comprise the dilution cycle for Template 1.

Dilution cycles differ across templates in the number of steps and types of operations they perform. You can program the autosampler to repeat dilution cycles up to four times within a template to carry out more complex procedures. Well see how this works in the section titled "Template Descriptions" on page 62. But first we'll discuss what happens in the simple dilution cycle used in Template 1 because its steps are used in all four templates. Once you understand how these steps control the autosampler's operation, you'll be able to apply the same principles to Templates 24.

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Dilution Cycles 1
1—Load 500 µL Solv s – 1
2—Pickup 100 µL Sample
3—Pickup 0 µL Sample
4—PickLG 0 µL Sample
5—Add 500 µL to Sam+1
6—Mix for 0.5 minute
Overlap Enable: NO
This Procedure requires
That 1 Extra Vial be
Placed immediately after
Each Sample Vial.

Figure 4.2 Template 1

Your autosampler's simple dilution cycle is easily understood by comparing it to a manual dilution procedure. In a manual dilution, you draw a specific amount of sample into a pipet and expel a measured volume into a volumetric flask. Next you dilute to final volume with a desired amount of solvent and mix your sample/solvent mixture for a specific amount of time.

In the manual dilution we've just discussed, solvent and sample are added to the target container one at a time. In contrast, the autosampler first draws all of the resources for a given dilution into its solvent-holding loop and sample/needle lines. An air bubble is drawn before and after each resource to isolate it in the loop. Once all of the resources have been drawn into the lines, the autosampler delivers them to the target vial simultaneously. The vortex mixer then mixes the target vial for the specified amount of time.

All of the templates use Us dilution routine. The next section discusses how the individual template steps carry out this general dilution procedure.

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How Dilution Cycles Work

Delivery lines transport liquid and air through the sample preparation system as programmed in the template steps. Each step influences the operation of one or more of the six major components in the sample preparation system:

1) Prep syringe
2) Sample syringe with 6-port flush valve
3) Solvent holding loop
4) Six-port solvent selection valve with your four solvent bottles
5) Heater/vortex mixer
6) Needle

Refer to Figure 4.3 and keep these components in mind as you learn the functions of each step in a simple dilution cycle.

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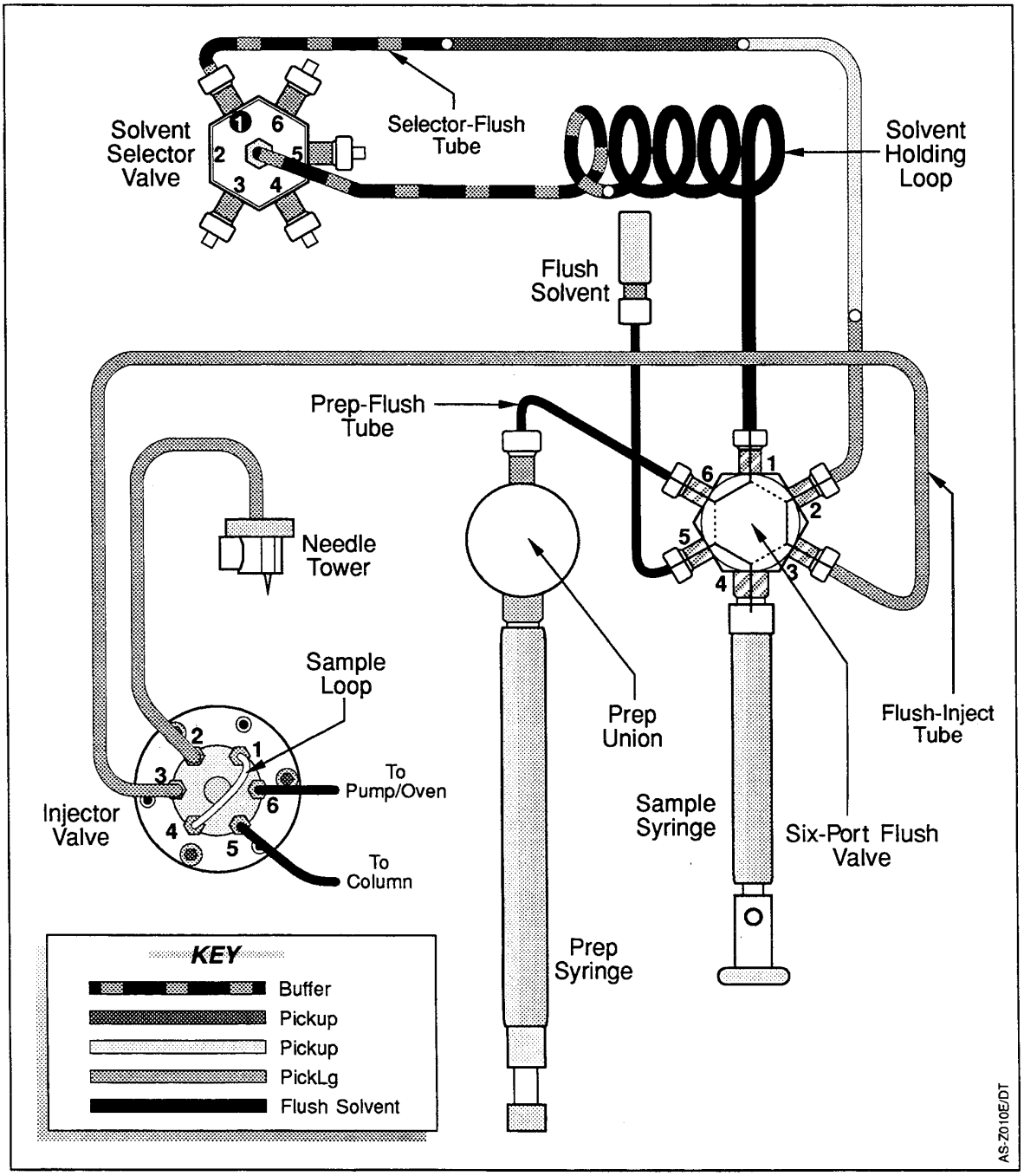


Figure 4.3 The sample preparation dilution process

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THE SAMPLE DILUTION CYCLE

Before the dilution cycle begins, the autosampler' s lines contain flush solvent. The simple dilution cycle is made of five different steps: Load, Pickup, PickLG, Add, and Mix. Each of these steps is described below.



***NOTE**: Some of these steps are repeated or skipped in the default methods. We'll see how this works in the sections that describe each template's dilution cycle.*

LOAD

The Load step flushes the lines with the selected solvent in a six-step process:

1) The 6-port solvent-selection valve rotates to the air position to draw an air bubble into the solvent holding loop.

2) The solvent selection valve then rotates to the selected solvent position (S-1, S-2, S-3, or S-4).

3) The preparation (prep) syringe draws the specified amount of solvent (0-1500 gQ plus a constant excess amount into the solvent holding loop. The excess solvent will be used in step 6 to flush the dead volume from the solvent-selection valve to the needle tip. The air bubble drawn into the solvent-holding loop in step 1 isolates the selected solvent (this step) from the flush solvent already present in the lines.

4) The solvent-selection valve moves back to the air position and the prep syringe draws another air bubble.

5) The solvent-selection valve rotates to the sample/needle line.

6) The prep syringe moves forward and expels solvent, thus flushing the dead volume with the selected solvent and expelling the second air bubble leaving only the selected solvent behind.

Pickup

The Pickup step causes the sample syringe to withdraw the specified volume of liquid from the source vial. The source can be the vial in the Sample (active) position, the vials in the Reagent A, B, or C locations (anywhere in the trays), or vials in the three positions adjacent to the Sample vial: Sam+l, Sam+2, or Sam+3. For example, if the Sample vial position is A01, Sam+l would be A02, Sam+2 would be A03, etc. The following operations occur during Pickup:

1) The hook moves to the selected vial position and picks up the vial.

2) The autosampler' s arm brings the vial to the sample tower and pushes the funnel out of the way.

3) The sample syringe draws an air bubble to isolate the sample or reagent from the selected solvent already present in the solvent holding loop (from the Load step).

4) The vial is raised in the sample tower. Sample is then drawn into the sample/needle line.

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5) The vial is lowered and the sample syringe draws an air bubble to isolate the sample slug and to prevent sample loss.

6) The hook returns the vial to the sample tray.

This process is repeated if a second Pickup step is used.



NOTE: Each pickup step allows you to pick up a maximum of 100 µL. If you want to pick up more than 100 µL, use either the second pickup step or the PickLg step.

PickLg

The PickLg (Pickup Large) is the same as Pickup except that it uses the solvent syringe to transport larger volumes (0 - 1000 µL).

Add

During the Add step two events happen:

1) The hook moves to the designated "target" vial position (Sample, Sam+1, Sam+2, or Sarn+3) and transports the vial into the sample tower.

2) The prep syringe expels the desired volume (0 - 1000 gL) of the liquids drawn into the solvent-holding loop and sample/needle lines, during the Load, Pickup, and PickLG steps, into the "target" vial.



NOTE: Because the Add step uses the prep syringe, the maximum volume that can be added is the sum of the Load volume and the PickLG volume. That is, you can't add (deliver to the target vial) more than you've withdrawn into the large (prep) syringe in the Load and PickLg steps.

Mix

The final step in the simple dilution cycle is the Mix step. During this step the target vial is carried to the mixer and vortex mixed for the specified length of time (0.0 to 9.9 minutes). In templates 1 and 2, the vial is returned to the sample tray after the Mix step. In templates 3 and 4, you have the option of doing an extraction operation before returning the vial to the tray.

After each dilution cycle, the autosampler automatically flushes its lines and syringes with flush solvent. This operation (not shown in the templates) returns the instrument to a "known" state. Any residual sample, reagents, or solvents from a previous cycle is flushed from the lines so that the instrument is ready for the next cycle or injection.



CAUTION! If you are using immiscible solvents, be sure to flush the preparation syringe (prep flush) between solvents to minimize contact between the immiscible solvents in the autosampler's lines.

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Template Programming Rules

Just as there were rules for creating regular files, there are rules that you need to remember when creating sample preparation procedures. These nine rules are also included on your Sample Preparation Quickstart Card for future reference.



***NOTE**: You may wish to read through these guidelines briefly and then refer back to them as you read the section titled, "Template Descriptions. "*

1. Extra vials are placed in the three vial positions subsequent to the Sample vial position (Sam+l, Sam+2, Sam+3). For example, if the Sample position is A02, Sam+1 is A03, Sam+2 is A04, etc.

2. It is impossible to add, delete, or change the order of steps within a template, but you can skip any step (except an Add step) by setting its volume parameter to 0 µL.

3. Specify one dilution cycle for each delivery of chemicals to, or for each operation carried out on, the target vial.

4. The desired final volume is specified in the Add step.

5. To perform an operation on, but not make a delivery to, the target vial, set the Add step's volume parameter to 0. This approach is useful, for example, when you want to mix or heat the contents of a vial.

6. The volume specified in the Add step in a given dilution cycle must be less than or equal to the sum of the PickLG and the LOAD volumes.

7. The Add step makes deliveries to the target vial on a last-in first-out basis.

8. Leave the Overlap Enable function turned on (Yes) if sample preparation for the next vial to be injected can begin while the current vial is still running.

9. The autosampler injects only from the last vial referenced in the method template's last Add step.

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Sample Preparation Menu Differences

Before we describe each template in detail, we'll discuss how to do a sample preparation run.

Performing a sample preparation run is essentially the same as doing a run without sample preparation. You create and load your file, build your sample queue, and start your run just as you did in Chapter 3. However, with the Sample Preparation Option installed, the Edit and Samples Menus, and Status Screens are expanded to allow you to enter and monitor your sample preparation parameters. The "new" lines that are dedicated to sample preparation in each of these menus and screens are discussed below.

EDIT MENU

With the Sample Preparation Option installed, the Edit Menu has the Sample Prep field that you saw in Figure 4. 1.

SAMPLE PREPARATION MENU

Selecting /Sample Prep/ from the Edit Menu accesses the Sample Prep Menu from which you can use the [+] and [-] keys to select one of the four sample preparation templates (Fig. 4.4):

```
Use Template                    1
Description              Dilution
(The Templates start here)
```

Figure 4.4 The Sample Prep Menu

SAMPLES MENU

The Samples Menu (Fig. 4.5) has several additional lines with the Sample Preparation Option installed. These new lines reflect the number of reagent and extra vials used by the method.

Reagent Vials (Reag-A, B, and C)

If reagents (A, B, and C) are used in the sample preparation method, a line is added on the Samples Menu for each reagent used by the method to allow you to edit its vial location. If one reagent is used, only the Reag-A appears; if two reagents are used, the Reag-B line appears. Figure 4.5 shows the Samples Menu when all three reagents are used.



***NOTE**: Reagent vials can be placed in any vial position in any of the vial trays. They don't have to be in proximity to the sample vial.*

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```
Sample Set                  1
File 1:
Injection Volume           10
Injections/Sample           1 (If injection is used)
Cycle Time                0.1
Reag-A                    C23  [If reagent vial(s) is (are) used]
Reag-B                    C24
Reag-C                    C25
Calib Vial 1 (of X)       A01  (If calibration is used)
First Sample Vial         A04
Number of Samples           1
File requires n extra
vials after each sample
Add to Queue?             Yes
```

Figure 4.5 The Sample Menu with reagents, calibration, and extra vials used in the sample preparation method

File requires n extra vials after each sample

If extra target vials are used after each sample vial, the two lines indicating the number of extra vials required are copied from the sample preparation template into the last two lines of the Samples Menu.

The "n" represents the number of extra (empty) vials placed after the Sample vial (the Sam+1, Sam+2, and Sam+3 vials). An extra vial (one in which sample is mixed with solvent, where a reaction takes place), or a pickup (PickLg) is made. "n" is selected in the Add steps of each template. Figure 4.5 illustrates the Samples Menu for a file that uses calibration with the Sample Preparation Option installed.



***NOTE**: The screen actually displays a number (1, 2, or 3) in the "n" field. We've represented this value as "n" because it changes depending on whether you've chosen Sam+1, Sam+2, or Sam+3 during your run.*

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Changes to real-Time editing

As described in Chapter 3, you can make changes to the run file for the run in progress from the Samples Menu. Table 4.1 summarizes when Samples Menu changes to the run in progress take effect.

Table 4.1 When Changes Affect the Run in Progress

File #	Can't edit
Injection Volume	Next injection*
Injection/Sample	Next injection
Cycle Time	Current injection
Reagent A(B,C)	Next sample
Reagent A-C	Next sample
Calib Vial 1 of (x)	Next recalibration
First Sample Vial	Can't edit
Number of Samples	Next injection

*Changes to this field affect sample vials only, not calibration vials.

Protecting files from real-time editing

As described in Chapter 3, a file can be protected from real-time editing by turning on the Protect function in the Configuration Menu. File Protect prevents you from editing the injection volume, injections per sample and cycle time and eliminates some lines from the Sample Menu for a protected file that uses sample preparation just as it did for a regular file. Figure 4.6 shows the Sample Menu for a protected file that uses sample preparation and calibration.

```
Sample set          1
File  1:  VITAMINS
Calib Vial  1  (of 3)        A01
First Sample Vial            A04
Number of Samples            10
Already in Queue
```

Figure 4.6 The Samples Menu for a Protected File that uses sample preparation and calibration

STATUS SCREEN

With the Sample Preparation Option installed, and additional Status Screen is included to give you information on the current template step, the heater/mixer status and the resource locations for the active file. Figure 4.7 shows the Status Screen for a file that contains a sample preparation procedure and that uses several reagents and calibration vials.

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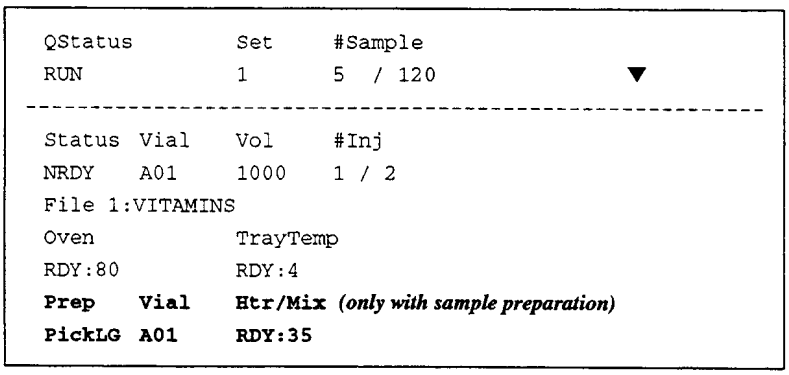


Figure 4.7 The Status Screen for a file with sample preparation

Prep

This line tells you which template step is in progress (Load, Pick.Up, Pick_Lg, Add, Mix, Wait, or Remove). For example, Figure 4.7 indicates that even though the sample queue is running (RUN), since a Mix step is in progress, the autosampler is not ready (NRDY) to inject.

Vial

This field indicates the position of the vial currently undergoing sample preparation.

Htr/Mix

This field indicates the status (OFF, RDY, AND NRDY) of the heater/mixer and its actual temperature, if turned on and set in the Heater field in templates 2 and 4. If not in use, this field reads "OFF" followed by the actual temperature.

***NOTE**: The temperature is always displayed whether or not the heater/mixer is in use.*

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Template Descriptions

You are now familiar with the basic template steps and the additional menu selections you have to make when using automated sample preparation. In this next section we'll describe the specifics of each template.

All four templates are based on the liquid transport and delivery process described in the section titled "The Simple Dilution Cycle" above. In fact, Template I (simple dilution) contains only the steps described above (Fig. 4.2). If your protocol has more than two dilutions, simply specify the number of dilution cycles you need in the line before step 1. The autosampler will automatically replicate the template's dilution cycle a total of four times.

Each template will be discussed in two ways: with a boxed Figure showing how the display looks, and with a line-by-line description-of each step. You can use the line-by-line description to assist you in creating a customized method file for your own applications. Each example uses the template's default values.

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TEMPLATE 1: DILUTION

Template 1 (Fig. 4.8) is a dilution program that you can use to perform a 1:5 v/v dilution procedure.

```
Use Template  1
Description  Dilution
# of Dilution Cycles  1
1- Load 500 µL Solv S-1
2- Pickup  100 µL Sample
3- Pickup  0 µL Sample
4- PickLG  0 µL Sample
5- Add 500 µL to Sam+1
6- Mix for 0.5 minute
Overlap Enable:       Yes
This procedure requires
that 1 Extra Vial(s) be
placed immediately
after each Sample Vial.
```

Figure 4.8 Template 1: Dilution

Line-by-Line Description of Template 1

`Use Template 1`	Template I is selected.
`Description  Dilution`	Template I is the Dilution Template.
`# of Dilution Cycles 1`	One dilution cycle requested for delivery to Sample vial.
`1- Load 500 µL Solv S-1`	The prep syringe draws 500 µL Solvent I (buffer) into solvent holding loop
`2- Pickup 100 µL Sample`	The sample syringe draws 100 µL sample into the sample lines.
`3- Pickup 0 µL Sample`	Skip this step; the sample syringe is not needed.
`4- PickLG 0 µL Sample`	Skip this step; the prep syringe not needed.
`5- Add 500 µL to Sam+1`	Dilute 100 µL sample to 500 µL and deliver to Sam+ 1.
`6- Mix for 0.5 minute`	Mix sample and buffer for 0.5 minute.
`Overlap Enable Yes`	Turn on the Overlap Enable function to begin preparation after the last injection and flush of the previous vial (sample or calibration).
`This procedure requires that 1 Extra Vial(s) be placed immediately after each Sample Vial.`	One Extra (empty or extra) vial is required since mixing takes place in the Sam+ 1 vial position.

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HOW THE SIMPLE DILUTION STEPS WORK TOGETHER

Before continuing to the other three templates, it is important to understand how the Load, Pickup, PickLg, and Add steps affect the contents of the Add step target vial. Remember that the Add step makes deliveries to the target vial on a last-in-first-out basis. Consider the following examples to see how this works.

```
Use Template  1
Description  Dilution
# of Dilution Cycles   1
1- Load 1000 µL Solv S-1
2- Pickup   0 µL Sample
3- Pickup   0 µL Sample
4- PickLG   0 µL Sample
5- Add 1000 µL to Sam+1
6- Mix for 0.5 minute
Overlap Enable:        Yes
This procedure requires
that 1 Extra Vial(s) be
placed immediately
after each Sample Vial.
```

Figure 4.9 Template 1: Dilution Cycle with no Pickup or PickLg

In Figure 4.9, 1000µL of solvent is loaded into the holding loop and delivered into the target vial since no other reagents are drawn into the holding loop in the Pickup or Picklg steps.

In contrast, in Figure 4.10, 1000µL of solvent and 100µL sample are loaded into the holding loop. The Add step delivers the last 1000 µL of liquid drawn: 100µL sample from the first Pickup step but only 900 µL solvent from the Load step. The Add step designates the desired final target-vial volume.

```
Use Template  1
Description  Dilution
# of Dilution Cycles   1
1- Load 1000 µL Solv S-1
2- Pickup   100 µL ReagA
3- Pickup   0 µL Sample
4- PickLG   0 µL Sample
5- Add 1000 µL to Sam+1
6- Mix for 0.5 minute
Overlap Enable:        Yes
This procedure requires
that 1 Extra Vial(s) be
placed immediately
after each Sample Vial.
```

Figure 4.10 Template 1: Dilution Cycle showing a 1:10 dilution

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TEMPLATE 2: DABS-CL

Template 2 (Fig. 4.11 is a program to perform various chemical reactions. The default method is specifically designed to perform a (Dimethylamino)azobenzenesulfonyL chloride (DABS-C1) derivatization procedure. This template contains the same steps as Template 1, plus three new steps described here.

Heater Temp

This line allows you to turn the heater on and off and set the temperature from 30 - 100 'C.

Heat

In this field you can specify a heating time from 0.0-99.9 minutes.

Mix

This field allows you to remix the reagents for a specified amount of time (0.0 - 9.9 minutes) after heating. Figure 4.11 shows the first Template 2 dilution cycle.

```
Use Template 2
Description  DABS-CL
# of Dilution Cycles   2
Heater: On      Temp: 65 C
1- Load 200 µL  Solv S-1
2- Pickup 100 µL Reag-A
3- Pickup  40 µL Sample
4- PickLG   0 µL Sample
5- Add 140 µL to Sam+1
6- Mix for 0.3 minute
7- Heat for 4.5 minute
8- Mix for 0.3 minute
Overlap Enable  Yes
```

Figure 4.11 The first dilution cycle of Template 2

The default method's values are those used to perform a simple DABS-C1 derivatization of amino acids. For the exact reagents, samples, and concentrations used for this procedure, please refer to Thermo Separation Products' Application Note 2: Automated DABSYL Derivatization.

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Line-by-Line description of Template 2

`Use Template 2`	Template 2 selected.
`Description DABS-CL`	Template 2 is DABS-Cl derivatization.
`# of Dilution Cycles 2`	Two dilution cycles is default value: one dilution cycle per delivery to Extra vial (Sam +1).
`Heater: On Temp: 65`	Turn on heater; set the temperature.
`1- Load 200 gL Solv S-1`	Draw buffer into the solvent-holding loop.
`2- Pickup 100 gL Reag-A`	Sample syringe draws Reag-A into sample/needle line (e.g. DABSCI).
`3- Pickup 40 gL Sample`	Sample syringe draws 40 p.L Sample into sample/needle line (e.g. Amino Acids).
`4- PickLG 0 gL Sample`	Skip step.
`5- Add 140 JIL to Sam+1`	Deliver Reag-A and Sample into first extra vial (e.g.DABS-CI).
`6- Mix for 0.3 minute`	Mix contents of the first Extra vial for 0.3 minutes to bring vial to temperature.
`7- Heat for 4.5 minute`	Heat vial for 4.5 minutes.
`8- Mix for 0.3 minute`	Nfix reaction vial for 0.3 minute.
`Repeat Dilution Steps`	
`9- Load 1000 IiL Solv S-1`	Draw buffer into the solvent-holding loop.
`10- Pickup 0 gL Sample`	Skip this step.
`11- Pickup 0 gL Sample`	Skip this step.
`12- PickLG 0 gL Sample`	Skip this step.
`13- Add 860 RL to Sam+l`	Deliver 860 pL Solv S- I into reaction vial (e.g. acetate buffer).
`14- Mix for 0.3 minute`	Nfix reaction vial for 0.3 minutes.
`15- Heat for 0.0 minute`	Skip this step.
`16- Mix for 0.0 minute`	Skip this step.
`Overlap Enable Yes`	Turn on Overlap Enable function.
`This procedure requires that 1 Extra Vial(s) be placed immediately after each Sample Vial.`	The protocol requires one reaction vial for vortex mixing Reag-A, sample, and Solv S-1.

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TEMPLATE 3: RAINBOW

Template 3 (Rainbow) allows you to do a more complex dilution process or an extraction process and is the same as Template 1 (dilution) with three additional steps. Wait and Remove steps are added after the Mix step at the end of each dilution cycle. The FlushP step has been added after the last dilution cycle before the Overlap Enable step. These new steps are described here.



NOTE: The default values in Template 3 are designed to illustrate a dilution procedure that creates a calibration series with two components (Reagents A and B) and four calibration levels. For this illustration, the resource locations are as follows: ReagA contains you first standard, ReagB contains your second standard, and Sample, Sam + 1 -3 are empty.

Wait

The Wait step programs a reaction time for the vial contents or to allow sufficient time for the liquid layer in an extraction to separate. Wait pauses the autosampler for the specified amount of time (0.0 -99.9 minutes).

Remove

The Remove step positions the bottom of the vial at the desired distance (0.0 - 20.0 mm) from the needle tip, withdraws a desired volume of solvent (0 - 1500 µL), and delivers it to waste.

FlushP

As you know, the autosampler automatically flushes the lines and syringes with flush solvent at the end of each dilution cycle. (This step is not shown on your templates.) The FlushP step allows you to fill the autosampler's lines with any of the four solvents (or buffers) in preparation for the sample injection. You can select any of the four external solvents (S- 1, S-2, S-3, S-4), or you can flush the lines with flush solvent by selecting Solv Btl. After flushing the lines, FlushP returns the prep syringe to its initial position in preparation for the next injection. Figure 4.12 illustrates the last dilution cycle for Template 3 in order to include the FlushP step.

```
Repeat Dilution Steps
  25- Load  1000 µL  Solv  S-1
  26- Pickup 0 µL Sample
  27- Pickup 30 µL Reagent A
  28- PickLG 0 µL Sample
  29- Add 1000 µL to Sam+3
  30- Mix for 0.5 minute
  31- Wait for 0.0 minute
  32- Remove 0 µL to
  waste at 2.0 mm height
  FlushP  0 µL  Solv  S-1
  Overlap Enable No
  This procedure requires
  that 3 Extra Vial(s) be
  placed immediately
  after each Sample Vial.
```

Figure 4.12 The last dilution cycle of Template 3

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Line-by-Line description of Template 3

`Use Template 3`	Template 3 selected.
`Description Rainbow`	Template 3 is Rainbow Dilution example.
`# of Dilution Cycles 4`	Specify a dilution cycle for the reagent(s)/solvent delivery to each of the four calibration vials.
`1- Load 1000 µL Solv S-1`	Draw solvent into the solvent holding loop.
`2- Pickup 3 0 µL Reag-B`	Sample syringe draws Reagent B into the sample/needle line.
`3- Pickup 0 µL Reag-A`	Skip step (no Reagent A is used in the first calibration vial).
`4- PickLG O µL Sample`	Skip this step.
`5- Add 1000 µL to Sample`	Dilute 30 µL Reagent B to 1000µL with solvent S- I and deliver to sample vial.
`6- Mix for 0.5 minute`	Mix contents of sample vial for 0.5 minutes (first calibration level).
`7- Wait for 0.0 minute`	Skip this step.
`8- Remove 0 µL to waste at 2. Omm height`	Skip this step.
`Repeat Dilution Steps`	Flush lines and solvent loop with the desired volume of solvent (S-1).
`9- Load 1000 µL S-1.`	Sample syringe withdraw 20 µL Reagent B into sample/needle line.
`10- Pickup 20 µL Reag-B`	Sample syringe withdraw 10 µL- Reagent A into sample/needle line.
`11- Pickup 10 µL Reag-A`	Skip this step.
`12- PickLG 0 µL Sample`	Dilute 20 4L Reagent B and 10 µL Reagent A to I mL with solvent S- 1 and deliver to first Extra vial (second calibration level).
`13- Add 1000 µL to Sam+l`	Mix contents of first extra for 0.5 minutes.
`14-Mix for 0.5 minute`	Skip this step.
`15- Wait for 0.0 minute`	Skip this step.
`16- Remove 0 µL to waste at 2.0mm height`	Draw solvent into the solvent holding loop.
`17- Load 1000 µL Solv S-1`	Sample syringe draw 10 µL Reagent B into the sample/needle line. c.
`18- Pickup 10 µL Reag-B`	Sample syringe draw 20 VL Reagent A into the sample/needle line.
`19- Pickup 20 RL Reag-A`	Skip this step.
`20- PickLG 0 µL Sample`	

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21- Add 1000 µL to Sam+2	Dilute 10 µL Reagent B and 20 µL Reagent A to I mL with solvent S- I and deliver to second Extra vial (third calibration level).
22- Mix for 0.5 minute	Mix contents of second Extra for 0.5 minutes.
23- Wait for 0.0 minute	Skip this step.
24- Remove OµL to waste at 2.0mm height	Skip this step.
Repeat Dilution Steps	
25- Load 1000 µL S-1.	Flush lines and solvent loop with the desired volume of solvent (S-1).
26- Pickup 0 µL Reag-B	Skip this step (no Reagent B needed in the last calibration level).
27- Pickup 30 µL Reag-A	Sample syringe withdraw 30 µL Reagent A into sample/needle line.
28- PickLG 0 µL Sample	Skip this step.
29- Add 1000 µL to Sam+3	Dilute 30 µL Reagent A to 1 mL with solvent S-1 and deliver to third Extra vial (fourth calibration level).
30-Mix for 0.5 minute	Mix contents of third extra for 0.5 minutes.
31- Wait for 0.0 minute	Skip this step.
32- Remove 0 µL to waste at 2.Omm height	Skip this step.
FlushP 0 µL Solv S-1	Skip this step.
Overlap Enable No	Turn off the Overlap Enable function.
This procedure requires that 3 Extra Vial(s) be placed immediately after each Sample Vial.	One extra vial is required for each standard level. The sample vial contains the first level; levels 2-4 are in positions Sam+1, 2, and 3, respectively.

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TEMPLATE 4: LINEAR

Template 4 template is also named for its default method; it allows you to do linear dilutions. This template (Fig. 4.13) contains the heating and mixing steps added to Template 2 and the Wait, Remove and FlushP steps used in Template 3. Figure 4.13 shows the last dilution cycle of Template 4.

```
Use Template 4
Description Linear
# of Dilution Cycles   3
Heater: On      Temp: 30 C
21- Load 1000 µL Solv S-1
22- Pickup 5 µL Sample
23- Pickup  0 µL Sample
24- PickLG  0 µL Sample
25- Add 1000 µL to Sam+1
26- Mix for 0.3 minute
27- Heat for 0.0 minute
28- Mix for 0.0 minute
29- Wait for 0.0 minute
30- Remove 0 µL to
waste at 2.0 mm height
FlushP  0 µL Solv S-1
Overlap Enable  No
This procedure requires
that 3 Extra Vial(s) be
placed immediately
after each Sample Vial.
```

Figure 4.13 The last dilution cycle of Template 4: Linear

This template can be used to perform any function or application performed by any of the other templates. The default values can be used to perform a linear dilution procedure, for example, to prepare calibration vials. If you use the default values, you diluent is Solvent S-1, Sam+1 will hold your Level 2 standard, and Sam+3 will hold your Level 3 standard.

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Line-by-Line description of Template 4

Use Template 4	Template 4 is selected.
Description Linear	Template 4 does linear dilution.
Dilution Cycles 3	One cycle per delivery to a target vial.
Heater: On Temp: 30 C	Turn on heat: set temperature.
1 – Load 1000 µL Solv s-1	Load 1000 µL buffer into solvent holding loop.
2 – Pickup 5 µL Sample	Sample syringe draw sample (for the Level 1 standard) into the sample/needle line.
3 – Pickup 0 µL Sample	Skip this step.
4 – PickLG 0 µL Sample	Skip step.
5 – Add 1000 µL to Sam+1	Deliver buffer and sample to the first Extra vial (Level 1 standard).
6 – Mix for 0.3 minutes	Mix the first Extra vial for 0.3 minutes.
7 – Heat for 0.0 minute	Skip step.
8 – Mix for 0.0 minute	Skip step
9 – Wait for 0.0 minute	Skip step
10 – Remove 0 µL to waste at 2.0 mm height	Skip step
Repeat Dilution Steps	Begin dilution cycle 2
11 – Load 1000 µL Solv s-1	Draw 1000 µL buffer into the solvent holding loop.
12 – Pickup 0 µL Sample	Skip step.
13 – Pickup 0 µL Sample	Skip step
14 – PickLG 0 µL Sample	Skip step
15 – Add 1000 µL to Sam+2	Add buffer and sample to second Extra vial (Level 2 calibration vial).
16 – Mix for 0.3 minute	Mix the Level 2 calibration vial.
17 – Heat for 0.0 minute	Skip step.
18 – Mix for 0.0 minute	Skip step.
19 – Wait for 0.0 minute	Skip step.
20 – Remove 0 µL to waste at 2.0 mm height	Skip step.
21 – Load 1000 µL Solv s-1	Load 1000 µL buffer into the solvent holding loop.

22 – Pickup 100 µL Sample	Sample syringe draws 100 µL sample into the sample/needle line.
23 – Pickup 0 µL Sample	Skip step.
24 – PickLG 0 µL Sample	Skip step.
25 – Add 1000 µL to Sam+3	Deliver sample and buffer for the Level 3 calibration vial into the third Extra vial.
26 – Mix for 0.3 minute	Mix Level 3 calibration vial for 0.3 minute.
27 – Heat for 0.0 minute	Skip step.
28 – Mix for 0.0 minute	Skip step.
29 – Wait for 0.0 minute	Skip step.
30 – Remove 0 µL to waste at 2.0 mm height	Skip step.
FlushP 0 µL Solv s-1	Flush lines with selected solvent.
Overlap Enable No	Do not begin sample preparation on next set until current set has completed injection.
This procedure requires that 3 Extra Vial(s) be placed immediately after each Sample Vial.	You need an extra vial for each calibration vial.

Starting a Sample Preparation Run

Once you have filled in all of the parameters in one of the four sample preparation templates, you're ready to enter the rest of your parameters into the Edit Menu. Before you start your run, you need to:

- Enter your Timed Events Menu parameters
- Enter your More Menu parameters
- Load your file
- Press [RUN]

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Preparing And Running Your Samples Automatically

A SIMPLE DILUTION RUN

If you are doing a simple dilution, you can put your concentrated sample, diluent, reagent (if used) and extra vial in one sample set. The autosampler will automatically inject the last vial used during sample preparation, in this case, the contents of your extra vial. Figure 4.14 illustrates this example. For this example, vial A01 is a reagent (Reag-A), vial A02 contains the sample concentrate, and vial A03 is the extra vial.

```
Sample Set                  1
File 1: EXAMPLE
Injection Volume           10
Injections/Sample           1
Cycle Time                0.1
Reag-A                    A01
First Sample Vial         A02
Number of Samples
File requires 1 extra
vial(s) after each sample
Add to Queue?             Yes
```

Figure 4.14 An example of a simple dilution run using sample preparation and injection in the same sample set



***NOTE**: In Figure 4.14, the vial in the First Sample Vial position (A01) is your first preparation vial, <u>not</u> your first injection vial.*

PREPARING CALIBRATION VIALS AND INJECTING THEM

If you want to prepare several calibration vials and then inject them, if s easiest to set up two sample sets. The first set will be your sample preparation set without injections; the second set will be for your actual run with injections.

For the first set, you set Injections/Sample to 0 in the Edit and Samples Menus. For the second set, you set Injections/Sample to the desired number of sample injections in both menus. Figure 4.15 illustrates the Samples Menu for the preparation set; Figure 4.16 illustrates the Samples Menu for the injection set.

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```
Samples Set             1
File 1:  PREP
Injection Volume        10
Injections/Sample       0
Cycle Time              10.0
First Sample Vial       A01
Number of Samples       10
Add to Queue            Yes
```

Figure 4.15 The Samples Menu for the Prep Set



***NOTE**: You can decrement the Injections/Sample field to 0 only if you have first selected a sample preparation template.*

```
Samples Set             2
File 2:  INJECT
Injection Volume        10
Injections/Sample       5
Cycle Time              10.0
First Sample Vial       A01
Number of Samples       10
Add to Queue            Yes
```

Figure 4.16 The Samples Menu for the Injection Set



***NOTE**: If you enter your sample preparation and other run parameters, load your file and then start your run, the autosampler will prepare your samples as you would expect, but inject only the vial used by the last Add step in the sample preparation template.*

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Stopping A Run

Similar to operation without sample preparation, the [STOP] key has several functions depending upon the instrument! s status when the [STOP] key is pressed. However, the [STOP] sequence discussed in Chapter 3 is slightly different when sample preparation is used. Refer to Figure 4.17 as you read about the STOP/ABORT sequence for sample preparation.

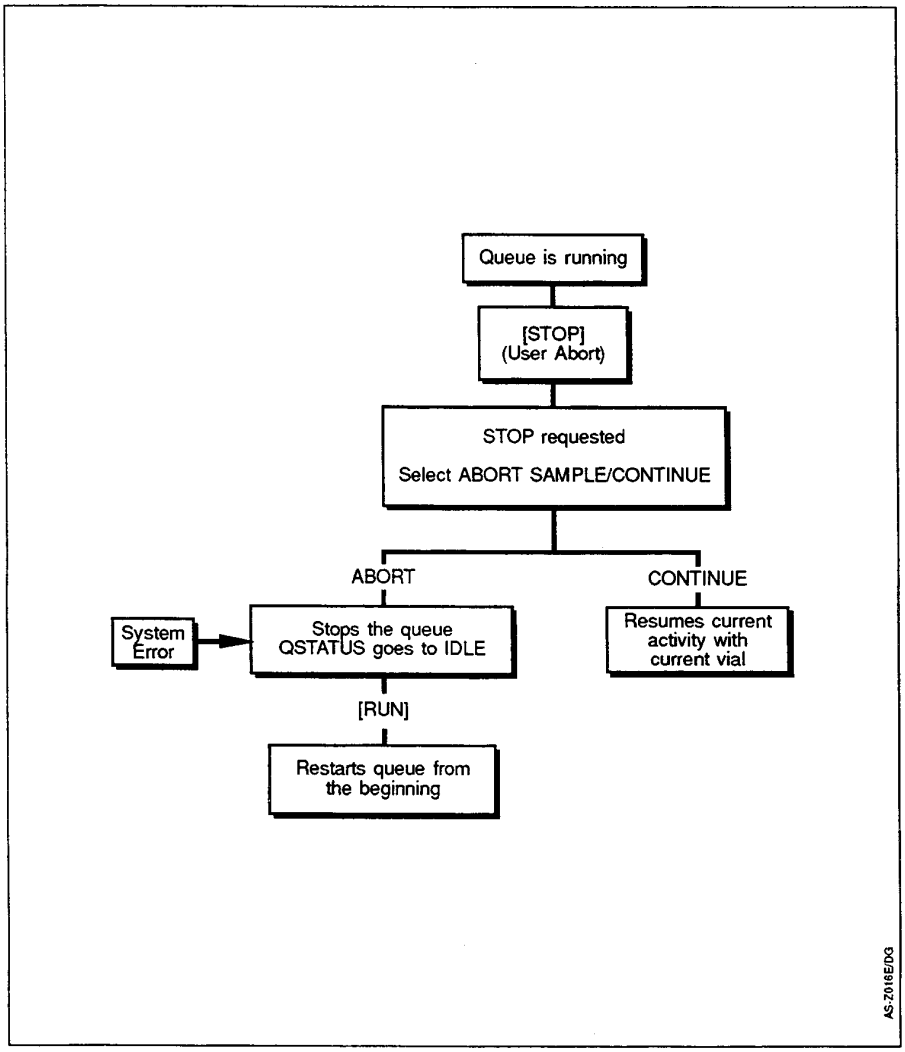


Figure 4.17 The Queue Stop/Abort Sequence

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If the queue is in RUN when you press the [STOP] key, the autosampler suspends its current operation and waits for you to make a selection from the ABORT/Continue Menu as described in Chapter 3.

If you press [+J to select Continue, the autosampler returns the queue to the ACTIVE state and resumes the motor activity (as if you had not pressed the [STOP] key), just as it did without sample preparation.

If you select ABORT or if a system error occurs, the autosampler:

- Aborts the processing of the current sample
- Returns the active vial to the vial tray
- Flushes the lines (both syringes)
- Puts the queue in the IDLE state (not PAUSE)

You can then press [RUN] to restart processing from the beginning of the queue.

This [STOP] sequence allows you to resume the run from the ABORT/CONTINUE menu. But, if you press [STOP] and select ABORT, you can only restart (not resume) the queue from the beginning.

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Customizing the Templates

GUIDELINES

In our examples so far, the contents of the vials in the template positions have matched the autosampler's names for those positions. For example, we have placed the sample in the Sample position and stock standards in the Reagent A, B, and C positions. We have also used the vial positions in a sequential order. However, the autosampler does not limit you to this approach. In customizing the templates to your own applications, the following more general rules may help:

1. In each dilution cycle, you have seven vial positions to work with: three source-vial positions (Reagents A, B, Q and four vial positions that may be either source or target vials (Sample, Sample + 1, Sample + 2, and Sample + 3).

2. You can arrange your Pickups and Add vials in any order you wish within a dilution cycle as long as you remember that the autosampler locates the extra vials (Sample + 1 - 3) in relationship to the vial you've assigned to the Sample position. For example, you could Pickup from the Sam + 3 vial and Add to the Sample vial. (But, you couldn't Add to the ReagentA vial since, according to rule 1, it cannot be a target vial.)

3. You can use the vial positions in any order you wish within a dilution cycle. For example, even though the autosampler cannot skip Sample vial positions, you can program it to prepare interspersed calibration vials by Adding to the Sam + 2 position, without using the Sam + I position in any of the steps in a given dilution cycle.



HINT. In loading your tray, if you want yourfirst calibration vial to be A01, then you can use rule 3 and the autosampler's "wrap "feature by assigning the First Sample Vial as C39 (Sam + 2 will then be A01). However, you will still need to create two sample sets: one to prepare and the other to run your calibration and sample vials.

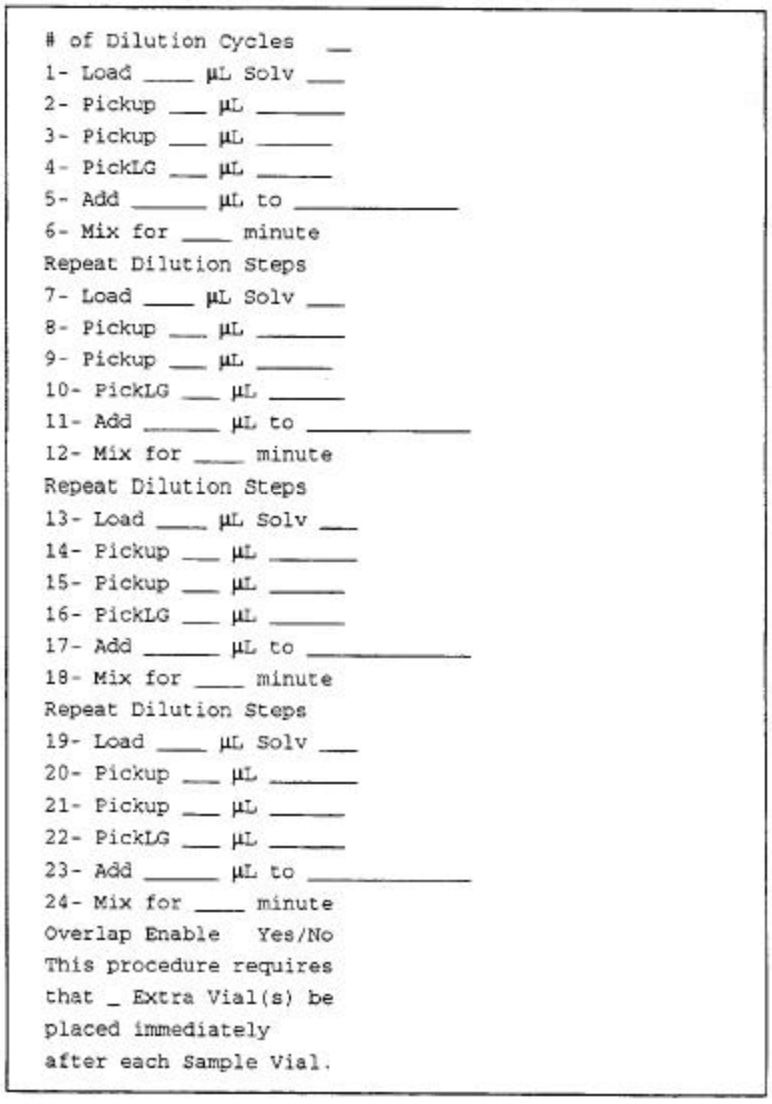
You'll discover other shortcuts as you integrate your autosampler into your laboratory procedures.

BLANK FORMS

After completing this chapter, you are now ready to begin developing your own sample preparation procedures. To assist you in documenting your sample preparation protocols, we have included a blank form (without the default values) for each of the four templates We have provided four dilution cycles (the maximum available) for each template. We hope these forms will be helpful to you as you incorporate your SpectraSYSTEM autosampler into your regular laboratory procedures.

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TEMPLATE 1:
DILUTION



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TEMPLATE 2:
DILUTION

```
# of Dilution Cycles   __
Heater: ___ Temp: ___
1- Load ____ µL Solv ___
2- Pickup ___ µL _______
3- Pickup ___ µL ______
4- PickLG ___ µL ______
5- Add ______ µL to _____________
6- Mix for ____ minute
7- Heat for ___ minute
8- Mix for ____ minute
Repeat Dilution Steps
9- Load ____ µL Solv ___
10- Pickup ___ µL _______
11- Pickup ___ µL ______
12- PickLG ___ µL ______
13- Add ______ µL to _____________
14- Mix for ____ minute
15- Heat for ___ minute
16- Mix for ____ minute
Repeat Dilution Steps
17- Load ____ µL Solv ___
18- Pickup ___ µL _______
19- Pickup ___ µL ______
20- PickLG ___ µL ______
21- Add ______ µL to _____________
22- Mix for ____ minute
23- Heat for ___ minute
24- Mix for ____ minute
Repeat Dilution Steps
25- Load ____ µL Solv ___
26- Pickup ___ µL _______
27- Pickup ___ µL ______
28- PickLG ___ µL ______
29- Add ______ µL to _____________
30- Mix for ____ minute
31- Heat for ___ minute
32- Mix for ____ minute
Overlap Enable   Yes/No
This procedure requires
that _ Extra Vial(s) be
placed immediately
after each Sample Vial.
```

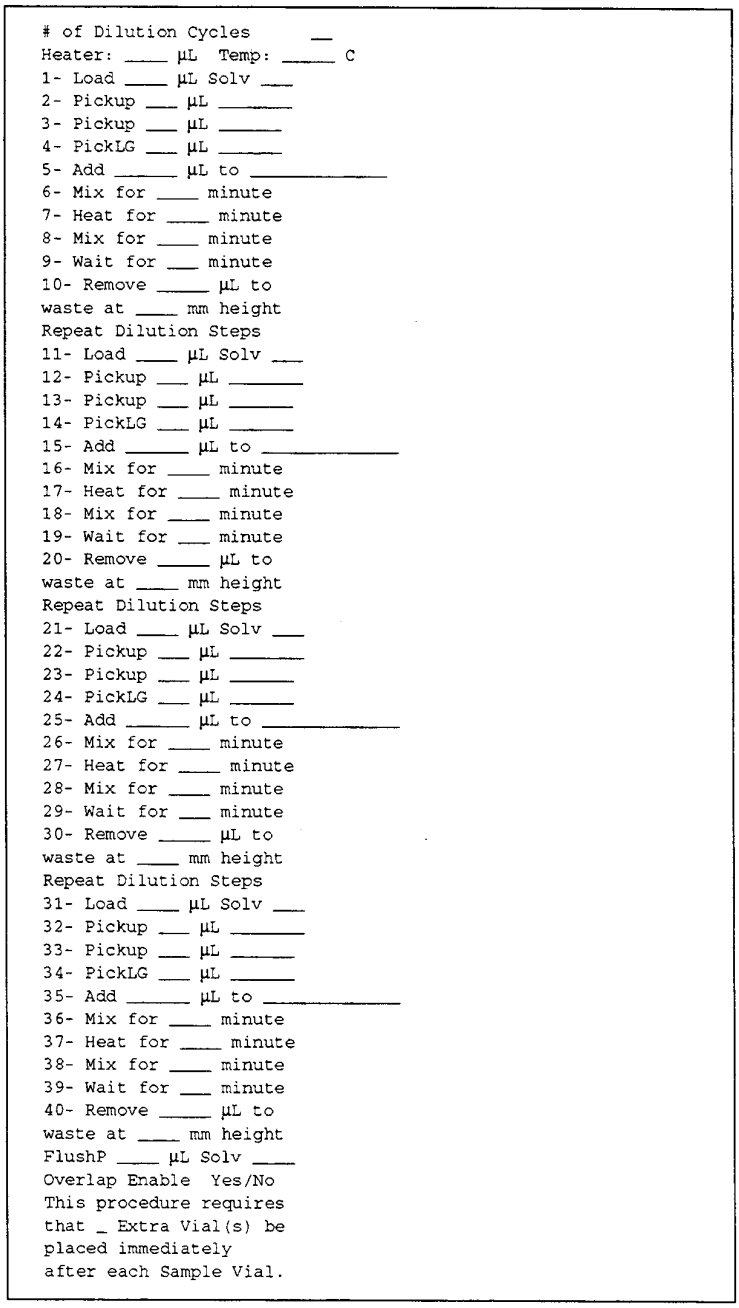
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TEMPLATE 3:
DILUTION

```
# of Dilution Cycles   __
1- Load ____ µL Solv ___
2- Pickup ___ µL _______
3- Pickup ___ µL ______
4- PickLG ___ µL ______
5- Add ______ µL to _____________
6- Mix for ____ minute
7- Wait for ___ minute
8- Remove _____ µL to
waste at ____ mm height
Repeat Dilution Steps
9- Load ____ µL Solv ___
10- Pickup ___ µL _______
11- Pickup ___ µL ______
12- PickLG ___ µL ______
13- Add ______ µL to _____________
14- Mix for ____ minute
15- Wait for ___ minute
16- Remove _____ µL to
waste at ____ mm height
Repeat Dilution Steps
17- Load ____ µL Solv ___
18- Pickup ___ µL _______
19- Pickup ___ µL ______
20- PickLG ___ µL ______
21- Add ______ µL to _____________
22- Mix for ____ minute
23- Wait for ___ minute
24- Remove _____ mL to
waste at ____ mm height
Repeat Dilution Steps
25- Load ____ µL Solv ___
26- Pickup ___ µL _______
27- Pickup ___ µL ______
28- PickLG ___ µL ______
29- Add ______ µL to _____________
30- Mix for ____ minute
31- Wait for ___ minute
32- Remove _____ µL to
waste at ____ mm height
FlushP ___ µL Solv ___
Overlap Enable  Yes/No
This procedure requires
that _ Extra Vial(s) be
placed immediately
after each Sample Vial.
```

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TEMPLATE 3:
DILUTION



```
# of Dilution Cycles      __
Heater: ____ µL  Temp: _____ C
1- Load ____ µL Solv ___
2- Pickup ___ µL _______
3- Pickup ___ µL ______
4- PickLG ___ µL ______
5- Add ______ µL to _____________
6- Mix for ____ minute
7- Heat for ____ minute
8- Mix for ____ minute
9- Wait for ___ minute
10- Remove _____ µL to
waste at ____ mm height
Repeat Dilution Steps
11- Load ____ µL Solv ___
12- Pickup ___ µL _______
13- Pickup ___ µL ______
14- PickLG ___ µL ______
15- Add ______ µL to _____________
16- Mix for ____ minute
17- Heat for ____ minute
18- Mix for ____ minute
19- Wait for ___ minute
20- Remove _____ µL to
waste at ____ mm height
Repeat Dilution Steps
21- Load ____ µL Solv ___
22- Pickup ___ µL _______
23- Pickup ___ µL ______
24- PickLG ___ µL ______
25- Add ______ µL to _____________
26- Mix for ____ minute
27- Heat for ____ minute
28- Mix for ____ minute
29- Wait for ___ minute
30- Remove _____ µL to
waste at ____ mm height
Repeat Dilution Steps
31- Load ____ µL Solv ___
32- Pickup ___ µL _______
33- Pickup ___ µL ______
34- PickLG ___ µL ______
35- Add ______ µL to _____________
36- Mix for ____ minute
37- Heat for ____ minute
38- Mix for ____ minute
39- Wait for ___ minute
40- Remove _____ µL to
waste at ____ mm height
FlushP ____ µL Solv ____
Overlap Enable  Yes/No
This procedure requires
that _ Extra Vial(s) be
placed immediately
after each Sample Vial.
```

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5 Required Maintenance

Introduction

SPECIFIC HAZARDS

This chapter contains the maintenance schedule and procedures for your autosampler.

For your own safety, please familiarize yourself with and follow these safety precautions when using this instrument:



WARNING -- High Voltage! To avoid electrical shock, do not remove the top cover or remove parts beyond the descriptions in the operation portions *of* this manual or in the maintenance section.



WARNING! Disconnect the power cord before replacing fuses.



WARNING! When replacing fuses, use only fuses *of* the type and current rating specified. Do not use repaired fuses and do not short - circuit the fuse holder.



WARNING! Failure to heed these or any other cautions or warnings described in this manual may invalidate your warranty.

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Maintenance Schedule

Your autosampler requires only a few simple maintenance procedures to keep it in optimal working condition. Table 5.1 contains the Maintenance Schedule for the autosampler. The yearly items should be performed only by a LabAlliance service representative or other qualified person.

Table 5.1 Maintenance Schedule

Frequency	Procedure	Performed By
Daily	Empty flush solvent tray	User
Monthly	Inspect the sample needle module.	User
	Check the solvent tubing and connections for leaks.	User
	Run a standard sample.	User
	Clean the drop catch.	User
Semiannually	Replace the flush-solvent inlet filter.	User
Annually	Replace the injector valve's rotor seal.	User
	Clean and lubricate the syringe drive mechanism.	Service
	Clean and lubricate the XYZ arm.	Service
	Verify the XYZ arm alignment.	Service



***NOTES**: Maintenance of the autosampler is the responsibility of the user. Routine maintenance is not provided under warranty; however, planned maintenance contracts are generally available. Please contact your local representative if you are interested in purchasing a planned maintenance contract.*

This chapter contains recommended maintenance intervals based upon operation of the autosampler under typical conditions. Operation of the autosampler under certain harsh conditions (corrosive solvents, extreme temperatures, etc.) may require more frequent maintenance than indicated in this chapter.

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Maintenance Schedule

EMPTYING THE FLUSH-SOLVENT TRAY

For normal operation, you may need to empty the tray at least once or twice a day. To empty the tray:

1. Be sure the autosampler is in idle status.
2. Carefully slide the tray out without spilling the collected solvent.
3. Empty the tray, rinse it off with water, and slide it back into place under the black elbowed, barbed piece of tubing.



WARNING! Dispose of hazardous waste according to local, state, and federal regulations.



CAUTION! Be sure that the level of the liquid in the waste container is below the level of the instrument or waste *will* collect in the lines and could back flush into the instrument.

REPLACING THE ROTOR SEAL

Replacing the rotor seal includes the following procedures:

- Removing the injector valve
- Disassembling the valve
- Cleaning the stator
- Reassembling and replacing the valve

NOTE: If you are only replacing the rotor seal, you don't have to remove the injector valve from the instrument; however, it's easier. If you wish to replace the rotor seal with the valve installed, proceed to the section entitled, "Disassembling the Injector Valve" on page 87.

Removing and reinstalling the Injector Valve

To disassemble the injector valve (Fig. 5. 1):

1. Using a 1/4" open-end wrench, disconnect tubing from ports 2, 3, 5, and 6.
2. If you have the Column Oven Option and are using the 100 pL loop shipped with your instrument, you remove the sample loop. Otherwise, proceed to step 3.
3. If you have purchased the column oven, loosen and remove the two Phillips-head screws that secure the injector valve collar to the front of the oven. Then remove the collar.
4. Remove the two Phillips screws that secure the valve mounting plate.
5. Carefully pull the valve out of the instrument.
6. Reverse these steps to reinstall the valve.

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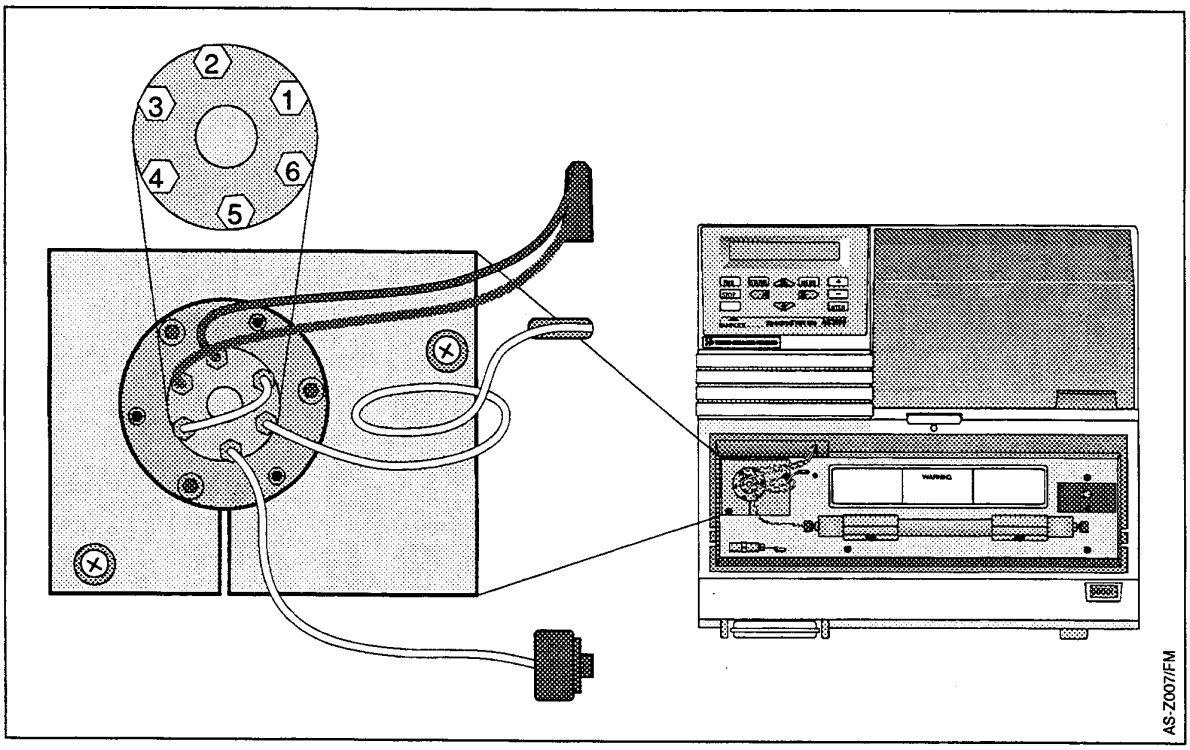


Figure 5.1 The injector valve

CAUTION! When reinstalling the bracket/valve assembly, be sure to orient the bracket with the "locating-hole" corner in the lower right as shown in Figure 5.2. You should be able to see the pin through the locating hole.

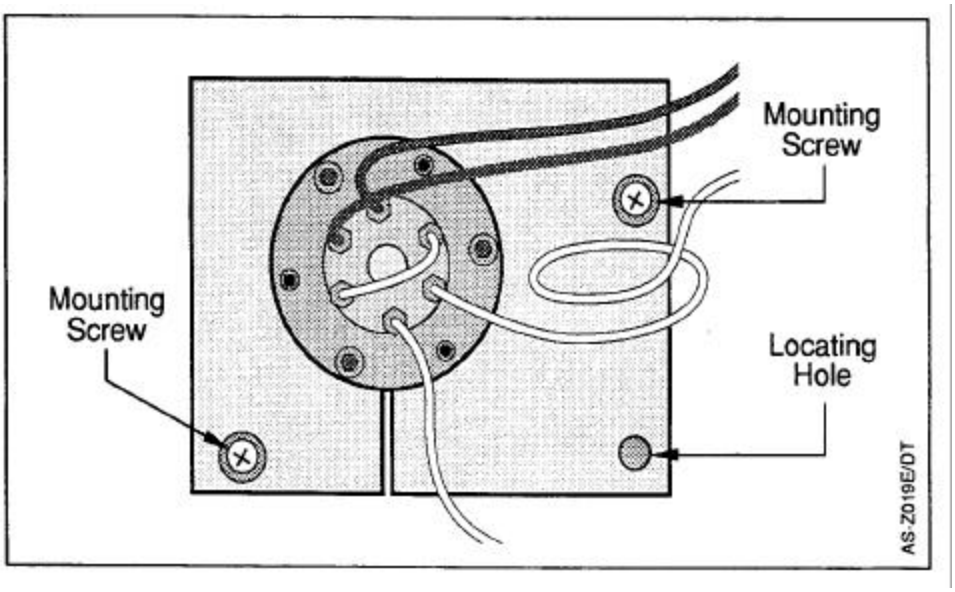


Figure 5.2 The correct injector valve bracket orientation

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Disassembling the Injector Valve

***NOTE**: This repair must be done in a clean environment. Component cleanliness will affect the useful life of your injector valve.*

CAUTION! Do not activate the valve mechanism with the injector valve removed from the instrument or valve mechanism damage could occur.

To disassemble the injector valve:

1. Follow the procedures above for removing the injector valve.
2. Using an Allen wrench, remove the 9/64" hex cap screws from the top of the valve (Fig. 5.3).
3. Carefully lift the stator from the valve body and set it onto a clean surface.

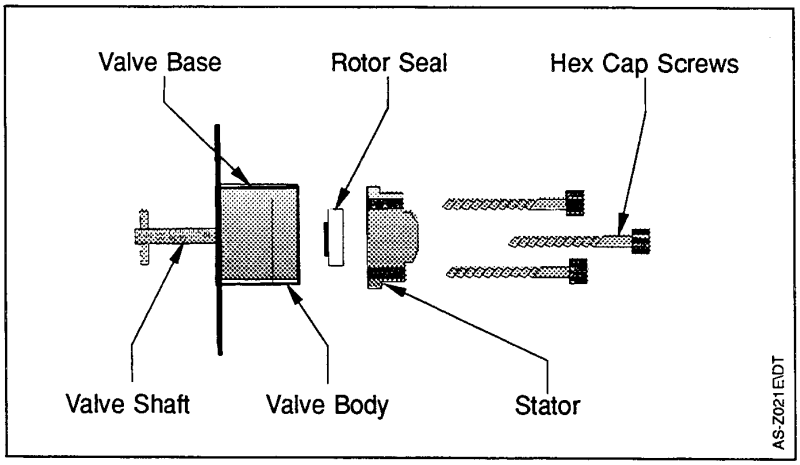


Figure 5.3 The injector valve's components

***NOTE**: Be careful not to drop the two rotation stop pins that protrude from the valve base (Fig. 5.4).*

4. Remove the valve body.
5. Before removing the worn rotor seal, note the following on the rotor seal (Fig. 5.4): the three circular port grooves on one side of the rotor (the other side is smooth) the small locating notch on the rotor's rim (not the V stamped on the seal).

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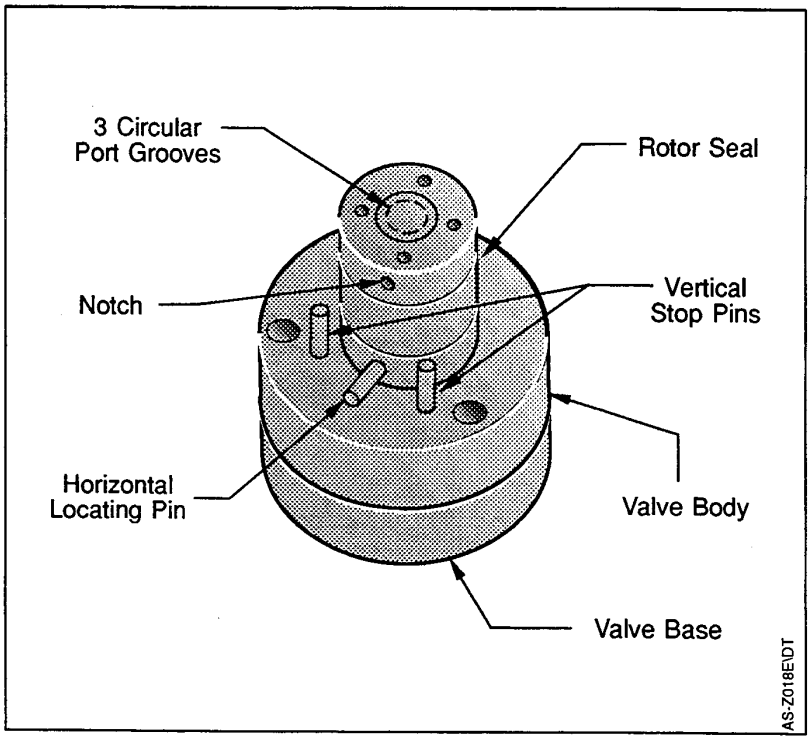


*Figure 5.4 The injector valve with valve body removed and rotor seal exposed (*NOTE*: The mounting bracket is not shown in this picture.)*

6. Remove the old rotor seal. A thin, flat-bladed tool may be needed.

Clean the Stator

Clean the stator as follows:

1 . Inspect the stator to determine if it requires cleaning.

2. If it is dirty or greasy, swab it with BPLC-grade methanol. If more stringent cleaning is required, use a sonicator.

3. Inspect the remaining valve components for contamination. Clean as necessary.



CAUTION! If the stator is scratched, replace it. Scratches can damage the rotor seal and cause valve leaks.

Installing the Rotor Seal

To install the rotor seal:

1. Install the new rotor seal in exactly the same orientation as the old one. Be sure that the rim notch is in line with the horizontal locating pin and that the circular port grooves are visible.

2. Install the valve body.

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3. Install the stator onto the valve body carefully, observing that the two locating pins in the valve body (Fig. 5.4) line up with the two holes on the bottom side of the stator assembly (Fig. 5.5).

CAUTION: Be sure to hold the valve shaft so that the horizontal locating pin touches the left stop pin as you reassemble the valve. This puts the valve into the INJECT mode (the same mode as when removed from the instrument). If the pins are not in the correct orientation, the valve shaft may not be in the proper orientation for reinsertion into the instrument.

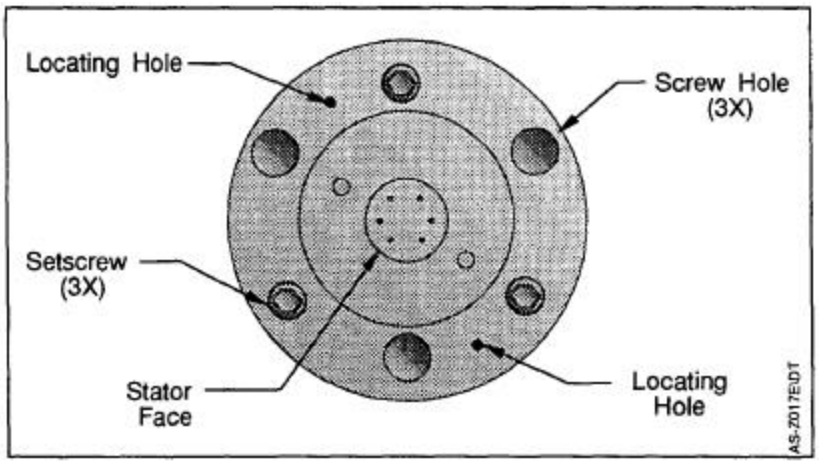


Figure 5.5 The bottom of the stator assembly

***NOTE**: These locating pins orient the stator assembly in one of two positions, but only one position aligns the mounting holes in the valve body with the screw holes in the stator.*

3. Insert the three cap screws so that each one just contacts the stator.

***NOTE**: There should be a uniform gap between the stator and the valve body to maintain the necessary pressure for proper valve operation and to avoid valve binding. Do not over-tighten the valve.*

4. Tighten each cap screw an equal amount until all set screws are in equal contact with the valve body.

REPLACING THE FLUSH SOLVENT INLET FILTER

Properly filtering your flush solvent extends the life of your injector valve and column. Replace the inlet filter as follows:

1. Be sure the autosampler is in the IDLE mode.

2. Remove the flush-solvent bottle cap and lift out the inlet tubing.

CAUTION: Be sure to follow all of the safety precautions for each solvent as prescribed by the manufacturer's MSDS.

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3. Put on a new filter cartridge and put the line back into the bottle. Tighten the solvent cap.

4. Flush the inlet filter and lines with an appropriate volume of solvent (~5 µL).

SAMPLE NEEDLE

Module

The sample-needle module resides in the sample tower and is responsible for piercing the vial septum and removing sample from the vial. It easily snaps out of the needle mount and is very easy to maintain.

Removal

Use the following steps to remove the sample needle module:

1. Remove the sample needle fitting from port 2 of the injection valve using a 1/4" open-end wrench.

2. Carefully (to avoid damage to the sample tubing) release the sample needle module from the tower by grasping the module edges and pulling towards the rear of the autosampler.

3. Pass the needle fitting through the autosampler' s front bulkhead and remove the needle module from the autosampler.



***NOTE**: If you are replacing (not inspecting) the needle, you may wish to cut the used needle to make it easier to pass the fitting through the bulkhead.*

Cleaning

To clean the sample needle module:

1. Inspect the needle module.

2. Rinse in water or sonicate if contamination is present.

3. If internal plugging is suspected, pass a cleaning wire through the needle and flush the needle with high flow from an LC PUMP.



CAUTION! The sample needle module is glued during manufacture and cannot be disassembled. Attempts to do so *will* damage the module.

Installation

Replace the sample needle module every six months. The needle module is easy to replace because it is designed to snap into place.

I . Remove the new module from the replacement kit.

2. Pass the valve end of the needle through the hole on the underside of the column compartment.

3. Snap the needle assembly into the mounting brackets on the backs ide of the sample tower (Fig. 5.6).

4. Place the tube fitting and ferrule onto the needle and install it into port 2 of the injection valve (Fig. 5. 1).

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Drop Catch

The drop catch (Figure 5.6) removes the last drop of sample from the end of the needle after injection. It must be flushed monthly as follows:

1. Remove the needle module assembly as described above, but leave the drop catch installed.

2. Flush the drop catch with water, an organic solvent, an acid or base (as appropriate for the sample), and once more with water. Then dry it with a clean tissue.

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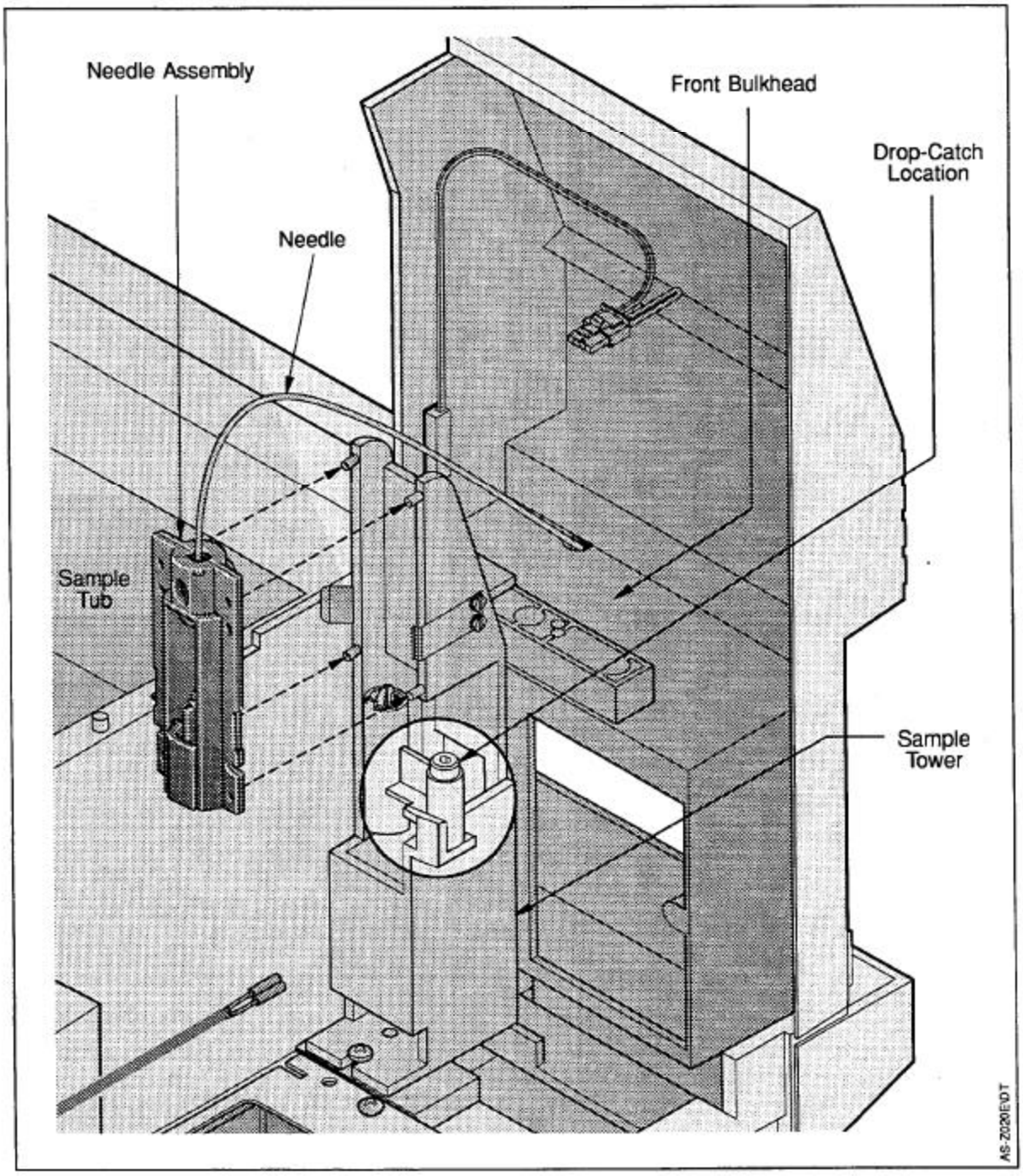


Figure 5.6 Replacing the sample needle module

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SYRINGE DRIVE ASSEMBLY

The section describes how to replace the sample (250 KQ syringe and the large (2.5 mL) sample preparation syringe.

250 mL Sample Syringe

You can replace the 250 µL sample syringe without removing the syringe drive assembly, but you must move the syringe drive about two inches out by interrupting a flush cycle. Replace the sample syringe as follows:

1. From the Main Menu, choose /Commands/, and /Flush Sample Syringe/.

2. When the plunger is lowered, turn off the power.

3. Hold the silver base of the plunger to prevent it from turning, and loosen the knurled syringe set screw under the syringe's plunger.



HINT *You may need to insert a screwdriver or coin into the slot in the set screw to get the screw started and then lower the plunger.*



NOTE*: The syringe set screw is "captive" and won't all down into the instrument's chassis.*

4. To loosen the top of the syringe, hold the metal tip on the top of the syringe barrel, unscrew the used syringe barrel and lift it out of the instrument.



CAUTION! Don't try to loosen the syringe by holding the barrel. The syringe could break between the glass barrel and the metal tip.



HINT*: Push up on the syringe plunger while lifting it out of place.*



HINT: *Install the syringe in its fully expelled position to prevent an air bubble in the syringe barrel.*

5. Reverse steps 4 and 5 to install your new syringe.

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2.5 mL Preparation Syringe

If you have the Sample Preparation Option, you can position the large (2.5 mL) syringe's plunger in the down position by interrupting a flush cycle, or you can remove the prep syringe without using the display and with the plunger in the normal operating position. The two procedures are detailed here.

Interrupting a FlushPrep Cycle

1. Be sure the autosampler is in the IDLE mode.
2. Move the XYZ arm out of your way by pressing [MENU], Commands, /Ann/, /Align Arm/, and selecting position A01. Then press [ENTER].
3. To start the flush cycle, from the Main Menu, choose Commands, and Flush Prep Syringe.
4. When the plunger has lowered, turn off the power.
5. Hold the plunger to prevent it from turning, and loosen the knurled syringe set screw under the syringe's plunger.



HINT: You may need to insert a screwdriver or coin into the slot in the set screw to get the screw started.



***NOTE**: The syringe set screw is "captive" and won't fall down into the instrument's chassis.*

6. To loosen the top of the syringe, hold the metal tip on the top of the syringe barrel, unscrew the used syringe barrel and lift it out of the instrument.



CAUTION! Don't try to loosen the syringe by holding the barrel. The syringe could break between the glass barrel and the metal tip.



***HINT**: Push up on the syringe plunger while lifting it out of place.*

7. Reverse steps 5 and 6 to install your new syringe.



***HINT**. When reinserting the bottom set screw, you may need to pull the plunger down slightly so that the end of the screw will reach the bottom of the plunger.*

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Removing Syringe with Plunger in Operating Position

Use the following procedure to remove the prep syringe without accessing the instrument's display:

I . Be sure the autosampler is in the IDLE mode or turned off.

2. Move the arm and hook to position AO I so that you can easily access the syringe assembly.



***HINT**: You can move the arm out of the way manually, or press [MENU], /Command/, /Arm/ , /Align Arm/, select position A01, and press [ENTER].*



CAUTION! Before moving the arm manually, be sure the pod (the triangular assembly that moves along the XYZ arm) is pushed all the way to the left. If you try to move the arm with the sample tower pushed toward the right side of the sample tub, the arm may bind and not move easily.

3. Hold the plunger to prevent it from turning, and loosen the knurled syringe set screw under the syringe's plunger.



NOTE: The syringe set screw is "captive" and won't fall down into the instrument's chassis.

4. Holding the metal tip on the top of the syringe barrel, unscrew the used syringe barrel and lift it out of the instrument.



***HINTS:** Push up on the syringe plunger while lifting it out of place.*

Install the syringe in its fully expelled position to prevent an air bubble in the syringe barrel.

5. Reverse steps 3 and 4 to install your new syringe.



***HINT**: When reinserting the bottom set screw, you may need to pull the plunger down slightly so that the end of the screw will reach the bottom of the plunger.*

SAMPLE LOOP

A number of different size sample loops are available for the SpectraSYSTEM autosamplers. Refer to the list of spare parts and consumables in the front of your manual. To change the sample loop:

1. Be sure that the autosampler is in the IDLE mode.

2. Using a open-end wrench, loosen the fittings at ports 1 and 4, and pull out the two ends of the sample loop.

3. Insert the ends of the new sample loop in the same orientation as the old one and tighten the fittings.



***NOTE**: If the Sample Preparation Option is installed, to ensure maximum reproducibility, do not perform small-volume injections with the large (preparation) syringe.*

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FUSES

Instrument power is supplied by two 5.0-amp SB fuses housed in the fuse compartment on the lower left comer as you are facing the instrument's rear panel (Fig. 5.7).

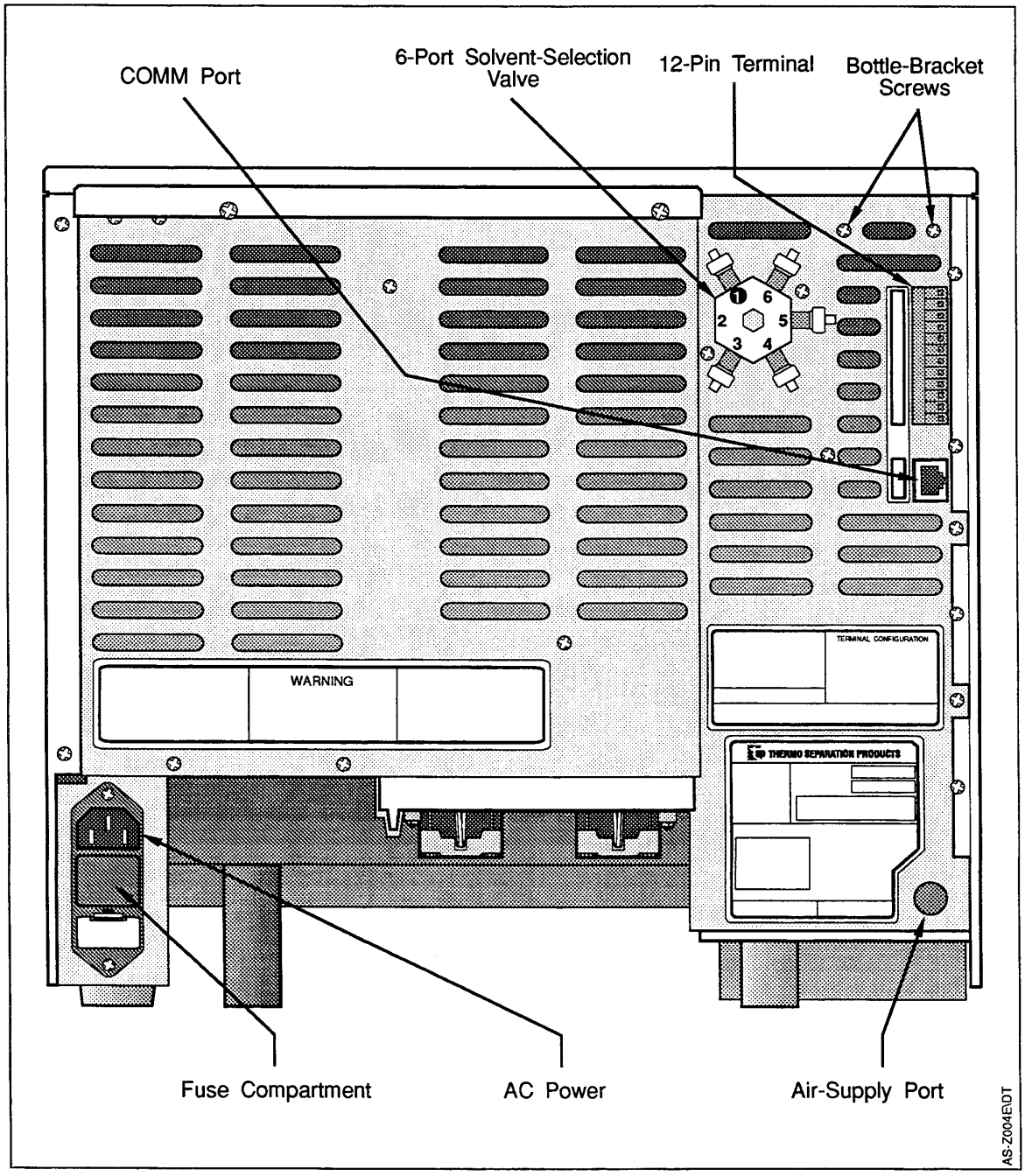


Figure 5.7 The autosampler's rear panel

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To replace the fuses:

1. Use a flat blade to press down on the plastic tab at the top edge of the fuse compartment and pull out the fuse holder.

2. Pull both fuses out of the holder and discard. Place the new fuses into the holder with the metal end visible.

3. Snap the fuse holder back into place.

Maintenance Options

If the autosampler is not used for a period of time (after completing an oven-light or over-the-weekend run), you can set it to turn off automatically. You enter the shutdown parameters in the OPTIONS Shutdown Menu (Fig. 5.8).

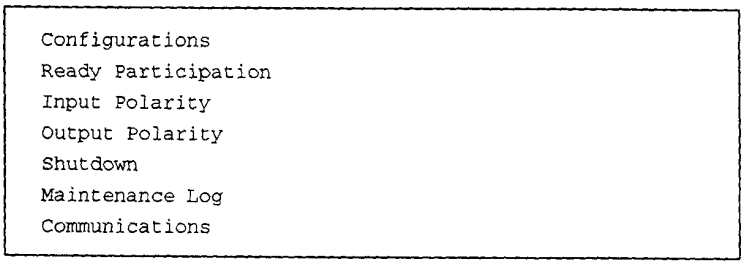


Figure 5.8 The Options Menu

Figure 5.9 illustrates autosampler's Shutdown Menu.

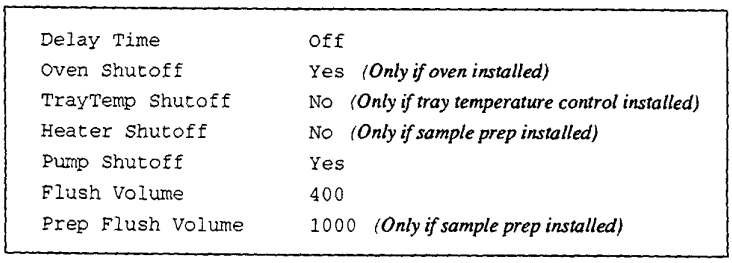


Figure 5.9 The Shutdown Menu

Delay Time

In this field you specify the desired amount of time that the autosampler should wait before initiating the shutdown procedure (Off, 5, 10, 20, 30, 45, 60, 90, 120, 240, 480 minutes).

Oven Shutoff

Use the [+] and [-] keys to select Yes or No. Selecting Yes causes the autosampler to automatically turn off the oven after the Shutoff Delay time has elapsed.

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***NOTE**: If you are using the Shutdown function with methods that call for heating and cooling, be sure to allow a long enough delay time (not less than 10 minutes)for thermal equilibrium to occur.*

TrayTemp Shutoff

Use the [+] and [-] keys to select Yes or No. Selecting Yes causes the autosampler to automatically turn off the Tray Temperature Control Option after the Shutoff Delay time has elapsed. You may or may not wish to turn off tray temperature control depending upon your sample requirements.

Heater Shutoff

Use the [+] and [-] keys to select Yes or No. Selecting Yes causes the autosampler to automatically turn off the heater after the Delay Time has elapsed. This field appears only if the Sample Preparation Option is installed.

Pump Shutoff

Use the [+] and [-] keys to select Yes or No. Selecting Yes causes the autosampler to automatically activate the Pump Stop output after the Delay Time has elapsed. Turning off the pump retains your solvent supply and prevents air from being pumped into the lines should the solvent reservoirs become depleted.

Flush Volume

This field allows you to flush the lines using the 250 PL sample syringe. Specify the flush volume in microliters (200 - 5000). The default value is 400 µL.

Prep Flush Volume

This field allows you flush the lines using the 2.5 mL sample preparation syringe. Specify the flush volume in microliters (0 - 6500). This field appears only if the Sample Preparation Option is installed.

MAINTENANCE LOG

The Maintenance Log Menu (Figure 5. 10), accessible from the OPTIONS Menu, monitors the instrument's maintenance status and counts and displays the number of activations for each of the autosampler's routine maintenance parts listed in the menu.

```
Total System Cycles
Injector Valve
Needle Wear
Sample Syringe
Prep Syringe
```

Figure 5.10 The Maintenance Log Menu

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Instrument Maintenance Status

The Maintenance Log Menu provides a convenient way to keep track of autosampler maintenance. When you first access the Maintenance Log Menu from the OPTIONS Menu, the "Maintenance Required" confirmation message is displayed if instrument maintenance is required. This message is displayed if any of the #Cycles field values in any of the Maintenance Log menus is greater than the number of cycles entered in the Due fields. See below for more information on these fields.

No message is displayed if maintenance is not required. Pressing the down-arrow key clears the confirmation message and returns you to the Maintenance Log Menu.

Total System Cycles Menu

The Total System Cycles Menu (Figure 5.11) displays the total number of instrument cycles in the Total Cycles field. This field value is set to zero at the factory and cannot be reset. The Note field is a six-character, alphanumeric, user-programmable field. You can use the [+]/[-] keys to enter the last service date or other comments regarding the maintenance of your instrument.

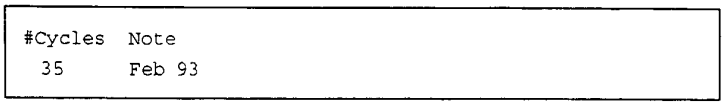


Figure 5.11 The Total System Cycles Menu with the Note field used to indicate the next maintenance due date

Injector Valve Menu

With the exception of the Total System Cycles Menu, all of the Maintenance Log menus are identical to the Injector Valve Menu (Figure 5.12). Therefore, only the Injector Valve Menu will be described in detail in this chapter. Refer to Appendix B for definitions of the other Maintenance Log menus.

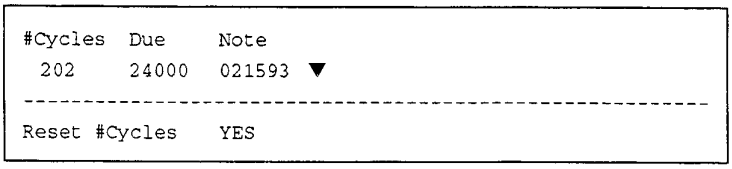


Figure 5.12 The Injector Valve Menu

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#Cycles: This field displays the number of activations of the specific autosampler part since the last time this field (counter) was reset (0 -999,999). The #Cycles value for each of the Maintenance Log menus is included in the menu descriptions below.

Due: This is a six-character numeric field where you enter the number of cycles before maintenance is due. If the #Cycles field value is larger than the Due field value, the "Maintenance Due" message is displayed when you access the Maintenance Log Menu from the Options Menu. The default maintenance Due field value is 12,000 (approximately once a year). The default values for the other Maintenance Log menus are included in the menu descriptions below.



***NOTE**: The Due field default values for all of the Maintenance Log menus are estimates only. The actual values appropriate for your instrument will vary with the type of applications and the specific conditions being used with your LC. If the "Maintenance Required" message is displayed, parts replacement is recommended. However, you may wish to increase the Due value and experiment with extending the time before maintenance is performed. Conversely, if instrument performance indicates, you may wish to decrease the Due field value for some of the Maintenance Log menus.*

Note: Similar to the Total System Cycles Menu, this is a six character, alphanumeric, user-programmable field. We recommend that you use the [+]/[-] keys to enter the last service date in this field. However, you can also use this field to document contact information or other comments regarding the maintenance of the specific autosampler part.

Reset #Cycles: Pressing the down-arrow key displays the Reset #Cycles field used to reset the #Cycles counter for each autosampler part. To reset the counter to 0 after maintenance is complete, press the [+]/[-] keys to select YES, and press [ENTER]. The confirmation message ** Counter Reset ** is displayed. Most likely, your service representative will reset the #Cycles field value when the instrument is serviced.

Needle Wear Menu

The #Cycles field in this menu is incremented each time the needle pierces a vial. The Due field's default value is 10,000, which corresponds to approximately every six months.

Sample and Prep Syringe Menus

The #Cycles field in both of these menus displays each fun syringe stroke. However, the instrument tracks the actual distance of linear travel, thus compensating for different injection (stroke) volumes. The default Due field value for both menus is 24,000, which corresponds to approximately once a year.

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A Basic Installation, Specifications, and Warranty

Introduction

This appendix describes the site requirements, basic installation procedures, and performance testing for the AS 1000 and AS 3000 SpectraSYSTEM autosamplers. Appendix D contains instructions for the advanced installation procedures and instrument configuration and initialization procedures.



***HINT**: If you are setting up your autosampler for the first time, you may wish to follow the procedures in the basic installation section and skip the advanced section until you are more familiar with your instrument.*

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Basic Installation

The easiest way to install your SpectraSYSTEM autosampler is to unpack it and set it on the bench, complete the procedures that require access to the back of the instrument, and then complete those that require access to the front. Additional or modified procedures required for instruments with one or more of the integrated options are incorporated into this section.



***NOTE**: For a list of all available options, upgrades, and accessories for your autosampler, see the insert at the front of this manual or contact your LabAlliance sales representative. Note that all upgrades require specialized installation. Contact your LabAlliance field service representative for further information.*

If you purchased the Sample Preparation Option, there are several additional steps in making the rear panel connections. Similarly, if you purchased the Column Oven Option or one of the inert/biocompatible versions, there is a slightly different installation procedure for making the front panel connections. These changes to the normal setup procedure are included in the rear- and front-panel sections, respectively. The sample Tray Temperature Control Option comes completely installed and requires no special user installation procedures.

BEFORE YOU START

Before you start, review the site requirements and assemble the tools you'll need to install your instrument: One Phillips screwdriver Two 1/4" open-end wrenches One small, standard screwdriver

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Site Requirements

SPACE

The standard autosampler weighs 16 kg. with all options installed, the autosampler weighs 24 kg. The instrument requires a bench space at least 37 cm by 41 cm by 51 cm. Be sure to allow adequate ventilation away from heating or air conditioning ducts. Avoid exposure to direct sunlight. If used with the SpectraSYSTEM pump and detector, place the autosampler in the middle (Fig. A. 1).

HINT: You may wish to set your instrument on the bench such that you have easy access to the rear panel.

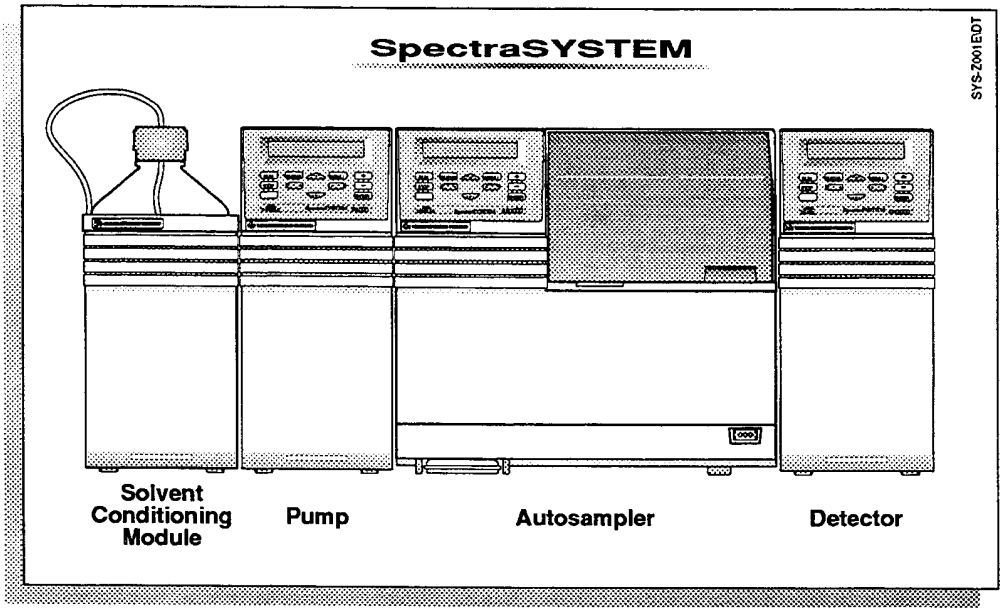


Figure A.1 The SpectraSYSTEM configuration

CAUTION! Be sure to use the proper venting apparatus if you use hazardous solvents.

Your autosampler automatically configures itself to operate from the local line voltage (100 - 260 VAC at 50 or 60 Hz). You do not need to set the line voltage. The instrument is shipped with the necessary fuses installed.

You should provide a stable, spike-free power source. This will avoid intermittent failures and unexplained occurrences (lockup, loss of memory, incorrect operation) that are directly related to poor power. We also recommend that you use an uninterruptable power supply (UPS) for maximum protection from power-related failures.

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Unpacking

Your autosampler was shipped in a specially designed shipping container to protect it from damage during transit. Upon arrival, inspect all containers and immediately report any damage in transit to the transportation company. Have the carrier note the shipping crate's condition on both the delivery receipt and the freight bill. The carrier is responsible for all damage incurred in shipment.

All SpectraSYSTEM autosamplers are shipped with the sample trays and syringe(s) installed. A cardboard shipping insert on top of the sample trays restrains the XYZ arm and holds the sample trays in place during shipment. Remove the shipping insert. Retain both the shipping insert and shipping crate should future shipment become necessary.

After unpacking, inspect your autosampler and its accessories for missing parts and/or physical damage. If damage is found, notify both the carrier and your LabAlliance representative. Please do not return goods without prior authorization (either a Returned Goods Authorization number or a Returned Materials Authorization number) from LabAlliance.

The contents of your accessory key will vary with the model and options

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you ordered (refer to Table A.1). Notify your LabAlliance representative of any shortages or damages.

Table A.1 Accessory Kit Contents

Part No	Qty	Description
Standard Accessory Kit		
1414-0300	1	250 mL glass bottle, screw top
2522-0066	3	extra long Rheodyne fitting/bushing
2522-3830	3	Rheodyne ferrule
5101-1851	1	6.3A fuse, 250V, SB
A0941-010	1	Tube, Stainless steel, .060DX.01 I.D. 12 inches
A0941-060	1	Tube, .06X.01X4.0
A2203-020	1	Vial kit (100)
A3563-010	1	Flush waste container
A3651-010	1	Bracket, flush solvent bottle
A4095-010	1	Cable assembly (4-donductor)
A4109-010	1	Solvent line preparation kit (cap and lines)
A4162-020	1	Label set
7111-0605	2	Screws for bottle bracket
Sample Preparation Accessory Kit (AS3000)		
A4094-010	4	Solvent filter
A4063-010	4	.125 tubing assembly with Cheminert™ fittings
Inert/Biocompatible Accessory Kit		
1413-0030	1	250 mL glass bottle, screw top
2103-9119	1	connector, plug, cable, (12FM, 5MM)
5101-1851	1	6.3A fuse, 250V, SB
A2203-020	1	Vial kit (1 package contains 144 vials)
A3563-010	1	Flush waste container
A3651-010	1	Bracket, flush solvent bottle
A3786-010	1	PEEK tubing, 3.94 x .010 I.D.
A3788-010	3	PEEK tubing fitting, 1/16 O.D.
A4095-010	1	Cable assembly 4-conductor
A4109-010	1	Solvent line preparation kit
A4162-020	1	Label set
7111-0605	2	Screws for bottle bracket

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Installation

REAR PANEL

Installation is easiest if you make the rear panel connections first. The rear panel installation procedures include plumbing (flush bottle), system communications and AC power.

Plumbing

Your accessory kit includes a 250 mL flush-solvent bottle and cap and a bottle bracket. The bracket attaches to the upper-right corner of the rear panel (as you are facing the rear of the instrument) and holds the flush bottle in the correct position and elevation for optimal flow through the lines.



***NOTE**: If you wish to use your own solvent bottle or other container, be sure that it is clean and chemically inert. We recommend using glass bottles.*



***HINT**: If you purchased the SpectaSYSTEM Solvent Conditioning Module (SCM400), you may want to store your flush solvent in one of the bottles in your SCM instead of installing a separate flush bottle and bracket.*

Bottle Bracket

The bottle bracket fastens to the rear panel with two Phillips-head screws included in your accessory kit. To install the flush-bottle brackets:

1. Align the bracket's mounting holes with the corresponding screw holes on the autosampler's rear panel (Fig. A.2).

2. Insert the screws into the holes and tighten them in place. Be sure that the bracket is fastened securely onto the back of the instrument.

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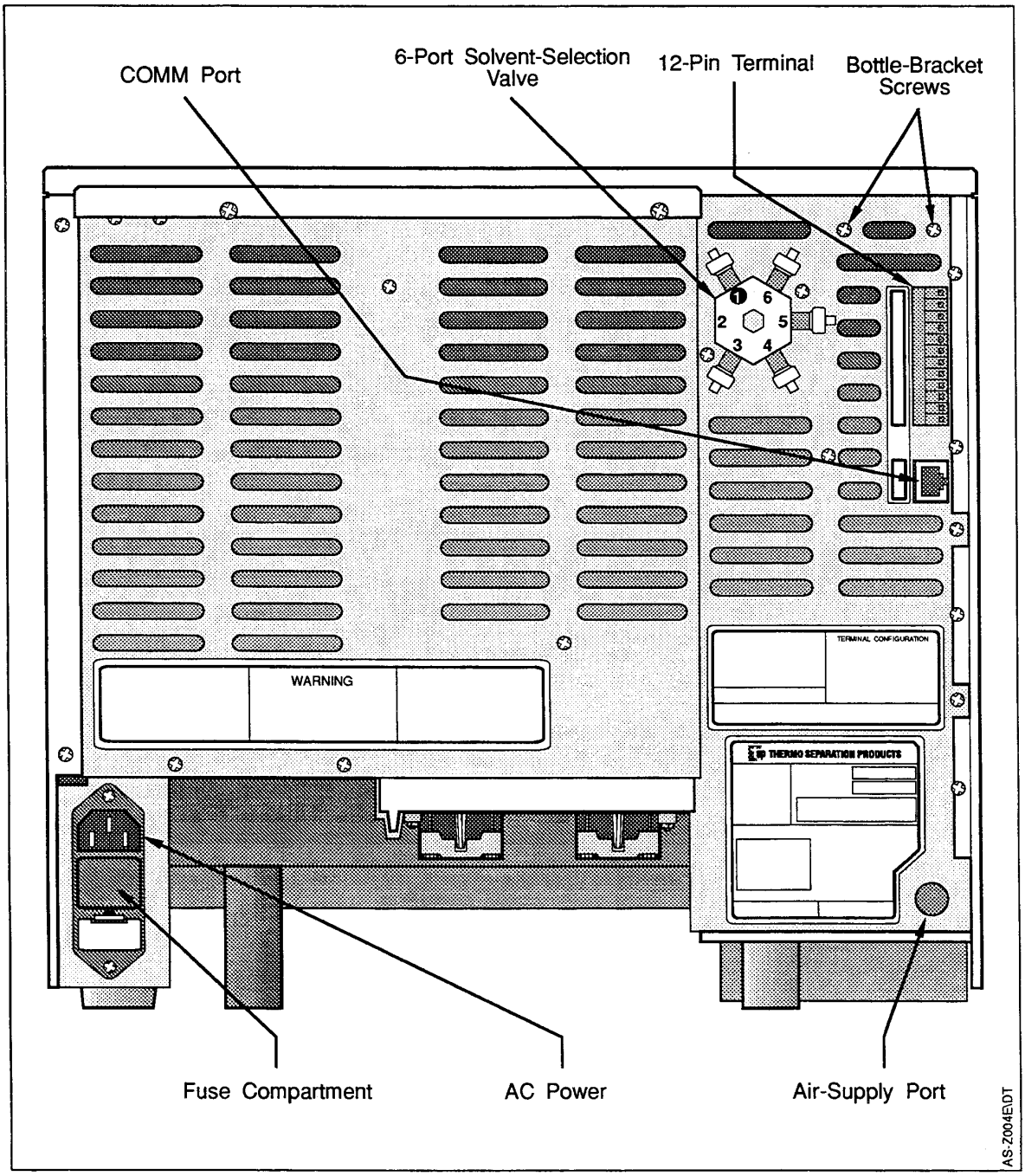


Figure A.2 The autosampler's rear panel connections (with the Sample Preparation Option installed)

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Flush Bottle

The flush bottle rests in the bracket and connects to the factory installed flush line via a Cheminert® union. The cap assembly comes with the 1/8 " OD x 1/16" ID Teflon inlet line and high-molecular weight polyethylene filter attached.

Use the following steps to install the flush bottle:

1. Rinse the bottle with LC-grade solvent to remove any dust. Then fill with appropriate LC-grade solvent.

2. Insert the filter into the solvent bottle and screw on the cap. Make sure that the inlet filter rests on the bottom of the solvent bottle.

3. Run the vent line to an appropriate exhaust apparatus, particularly when solvents with harmful vapors are used.



WARNING -- Chemical Hazard! Many LC solvents are hazardous and must be handled properly. Always dispose of waste solvents in an environmentally correct manner.

4. Place the flush bottle in the bracket.



NOTE: If you did not purchase the Sample Preparation Option, proceed to "System Communication" on page 109.

If you purchased the Sample Preparation Option, you will also need to install your external solvent bottles and lines and connect the heater/mixer to an air source.

External Solvents (S1-S4) (Sample Preparation Option)

If you purchased the Sample Preparation Option, the internal solvent line plumbing is already installed. You only need to set up your external solvent bottles and cap assemblies (not supplied), and connect the inlets and filters to the solvent-line extensions on the rear panel (Fig. A.2). Your accessory kit contains four solvent lines and four filter assemblies.

Each solvent line has a flared (flanged) Cheminert nut on one end; the other end is flareless. To connect a solvent line, you simply connect the Cheminert nut to the Cheminert union on the appropriate solvent line extension and attach the filter assembly to the other (flareless) end.

Use the following procedure to connect each external solvent line to its extension.

1. Designate four bottles as your solvent bottles (S 1 -S4), and plan the required space near your autosampler.

2. Remove the solvent lines and numbered labels from your accessory kit.

3. Clip the tie-wrap that coils the solvent-line tubing during shipment.

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4. Remove the red shipping cap from the end of the appropriate solvent-line extension.



***NOTE**: You don't have to use all four solvent bottles. Leave the red shipping cap on the end of any solvent line you're not planning to use.*

5. Connect the Cheminert union to the Cheminert nut on the end of the appropriate solvent-line extension.



***NOTE**: If you are going to use bottle caps, thread the other (flareless) end through the bottle cap.*

6. Remove a filter assembly from the accessory kit.

7. Slide the filter over the tubing with the threaded portion toward the end of the tubing.

8. Repeat steps 3-7 for the remaining solvent bottles and lines.

Heater/Mixer Air Supply

The Sample Preparation Option also requires that you connect the heater/mixer to an air supply. The heater/mixer requires a clean, filtered, regulated air supply (40 - 100 psi) to prevent particulates from obstructing the narrow tubing in the heater.



***NOTE**: You can also use dry ,filtered house air.*

Connect the autosampler's air input line to your air supply. The air irdet line is located in the lower right-hand comer of the rear panel (Fig. A.2). It is flared and has a Cherninert nut on the end.

Use the following steps as guidelines and adapt as necessary when connecting your autosampler to your air supply.

1. Remove the red cap from the end of the air input line.

2. Attach the flared end of the air inlet line to your air supply.



***NOTE**: Depending upon your regulator, you may need to cut off the Cheminert nut and flared end of the air supply line and use a standard 114" brass fitting in order to make the connection.*

3. Connect the brass connector to your regulator and use a wrench to tighten it in place.

System Communications

Your SpectraSYSTEM autosampler can be connected to other SpectraSYSTEM modules or to non-Then-no Separation Products modules via the rear panel's 12-pin terminal. Refer to Appendix D to connect your autosampler to other LC modules.

AC Power

Locate the power cord in the shipping container (not in your accessory kit). Insert one end into the AC power receptacle on the rear panel and the other end to a grounded power outlet. You don't have to worry about the fuses. Your autosampler is shipped with the correct fuses installed.

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FRONT PANEL

After you've completed all of the rear-panel connections, you can move to the front panel.

The front panel connections include:

- Solvent tray/waste container installation
- Pump-autosampler connection
- Column installation
- Autosampler-detector connection

The 250 µL syringe, 40-vial sample trays, sample needle, and a 100 pl, sample loop are already installed.



***NOTE**: The AS1000 comes with the 20 mL sample loop installed.*

If you purchased the Sample Preparation Option, the large (2.5 µL syringe) is also factory installed. If you purchased the Tray Temperature Control Option, your trays have only 35 vial positions (to accommodate the tub insulation). If you purchased a inert/biocompatible instrument, the inert/biocompatible needle is already installed.



CAUTION! If you purchased the column oven, you'll connect your pump outlet line to the autosampler's solvent preheating tube (rather than directly to the injector valve). Be sure to use the correct autosampler-pump connection procedure when installing your system.

Solvent Tray/Waste-Collection Container

A solvent-collection tray is provided for small-volume applications. For larger-injection applications, or for those requiring frequent flushing, you may wish to install a carboy or other large container for waste-collection. Install a waste-collection container using one of the following procedures.

Solvent Tray

The solvent tray (the flat, white plastic container included in your accessory kit) can hold 100 mL of waste. To install it, simply slide it into the compartment under the front-left side of the instrument.



***NOTE**: The back of the compartment prevents the trayfrom sliding toofar under the instrument.* Orient the tray so that the black-plastic, barbed elbow fitting is directed through the hole in the liquid-collection trough (across the front of the instrument) (Fig. A.3).



***NOTE**: Sometimes the barbedfitting may become dislodged during shipping. Be sure that the end of the barbedfitting is directed through the hole (not into the trough).*

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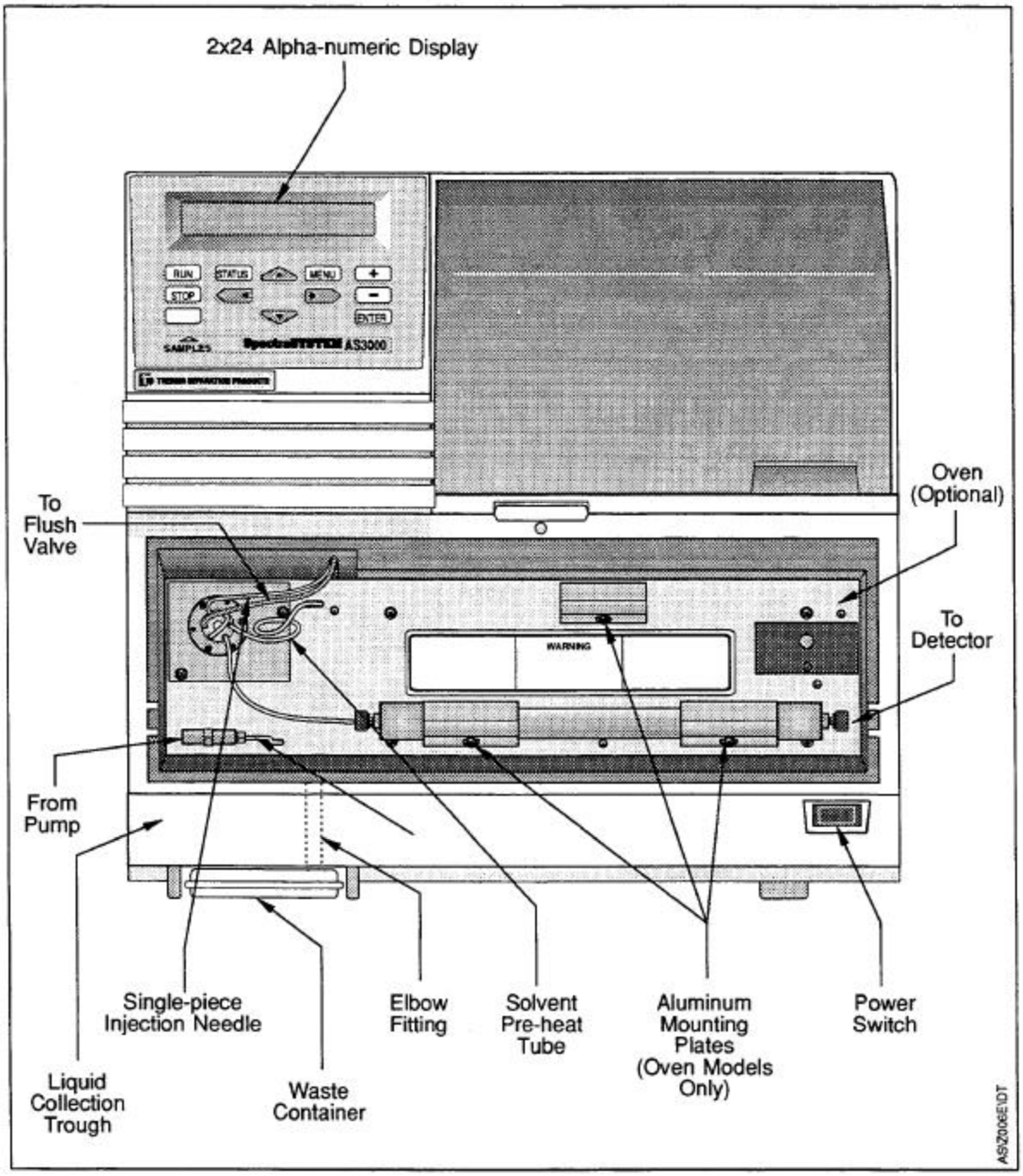


Figure A.3 The autosampler's front panel connections (the optional column oven installed)

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Other Waste-Collection Container

If you elect not to use the autosampler's solvent tray, you'll need to provide some other waste-collection device.

To hook up your waste container, locate the Tygon® tubing included in your accessory kit and connect it to the end of the black, barbed fitting. To prevent back-flushing into the lines, place the container lower than the instrument. Direct the Tygon tube into your container.

Pump-Autosampler Connection

The way you connect the pump depends upon whether or not a column oven is installed. For instruments without the column oven or for inert/biocompatible instruments, the pump is connected directly to the injector valve. If the column oven is installed, the pump is connected instead to the factory-installed solvent-preheating tube, a tube that routes behind the heating mantle (plate), up through the service loop, and then to the injector valve.



NOTE: *Your standard accessory kit contains two pieces of 0.010 ID stainless-steel tubing (one 4-inch piece and one 12-inch piece). The 12-inch piece is included to make the column-detector connection, but may be used instead to make the pump-autosampler connection.*

Without the Column Oven Installed

The instrument is shipped with the needle and flush lines installed. To connect your pump to the autosampler's injection valve, use the following steps:

1. Remove the column compartment cover (if you have not already done so) and set it aside.



NOTE*: The cover fits snugly. To remove, place your fingers or thumb in the indentation on the top edge of the column door and pull back toward you* ***firmly****.*

2. Route the pump outlet line through one of the two notches on the left side of the autosampler.

3. Connect the line to port 6 (Fig. A.4) on the injector valve. Finger-tighten, then use a 3/16" wrench to tighten the fitting an extra 1/8 turn.



NOTE*: Do not under- or over-tighten the screws, or damage to the connections could occur. Undertightening causes leaks; overtightening may cause leaks or damage the connections.*

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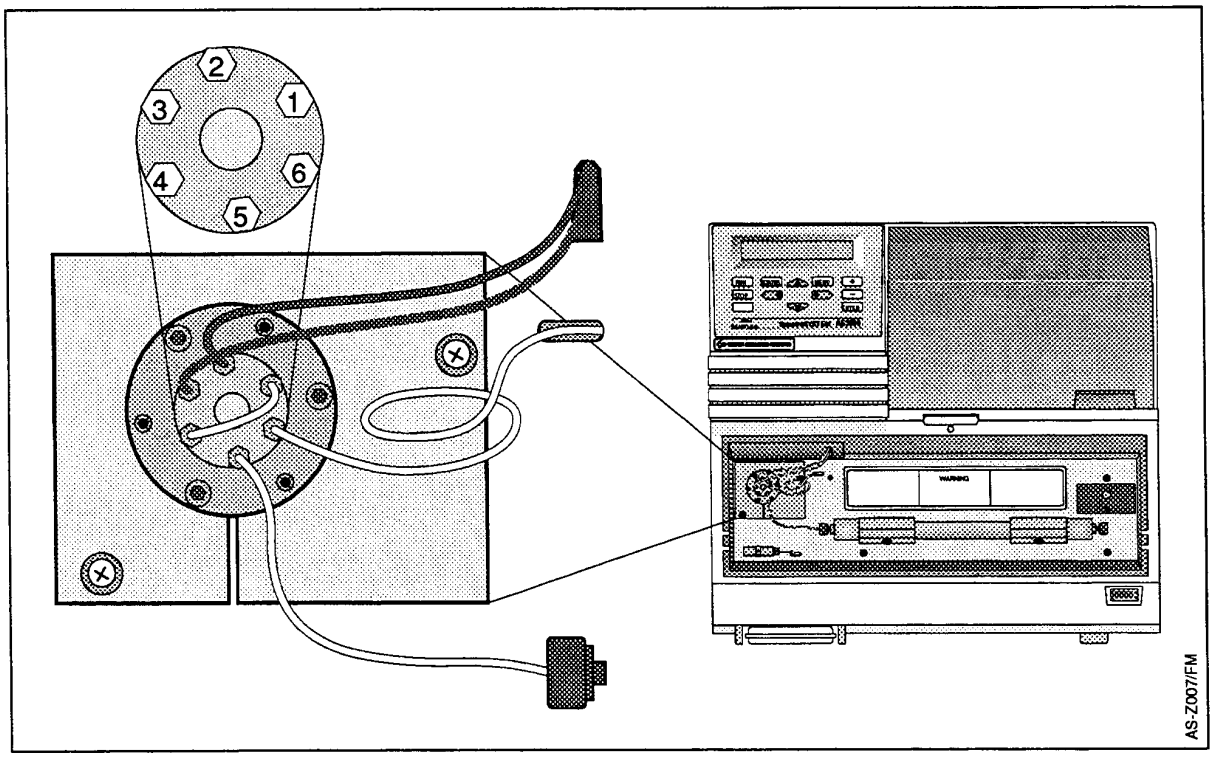


Figure A.4 The autosampler's injector valve

With the Column Oven Installed

The instrument is shipped with the column oven assembly and the flush and needle lines already installed. The stainless-steel, low-dead-volume union that connects the pump outlet tubing is also factory-installed. You will have to connect the pump to the preheat tube, however. The preheat line runs behind the column oven, through the oven's mantle, up through the service loop, and into the injector valve. This provides approximately 180 µL of preheated solvent.

Connect the pump to the preheat tube (Fig. A.5) as follows:

1. Remove the column oven cover (if you have not already done so) and set it aside.

   ***NOTE**: The cover fits snugly. To remove, place your fingers or thumb in the indentation on the top edge of the column door and pull back toward you **firmly**.*

2. Route your pump outlet line through the lower notch on the left side of the autosampler.

3. Connect the line to the low-dead-volume union on the preheat tube as follows:

   a. Locate the ferrule included in your accessory kit.
   b. Thread the tubing through the fitting, and place the ferrule on the end of the preheat tube (Fig. A.5).

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c. Then use two ¼” wrenches to tighten the connection. Use one wrench to hold the union body and one to tighten the nut 1/8-turn past the stop.

Refer to Chapter 3 for instructions on operating the oven.

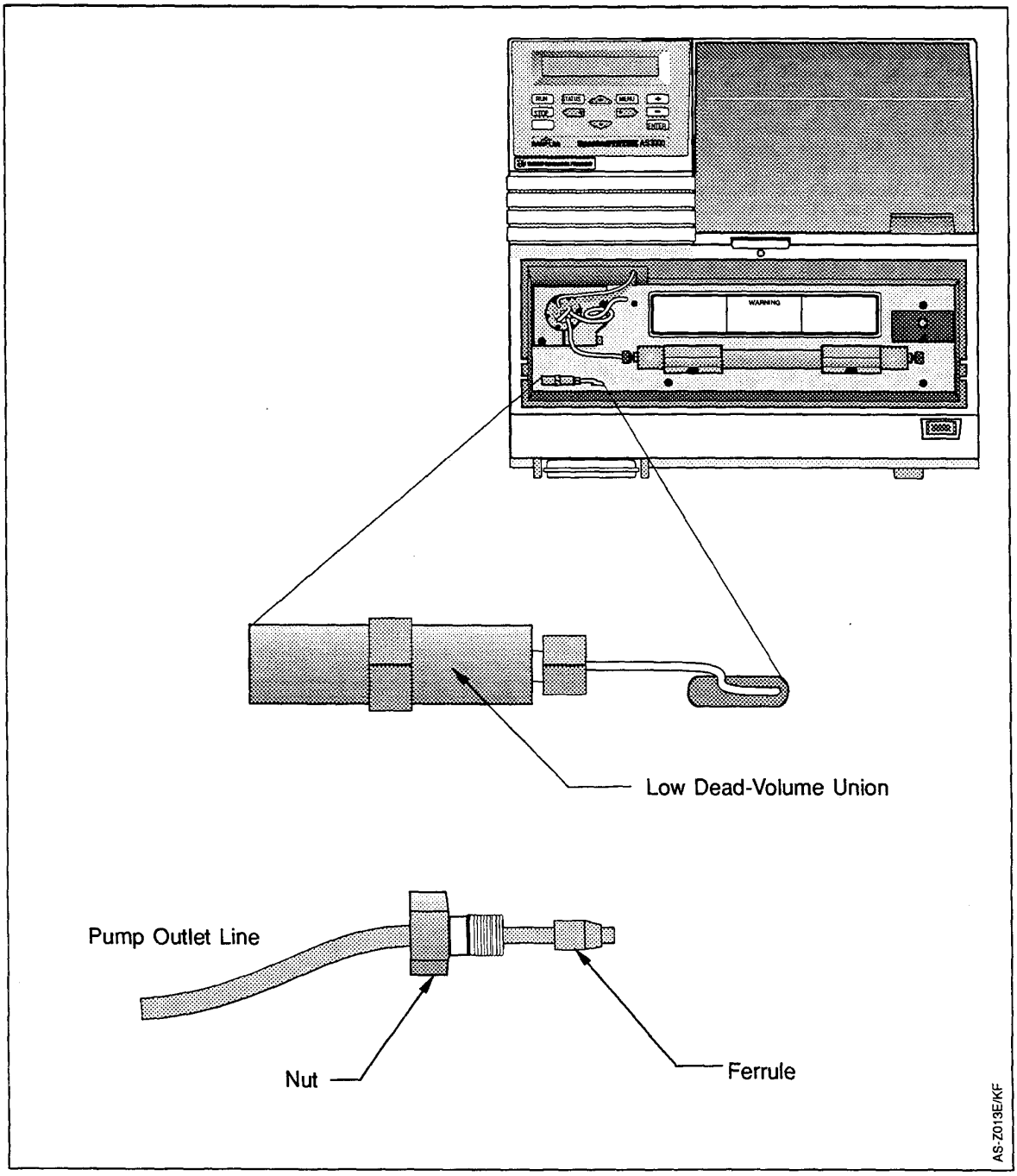


Figure A.5 The pump-to-preheat tube connection

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Sample Loop Installation

The AS3000 autosamplers come with a 100 µL sample loop installed; the AS 1000 aut6sampler comes with a 20 µL loop. If you wish to change sample loops, refer to Chapter 5, page 95.

Column Installation

Without the column oven installed, the column requires no special installation. If the column oven is installed, the column is placed between two aluminum column mounts that attach to the column oven's plate. The aluminum mounts increase the oven's resistance to ambient temperature changes, thereby ensuring a stable column temperature. They also provide excellent heat transfer from oven to column. Both mounting procedures are described here.

Without the Column Oven Installed

Use this procedure for mounting a column on instruments without the Column Oven Option and for inert/biocompatible versions.



***NOTE**: Flush/purge the pump and autosampler lines before connecting the column. This will ensure that no air gets into the column. Mount a column on your autosampler as follows:*

1. Locate the 4-inch length of stainless steel tubing (0.010) and fittings included in your accessory kit.
2. Connect the 4-inch tubing to the inlet end of the column.
3. Connect the other end of the 4-inch tubing to port 5 on the injector valve (Fig. A.3).

***NOTE**: Thermo Separation Products columns are connected such that liquids flow from left to right as you read the label. If you're not using a Thermo Separation Products column, refer to your column documentation to orient your column in the correct direction of flow.*



4. Use a 1/4 - 3/16" wrench to tighten the fittings. If you're installing the column onto the autosampler for the first time, tighten the fitting 1/8- turn past finger-tight. Otherwise, tighten the fittings 1/1 6-turn past finger-tight.

CAUTION! Do not over tighten the fittings. You could damage, or cause leaks to form around, the column connections.



With the Column Oven Installed

If you purchased the column oven, the column secures directly onto the front of the oven plate. The instrument is shipped with the column mounts already installed. To remove the front portion of the mounts:

NOTE: Flush1purge the pump and autosampler lines before connecting the column. This will ensure that no air gets into the column.



1 Use a flat-blade screwdriver to loosen the mounting screws and to remove and separate the column mounts.

***NOTE**: Thermo Separation Products columns are usually connected such that liquids flow from left to right as you read the label. If you are not using a Thermo Separation Products column, refer to your column documentation to orient it in the correct direction of flow.*

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Sample Loop Installation

The AS3000 autosamplers come with a 100 µL sample loop installed; the AS 1000 aut6sampler comes with a 20 µL loop. If you wish to change sample loops, refer to Chapter 5, page 95.

Column Installation

Without the column oven installed, the column requires no special installation. If the column oven is installed, the column is placed between two aluminum column mounts that attach to the column oven's plate. The aluminum mounts increase the oven's resistance to ambient temperature changes, thereby ensuring a stable column temperature. They also provide excellent heat transfer from oven to column. Both mounting procedures are described here.

Without the Column Oven Installed

Use this procedure for mounting a column on instruments without the Column Oven Option and for inert/biocompatible versions.



***NOTE**: Flush/purge the pump and autosampler lines before connecting the column. This will ensure that no air gets into the column. Mount a column on your autosampler as follows:*

1. Locate the 4-inch length of stainless steel tubing (0.010) and fittings included in your accessory kit.
2. Connect the 4-inch tubing to the inlet end of the column.
3. Connect the other end of the 4-inch tubing to port 5 on the injector valve (Fig. A.3).

***NOTE**: Thermo Separation Products columns are connected such that liquids flow from left to right as you read the label. If you're not using a Thermo Separation Products column, refer to your column documentation to orient your column in the correct direction of flow.*



4. Use a 1/4 - 3/16" wrench to tighten the fittings. If you're installing the column onto the autosampler for the first time, tighten the fitting 1/8- turn past finger-tight. Otherwise, tighten the fittings 1/1 6-turn past finger-tight.

CAUTION! Do not over tighten the fittings. You could damage, or cause leaks to form around, the column connections.



With the Column Oven Installed

If you purchased the column oven, the column secures directly onto the front of the oven plate. The instrument is shipped with the column mounts already installed. To remove the front portion of the mounts:

NOTE: Flush1purge the pump and autosampler lines before connecting the column. This will ensure that no air gets into the column.



1 Use a flat-blade screwdriver to loosen the mounting screws and to remove and separate the column mounts.

***NOTE**: Thermo Separation Products columns are usually connected such that liquids flow from left to right as you read the label. If you are not using a Thermo Separation Products column, refer to your column documentation to orient it in the correct direction of flow.*

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Figure A.6 The Commands Menu

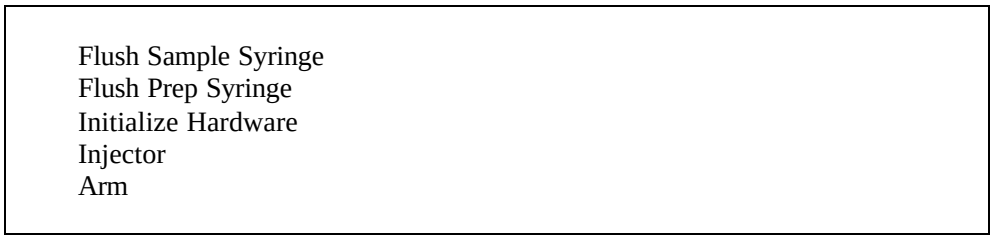


Figure A.6 The AS3000 Commands Menu with the Sample Preparation Option installed

3. Select/Flush Sample Syringe/ to access the menu shown in Figure A.7
4. Use the [+] and [-] keys to select 6500 µL. Then press [ENTER] to initiate the flush operation.



Figure A.7 The Flush Sample Syringe Menu

The autosampler automatically flushes the sample syringe and flush lines with the specified volume of flush solvent and returns the syringe to its starting position. The recommended 5000 µL flush takes approximately ten minutes. During the flush sequence, a confirmation message is displayed.

2.5 mL Syringe

If you purchased the Sample Preparation Option, you must also flush the large (2.5 mL) sample preparation syringe and external solvent lines as follows.

1. Fill each solvent bottle with an appropriate solvent.
2. Press [MENU] and select /COMMANDS/.
3. Select/Flush Prep Syringe/ (Fig. A.6)
4. Use the [+] and [-] keys to select 6500 µL. Then move the cursor to the Solv field and select /Btl/. Press [ENTER] to initiate the flush sequence.



Figure A.8 The Flush Prep Menu

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CAUTION! If there is residual air in the solvent-holding loop, flushing may expel solvent out the open port. To eliminate residual air that may be present during shipping, be sure to flush the lines with Btl selected before flushing with S-1 through S-4 selected.

5. Repeat this step for S-1 through S-4 with 1000 µL from the flush bottle (Btl).

The autosampler automatically flushes the selected solvent line with the specified volume of flush solvent and then returns the syringe to its starting position.

CAUTION! If you are replacing syringe(s), be sure to install the 2.5 mL syringe in on the left. Injecting small sample volumes with the larger syringe can adversely affect reproducibility.

Performance Verification

After you have installed your SpectraSYSTEM autosampler, we suggest that you run a standard sample, such as a 0.5% mixture of toluene and methanol, to verify the instrument's proper operation. In addition, you can use the following procedures to test your instrument's reproducibility and sample carryover, and to troubleshoot and maintain the instrument in the future.

REPRODUCIBILITY

Use this procedure to measure your autosampler's reproducibility. In this procedure, you will inject several volumes of a standard "sample" of 0.5% of toluene:MeOH, determine the percent relative standard deviations (%RSD) for the injection volumes, and compare them with the desired results included in this section. The test will require approximately three hours. Retain these results for comparison should troubleshooting be necessary.

Use the following HPLC column, solvents, and test "sample":

Column:	4.6 x 100 mm Spheri-5 RP18, 5 micron
Flow rate:	1.5 mL/minute
Mobile phase:	100% HPLC-grade methanol
Flush solvent:	100% HPLC-grade methanol
Test sample:	0.5% toluene in methanol
Detection:	254 mm

Test Setup

Set up the test run as follows:

1. Fill five vials with the test sample and place them in tray positions A01-A05.

2. Create a file using the Injection Menu parameters shown in Figure A.9.

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Injection Volume				10
Injection/Sample				10
Cycle Time				1.5
Col Oven	OFF	Temp	18	
TrayTemp	OFF	Temp	0	

Figure A.9 The Injection Menu performance verification parameters

3. Return to the Edit Menu.
4. Access the More Menu and verify that the Needle Height is set to 2.0. (Do not change any other parameters in this menu.)
5. Return to the Main Menu and Load your file.
6. Press the [SAMPLE] key and set up a sample queue that contains five sample sets of one vial each. For each sample set, enter the following Sample Menu values:

Inj/Sample	10
Cycle Time	1.5
No. Vials	1

Vary the injection volume (Inj Vol) for each set using the values listed in Table A.2.

Table A.2 Reproducibility Test Sample Queue Parameters

Sample Set	Inj Vol. (mL)	Sample Vial
1	10	A01
2	1	A02
3	3	A03
4	7	A04
5	10	A05

7. Return to the Main Menu.
8. Flush the syringe with 500 µL of flush solvent. (Select /COMMANDS/, /Flush Sample Syringe/).
9. Inspect all sample lines for bubbles. (Be sure to check the Teflon line that connects to port 3 of the injection valve.) If bubbles are present, repeat step 8 until all bubbles are removed.
10. Check for leaks.
11. Initialize your pump.
12. Prepare your integrator for a run.
13. Press [RUN].

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The instrument should perform 50 injections. The first group of ten injections is used to equilibrate the system, so don't use the data from these injections.

%RSDs

Determine the percent relative standard deviations (%RSD) for the injection volumes and compare them with the desired results in Table A.3.

Table A.3 %RSD Results for the Reproducibility Test

Volume (in mL)	%RSD
1	<1*
3	<0.7*
7	<0.5*
10	<0.5**

* Typical value
** Instrument specifications

If your results are other than expected, refer to Appendix C for troubleshooting.

SAMPLE CARRYOVER

This procedure will allow you to verify that the sample carryover is below the 0.01 % specification for your autosampler. It requires the use of silicon septa, a deflected point needle (p/n 4719-010, -020), the appropriate volume of the required flush solvent, and clean (not previously used) blanks. The procedure minimizes the effects of sample concentration or chemistry, chromatographic conditions, detector linearity, or other hardware characteristics that might affect sample carryover.

This procedure is <u>independent</u> of sample- and system-specific characteristics. It reduces sample effects including extinction coefficients, chemical interaction with other sample or system components (the column), and sample degradation. It also minimizes system effects including detector linearity, pump and mixing anomalies, and column and guard-column contamination.

In this procedure you will inject your sample, a subsequent blank (BLANKI), additional blanks to return the system to baseline, and then a standard (STANDARD) that is 0.01% of your sample. You will determine the % carryover by multiplying the ratio of the carryover by the 0.0 1 % specification:

$$\text{Carryover} = [\text{Peak Area}_{\text{BLANK1}} / \text{Peak Area}_{\text{STANDARD}}] \times 0.01\%$$

If desired, run this procedure three times to replicate the results.

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Materials

To perform this procedure you'll need:

1. Your sample
2. 7 vials, septa, and caps for each run
3. Autosampler with deflected point needle
4. Any HPLC pump, detector, and data system

Sample Preparation

Prepare your SAMPLE and STANDARD as follows:

1. Place appropriate volumes of sample and starting mobile phase into a vial labeled SAMPLE.
2. Dilute your sample 10,000 times in starting mobile phase and pipette into a vial labeled STANDARD.

Example:

1. The sample is 100 mg Protein in I mL Phosphate buffer (SAMPLE)
2. Transfer 1 µL into 9.99 mL of Phosphate buffer (STANDARD)

Blank Preparation

Prepare your blanks as follows:

1. Pipette fresh starting mobile phase into five vials.
2. Label vials as BLANK, BLANKI, BLANK2, BLANK3, and BLANK4 for each test set.

Test Procedure

System Setup

Set up your system as follows:

1. Prepare the appropriate mobile phase(s).
2. Set up the appropriate chromatographic conditions for your sample.
3. Set up your integrator/data system so that it displays retention times and peak area information.

Autosampler Setup

Set up your autosampler as follows:

1. Set the flush volume to 2000 mL:
   a. Press [MENU], /Files/Flush Volume/
   b. Select 2000 Ml



***NOTE**: The flush solvent should be the starting mobile phase or other solvent that completely dissolves the sample.*

2. In the Injection Menu, set the injection volume (loop size) to 5, 10, or 20 µL as appropriate.
3. Set the number of injections to 1.

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4. In the /Files/More/ Menu, set the injection type to Push.
5. Set the vial positions as follows:

   A01: Blank
   A02: SAMPLE
   A03: Blankl
   A04: Blank2
   A05: Blank3
   (Add additional Blanks as necessary to allow the system to return to baseline before running the STANDARD.)
   BOI: STANDARD
   B02: Blank4
   (Add additional Blanks as necessary to allow the system to return to baseline before replicating the test.)

6. Set up two additional groups of seven vials to run the carryover test in triplicate (optional, but recommended).

7. Run your HPLC system using the appropriate chromatographic conditions for your sample.

Carryover Calculation

To calculate the % sample carryover:

1. Divide the peak area of Blankl by the peak area of the STANDARD. This gives you the ratio of the carryover to the 0.01% specification.

2. Multiply this value by 0.01% to obtain the percent carryover.

Example:

If the peak area for Blank 1 is 110,95 8 counts, and the Peak area for the STANDARD is 656,283 counts, the ratio of the carryover to the specification is

110,958/656,283 or 0. 1691
% carryover = 0. 1691 x 0.0 1 %
% carryover = 0.00 1691

Specifications

Since 0.001691% < 0.01%, the autosampler is within specification.

DILUTION REPRODUCIBILITY

This procedure is used to determine your autosampler's dilution reproducibility. In this procedure you will prepare and inject five 1/100 dilution from a standard sample of 50% Toluene:Methanol. The percent relative standard deviation of peak areas will then be determined across the five samples and compared to the specification presented in this section. The test will require approximately 1.5 hours. These results should be retained for comparison should troubleshooting be necessary.

Use the following BPLC column, solvents, and "test" sample:

Column: Spheri-5 RP1 8, 5 micron, 100 x 4.6 mm

Flow Rate: 1.5 ml/min.

Mobile Phase: 100% BPLC grade Methanol (MeOH)

Flush Solvent (Btl & S- 1): 100% HPLC grade Methanol (MeOH)

Test Sample: 50% Toluene in Methanol

Detection: 254run

Test Setup

Setup the test run as follows:

1. Fill five vials with the test sample and place them in tray positions A01, A03, A05, A07, and A09.

2. Place empty vials in tray positions A02, A04, A06, A08, and A010.

3. Advance to the Sample Prep menu in Method 1. Enter I for the dilution template and setup the parameters as follows:

   - Template 1
   - Dilution
   - # of dilution cycles 1
   - 1 - Load 1000 ml Solv S-1
   - 2 - Pickup 10ml Sample
   - 3 - Pickup 0ml Sample
   - 4 - PickLG Oral Sample
   - 5 - Add 1000ml to Sam + 1
   - Mix for 0.5 minute
   - Overlap enable - Yes

4. Flush sample syringe with 500ml. Next flush the prep syringe with 4800 ml from Btl and S- 1. Check lines for air bubbles, repeat if necessary.

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5. Press the [SAMPLES] key and setup a sample queue that contains five sample sets of one vial each. For each sample set, enter the following Sample Menu values,

Inj/Sample	3
Cycle Time	1.5
No. Vials I	

Vary the sample vial position for each sample set:

Sample Set	Inj. Vol.	Sample Vial
1	10	A01
2	10	A02
3	10	A03
4	10	A04
5	10	A05

6. Initialize your pump and check for leaks.

7. Prepare the integrator for a run.

8. Press [RUN].

The autosampler will prepare 5 dilution of the test sample that make a 0.5% Toluene in methanol sample. It will then inject 10ml of the diluted sample three times.

When the test run is complete, gather the area counts for each of the sample set's third injection. Calculate the average of these five area j counts and their standard deviation. Divide the standard deviation by the average area count and multiply by 100 to obtain the % RSD.

The specification is < 1%.

If your results are not within the specification range, refer to Troubleshooting in Appendix C.

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Specifications and Warranty

Specifications

Vial capacity
- 120 vials in three removable trays
- 105 vials with tray temperature control

Variable-Volume

Injection Precision
- <0.5% RSD @ 10 µL, or greater

Injection Volume
- 0.1 - 100 µL injection standard (250 µL syringe)
- up to 1000 µL injections with larger loops and syringes

Fixed-Loop

Injection Precision
- <0.5% RSD @ 10 µL or greater

Injection Volume
- 20 µL, standard (250 µL syringe)
- 1000 µL injections with larger loops and syringes

Sample Carryover
- <0.0 1 % at 300 µL flush volume
- 5 mL flush volumes can be set

Minimum Sample Volume
- 1 µL can be injected from 5 µL with an end-port needle

Needle Height
- Programmable in 0. 1 mm increments

Column Oven (optional)
- Settable in V increments from 20' - 80'C, starting at 5'C above ambient
- Temperature stability ± 0.2'C
- Accommodates up to 30 cm columns

Tray Temperature Control (optional)
- Settable in VC increments from 0' - 60'C
- Temperature stability ± 10C
- Typical vial temperature from 4' - 50'C

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Sample Preparation (optional)

Reagent Vials
Three vials can be specified per file

Dilution Solvents
Four external solvents can be selected

Heating/Mixing
Single vial, vortex heater/mixer
Settable in VC increments from 30' - 100'C

Dilution Precision
Typically <1.0% RSD for a 1/100 dilution (combined dili.

Dispensing Precision
Typically <0.2% RSD for I mL

Method Files
Protected in non-volatile memory

Communications

Remote Inputs
Pump Ready, Inject Hold

Outputs
Pump Stop
Autosampler Ready
Inject
Gradient Start
Four timed-event outputs
BCD (optional)
SpectraNETIRS-232 port

Ambient Environment
100 - 400C, 5 - 95% relative humidity

Dimensions
37 cm x 41 cm x 51 cm (H x W x D)

Weight
16 kg standard; 24 kg with all options installed

Power requirements
100/120/220/240 VAC nominal; 80 - 450 VA
50/60 Hz

Product Certification
CSA, TOV/GS, FCC (EMI), VDE (EMI) applied for

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Menu Reference

Introduction

This appendix provides You with your autosampler's menu tree and menu reference. It is not necessary to read this appendix in order to learn how to use your autosampler. It is included in the manual as a quick reference and aid to using your instrument.

The Menu Trees represent the overall AS1000 and AS3000 menu structures. They show the location and interrelation of all the menus for your autosampler. The menu trees will also help if you become "lost" while moving through the autosampler's menus.

The Menu Reference is an alphabetical listing of each menu field, its definition, and, where appropriate, its allowable and default values.

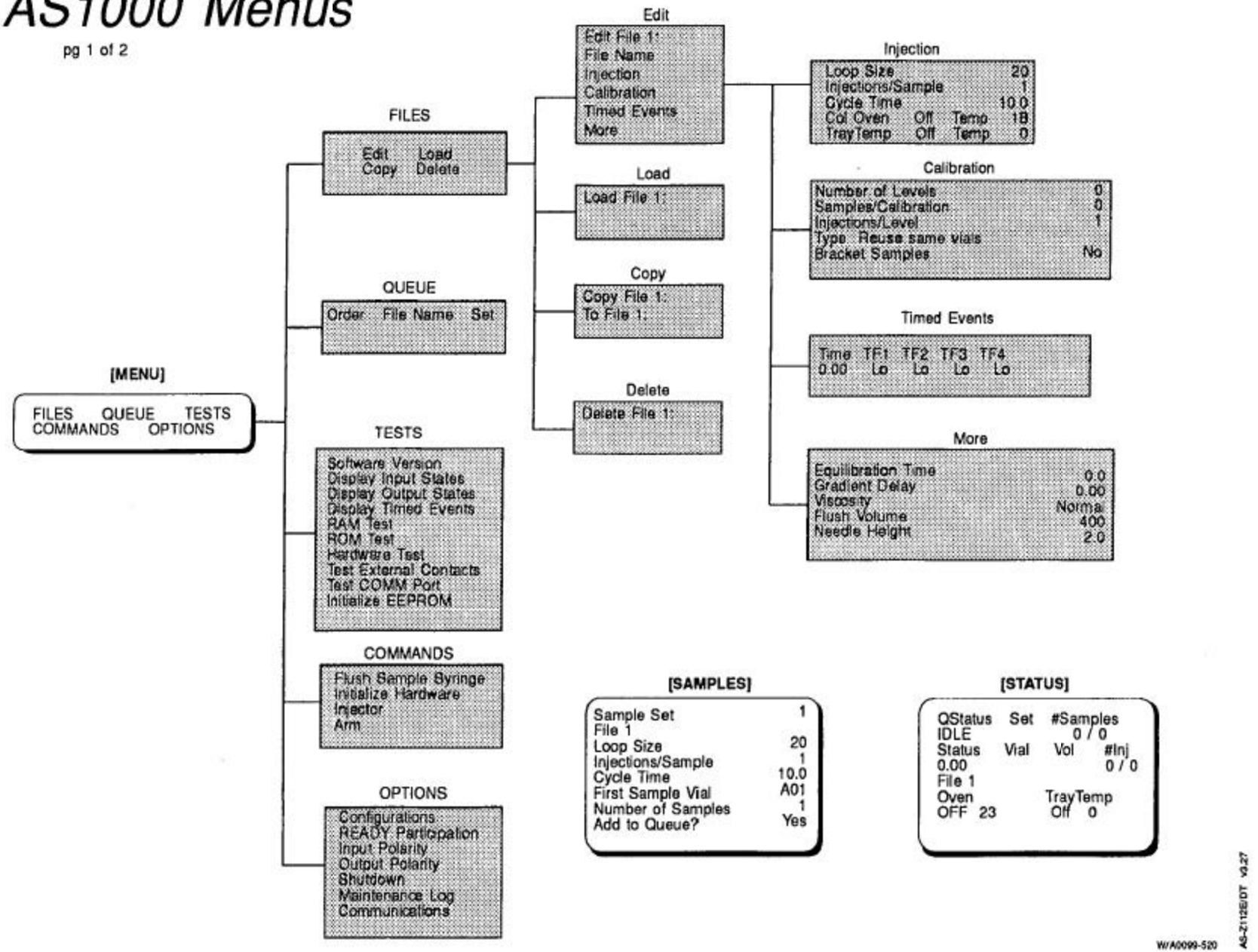
Menu Trees

The Menu Tree for your particular instrument is a useful reference guide to learning your way around your AS1OOO or AS3000 autosampler. You may wish to keep it open while you learn where each display is located in the overall menu structure and as you work through the operating instructions in Chapters 3 and 4. Additional copies are provided in the front pocket of this manual.

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AS1000 Menus

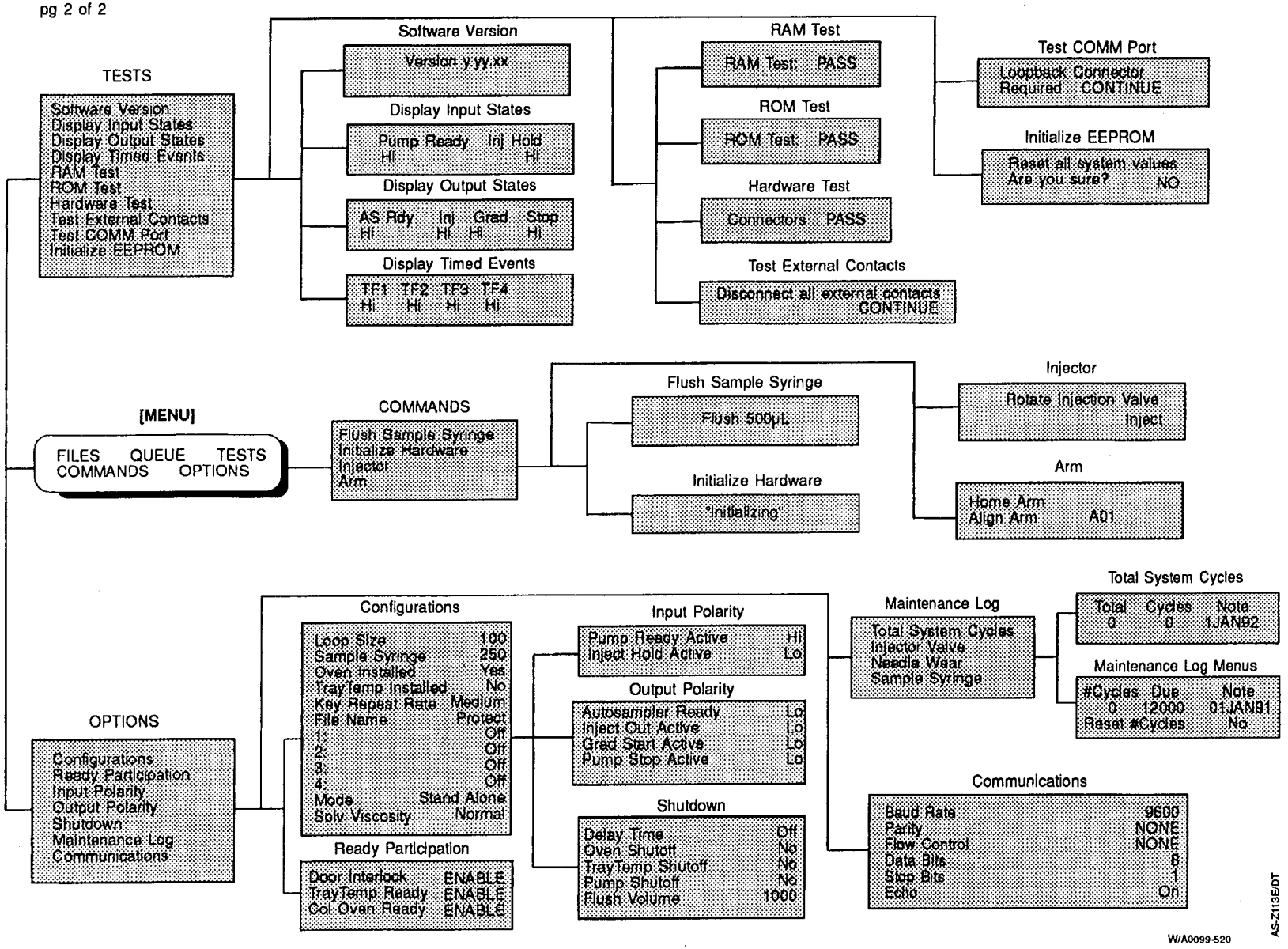
pg 1 of 2



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AS1000 Menus

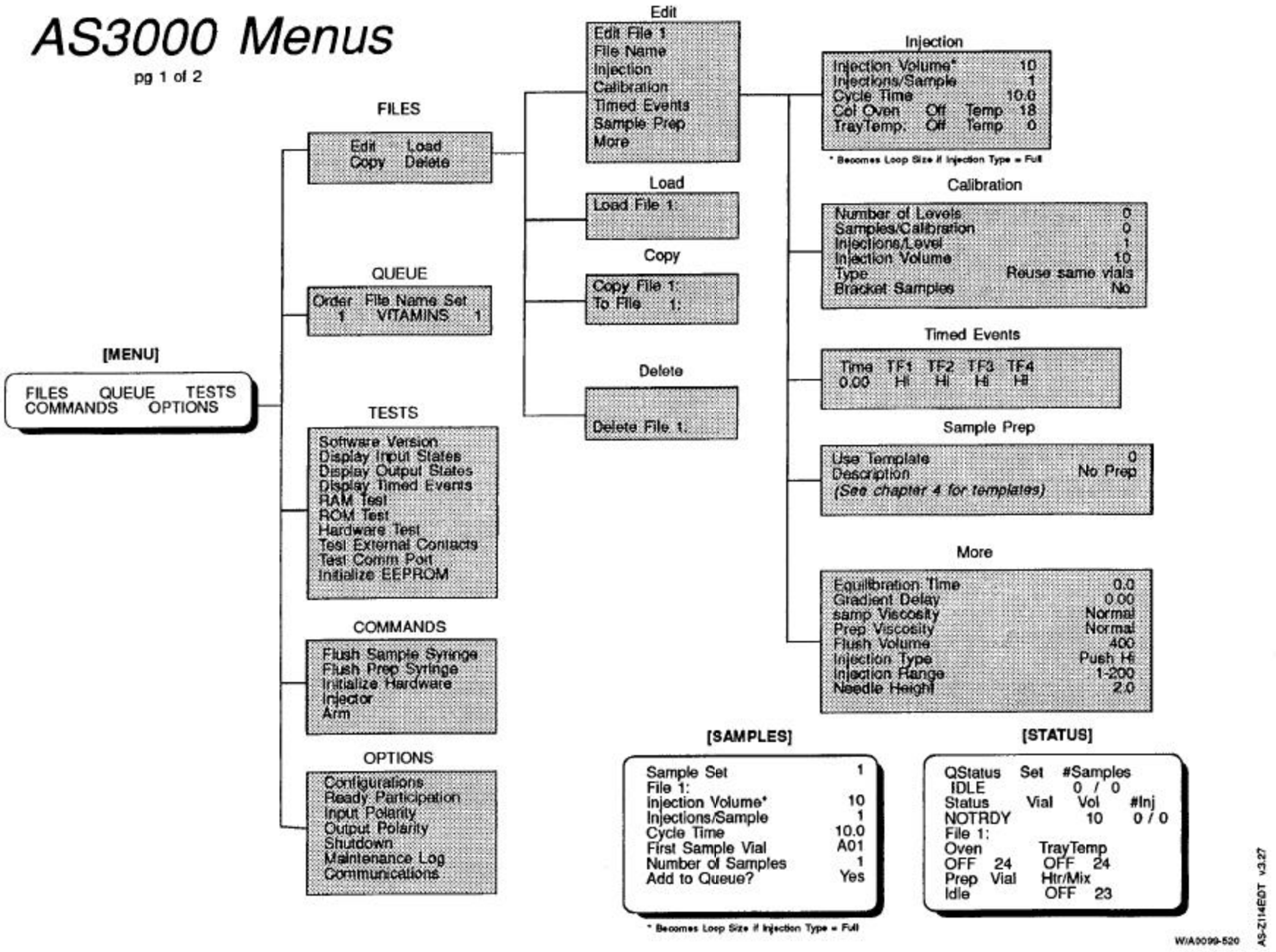
pg 2 of 2



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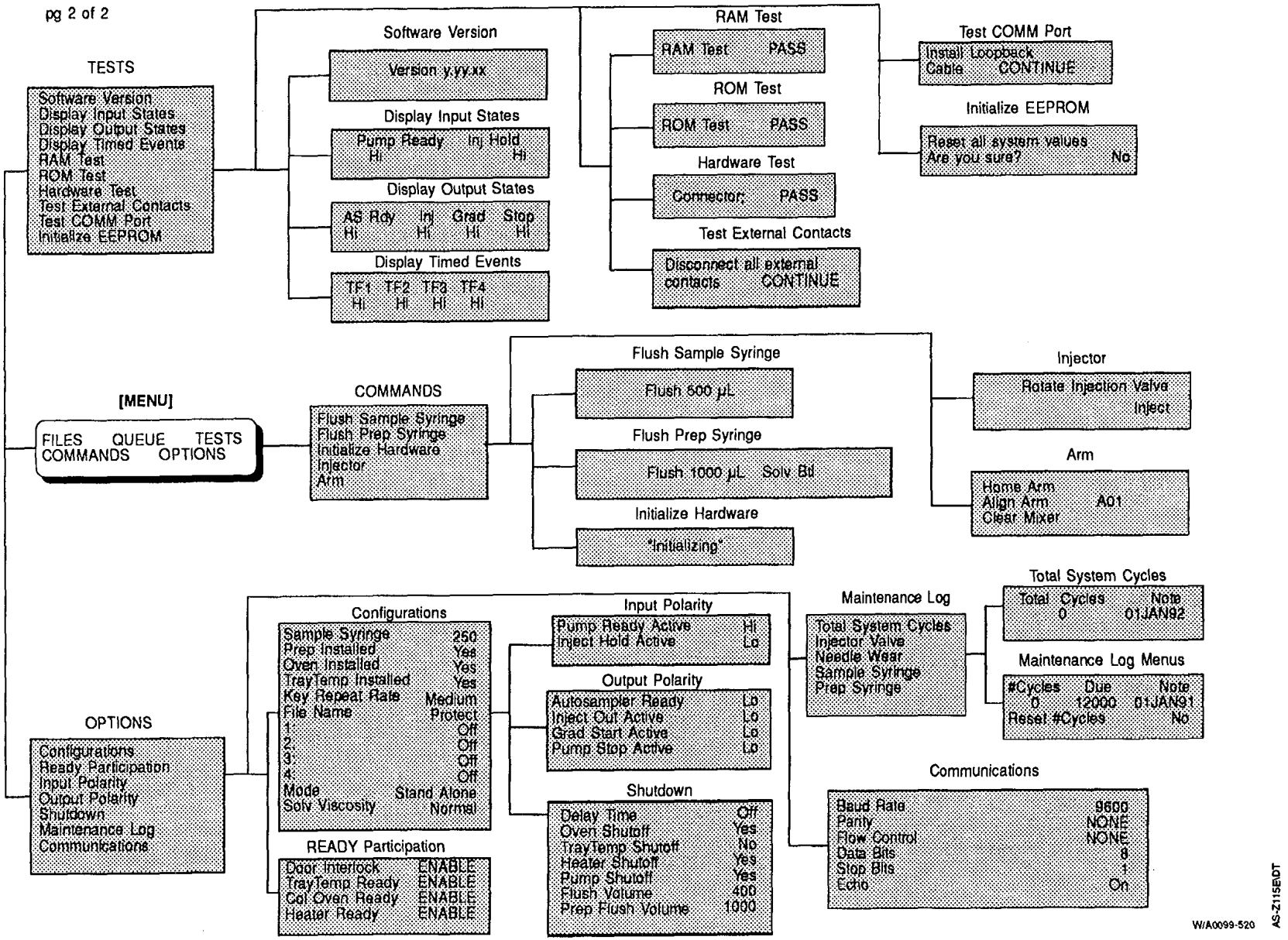
AS3000 Menus

pg 1 of 2



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AS3000 Menus



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Bracket Samples

In the Calibration Menu, this prompt asks (Yes[No) if you want to reinject the calibration vials (recalibrate) after all of the sample vials in the sample set have been injected. The default value is No.

Calib Vial I(of X)

In the Calibration Menu, this field specifies the position (AO1-C40) of the first calibration vial within the sample queue. X (1-9) is the number of calibration levels in the run. The vial position default value is A01; the calibration levels (X) default value is 1.

Calibration

This Edit Menu choice accesses the menu used to enter the calibration parameters.

Col Oven

In the Injection Menu, this field sets the column oven to turn on or off once the file is loaded. The default value is Off. This field also indicates the column temperature (15' - 80'C). The default setting is Off.

Col Oven Ready

When this function is enabled in the Ready Participation Menu, the autosampler will not indicate a "ready" status until the column oven has reached its set temperature. Pressing the [+] and [-] keys lets you enable or disable this function.

Commands

The Main Menu choice that displays the Commands Menu used to access the autosampler's built-in procedures that let you manually flush the syringe(s), initialize the hardware, control the injector valve and XYZ arm, and reset the software.

Configurations

Displays the menu used to set or change the instruments configuration, key repeat rate (cursor speed), and to protect files from being edited.

Connector Test

This Hardware Test diagnostic verifies all of the cable connections and instrument sensors. See Appendix C for more information on running this test.

Copy

Accesses the menu that lets you copy files.

Copy File #

Specifies the number of the file (1-4) to copy from.

Cycle Time

Specifies the time (0-655 minutes) between injections (run time). The default setting 10.0 minutes.

Delay Time

The time entered into the Shutdown Menu that the autosampler should wait before initiating the shutdown sequence. 'Me default setting is off.

Delete

Accesses the menu used to delete files.

Delete File #:

Specifies the number of the file to be deleted (1-4). Pressing [ENTER] restores the current file parameters to their default settings and erases the user-specified file name.

Description

Specifies the name of the sample preparation template in use (No Prep, Dilution, DABS-CL, Rainbow, and Linear).



NOTE: This field appears only if the Sample Preparation Option is installed.

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Display Input States

Accesses the menu that displays the input signal levels of the Pump Ready and Inject-Hold inputs from other LC system modules.

Display Output States

Accesses the menu that displays the output-signal levels of the Autosampler Ready, Inject Out, Gradient and Stop output (Hi/Lo).

Display Timed Events

Accesses the menu that displays the time-function-output signal states (Hi/Lo). The default setting is Lo (active Hi).

Door Interlock

When enabled, prevents the autosampler from indicating a "ready" status until the door is closed. Pressing the [+] and [-] keys lets you enable or disable this function. The default setting is ENABLE.

Due

This is a 6-character numeric field that appears in each Maintenance Loa, menu (with the exception of the Total System Cycles Menu) where you enter the number of cycles indicating that maintenance is due. This number varies for each autosampler component. Use the [+1 and [-1 keys to enter or edit the Due field value. Refer to Chapter 5 "Maintenance Log" for the default values for this field.

Edit

Displays the menu used to edit/change a file that is not yet been added to the sample queue.

Equilibration Time

This More Menu field lets you specify the time (0-99.9 minutes) required for the system to re-equilibrate after initializing the new HPLC method. The default setting is 0.0.

File #

Lets you select the number of a file to edit.

File Name

A name that you designate for the file that contains your run parameters. File names can be up to eight characters long and may contain only the following characters: A-Z, 0-9, /,-, or blank. Once entered into the Edit Menu, the autosampler automatically copies it to the Samples and Queue Menus, and Status Screen.

File requires Z Extra vials after each Sample vial.

Is supplied automatically by the sample preparation software and indicates the required number of empty vials (1 -3) after each sample vial in a set. If Sam is used as a target vial, Z = 0; if Sam and Sam + I are used as target vials, Z = 1; if Sam, Sam + 1, and Sam + 2 are used as target vials, Z = 2; if Sam, Sam + 1, Sam + 2, and Sam + 3 are used as target vials, Z = 3. The default value differs with each template.

FILES

This Main Menu choice provides access to the menus used for editing, loading, copying, and deleting files.

Fill

Indicates that the injector valve is in the Fill position. Pressing [+] and [ENTER] switches the valve between the Fill and Inject positions.

Flush mL

Located in the Commands Menu, this field specifies the volume of a manual flush cycle (100 - 5000 µL) for the sample syringe. The default value is 500 µL. Pressing [ENTER] in this field manually flushes the lines, sample loop and syringe(s).

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Flush Prep Syringe

This Commands Menu choice lets you manually flush the lines and the 2.5 mL prep syringe with a specified volume (1000 - 5000 JIL) of one of the four external solvents (S-1 to S-4) or your flush solvent (Btl). 1000 µL is the default setting.

Flush Sample Syringe

This Commands Menu choice lets you manually flush the lines and sample syringe with a specified volume (0 – 1500 µL) using the 250 µL (sample) syringe. 500 µL is the default setting.

Flush Volume

In the More Menu, this field specifies the desired volume of flush solvent (100 - 5000 p-L) for the automatic flush cycle that occurs after each injection. The default value is 400 µL.

Grad

In the Shutdown Menu, this field specifies the desired volume of flush solvent (0 - 5000 µL) for the sample-syringe flush that occurs as a part of the shutdown sequence. The default value is 400 µL.

Grad Start Active

This Display Output States Menu field indicates the level of the Gradient Delay output signal. You can't change the signal level from this menu. The signal must be changed from the Options, Output Polarity, Gradient Start Active field.

Gradient Delay

This Output Polarity Menu choice lets you specify the active level (Hi/Lo) of the gradient start output.

Hardware Test

This More Menu choice lets you Specify the time (0.0-99.99 minutes) required for the mobile phase to travel from the proportion valve to the head of the column. The default is 0.00 minutes (no delay).

Heat

Accesses the Connector Test Menu used to verify that instrument and sensor connections are made. (See also Connector Test.)

Heater

Used in sample preparation to specify a desired heating time (0 - 99 minutes).

Heater Ready

This sample preparation template field turns the heater/mixer on/off. The default is Off. TENT, in the same line, specifies the heater temperature (30- - 100-C).

Heater Shutoff

When enabled, this Ready Participation Menu field prevents the autosampler from indicating a "READY" status until the heater/mixer has reached its set temperature. Pressing the [+] and [-] keys lets you enable or disable this function. This field appears only with the Sample Preparation Option installed-

Home Arm

The field in the Shutdown Menu that lets you program the autosampler to automatically turn off the heater/mixer during the Shutdown sequence.

Returns the XYZ arm to its "home" position alongside the rear-left comer of the sample tub.

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Display Input States

Accesses the menu that displays the input signal levels of the Pump Ready and Inject-Hold inputs from other LC system modules.

Display Output States

Accesses the menu that displays the output-signal levels of the Autosampler Ready, Inject Out, Gradient and Stop output (Hi/Lo).

Display Timed Events

Accesses the menu that displays the time-function-output signal states (Hi/Lo). The default setting is Lo (active Hi).

Door Interlock

When enabled, prevents the autosampler from indicating a "ready" status until the door is closed. Pressing the [+1 and [-1 keys lets you enable or disable this function. The default setting is ENABLE.

Due

This is a 6-character numeric field that appears in each Maintenance Log menu (with the exception of the Total System Cycles Menu) where you enter the number of cycles indicating that maintenance is due. This number varies for each autosampler component. Use the [+1 and [-] keys to enter or edit the Due field value. Refer to Chapter 5 "Maintenance Log" for the default values for this field.

Edit

Displays the menu used to edit/change a file that is not yet been added to the sample queue.

Equilibration Time

This More Menu field lets you specify the time (0-99.9 minutes) required for the system to re-equilibrate after initializing the new HPLC method. The default setting is 0.0.

File #

Lets you select the number of a file to edit.

File Name

A name that you designate for the file that contains your run parameters. File names can be up to eight characters long and may contain only the following characters: A-Z, 0-9, /,-, or blank. Once entered into the Edit Menu, the autosampler automatically copies it to the Samples and Queue Menus, and Status Screen.

File requires Z Extra vials after each Sample vial.

Is supplied automatically by the sample preparation software and indicates the required number of empty vials (1-3) after each sample vial in a set. If Sam is used as a target vial, Z = 0; if Sam and Sam + 1 are used as target vials, Z = 1; if Sam, Sam + 1, and Sam + 2 are used as target vials, Z = 2; if Sam, Sam + 1, Sam + 2, and Sam + 3 are used as target vials, Z = 3. The default value differs with each template.

FILES

This Main Menu choice provides access to the menus used for editing" loading, copying, and deleting files.

Fill

Indicates that the injector valve is in the Fill position. Pressing [+] and [ENTER] switches the valve between the Fill and Inject positions.

Flush mL

Located in the Commands Menu, this field specifies the volume of a manual flush cycle (100 - 5000 µL) for the sample syringe. The default value is 500 µL. Pressing [ENTER] in this field manually flushes the lines, sample loop and syringe(s).

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Flush Prep Syringe

This Commands Menu choice lets you manually flush the lines and the 2.5 mL prep syringe with a specified volume (1000 – 5000 µL) of one of the four external solvents (S-1 to S-4) or your flush solvent (Btl). 1000 µL is the default setting.

Flush Sample Syringe

This Commands Menu choice lets you manually flush the lines and sample syringe with a specified volume (0 - 1500µL) using the 250 µL (sample) syringe. 500 µL is the default setting.

Flush Volume

In the More Menu, this field specifies the desired volume of flush solvent (100 - 5000 µL) for the automatic flush cycle that occurs after each injection. The default value is 400 µL.

In the Shutdown Menu, this field specifies the desired volume of flush solvent (0 - 5000 µL) for the sample-syringe flush that occurs as a part of the shutdown sequence. The default value is 400 µL.

Grad

This Display Output States Menu field indicates the level of the Gradient Delay output signal. You can't change the signal level from this menu. The signal must be changed from the Options, Output Polarity, Gradient Start Active field.

Grad Start Active

This Output Polarity Menu choice lets you specify the active level (HiA.0) of the gradient start output.

Gradient Delay

This More Menu choice lets you Specify the time (0.0-99.99 minutes) required for the mobile phase to travel from the proportion valve to the head of the column. The default is 0.00 minutes (no delay).

Hardware Test

Accesses the Connector Test Menu used to verify that instrument and sensor connections are made. (See also Connector Test.)

Heat

Used in sample preparation to specify a desired heating time (0 - 99 minutes).

Heater

This sample preparation template field turns the heater/mixer on/off. The default is Off. TENT, in the same line, specifies the heater temperature (30- - 100-C).

Heater Ready

When enabled, this Ready Participation Menu field prevents the autosampler from indicating a "READY" status until the heater/mixer has reached its set temperature. Pressing the [+) and [-I keys lets you enable or disable this function. This field appears only with the Sample Preparation Option installed.

Heater Shutoff

The field in the Shutdown Menu that lets you program the autosampler to automatically turn off the heater/mixer during the Shutdown sequence.

Home Arm

Returns the XYZ arm to its "home" position alongside the rear-left comer of the sample tub.

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Htr/Mix

In the sample preparation status line (Prep), this field displays the current state (on/off) of the heater/mixer and its actual (not set) temperature. The temperature is always displayed whether or not the heater/mixer is in use.

Initialize EEPROM

This Tests Menu choice erases the current EEPROM values, restores all file parameters to their default values, and erases all hardware configuration information. (Any installed options must be reset to "YES" in the Configurations Menu.)

Initialize Hardware

Lets you manually reset the autosampler's syringe(s), solvent selector, flush, and injector valves to their initial pre-injection positions.

Inject

In the Display Outputs Menu, Inject indicates the level of the Inject Hold output signal (Hi/Lo).

In the Injector Menu, Inject indicates that the injector valve is in the Inject position. Pressing [+1 and [-] switches the valve between the Fill and Inject positions.

Inj Hold

This Input Polarity Menu choice indicates the current level of the Inject Hold input signal (FE/Lo) from your pump.

Inject Hold Active

This Input Polarity Menu field lets you specify the active level (polarity) of the Inject Hold input signal (Hi/Lo).

Inject Out Active

This Output Polarity Menu choice lets you specify the active level (Polarity) (11i/Lo) of the Inject Out output signal.

Injection Type

This Injection Menu choice specifies the type Of injection method: Push (PushLoop), Pull (puff-loop), and Full (full-loop). The default is Push. The Injection Type value determines the Injection Range. (See also Injection Range.) If this field entry is Full, the Injection Menu's Injection Volume field becomes Loop Size. This field does not appear on the AS 1000 instrument.

Injection Range

This Injection Menu field indicates the allowable injection volume range (in microliters) as determined by the injection method (Injection Type) selected. The allowable ranges for each injection type are as follows: Push Hi (1-200), Push Lo (0.1-10.0); Pull (1-1500); Full (5-1000). This field does not appear on the AS 1000 instrument.

Injection Volume

Selects the total sample- or calibration-vial injection volume (1-1500 microliters). 10 µL is the default value.

Injections/Level

The Calibration Menu choice allows for multiple injections at a given calibration level. Specifies the number of injections (0-99) for each calibration level. The default value is 1.

Injections/Sample

This Injection Menu choice specifies the number of injections for each sample vial (1-99). A value greater than I allows for multiple injections. The default value is 1. A value of 0.0 allows for sample preparation without injection.

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Injector

This Commands Menu choice accesses the menu that allows you to manually control the injector's position.

Injector Valve

This Maintenance Log menu tracks the #cycles (activations) of the injector valve since #Cycles field value was last set. An activation occurs each time the valve changes direction. For example, an activation takes place when the valve changes from the Inject to the Fill position. When the #Cycles value exceeds the Due field value, the "Maintenance Required" message is displayed when the Maintenance Log Menu is accessed. The default maintenance Due field value is 12,000 (approximately once a year).

Input Polarity

This Options Menu choice lets you specify the active signal level (Hi/Lo) for a specific autosampler input. For more information, refer to Appendix D.

Intersperse Cals

This Calibration Menu field lets you specify a calibration sequence in which calibration vials are placed at regular intervals between sample vials. (See also TYPE.)

Key Repeat Rate

This Configuration Menu field is used to adjust the cursor scrolling speed: Fast [8 pulses per second (pps)], Medium (6 pps), and Slow (4 pps). Default: Fast.

Load

This Files Menu field accesses the Load Menu for. activating (loading) files.

Load File

Copies the edit file into the run file, which activates any options (oven, tray temperature control, heater) set in the Edit Menu.

Load _mL Solv S-1 (2,3,4)

A command used in sample preparation that initializes the solvent syringes, flushes the sample lines with the specified solvent (S 1 -S4), and loads the holding loop with the specified volume (0-1500 µL). The volume default is different for each template; the solvent default is S 1.

Loop Size

A field in the Injection Menu where you can specify your sample loop size fixed-loop injection. Allowable values are: 5, 10, 20, 50, 100, 200, 500, and 1000 µL. For an AS3000, if the More Menu's Injection Type is set to Full, this field appears on the Injection Menu in place of Injection Volume. For an AS 1000, this field appears in the Injection Menu in place of the Injection Volume field.

Maintenance Log

This Options Menu item is used to access autosampler maintenance status, use, and service information.

Mix

This sample preparation template command is used to vortex-mix, heat, and then remix for a specified period of time (in minutes).

More

This Edit Menu choice accesses the menu used to set the equilibration time, gradient delay, sample viscosity information, flush volume, injection method, and needle height.

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Needle Wear

This Maintenance Log menu tracks the #cycles (vial pierces) of the sample needle since #Cycles field value was last set. When the #Cycles value exceeds the Due field value, the "Maintenance Required" message is displayed when the Maintenance Log Menu is accessed. The default maintenance Due field is 10,000 vial pierces (approximately six months).

Normal

This More Menu Viscosity choice indicates a standard syringe speed appropriate for most sample viscosities.

Note

This 6-character alphanumeric field appears in all Maintenance Log Menus and is programmable using the [+]/[-] keys. It is designed to hold the date of the last instrument maintenance, but may also be used to hold any service-related information on the autosampler or one of its parts.

Number of levels

This Calibration Menu field specifies the number of calibration (concentration) levels (0-9) in the calibration series for a given sample set. A value of 0 indicates that there are no calibration vials in the sample set. The default is 0.

Number of Samples

This Sample Menu field specifies the total number of sample vials ((1-20) in the sample set. The default is 1.



NOTE: *With the Tray Temperature Option installed, the total number of vials is 105.*

OPTIONS

This Main Menu field accesses the Options Menu for setting/changing the instrument configuration, for performing error recovery and instrument shut-down, and for monitoring the maintenance log.

Order

This Queue Menu field is used to edit the processing order of sample sets within the sample queue. You can reorder any sets that are not currently running. The default order value is 1 (first, except when a priority set is added to the queue).

Oven

This Status Screen field displays the oven's current state (On/Off) and its actual (not set) temperature. The actual temperature is displayed even if the oven is turned off.

Oven Shutoff

The Shutdown Menu field lets you program the autosampler to automatically turn off the Oven during the Shutdown sequence.

Output Polarity

This Options Menu choice lets you specify the active signal level (Hi/Lo) for a specific autosampler output. For more information, refer to Appendix D.

Oven Installed

This Configuration Menu field indicates whether or not the Column Oven Option is installed (Yes/No). The default setting is No.

Overlap Enables

This last line in each sample preparation template allows sample preparation for the next sample to begin while the current sample is still running. The default is Yes. No delays sample preparation of the subsequent sample until the current sample's run is complete.

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P

This Queue Menu Order field entry indicates that the set has been assigned a priority status (from the Sample Menu). See Chapter 3 for more information on managing priority sample sets.

Pickup _mL Sample

This sample preparation command causes the sample syringe to withdraw the specified volume from the specified source vial (Sample, Sam + 1, Sam + 2, Sam + 3, Reag-A, Reag-B, or Reag-C). The volume and source-vial default values are different for each template.

PickLg _mL Sample

This sample preparation command causes the prep syringe to withdraw the specified volume from the specified source vial (Sample, Sam + 1, Sam + 2, Sam + 3, Reag-A, Reag-B, or Reag-C). The volume and source-vial default values are different for each template.

Prep Installed

This Configuration Menu field indicates whether or not (Yes/No) the Sample Preparation Option is installed. The default setting is No.

Prep

This Status Screen field displays the current sample preparation template step in progress.

Prep Flush Volume

This Shutdown Menu field specifies the desired volume of flush solvent (0 – 5000 µL) for the prep-syringe flush that occurs as a part of the shutdown sequence. The default value is 1000 µL.

In the Maintenance Log Menu, this field tracks the #cycles (prep syringe strokes) since #Cycles field value was last set. When the #Cycles value exceeds the Due field value, the "Maintenance Required" message is displayed when the Maintenance Log Menu is accessed. The default maintenance Due field value is 24,000 strokes (approximately once a year).

Prep Viscosity

This More Menu field indicates the viscosity of the sample preparation resource (sample/reagent) to be picked up in the PickLg step(s) by the 2.5 mL prep syringe. Allowable values are Normal, Medium, and Viscous. This value affects the overall syringe rate.

Protect

This Configuration field allows you to restrict editing of selected files as named in the Files Menu. If Protect is turned on (Yes) for a particular file, you will be unable to edit or view a particular file until Protect is turned off (No). The default is No.

Pump Ready Active

This Options, Input Polarity Menu field indicates the active level (polarity) of the pump ready input signal (Hi/Lo).

Pump Shutoff

This Shutdown Menu field lets you program the autosampler to automatically turn off the pump during the Shutdown sequence.

Pump Stop Active

The Output Polarity Menu field lets you specify the active level (polarity) (Hi/Lo) of the Pump Stop output signal.

Push Hi

One of the PushLoop injection types in the Injection or Calibration Menus. This choice corresponds to an injection range of 1-200 µL.

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Push Lo

One of the PushLoop injection types in the Injection or Calibration Menus. This choice corresponds to an injection range of 0.1-10µL.

QUEUE

This Main Menu entry displays and allows you to edit the items in the sample queue. (See also Order).

QStatus

This first status screen field lists the current state of the sample queue (RUN, IDLE, EQUIL, etc.). Refer to Chapter 3 for a description of the various autosampler states.

RAM Test

This Tests Menu choice initiates the autosampler's RAM test to verify proper operation.

READY Participation

This Options Menu choice lets you define the "Autosampler Ready" requirements and set the input- and output-signal polarities.

Reagent A (B, C)

These sample preparation template fields specify the reagent vial position(s) (A01-C40, without tray temperature control; A01-C35, with tray temperature control) required by the sample preparation method.

Remove _mL To Waste At _mm Height

This sample preparation command withdraws a specified volume (0-1500 µL) from a specified source vial (Sample, Sam + 1, Sam + 2, Sam + 3) at a specified distance (height 0-20 mm) between the needle tip and the bottom of the source vial and delivers it to waste. This function enables you to select a given layer of sample for extraction. Volume and source defaults are different between Templates 3 and 4. (See Chapter 4.)

Reset #Cycles

This Maintenance Log Menu field lets you reset the #Cycles counter for each autosampler part. To reset the counter to 0 after maintenance is complete, press the [+]/[-] keys to select YES, and press [ENTER]. The confirmation message* * Counter Reset * * is displayed

Reuse same vials

This Calibration Log Menu choice lets you select the calibration sequence type that programs the autosampler to use the same set(s) of calibration vials throughout the run. The autosampler will automatically (Yes) reinject from the same calibration vials according to the calibration interval specified. (See also Type and Samples/Calibration.)

ROM Test

This Tests Menu choice initiates the autosampler's ROM test to verify proper operation.

Rotate Injection Valve

This Commands, Injector Menu choice lets you manually (outside of a run) rotate the injection valve between the Inject and Fill positions.

S-1 (2, 3, 4)

Indicates one of the four external solvents used in sample preparation.

SAMPLES

Is the autosampler's "special" key that displays the Sample Menu, which lets you define the resources and file for each sample set and add sample sets to the sample queue. For more information, see Chapter 1.

Sample Prep

This Files, Edit Menu choice accesses the sample preparation templates (menus) and appears only if the Sample Preparation Option is installed.

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Sample Set

This Samples Menu field indicates the sample set number (1-39) in the sample queue. A set is a group of sample vials and associated calibration vials.

Sample Syringe

This Maintenance Log Menu tracks the *cycles (sample syringe strokes) since the #Cycles field value was last set. When the #Cycles value exceeds the Due field value, the "Maintenance Required" message is displayed when the Maintenance Log Menu is accessed. The default maintenance Due field value is 24,000 strokes (approximately once a year).

Sample Syringe Size

This Configuration Menu field specifies the size of the sample (small) syringe (250, 500, 1000, 2500, and 5000 µL). The default is 250 µL.

Samples/Calibration

This Calibration Menu field specifies the number of sample vials (0-99) between calibration vials (the calibration interval). A value of 0 indicates that there are no calibration vials in the set. The default setting is 0.

Set

In the Queue Menu, Set indicates the sample set assigned to the file in the same line.

Shutdown

This Options Menu choice lets you specify the power-down sequence that turns off the temperature zones, zeroes the detector, turns off the pump, and flushes the autosampler's lines before putting the autosampler in the Idle mode.

Software Version

This Tests Menu field displays the current software version installed in the instrument.

Solv Viscosity

This Configuration Menu field allows you to adjust the prep syringe speed (draw rate) to accommodate the viscosity of your solvent. Range: Normal, Medium, Viscous. Default value: Normal.

Status

This key accesses the Status Menu that displays the queue, injection, temperature zones, and sample preparation status information.

Temp

This Injection Menu field lets you specify the column oven temperature (15º - 80ºC) and the tray temperature (0º - 60ºC), in installed.

Test COMM Port

This Tests Menu choice lets you to perform a read/write test to verify proper operation of the communications (COMM.) port. A loop-back cable is required for this test.

Test External Contacts

This Tests Menu choice activates all of the external contacts and tests them for proper activation. This menu displays the current levels (Hi/Lo) of the autosampler's input and output signals.

TESTS

This Main Menu choice accesses the autosampler's build-in diagnostic routines.

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TF1 (2, 3, 4)

If the Timed Events Menu, this field indicates one of the four open-collector outputs cable of handling 500 mA at 28V. The default state is Lo ("open"). This output can be set to Hi or Lo to activate an external device such as a fraction collector or column-switching device.

Time

In the Display Timed Events States, This field is used to specify a relay activation time (0.01-655.0 minutes).

Timed Events

This Edit Menu choice accesses the menu used to program external outputs. [See also TF 1 (2, 3, 4)].

To File #

Located in the Copy Menu, this field lets you specify the destination file number (1-4) for copying a file. The default is 1.

Type

This Calibration Menu field lets you specify the type of calibration sequence: Reuse same vials or intersperse calibration vials (Intersperse Cals).

Total Cycles

This unprogrammable Maintenance Log Menu field contains instrument usage information.

TrayTemp

In the Edit Menu, this field lets you turn on/off the sample tray heater/cooler. The default setting is No. This field also indicates the tray temperature (0º- 60ºC).

In the Status Screen, this field displays the sample tray heater/cooler's current state (On/Off) and is actual (not set) temperature. The actual temperature is displayed even if the tray heater/cooler is turned off. This field appears only if the Tray Temperature Control Option is installed.



NOTE: This field appears only if the Tray Temperature Control Option is installed.

TrayTemp Shutoff

The field in the Shutdown Menu that lets you program the autosampler to automatically turn off the heater/cooler during the Shutdown sequence. This field appears only if the Tray Temperature Control Option is installed. The default is Yes.

TrayTemp Installed

Indicates whether or not the Tray Temperature Control Option is installed (Yes/No). The default setting is No.

Use Template

Used to select the number of a desired sample preparation template file: 0:No Prep, 1:Dilution, 2:DABS-CL, 3:Rainbow, and 4:Linear. The default value is 0:No Prep.

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Vial

In the injection Status Screen (Status), the vial field specifies the injection-vial position and vial type (A01-S). The vial position falls in the A01-C40 range without the Tray Temperature Control Option installed and in the A01-C35 range with the Tray Temperature Control Option installed. The vial type is either S (sample) or C (calibration vial).

Viscosity

In the sample preparation Status Screen (Prep), this field displays the vial currently undergoing sample preparation.

Vol

This Edit, More Menu choice is used to specify the sample viscosity (Normal, Medium, or Viscous), which affects the syringe drawrate.

Wait for _minutes

This sample preparation command returns the vial to the sample tray and pauses all sample preparation activity for the specified period of time (0-99.9 min). The default value is 0.0 minutes.

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C Troubleshooting

Introduction

This appendix provides information to help you troubleshoot possible autosampler problems. We have divided it into five sections:

- a brief theory of operation,
- a troubleshooting guide to general chromatographic problems
- an autosampler-specific troubleshooting table that lists symptoms, possible problems, remedies,
- a list of possible error messages, and
- a description of the autosampler's diagnostic tests.

If, after reviewing the troubleshooting table and diagnostics included in this chapter, you would like additional assistance in troubleshooting a particular problem, don't hesitate to contact the Thermo Separation Products Technical Support Group or your service representative. The telephone numbers are found on the back cover of this manual.

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Theory of Operation

BASIC AUTOSAMPLER

The autosampler consists of a number of subsystems. It is important for you to be familiar with how these subsystems contribute to the instrument's operation in order for you to be able to trace a problem or symptom to its probable cause.

The basic autosampler consists of the following components: 1) flush valve, 2) small (250 µL) syringe, 3) injector valve, 4) sample loop, 5) needle and hook, 6) flush funnel, 7) pump output line, 8) column input line, and 9) sample vial. Figure C.1 illustrates the autosampler's major components without the Sample Preparation Option. Figure C.2 illustrates the major components with sample preparation installed.

Vials filled with samples to be analyzed are loaded into three trays that hold up to 40 vials each (35 with the Tray Temperature Control Option installed). During operation, a mechanical are (the XYZ arm) locates a sample vial. The hook on the end of the arm moves under the selected vial and lifts it from the sample tray. The XYZ arm transports the vial to the sample tower and the sample needle for sample injection. The injection valve switches between the Inject and Fill positions (Figure C.3) as necessary to draw sample or solvent into the autosampler lines and to inject sample onto the column. All of the SpectraSYSTEM autosamplers perform full-loop injections. The AS3000 offers both pull-loop injection and Thermo Separation Products' PushLoop injection. These injection routines are described here.

Full-loop injection

In full-loop injection, an air bubble is drawn into the needle to isolate the incoming sample slug from the flush solvent already present in the lines (Fig. C.4). With the injector valve in the FILL position, the syringe retracts to pull the sample from the vial into the sample loop. The injector valve rotates to the INJECT position, and the pump pushes the sample from the sample loop onto the column.

Pull-loop injection

The pull-loop injection routine is useful when you have a limited amount of sample. Only the desired sample amount is pulled from the vial into the sample loop. Specifically, an air bubble is drawn into the needle to isolate the incoming sample slug from the flush solvent already present in the lines (Fig. C.5). With the injector valve in the FILL position, sample is drawn into the needle. The sample vial is lowered from the end of the needle and sufficient air drawn into the needle to pull the sample slug into the sample loop. The injector valve then rotates to the INJECT position and sample is expelled from the sample loop onto the column.

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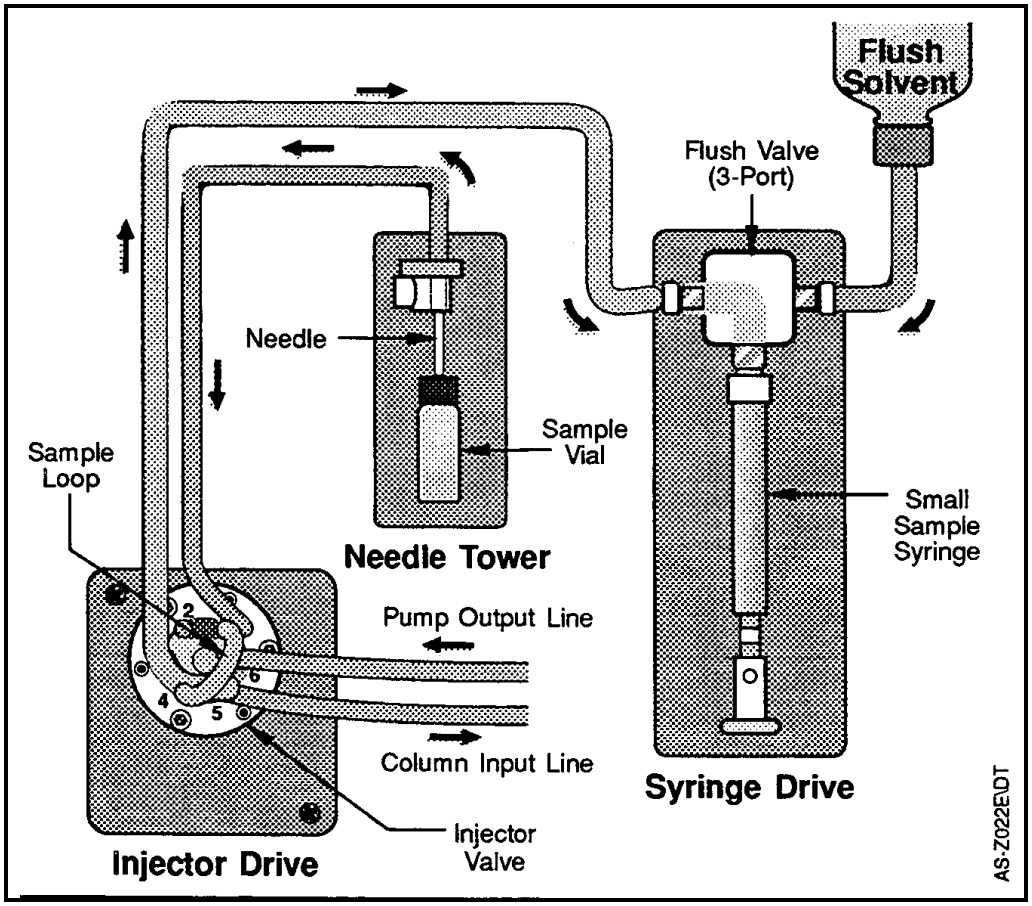


Figure C.1 The autosampler's major components without the Sample Preparation Option

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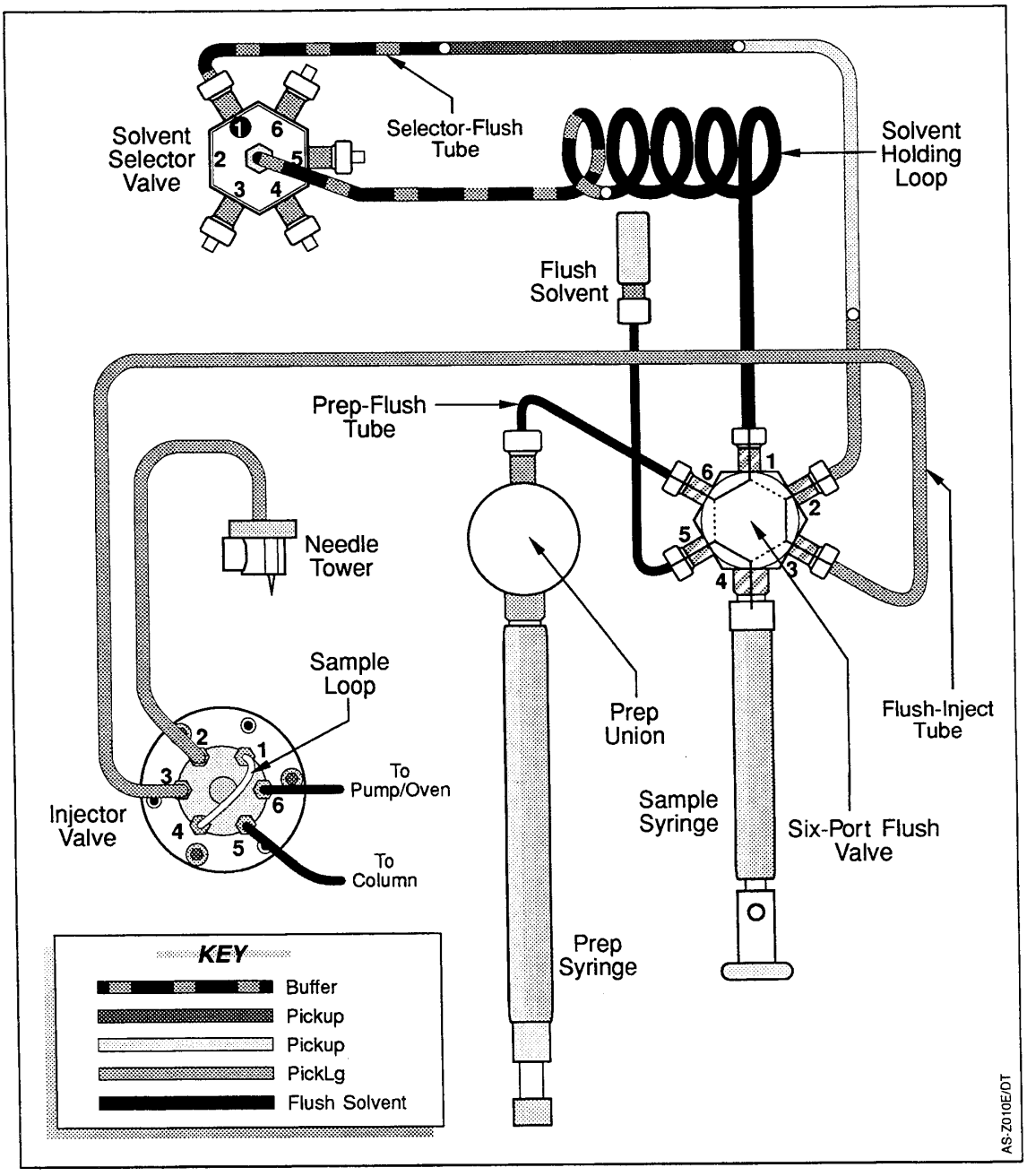


Figure C.2 The autosampler's major components with the Sample Preparation Option installed

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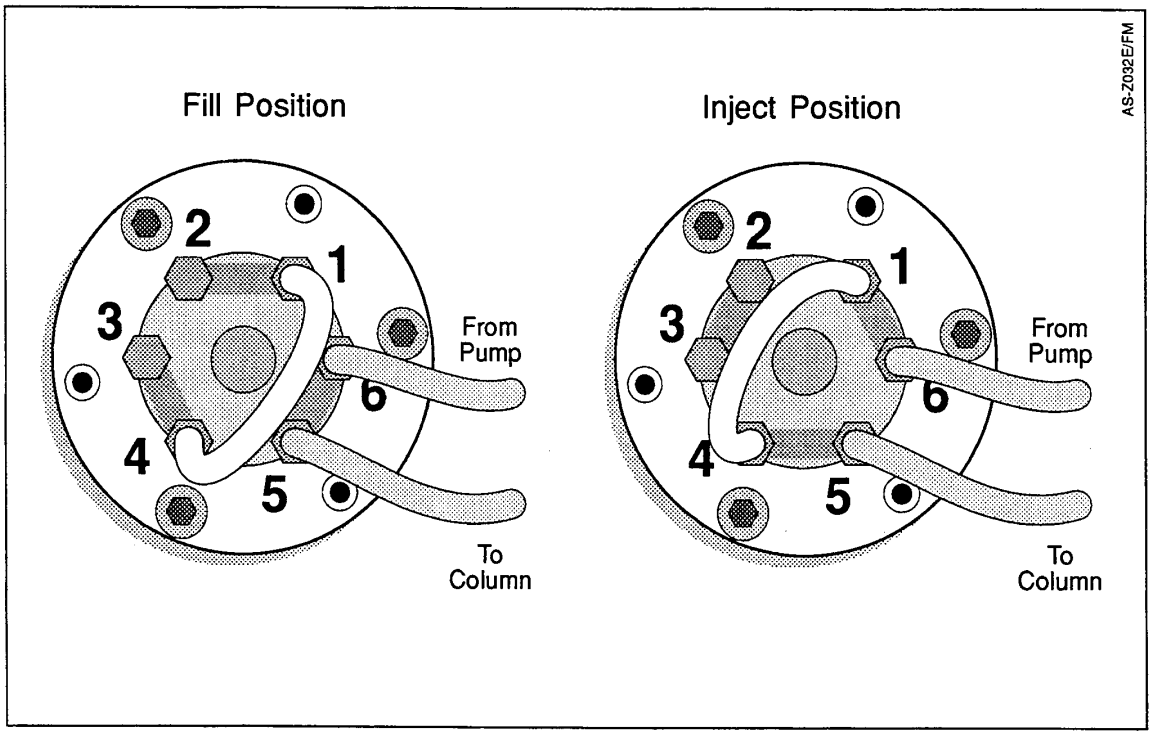


Figure C.3 The injector valve's inject and fill positions

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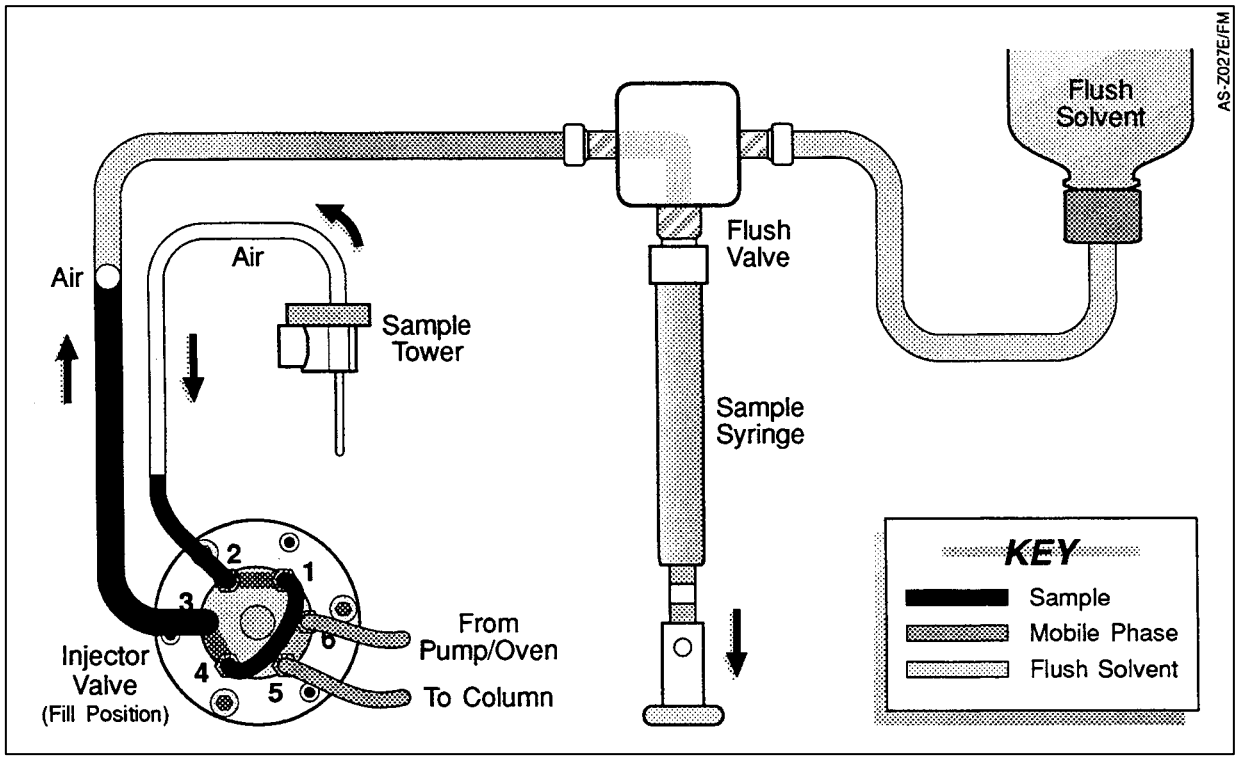


Figure C.4 Full-loop injection just prior to sample injection

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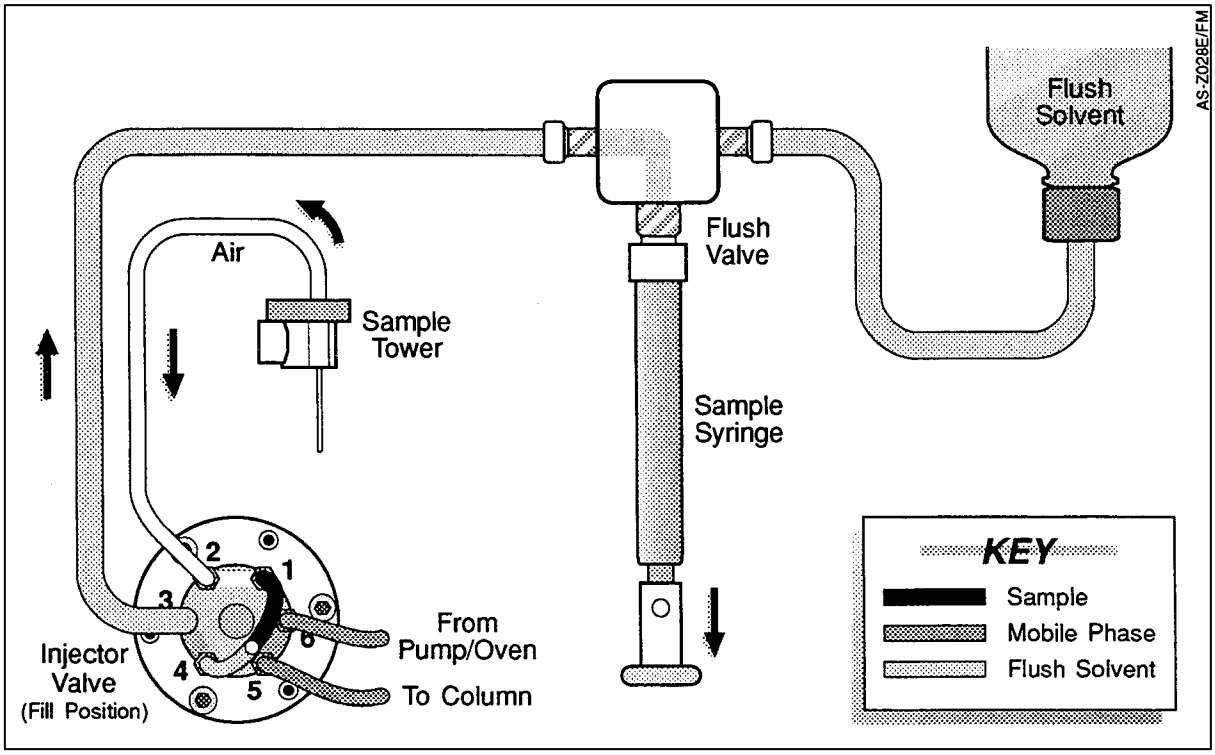


Figure C.5 Pull-loop injection sequence

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PushLoop Injection

The concept behind PushLoop injection is that it is more accurate to push a column of fluid than it is to pull it. Pulling liquids through a small tube can cause "cavitation" within the tubing. Cavitation is the outgassing of dissolved gases caused by reducing the pressure on a column of liquid. This forms bubbles within the column of liquid, resulting in inaccuracies and nonreproducable volumes.

With PushLoop injection, sample volume is separated from the previous flush solvent by an air bubble. With the valve in the Inject position, the sample slug is pulled up to and past the injection valve. After compensating for the syringe backlash, the valve is switched to the Fill position and the desired volume of sample is pushed into the sample loop. In PushLoop injection, sample volumes are "pushed" into the sample loop from solution directly adjacent to the loop. The sample solution is essentially at the "bottom" of the column of liquid. Any residue from the previous solvent has been minimized. The starting boundary of the delivered sample slug is well defined by the valve. There is no uncertainty about the air bubble or solvent droplets that may affect accuracy or reproducibility. Finally, the injector valve is then switched back to the Inject position, and sample is expelled from the sample loop onto the column. Figures C.6, C.7, and C.8 illustrate this routine.

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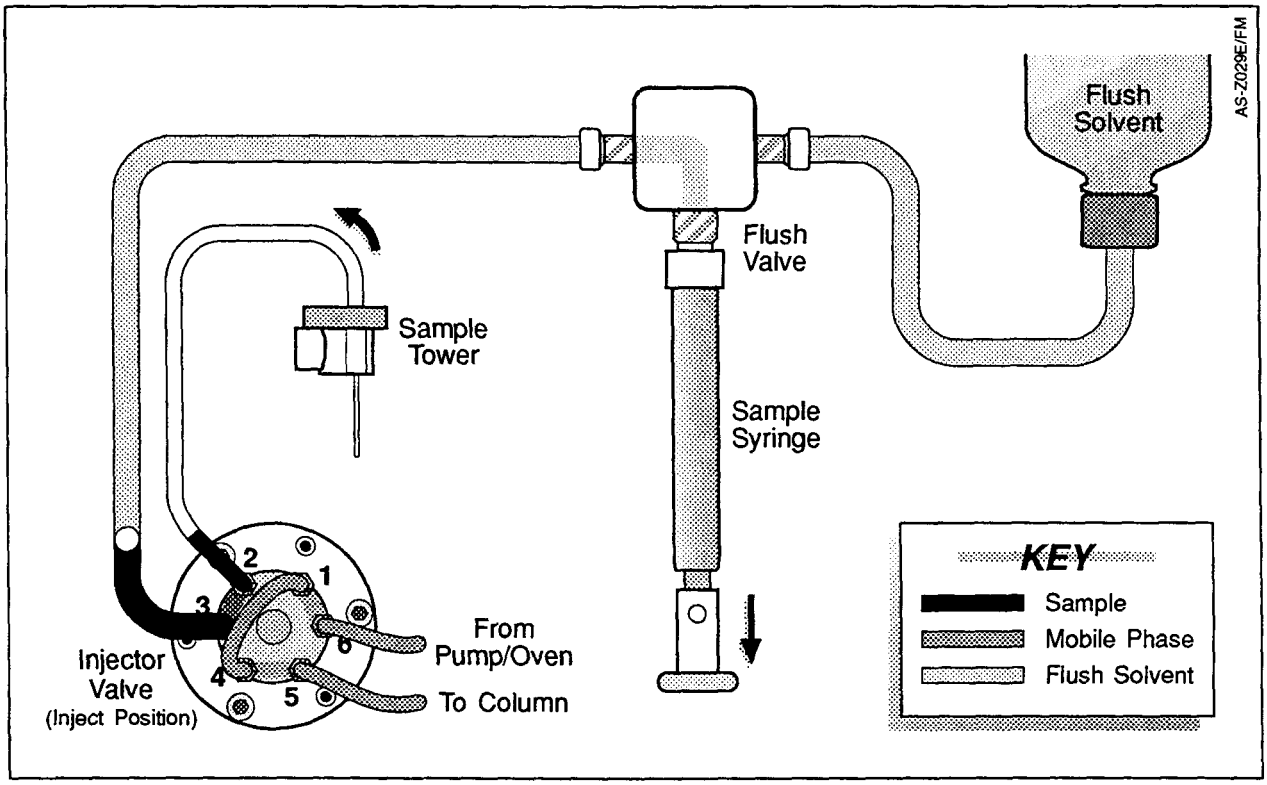


Figure C.6 PushLoop injection
(step 1: The sample is pulled up to and past the injector valve.)

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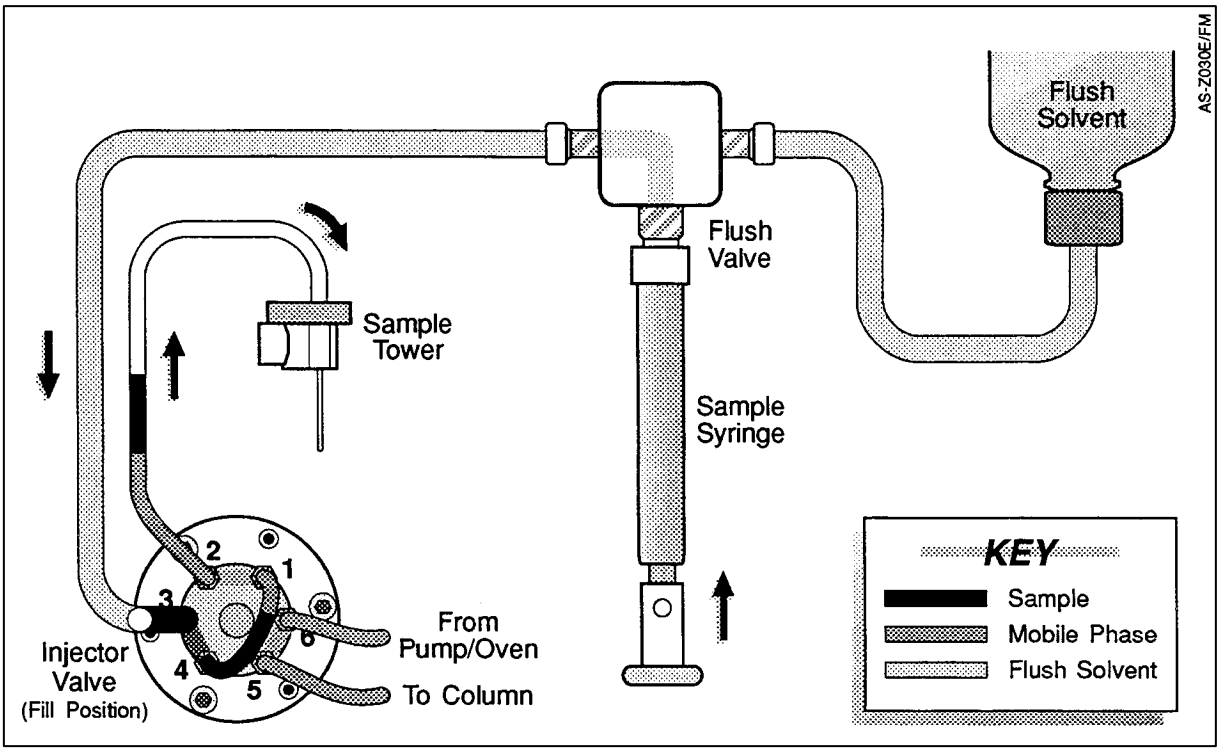


Figure C.7 PushLoop injection
(step 2: The injection valve is switched to the Fill position, and desired volume of sample is pushed into the sample loop.

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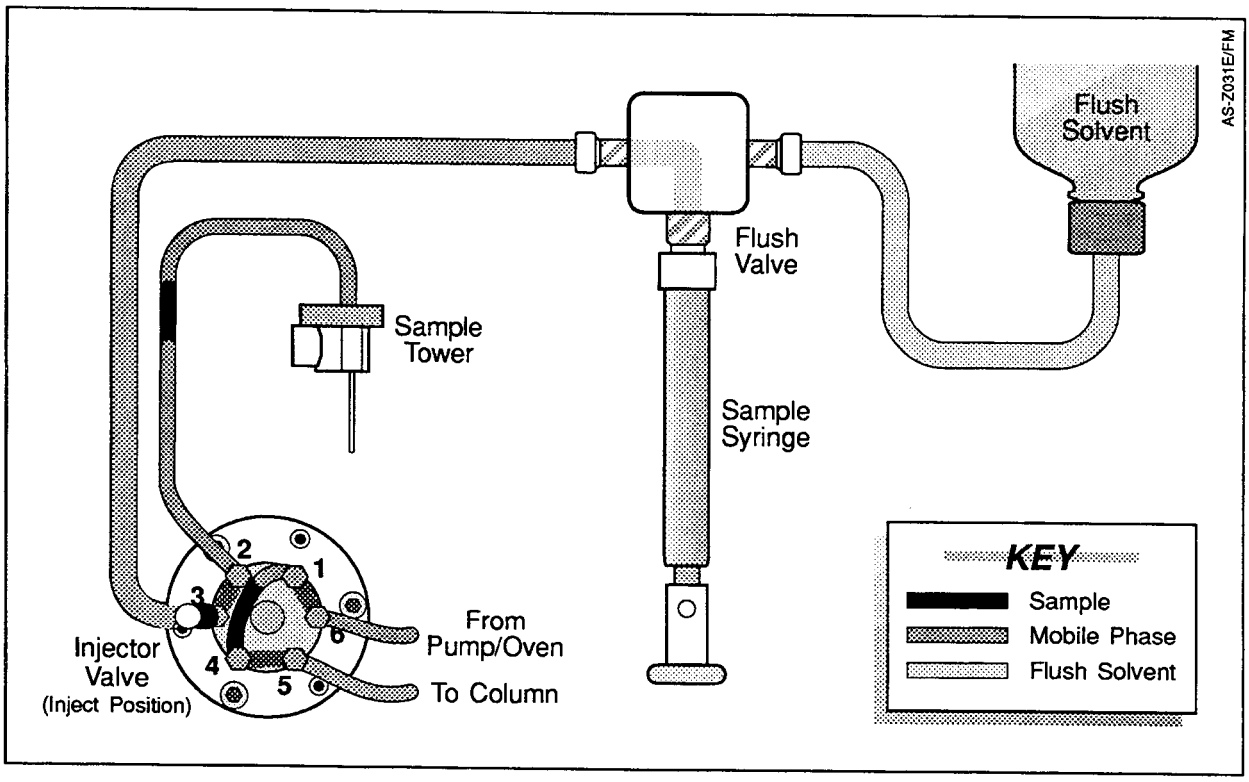


Figure C.8 PushLoop injection
(step 3: The injection is switched back to the Inject position, and sample is expelled from the sample loop onto the column.)

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OPTIONS

Column Oven

The column oven contains a resistive thermal device that heats up to stabilize the column temperature. A temperature sensor detects the temperature and sends the temperature reading back to the microprocessor, which turns the resistive device on or off to regulate the temperature. This is a closed assembly and requires no routine maintenance.

Tray Temperature Control

The tray temperature control unit consists of four Peltier devices, two high-speed fans, and a heat sink. Heat is pumped from the sample compartment into the heat sink and then blown out the back of the instrument by the two fans. A temperature sensor sends the temperature reading back to the microprocessor, which controls the operation of the four Peltier devices to regulate the sample compartment temperature. Similar to the column oven, the tray temperature control unit is a closed assembly and requires no routine maintenance.

Sample Preparation

The sample preparation hardware consists of the solvent selection system and the heater/vortex mixer. (Refer to Chapter 4 for information on the sample preparation software.)

The solvent selection system is located at the back of the instrument and includes the sample preparation syringe(s) and the solvent selection valve.

The heater/mixer or vortex mixer, located behind the sample tower, uses compressed air to spin the sample vial creating the vortex inside the vial that mixes the vial's contents. The microprocessor clock tracks the run time and turns the mixer on and off at the desired times. A resistive thermal device (heater) and a temperature sensor, similar to the column oven, maintain the desired temperature during the vortex-mixing process.

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General Chromatography Troubleshooting

This section focuses on general chromatographic problems and is designed to help you define the particular instrument or condition causing the problem. Once you have ascertained the cause, use the ordered outline to fix the problem or refer to the individual SpectraSYSTEM instrument's troubleshooting guide. Each of these problems can be detected by monitoring the chromatography running conditions or by observing the chromatogram.

SYMPTOM	CAUSE/REMEDY
1. No flow.	a) Check mobile phase connections b) Check for leaks c) Check pump troubleshooting guide
2. High Backpressure	a) Check flowrate and system/column specifications b) Check for tubing or column blockage c) Check pump troubleshooting guide
3. Unstable baseline or drift	a) System/column not equilibrated; allow more time b) Check detector troubleshooting guide c) Check pump troubleshooting guide
4. Baseline noise.	a) Check for air bubbles in system, degas solvents b) Check for system/solvent contamination c) Check pump troubleshooting guide d) Check ISM or PC 1000 troubleshooting guides
5. No peaks.	a) Check detector and data system connections b) Check autosampler troubleshooting guide c) Check sample retention with chromatographic conditions
6. Contaminating/ghost peaks	a) Clean system and column b) Check autosampler troubleshooting guide c) Check pump troubleshooting guide

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7. Porr peak shape.	a) Check system for leaks b) Check fittings and tubing lengths c) Check column performance d) Check autosampler troubleshooting guide e) Check pump troubleshooting guide f) Check detector troubleshooting guide
8. Poor retention time reproducibility	a) Check system for leaks and bubbles b) System/column not equilibrated, allow more time c) Check column performance d) Check pump troubleshooting guide e) Check autosampler troubleshooting guide f) Check ISM or data system troubleshooting guide
9. Poor peak area reproducibility.	a) Check column performance b) Check autosampler troubleshooting guide c) Check ISM or data system troubleshooting guide
10. Non integrated or too many peaks	a) Check integrator, ISM, or data system troubleshooting guide
11. No instrument of device control.	a) Check cable connections b) Check system configuration c) Check individual instrument troubleshooting guide d) Check integrator, ISM, or data system troubleshooting guide

For more detailed chromatographic troubleshooting, refer to any BPLC troubleshooting reference book or call your local sales or service representative.

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Autosampler-specific Troubleshooting

Chromatographic Problems

SYMPTOM	POSSIBLE PROBLEM	REMEDY
1) Baseline drift when the injector valve changes from INJECT to FILL.	a) Lack of thermal stability in the column oven compartment, detector, or mobile phase.	c) Eliminate drafts around the column compartment. Wait until the column oven has reached thermal equilibrium. If the problem persists, contact your local Thermo Separation Products service representative.
2) Shifting retention times.	a) Temperature variations in the lab.	a) Stabilize temperature.
	b) Column-heater problems.	b) Call your Thermo Separation Products service representative.
3) Sensitivity changes.	a) Inconsistent sample injection volumes.	a) Check for a partially blocked injector valve and observe the pressure. If the problem persists, replace the injection valve (refer to Chapter 5).
	b) Leaks between the injector valve and the column causing inconsistent sample-volume injection.	b) Tighten/remake fitting.
	c) Air bubbles aspirated into the lines along with sample.	c) Replenish/prepare fresh sample and repeat the injection.
		c) Slow down syringe speed.
	d) Incomplete sample mixing during sample preparation.	d) Check mix time (Sample Preparation Option, only). Thaw frozen samples completely prior to sample preparation to prevent sample "layering."
	e) Sample adsorption to the walls of the injector valve or sample loop.	e) Change the mobile phase concentration.

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Chromatographic Problems, con't.

SYMPTOM	POSSIBLE PROBLEM	REMEDY
3) Sensitivity changes, con't	f) Sample solvent incompatible with the mobile phase.	f) Change solvent, use mobile phase if possible.
	g) Sample insoluble in solvent.	g) Be sure sample solvent is lower elution strength than mobile phase.
	h) Air in syringe/sample lines.	i) Flush sample lines.
4) Irregular peak shapes.	a) The volume sample injected exceeds the column's capacity.	a) Decrease the volume injected or dilute the sample.
	b) The strength of the solvent used to dilute the sample exceeds the initial concentration of the mobile phase.	b) If appropriate for your application, ensure that the flush solvent is of the same composition as the solvent used to dissolve the sample and that it never exceeds the initial composition of the mobile phase.
	c) Degradation of sample component(s).	c) Prepare fresh sample and repeat injection.
5) Band broadening.	a) Fitting problem at the injector-column connection.	a) Inspect for damage, mismatch, incorrect assembly.
	b) Tubing I.D. too large.	b) Use 0.010-inch I.D. tubing.
6) Smaller than expected peak heights.	a) Small injection due to wrong-size syringe.	a) Check syringe size.
	b) Wrong injection.	b) Check injection volume value in file.
	c) Air in lines.	c) Flush.
	d) Misadjusted syringe-drive mechanism.	d) Call your Thermo Separation Products service representative.

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Chromatographic Problems, con't.

SYMPTOM	POSSIBLE PROBLEM	REMEDY
7) Peaks during a blank injection (from a previous injection).	a) Sample carryover due to residual sample in the sample loop.	a) Insufficient flush volume. Increase Injection Volume in Edit Menu; add a blank vial between sample vials; or use a stronger flush solvent.
	b) Sample carryover due to incorrectly assembled sample-loop fittings (adding dead volume that can contain residual sample).	b) Check and remake fittings.
	c) Sample too concentrated.	c) Dilute sample.
8) No peaks.	a) Plugged or bent needle.	a) Clean/replace needle. Tighten fittings and syringe.
	b) Leaking flush valve fitting.	b) Tighten fitting.
	c) Plugged or leaking tubing.	c) Replace faulty tubing.
9) Incorrect precision and/or accuracy problem on a sample of known content-random error.	a) Air leak.	a) Tighten all fittings and repeat the run.
	b) Worn out syringe.	b) Replace.
10) Irreproducibility of peak areas.	a) Plugged or bent needle.	a) Clean/replace needle. Tighten fittings and syringe.
	b) Leaking flush valve fitting.	b) Tighten fitting.
	c) Plugged or leaking tubing.	c) Replace faulty tubing.
	d) Sample viscosity too low or too high.	d) Change viscosity setting in the FILE/Edit/More Menu.
	e) Solvent viscosity too low or too high.	e) Change Solv Viscosity setting in the OPTIONS/Configuration Menu.

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Hardware Problems

SYMPTOM	POSSIBLE PROBLEM	REMEDY
Startup		
1) Nothing works when instrument is turned on.	a) Power cord unplugged or faulty.	b) Plug in/replace cord.
	b) Blown Fuse.	c) Replace fuse.
	c) Defective power switch, driver, transformer.	d) Call your local Thermo Separation Products service representative.
2) Fan is running but display doesn't work.	a) Display component(s) failure.	a) Call your local Thermo Separation Products service representative.
3) No response from keypad but display works.	a) File isn't loaded.	a) Call your Thermo Separation Products service representative.
4) Column oven and/or tray temperature control won't turn on.	a) File isn't loaded.	a) Load file.
Communication		
5) No RS-232-C communication.	a) Further troubleshooting required.	b) Call your Thermo Separation Products service representative.
6) EEPROM fails to retain memory.	a) Reinitialize the EEPROM.	a) If unsuccessful, may be a defective PCB. Call your Thermo Separation Products service representative.
7) External devices (timed events) don't work.	a) Wrong settings.	a) Check Appendix D for proper pinouts.
	b) Interface equipment problem.	b) Refer to respective instrument manuals.

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Hardware Problems con't

SYMPTOM	POSSIBLE PROBLEM	REMEDY
Vials		
8) Vial stuck in tower.	a) Needle bent.	f) Replace needle module (see Chapter 5).
9) Vial stuck in mixer.	a) Run Clear Mixer routine in the Commands Menu.	a) See page 182.
10) Instrument can't locate vial.	a) Incorrect vial position entered into Samples Menu.	a) Check Samples Menu.
	b) TrayTemp Option incorrectly selected in Configurations Menu. (Instruments with tray temperature control have fewer tray rows.)	b) Check configuration.
	c) Sample incorrectly placed in sample tray.	c) Check tray arrangement.
	d) Arm misaligned.	d) Home arm. See "Special Commands" on page 179.
	e) Arm movement restricted.	e) Remove restriction and home arm.
11) Vials don't fit in tray.	a) Incorrect vials.	a) Vial diameter should be 0.44 – 0.47 inches.
12) Broken vials.	a) Tray not aligned or not seated properly.	a) Seat tray completely in tray chamber and **pull handle back toward you**.
13) Torn septa.	a) Bent needle.	Replace needle.

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Hardware Problems con't

SYMPTOM	POSSIBLE PROBLEM	REMEDY
Injector		
14) Injector valve won't switch.	a) Slipping injector drive.	a) Call your Thermo Separation Products service representative.
15) Injector valve continues to rotate until fault is generated.	a) Loose or faulty sensor cable.	a) Call your Thermo Separation Products service representative.
16) Injector valve does not rotate to the proper position.	a) Valve requires excessive torque.	a) Call your Thermo Separation Products service representative.
17) Motor fails to stop after syringe is fully returned.	a) Possible defective sensor.	a) Call your Thermo Separation Products service representative.
18) Sample loop won't fill.	a) Blocked Injector valve.	a) Follow procedures on page 182 to isolate blockage and flush. Or take apart valve and sonicate. DO NOT clean valve with a cleaning wire or you could damage it.
	b) Blocked sample loop.	b) Backflush loop. If problem persists, replace the loop.
	c) Worn syringe.	c) Substitute a "known-good" syringe and determine if symptoms persist.
	d) Blocked needle.	d) Clean with a cleaning wire.
19) Rapid valve-seal wear.	a) Too much valve seal pressure against valve.	a) Decrease LC system pressure.
	b) Particulate matter in valve.	b) Use an in-line filter before the injector valve.
	c) Inadequately filtered sample.	c) Filter with a 0.5 gm filter before injection.

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Hardware Problems con't

SYMPTOM	POSSIBLE PROBLEM	REMEDY
Injector, con't		
20) Rapid valve-seal wear con't	d) Buffer crystallization.	e) Don't allow buffers to stand in system.
21) Injection from incorrect vial.	a) Incorrect vial position entered in Sample Menu.	a) Check Samples Menu.
	b) Sample placed in incorrect position in vial tray.	b) Check tray arrangement.
22) Samples injected incorrectly in a pattern.	a) Error in file (calibration vials placement/sample preparation resource placement).	a) Check file (calibration/sample preparation template).
Needle		
23) Needle bent or broken.	a) Incorrect vials.	a) See list of recommended replacement parts in the front of the manual.
	b) Alignment problem.	b) Call Thermo Separation Products.
	c) Arm sticking.	c) Call your Thermo Separation Products service representative.
24) Needle plugging.	a) Multiple/incorrect septa.	a) Verify a single septum of type recommended.

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Hardware Problems con't

SYMPTOM	POSSIBLE PROBLEM	REMEDY
Syringe		
25) Loud buzzing from syringe drive.	a) Defective limit switch.	a) Call your Thermo Separation Products service representative.
26) Flush valve won't activate.	a) Defective valve/coupler/motor.	a) Call your Thermo Separation Products service representative.
27) Flush valve actuated, but syringe can't draw flush solvent.	a) Plugged inlet filter in solvent reservoir.	a) Replace inlet filter (Chapter 5)
Arm		
28) Hook doesn't lift vial.	a) XYZ arm motor stepping failure.	a) Call your Thermo Separation Products service representative.
Leaks		
29) Leakage during loop filling.	a) Blocked loop or waste line or valve not deflected far enough.	a) Flush sample loop or replace; check and flush waste line. Check the sensor.
30) Cross-port leakage.	a) Worn rotor seal.	a) Replace according to instructions in Chapter 5.
31) Liquid on vial caps.	a) Leaky flush valve.	a) Replace flush valve.
	b) Leaky injector valve.	b) Replace rotor seal (Chapter 5).

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Hardware Problems con't

SYMPTOM	POSSIBLE PROBLEM	REMEDY
Other		
32) An automatic sequence is interrupted, or instrument will not start.	a) Column-oven, heater, door interlock(s) activated.	a) Check the Ready Participation Menu.
33) In Stand Alone mode, Status Screen indicates a run in progress, but the instrument is locked up.	a) Configuration is incorrectly set to SpectraNET mode.	a) In the Configurations Menu, set Mode to Stand Along.
34) Contaminated sample.	a) Dirty Vials.	a) Replace vials.
	b) Improper septa made of silicone or other rubber-like material.	b) Use recommended septa. *NOTE: Thermo Separation Products septa are made of Teflon-faced silicone.*
35) Elevated system pressurs.	a) Block between column and autosampler.	a) Crack each fitting and observe pressure. See instructions in Appendix C "Testing for Tubing Restrictions" on page 182.

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Display Messages

As we mentioned in Chapter 1, **there are three different kinds** of messages that can appear on your autosampler's display: user messages, confirmation messages, and error messages. This section describes the possible conditions that generate these messages.

USER MESSAGES

User messages are displayed as a result of an incorrect operation on the part of the user.

Autosampler Busy

You can't issue a command from a menu or press a key while the autosampler is in the middle of a previously requested operation.

Autosampler Busy No Loading Allowed

You can't load a sample set while the queue is running.

Can not add Priority Set during final calibration

You cannot add a priority set during bracketing (after all the sample vials have been injected).

Can not add Priority Set at this time

You cannot add a priority set to other than set 1. You cannot add a priority set unless the file's Calibration Menu calls for interspersed calibration vials (you must select reuse calibration vials in order to add priority sets to the queue).

File in Queue Cannot be Copied to

You cannot copy to a file assigned to a sample set in the queue.

File in Queue Cannot be Deleted

You cannot delete a file assigned to a sample set in the queue.

File in Queue Cannot be Edited

You cannot edit a file assigned to a sample set in the queue from the Edit Menu.



NOTE: You can edit the run-file copy of the active file from the Samples Menu.

File Protected Cannot Be Copied To

You can't copy to a file that is protected (Protect is set to On) in the Configurations Menu.

Use SAMPLES key to Add Samples to Queue

You can't start a run by pressing the [RUN] key when the queue is empty. You must first load or add sample set(s) to the queue.

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CONFIRMATION MESSAGES

Confirmation messages inform you that the autosampler is completing a requested operation or command. These messages disappear when the operation is complete.

Aligning Arm	The Align Arm command is in progress.
Clearing Mixer	The Clear Mixer command is in progress.
File Deleted	The requested file has been deleted.
File Loaded	The requested file has been loaded into the queue.
Flush Samp Syringe	The Flush Sample Syringe command is in progress.
Flush Prep Syringe	The Flush Prep Syringe command (page 180) is in progress.
Homing Arm	The Home Arm command is in progress.
Initializing	The Initialize Hardware command (page 180) is in progress.
Set Added to Queue	The requested sample set has been added to the sample queue.

ERROR MESSAGES

There are three kinds of errors: 1) hardware errors, 2) system errors, and 3) diagnostics errors. Hardware and system errors are discussed below. Diagnostic errors are discussed in the diagnostics section on page 174.

Hardware Errors

Hardware errors occur during the normal use and operation of the instrument. Most of the time you will be able to clear them. For some, you may need to call your Thermo Separation Products service representative. Pressing any key clears the error message. Then take the steps suggested for each message to resolve the problem.

Arm Failed to Home X, Y, Z	The XYZ arm could not make the final X-, Y-, or Z-axis motion required return to the home position. Check for a dislodged vial, tray, or other obstruction. Run the Initialize Hardware routine. If the error persists, contact your Thermo Separation Products service representative.
Arm Jam on X, (Y, 2 Axis	The XYZ arm is obstructed on its left-to-right (X) axis; its front-to-back (Y) axis; or its vertical (Z) axis. Check for a dislodged vial, tray, or other obstruction. Run the Initialize Hardware routine. If the error persists, contact your Thermo Separation Products service representative.
Flush Valve Misaligned	The flush valve did not turn all the way to the required position. Perform the Initialize Hardware routine. If the error persists, run the Test Connectors routine. If the error persists, contact your Thermo Separation Products service representative.

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Hardware Failure	A problem occurred with the valves or syringes. Run the Initialize Hardware diagnostic on page 180 and/or the Injector Test (page 180). If you are unable to clear the error, contact your Thermo Separation Products service representative.
Hardware Not Homed	The solvent valve was requested to move to a certain position, but the hardware was not initialized properly. Or, on powerup, the solvent valve could not home properly. Run the Initialize Hardware diagnostic on page 180. If you are unable to clear the error, contact your Thermo Separation Products service representative.
Hardware Timeout	A requested mechanical operation was not completed in the allotted time. The problem could be with the arm, valves, or syringe(s). Run the Initialize Hardware routine on page 180. If the problem persists, try manually flushing the syringe(s) using the Flush Sample (page 179) and Flush Prep Syringe (page 180) commands. If the problem persists, contact you Thermo Separation Products service representative.
Injector Valve Failure	The injector valve failed to move (between the INJECT and FILL positions, or vice versa) for the requested operation. Issue the Injector command from the Commands Menu. If the error persists, call your Thermo Separation Products service representative.
Inject Valve Misaligned	The injector valve is positioned between the INJECT and FUL positions. Run the Injector test (page 180), and call your Thermo Separation Products service representative if the error persists.
Ivalve Move Is Impossible	The injector valve cannot move to the requested position from its current position. Run the move the injector valve to another position using the Injector command, and call your service representative if the error persists.
Ivalve Move Not Detected	The system did not detect a requested movement to the required position. Check for a loose connection to the injector. Try to manually move the injector to another position. If the error persists, call your service representative.
Inject Valve Overshoot	The injector valve moved past the desired position. Run the Injector test (page 180), and call your Thermo Separation Products service representative if the error persists.
Injector Valve Undershoot	The injector valve failed to move far enough to the desired position. Run the Injector test (page 180), and call your Thermo separation Products service representative if the error persists.
Parameter Out of Range	A value too large or too small was entered. For example, an injection volume too large for the installed syringe was entered. Check the installed syringe/sample loop and versus the edit file parameters.
Solvent Valve Misaligned	The 6-port solvent selection valve failed to turn far enough to reach the next required port or turned past the next required port. Issue the Initialize Hardware command on page 180. If the error persists, issue the Flush Prep Syringe (page 180) using various external solvent positions (S-1, S-2, S-3, S-4). This message appears only on instruments with the Sample Preparation Option installed.

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Svalve Not Present	A problem occurred with the valves or syringes. Run the Initialize Hardware diagnostic on page 180 and/or the Injector Test (page 180). If you are unable to clear the error, contact your Thermo Separation Products service representative.
Syr 1(2) Not Enough to Expel	One of the syringe(s) is not retracted far enough to expel the requested volume in the ADD step (sample preparation syringe) or flush operation (both syringes). Run the Initialize Hardware test (page 180), and restart the run (sample preparation) or the flush cycle.
Syringe Failure	A syringe failed to moved to the appropriate position required for an operation. Run the Initialize Hardware test (page 180). If the error repeats, call your Thermo Separation Products service representative.
Syringe Overfill	A volume has been requested for injection or ADD that is too large for the size of the installed syringe. Check the method and syringe volume(s).
TrayTemp Failure	The Tray Temperature Control Option was unable to reach the desired set point and has turned itself off returning the instrument to a READY state. Any method loaded will be executed without the Tray Temperature Control Option. Pressing any key clears the error. This error indicates a problem with the Tray Temperature Control Option. Call your Thermo Separation Products service representative for assistance.
Unexpected vial in Hook	This message appears if a vial is found in the hook where no vial is expected for the requested operation. To clear the error, remove the vial and restart or resume the requested operation.
Vial Not Found in Hook	This message appears if a vial is not found in the hook when a vial is expected. Verify that a vial exists in the requested tray location, check the file and vial assignments in the Samples Menu, and check to see if a vial is lodged in the sample tower. (If so, the needle may be bent.)

System Errors

System errors occur very rarely and abort normal operation. Since they are not usually problems you can correct, we will list some of them, but not describe them in detail. Should you see any of the following messages or other messages not described in this section, document them, and contact your Thermo Separation Products service representative for assistance.

Invalid Current Location
Invalid Command
Not in GET/RET Sequence
Z Must be Up/No Vial
Move Z Not Allowed
Get LocNum: Bad Row, Col
Zup Only in Serv Block
Invalid Target Location
Svalve Extra Interrupt
Bad Vial Number
Unexpected Syr Event

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Diagnostics

The SpectraSYSTEM autosamplers have built-in diagnostic routines accessible from the Tests Menu (Fig. C.11). These tests enable you to access the operation of your autosampler. Before describing each of these tests, we'll describe how the diagnostic tests are run and how to interpret the error screen information, should a test fail.

All of the test screens are arranged similarly. A test is initiated by pressing [ENTER]. While a test is in progress, the name of the test is displayed along with three dots (Fig. C.9)

Test External Contacts: . . .

Figure C.9 The In-progress Screen

INTERPRETING THE RESULTS

When the test is complete, either a PASS or FAIL result is displayed. Should a test fail, an error screen similar to that shown in Figure C.10 will appear that contains the following information:

- Test identification
- Fault code (diagnostic error)
- Module identification
- [RUN]/[STOP]

Conector:	ERROR # 3
Syringe1	[RUN] / [STOP]

Figure C.10 An example of an error screen



***NOTE**: The error number in this screen is just an example to show you the screen layout.*

Test Identification

The test identification is the name of the test being run.

Fault Code

The fault code is the error number associated with the specific failed result. Document and report the fault code to your service representative to receive further assistance in diagnosing and solving the problem.

Module Identification

This field displays the specific autosampler board or assembly where the problem occurred.

[RUN]/[STOP]

This field prompts you to press [RUN] to repeat the test or to press [STOP] to exit the current test and return to the Tests Menu.

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TEST DESCRIPTIONS

All diagnostics and built-in assessment routines are accessible from the Tests Menu (Fig. C.11). Each of the routines is described here.

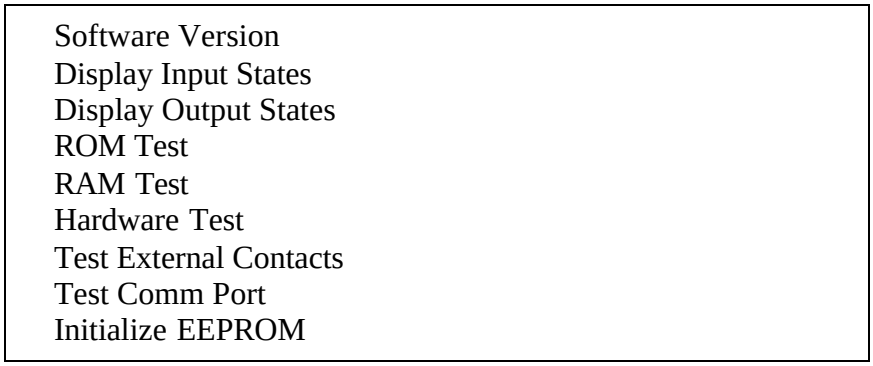


Figure C.11 Tests Menu

Software Version

Selecting this field displays the current software version number, x.yy.zz.

Display Input States

This menu (Fig. C.12) shows the current states of the TTL-input device signals (Hi or Lo). This information can be used to check the level of the Pump Ready and Injection Hold input lines from other LC system modules. You can use this information to diagnose situations where the autosampler is waiting for ready or waiting to inject. Make sure the Input Polarity is set to the proper level for each of these signals. (See Appendix D for more information on the signal levels.) Pressing the up- or down-arrow keys returns you to the Test Menu's Display Input States field should you wish to repeat the test.

NOTE: You can change the active polarity of a specific input signal from the OPTIONS Input Polarity Menu.



Figure C.12 Display Inputs States Menu

Display Output States

This menu shows the current states of the TTL-output device signals (Hi or Lo). This information is used when hardwiring your autosampler to other LC system modules. (See Appendix D for more information.) Pressing the up- or down-arrow keys returns you to the Test Menu's Display Output States field should you wish to repeat the test.

NOTE: You can change the active polarity of a specific input signal from the OPTIONS Input Polarity Menu. This menu shows the states of the TTL output device signals (Hi or Lo) as shown in Fig. C.13.

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Display	Output	States	
As Rdy	Inject	Grad	Stop
Lo	Lo	Lo	Lo

Figure C.13 Display Output States Menu

Pressing the up- or down- arrow keys returns you to the Test Menu's Display Output States field should you wish to repeat the test.

Display Timed Events

This menu (Fig. C.14) displays the current states of the timed events outputs. This information is used when hardwiring your autosampler to other LC system modules. Refer to Appendix D "System Communication" for more information.

TF1	TF2	TF3	TF4
Lo	Lo	Lo	Lo

Figure C.14 Display Timed Events Menu

RAM Test

This test (Fig. C.15) writes to and then reads the contents of memory. If the write information is the same as the read information, the test is successful. You may wish to run this test once a month to verify proper software operation.

Short Memory Test:

Figure C.15 RAM Test Menu

If successful, the autosampler returns a PASS result (Fig. C.16). If unsuccessful, the autosampler returns a "FAIL" result and displays an error message. Document the test results and error message, and call your Thermo Separation Products service representative.

Short Memory Test: PASS

Figure C.16 Successful RAM Test

Press [STOP] to return to the Test Menu.

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ROM Test

It evaluates check-sums on the contents of the instrument's ROM and compares the results to known values.

If successful, the autosampler returns a PASS result. If unsuccessful, the autosampler displays the number of the failed chip(s) along with the regular error screen information (Fig. C.17). If the test fails, document the test results (if failed) and call your Thermo Separation Products service representative.

ROM Test:	Error #: U78, U79
CPU	[RUN] / [STOP]

Figure C.17 Failed ROM Test

Hardware Test

This is the most important autosampler diagnostic. It checks all of the cable connections, sensors, motors, circuitry, and attached components.

Before running this test, be sure that the XYZ arm is in the home position (at the left rear of the sample compartment), or false errors may be reported. You can use the Home Arm command in the Commands Menu (see "Special Commands" on page 179) if the arm is not at home.

If the Hardware Test fails, the screen displays the test identification, fault code, applicable module or connection, and the [RUN] / [STOP] fields. For example, the message "J10/J40/J45" in Figure C.18 indicates that further troubleshooting is required. Document the screen information and report it to your Thermo Separation Products service representative when you call for assistance.

Connector:	ERROR # 3
J10/J40/J45	[RUN] / [STOP]

Figure C.18 An example of an error screen

Pressing [STOP] returns you to the Test Menu

Most of the failed Hardware Test results require that you contact your service representative for assistance. However, there are several messages that indicate problems that you can most likely resolve without a service call. These are described here.

Opt Config

This message indicates an incorrect configuration. Check the Configurations Menu (under OPTIONS) and verify that the options field entries match your installed options.

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Arm,*

This message indicates that the XYZ arm was not in its 'home" position when the Hardware Test was initiated. Select /COMMANDS/ , /Arm/ , /Home arm/.

Test External Contacts

This test activates all of the external contacts and tests for proper activation. It should not be run while the external contacts are wired to other equipment, or the test will report false errors. Before running this test, disconnect the 12-pin connector block from the rear of the instrument. After the test is complete, plug the connector back in to resume normal instrument operation.

Pressing [ENTER] with the cursor in the Test External Contacts field displays a warning prompt (Fig. C.19).

Disconnect all external contacts	CONTINUE

Figure C.19 The External Devices Menu

From this menu, you can use the [+] and [-] keys to select CONTINUE or ABORT. Selecting Continue starts the test.

Selecting ABORT discontinues the test and returns you to the Test External Contacts field of the Tests Menu. If ABORT is displayed and you want to resume to test, press the left- or right-arrow keys. The display will again show CONTINUE.

Test COMM Port

A loop-back connector is required to run this test used to assess the operation of the communications port located on the instrument's rear panel. This test sends a signal to the communications port and expects a signal in return. A successful result is reported as a PASS; an unsuccessful result is indicated by the regular error screen.



NOTE: If you wish to perform this test, contact your Thermo Separation Products Field Service Engineer for the procedure and necessary parts information.

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Initialize EEPROM

The Electronically Erasable Programmable Read Only Memory (EEPROM) stores all files, user-set variable values, and system configuration information when the power is turned off. Reinitializing the EEPROM restores the default values to all files and erases the system configuration information. Pressing [ENTER] in this field displays the message shown in Figure C.20:

Reset all system values YES

Figure C.20 The Initialize EEPROM Menu

If you want to exit this routine before running it, you can:

- Press the [+] [-] keys to select No
- Press [MENU] to call up the Main Menu
- Press [Status] to display the Status Screen
- Press the up- and down-arrow keys to return to the Tests Menu



CAUTION! This routine erases any current file values and returns all field values to their default values. Initialize EEPROM also erases all configuration information. After running this routine, be sure to reset all installed options to "YES" in the Configuration Menu.

Special Commands

The autosampler has several built-in commands that let you manually manipulate some of the autosampler's components (the arm, injector valve, etc.) outside of a run. These commands, useful at times during maintenance and troubleshooting procedures, are accessible from the Commands Menu (Fig. C.21).

Flush Sample Syringe Flush Prep Syringe Initialize Hardware Injector Arm

Figure C.21 The Commands Menu

FLUSH SAMPLE SYRINGE

This command lets you manually flush the 250 µL sample syringe. You used this command when you primed your autosampler. It can also be useful for maintenance and troubleshooting. To access this test:

1. Fill your flush solvent bottle, if you have not already done so.
2. Press [MENU] and select /COMMANDS/, (Fig. C.21).
3. Select /Flush Sample Syringe/ to bring up the menu shown in Figure C.22.
4. Use the [+] and [-] keys to select the desired flush volume in microliters. Then press [ENTER] to initiate the flush operation.

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Flush 5000 µL

Figure C.22 Flush Syringe Menu

The autosampler automatically flushes the sample syringe and flush lines with the specified volume of flush solvent and returns the syringe to its starting position. During the flush sequence a confirmation message is displayed.

Flush Prep Syringe

If you purchased the Sample Preparation Option, you can also flush the large (2.5 mL) sample preparation (prep) syringe and external solvent lines manually as follows:

1. Fill each solvent bottle with an appropriate solvent.
2. Press [MENU] and select /COMMANDS/.
3. Select Flush Prep Syringe (Fig. C.23).
4. Use the [+] and [-] keys to select the desired flush volume in microliters. Then move the cursor to the Solv field and select the desired solvent (S-1, S-4, Bt.). Press [ENTER] to initiate the flush sequence.

Flush 5000 µL Solv Btl

Figure C.23 Flush Prep Menu

The autosampler automatically flushes the selected solvent line with the specified volume of flush solvent and then returns the prep syringe to its starting position.

INITIALIZE HARDWARE

The Initialize Hardware command allows you to manually control the autosampler's syringes and injector valves. This command brings the syringe(s) to the initial position(s) and removes any backlash.

INJECTOR

The Injector commands allows you to manually switch the injector valve between the inject and fill positions. Selecting /Injector/ displays the menu shown in Figure C.24.

Rotate Injection Valve INJECT

Figure C.22 Flush Syringe Menu

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ARM

Pressing the [+] or [-] key and then pressing [ENTER] changes the injector valve's position.

The Arm Menu contains two routines for returning the XYZ arm to its home position and for aligning the arm. Figure C.25 shows the Arm Menu. The two routines are described below.

Home Arm Align Arm Clear Mixer

Figure C.25 The Arm Menu

Home Arm

Pressing [ENTER] with the cursor in this field moves the arm from its present position to its home location to the left of the rear-left corner of the tub (Fig. C.26).

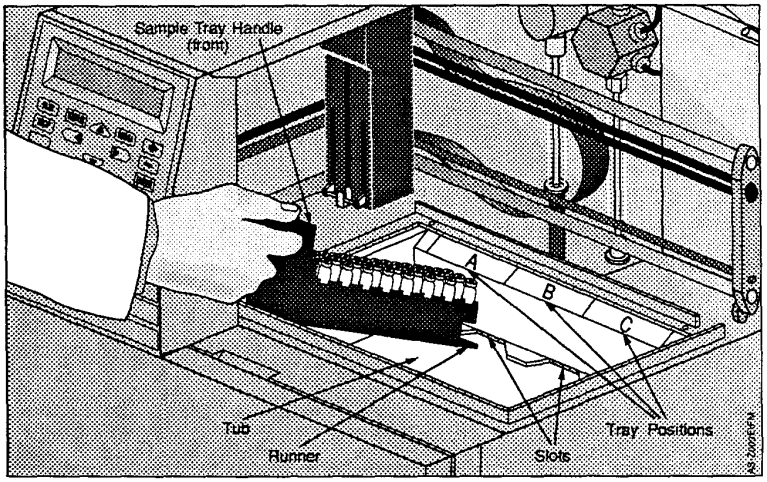


Figure C.26 The arm in home position

Align Arm

This routine moves the arm to the vial position requested (Fig. C.27). It is used primarily by Thermo Separation Products service engineers to properly adjust the arm's x-y-z reference points. If you suspect that the arm's alignment is incorrect, call your Thermo Separation Products Service representative.

Align Arm	A01

Figure C.27 Align Arm Menu

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NOTE: The arm "senses" its position relative to its home position. Therefore, if you request the arm to go to position A01, and it goes to position A06, the system configuration may incorrectly indicate that the Tray Temperature Control Option is installed. Check the system configuration in the OPTIONS Configuration Menu against the installed options.

Clear Mixer

This routine is used to pull a vial from the mixer if a power failure occurs during a mix step in a sample preparation procedure. The arm picks up the vial from the mixer and returns to the home position. You must manually return to the vial to its proper location in the sample tray.

NOTE: This routine appears on the Arm Menu only with the Sample Preparation Option installed.

Testing for Tubing Restrictions

If there is a restriction in the solvent lines, you will probably hear a loud grinding sound from the syringe-drive mechanism due to increased resistance to syringe travel.

NOTE: Before beginning this procedure, be sure to set up a beaker or other container to catch the solvent.

To isolate a block to a section of tubing:

1. Place the Autosampler injection valve into the FILL position (press [MENU], COMMANDS, Injector Test, and use the [+] and [-] keys to select /Fill/.
2. Disconnect the injection valve-to-flush valve tubing at the injection valve. Initiate a flush sequence (press [MENU], /COMMANDS/ , / Flush Sample Syringe/, page 179 or /Flush Prep Syringe/, page 180. Solvent will flow freely on the return stroke of the plunger if there are no restrictions present. Flow restriction is unlikely at this point due to the large internal tubing diameter.
3. Reconnect the tubing.
4. Disconnect the needle.
5. If no restriction is found, reattach all of the tubing, and flush the syringe to verify that the restriction still exists.
6. If a restriction is still present, remove and clean the sample needle as described in Chapter 5, page 90.

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D Advanced Installation and Features

Introduction

This section contains advanced installation procedures as wen as instructions for using some of the advanced autosampler functions. The installation procedures covered in this chapter are:

- System Communication
- Communication Modes
- Setting the Polarity
- Ready Participation

The advanced functions included in this chapter are:

- Instrument Configurations and Other Features
- Gradient Delay
- Needle Height
- Optimization (injection volume and solvent viscosity)

We recommend that you familiarize yourself with Chapters 2 and 3 before trying the procedures in this appendix.

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System Communication

Your autosampler can operate as a separate unit or as part of an automated LC system. The 12-pin, in-line connector on the instrument's rear panel allows communications between your autosampler and other components in an LC system (Fig. D. 1). This simplistic communications system, known as "hardwiring," provides injection synchronization between your autosampler and the other components of your LC system.

A mating connector (provided in the accessory kit) allows you to attach control wires from other LC devices to the 12-pin terminal by loosening the appropriate screw, inserting the wire, and then tightening the screw. Once all connections are made, you can quickly make and break connections without disturbing individual wires.

Hardwire communications allow the autosampler to start and stop a pump or detector, to begin a gradient program (gradient pumps only), or to control other features of the externally connected instruments. In addition, the terminal has four timed-function outputs that can be used to control valve-switching devices or other devices on a timed basis. (Refer to Chapter 3, "Timed Events Program," page 26 to set up a timed-events program.)

This section describes the pin assignments, the recommended wiring configuration, and some optional wiring configurations.

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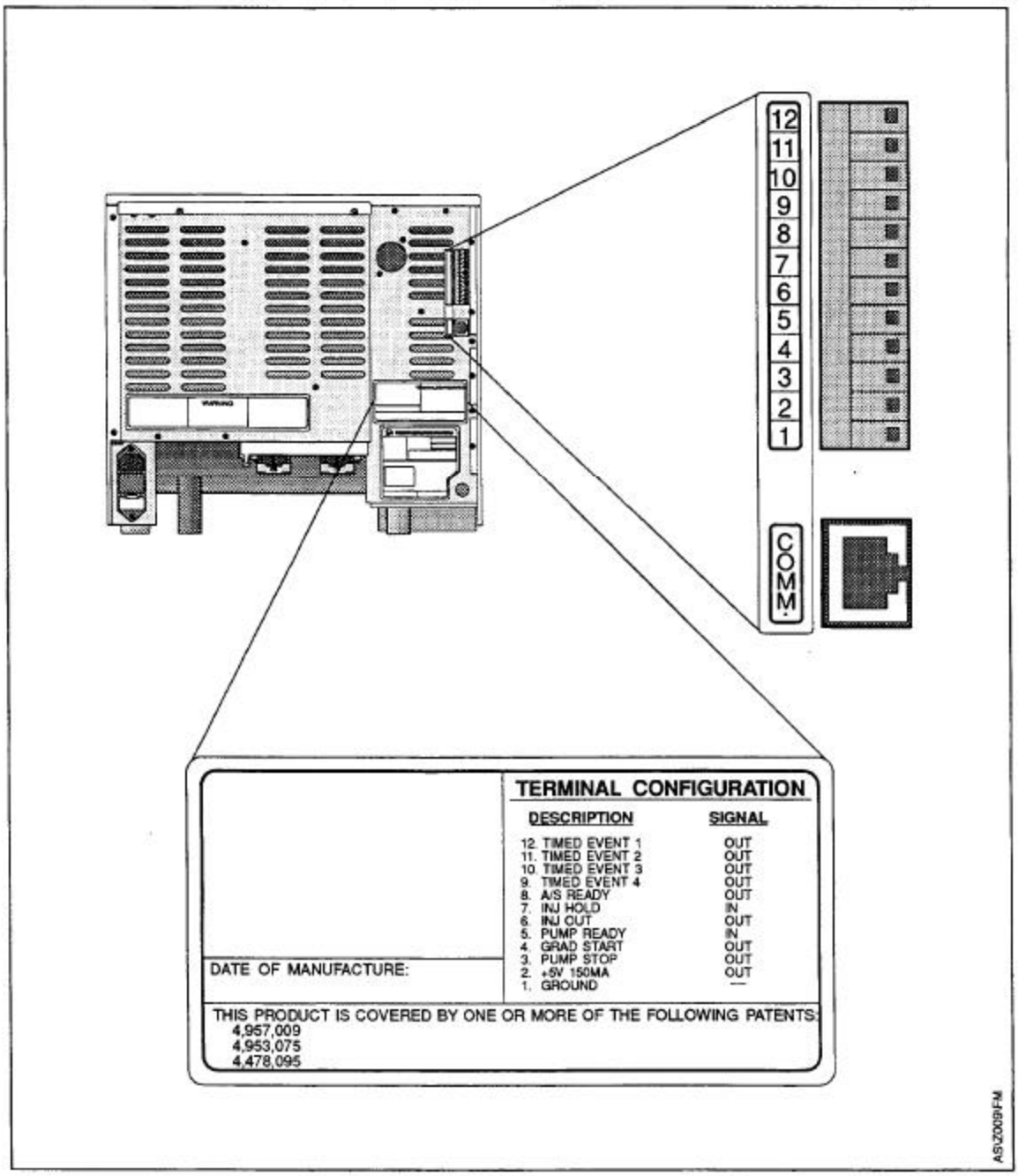


Figure D.1 The 12-pin terminal

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PIN ASSIGNMENTS



NOTE: All inputs and outputs are referenced to ground.

Pin 1 - GROUND

This is the ground reference used for all inputs and outputs.

Pin 2 - +5VDC

This regulated +5 VDC supply can be used to power pull-up resistors or external relays. The current is limited to a maximum of 300 mL to protect against external short circuits affecting the autosampler's operation.

Pin 3 - PUMP STOP (output)

This pulsed output is used to stop the pump after the last sample has been processed. It is activated when the last sample in the queue has completed its last injection, or when the shutdown file has been activated (using the special shutdown command). See Chapter 5 for more information on the Shutdown command.

Pin 4 - GRADIENT START OUTPUT

This signal activates the pump's gradient program. It occurs at a specified amount of time before the Inject Out line is activated. The time is specified as a Gradient Delay value in the More Menu (under Files). This momentarily active, pre-injection signal permits the gradient to start prior to sample injection to compensate for system delay volume. The result is an injection that occurs just as the gradient front reaches the injection valve. See the last section in this appendix for more information on the gradient delay function.

Pin 5 - PUMP READY (input)

Through this input, the pump signals the autosampler that the mobile-phase flowrate is stable and that the pump is ready for an analysis to begin. (Other devices may also be connected here.) This input must be continuously active for the autosampler to proceed with injections. From the Input Polarity Menu you can select whether a high or low level on this line means "ready." The default is Hi.

Pin 6 - INJECT OUT (output)

This line sends a momentary signal each time the autosampler makes an injection. This signal allows the synchronization of external devices (pumps, detectors, integrators, etc.) to the autosampler s injection. See "Outputs" below for the specifications of this output.

Pin 7 - INJECT HOLD (input)

This signal puts the autosampler in the hold state with sample in the loop. Releasing the hold will cause an immediate injection. The signal must remain continuously active to hold injections. That is, when active, this signal will delay an imminent injection until this line goes to the inactive state. This function can be used with external equipment to precisely control the timing of the injection valve activation. The active level (Hi or Lo) of this input can be selected in the Input Polarity Menu (see Advanced Installation) at the end of this appendix.

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Pin 8 - A/S READY

This line is used to signal to other devices that the autosampler is in a READY condition. See "Outputs" below for the specifications of this output.

Pins 9 thru 12 - TH - TF4 (timed outputs)

These user-programmed outputs are useful for triggering column switching valves or other external hardware. These outputs are controlled by the time functions defined in the currently active file (see Chapter 3 "Timed Events" page 26).

OUTPUTS

All output signals, including Time Functions 1 through 4 (TFl-4), are open-collector outputs capable of sinking (handling) currents up to 600 mL at voltages up to 24 VDC. The active state is user programmable in the Timed Events Menu (see Chapter 3).

INPUTS

All inputs accept TTL-level inputs, with a transition activating each. The two inputs are tied to +5 VDC through pull-up resistors. A "Lo" level is from 0 to 0.8V; a "Hi" level is from 2 to 5.5V.

RECOMMENDED CONFIGURATION

The recommended configuration requires a pump, autosampler, UVIVIS or fluorescence detector, and an integrator. We recommend that you connect the following signals:

- Pump ready
- Inject hold (detector ready)
- Detector run/start
- Integrator run/start

Use Table D. I to wire the appropriate pin connections, Pin connections for the SpectraSYSTEM modules are indicated in parentheses. Optional connections are indicated by asterisks (*).

Table D.1 Pin Connections for the Recommended Configuration

Autosampler (Pin Connection)	Pump	Detector	Integrator
Ground (1)	Ground (3)	Ground (9) (11)	Ground (B1)
*Pump stop (3) (pulse output)	Pump Stop (5)	N/A	N/A
Pump ready (5) (input)	Pump ready (1)	READY (4)	N/A
Inject out (6)	N/A	RUN/START (10)	RUN/START “A” (B5) RUN/START “B” (B14)
*Inject hold (7)	Sync (8)	N/A	N/A
Gradient start (4)	Run grad (7)	N/A	N/A

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Figure D.2 illustrates the hardwire connections for the SpectraSYSTEM modules.

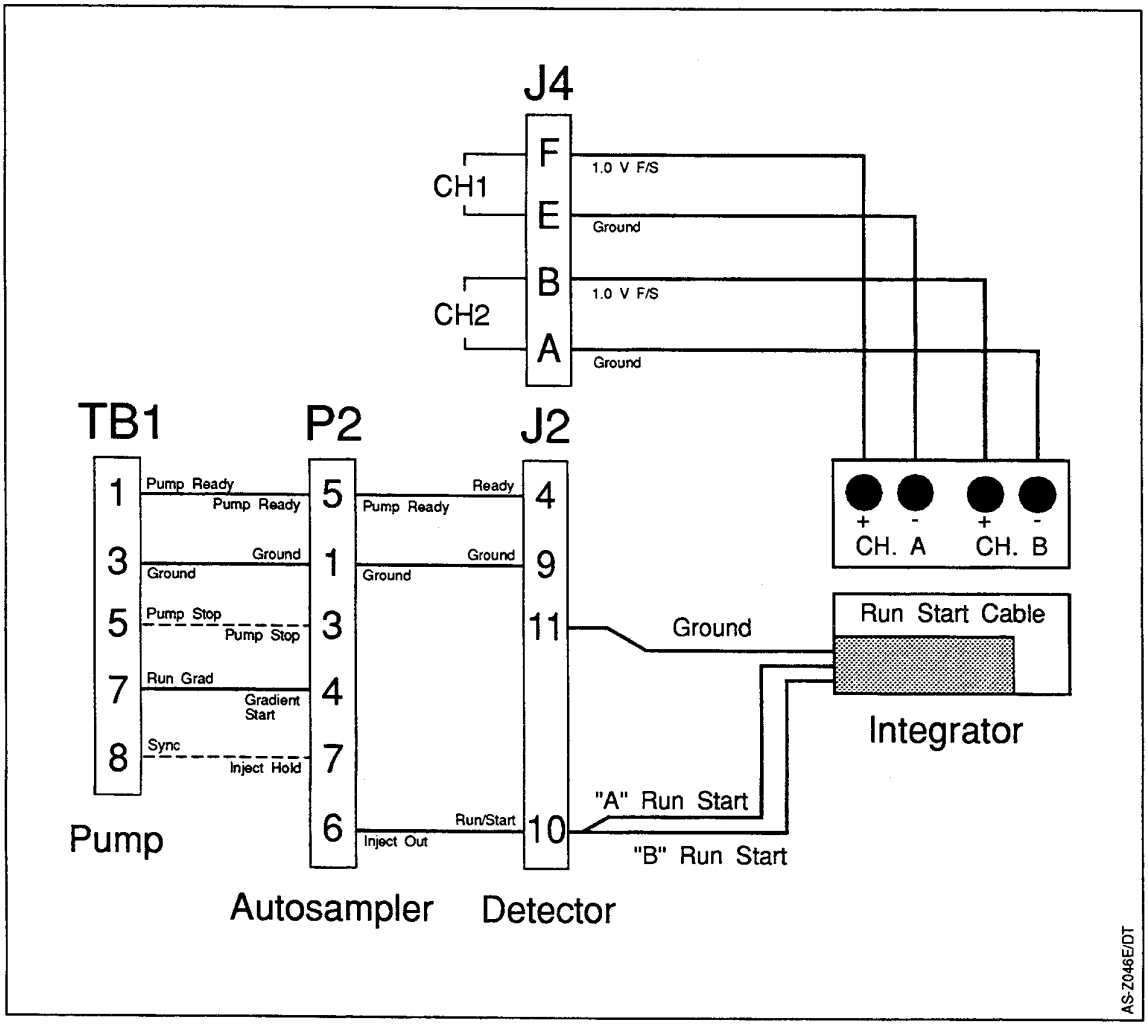


Figure D.2 Hardwire connections for SpectraSYSTEM Modules

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Communication Modes

The SpectraSYSTEM autosamplers can be configured to send information to a system interface that communicates with the PC1000, ISM100, or other SpectraSYSTEM modules. To achieve these data communications options, two autosampler configurations are available:

- SpectraNET mode as a part of a **SpectraSYSTEM** chromatograph connected to other **SpectraSYSTEM** modules through the SN4000, and controlled by PC 1000 (PC-based system) or the Intelligent System Manager (ISM)
- Stand-alone mode connected to other non-SpectraSYSTEM BPLC modules through the remote input/output connectors.

SPECTRANET MODE

This section describes each of these configurations.

Configured as a SpectraSYSTEM module using SpectraNET communication and the SN4000, your autosampler is automatically controlled by PC 1000 or the Intelligent System Manager (ISM 100). The PC 1000 or ISM assumes almost all keyboard control and coordinates the majority of the autosampler's operations. To configure your autosampler to use SpectraNET communication:

I . Turn off your autosampler.

2. Connect your autosampler to the SpectraNET Interface Mc as described in your PC 1000 or ISM 100 reference manuals.

3. Turn on your autosampler, wait for the powerup sequence to complete, and then press /OPTIONS/Configurations/ to access the Configurations Menu (Figure D.3).

Sample Syringe	250	
Prep Installed	Yes	(With sample prep)
Oven Installed	Yes	
TrayTemp Installed	Yes	
Key Repeat Rate	Medium	
Door Interlock	On	
File Name	Protect	
1: VITAMINS	On	
2: BARBITUA	Off	
3: EXPER 3	Off	
4: STAT	Off	
Mode	**SpectraNet**	
Solv Viscosity	Normal	

Figure D.3 The Configurations Menu in SpectraNET mode

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***NOTE**: If you are running the RAU Hardware Test with "No System Control" selected in the Mode Menu (38), the Controller (ISM100) will cause the autosampler to reinitialize the hardware.*

4. Move the cursor to the Mode field, and use the [+] and [-] keys to select SpectraNET. Then press [ENTER] to accept the field value and exit the Configurations Menu.

Your autosampler is now configured as a SpectraSYSTEM module.

STAND ALONE MODE

Stand alone mode is used to **operate SpectraSYSTEM modules** without **ISM** or PCIOOO control. Stand alone mode is also used to connect older and non-Thermo Separation Products laboratory equipment. In this mode, two specialized communication setups are available:

- Via an autosampler-to-integrator cable to ChromJet or DataJet integrators
- Via BCD interface to older Thermo Separation Products integrators or to non-Thenno Separation Products Integrators

Before using either of these communications setups, it is necessary to configure your autosampler as a stand-alone module as follows:

1. Turn on your autosampler, wait for the powerup sequence to complete, and then press /OPTIONS/Configurations/ to access the Configurations Menu (Fig. DA).

Sample Syringe	250
Prep Installed	Yes
Oven Installed	Yes
TrayTemp Installed	Yes
Key Repeat Rate	Medium
Door Interlock	On
File Name	Protect
1: VITAMINS	On
2: BARBITUA	Off
3: EXPER 3	Off
4: STAT	Off
Mode	**Stand Alone**
Solv Viscosity	Normal

Figure D.4 Configurations Menu in stand-alone mode

2. Move the cursor to the Mode field, and use the [+1 and [-] keys to select Stand Alone. Then press [ENTER] to accept the field value and exit the Configurations Menu.

Your autosampler is now configured as a stand-alone LC module.

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Normal Operation

For normal stand-alone operation, you do not need to change any of the values in the Communications Menu. Leave the communications parameters set to the default values (Fig. D.5). Details are provided for information only.

Baud Rate	9600
Parity	NONE
Flow Control	NONE
Data Bits	8
Stop Bits	1
Echo	On

Figure D.5 The Communications Menu default values used for normal stand-alone operation.

The allowable entries and default values are listed below for each of the communications parameters.

Baud Rate: Allowable entries: 1200, 2400, 4800, or 9600 Bd; default: 960013d.

Parity: Allowable entries: None, Even, or Odd; default: NONE.

Flow Control: Allowable entries: NONE or XON/OFF; default: NONE.

Data Bits: Allowable entries: 8 or 7; default: 8.

Stop Bits: Allowable entries: I or 2; default: 1.

Echo: Allowable entries: On or Off, default: On.



***NOTE**: If the Communications Menu doesn't appear as one of the OPTIONS Menu choices, return to the OPTIONS Configurations Menu, and verify that Mode is set to Stand Alone. The Communications field will not appear in the OPTIONS Menu if Mode is set to SpectraNet.*

Autosampler-Integrator Cable

With this option, you can set up your autosampler to send vial information to a Thermo Separation Products ChrornJet (SP4400) or a DataJet (SP4600) integrator. For this connection you will need an RJ-45-to-RJ-45 cable and an integrator adapter (p/h A3981-010).

To make this connection:

1. Turn off your integrator.
2. Insert the RJ-45 connector (telephone connector) into the COMM. Port (telephone jack) on the autosampler's rear panel.
3. Connect the adapter to the other end of the cable. It orients in only one direction.
4. Plug the adapter-cable end into your DataJet or ChrornJet integrator.

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5. Turn on your integrator and type in the following:
   BAUD 9600
   POKE#Cl2D,#FF

6. Hold the [CrL] key and press [CALIBI.

CAUTION! You must reenter this sequence after a= soft reset!

This sequence provides you with the four-line report from your autosampler shown in Figure D.6.

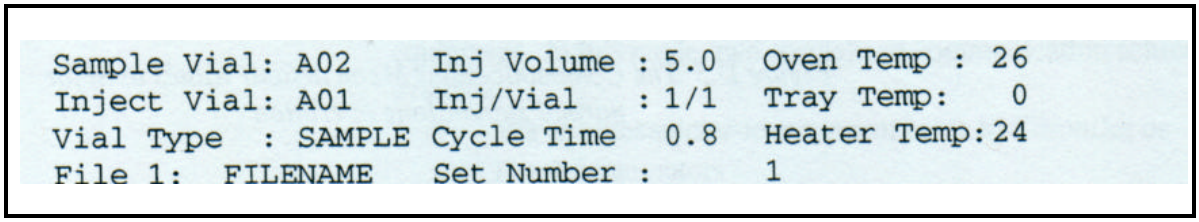


Figure D.6 The vial location and injection report

Once you have completed your hardwiring connections, return to the Basic Installation Procedures section (page 110) and complete the front-panel autosampler installation procedures, if you have not already done so.

BCD Communication

The BCD Communication Option provides BCD communication between the AS1000/AS3000 SpectraSYSTEM autosamplers and older Thermo Separation Products and non-Thermo Separation Products integrators and computers. This option provides tray- and/or vial-location information to devices requiring either binary or binary-coded decimal data. The BCD assembly must be installed by a Thermo Separation Products service representative if ordered after initial instrument delivery. Installation instructions are provided in the BCD Option Kit. Contact your representative for further information.

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Setting the Polarity

From the Options Menu you can change the sense of your autosampler's input and output signals as required by your other LC system modules. For example, when shipped, the Autosampler Ready Output is set to send a positive voltage (Hi) when the instrument is ready. If you change the Autosampler Ready Output Polarity to Lo, the instrument will send a 0-volt signal (Lo) when it's ready. The procedure for changing the polarity of the input and output signals is described below.

INPUT POLARITY

To change the polarity of an input signal, select /Input Polarity/ from the Options Menu. Use the [+] and [-] keys to select either Hi or Lo for the desired input signal shown in Figure D.7.

Pump Ready Active	Hi
Inj Hold Active	Lo

Figure D.7 The Input Polarity Menu

This menu shows the level for the active state for each input signal.



NOTE: If you want to determine the current state of a particular signal, return to the Main Menu, and select /TESTS/ Display Input States/.

OUTPUT POLARITY

To change the polarity of an output signal, select /Output Polarity/ from the Options Menu. Use the [+] and [-] keys to select either Hi or Lo for the desired output signal shown in Figure D.8.

Autosampler Ready	Lo
Inject Out Active	Lo
Grad Start Active	Lo
Pump Stop Active	Lo

Figure D.8 The Output Polarity Menu

This menu shows the level for the active state for each output signal.



***NOTE**: If you want to determine the current state of a particular signal, return to the Main Menu, and select /TESTS/ Display Output States/.*

Tables D.2 and D.3 show the output signal levels relative to the polarity settings and instrument status for the autosampler's input and output signals, respectively.

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Table D.2 Input Levels of Autosampler Input Signals			
		--------Polarity--------	
		Hi	Lo
Pump Ready	Ready Not Ready	Hi* Lo	Lo Hi
Inj Hold	Active Inactive	Hi Lo	Lo* Hi
Note: The asterisked (*) values indicate default settings.			

Table D.3 Output Levels of Autosampler Output Signals			
		--------Polarity--------	
		Hi	Lo
Autosampler Ready	Ready Not Ready	Hi* Lo	Lo Hi
Inject Out	Active Inactive	Hi Lo	Lo* Hi
Gradient Start	Active Inactive	Hi Lo	Lo Hi*
Pump Stop	Active Inactive	Hi Lo	Lo Hi*
Note: The asterisked (*) values indicate default settings.			

Ready Participation

When the instrument is shipped, the following conditions must be satisfied in order for the Status Screen to indicate that the autosampler is “ready” for operation:

- The door must be closed.
- The oven must be at the set temperature (if installed).
- The heater must be at the set temperature (with the Sample Preparation Option only).
- The tray must be at the set temperature (the the Tray Temperature Control Option installed).

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The Options Ready Participation Menu allows you to customize the "autosampler ready" requirements to fit your own needs. For example, you may wish to prepare your first sample set (without injection) while the column oven is still warming up. In this case, you would want to disable the column oven condition in order to complete the sample preparation run. Or, you may wish to run your autosampler with the door removed. In this case you would want to disable the door interlock.

To access the Ready Participation Menu (Fig. D.9), select /OPTIONS/Ready Participation/.

Door Interlock	ENABLE
Col Oven Ready	ENABLE
Heater Ready	ENABLE

Figure D.9 The Ready Participation Menu

To change any of the above requirements, move the cursor to the appropriate line, and use the [+] and [-] keys to change ENABLE to DISABLE.

Instrument Configuration and Other Features

Your autosampler is shipped with a 100 µL sample loop (the AS1000 series instruments come with a 20 µL loop), a 250 µL sample syringe, and a 2.5 mL sample preparation syringe (with the Sample Preparation Option installed). Should you wish to change your syringe sizes, you can do so from the Configuration Menu (Fig. D.10). This menu also reflects your other installed options (oven and tray temperature control). If you would like to adjust the rate at which fields cycle through the available choices, you can change the Key Repeat Rate entry. Chapter 3 discusses the File Protect function also available from this menu.

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Sample Syringe Size	250
Prep Syringe Size	2500
Prep Installed	Yes
Oven Installed	Yes
TrayTemp Installed	Yes
Key Repeat Rate	Medium
File	Protect
1:	Off
2:	Off
3:	Off
4:	Off
Solv Viscosity	Normal

Figure D.10 The Configuration Menu

DISPLAY CONTRAST

You can vary the display's contrast to make it easier to read.

To change the display's contrast, first press [STATUS] to access the Status Screen. Then simultaneously press [>] and [+] to *increase* the contrast, or [>] and [-] to *reduce* the contrast.

Gradient Delay

Gradient delay is a user-defined value that delays the injection of a sample. The delay is the amount of time required for a change in the mobile phase to travel from the gradient proportioning valve to the injection valve. The volume of mobile phase contained between the two valves is also referred to as the precolumn volume (PV).

Although not required for normal autosampler operation, a gradient delay is recommended when using large volume pre-filters or low flow rates (<0.5mL/min). The gradient delay time can be found under the FILE/Edit/More Menu. You then use the [+] or [-] keys to enter the desired time in minutes (Fig. D.11).

The following procedure is used to calculate the gradient delay time for your HPLC System. (See Figure D.12.)

1. Inject an unretained sample into your LC system with a column in-line and using 100% A as the mobile phase (e.g., MeOH).
2. Change to 100% B (e.g., 0.1% Acetone in MeOH) as fast as possible (0.1 minutes for Thermo Separation Products pumps).
3. Subtract the retention time (R_t) of the unretained peak from the retention time of the start of the baseline shift (T_2-T_1).
4. Subtract the tmie required to switch mobile phases from 100% A to 100% B.
5. The resulting time is the gradient delay time.

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EXAMPLE

The following example illustrates how to apply this procedure.

An unretained peak has an Rt of 3.0 min. with a baseline shift at 4.5 min. and a complete mobile-phase switch time of 0. 1 min. The gradient delay is calculated as follows:

Gradient Delay = (T2-TI) - 0. 1 min = (4.5 - 3.0) - 0.1 = 1.4 minutes

Equilibration Time	0.0
Gradient Delay	**0.00**
Viscosity	Medium
Flush Volume	250.0
Injection Type	Push
Injection Range	1-1500
Needle Height	2.0

Figure D.11 The More Menu (Gradient Delay)

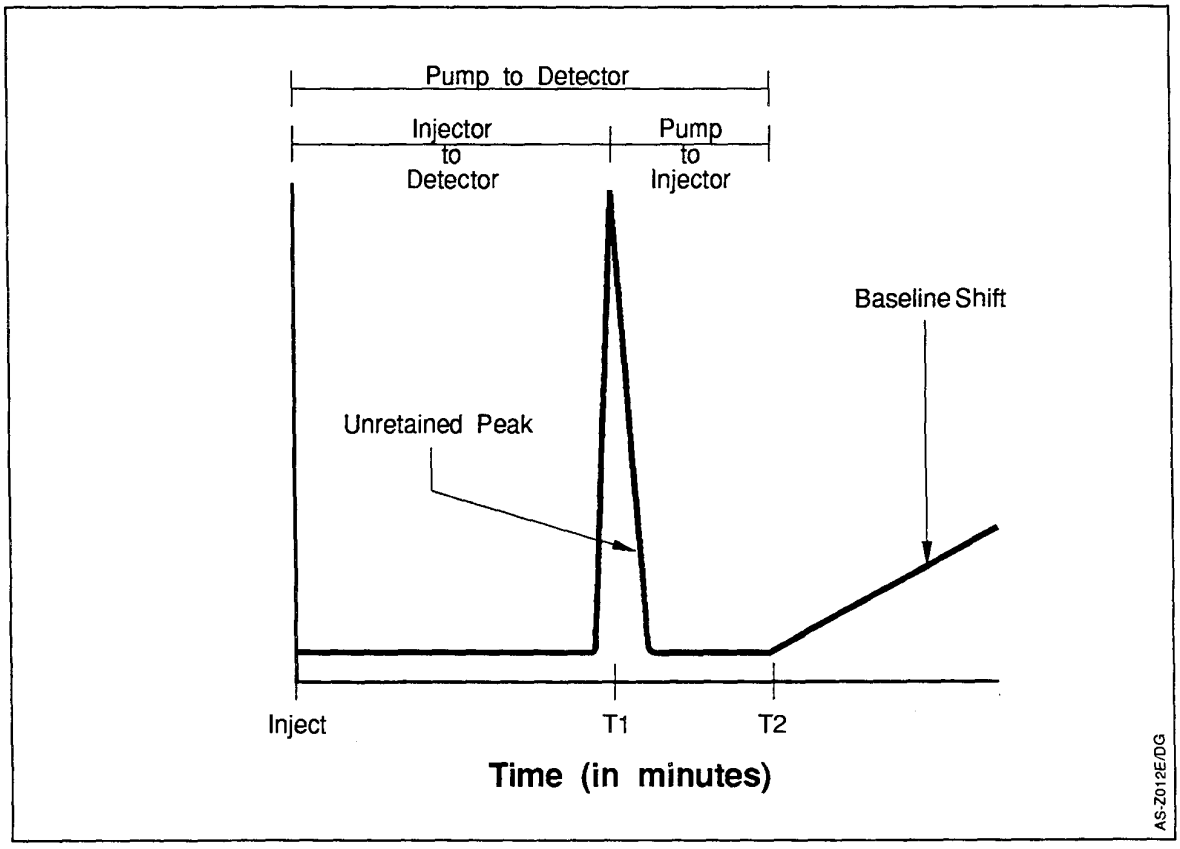


Figure D.12 The delay time calculation using a hypothetical chromatogram

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Needle Height

For some applications, such as liquid-liquid extractions and low-injection volumes, you may need to change or optimize the "needle height." On SpectraSYSTEM autosamplers, the needle doesn't move. Instead, the bottom of the *vial* moves relative to the needle tip. The "needle height" is the approximate distance between the needle tip and the bottom of the vial (0 – 20 mm).

The More Menu's default setting of 2 mm (Fig. D.13) is sufficient for most applications and accommodates a wide variety of vials. However, in cases where you have very limited sample volumes, further optimization may be required.

NOTE: The thickness of the bottom of the vial varies considerably between vial types (1.8 mL vials, microvials, and vials with mocro-inserts, etc.) and materials (glass, plastic, etc.).

Equilibration Time	0.0
Gradient Delay	0.00
Viscosity	Medium
Flush Volume	250.0
Injection Type	Push
Injection Range	1-1500
Needle Height	**2.0**

Figure D.13 The More Menu (Needle Height)

CAUTION! Operating the autosampler with a needle height different from the default height may bend the needle. Do not adjust the needle height without a spare needle available.

Use the following procedure to optimize the needle height for your sample vials:

1. Place 200 – 300 µL water in a vial in position A01.

2. Create an autosampler file that will make two 200 µL injections (see Chapter 3 for more information).
   a. Set the Injection Menu's Injection Volume field equal to 200.
   b. Set the Injection Menu's Injection/Sample to 2.

3. Run the file and observe the amount of fluid remaining in the vial. The fluid height indicates the position of the needle tip from the vial's bottom.

4. Adjust the More Menu's Needle Height value until the needle tip is located approximately 1 – 2 mm from the bottom of the vial.

5. Repeat steps 1 – 4 to confirm the needle height.

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Optimization

Injection Volume

This section describes how to select the sample loop and syringe size to obtain maximum reproducibility when performing large-volume injections.

The maximum injection volume depends upon:

- The volume of the syringe currently installed as designated under /OPTION/Configuration/Syringe Size/;
- The Injection Mode selected under /FILE/More/Injection Type; and
- The volume of the installed sample loop.

By changing the sample syringe and/or the sample loop, you can set up your autosampler to deliver injection volumes larger than can be delivered by the 250 µL syringe shipped with the instrument. Use the following steps to prepare your instrument for large-volume injections:

1. Determine the correct sample loop size for the injection volume desired. (See Table D.4.)
2. Use the injection volume and algorithms outlined below to determine the required syringe size. (See Table D.5.)

   The following algorithms define the amount of sample needed for your injection volume:

Push Loop	(Injection Volume + 15 µL)
Pull Loop	(Injection Volume + 1.1 µL)
Full Loop	(Injection Volume X 1.33) + 70 µL

3. Change your sample loop and syringe. (Refer to Chapter 5 for instructions.)
4. Enter the syringe size in the /FILE/Configuration/Syringe Size field.
5. Enter the Injection Mode in the /OPTIONS/More/Injection Type/field.
6. Enter your injection volume in the Injection Menu.



NOTE: If the Full-loop mode is chosen, the maximum value depends only on Loop Size (assuming that the syringe size is larger than the loop size). (Loops come only in the sizes included in the Parts and Accessories list located at the front of your Reference Manual).



NOTE: For Pull and PushLoop injection, to ensure maximum reproducibility, we recommend that you do not inject more than half of your loop volume.

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Table D.4 Recommended Injection Size for Selected Sample Loop Sizes*				
		Injection Volume (mL)		
		Injection Mode		
Sample Loop Size (mL)		Push Loop	Pull	Full
5		2.5	2.5	5
10		5.0	5	10
20		10	10	20
50		25	25	50
100		50	50	100
200		100	100	200
500		200	250	500
1000		200	500	1000

- These recommended volumes will provide the best chromatographic performance. Maximum injection sizes will be determined by the particular system being used.

The syringe size needed will be the total volume determined by these algorithms. For example, for a 500 µL full-loop injection (using a 1000 µL loop), the Total Volume (TV) needed is

TV = (500 µL x 1.33) + 70 µL
TV = 735 µL

Therefore you will need a 1000 µL syringe since there are no 735 µL syringes available. (See Table D.5.)

Table D.5 Maximum Injection Size for Syringe Size					
	Syringe Size (in mL)				
Injection Type	250	500	1000	2500	5000
Push Hi	200	200	200	200	200
Pull	212	461	956	1500	1500
Full	100	200	500	1000	1000

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SOLVENT VISCOSITY

You can adjust the syringe speed (draw rate) for your solvent viscosity from the Solv Viscosity field in the Configuration Menu (Fig. D.14).

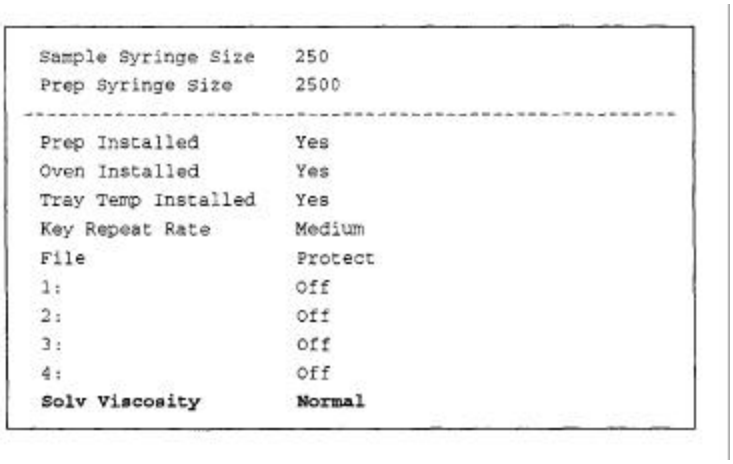


Figure D.14 Configurations Menu -- Solv Viscosity field

Using the [+]/[-] keys you can choose among three different syringe speeds: 1) Normal (fastest), 2) Medium, and 3) Viscous (slowest). "Normal" lets you optimize your cycle time and is optimal for most applications. With either very viscous solvents or normal-phase (low viscosity) solvents, you may wish to use the "Viscous" setting to slow down the syringe rate to prevent cavitation and unreproducible injection volumes.

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E Glossary

Introduction

We have included a glossary to define certain technical terms used throughout the manual's text. These terms should be consistent with standard definitions used throughout the analytical industry, and are added here as a quick reference only.

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A

Active set/vial — The sample set or vial currently being processed. The active vial refers to the vial currently being injected or currently undergoing sample preparation.

Add — 1) A step used in sample preparation used to deliver all Pickup volumes and the Load volume to the target vial. 2) The process of putting a sample set into the sample queue.

B

Backlash — The slack in the syringe and its drive mechanism.

C

Calibration interval — The sample set or vial currently being processed. The active vial refers to the vial currently being injected or currently undergoing sample preparation.

D

Dead volume — The volume from the solvent-selection valve to the needle tip.

Decrement — Press the [-] key to decrease the value of a parameter.

Diluent — The solvent or buffer used to dilute a sample. On the autosampler, the volume of diluent is specified in the Load setup of all of the sample preparation templates.

Dilution cycle — A preset sequence used in the sample preparation templates that transfers solvents, sample, and reagents from the solvent

E

Extra vials — In sample preparation, an empty or extra reagent vial where samples and solvents are mixed; an empty reaction vial. It is always placed in relationship to the sample vial (Sam+1, Sam+2, Sam+3).

F

File/method — "File" and "method" are used interchangeably to describe the autosampler run parameters. However, the word "file" is often used in a more specific sense to mean the autosampler files in which the parameters are stored.

Flush solvent — The solvent used to wash the internal tubing, syringes, and sample needle after an injection. This solvent should be of sufficient strength to easily remove the small amounts of sample that can cling to the tubing walls.

Full-loop injection — A technique that injects a sample volume equal to the loop volume.

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G

Guard column — A small column placed just before the separation column. The guard column is used to remove contaminants that might otherwise damage the main column or interfere with the separation.

H

Holding loop — The isolated section of connecting line used in sample preparation to hold measured amounts of sample, reagents, and solvents until they are delivered into a vial.

Home — The position to the left of the rear-left comer of the sample tub where the XYZ arm resides when it is not transporting a vial.

Hook — The attachment on the end of the pod that moves a vial up and down (along the Z axis). The hook lifts the vial from the sample tray and holds it during transport to the sample tower for injection.

I

Increment — Press the [+] key to increase the value of a parameter.

Initialization — The process that takes place between dilution cycles that brings the autosampler to a "known" state. During this process the lines and syringes are automatically flushed with flush solvent. Any residual resources present from a previous dilution cycle are flushed out of the lines to prepare the autosampler for the next injection.

J – L

Linear dilution — A dilution sequence in which resource A (buffer) is added to resource B, C, and D.

Loading file — Loading a file copies the original edit file into the run-file location to be run. The original edit file remains.

M

Method — A group of parameters applied to one or more vials.

Mobile phase — In HPLC, the liquid used to move the sample through the column.

N – O

Open-collector outputs — An output, usually a transistor, that has no connection to the collector.

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P

PickLg — A step used in the sample preparation templates that retracts the prep syringe to draw a large volume of solvent or buffer into the holding loop.

Pickup — A step used in the sample preparation templates that retracts the sample syringe to draw a small volume of sample or reagent into the holding loop.

Prep syringe — The large 2.5 mL sample preparation syringe used in to transfer large volumes of liquid (usually buffer or solvents).

Pod — The autosampler component attached to the XYZ arm that contains the hook.

Precision — The degree to which results are reproducible (analysis of the same sample yields similar results).

Prime — The process that flushes lines with flush solvent, etc. Priming is done during installation and whenever a syringe is replaced.

PCB — A printed circuit board.

Pull-loop injection — Variable-loop injection.

Pull-up resistor — Resistors used to pull inputs or nonactive open-collector outputs to "Hi" states.

Q

Queue — One or more vial sequences/sets subjected to one or more methods run in a series that use(s) the same column and solvents. The sample queue lists the order and file name for each sample set to be processed during a given run.

R

Reagent transfer step — In a sample preparation program, a step used to deliver solvents, reagents, or samples from one location (vial or solvent bottle) to another (target or sample vial).

Real-time editing — The ability to edit the run file for a run currently in progress. On the SpectraSYSTEM autosamplers, this function is provided by the [SAMPLES] key; on other SpectraSYSTEM instruments, this function is accessed by pressing the (STATUS] key.

Recalibration — The reinjection of the calibration vial(s) after the injection of each series of sample vials within a sample set.

Recalibration interval — The number of sample vials injected between each calibration series injection. The value in the Samples/Calibration field.

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Resource One of the samples, calibration vials, reagents, or solvents used in an autosampler run.

Routine The type of order in which calibration vials are arranged within a sample set. Calibration vials may be reused throughout the set (Reuse same vials) or they may be interspersed (Intersperse) among the sample vials in the sample tray.

Run file The copy of the edit file that has been loaded and the file that is currently running. From the Samples Menu, changes can be made to the run file during a run, but those changes can't be saved until after the run has completed. Also, changes can't be copied to a file that is in use (assigned to a sample set in the queue).

S

Sample needle The needle that pierces the septum of the vial cap and through which vial contents are drawn into the holding loop.

Sample syringe The 250 µL syringe. In sample preparation, this syringe is used in sample preparation to transfer small volumes of liquid (usually samples or reagents).

Sample tower The autosampler location and component where injection takes place.

Sample tub The autosampler compartment that holds the sample trays.

Sample tray One of the three racks that holds the sample vials in position in the autosampler.

Sample valve The valve through which sample is injected onto the column.

Sample vial The container that holds the sample in the sample tray. Sample vials must have a 12 mm diameter.

Set A group of samples vials and associated calibration vials run with a particular method.

Solvent-selection valve The 6-port valve that regulates the flow of solvents and air into the sample holding loop. This valve allows the flow of waste out of the loop.

Solvent reservoir One of the four bottles on the back of the autosampler that contains the external solvents used in sample preparation. The solvent in the first position usually contains the buffer.

Standard A vial that contains a known concentration of sample. Peaks generated from this vial are used to plot the calibration curve used to determine the concentration of the compound of interest. (See also calibration vial.)

Syringe refill and loop-isolation valve The valve in the sample preparation system that regulates the flow of liquid from the syringes.

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T – V

Template

In sample preparation, one of the four file formats that contain a unique, preset, unchangeable sequence of steps used to carry out a series of sample preparation procedures. The four types of templates include simple dilution, heating, extraction, and heating and extraction.

W –X

Wait

A step used in the sample preparation templates that pauses all autosampler activity. This step may be used to allow a mixture to settle or to react.

X

In the Calibration Menu's Calib 1 (of X) field, X indicates the number of calibration levels in the calibration sequence. X is not visible on the screen; it is used for discussion purposes only.

XYZ arm

Also referred to as the "arm," this component carries the sample vials to and from the sample tray and to the various workstations (sample tower, heater mixer) in the autosampler.

Y – Z

Z

In the sample preparation templates, the variable used to represent the number of clean (extra) vials subsequent to the sample vial. Z equals 1, 2, or 3.

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