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Model 718AL Autosampler

Operators Manual

Software Version 2.0
July 99

Part Number 719-42801-01

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APPENDICES

Appendix A: The Proper Use of Tube Fittings
Appendix B: Spare Parts List and Accessories
Appendix C: Factors Which Affect Injection Precision and Accuracy
Appendix D: Alcott Chromatography Inc. Model 718 Checkout Procedure

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INSTALLATION

This section describes how to install and verify the operation of the Model 718AL Autosampler.

Using This Manual

This manual contains instructions for installing, operating and performing routine maintenance on the Model 718AL Autosampler.

This manual uses Grayed Text Boxes shown below to identify notes of importance, cautions, and warnings.

Notes of Importance, Cautions, and Warnings contain information to help you prevent actions which could cause personal injury.

The keypad and the use of the keys are discussed in many sections of this manual. The following convention is used in all keypad references. The face name of the key is enclosed in square brackets such as [ENTER] or [1] for the "Enter" or "1" keys, respectively. Some keys, such as the [7] key, have secondary functions much like some scientific calculators. The secondary function of [7] is [METHOD]. These secondary functions are accessed by first pressing the [FUNCTION] key and then the face key of the desired function. For example, to access the [METHOD] key, first press [FUNCTION], release it, and then press [7]. It is not necessary to hold [FUNCTION] in while pressing the [7] key. Secondary function keys are always referenced by the name of the secondary function, however, the key sequence is given in parenthesis, *e.g.* [METHOD] ([FUNCTION] [7]).

Unpacking

Visually inspect the Autosampler and its accessories to ensure that all items have been received and none has sustained physical damage. A packing list can be found in an envelope supplied with the accessories. The carrier and your local Alcott Chromatography representative, or the factory, should be notified immediately if shipping damage is found. **An inspection report must be obtained from the carrier for insurance purposes.**

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Selecting The Location

The Model 718AL Autosampler (Figure 1-1) consists of a single controller/sampler module. The Autosampler should be located as close as possible to the Liquid Chromatograph, especially the column, to minimize dead volume. Refrigerated units should be situated so as to allow adequate air flow through the instrument's ventilation holes.

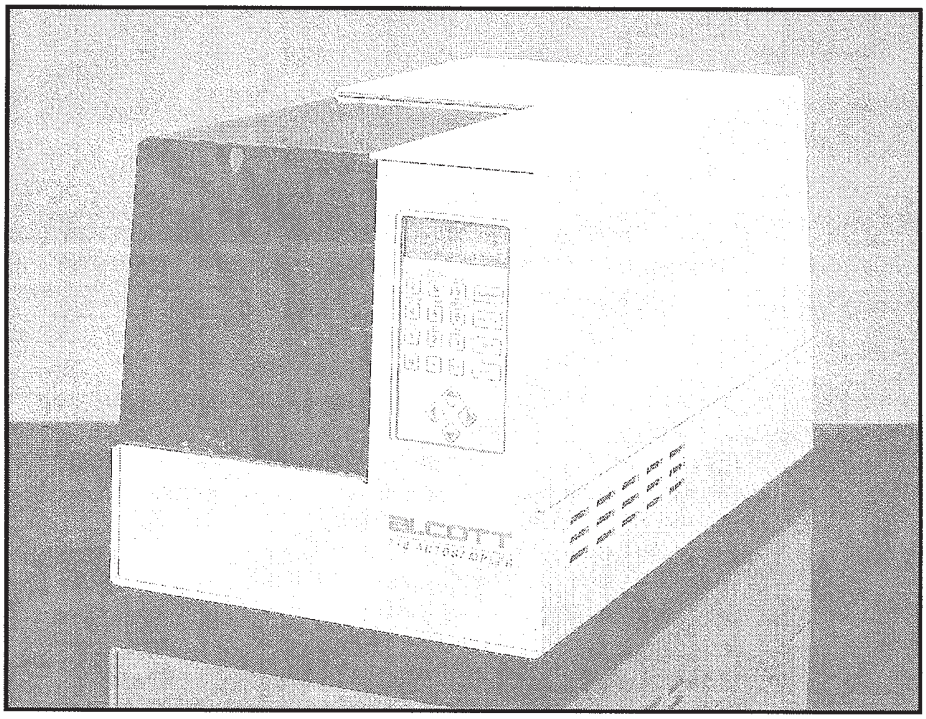


Figure 1-1: The Model 718AL Autosampler

Plumbing

The proper use of tube fittings is essential for optimum performance. APPENDIX A covers the cutting and swaging of stainless steel tubing. Improperly cut and swaged tubing can be a source of poor reproducibility and carryover. Note that all plumbing fittings used in the Model 718AL Autosampler are Valco brand. Although it is possible to use swaging screws and ferrules from other manufacturers, it is not recommended.

The Model 718AL incorporates a Valco six-port sampling valve, fitted with a special, Valco Loop Filler Port. Figure 1-2 shows the Model 718AL with a Valco Model C3 valve installed. The HPLC pump is connected to Port 2 while the column is attached to Port 3.

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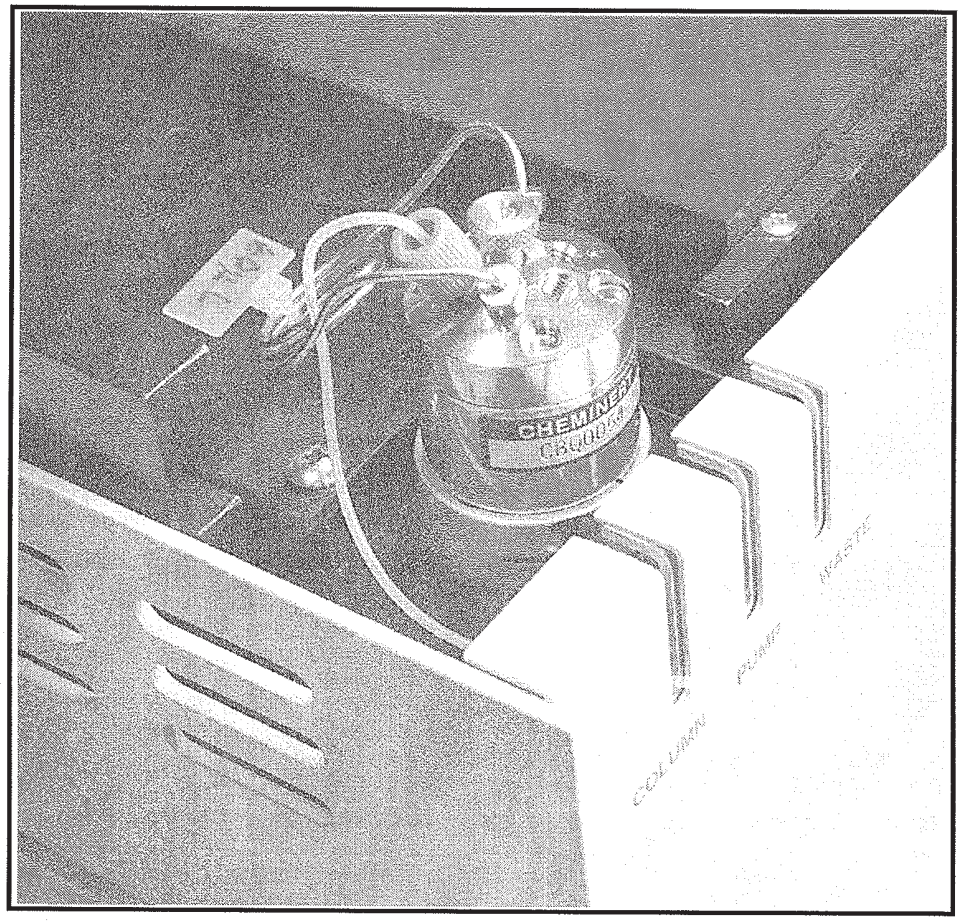


Figure 1-3 Autosampler with the Valco C3 Valve installed

Prime the syringe with Mobile Phase using one of the two methods described in **Chapter 5, Syringe Maintenance and Replacement**. The 250 and 2000 µL syringes usually require only automatic priming using one or two rinse strokes to render the tubing and syringe air free. The 50 µL syringe may require as many as twenty rinse strokes to remove air bubbles and manual priming may be necessary for complete air bubble removal.

Electrical Requirements

The Autosampler requires 100- 240 VAC ±10% at 50/60 Hz. The power entrance automatically senses the available voltage and frequency once it is plugged into the power mains and adjusts itself accordingly. The fuses used are two (2) × 3AG 2 Amp SLOW BLOW, 250 VAC for the regular unit or two (2) × 3 Amp SLOW BLOW, 250 VAC for the refrigerated Model for both 115 VAC or 230 VAC operation. The fuses are installed by first removing the power cord and then prying the fuse holder out with the aid of a flat edged screw driver. Insert the appropriate fuse

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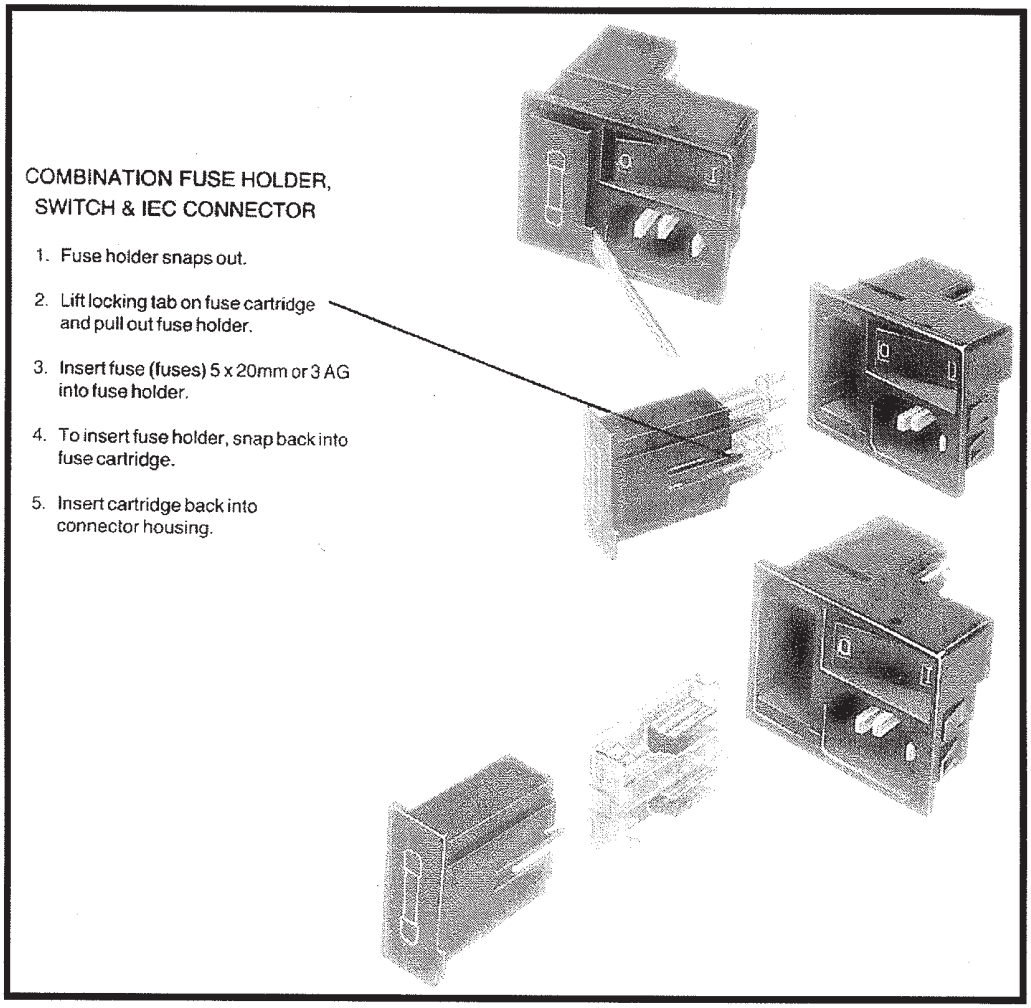
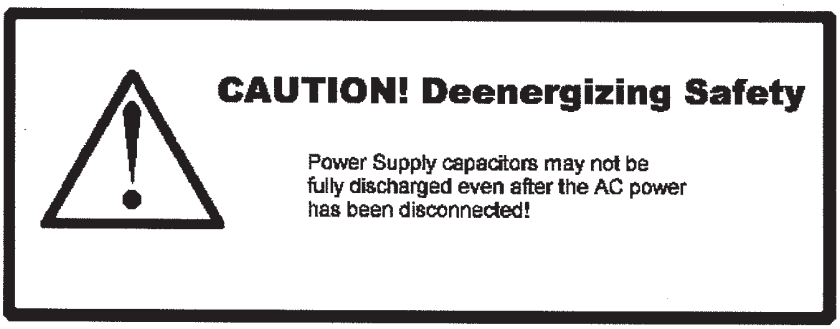


Figure 1-4: Fuse Installation

size, reinstall the fuse holder and reattach the power cord. Figure 1-4 illustrates the process.

NOTE: Use only the three-pronged Power Cord supplied with the instrument in the accessories for connecting the unit to the Main AC Power. This is to insure that proper electrical grounding has been made.

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Sample Tray Installation

The Model 718AL can be fitted with one of four sample tray sizes. The sizes are 128, 8 mm vials; 72, 12 mm vials; 50, 15 mm vials; or one, 96-well microplate. The 12 mm vial tray is standard size. Vial position number one should be in the left front of the instrument. Also note that there are two positioning pins located on the left side of the sample tray compartment. The tray is properly installed when these pins are inserted into the corresponding holes in the bottom of the sample tray. Once the tray is installed, the Autosampler must be configured for the new tray size. Tray size configuration is described under **The System Settings Key** in **Chapter 2, Theory of Operation**

Chapter 5, Routine Maintenance and Parts Replacement contains additional information about the sample tray and its options.

Remote Interface Options

The BCD, RS-232-C, and IEEE-488 (GPIB) interfaces are standard on every Model 718AL. It is recommended that basic operation of the Autosampler be verified, as described below, before attaching any external interface.

Initial Checkout

Initial checkout will prove the general operation of the instrument and show that no shipping damage or installation problems exists which would prevent normal operation. This procedure assumes all connections specified in the Installation section are made. An HPLC pump and column should be attached at this time.

The use of any suitable HPLC test mixture, mobile phase, and column combination is acceptable for the Initial Checkout. The suggested system is a mixture of alkyl parabens (methyl, ethyl, and propyl). These can be easily separated on a C18 column using a

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70/30 mixture of Methanol and Water. After the mobile phase is prepared, start solvent flow through the column. Place a vial filled with sample into vial position 1. Prime the syringe with Mobile Phase as described in **Chapter 5, Routine Maintenance and Parts Replacement**. A short Method will be entered to insure proper operation of the instrument. The Method will be programmed to make one, 10 µL injection from vial number one with a ten minute run time. The Autosampler's default Injection Parameters will be used.

Step 1: Shift the POWER switch on the Autosampler to the ON position. The following screen will be displayed after power up and initialization:

```
Ready

Select a Function
```

Step 2: From this screen, press the [METHOD] key ([FUNCTION] [7]). The following screen will appear:

```
   METHOD MENU
→Edit Method
 Run Method
 Copy Method        ↓
```

Step 3: After this screen appears, press the [ENTER] key. The screen will now change to:

```
Enter Method Number

          #  ■
```

Step 4: At this prompt, press the [1] key then press the [ENTER] key. This results in the following display:

```
METH  1 INJECT PARAM
→Inject Type    PART>
 Withdraw Rate    50
 Loop Fill Rate   50↓
```

Step 5: When this screen appears, press [FUNCTION] and then the [↑], Up Arrow key. This will accept the Autosampler's defaults. The Autosampler will then display:

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```
   EDIT METHOD # 1
→Edit Step
 Append Step
 Insert Step        ↓
```

Step 6: At this prompt, press the [ENTER] key. The display should now read:

```
METH  1 STEP  1 PART
→Start Vial        1
 Final Vial        1
 Standard Vial NONE↓
```

Step 7 Press the [↓] Down Arrow key five times. The Display will then read:

```
METH  1 STEP  1 PART
 Injections       1↑
 Inject. Vol.    10
→Run Time       1.0↓
```

Step 8 Press [1] [0] [.] [0] and then [ENTER]. The Display will show:

```
METH  1 STEP  1 PART
Inject. Vol.    10↑
 Run Time       10.0
→Event1 Time    0.0↓
```

Step 9 Press [FUNCTION] [CLEAR] which will return the Display to the Default Screen. Then press the [RUN/STOP] key. The Display will read:

```
Enter Method Number

        #  1
```

At this screen, press the [ENTER] once more and the Autosampler will make a 10 µl injection from the vial at position 1 with a ten minute Run Time. Observe the chromatogram and record the results. Refer to **Chapter 3, Operation**, section **Entering a Method** for a detailed explanation of the steps just performed.

Refer to **Chapter 4, In Case of Difficulty**, or contact Alcott Chromatography at (800) 229-5052, should any problems occur while entering these steps or while trying to execute this Method.

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Specifications

Characteristic	Specification
Operation	
Number of Samples	Four Vial tray sizes: 128, 8 mm Vials 72, 12 mm Vials (Standard Tray) 50, 15 mm Vials Standard 96-Well Microplates
Number of Injections per Vial	Essentially Unlimited
Minimum and Maximum Injection Size	1 µL and 2 mL
Syringe Sizes Available	50 µL, 250 µL, and 2000 µL (250 µL Standard)
Injection Precision	Less than ± 1.0 % Relative Standard Deviation , (RSD) Peak Area for 10 µL Partial Loop Fill Injections (typically better than ±0.5%.) (Using 250 µL syringe and loop under standard Alcott Chromatography QC Test conditions: Sample 0.005 mg/mL Propyl Paraben in 70/30 Methanol/Water, Sperisorb ODS II, 5 µm × 250 mm L × 4.6 mm ID column, 70/30 Methanol/Water Mobile Phase, UV detection at 250 nm.)
Minimum Carryover	Less than 0.01% with rinse. (Using Alcott Chromatography's standard test sample and conditions and pure mobile phase as the rinse solution.)
Programmable Needle Depth Range	1 to 48 mm from the Needle Home Position.

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Characteristic	Specification
Materials	
Valves and Plumbing:	316 Stainless Steel, Nitronic 60, Borosilicate Glass, PEEK, PTFE, and PTFE filled PEEK. Alternate materials available by request.
Vials and Caps:	Standard 8 mm, 12 mm, or 15 mm vials, 45 mm tall and shorter. Snap caps, screw caps or crimp top caps can be used. Low volume inserts can be used.
Exposed Materials:	Aluminum, Stainless Steel, Nitronic 60, Mylar, Polane Paint, Polypropylene and Delrin®
Displays, Indicators, and Controls	
Front Panel:	20-key keypad. Alphanumeric, 20 × 4 Character, Back Lit, Liquid Crystal Display.
Programming:	Storage for 10, 128 Step Methods.
Remote Control:	4 user selectable normally open or normally closed Contact Closures with user selectable closure duration time. External Injection Input BCD interface RS-232-C IEEE-488 I^2C Accessory Bus Connector Parallel printer interface.

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Characteristic	Specification
Cabinet	
Weight:	12.7 Kg (28 lb.) 14.5 Kg (32 lb. Refrigerated)
Dimensions:	33.0 cm H × 32.4 cm W × 55.3 cm D (13 inches H × 12.75 inches W × 21.75 inches D)
Color:	Dark gray, Light gray, light blue and black.
Refrigeration Option	
Range:	4 to 40°C (ΔT of 24°C)
Precision:	± 1°C
Accuracy:	± 1°C of Setting
Control:	Keyboard entry and front panel display of cold plate temperature.
Equilibrium:	Ambient to set point in 45 minutes.

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Characteristic	Specification
Power Requirements	
Voltage:	Autoselectable; 100/120/220/240 VAC ± 10%, 47-63 Hz
Fuse Sizes, two each:	Non refrigerated: 100-240VAC, 85VA, 50-60HZ (2A SLOW BLOW) Refrigerated: 100-240VAC, 195VA, 50-60Hz (3A SLOW BLOW)
Power Consumption Non-refrigerated Refrigerated	 80 watts, max. 200 watts, max.
Operating Environment (Temperature)	15°C to 30°C

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GENERAL DESCRIPTION

Theory of Operation

The Model 718AL Autosampler is a microcomputer-controlled, automatic sampling and injection device, designed for use with all commercially available High Performance Liquid Chromatographs (HPLCs), Supercritical Fluid Chromatographs (SFCs) and Ion Chromatographs (ICs). The unit essentially performs an unlimited number of partial loop or fixed loop injections per vial. Sample trays are available to contain either 128, 8 mm vials: 72, 12 mm vials; or 50, 15 mm vials and 96-well microplates. The 12 mm vial tray is standard In addition, the Autosampler has four permanent, 12 mm vial positions, separate from the Sample tray, which can be used for standards or reagents. The Autosampler can be interfaced to an assortment of laboratory data systems and computing integrators. The instrument accommodates partial remote control of injection time by contact closure. Complete remote control of injection time, number of injections per vial, syringe drive, three auxiliary contact closures, and internal injection valve actuation can be accomplished by means of either the RS-232-C serial interface or the IEEE-488 (GPIB) parallel interface. The BCD interface allows vial and injection identification numbers to be passed to most computing integrators. A separate refrigerated unit, the Model 718ALR is available with solid state temperature control for maintaining vial temperature in a range from 4° C to 40° C.

The mechanical components and operational schematic of the Model 718AL are shown in Figure 2-1. The basic components are a six port HPLC injection valve (equipped with a sample loop and a Loop Filler Port), a syringe drive (equipped with a priming valve) and a needle. The needle is connected to the syringe by a length of PTFE tubing whose length and inner diameter are a function of the syringe size. The injection valve can be fitted with a variety of loop sizes. The standard loop is 250 µL. The syringe drive can be fitted with 50, 250, and 2000 µL syringe sizes. The 250 µL syringe is standard. The syringe size is chosen to work in conjunction with a given sample loop size.

The basic injection process mechanically emulates how the chromatographer would make an injection manually. First the injection valve is placed in the LOAD position. Next, the needle is placed in a vial of sample. The syringe withdraws the desired amount of sample into the needle after which the needle is removed from the sample vial. The needle is then put into the Loop Filler Port of the injection valve and the syringe's contents are expelled into the sample loop. The injection valve is then switched to the INJECT position where the sample loop contents are flushed into the column. While the injection valve is still in the INJECT position, the syringe is rinsed and filled with a suitable solvent (usually Mobile Phase) to rinse the Loop Filler Port. After

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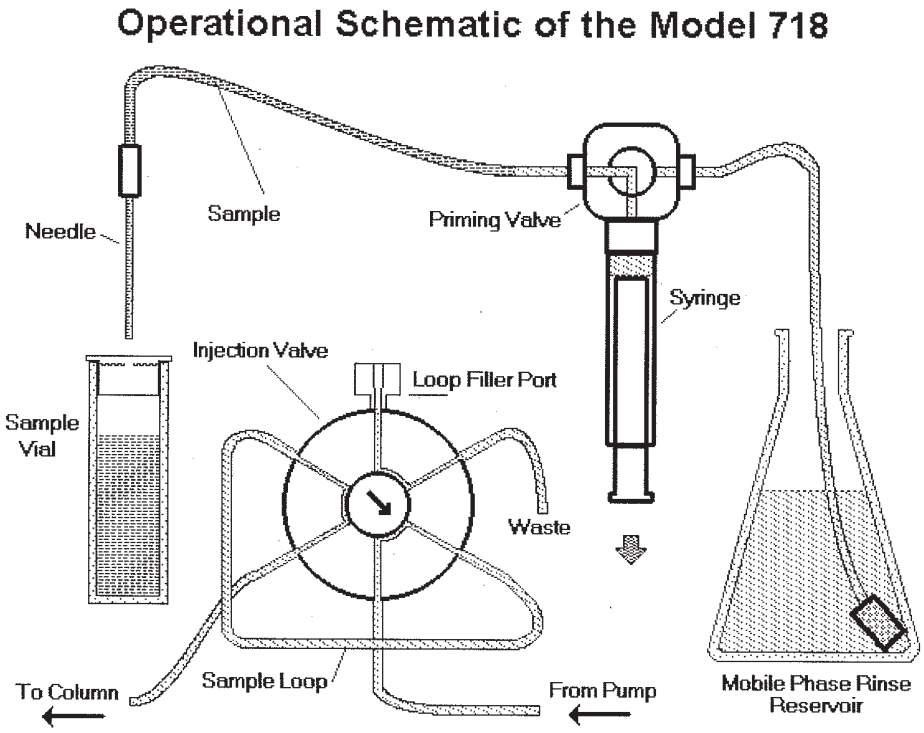


Figure 2-1: Model 718AL Operational Schematic

the rinsing has completed and the chromatogram is finished, another sample can be injected.

Both Partial Fill Loop Injections and Fixed Loop Injections can be used with the Model 718AL. The former allow the chromatographer to vary the sample injection size without changing the sample loop with an injection precision which is typically ±0.5% RSD. The latter offers the highest injection precision which is typically better than ±0.3% RSD. The two procedures are described below.

PARTIAL LOOP FILL INJECTIONS

Partial loop injections use the following steps:

(1) The Needle is indexed to the desired vial, but not lowered. If the Pre-Pierce option is selected, the vial cap is vented at this time.

(2) The Priming Valve is rotated to PRIME.

(3) The Syringe withdraws the designated PRE-INJECTION VOLUME of Mobile Phase.

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(4) The Priming Valve rotates to RUN.

(5) The Syringe withdraws the designated PRE-INJECTION AIR BUBBLE.

(6) The Needle is lowered into the sample vial.

(7) The Syringe withdraws the specified volume of sample. Once sample is withdrawn, the instrument pauses for the specified DELAY TIME to compensate for sample viscosity and compressibility

(8) The Needle is raised from the vial.

(9) The Syringe withdraws the designated POST-INJECTION AIR BUBBLE.

(10) If the optional External Needle Rinse is used, the Needle is indexed to the desired rinse vial and the exterior surface of the Needle is washed.

(11) The Needle is indexed to the Loop Filler Port of the Injection valve.

(12) The Needle is lowered into the Loop Filler Port.

(13) The Injection Valve is rotated to the LOAD position.

(14) The entire syringe contents are injected into the LOOP. The instrument then pauses for the specified DELAY TIME to compensate for sample viscosity and compressibility.

(15) The Injection Valve is rotated to the INJECT position.

(16) The Syringe performs the user specified number of RINSE strokes.

The bolus of Mobile Phase drawn into the syringe in steps 3, 4, and 5 compensates for the extra volume in the Loop Filler Port and pushes the sample slug completely into the loop. The air bubbles drawn into the syringe in steps 5 and 9 are used to disrupt the laminar flow of the sample slug as it is injected into the Loop Filler Port in step 14. This allows up to 80% of the sample loop to be used for partial loop fill injections. Without the air bubbles, only about 50% of the loop could be used for Partial Loop Fill injections. *Note that these steps are optional and can be set to zero at the user's option.* **Appendix C, Factors Which Affect**

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Injection Precision and Accuracy, describes the advantages and disadvantages of these steps.

FIXED LOOP INJECTIONS

Fixed loop injections incorporate a "Heart Cut" injection technique. The following steps describe the process.

(1) The Needle is indexed to the desired vial, but not lowered. If the Pre-Pierce option is selected, the vial cap is vented at this time.

(2) An optional pre-injection volume of Mobile Phase and/or a small air bubble can be introduced at this time. Usually a 1 µL air bubble is recommended.

(3) The Needle is lowered into the sample vial.

(4) The Syringe withdraws a volume of sample, usually 4 × the Loop volume plus an additional 5 µL for the needle. The instrument then pauses for the specified DELAY TIME to compensate for sample viscosity and compressibility.

(5) The Needle is raised out of the vial.

(6) An optional post injection air bubble is introduced at this time.

(7) If the optional External Needle Rinse is used, the Needle is indexed to the desired rinse vial and the exterior surface of the Needle is washed.

(8) The Needle is indexed to the Loop Filler Port of the Injection valve.

(9) The Needle is lowered into the Loop Filler Port.

(10) The Injection Valve is rotated into the LOAD position.

(11) The volume of sample corresponding to the 4 × the Loop volume is injected into the valve. The excess 5 µL is left in the needle and transfer line. The instrument then pauses for the specified DELAY TIME to compensate for sample viscosity and compressibility.

(12) The Injection Valve is rotated to the INJECT position.

(13) The Syringe performs the user specified number of RINSE strokes

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Here the sample loop is flushed with 4 × the LOOP volume of sample. The 5 µL excess is added to insure a contiguous liquid column of sample through the Needle Transfer Line, Loop Filler Port, Needle, Sample Loop and Waste line. For example, if the Sample Loop size is 20 µL, then the syringe needs to withdraw 85 µL of sample (4 × 20µL + 5µL). No pre-injection of Mobile Phase is generally needed, but can be used. Additional information is given in **Appendix C, Factors Which Affect Injection Precision and Accuracy**.

Needle Port Rinsing

The last step in both procedures is the rinsing of the Needle Port. The steps used are outlined below:

(1) The Injection Valve is rotated to the INJECT position. (Which it should be after an injection).

(2) The needle is then seated in the Loop Filler Port. (Which it should be after an injection).

(3) The Priming Valve on the syringe drive is rotated to the PRIME position.

(4) The syringe plunger is withdrawn the full stroke length, to fill with rinse solvent.

(5) The Priming Valve is then rotated back to RUN.

(6) The contents of the syringe are forced through the Loop Filler Port, valve and then out the WASTE PORT.

(7) Steps 3 through 6 are repeated for the total number of user designated Rinse Strokes.

The main function of the RINSE is to flush the Needle and the Loop Filler Port after an injection to reduce carry-over and to reprime the syringe. The number of rinses required depend on the nature of the sample being injected and the syringe size. For example, sample which is a concentrated sugar solution would require about 20 rinse strokes if a 50 µL syringe is used.

External Needle Rinsing

Some samples, such as concentrated sugar sample for example, can leave a sticky residue on the outer surface of the needle. This residue can contribute significantly to injection carry-over. The Model 718AL provides for rinsing of the needle's external surface. The external rinse vial and the number of external rinse strokes are programmed by the user.

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Vial Numbering Conventions

The Model 718AL Autosampler can be fitted with four different sample tray types: 128, 8 mm vial tray, 72, 12 mm vial tray, 50, 15 mm vials or a 96-well, microplate tray. The Autosampler must be told which tray is installed. This setting is made through the System Settings Key (see section entitled **System Settings** in this chapter for details). The 8 mm, 12 mm, and 15 mm vial trays are usually accessed using a **Row Major** convention as shown in Figure 2-2A for the 12 mm vial tray. The first vial begins at the lower left-hand corner with subsequent vial numbers moving to the right. Vial trays are silk-screened with the appropriate vial number to prevent confusion.

The 96 well, microplate positions can be accessed in either the **Row Major** convention or the **Column Major** convention as shown in Figure 2-2B. The reason for this change in conventions is that the microplate is an industry standard sample container. As also shown in Figure 2-2B, rows are labeled numerically while

12 mm Vial Tray Assignments

Row Major

67	68	69	70	71	72
61	62	63	64	65	66
55	56	57	58	59	60
49	50	51	52	53	54
43	44	45	46	47	48
37	38	39	40	41	42
31	32	33	34	35	36
25	26	27	28	29	30
19	20	21	22	23	24
13	14	15	16	17	18
7	8	9	10	11	12
1	2	3	4	5	6

A

MicroPlate Vial Assignments

Column Major

	A	B	C	D	E	F	G	H
12	12	24	36	48	60	72	84	96
11	11	23	35	47	59	71	83	95
10	10	22	34	46	58	70	82	94
9	9	21	33	45	57	69	81	93
8	8	20	32	44	56	68	80	92
7	7	19	31	43	55	67	79	91
6	6	18	30	42	54	66	78	90
5	5	17	29	41	53	65	77	89
4	4	16	28	40	52	64	76	88
3	3	15	27	39	51	63	75	87
2	2	14	26	38	50	62	74	86
1	1	13	25	37	49	61	73	85

B

Figure 2-2: Vial Numbering Conventions
A Row Major B Column Major

columns are labeled alphabetically. The **Column Major** convention allows the user to access the well positions in a manner

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consistent with other instruments which use microplates as their standard sample container. In addition, vial positions can be alternatively addressed in a Alpha-Numeric fashion when using the Column Major convention.

Note that all trays can be configured for either **Row Major** or **Column Major** conventions although the former is best used with the 8 mm, 12mm, or 15mm vial trays while the latter is more suitable for the microplate containers.

The Front Panel User Interface

The Front Panel User Interface is a 20 character, LCD Display with a 20 key Keypad. It is illustrated in Figure 2-3.



Figure 2-3: The Front Panel

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Keypad

The Keypad consists of 20 keys. Some of the keys serve two functions. The primary function is printed on the key itself. The secondary function is printed on the front panel facia just above the key. The secondary function is accessed by first pressing the [FUNCTION] key, releasing it, and then pressing the key whose secondary function has the desired action. The keys and their secondary functions are described in Table 2-1.

The Main Screen

After the Autosampler has powered up and initialized, the following screen is displayed:

```
Ready

Select a Function
```

This screen is called the Main Screen. It is always displayed any time the instrument is idle. Note that the line "Select a Function" flashes.

Display, Keypad, and Manual Conventions

Methods, System Settings, and other functions consist of a menu of parameters which can be edited for different sample requirements. The complete parameter menu, in many instances, cannot be shown on the Display at one time due to the available number of display lines. The last entry in most menus is Exit unless other instructions are given on the displayed screen. The absence of the word Exit or the display of the characters, ↑ and ↓, indicates that the [↑] and [↓] Arrow Keys are used to scroll the menu either up or down in order to view and edit the different parameters. A right pointing arrow, →, in the leftmost margin of the Display indicates the menu item currently available for selection.

The names of all keys referred to in this manual are written in upper case type and enclosed in square brackets "[ ]". Parameters in each function menu require either a numerical entry, or the selection of an item from a second list. Numerical entries are indicated in this manual by the pound sign, #. The number of pound signs indicates the number of digits available for that particular parameter. The [ENTER], [CLEAR], and Number keys are used for numerical entry purposes. In addition, the [→] or [←] keys may be used to increment or decrement numerical values respectively. If it is desired to keep the existing numerical entry, press either the [↑] or [↓] to scroll the menu to the next parameter or press [ENTER], to preserve the previous entry.

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If a numerical entry is made which is outside of the range possible for that parameter, the Autosampler will issue a warning alarm, followed by the display of the following error message:

```
      Error
Value out of range
Enter value between
    #  -   ##
```

where # and ## are the appropriate numerical values. The Error Message will remain on the Display until the [ENTER] key is pressed.

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Table 2-1: Key Descriptions

KEY	DESCRIPTION
[↑] (up arrow)	Moves the Display cursor up a line.
[↓] (down arrow)	Moves the Display cursor down a line.
[←] (left arrow)	Moves the Display one selection item to the left. Decrements numerical values.
[→] (right arrow)	Moves the Display one selection item to the right. Increments numerical values.
[.],[0],[1],[2],[3],[4], [5],[6],[7],[8],[9]	Numerical value entry.
[FUNCTION]	This key allows selection of overlay functions.
[RUN/START]	A toggle key. If a Method is not running, the [RUN/STOP] key allows the selection and execution of a Method. If a Method is running, the [RUN/STOP] key stops the Method and all mechanical movement.
[PRIORITY]	Allows Method interrupt editing and injection of "Priority" sample.
[ENTER]	Allows edited parameter to be logged into Method.
[CLEAR]	Clears the value of the highlighted parameter.
[METHOD] ([FUNCTION] [7])	Calls up the Method Editing Menu from Main Screen. Allows Methods to be edited or deleted. Resets all Methods to default values if pressed while in System Settings Menu. See the section **Editing A Method**, for details.
[SYSTEM] ([FUNCTION] [4])	Allows editing of system parameters such as contact closure duration times and loop size.
[RINSE] ([FUNCTION] [9])	Rinses the Needle Port and syringe in the Model 718AL.
[MANUAL INJECTION] ([FUNCTION] [8])	Allows a manual injection to be made

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Table 2-1: Key Descriptions, Continued

Key	Description
[TEMP] ([FUNCTION] [5])	Allows the selection of the sample tray temperature if the Cooling Option is installed. An Error Message will result if an attempt is made to enter a temperature if the Cooling Option is not installed.
[REM/LOC] ([FUNCTION] [6])	Allows selection of the type of Remote Control if installed. If RS-232-C is selected, user will also select Baud rate If unit is in the Remote Mode, this function will return it to the Local Mode. If unit is in Remote Mode and Power is switched to OFF or if there is a Power failure, the unit will power up in Remote Mode. If no Remote interface is installed, an Error Message will result if the [REM/LOC] key is pressed.
[F1], ([FUNCTION] [1])	This key toggles the Auxiliary Power Controller On or OFF.
[F2], ([FUNCTION] [2])	This key allows all existing Methods to be erased at once.
[F3], ([FUNCTION] [3])	This key is reserved for future use.
[FUNCTION] [CLEAR]	This acts as the Autosampler's operating system's "Escape" key. It allows the user to jump out of current menu to the Main Screen.
[FUNCTION] [UP ARROW]	Allows the user to jump out of current menu to next higher menu.
[FUNCTION] [0]	Moves the Needle to the HOME (over Injection Valve) position.

Parameter lines which contain the characters < and > on either side of the parameter value indicate that a selection must be made from a discreet menu of items. Pressing either the [←] or [→] key allows scrolling through the selection list. When the desired selection is displayed, press either the [ENTER], [↑], or [↓] keys to confirm the selection.

System Settings

The System Settings are global parameters which affect the operation of the Autosampler, but are not required to be programmed for each sample or Method. They are accessed by

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pressing the [SYSTEM] key ([FUNCTION] [4]) The System Settings menu selections are described below.

The following screen results upon pressing the [SYSTEM] key ([FUNCTION] [4]):

```
   SYSTEM SETTINGS
→Park Needle
 Contact Closures
 Needle Setup        ↓
```

There are eleven choices: Park Needle, Contact Closures, Needle Setup, Key Beep, Syringe Size, Sample Vial Size, Time/Date, Addressing Mode, Initialize Valve, Exit, and Status. The [↑] and [↓] arrow keys are pressed to move the right pointing arrow, →, to the appropriate choice. Note that Exit is outside of the Display window. After the desired choice is pointed to, the [ENTER] key is pressed. The down arrow, ↓, in the lower right margin indicates that additional selections are available.

Park Needle

The Park Needle command causes the Autosampler to move the Needle Drive Carriage to the left rear of the instrument. It moves the Needle Drive Carriage out of the way to provide easy access to the sample tray.

Contact Closure Time

There are four, user settable Normally Open or Normally Closed, contact closures. The closure duration time for each is individually programmable from 1 second (the Default Time) to 32000 seconds. The individual contact closures are:

1. Injection Mark
2. Event 1
3. Event 2
4. Event 3

The Injection Mark always closes upon the injection of a sample regardless of whether the injection was from a Method or a Manual Injection. The three remaining switches, Event 1 , Event 2, and Event 3 have programmable actuation times which are set in a Method.

Selection of the Contact Closure item results in the following screen:

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```
  CONTACT CLOSURES
→Injection
 Event 1
 Event 2                ↓
```

The items not shown on the Display window are Event 3 and Exit. These will be scrolled up after the [↓] key has reached the bottom line and is pressed. A numerical value between 1 and 32000 seconds may be entered for the four Contact Closures. After the number is keyed in, the user presses [ENTER] to set the time. After all the desired settings are made, the Exit selection is made to save the settings and to exit the routine. The Default value for all four contact closures is 1 second.

Needle Setup

The Needle Setup command allows the user to set the depth at which the Needle penetrates the Injection Port on the Injection Valve. Ideally, the Needle needs to make firm contact with the bottom of the Injection Port in order to ensure good fluid transfer and hence good injection reproducibility. The standard Needle is 58.42 mm ± 0.25 mm (2.30 in. ± 0.010 in.) in length. From these specifications, it can be seen that Needle can vary as much as 0.5 mm (0.020 in) in length. The Needle Setup command allows the instrument to compensate for this difference in length from one Needle to the next.

When the command is activated, the following screen appears:

```
    NEEDLE SETUP
→Depth
 Stall
 Exit
```

The menu item, Depth, is use to set the Needle Depth. The menu item, Stall, has no function at this time. Upon selecting Depth, the Needle Carriage is move to the Injection Port Position, the Needle is lowered, and the following screen is shown:

```
 Enter Needle Depth
   for valve port.
<↑> or <↓> to Adjust
       XX.X mm
```

where XX.X mm is the currently set Needle Depth. This value is typically ~25.0 mm. The depth can be set using one of two methods described below.

The first method involves the use of the [↑] and [↓] keys. Pressing the [↑] key raises the Needle 0.1 mm. Conversely, pressing the [↓] key causes the Needle to move downward 0.1 mm. Each 0.1 mm

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increment or decrement in Needle Depth corresponds to one press of the appropriate key. The proper Needle Depth is set when the Needle is lowered deep enough to deflect the entire Needle Carriage up a few millimeters. Do not lower the Needle down so far as to cause the Needle to bow or bend. **Note: it is a good laboratory practice to record and store this Needle Depth value, once it is determined, for future reference**.

The second method for setting the Needle Depth to first press the [ENTER] key, and then to key in the Needle Depth value directly using the Number Keys followed by the [ENTER] key. Note that while in this mode, the cursor will flash at the Needle Depth value on the screen. Once the value is entered, the Needle will move to the entered depth.

Once the desired Needle Depth has been set by either of the two methods described above, exit the Needle Setup command by first pressing the [FUNCTION] key followed by the [↑] key.

Key Beep

This function allows the user to configure the autosampler to emit an audible beep whenever a key is pressed. **ON** turns the audible beep on and **OFF** turns it off.

Syringe Size

The Syringe Size Parameter allows the selection of one of two available syringe sizes. The available sizes are 50 µL, 250 µL, and 2000 µL. When this menu item is selected, the following screen is displayed:

```
Select Syringe Size

<250µl>
```

Choices are made by pressing the [←] or [→] keys as prompted by the < and > symbols next to the syringe size value. After the desired value is displayed, the [ENTER] key is pressed to verify the value. The default size is 250 µL.

Sample Vial Size

As mention in the introduction to this chapter, the Model 718AL can utilize sample trays to contain either 128, 8 mm vials, 72, 12 mm vials or 50, 15 mm vials or one, 96-well, microplate. **Regardless of which tray is in use, the Autosampler must be**

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informed as to the tray size installed. The Sample Vial Size command is used for this purpose.

Upon selection of the Sample Vial Size command, the following screen is shown:

```
  Select Vial Size

      <12 mm>
  <ENTER> to Exit
```

The appropriate vial size is selected by pressing the [←] or[→] Keys and then pressing [ENTER]. The choices are 8 mm, 12 mm, 15 mm, Microplate, and Special.

Time/Date

This function allows the user to set the Time and Date of the microprocessors Real Time Clock. Note that it is Year 2000 compliant. Upon selecting this function, the following Menu is presented:

```
     Time/Date
 03/13/98  12:54 PM
→Set Date
 Set Time          ↓
```

The Date and Time are entered by selecting the appropriate menu item and then pressing the [ENTER] key. Note the that Model 718AL is year 2000 compliant.

Addressing Mode

The Addressing Mode command is used for setting the manner in which vial positions are accessed. See the section entitled **Vial Numbering Conventions,** above, for a detailed discussion.

The Addressing Mode Menu is shown below.

```
  Addressing Mode
→8 mm>
 Column Major
 Numeric           ↓
```

There are three items in this menu plus Exit. The first item, shown here as 8mm>, allows selection of the vial tray size. The choices are 8 mm, 12 mm, 15 mm, Microplate, and Special. The remaining menu items let the user set how that particular tray size is accessed.

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The menu item Column Major allows selection of either Column Major or Row Major sample access. The default is Row Major for the 8 mm, 12 mm, and 15 mm vials and Column Major for the Microplate.

The menu item Numeric, allows the Autosampler to interpret vial positions as numbered either numerically or alphanumerically. The defaults are Numeric for the 8 mm, 12 mm, and 15 mm vials and Alphanumeric for the Microplate. With this command, an 8 mm vial tray, for example, can have its vial's positions number alphanumerically just like a Microplate.

Since there are no letter Keys on the Keypad, special entry screens are provided to the Method Editing Screen for entering Alphanumeric vial assignments.

Initialize Valve

The Initialize Valve function takes the user through the valve installation process and allows calibration of the valve controller. The Model 718AL is equipped with an electric Valco Two Position Valve Actuator. The actuator can be used with all Valco six, eight, ten, twelve, and fourteen port valves. The actuator senses which valve is present during initialization, and calibrates itself accordingly.

When the Initialize Valve selection is chosen, the following screen is presented:

```
VALVE INITIALIZATION
  Disconnect valve
    from actuator
<ENTER>  to Continue
```

at this point the user must physically remove the valve from the actuator, using the procedure described in **ROUTINE MAINTENANCE AND PARTS REPLACEMENT**. If the valve is not removed, no recalibration will occur. After the valve has been removed, press the [ENTER] key which results in the "Cycling" message:

```
VALVE INITIALIZATION

      Cycling
```

Which then results in the following screen:

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```
VALVE INITIALIZATION
Firmly install valve
   onto actuator
<ENTER>  to Continue
```

At this point, the valve is physically reinstalled and the [ENTER] key is pressed. The actuator will cycle eight times in two sets of four to calibrate itself. Each set is a different speed. Once completed, the Display will return to the Main Screen.

Status

This function is for trained, Alcott Service Personal and is not intended for normal, User operation.

Temperature Setting (Optional)

To set the temperature of the sample tray, the [TEMP] key ([FUNCTION] [5]) is pressed. The following screen is given:

```
 SAMPLE TEMPERATURE
→Set Temp.
 Actual Temp.    TT.T
 Temp. Control   ON.
```

The temperature is entered on the first line. The range is from 4 °C to 40 °C. Values outside of this range will result in the error message: **VALUE OUT OF RANGE. TEMPERATURE CAN ONLY BE SET FROM 4 °C to 40 °C.**

The third line allows the Temperature Control circuitry to be turned ON or OFF. When OFF, the large internal Heat Pump Fan is turned off as well.

The following screen appears if no cooling option is available and the [TEMP] key is pressed:

```
Temperature Control
    Module  is
NOT  installed
```

Rinse

This command allows the rinsing of the needle port and syringe and is also used to prime the syringe. The general procedure is

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described in the section **Needle Port Rinsing**. When the [RINSE] key ([FUNCTION] [9]) is pressed, the following screen appears:

```
 SYRINGE RINSE MENU
→Rinse Strokes      1
 Rinse Rate        <20
 Begin Rinses       ↓
```

There are two parameters to select before using the Begin Rinses command on this menu.

The Rinse Strokes parameter is the number of times the syringe is to be filled and emptied. The Rinse Rate parameter is the flow rate at which the syringe delivers the rinsing solvent. There are three flow rates, slow, medium and fast. Actual flow rate values depend on the size of the syringe installed. Table 2-2 lists these flow rate ranges for the three available syringe sizes.

Table 2-2: Rinsing Flow Rates

Syringe Size	Rinse Rate Ranges (µL/sec)
50 µL	1 to 20
250 µL	1 to 100
2000 µL	1 to 400

After the desired rinse parameters are entered and the Begin Rinses command is entered, the rinse cycle will start. The following screen will be displayed:

```
  SYRINGE RINSING

Stroke:  n of  m
```

where n is the current active rinsing stroke and m is the total number of rinsing strokes. After the Rinse is executed, the screen returns to the Main Screen.

Manual Injection

The [MANUAL INJECTION] key ([FUNCTION] [8]) allows the user to manually load the injection valve using a syringe equipped with a 22 gauge needle. When pressed, the following screen:

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```
  MANUAL INJECTION
 <←> OR <→> TO Move
 <RUN/STOP> TO Exit
POSITION:      LOAD
```

is displayed and the Injection Valve is placed in the LOAD position. While in the Manual Injection mode, pressing the Left Arrow key, [←], will place the valve in the INJECT position. Pressing the Right Arrow key, [→], will place the valve in the LOAD position. Pressing the [RUN/STOP] key will return the instrument to the Main Display.

To make a Manual Injection, remove the Needle Guide from the Loop Filler Port of the Injection Valve. Next, put the Valve in the LOAD position by pressing the Right Arrow key, [→]. Then load the sample into the Sample Loop by using a syringe equipped with a 22 gauge needle. The Sample Loop can either be partially filled or totally filled. Finally, once the sample is in the loop, press the Left Arrow key, [←], to put the Valve into the INJECT position. For best results, leave the syringe in the Loop Filler Port when switching the Valve from the LOAD to the Inject position.

To leave a Manual Injection session, replace the needle guide and press the [RUN/STOP] key.

Injection Parameters

Injection parameters are set for each Method and remain unchanged within each Step of that Method. They can be edited, but the new changes affect the whole Method. These parameters are global for all Steps of a Method. The parameters, listed below, affect the overall reproducibility of an injection for a given sample and solvent combination. Further guidelines are given in **Appendix C, Factors Which Affect Injection Precision and Accuracy**.

Inject Type — The choices are **PART>** for Partial Loop Fill injections or **<FIX** for Fixed Loop Injection. The default is **PART>**. When **<FIX** is selected, the **Withdraw Vol** parameter, described below, is enabled.

Withdraw Rate
Loop Fill Rate — These are the flow rates of the syringe as it **withdraw**s sample from the vial and then **fill**s the loop with sample. The flow rates for each syringe size are given in Table 2-2.

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Delay Time

This is the waiting time, in seconds that the Autosampler pauses after it has drawn sample up into the needle. The range is from 0 to 60 seconds. The default Delay Time is 5 seconds.

Tray Vial Depth

This is the depth, in millimeters, at which the needle penetrates the vial to withdraw sample. The range is from 1 to 48 mm. A value of 48mm will place the needle to the very bottom of the sample vial. The default value is 40 mm. Care should be exercised when setting this parameter, especially when using vials equipped with low volume inserts.

Ext. Rns Depth

This is the depth, in millimeters, at which the needle penetrates the four External Rinse vials to rinse the needle. The range is from 1 to 46 mm. A value of 46mm will place the needle to the very bottom of the sample vial. The default value is 40 mm. Care should be exercised when setting this parameter, especially when using vials equipped with low volume inserts.

Pre-Injection Vol

This is the volume of rinse solvent, usually Mobile Phase, that the syringe will withdraw from the Rinse Reservoir prior to withdrawing sample to compensate for the volume of the needle port. The range is from 0 to 10 µL. The default is 5 µL.

Pre-air Vol

This is the volume of the optional air bubble which can be placed after the Pre-Injection Volume of Mobile Phase and before the Sample. The range is from 0 to 10 µL. The default is 1 µL.

Withdraw Vol

This parameter is the total volume of sample withdrawn for a "Heart Cut", Fixed Loop Injection. It is activated when the **Injection Type** is set to **<FIX** but is inactive when the **Injection Type** is set to **PART>**.

Post-air Vol

This is the volume of the optional air bubble which can be placed after the

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Sample. The range is from 0 to 10 µL. The default is 1 µL.

Rinse Strokes This is the number of Rinse Strokes the Autosampler will use to rinse the needle port after each injection. The range is from 0 to 100 strokes and the default value is 1 stroke.

Rinse Rate This is the flow rate used by the syringe to rinse the Needle and Needle Port. The flow rates are show in Table 2-2 for each of the three available syringe sizes.

Pre-Pierce Some types of vial septa can cause a very tight, air-impermeable seal around the Needle the first time the Needle penetrates the septum. This tight seal can cause the syringe to cavitate on the first injection. The Pre-Pierce option allows the vial septum to be pierced twice before sample is withdrawn in order to reduce the risk of syringe cavitation by providing a small amount of venting.

Ext. Rinse This is the number of External Rinse Strokes the Autosampler will use to rinse the needle port Seat after each injection. The range is from 0 to 100 strokes and the default value is 0 stroke.

Method

A Method is a user defined program under which the Autosampler operates through normal front panel control. The Autosampler can store up to ten Methods and each Method is composed of from 1 to 128 Steps.

The [METHOD] key ([FUNCTION] [7]) can be pressed at any time to edit a new Method or to make Method changes, even as another Method is running. This feature is known as "On-The-Fly-Programming". A Method can be edited while it is executing, however, the changes will not affect the current vial. Pressing the [METHOD] key renders the following screen:

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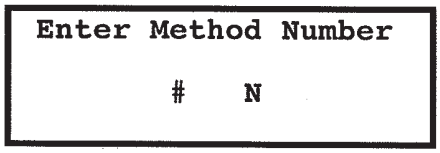
This Display is called the Method Menu Screen.

There are seven menu items in the Method Menu Screen. A brief description for each is given below.

Edit Method	Allows Methods and Steps to be entered or edited.
Run Method	Executes the selected Method.
Copy Method	Allows the Steps from one Method to be copied into an unused Method block.
Delete Method	Allows the designated Method to be deleted.
Display Method Use	Shows which of the ten Method blocks are filled.
Print Method	Allows the Autosampler to print out a Method listing to a Centronics type printer.
Exit	Returns the Display back to the Main Screen.

Editing a Method

To edit a Method, select Edit Method from the Method Menu Screen. After in the editing mode, the following screen appears:



In this example, N represents the Method number selection in a range from 1 to 10. After the Method number is keyed in and the [ENTER] key is pressed, the Step Menu Screen is displayed:

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As mentioned above, a Method is composed of Steps. This screen is a menu of operations which can be performed on a STEP. Listed below are each of these Edit Menu Items and their function:

Edit Step — Calls up a Step and allows it to be edited. Editing details are discussed below.

Append Step — Causes a new Step to be added to the end of a Method. After invoked, the user is immediately displayed the new step for editing.

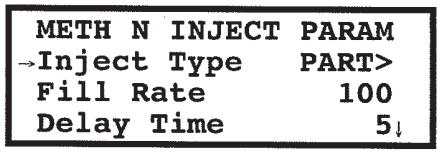
Insert Step — Causes a new Step to be inserted between two existing Steps in a Method. The screen prompts for the Step number to be inserted. The user is immediately displayed the new step for editing.

Delete Step — Prompts the user for the number of the Step to be removed from the Method. A verification prompt is given to insure that the Step Deletion is intentional.

Edit Inject Param. — Calls the Injection Parameters editor.

Exit — Returns control back to the Method Menu Screen.

When a new Method is to be edited, the first screen shown is:



This menu and the parameters have been discussed in the previous section, **INJECTION PARAMETERS**. Note that the Injection Parameters are set once for each Method and are the same for each Step in that Method. The Injection Parameters may be re-edited.

The following screen appears if either Edit Step, Append Step or Insert Step is chosen:

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The Method and Step numbers are given at the top of the screen. In the example shown here, the Method number is NN and the Step number is M. There are ten parameters available for editing. They are listed below:

Start Vial

This is the first vial in a sequence of vials to be injected. It can be any vial from 0 to the maximum number of vials for a given vial size. The maximum values are 128 for 8 mm vials, 72 for 12 mm vials and 50 for 15 mm vials. The Default is vial 1. A 0 vial number allows injection from one of the four 12 mm vials located just behind the injection valve.

Final Vial

This is the last vial in a sequence of vials to be injected. It can be any vial from 1 to the maximum number of vials for a given vial size. The Default is vial 1

Note **Microplates default to a Column Major sample position access convention (see Section entitled "Vial Numbering Conventions"). Sample positions are numbered alphanumerically. Since alphabetical keys are not available on the Keypad, a special dialog screen is used to assist in editing alphanumeric vial positions. To access this screen, select "Start Vial" and press the [ENTER] key, then follow the on screen instructions. Repeat for the Final Vial.**

Standard Vial

This is one of the four 12 mm vials located just behind the injection valve. These are used to hold a standard solution used for calibration purposes. The choices are from 1 to 4.

Injections

This parameter is the number of injections to be made from a vial.

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The selections are from 1 to 99 per Step. A vial may be resampled with each step. The Default is 1 injection per vial.

Inject. Vol.

This is the volume of sample to be injected. The range is from 1 µL to the syringe size maximum. The available syringe sizes are: 50, 250, and 2000 µL. Note that when the **Injection Type** is **<FIX**, the **Inject. Vol.** is the portion of the total **Withdraw Vol** actually injected into the sample loop. The **Withdraw Vol.** is set under the **Injection Parameters Menu**.

Run Time

This is the chromatographic analysis time per injection. The range is from 0.1 minutes to 999.9 minutes. A value of 0.0 sets up the External Inject signal. In this condition, the Run Time is determined by external control. The Autosampler will only make an injection when it receives a remote switch closure. The Default value is 1.0 minute.

Event1 Time
Event2 Time
Event3 Time

These are the user programmable Contact Closures, also called Timed Events. Their range is from 0.0 minutes to 999.9 minutes. This parameter must be less than or equal to the Run Time in order for the Contact Closure to actuate. If the Timed Event is greater than the Run Time, the Contact Closure will not actuate. The Default time is 0.0.

Rinse Vial

This is the External Needle Rinse Vial. It is one of the four 12 mm vials located in the left rear of the sample compartment behind the injection valve. Note that the Autosampler will not let you use a vial for the External Needle Rinse if that vial has been assigned as a Standard Vial as described above.

Rinse Strokes

This is the number of times the Needle is immersed in the External Needle Rinse Vial.

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Add Step	This allows the addition of a new Step.
Exit	This saves the currently edited Method, and returns the display to the previous screen.

Chapter 3, OPERATION gives a step by step example of how to edit and run a Method.

Running A Method

A Method can be initiated in one of two ways. From any Menu Screen, pressing the [RUN/STOP] key will cause the Autosampler to prompt the user for a Method number. After the desired number is keyed in and the [ENTER] key is pressed, the Method will begin.

Methods can also be started from the Method Menu Screen. The Method Menu Screen is accessed by pressing the [METHOD] key ([FUNCTION] [7]). The following screen will appear:

```
    METHOD MENU
→Edit Method
 Run Method
 Copy Method          ↓
```

From this screen, the user selects the Run Method item and presses [ENTER]. The Autosampler will prompt the user for a Method number. After the desired number is keyed in and the [ENTER] key is pressed, the Method will begin.

As a Method is running, the following screen is displayed:

```
METHOD  STEP  METHTIME
  NN      M     XX.X
 VIAL    INJ    RTIME
   n      m     xx.x
```

This screen displays the current Method number, NN, and Step, M, which is executing, the Vial, n, from which the injection is made, and which Injection, m, is currently running. In addition, the chromatographic Run Time, RTIME, is displayed along with the total elapsed time of the Method, METHTIME in minutes.

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A Method can be stopped at any time by pressing the [RUN/STOP] key. The Autosampler will verify that the user's intention is to abort the Method by giving the following prompt:

```
   METHOD STOPPED
<RUN/STOP> to Resume
<ENTER>    to  Abort
<FUNC 7>   to   Edit
```

To resume the Method, press the [RUN/STOP] key. To completely stop and abort the Method, press the [ENTER] key. To edit the Method, press [FUNCTION] [7].

Chapter 3, OPERATION gives a step by step example of how to edit and run a Method.

Priority Sample Interrupt

A Priority Sample Interrupt is invoked by pressing the [PRIORITY] key. It is used to interrupt a currently active Method to allow the editing of conditions for a "Priority Sample". All injections for the Priority Sample will be made immediately after the current injection for the present vial in the currently active Method has been completed. After the Priority Sample is completed, the Autosampler will return to the previous vial and continue with the next injection.

The following prompt screen is given when the [PRIORITY] key is pressed:

```
  PRIORITY  SAMPLE
→Sample Vial      1
 Standard Vial NONE
 Injections       ↓
```

Additional parameters not shown include: Inject. Vol., Run Time, Event 1, Event 2, Event 3, Run Sample, and Exit. These parameters have the same descriptions as those of the same name found in the section **Editing a Method** except for the Run Sample parameter which is described below.

After all the parameters have been edited, the user selects Run Sample and presses [ENTER]. The Autosampler then waits until the current injection Runtime is complete, then the Priority Sample is injected. After all the injections from the Priority Sample vial are completed, the suspended Method is resumed at the point it was interrupted.

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Remote/Local

The [REM/LOC] key ([FUNCTION] [6]) allows selection of the mode of operation of the Model 718AL Autosampler. Note that the Autosampler must be equipped with either of these optional remote control interfaces. Choices are Local or normal front panel control, RS-232-C serial interface control. The IEEE-488 (GPIB) parallel interface control is not functional at this time. Should one of the last two items be selected, the Autosampler will prompt for additional operational information as described below. After these parameters are entered, the Autosampler will be placed in the Remote mode. All control will be by the host computer or controlling device. The Autosampler will stay in the Remote mode until the host computer returns it to Local, or until the [REM/LOC] key is pressed on the front panel. The Autosampler will remain in the Remote mode after a Power failure. The following screen is displayed when the [REM/LOC] key is pressed:

```
    REMOTE/LOCAL
→Local
 RS-232
 IEEE-488              ↓
```

The menu item not shown is Exit.

RS-232-C

The following screen is displayed if the RS-232-C mode is selected:

```
Select Baud Rate

      <4800>
```

This screen allows selection of the baud rate at which the RS-232-C interface will operate. The interface is configured for 8 bit data with no parity. Baud rate selection is made using the [←] or [→] keys. Available baud rates range from 300 to 38400 bits per second. Additional screens are presented to allow the user to choose the Parity, the Number of Data Bits, and the number of Stop Bits. After the number of Stop Bits is selected and the [ENTER] key is pressed, the Autosampler is placed in the Remote Mode and the following default screen is shown:

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```
REMOTE MODE

RS-232
```

This screen may be changed from within the program of the controlling host computer. The section, **Remote Mode Instruction Set** gives instructions on changing this screen from within a controlling host's program.

The RS-232-C Mode may be exited from either the user's remote control program, or by pressing the [REM/LOC] key ([FUNCTION] [6]).

IEEE-488 (GPIB)

This option is not functional at this time.

Remote Control Instruction Set

The RS-232-C interface allows remote control of the Autosampler by a host computer. The controlling host computer requires a user written program, containing the sequence of instructions the Autosampler is to follow. The Remote Control Instruction Set is a group of mnemonic commands that the user incorporates into his host computer's program. These mnemonic commands are shown in below:

@ — **Causes the Autosampler to go into the Remote Mode**
The instrument must have be previously configured for the BAUD rate and other communications protocols currently used by the Host computer.

@AUX #, $$$ — **Set state of Contact Closure [0-3] [ON OFF] [-1 for all]**
Expects a number followed by ON or OFF. Turns that contact on or off, 0 is the Inject Signal 1-3 are the events, -1 sets all Contact Closures at once

Examples:
```
@aux 1 ON
@aux -1 OFF
```

@BCD ##### — **Output to the BCD (5 digits, i.e.: 01034)**
Outputs to the 5 digits of the BCD, expects 5

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contiguous digits

Example: `@bcd 10344`

@BZR $$$ **Set Buzzer to: [ON OFF CLK ERR ALM PNC]**
Allows the internal buzzer to make on the self describing annoying sounds.

Example: `@bzr ALM` Turns ALARM sound ON
`@bzr OFF` Turns the sound OFF

@CLM # **Clear Method**
Expects a Method number to erase. Empty methods are valid choices.

Example: `@clm 7`

@DLM # **Download Method number # FROM the 718AL TO the client computer**
Downloads the Method structure for Method # from the autosampler to the client computer. The c/c++ structure is as follows:

```
typedef struct
{
    uint_8      bMethodNum;
    uint_8      bNumberOfSteps;
    uint_8      bReserved (0xff);
    uint_16     uiVersion (0x02);
    SRemoteInjParms sInjParms;
} SRemoteMethod718;

where SRemoteInjParms is:

typedef struct
{
    uint_8      bInjectionType;
    uint_16     uiWithdrawRate;
    uint_16     uiLoopFillRate;
    uint_8      bDelayTime;
    uint_8      bNeedleDepth;
    uint_8      bNeedleDepthRinse;
    uint_8      bPreInjectVol;
    uint_8      bAir_1_Bubble;
    uint_16     uiWithdrawVolume;
    uint_8      bAir_2_Bubble;
    uint_8      bRinseStrokes;
    uint_16     uiRinseRate;
    uint_8      fPrePierce;
    uint_8      bTrayType;
    uint_16     uiInjectValveOpenTime;
```


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```
} SRemoteInjParms;
```

followed by as many steps structures as were indicated by bNumberOfSteps, step structure is as follows:

```
typedef struct
{
    uint_16     uiStartVial;
    uint_16     uiFinalVial;
    uint_8      bStandardVial;
    uint_8      bNumberOfInjections;
    uint_16     uiInjectionVolume;
    uint_16     uiRunTime;
    uint_16     uiEvent1Time;
    uint_16     uiEvent2Time;
    uint_16     uiEvent3Time;
    uint_8      bRinseVial;
    uint_8      bRinseStrokes;
} SRemoteStep718;
```

Example: `@dlm 7`
will return: ~~~~~~~~ where '~~~' represent as much binary data as is appropriate for Method 7.

@EXM # **Does Method Exist**

Expects a method number, will confirm or deny the existence of that Method in memory

Example: `@exm 7`

@GAM # **Get tray addressing mode**

Expects a tray number, then reports the addressing parameters for it. This command works independent of the installed tray. It can be used to query the configuration for all possible tray types.
Arguments:
8_mm 128 × 8 mm vial tray
12_mm 72 × 12 mm vial tray
15_mm 50 × 15 mm vial tray
22_mm 28 × 22 mm vial tray
micro 1 × 96 well microplate
Returns:
R,N for row, numeric
C,A for column, alphanumeric

Example: `@gam 12_mm`

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@GERS **Send error value and type**
Returns last error code generated

Example: `@gers`

@GSYS **Send system information**
Returns full system spread, including PIC codes in ASCII format

Example: `@gsys`

@GTD **Show the time and date (binary form)**
Returns time/date structure (in binary). Note that the time/date structure used is the same one used by most ANSI Standard c/c++ compilers and is describe in the time.h header file of the compiler.

Example: `@gtd`
returns: ~~~~~~~~~~ where '~' is as much binary data as is appropriate

@GTDA **Show the time/date (ASCII form)**
Same as @gtd, but output formatted in ASCII

Example: `@gtda`

@GTV ### **Go to Vial Position ###**
Expects a vial number, ## (1 to Maximum Tray Size, 201-204 for Standard/Rinse Vials, 0 for Injection Port)

Example: `@gtv 10`

@HOME **Move Needle carriage to HOME Position**
Moves the needle carriage to HOME position (same as Injection Port)

Example: `@home`

@INIT **Initialize to defaults**
Resets all stepper motors and internal settings as if a boot-up (POWER-UP) had occurred

Example: `@init`

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@LOC **Return to local control**
Places the autosampler back in Front Panel control.

Example: `@loc`

@MSG #,# "$"** **Display message "$" on the screen at #,#
Expects row and column (separated by comma) and then a text message in "" displays that message on the screen at that location

Example: `@msg 0,5 "Alcott"`

@MVX **Step X motor to**
@MVY **Step Y motor to**
@MVZ **Step Z motor to**
Expects a positive or negative displacement (in steps), to step the motor (negative is toward home)

Examples:
```
@mvx 500
@mvy -200
```

@NDL ## **Move needle to Position ##**
Expects a position in mm to go to. max is 50 a value of 99 is interpreted as 'Inject Port Depth' and it will drop to the pre-programmed Injection-Port setting

Example: `@ndl 40`

@PRK **Parks needle carriage**
Parks the needle carriage to the far left rear of the instrument for tray access

Example: `@prk`

@PVL $$$$$ **prime valve to PRIME/RUN**
Switches the Syringe's Priming Valve to PRIME or RUN Positions

Example: `@pvl PRIME`

@PWR **Toggle the external power control**
Toggles the Auxiliary Power Control Box ON or

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OFF

Example: `@pwr`

@QRY **Query run status**
Returns the current status of the autosampler in binary format of the form:

Example: `@qry\n~~~~~~~` '~' = binary data

```
typedef struct
{

uint_8   bMethodNum; // method number being executed
uint_16  uiCurrentVial;  // Sample Vial (1-128, 201-204)
uint_8   bCurrentInjection;  // Injection # (1-99)
uint_8   bCurrentRinseStroke; //Indicate rinses completed
uint_16  uiMethodTime;  // how long method has been excuting
uint_16  uiRunTime;  // how long this injection has been executing
uint_16  uiStepNum;   // Current step
uint_8   bCurrentNeedleStroke; // Needle Rinse
uint_8   bStandardVial;   // Standard vial being accessed
uint_8   bRinseVial;   // RinseVial being accessed
uint_8   bWaitingForExternal; // waiting for an external
                                 injection?

} SRemoteQuery;
```

@QRYA **Query run status (return ASCII)**
Same as @qry but in ASCII format

Example: `@qrya`

@RDX **Read X-Axis position**
@RDY **Read Y-Axis position**
@RDZ **Read Z-Axis position**
Returns axis position in steps

Example: `@rdx`

@RMD # **Run Method # (1 to 10)**
Expects a Method number (from 1 to 10) to run. Method must be in memory. Checks the syringe size and tray type of the Method and then runs it

Example: `@rmd 7`

@RNP **Read needle position**
Returns the needle position in millimeters.

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Example: `@rnp`

@RPP **Read Priming Valve Position**
Returns RUN or PRIME

Example: `@rpp`

@RSIR **Read flow rate**
Returns the current syringe flow rate (in µL/sec)

Example: `@rsr`

@RSP **Read syringe position**
Returns the current syringe position in µL

Example: `@rsp`

@RSR **Read flow rate**
Returns the current syringe flow rate as either Slow, Med, or Fast. Used in conjunction with @ssp for Legacy code purposes.

Example: `@rsr`

@RSS **Read syringe size**
Returns current syringe size (programmed value).

Example: `@rss`

@RTS **read tray size**
Returns the current tray size (programmed value, it cannot read this directly). Returned values are:
128 for the 8 mm vial
72 for the 12 mm vial
50 for the 15 mm vial
28 for the 22 mm vial
MICROTITER for a microplate

Example: `@rts`

@RTT **Read tray temperature**
Returns the current tray temp in °C

Example: `@rtt`

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@RVN **Read Vial Number (1-max/201-204)**
Returns the current vial number (or none).

Example: `@rvn`

@RVP **Read Injection Valve position**
Returns INJECT or RUN.

Example: `@rvp`

@SAM **Set tray type and address mode**
Expects tray type, row or column major (R/C) and alphanumeric or numeric entry(A/N)
Valid Tray Types are:
8_mm 128 × 8 mm vial tray
12_mm 72 × 12 mm vial tray
15_mm 50 × 15 mm vial tray
PCS8_mm 7 × 11 8 mm vial tray
PCS12_mm 5 × 8 12 mm vial tray
22_mm 28 × 22 mm vial tray
micro 1 × 96 well microplate

Example: `@sam 12_mm,r,n`

@SIZ #### **Set syringe size (50, 250 or 2000)**
Tells the Autosampler what Syringe size is installed

Example: `@siz 50`

@SMD # # # **Stop Method**
Expects 3 parameters with spaces between each argument: Vial position of where to dump a (possibly) full syringe, how any rinses to do and at what rate

Example: @smd 0 1 20
Will dump to the Injection Port then do 1 Rinse at 20 µL/sec

@SSIP ### **Set flow rate to ### µL/sec**
expects a flow rate, ###, from 1 to the maximum for the syringe

Example: `@ssp 40`

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@SSP # **Set flow rate to S/M/F**

Set the syringe flow rate to **S**low, **M**edium, or **F**ast Speeds. Actual syringe flow rates are as follows:

Fast = Maximum Syringe Speed
Medium = (Maximum Syringe Speed) / 2
Slow = (Maximum Syringe Speed) / 4

Example: `@ssp F`

@STD **Set time and date**

Expects a time/date struct in binary with which to update its internal clock

Example: `@std\n~~~~~~~~` where '~' is as much binary data as is appropriate

@STT ## **Set tray temp to ## degrees**

Expects a temperature between 4 and 40C

Example: `@stt 21`

@SYR #### **Move syringe to ### µL position**

Move the syringe to an absolute volume, ###, (in µL). Expects a number corresponding to the µL position (0-Syringe max)

Example: `@syr 40`

@TPCL X **Turns temperature controller ON or OFF**

The argument X is ON or OFF. Allows Power to the built-in Temperature Controller to be controlled remotely

Example: `@tpcl OFF` turns the Temperature Controller OFF.

@ULM # **Upload method # FROM the client computer TO the autosampler**

Same as @dlm but other direction. Note that the form of the Method structure to send is describe under the @DLM command description

Example: `@ulm 9\n~~~~~~~~` where '~~~' represent as much binary data as is appropriate

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@VAL **Return validation status**
returns whether or not the software is validated.

Example: `@val`

@VLV $$$ **Set injection valve to Position $$$**
Expects a position LD or INJ (load/inject respectively)

Example: `@vlv LD`

@VRB # **Verbose**
Expects a 0 or 1 to turn off or on (respectively) verbose error reporting, so instead of getting -6 you get Err: Unknown Command

Example: `@vrb 1`

Note that the Autosampler can be placed in the Remote Mode by sending the ASCII "@" character. Since the instrument will assume the baud rate at which it was previously set, the controlling Host computer must also be set for this baud rate. The factory default baud rate is 4800.

Rear Panel

The Model 718AL Autosampler was designed to be interfaced with many different types of HPLC instrumentation, data systems, and controlling devices. Figure 2-4, The Rear Panel, shows the five different types of interfaces available on the Model 718AL plus the External Power Control Switch. These interfaces are the Contact Closure Strip, the BCD Connector, the RS-232-C Serial Connector/Centronics parallel printer port, and the Accessory Bus. Each of these is discussed below.

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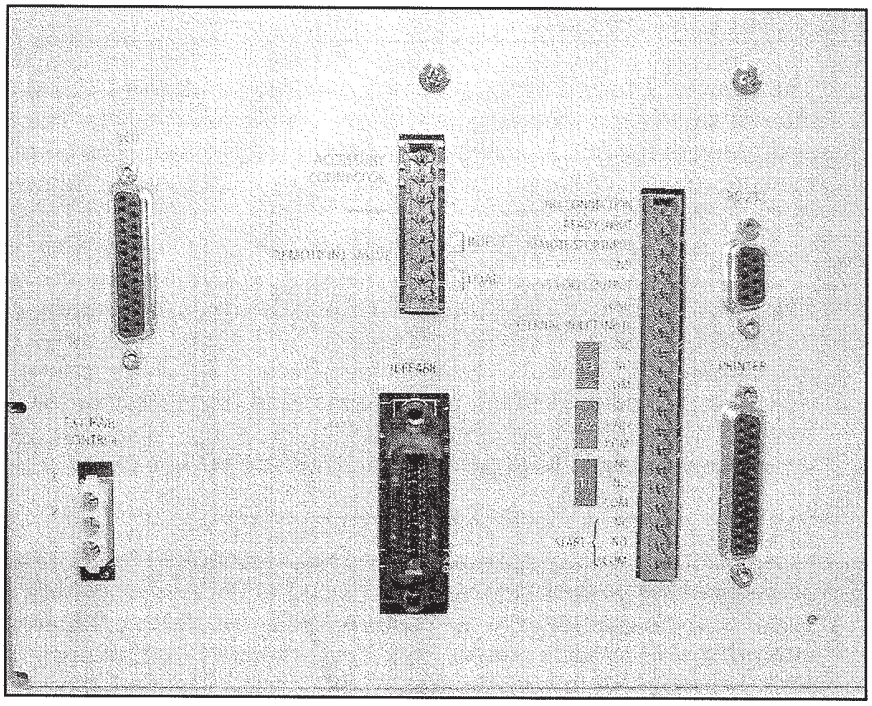


Figure 2-4: The Rear Panel

The External Power Control Switch

The External Power Control outlet on the rear panel provides an electrical signal for actuating a solid state relay which turns on AC Power to external hardware such as a strip chart recorder or an HPLC pump by means of the External Power Control Accessory (ACI P/N 728-33010-00). The External Power Control outlet is primarily controlled by the POWER switch. It is activated when the Autosampler's Power switch is turned ON. It will be turned OFF by one of four conditions: (1) when the Power switch is turned OFF, (2) when the Autosampler completes the last injection from the last vial in a Method, (3) when it is toggled OFF by pressing the [F1] ([FUNCTION] [1]) Key or (4) when a hardware failure occurs with the Autosampler. It is turned back ON when a Method is started. Note that the External Power Control Switch stays ON if a Method is manually terminated by pressing the [Run/Stop] Key while the Method is running. Also note that the [F1] ([FUNCTION] [1]) Key, toggles the External Power Control Switch ON or OFF.

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The Contact Closure Strip

The Contact Closure Strip is the nineteen pin connector located on the left side of the group of connectors on the Rear Panel. The pin assignments for this connector are given in Table 2-6. The Contact Closure may be either normally open or normally closed. Selection is made by using the appropriate pairs of pins as shown in Table 2-6. Note that Pin 1 is at the bottom of the Contact Closure Strip.

The time duration of the Contact Closures is adjustable within a range of 1 to 32000 seconds in 1 second increments. For most instruments, the 1 second duration is sufficient; however, consult the operator's manual of the instrument being interfaced for the best duration time specification. The duration time is set by editing the System Settings as detailed in the [SYSTEM] key description.

The INJECTION MARK or **START** Contact Closure, located at pins 1 and 2 normally open or pins 1 and 3 normally closed, always actuates at the moment the valve is placed in the Inject position and is not user programmable. Its primary use is for starting integrators and other data systems, or for initiating gradient programmers. Pins 4 through 12 are the programmable Timed Events 1 through 3. These switches can be used for an assortment of applications, such as valve switching and fraction collector control.

Pins 13 and 14 are the External Inject Control. These accept an external contact closure from another device such as an integrator laboratory computer, or HPLC Gradient Controller and are used to let an external device control the time when the Autosampler loads and injects a sample. However, a Method must still be edited in order to use the External Inject Control Mode. To activate the External Inject Control, enter a "0" for the Run Time parameter (see Section **Editing a Method**). When in the External Inject Mode, the Timed Event 3 relay will energize and can be used as a "Ready" signal for HPLC controllers which require such a "Ready" signal before sending the Remote Start signal. Thus Timed Event 3 should not be used for other purposes when performing External Injections.

Pin 18 is called READY. It is pulled high internally. Any device in the chromatographic system may pull this signal low to suspend injections by the Autosampler. The Autosampler will resume when READY is released. This signal will work whether the Autosampler is controlled Internally or Externally.

Pins 15 and 16 are a +5 volts and Ground source, respectively. They may be used in conjunction with the Contact Closures for those devices which require a +5 volt pulse for actuation.

Finally, a remote switch closure across pins 17 and 14 will cause the Autosampler to stop. The unit will remain off until the remote switch is opened. The Autosampler will then prompt the user in

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the same fashion as a [RUN/STOP key] aborted run as described in **Chapter 2, Running a Method**.

The BCD Connector

The Binary Coded Decimal or BCD Connector is a 25 pin, D-type connector located on the Rear Panel. The BCD Connector provides vial code information for integrators and other similar devices. Table 2-7 lists the pin assignments for the BCD Connector.

An eight bit, BCD code representing the sample vial number is present on pins 1 through 6 and 14 through 19. Pins 10 through 13 and 22 through 25 represent injection number. The code is positive-true, TTL compatible. It is 5 volt CMOS compatible if 4.7 kΩ resistors are used. Table 2.8 illustrates its use.

The RS-232 Connector

The RS-232 connector is a industry standard, 9 pin, D type connector.

The Printer Port

The Centronics compatible parallel printer port allows any personal computer printer equipped with a parallel interface to be connected to the Autosampler. This capability allows the Autosampler to print out Methods.

The I^2C Accessory Bus Connector

The Accessory Bus Connector is used to attach other Alcott accessory products which can be controlled by the Autosampler. Refer to the particular accessory's manual for details.

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Table 2-6: The Contact Closure Pin Assignments

PIN	FUNCTION
	Top of Connector
19	No Connection
18	Ready Input
17	Remote Stop Input
16	Ground
15	+5 Volts
14	Ground
13	Ext. Inject Input
12	Timed Event 3-NC
11	Timed Event 3-NO
10	Timed Event 3-COM
9	Timed Event 2-NC
8	Timed Event 2-NO
7	Timed Event 2-COM
6	Timed Event 1-NC
5	Timed Event 1-NO
4	Timed Event 1-COM
3	Injection Start-NC
2	Injection Start-NO
1	Injection Start-COM
	Bottom of Connector

COM = Common, NO = Normally Open, NC = Normally Closed

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Table 2-7: The BCD Connector Pin Assignments

PIN	FUNCTION	PIN	FUNCTION
1	Vial units, 2^0	14	Vial units, 2^1
2	Vial units, 2^2	15	Vial units, 2^3
3	Vial tens, 2^0	16	Vial tens, 2^1
4	Vial tens, 2^2	17	Vial tens, 2^3
5	Vial hundreds, 2^0	18	Vial hundreds, 2^1
6	Vial hundreds, 2^2	19	Vial hundreds, 2^3
7	Ground	20	Ground
8	Ground	21	Ground
9	Ground	22	Injection units, 2^0
10	Injection units, 2^1	23	Injection units, 2^2
11	Injection units, 2^3	24	Injection tens, 2^0
12	Injection tens, 2^1	25	Injection tens, 2^2
13	Injection tens, 2^3		

Table 2-8: Vial Code Number

	RANGE							
	TENS				ONES			
Pin No:	17	4	16	3	15	2	14	1
Decimal Value:	80	40	20	10	8	4	2	1
*Example Code:	0	1	1	0	1	0	0	1
Decode	0 +	40 +	20 +	0 +	8 +	0 +	0 +	1
							Vial =	69

*0 is logic low, 1 is logic high

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OPERATION

This chapter describes the general operation of the Model 718AL Autosampler.

Loading Sample Vials

The Model 718AL can use any screw cap, snap cap or crimp top vial with 8 mm, 12 mm, or 15 mm vial diameters. Regardless of the type used, the vial should be filled to within ~ 6 mm (1/4 in.) of the top. Overfilling the vial can lead to cross contamination and carryover problems.

NOTE: Do not use the 8 mm caps designed for use with the Alcott Model 708 Positive Displacement Autosampler with the Model 718AL. The use of these caps will result in a bent needle! Use the correct 8 mm cap listed in Appendix B.

Note that vials must be placed into the Autosampler in the correct orientation. The trays for the 8 mm, 12 mm, or 15 mm vials are mounted into the Autosampler so that the numbers can be read when facing the front of the instrument. Make sure that the tray is firmly and squarely placed into the mounting space to prevent needle misalignment.

Sample Preparation

It is important that samples be free of particulate matter which may plug the needle or tubing. Metallic or gritty particles will cause damage to the valve and may plug the chromatographic system. Samples should be filtered prior to filling the vials using at least a 0.45 μm filter. For best results, make sure to use the filter type appropriate for the sample to be analyzed.

Operating Parameters

Operating parameters must be selected before running samples. The first decision to make is which type of injection process is to be used, either Fixed Loop Injections or Partial Loop Fill Injections. The former offers somewhat better injection reproducibility at the cost of high sample consumption, while the latter allows variable size injections with a minimum of sample.

Sample solution viscosity and/or its compressibility should be considered when determining the appropriate Injection Parameters. Table 2-2 in the previous chapter lists the flow rates associated with each Fill Rate for each syringe size. Liquids with low viscosity or which have a low solvent compressibility such as

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Acetonitrile, can use a faster Fill Rate than those which have a higher viscosity or which have a high degree of compressibility. The Injection Delay Time should be selected with the same degree of care. Both of these parameters may affect injection reproducibility.

Retention time is an important factor. The time setting should be sufficiently long for each injection to elute before the next injection is started. It may be necessary to allow time for baseline analysis and calculations when using a computing integrator.

Further information about operating parameters is given in **Appendix C, Factors Which Affect Injection Precision and Accuracy.**

Needle Depth

The Model 718AL allows the user to select the depth at which the Needle penetrates into the sample vial. The fully extended Needle Depth is 48 mm and the Autosampler can be programmed to extend the Needle any distance between 1 and 48 mm. A depth of 48 mm will place the tip of the needle to the very bottom of most flat-bottomed, 8, 12, or 15 mm (OD) sample vials. Care must be exercised in setting this parameter, especially when using coned bottom vials or vials equipped with low volume inserts. When in doubt, use a ruler to measure the distance, in mm, from the bottom of the vial to the bottom of the insert. Subtracting this distance from 48 mm provides the value to be use for the Needle Depth.

Power

The POWER switch must be set in the ON position to supply power to the Autosampler and to actuate the External Power Control connector. Additional information is given in **Chapter 2, The Rear Panel**.

Entering a Method

As an example of how to enter a Method, a sample program will be created which will make four, 20 µL, Partial Loop Fill injections per vial from ten samples. The Method will start with vial number 1 and will go to vial 10. The Run Time will be 15 minutes and Timed Event 1 will be used five minutes into the run. The syringe will be set up to operate at its fastest fill rate with a 5 second delay time. The needle will be programmed to enter the vial at a depth of 45 mm and a 5 µL Pre-injection Volume of Mobile Phase will be used to push the sample into the loop. No air bubble segments or External Needle Rinse will be used and the final Needle Port Rinse will consist of 2 syringe strokes. Method 5 will be used for this example and only one Step will be required.

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Turn the Autosampler ON by pushing the Power Switch into the ON position. The Autosampler will initialize itself by performing a series of self-tests. After initialization, the following screen will appear on the Display:

```
READY

SELECT A FUNCTION
```

To begin Method entry, press the Method key by first pressing the [FUNCTION] key followed by pressing the [7] key. The resulting screen on the Display will be:

```
   METHOD MENU
→Edit Method
 Run Method
 Copy Method        ↓
```

At this point, the desired menu item is Edit Method and since the right arrow,→, is already pointing at this item, only the [ENTER] key need be pressed. This results in the following screen:

```
Enter Method Number

          #  ■
```

As stated above, Method 5 will be used in this example. To use Method 5, press the [5] key followed by the [ENTER] key. The following screen results:

```
METH  5 INJECT PARAM
→Inject Type  PART>
 Withdraw Rate   50
 Loop Fill Rate  50↓
```

Since this is a new Method, the Injection Parameters must be edited first. The syringe Injection Type is the first parameter and the default type is PART for Part Loop Fill which is the type to be used in this example. Simply press the [ENTER] key to move to the next menu item:

```
METH  5 INJECT PARAM
 Inject Type  PART>
→Withdraw Rate   50
 Loop Fill Rate  50↓
```


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After the Injection Type is selected, the syringe Withdraw Rate, followed by the Loop Fill Rate are set. The default rate of 50 µL per second is to be used for both and the [ENTER] key is pressed twice:

```
METH  5 INJECT PARAM
 Withdraw Rate   50↑
 Loop Fill Rate  50
→Delay Time       5↓
```

Next, the Injection Delay Time is set. Again, the default time of 5 seconds is to be used and only the [ENTER] key need be pressed:

```
METH  5 INJECT PARAM
 Loop Fill Rate  50↑
 Delay Time       5
→Needle Depth    40↓
```

As stated in the opening paragraph, a Needle Depth of 45 mm will be used in this example. To change the default value of 40 to 45, press the [4] key and then the[5] key followed by the [ENTER] key. The resulting screen will be:

```
METH  5 INJECT PARAM
 Delay Time       5↑
 Needle Depth    45
→Pre-Inject Vol   5↓
```

The Pre-Injection Volume of Mobile Phase will be 5 µL in this example. This is also the default value for this parameter so that only the [ENTER] key need be pressed. After pressing [ENTER], the screen becomes:

```
METH  5 INJECT PARAM
 Needle Depth    20↑
 Pre-Inject Vol   5
→Pre-air Vol      1↓
```

No air bubble segmentation is used in this example, therefore, the Pre-air Vol (Pre-Injection Air Bubble Volume) is 0. Press [0] then [ENTER]:

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```
METH  5 INJECT PARAM
 Pre-Inject Vol    5↑
 Pre-air Vol       0
→Withdraw Vol     10↓
```

Since this is a Partial Loop Fill example, this parameter has no effect. Simply press the [ENTER] key to bypass.

```
METH  5 INJECT PARAM
 Pre-air Vol       0↑
 Withdraw Vol     10
→Post-air Vol      0↓
```

Again, since no air segmentation is used, press [0] followed by [ENTER]:

```
METH  5 INJECT PARAM
 Withdraw Vol     10↑
 Post-air Vol      0
→Rinse Strokes     1↓
```

The final Injection Parameter is the number of Rinse Strokes for ***internally*** washing the Needle Port. For the example given here, the value is two strokes. Press the [2] key and then [ENTER]. The screen will then show:

```
METH  5 INJECT PARAM
 Post-air Vol      0↑
 Rinse Strokes     2
→Rinse Rate      <20↓
```

Following Rinse Strokes is the Rinse Rate. This is the flow rate use by the syringe during ***internal*** rinsing. The default rinsing flow rate of 20 µL/sec will be used. Therefore, press [ENTER]

```
METH  5 INJECT PARAM
 Rinse Strokes     2↑
 Rinse Rate      <20
→Exit
```

The Rinse Rate is the last injection parameter. Pressing [ENTER] at this time will cause the Step editing menu to appear:

```
 METHOD  5   STEP  1
→Start Vial        1
 Final Vial        1
 Standard Vial NONE↓
```


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As previously stated, this example will make four injections per vial from ten samples starting with vial 1. The right arrow,→, is already pointing to Start Vial and the default value, position 1, is the desired value so press the [ENTER] key. The Display will show:

```
METHOD  5    STEP  1
Start Vial        1
→Final Vial       1
Standard Vial NONE↓
```

The Final Vial will be vial 10. Press [1] [0] and [ENTER]. The Display will show:

```
METHOD  5    STEP  1
Start Vial        1
Final Vial       10
→Standard Vial NONE↓
```

No Standard Vial is to be used, to skip this parameter, simply press [ENTER]. The Display will be changed to:

```
METHOD  5    STEP  1
Final Vial       10↑
Standard Vial NONE
→Injections       3↓
```

The number of injections per vial in this example is four. To enter, press the [4] key and press [ENTER]:

```
METHOD  5    STEP  1
Standard Vial NONE↑
Injections        4
→Inject. Vol     10↓
```

The Injection Volume in this example is 20 µL. To enter this value, press the [2] key, the [0] key and the [ENTER] key. The screen will change to:

```
METHOD  5    STEP  1
Injections        4↑
Inject. Vol      20
→Run Time       1.0↓
```


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The chromatographic Run Time in this example is 15.0 minutes. This is entered by pressing [1] [5] [0] and [ENTER]. The screen now becomes:

```
METHOD  5    STEP  1
Inject. Vol     20↑
Run Time       15.0
→Event1 Time    0.0↓
```

As per the example, Timed Event 1 will be set to 5.0 minutes. This is accomplished by pressing [5] [0] and [ENTER] resulting in the screen:

```
METHOD  5    STEP  1
Run Time       15.0↑
Event1 Time     5.0
→Event2 Time    0.0↓
```

Neither Timed Event 2 nor Timed Event 3 will be needed in this example. These can be conveniently skipped by pressing the [↓] key twice. The following screen will appear:

```
METHOD  5    STEP  1
Event2 Time     0.0↑
Event3 Time     0.0
→Rinse Vial   NONE>↓
```

Since no External Needle Rinse is to be used, the Method is essentially complete. No further Steps are required. Press the [↓] three times to the Exit command and then press the [ENTER] key. This will return the display back to the Edit Menu Screen:

```
 EDIT METHOD  #  5
→Edit Step
 Append Step
 Insert Step      ↓
```

None of the functions on this screen are needed. To escape from this screen press the [FUNCTION] key and then the [CLEAR] key. This will result in the display of the Main Screen:

```
        READY

SELECT A FUNCTION
```

At this point, the Method is ready to run.

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Running a Method

There are two ways to start a Method. Methods can be initiated from the Main screen or from the Method Menu Screen.

From the Main Screen, which is the default screen on power-up:

```
READY

SELECT A FUNCTION
```

press the [RUN/STOP] button. The following screen will appear:

```
Enter Method Number

#  ■
```

From this screen, simply key in the desired Method number and press [ENTER].

The alternative approach is to use the Method Menu Screen. At the end of the discussion in the previous section, **Editing a Method**, the editing session ended with the Autosampler in the Method Menu Screen:

```
   METHOD MENU
→Edit Method
 Run Method
 Copy Method        ↓
```

To execute a Method from this screen, press the [DOWN ARROW] key and then the [ENTER] key. This will result in the same screen as before:

```
Enter Method Number

#  ■
```


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to which the desired Method number is keyed in and the [ENTER] key pressed.

Making Manual Injections

The [MANUAL INJECTION] key ([FUNCTION] [8]) allows the user to manually load the injection valve using a syringe equipped with a 22 gauge needle. When pressed, the following screen:

```
 MANUAL INJECTION
<←> OR <→>  TO MOVE
<RUN/STOP>  TO EXIT
POSITION:      LOAD
```

is displayed and the Injection Valve is placed in the LOAD position. While in the Manual Injection mode, pressing the LEFT ARROW key, [←], will place the valve in the INJECT position. Pressing the RIGHT ARROW key, [→], will place the valve in the LOAD position. Pressing the [RUN/STOP] key will return the instrument to the Main Display.

To make a Manual Injection, remove the Needle Guide from the Loop Filler Port of the Injection Valve. Next, put the Valve in the LOAD position by pressing the RIGHT ARROW key, [→]. Then load the sample into the Sample Loop by using a syringe equipped with a 22 gauge needle. The Sample Loop can either be partially filled or totally filled. Finally, once the sample is in the loop, press the LEFT ARROW key, [←], to put the Valve into the INJECT position. For best results, leave the syringe in the Loop Filler Port when switching the Valve from the LOAD to the Inject position.

To leave a Manual Injection session, replace the needle guide and press the [RUN/STOP] key.

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Remote Control and Instrument Interfacing

The Contact Closure Interface

A Contact Closure is simply a remote ON/OFF switch. It is available in two varieties, NO for Normally Opened or NC for Normally Closed. Normally Opened means the switch is open (no electrical contact or current flow). When a NO contact closure is activated, the switch closes, allowing electrical contact for current to flow. Normally Closed contact closures act in just the opposite fashion. These switches are closed, allowing electrical contact for current flow. When actuated, the switch opens up, breaking electrical contact and halting current flow.

The type of contact closure used depends entirely on the external device the Autosampler is trying to control. The instruction manual for the external device indicates which type to use. The Autosampler supports both NO and NC contact closures, and although there is no standard in the industry, most chromatographic instruments use NO contact closures.

To illustrate how Contact Closures are used, the following example is presented. To start the integrator after the appropriate integration parameters have been entered, the user presses the Start Integration key on the front panel keyboard. After this key is pressed, the integrator begins taking and processing data. The User usually, but not always, enters an integration method which specifies among other things, the duration time of the data acquisition phase. After this designated integration time period, the integrator stops taking data and prints the integration results. In an automated system, the Autosampler will press the button for you. It does this by using the Injection Mark contact closure on the back of the Autosampler to close a switch across the "Remote Start" contacts on the back of the integrator. Most integrator companies offer a Remote Start cable for their instrument.

The length of time which a contact closure must stay closed (or open) depends entirely on the device to be controlled. The instruction manual for the device to be controlled should indicate how long a closure (or opening) time to use. A one second (1 sec) contact closure time is adequate for most integrators and data systems, although some older models may require up to two seconds (2 sec). Other devices, such as some fraction collectors, may require the contact closure to stay Closed (or Open) for the entire run. This parameter is adjusted using the [SYSTEM] key. Refer to the section **System Settings** in Chapter 2 for setting the Contact Closure time duration.

The Model 718AL can be interfaced to the data (or chromatographic) system in one of two ways. The simplest interface is to let the Autosampler control the system. Under these conditions, the Autosampler remotely signals the system when to start. Stopping the data system (or chromatographic system) can

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be handled in one of two ways. The first way is to program the system to stop after a specified length of time. For example, suppose an analysis requires 10 minutes for the separation to develop. The User would program the data system to take data for 10 minutes, after which it would stop and print out the integration results. The Autosampler in this case would be programmed to make an injection every 10.5 to 11 minutes. The extra time over 10 minutes between injections in this example is used to allow for the printing of results.

The second interfacing technique is to let the Autosampler stop the system as well as start it. In this case, one of the Autosampler's Auxiliary contact closures is connected to the "Remote Stop" signal of the system. To use our 10 minute example given above, the Autosampler will be programmed to make an injection every 10.5 to 11 minutes. The Auxiliary contact closure, which will stop the system, will be programmed to close after 10 minutes. As in the previous example, the extra time over 10 minutes between injections is used for the printing of results.

Although not as common, the system can also control the Model 718AL. A bi-directional line of communication must be established between the Autosampler and the controlling system. The system will send a signal to the Autosampler telling it to make an injection. This is done through the Autosampler's External Inject signal detection. After receiving this signal, the Autosampler will load the sample loop and will inject the sample into the chromatograph. Upon injection, the Autosampler can send a signal back to the system informing it that an injection has been made by using the START closure. The External Inject signal can be synchronized by using the Timed Event 3 contact. After this contact is energized, the Autosampler is ready for the External Inject Input from the controlling system.

The Autosampler still must be programmed for the number of vials, the number of injections per vial, and Rinses. Method Run Time is set to 0. After the Method is set up for the External Inject Mode, it is started as described in the section **Running a Method**. The Autosampler, although running at this point, will not do anything until it receives the External Inject signal from the controlling system.

When using the External Inject Mode, the system must establish two lines of communication with the Autosampler in which it:

1. Must send a signal to the Autosampler to make an injection.

2. Must suspend the data acquisition and/or the solvent gradient until it has received the injection signal back from the Autosampler in order to properly synchronize these events.

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The BCD Interface

The BCD Interface, an option with the Model 718AL, allows the data system to read the vial position and/or the injection number of the sample the Autosampler has just injected in order to print out the vial and injection number with the integration report. Pin assignments and a description of how the BCD interface works is given in the section **The BCD Connector** in Chapter 2.

Autosamplers equipped with the BCD Interface are shipped with an interface cable. The cable has a 25-pin "D" type connector on one end, which mates with the Autosampler's BCD receptacle, while the other end is bare cable. This allows users to fabricate their own interface cable. Alcott Chromatography offers complete BCD cables for integrators manufactured by Thermo Separations (the former Spectra Physics line) and Hewlett Packard. Part numbers for these cables are listed in the spare parts list given in APPENDIX-B. Alcott Chromatography will custom fabricate BCD cables for other instruments for a nominal fee. The user must supply complete schematics and connectors for the instrument to be interfaced. For details on custom BCD cables, contact the nearest Alcott Chromatography representative or the Factory at (800) 229-5052.

RS-232-C Control

The Model 718AL can be remotely controlled by a computer through an optional RS-232-C serial interface. This allows any computer, which supports these interfaces, to remotely control the Autosampler through a suitable program in any computer language of the choice. All aspects of the instrument can be controlled.

Table 3-2 is a very rudimentary RS-232-C control program for MSDOS, written in Microsoft BASIC. Note that it does no error checking, however, it does illustrate the basic steps used to control the Autosampler.

Alcott Chromatography offers a simple, Windows 95/NT based program for the remote control of the Autosampler.

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Table 3-2: Simple Microsoft BASIC RS-232-C Control Program

```
100 ' ***  OPEN COM1 FOR RS-232 ***
110 OPEN "COM1:4800,N,8,1,RS,CS,DS,CD" AS #1
120 ' ***  SEND CARRIAGE RETURN TO COM1 ***
130 PRINT #1, CHR$(13)
180 ' *** GO TO VIAL POSITION 25 ***
190 PRINT #1,"@GTV 25" + CHR$(13)
200 ' *** LOWER THE NEEDLE 30 mm ***
210 PRINT #1, "@NDL 30" + CHR$(13)
220 ' *** WITHDRAW 25 uL SAMPLE ***
230 PRINT #1, "@SYR 25" + CHR$(13)
240 ' *** RAISE NEEDLE  ***
250 PRINT #1, @NDL 0" + CHR$(13)
260 ' *** GO TO NEEDLE SEAT ***
270 PRINT #1,"@GTV 0" + CHR$(13)
280 '*** PUT VALVE IN LOAD POSITION ***
290 PRINT #1, "@VLV LD" + CHR$(13)
300 ' *** LOWER THE NEEDLE 19 mm ***
310 PRINT #1, "@NDL 19" + CHR$(13)
320 ' *** DEPOSIT SAMPLE IN VALVE***
330 PRINT #1, "@SYR 0" + CHR$(13)
340 '*** PUT VALVE IN INJECT POSITION ***
350 PRINT #1, "@VLV INJ" + CHR$(13)
360 ' *** CLOSE RS-232 PORT ***
370 CLOSE #1
380 END
```


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IN CASE OF DIFFICULTY

Non-Reproducible Injections

Non-reproducible injections are usually due to problems such as fitting leaks, plugged tubing, poorly swaged fittings, or submerged waste tubes. In some instances, non-reproducibility may appear to be injector related when, in fact, it is caused by other components in the chromatographic system. Systematically analyze the entire system to assure that efforts are expended on the proper component before undertaking any repair.

Leaks

Plumbing leaks are the simplest causes of non-reproducibility to detect. Leaks affect reproducibility by diverting solvent from the column in an unknown amount, often resulting with a longer sample elution time because of the reduced solvent flow. Places to check for leakage are the syringe, the transfer line between the needle and the syringe, the needle, injector loop, and injector seals. After the leak has been found, either tighten the fitting or replace the fitting, should tightening not be sufficient.

A leaky syringe can also indicate a restriction downstream from the syringe. Possible causes for the restriction are a plugged needle, needle port, or sample loop. The syringe will also leak if the loop tubing internal diameter (ID) is too small for the syringe size installed. For example, loops with ID's less than 0.020 inch (.508 mm) are too small to be used with the 2000 µL syringe. When using the 2000 µL syringe, the slowest fill rates are recommend

Vial Venting

The Model 718AL incorporates the same, standard 22 gauge (0.028 inch OD) needle found on all HPLC injection valve syringes. This type needle provides no venting. Some septa types, such as the PTFE-Silicon-PTFE sandwich type, can form a very tight seal around the needle, especially the first time the septum is pierced. This tight seal can cause some slight cavitation in the syringe on the first injection from a new vial. The symptom for this effect is that the first injection from a vial will have a noticeably smaller peak area or peak height for the first injection. To eliminate this problem, use the Pre-Pierce option in the Injection Parameters item in the Method Step Editing Menu and use thoroughly degassed priming solution.

Do not overfill Snap-Cap type, Shell vials of any diameter. These vials can intermittently exhibit larger first injections if they are over filled. The recommended fill level for these vials is 3/4 the height or less. Samples in solvents with high vapor pressures should not exceed 1/2 the height.

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Air in the Syringe

Air in the syringe or needle line, especially air bubbles larger than 10 µL, is the major cause of reproducibility problems because it changes the compressibility of the sample fluid being aspirated by the syringe. The main sources of air in the syringe are:

1. The syringe has not been screwed into the priming valve tight enough.
2. The o-ring which forms the seal between the priming valve and the syringe is worn and needs replacing.
3. The plunger tip is worn and air is being aspirated into the back of the syringe.
4. The sample is too viscous or the syringe fill rate is too fast. The syringe is cavitating and air is being aspirated into the back of the syringe.
5. The Priming Solvent is not degassed sufficiently.

Note: Number 3 and 4 above produce the same symptom. Number 3 should be suspected if the problem occurs on a sample and solvent system which previously caused no problem. If problem 4 is suspected, try a slower fill rate.

The syringe should be reprimed if any of these problems are suspected and repaired. Normal front panel priming should be sufficient in most incidences, however, the 50 µL syringe, because of its small volume and solvent surface tension effects, can be troublesome to reprime and may have to be removed from the instrument and manually reprimed. Consult **Chapter 5, Routine Maintenance and Parts Replacement** for details concerning manual priming.

The best way to eliminate air bubble problems is to use thoroughly degassed priming solution. The use of a continuous Helium system or a small inline Vacuum degasser is highly recommended.

Plugged Tubing

Plugs and partial plugs due to debris may occur in the needle, the needle transfer line, the injector loop, or the injector body. Plugs prevent proper loading of the syringe or sample loop. Disconnect both ends of the transfer line tubing and use a syringe to force solvent through to check for blockage. There should be no difficulty in forcing solvent freely through 0.010-in. ID tubing. Force solvent through the needle in the same fashion. There should be no difficulty in forcing solvent freely through the needle.

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Replace the needle or the tubing if either remains plugged and resume operation.

Remove the sample loop from the injector valve body and check it for blockage with a syringe if the problem persists. Partial blockage of the loop will prevent accurate and consistent sample loading. Follow the procedures in the instruction manual for the Valco Valve included in the accessories if loop replacement is necessary.

Certain types of septa, such as those made from natural rubber or silicon rubber, can sometimes cause the needle to plug due to coring. This can be a frequent occurrence if the needle tip is burred or flanged. Alcott offers a narrow bore needle, ACI P/N 718/25881/00 for use with septa susceptible to coring. Note that this needle has a very small, 0.006 inch ID bore. It is recommended that the slower syringe fill rates be used with the small bore needle, especially when the 250 and 2000 µL syringes are installed as the smaller bore cause an increased restriction in the needle/transfer-line flow path!

Poorly Swaged Fittings

Non-reproducibility due to dead volume caused by poorly swaged fittings usually occurs after incorrect installation of new loops or other connections or after changing previously swaged loops. A gap as small as 0.05 mm (0.002-in.) between the end of the tubing and the shoulder inside the female portion of the fitting will have a dead volume of 0.1 uL with 1/16-in. OD tubing. This type of irreproducibility always exhibits one of two peak height patterns. The patterns are observed by setting the Autosampler to make three injections per vial. In the first pattern, the heights of the peaks in the first of the three injections tend to be smaller than the heights of the peaks for the second two injections, which are usually about the same size. In the second pattern, larger peak heights on the first injection followed by smaller but equal peak heights for the second two injections are observed. Observation of either pattern requires complete replacement of the loop.

Carefully follow the procedures in **APPENDIX-A** for correct assembly of ferrule type fittings and the Valco Valve instruction manual for loop replacement procedures.

When using a previously swaged tube, be sure to use it with the fitting in which its ferrules were originally set. Small inconsistencies in the machining of the fitting are a potential source of dead volume.

Leaking Injection Valve

The probable cause of injection valve leakage is a worn or damaged seal. Consult the Valco Valve Users Manual included in the accessories for the Model 718AL for information on seal replacement.

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Missed Injections

A plugged needle should be suspected if an injection is missed. This can readily be determined by first removing the needle from the Autosampler as described in **Chapter 5, Routine Maintenance and Parts Replacement**, and checking the needle for blockage with a small wire such as a "cat whisker" or a light gauge, electric guitar string.

The injection valve should be checked if sample is not being injected or occasional injections are missed and the needle is not plugged. If the valve has had a recent rotor seal replacement, the problem is that the replacement rotor has been installed 180° out of phase. The remedy is to reinstall the rotor in the correct orientation. Should the valve rotate and sample is not placed on the column, refer to **Chapter 5, Routine Maintenance and Parts Replacement** and check for plugs or leaks. If the valve is at fault, consult the Valco Valve User's Manual located in the Accessories.

Carryover

Carryover is the result of remaining residue left either inside the Needle or on its external surfaces. A loose Injection Port will also result in carryover. Carryover manifests itself most when either concentrated samples are injected, or samples which have an affinity for any of the wetted parts in the Needle/Transfer Line network. If carryover is a problem, first check that the Injection Port is sufficiently tightened. If the Injection Port checks out fine, the next step is simply to incorporate additional rinsing into your Method. There are two types of Needle rinsing procedures used in the Model 718AL- Needle Port Rinsing and External Needle Rinsing.

Needle Port Rinsing

Needle Port Rinsing occurs immediately after the Injection Valve has switched to the INJECT position during an injection sequence. The Rinse Rate and the number of Rinse Strokes are set in the Injection Parameters Menu. This rinse washes the internal surfaces of the Needle, Transfer Line, the Loop Filler Port, and the Syringe.

External Needle Rinse

This rinse occurs after the Needle has withdrawn sample from the vial and before it is inserted into the Loop Filler Port. The External Rinse Vial and the number of Rinse Strokes are entered after the Timed Events in a Method Step. This rinse washes the external surface of the Needle only.

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Error Messages

There are two categories of Error Messages that the Autosampler can display. Entry Errors are errors usually caused by the user making an illegal keyboard entry or selection. These errors are identified by a flashing "ERROR" message displayed on the top line of the Display and an audible beep sound.
System Error Messages occur after a mechanical fault has been detected in the instrument. Service is usually indicated when a System Error occurs. An audible beep sound usually occurs. If the Autosampler is being operated under remote computer control by the RS-232 interface, the error message will be sent in the form of a coded number.

Entry Error Messages

"Unable to execute command while Method is running."

An attempt was made to select an operation that is not allowed to execute while a Method is in operation, e.g. a Manual Injection or a Manual Rinse.

"Temperature Control Module is NOT installed"

The Temperature option was selected, via [FUNCTION] [5], but the temperature controller module and card are not installed or are installed improperly.

"Value out of range. Enter value between xx - yy"

A value was entered for a parameter that is not in the parameter's allowable range. The value must be between xx and yy.

"A Method is NOT currently running"

The Method Status display was requested (from the Method Menu), but there is no Method running.

"Maximum number of Steps for Method is 50"

All 50 Steps in a Method have been programmed and an attempt was made to add or insert a Step.

"No Steps programmed!"

An attempt was made to delete a Step in an unprogrammed Method, i.e. the Method contains no Steps.

"Final vial # must be greater than or equal to start vial."

An attempt was made to program the final vial in a Step to a value less than the start vial. The final vial must always be ≥ the start vial.

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"Cannot delete a Method that is currently running"

An attempt was made to delete a Method while that Method was running.

"Cannot execute! Autosampler is currently running."

An attempt was made to begin an operation while the Autosampler is currently running an operation. For example, if a Manual Injection is running and an attempt was made to run a Method.

"Method Number does NOT exist!"

An attempt was made to run, delete, or copy a Method that is unprogrammed.

"Priority Sample currently running."

An attempt was made to begin a priority sample while a priority was already running.

"Maximum syringe volume exceeded"

The combined volume of the pre-injection Mobile Phase, pre-injection air bubble, post-injection air bubble, and sample exceeded the maximum volume of the selected syringe. Volumes must be reduced or a larger syringe installed.

Note: If a Method was programmed with a specific syringe installed and later the syringe size is changed to a smaller size, the Autosampler will not automatically check that the total volume programmed does not exceed the volume of the smaller syringe. If the sample volume is ever edited, the Autosampler will then notice that the volume is too large for the syringe and induce an error. If the Method is started without editing the sample volume, the Autosampler will first draw the appropriate amounts of pre-injection Mobile Phase and pre-injection air bubble. Second, the syringe will draw as much sample as possible without exceeding the syringe volume. Finally, no post-injection air bubble will be drawn.

"Must use Sample Vial or a Standard Vial, but not both"

When programming a Priority sample or a Manual Injection, only one sample vial may be selected, a normal sample vial or a standard vial.

"Unable to Print. Paper out!"

An attempt was made to print, but the printer is out of paper. Check the printer to assure it has paper before attempting to print again.

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"Unable to print. Printer is off line!"

An attempt was made to print but the printer is off line. Switch the printer to on line and assure that it does not have an error before attempting to print again.

"Unable to print. Printer is not ready!"

The printer is not ready to print. Check to see if power is on, printer is on-line and does not have an error condition before attempting to print again.

"Lost synchronization with printer."

Printer was printing but does not accept any more data. Check if printer is still on-line and error free. If printer is OK and problem persists, then printer buffer could be overflowing. Try a different printer with a larger printer buffer (more memory).

"Cannot print while motor is running"

The Autosampler can print while running a Method, but it will not send any characters to the printer while any motor is running. The unit will typically wait for the motor to stop running and then continue printing. If a motor is on for more than one minute, the Autosampler will time out and issue this error. Try printing again when a motor is not running.

"Temperature setpoint was not reached within 90 minutes"

The sample tray did not reach the desired operating temperature within 90 minutes. This error usually occurs when the instrument is asked to reach and maintain an operating temperature which exceeds the ΔT°C specification (24°C). For example, trying to maintain the tray at 4°C while the room temperature is 30°C.

"Source Undefined"

An error occurred but the origination is unclear or unknown. This error should not occur. If it does, then a problem probably exists with the Autosampler's internal memory.

System Error Messages

"Row X Drive Motor Stepping Error"
RS-232-C response: -28

The motor drive assembly that moves the needle carriage left and right is not working properly. This error generally occurs when the motor is trying to move the carriage toward the left side and either the carriage is binding when moving along the rods, or the carriage reached the left side but the home sensor did not sense the carriage. If the carriage is binding it may be caused by 1) an improperly installed belt, being too tight or too loose, 2) the motor connections have come loose and may need to be reconnected,

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or the leads are shorting, 3) the bearings in the carriage are not sliding properly on the guide rods, and the rods need to be **lightly** oiled (3-in-1® type), 4) the cable to the needle carriage is caught, not allowing the carriage to move, 5) the driver IC on the I/O board has failed, or 6) the motor is bad. If the home sensor is not working properly it may be 1) the photo-detector is bad, 2) the cable to the sensor has disconnected, or 3) the I/O board has an IC failure.

"Row Y Drive Motor Stepping Error"
RS-232-C response: -29

This error is similar to the Row X Drive Error above except it is for the needle carriage moving forward and backward. The error will most likely occur when moving forward towards the home sensor.

"Cannot determine vial position."
RS-232-C response: -30

The autosampler is unable to determine the coordinates of a vial location. This error is a software problem. Call Alcott Chromatography at (800) 229-5052.

"Needle Drive Motor Stepping Error"
RS-232-C response: -31

The needle drive assembly is not operating correctly. This usually occurs when moving the needle up but it never reaches the home position sensor. The needle may be binding as it moves or the sensor may not be operating properly. If the needle is binding it may be 1) the needle drive screw is not attached tightly to the needle, 2) the motor leads are shorting or become disconnected, 3) the needle assembly is not sliding properly on the guide rods, therefore the rods need to be **lightly** oiled (3-in-1® type), 4) there is an obstruction preventing the needle assembly from moving, 5) the driver IC on the I/O board has failed, or 6) the motor is bad.

"Needle Drive Motor Stalled"
RS-232-C response: -32

The needle assembly was trying to move up or down, but the encoder sensed that the needle was not moving. This may be caused by either the needle is not moving when the motor is stepping or the encoder is not working properly. If the needle is not moving it may be because 1) there is an obstruction such as a stuck vial cap, an improperly seated vial or tray, or debris in the needle path, 2) the motor is bad, 3) the motor leads have disconnected or shorted, 4) the needle drive screw is not firmly attached to the needle assembly, or 5) the needle has reached the top or bottom but is still trying to move (caused by a bad home sensor, bad encoder, or software problem). If the needle assembly moves freely but the error still occurs, then it may be an encoder problem. This may be the result of 1) a damaged encoder strip, 2) a faulty photodetector, 3) a cable disconnected, or 4) a failed IC on the I/O board.

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"Injection Valve Switch Failure"
RS-232-C response: -34

The injection valve attempted to switch to INJECT or LOAD, but never located the position. This may be caused by 1) a faulty valve actuator, 2) a bad valve, 3) a faulty valve controller module, or 4) poor cable connections. Call Alcott at (800) 229-5052.

" No RS-232-C response"

Receive errors on the **RS-232-C** response. This may be caused by 1) transmitting at the wrong baud rate (RS-232), 2) the wrong data bits length (RS-232), 3) the wrong stop bit length (RS-232), or 4) the wrong parity bit (RS-232). The 718AL Autosampler expects to transmit RS-232 with 8 data bits and no parity bit.

"Syringe Drive Motor Stepping Error"
RS-232-C response: -39

The syringe drive mechanism failed. This usually occurs when the syringe is moving to the empty position, but never reaches it. This may be caused by 1) attempting to push a high viscosity liquid too fast, causing the motor to stall. A slower syringe rate is needed, 2) the home sensor has failed, 3) the motor is bad, or 4) the connections to the syringe drive module are loose or shorted.

"Priming Valve Switch Failure"
RS-232-C response: -40

The priming valve attempted to switch to RUN or PRIME, but never located the position. This may be caused by 1) a position sensor has failed, 2) the motor to turn the valve has failed or is running too fast or too slow (which may be caused by a software problem or an I/O board problem), 3) the connections to the priming valve assembly are incorrect, or 4) a bad valve.

"SOURCE INDETERMINATE"
RS-232-C response: -35

The Autosampler experienced an error for an unknown reason. This may be caused by a possible software bug or noise in the electrical system. Call Alcott Chromatography at (800) 229-5052

Remote Control Error Codes

The following Return Codes are issued by the Autosampler after an error occurs. The right hand column lists the verbose messages issued by the Autosampler if the command "@vrb 1" is issued. The numerical codes are the default messages ("@vrb 0").

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Verbose Return String	Numerical Return
"Ok"	0
"Err: Invalid Aux Contact",	-1
"Err: Invalid Aux Output",	-2
"Err: Syringe Volume Invalid",	-3
"Err: Busy",	-4
"Err: Invalid Buzzer",	-5
"Err: Invalid Command",	-6
"Err: Method Exists",	-7
"Err: Display Request Out of Range",	-8
"Err: Invalid Step Num",	-9
"Err: Step Exists",	-10
"Err: Invalid Method",	-11
"Err: Needle Pos Unknown",	-12
"Err: Requested Needle Pos Invalid",	-13
"Err: Step Out Of Range",	-14
"Err: Method Not Running",	-15
"Err: Invalid External Power Request",	-16
"Err: Syringe Size Unknown",	-17
"Err: Syringe Pos Unknown",	-18
"Err: Invalid System",	-19
"Err: Invalid Syringe Pos",	-20
"Err: Invalid Vial Number",	-21
"Err: Needle Pos Invalid",	-22
"Err: Inject Not In Load",	-23
"Err: Inject Valve Unknown",	-24
"Err: Prime Valve Unknown",	-25
"Err: Tray Type Invalid",	-26
"Err: Invalid Valve Pos",	-27
"Err: X Motor Fail",	-28
"Err: Y Motor Fail",	-29
"Err: VialPos Unknown",	-30
"Err: Needle Drive",	-31

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```
"Err: Needle Stall",                    -32
"Err: Needle Encoder",                  -33
"Err: Inject Valve",                    -34
"Err: Origin Unknown",                  -35
"Err: Local Stop",                      -36
"Err: Syringe Speed Unknown",           -37
"Err: Syringe Rate Invalid",            -38
"Err: Syringe Drive",                   -39
"Err: Prime Valve",                     -40
"Err: SyringeSizeInvalid",              -41
"Err: Tray Param Invalid",              -42
"Err: Temp Not Installed",              -44
"Err: Temp Not Valid",                  -45
"Err: Temp Not Set",                    -46
"Err: Step Not Valid",                  -47
"Err: XY Not Valid",                    -48
"Err: Serial",                          -49
"Err: BCD String Length Invalid",       -50
"Err: Method Running",                  -51
"Err: Rinse Strokes Invalid",           -52
"Err: Still Busy",                      -53
```


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ROUTINE MAINTENANCE AND PARTS REPLACEMENT

The X,Y,Z Sample Tray Assembly is equipped with bushings and bearings made from materials chosen to minimize the need for lubrication, although it is recommended to lightly coat the X &Y Drive guide rods with a light machine oil such as "3-in-1 Oil" about every six months. The Needle Drive Carriage position is recalibrated at power-up and with the start of each Method. Therefore, there are no user serviceable parts in the X,Y,Z Sample Tray Assembly or the Needle Drive Carriage.

Buffered and Corrosive Samples

Always flush the Autosampler's plumbing with plenty of distilled water after using buffered or corrosive samples. This can be readily accomplished by filling the syringe Rinse Solvent Reservoir with distilled water and perform several rinses (50 or more) using the Front Panel **RINSE** command.

NOTE: The standard, internal Valco valve is composed of Nitronic 60, a form of stainless steel, chosen for its ease of machineability. It is not recommended for use with buffered samples or Mobile Phases, especially those using Halogenated salts. Valves made of Hastelloy C are recommended for use with buffered samples or Mobile Phases.

Solvents that will dissolve most polymers such as tetrahydrofuran, methylene chloride, and chloroform will attack the standard, silicone o-ring seal found between the syringe and the priming valve. An alternative o-ring made from Kalrez® is available from Alcott Chromatography (P\N 004-25036-00). The Kalrez® o-ring should be used when any of these solvents are employed.

Needle Replacement

To remove the Needle, stop any Method which may be running by pressing the [RUN/STOP] key and then [ENTER]. Park the needle drive by using the Park Needle command in the [SYSTEM] key menu. Turn the instrument off. Remove the needle using the 3/16-in. open end wrench supplied in the accessory kit to completely loosen the 3/16-in swaging screw. The old needle can then be removed by sliding the needle down from the bulkhead swaging port then pulling the needle forward and up so that it is freed from the Needle Guide/Vial stripper. The replacement needle is already fitted with a factory installed ferrule. Slide the 3/16-in. swaging screw onto the replacement needle, threaded end toward the ferrule. Next, place the tip of the needle into the guide hole of the Needle Guide/Vial Stripper and then lift up to push the needle into the bulkhead fitting. Tighten the 3/16-in. swaging screw until finger-tight. Finally, tighten the 3/16-in. swaging screw 1/2 turn with the 3/16-in., open-end

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wrench. For best results, always use an additional 7/16-in. wrench to hold the bulkhead fitting in place while the 3/16-in. wrench is used to tighten the needle in place. Instruct the Model 718AL to perform a Front Panel RINSE.

Syringe Maintenance and Replacement

Syringe Removal and Replacement

The Model 718AL can be fitted with one of three interchangeable syringes. The available sizes are 50 µL, 250 µL and 2000 µL. A photograph of the Syringe Drive is shown in Figure 5-1. The syringe, regardless of size, is fitted into the common port of the three way priming valve and is held in place by either two Phillips head or two 5/64 inch hex head allen screws as shown in Figure 5-1.

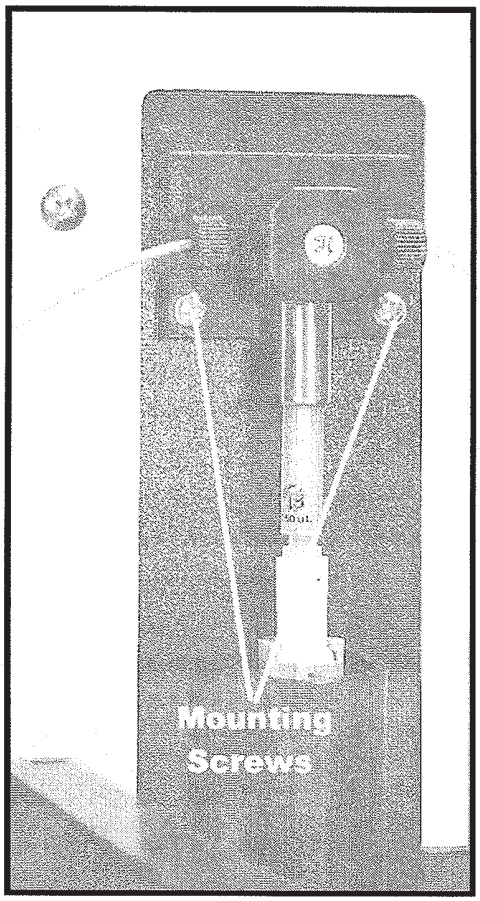


Figure 5-1: The Syringe Drive

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The procedure for removing and replacing the syringe is given in the following steps:

1. Loosen and remove the two Phillips head screws with a Phillips screwdriver. On some earlier Models, two 5/64 inch hex head screws are used to attach the syringe in place.

2. Lift the syringe with the attached priming valve up from the syringe drive.

3. Unscrew the syringe barrel out of the priming valve.

4. Replace or re-attach the syringe by reversing steps 1 to 3 above.

Once the syringe has been removed, inspect the red, silicone rubber o-ring which forms the seal between the syringe and the priming valve. Replace the o-ring if any sign of wear is present. If a different size syringe is being fitted, be sure to reconfigure the syringe size using the **SYSTEM** menu function on the Front Panel as described in **Chapter 2, General Description**.

Prime the syringe using one of the two methods given below. The 250 µL syringe usually requires only automatic priming using one or two rinse strokes to render the tubing and syringe air free. The 50 µL syringe may require as many as twenty rinse strokes to remove air bubbles and manual priming may be necessary for complete air bubble removal. This is due to the surface tension of the liquids used and the small geometry of the 50 µL syringe.

Syringe Priming--Automatic

Automatic syringe priming utilizes the Front Panel **RINSE** function to effect purging of the syringe, priming valve, needle, needle port, and all associated plumbing. The complete mechanical description is given in the introduction to **Chapter 2, General Description**.

Before using the automatic priming feature, obtain a small container such as a 250 mL Erlenmeyer flask and fill it with degassed solvent solution to be used for priming. The chromatographic Mobile Phase for the analysis to be used with the Autosampler is the ideal priming solution. Place the priming solution dipper line into this container. (The priming solution dipper line is the Teflon® tubing with the Teflon® filtering frit attached.) Automatic syringe prime is now ready to be used by pressing the [RINSE] button ([FUNCTION] [9]) and following the given menu commands. As mentioned in the previous section, the 250 µL and the 2000 µL syringes usually require only one or two rinse strokes to rid the tubing and syringe of air bubbles. The 50 µL syringe may require as many as twenty rinse strokes to remove air bubbles.

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Syringe Priming--Manual

Manual syringe priming may be necessary in cases where priming the syringe using the Front Panel **RINSE** function does not remove the air bubble from the syringe. This is more likely to occur when the 50 µL syringe is installed. Before beginning the procedure, park the needle by using **Park Needle** in the **SYSTEM** function menu. Place a small beaker or other small container under the Needle to catch the Rinse Solution overflow. Make sure the Rinse Solvent reservoir is full and the Rinse Solvent dipper line is well submerged and primed. Have an adequate supply of laboratory towels to wipe up possible solvent spills. Once these conditions have been met, use the following procedure to manually prime the syringe:

1. Turn the power off.
2. Remove the Syringe and Priming Valve assembly as described in the previous section, but do not remove the syringe from the priming valve.
3. Turn the Syringe/Valve assembly until the "T" handle in the priming valve is facing you.
4. Rotate this valve 90° **counter-clockwise**. The "T" handle should now be parallel to the syringe barrel.
5. Remove the plunger from the syringe barrel.

Note: At this point, solvent should flow out of the syringe barrel. If free flow is sluggish, or does not occur, raise the Rinse Solvent Reservoir to increase the head pressure to the solvent. A Pasture pipette bulb attached to the plunger end of the syringe barrel can be used to facilitate solvent flow. The solvent should produce a steady drip at this point. If not, check and make sure the dipper line to the Rinse Solvent is well primed and filled with solvent. If the dipper line is filled with solvent and no flow occurs, the "T" handle on the priming valve has been turned in the wrong direction and is 180° out of phase. Rotate the "T" handle 180° and repeat the instructions in this note.

6. Once a steady drip of priming solvent has been established, insert the plunger but **do not** push it all the way in.
7. Rotate the "T" handle 90° **clockwise**. The "T" handle should now be perpendicular to the syringe barrel.
8. Push the plunger completely in.

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Note: **Solvent should squirt out of the needle as the plunger is pushed all the way in. If solvent does not squirt out of the needle, or if it is difficult to push the plunger in, the "T" handle on the priming valve has been turned in the wrong direction and is 180° out of phase. Rotate the "T" handle 180° and push the plunger in.**

9. Check the syringe for air by repeating step 3 and retracting the plunger but not removing it from the syringe. If no air bubble is present, repeat Steps 6 and 7 and reinstall the Syringe/Priming Valve assembly. If a bubble is present, repeat Steps 4, 5, 6, and 7 until all the air is gone.

Note: **When reinstalling the Syringe/Priming Valve Assembly, the "T" handle should be perpendicular to the syringe barrel.**

10. Return power to the Autosampler and use the Front Panel **RINSE** function to check for bubbles.

Valve Maintenance and Replacement

General Maintenance

The Model 718AL Autosampler uses a Valco six-port injection valve, controlled by a built-in Valco Two Position Electric Valve Actuator. Maintenance and seal replacement information for these items are given in the Valco Valve User's Manual found in the Autosampler's accessories. The plumbing configuration is given in Table 5-1.

Loop Filler Port Replacement

The Loop Filler Port used in the Model 718AL part number is P/N 004/25709/00. Replacement is straightforward, simply unscrew the worn Loop Filler Port and screw in the new one. **Do not over tighten!**

Loop Replacement

The sample loop is connected between Ports 1 and 4 on the Valco Model C3 valve. If the ferrules have not been set at the factory, it is recommended to set the ferrules on the loop, one end at a time, before bending the tubing to fit the valve.

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Valve Removal and Replacement

The valve must be removed for major repairs such as the removal of a broken swaging screw or to replace the valve with a different type such as a ten-port Valco valve.

If the valve is to be replaced, remove the valve from the actuator by first loosening the 7/64 inch Allen head screw in the Valve Retainer C-Clamp located between the valve and the valve actuator (Figure 5-2). Once the clamp has been sufficiently loosened, the valve can be removed by pulling it up. Replacement is the reverse of this procedure.
Note that any time the valve is removed from the actuator or a different type valve is installed, the valve actuator must be reinitialized. The valve actuator calibration procedure is described in **Chapter 2, System Settings, Initialize Valve.**

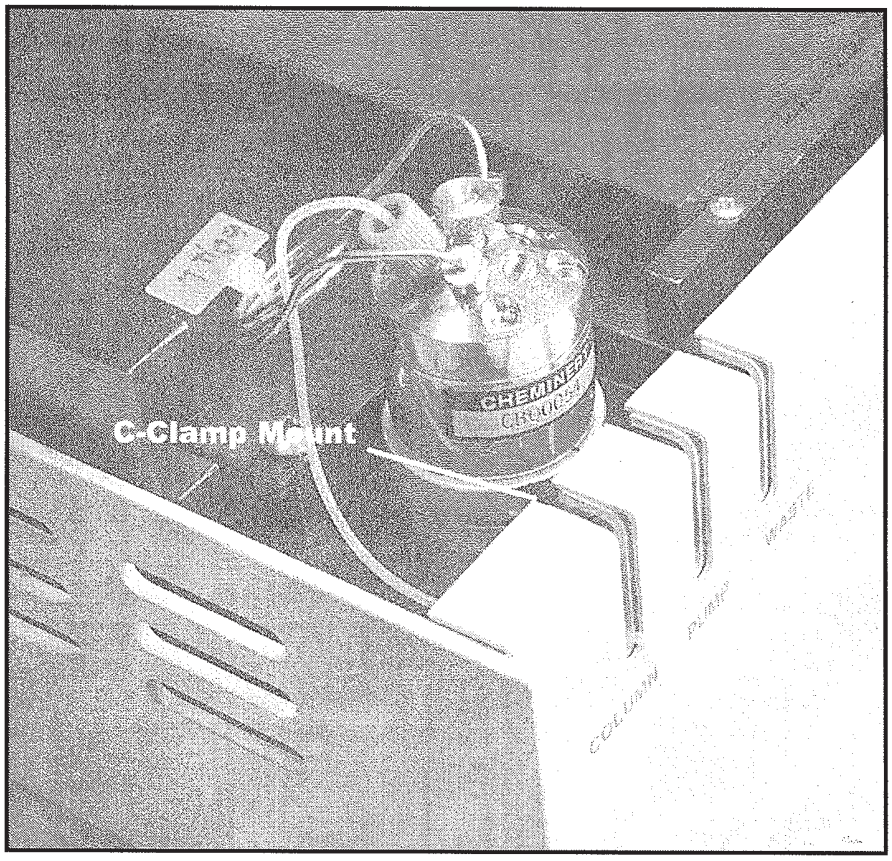


Figure 5-2: Valco C3 Valve C-Clamp Screw

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Table 5-1: Valco Valve Port Assignments

The VALCO Model C3 Valve	
Port Number	**Function**
1	Loop
2	Pump Inlet
3	Column Outlet
4	Loop
5 (Center)	Injection Port
6	Waste Line

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APPENDIX A

THE PROPER USE OF TUBE FITTINGS

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CORRECT TUBE CUTTING AND ASSEMBLY OF FERRULE-TYPE TUBE FITTINGS

Tube Cutting:

The use of tubing cutters is the preferred method of cutting tubing. Several types of tube cutters for cutting either plastic or metal tubing are commercially available and should be employed. Follow the instructions which come with the tubing cutter. After the tubing is cut, the tubing ends should be deburred and the tubing rinsed with solvent before use.

Nut Type (Two Ferrule) Fittings:

To assemble, insert the tubing against the shoulder of the fitting. Complete removal of the nut is not recommended -- just loosen the nut and insert the tubing until it seats against the shoulder, then retighten finger-tight. If the fitting is disassembled, slide a nut, a back ferrule, and a front ferrule onto the tubing and make sure they are correctly oriented. Seat the end of the tubing against the shoulder, slide the ferrules and nut into place and tighten the nut finger-tight. Then tighten the fittings with a wrench about one full turn. Do not over tighten, especially when using tubing made from PEEK® or Teflon®.

Swaging Screw Type (Single Ferrule) Fittings:

Many HPLC instrument and column manufacturers, and all of the stainless steel fittings used on the Model 718AL, use a swaging screw to swage ferrules on the tubing. External threads on the screw mate with internal threads in the shoulder recess hole machined into some part of the instrument.

To assemble this type of fitting, slide a swaging screw and a ferrule onto the tubing and insert the tubing into the shoulder recess hole until the end of the tube bottoms out against the shoulder. Make certain the ferrule and screw are in proper sequence and correctly oriented, then slide them along the tubing until the screw threads engage the hole threads. Tighten the screw finger-tight, then use a wrench to swage the ferrules and seal the connection which usually requires about 1/2 turn. Do not over tighten, especially when using tubing made from PEEK® or Teflon®.

Upchurch Light Touch Fittings

Alcott Chromatography uses Upchurch LiteTouch® two piece ferrule sets and Fingertight swaging screws for all plumbing using PEEK or PTFE tubing. The ferrule set consists of a PEEK ferrule,

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which can be either coned or flat, and a stainless steel lock ring. The coned ferrule is used in standard 1/16-inch fittings such as found in injection valves and column end fittings. The flat ferrule is used in all 1/4-28 type "Flange type" fittings. The stainless steel lock ring is the same for both fittings. The stainless steel lock ring is oriented such that the end with the wider opening is facing the ferrule. The end with the narrower opening is facing the swaging screw. These fittings require only finger-tight pressure to seal. Over tightening the fitting can damage the ferrule, making disassembly difficult.

Valco Injection Port Fittings

Valco Injection Port Fittings consist of a special stainless steel nut, a piece of Teflon® tubing, and a Teflon® ferrule. The nut needs to be tightened sufficiently to prevent leakage and carryover but not so tight as to cause the Teflon® ferrule to over-compress. Over compression will damage the fitting and it may not be readily removable. Always make sure the needle is inserted into the Injection Port before tightening. Tighten the Injection Port fitting figure tight, then using a pair of long nose pliers, tightened the fitting an additional 1/16th turn.

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APPENDIX B

SPARE PARTS LIST AND ACCESSORIES

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Spare Parts and Accessories List

Tools

004/54006/00	Wrench, open end, 1/4 x 5/16 inch
004/54109/00	Wrench, .050 Allen Hex
A04/54103/00	Wrench, Hex Head Allen, 5/64 inch

Electrical

003/51137/00	Fuse, 3AG REGULAR, 2 Amp
003/51132/00	Fuse, 3AG SLO BLOW, 3 Amp
003/51197/00	Fuse, IEC, SLO BLOW, 5 × 20 mm, 1.5 AMP
003/60101/00	Power Cord
708/33001/00	Interface, BCD with cable
708/33002/00	Interface, RS-232-C and Parallel Printer with cables
708/60820/00	Assembly, Cable, BCD
728/33010/00	Assembly, External power control, 120 VAC

Fittings

004/25541/00	Nut, Swaging, 1/16 inch (Valco)
004/25541/01	Ferrule, 1/16 inch (Valco)
004/25912/00	Ferrule, Lite Touch, 1/16" (transfer line and waste line)
A04/25023/00	Nut, Swaging, Lite Touch, 1/16" (transfer line and waste line)

Sample Trays

708/25876/00	Vial Tray, 8 mm × 128
708/25883/00	Vial Tray, 8 mm × 128, refrigerated
718/25863/00	Vial Tray, 12 mm × 72
718/25864/00	Vial Tray, 15 mm × 50
718/25876/00	Vial Tray, 12 mm × 72, refrigerated
718/34105/00	Vial Tray, 96 Well
718/34105/01	Vial Tray, 96 Well, Refrigerated

Vials and Caps

718/61608/00	Vial, 8 mm × 35 mm, Clear Shell, Clear Snap Cap (1000)
718/61612/00	Vial, 12 mm × 32 mm, Clear Shell, Snap Cap, (100)

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718/61612/01	Vial, 12 mm × 32 mm, Amber Shell, Snap Cap, (100)
718/61612/02	Vial, 12 mm × 32 mm, Polypropylene , Snap Cap, (100)
718/61615/00	Vial, 15 mm × 45 mm, Clear Shell, Snap Cap, (100)
718/61615/01	Vial, 15 mm × 45 mm, Amber Shell, Snap Cap, (100)
718/61615/02	Vial, 15 mm × 45 mm, Polypropylene Shell, Snap Cap, (100)
728/61601/01	Vial, 8 mm × 35 mm, Clear Shell (1000)
728/61600/01	Vial, 8 mm × 35 mm, Amber Shell (1000)
728/61604/00	Vial, 8 mm × 35 mm, Polypropylene Shell (1000)
718/61608/01	Cap, 8 mm, Clear Polypropylene
718/61608/02	Cap, 8 mm, Red Polypropylene
718/61608/03	Cap, 8 mm, Blue Polypropylene
718/61608/04	Cap, 8 mm, Green Polypropylene
718/61608/05	Cap, 8 mm, Yellow Polypropylene
738/61600/00	Clear Vial, cap with PTFE/SIL/PTFE Septum installed, 12 × 32 mm, (100)
738/61600/01	Amber Vial, cap with PTFE/SIL/PTFE Septum installed, 12 ×32 mm, (100)
738/61601/00	Clear Vial only, 12×32 mm, (100)
738/61601/01	Amber Vial only, 12×32 mm, (100)
738/61602/00	Cap only, Target, Screw Top, 12×32 mm, 100/pack
738/61603/00	Septum only, Target, Screw Top, PTFE/SIL/PTFE, 100/pack
738/61604/00	Insert only, Target, polyspring design, 300 µL, 100/pack
738/61605/00	Cap with PTFE/SIL/PTFE Septum installed, 12×32 mm, 100/pack

Loops

708/62600/00	Loop, 10 microliters, Valco
718/62600/00	Loop, 60 microliters, Valco
718/62601/00	Loop, 300 microliters, Valco
718/62602/00	Loop, 2500 microliters, Valco
728/62805/01	Loop, 5 microliters, Valco
728/62805/03	Loop, 20 microliters, Valco
728/62805/04	Loop, 50 microliters, Valco
728/62805/05	Loop, 100 microliters, Valco

Syringes and Needles

004/25036/00	O-ring, Syringe, Kalrez
004/25045/00	O-ring, Syringe, Silicone
004/25709/00	Loop Filler Port
718/25880/00	Autosampler needle, pre-swaged, 22 gauge, 0.006" wall (Standard 0.012 inch ID bore needle)
718/25881/00	Autosampler needle, pre-swaged, 22 gauge, 0.011" wall (Narrow 0.006 inch ID bore needle)

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A04/61602/00	Syringe, TCS, 50 µL
A04/61603/00	Syringe, TCS, 250 µL
A04/61604/00	Syringe, TCS, 2000 µL

Miscellaneous

708/25921/00	uv Cover, Sample Area
709/42802/00	Service Manual, 708/718AL Autosampler, with binder
719/42801/00	Instruction Manual, 718AL Autosampler, with binder

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APPENDIX C

FACTORS WHICH AFFECT INJECTION PRECISION AND ACCURACY

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Partial Loop Fill Injections

The Model 718AL Autosampler performs partial loop fill injections using the same steps that a chromatographer would employ when making manual injections. These steps are outlined in **Chapter 2, Theory of Operation**. According to one HPLC injection valve manufacturer, the precision of manual, partial loop fill injections is typically ±2.0% or better depending on the "technique and skill" of the chromatographer[1]. Because the Model 718AL is a mechanical device, the imprecision caused by "technique and skill" is automatically eliminated. The instrument is capable of making routine injections in a highly reproducible fashion, typically better than ±0.5%. Achieving this level or precision requires that the user optimize the Injection Parameters for the sample which is to be injected. Guidelines for maximizing performance are given below for each of the Injection Parameters.

Syringe Size

Although Syringe Size is a System Parameter and not an Injection Parameter, syringe size selection can greatly influence the instrument's reproducibility. For example, it is better to use the 50 µL syringe for making 5 µL injections rather than the 250 µL syringe. Table C-1 lists the two available syringe sizes, their injection volume ranges, and their recommended injection volume minimums.

Table C-1: Syringe Size Parameters, Partial Loop Fill Injections

Syringe Size	Volume Range	Minimum Volume	Recommended Minimum Volume	Loop Size
50	1 to 50	1	1	60
250	1 to 250	1	10	300
2000	2 to 2000	2	100	2500

All volumes in µL

The reason for these recommendations is the step resolution of the stepper motor used in the syringe drive. A 1 µL injection made with the 50 µL syringe requires more stepper motor pulses than a 1 µL injection made with the 250 µL. (It takes a longer plunger stroke to pull 1 µL on a 50 µL syringe than it does to pull 1 µL on a

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250 µL syringe!). The higher pulse requirement insures more precise volumetric delivery at the smaller volumes.

The final item in Table C-1 is the minimum sample loop size needed for the given syringe size to insure the most linear injection response. This will be discussed below.

Fill Rate

The **Fill Rate** is the flow rate at which the syringe drive withdraws sample from the vial and also the flow rate at which sample is introduced into the Sample Loop. Note that the **Fill Rate** is in µL/sec. Consult Table 2-3 for the **Fill Rates** of the individual syringes. Use the faster **Fill Rate** for solutions with viscosities less than 1.0 cp. This includes solvents such as Water, Methanol, Acetonitrile, and Hexane. The medium **Fill Rate** should be used with solutions with viscosities between 1 and 3 cp such as alcohol/water mixtures. The slower **Fill Rate** should be used with solutions with viscosities greater than 3 cp. Note also that sample loops made from tubing with less than 0.010 inch (0.25 mm) internal diameter can cause too much flow restriction and either the Loop Filler Port or the syringe may leak. Slower **Fill Rates** will eliminate the possible leakage.

These viscosity ranges and corresponding **Fill Rates** are suggestions only. The user may have to experiment with the **Fill Rate** to optimize the injection precision with his or her system. Experience has shown however, that most applications can be successfully done with the fast **Fill Rate**.

Delay Time

The **Delay Time** is the pause the Autosampler makes after the syringe has withdrawn the designated volume of sample, but before the needle is withdrawn from the sample vial to allow the pressure of the liquid in the needle to come to equilibrium with atmospheric pressure. The default time is 5 seconds, which is adequate for most applications. However, longer **Delay Time** periods may be used for sample solutions with very high solution viscosities or which are highly compressible. A concentrated sugar solution is an example of a highly viscous sample. Methylene Chloride solutions have a very low viscosity but are very compressible, and would benefit from a long **Delay Time**. **Delay Times**, as with **Fill Rates**, should be optimized empirically for each application. Generally speaking, the default values will handle most applications.

Pre-Injection Volume

The **Pre-Injection Vol**ume parameter is a user specified volume of Rinse Solvent, usually Mobile Phase, that is used to compensate for the "dead Volume" in the Loop Filler Port. A schematic of the Injection Valve with Loop Filler Port and Sample Loop are shown in Figure C-1. The Loop Filler Port has a volume of approximately

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5 µL. In order to place the sample exactly into the sample loop, a 5 µL addition of Mobile Phase (the Pre-Injection Vol) is drawn from the rinse reservoir by means of the priming valve and syringe. This slug or bolus of Mobile Phase is withdrawn first, followed by the sample. When the syringe deposits its contents into the Loop Filler Port and into the Sample Loop, the sample is the first solution to enter the valve, followed by the bolus of Mobile Phase. Ideally, all the sample is pushed into the Sample Loop while the bolus of Mobile Phase remains inside the Loop Filler Port.

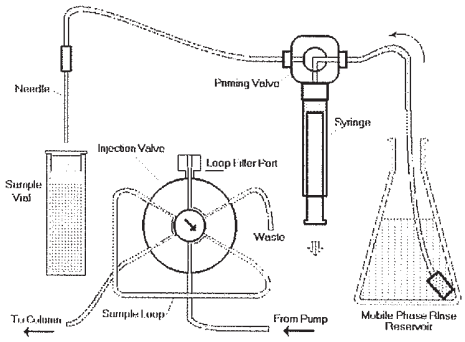
Another useful partial loop fill procedure technique, especially for small sample volumes, is to center the sample bolus into the middle of the Sample Loop. In this technique, a large volume of Mobile Phase from the Rinse Reservoir is programmed to push a small volume of sample into the middle of the Sample Loop. For example, a standard Model 718AL is equipped with 50 µL syringe and a 60 µL loop. One can program the Autosampler to have a Pre-Injection Volume of 30 µL to push a 3 µL sample approximately into the middle of the sample loop. This will insure that all of the sample is pushed into the loop.

Air Bubble Segmentation

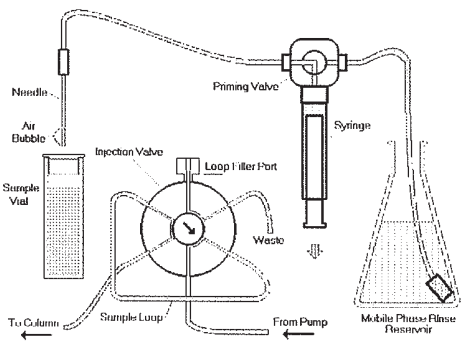
Both partial loop fill procedures described in the two previous paragraphs work on the assumption that "Plug Flow" is present during all phases of sample transfer. Unfortunately, this is not the case. Laminar Flow will exist for all Fill Rate ranges available to the Syringe Drive for the Model 718AL. In Plug Flow, the fluid velocity is constant across the tube's cross section, while in Laminar Flow, fluid velocities are distributed in a parabolic profile across the tube's cross section. In Laminar Flow, the fluid velocity at the tube's wall is practically zero while the fluid velocity at the center is about twice the average velocity. Figure C-2 illustrates the difference between Plug Flow and Laminar Flow.

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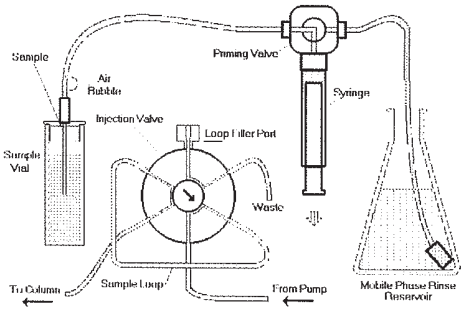
Step 1: Withdraw 5 µL of Mobile Phase



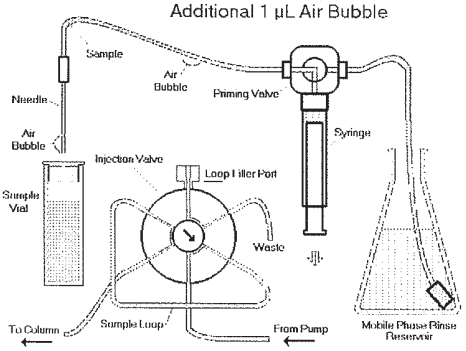
Step 2: Switch Priming Valve and Withdraw 1 µL Air



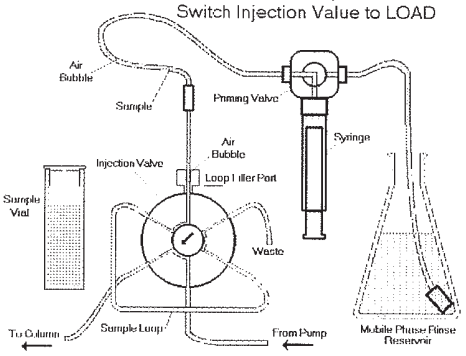
Step 3: Lower Needle and Withdraw Desired Volume of Sample



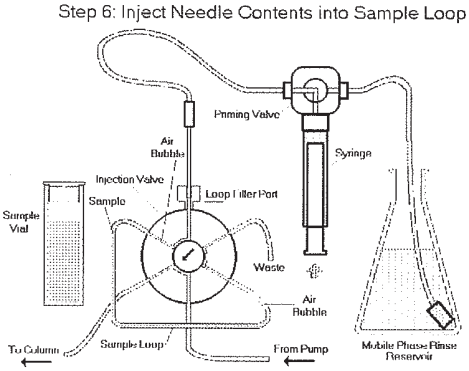
Step 4: Raise Needle and Withdraw an Additional 1 µL Air Bubble



Step 5: Insert Needle into Loop Filler Port and Switch Injection Value to LOAD



Step 6: Inject Needle Contents into Sample Loop



Step 7 : Switch Injection Valve to INJECT and Rinse Needle with Mobile Phase

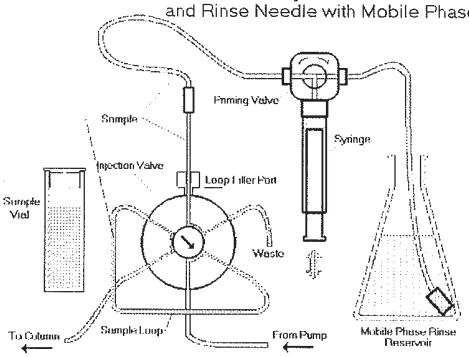


Figure C-1

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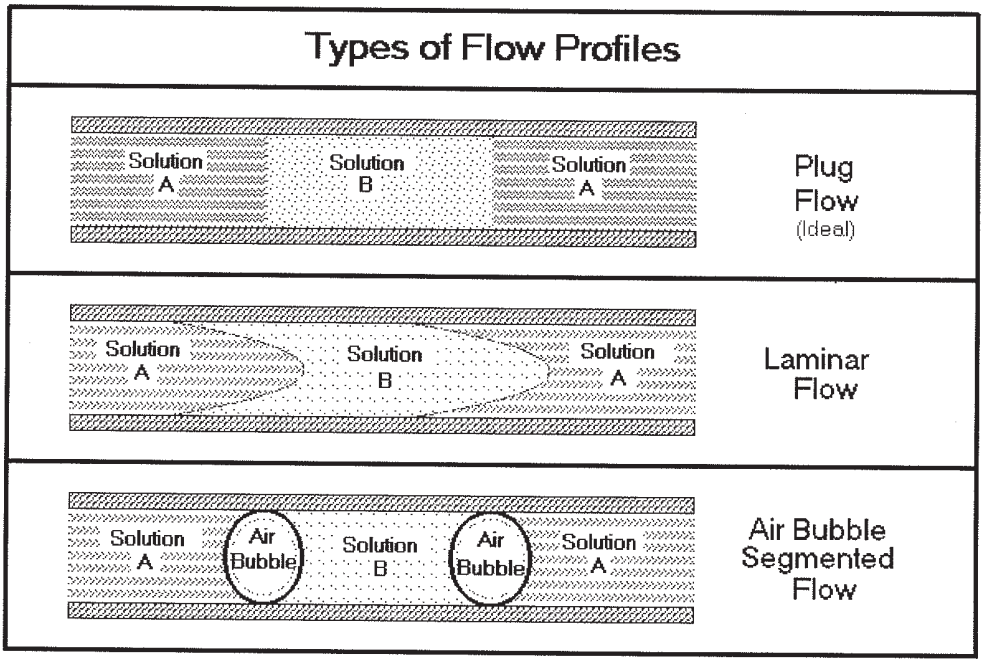


Figure C-2

As the bolus of sample travels down the tube in the presence of Laminar Flow, the sample in front, which travels faster, becomes diluted, as does the tailing portion. This dilution effect (coupled with sample diffusion) limits the accuracy of injections made by the partial loop injection technique as some of the sample may be lost due to these convective and diffusive forces. **Note that these natural processes do not affect injection precision!** In addition, only about 50% of the sample loop can be accurately used. A detailed study of this effect is given in Reference 1 and is shown graphically in Figures C-3. Accuracy would be greatly improved if Plug Flow were possible. (Some sample dilution would still occur due to diffusion!) Plug Flow can be approximated by using small air bubbles to disrupt the Laminar Flow process, a technique commonly used for manual injections[2]. This principle of air segmentation is also shown in Figure C-2.

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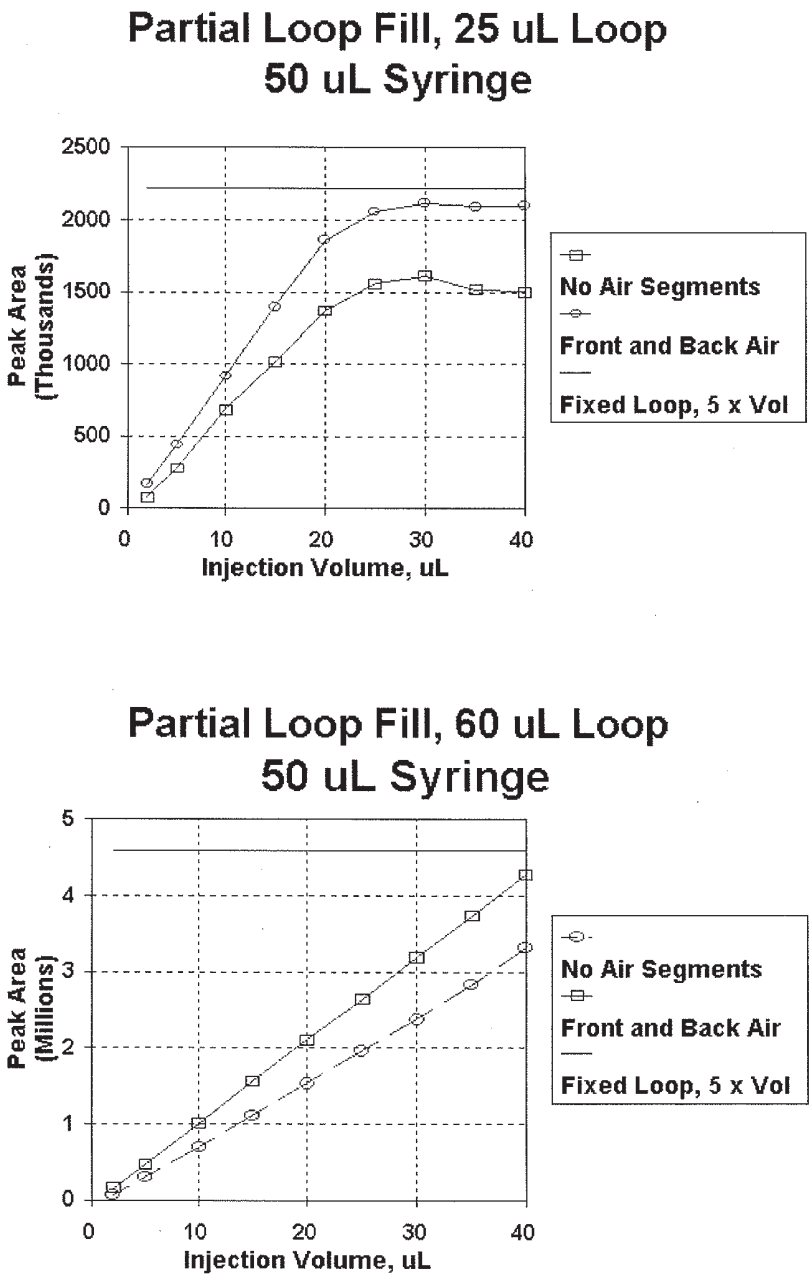


Figure C-3

The two Injection Parameters, **Pre-Air Vol** and **Post-Air Vol** allow the user to program the use of air segmentation. The **Pre-Air Vol** is the air bubble placed between the **Pre-Injection Volume** of Mobile Phase and the sample, while the **Post-Air Vol** is the air bubble placed between the sample and the Waste Port. The range for both values is 0 to 10 µL. The default volume is 1 µL,

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which is adequate for most applications. Some HPLC systems cannot tolerate the addition of air for various reasons. In these instances, a value of 0 can be used and no air bubbles will be incorporated. To restate the above discussion, the use of air segmentation improves the accuracy of a partial loop injection. The injection precision remains the same regardless of whether or not air segmentation is used. This is true for any type of HPLC injection device whether it is a manual injection valve or an autosampler. Note that the last parameter in Table C-1, Loop Size, was determined using air segmentation.

Fixed Loop Injections

Fixed loop injections provide the Model 718AL with the highest injection precision. A "Heart Cut" loop filling technique is used as is shown in Figure C-4 As with the partial loop fill injections, the steps for performing a fixed loop injection are outline in **Chapter 2, Theory of Operation**. Most of the discussion given in the previous sections for partial loop fill injection is applicable to fixed loop injections. Those areas which differ are discussed below.

Syringe and Loop Sizes

Table C-2 lists the maximum loop size that can be used for a given syringe size while performing fixed loop type injections. Note that these values were determined using air segmentation.

Table C-2: Maximum Loop Size for Fixed Loop Injections

Syringe Size (µL)	Loop Size (µL)
50	10
250	50
2000	490

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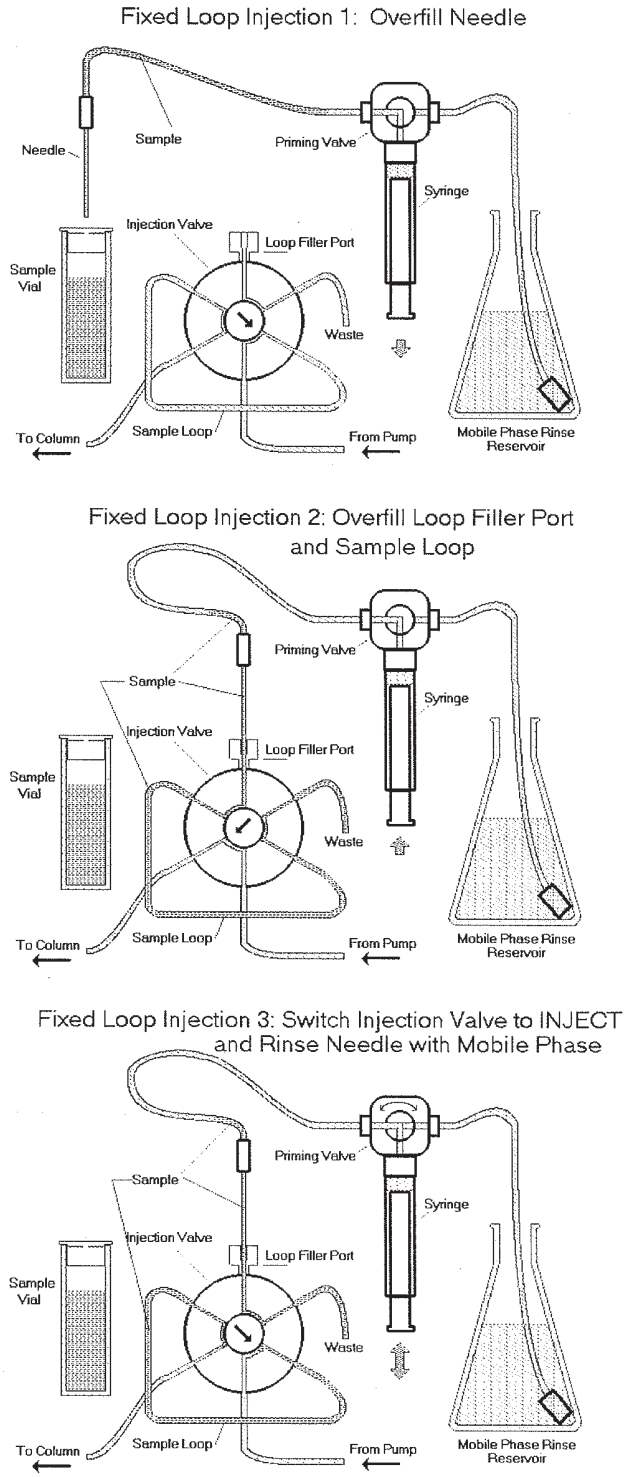


Figure C-4

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Pre-Injection Solvent and Air Bubble Volumes

The **Pre-Injection Vol**ume of Mobile Phase is not required when making fixed loop injections because excess sample will be used to compensate for the dead volume in the Loop Filler Port. Air segmentation can be used, but is also usually not required. A volume of 1 µL is recommended for both the **Pre-Air Vol** and the **Post-Air Vol**.

Withdraw Vol

The **Withdraw Vol**ume is the total volume of sample withdraw by the syringe. This large bolus of sample contains sufficient liquid to flush the sample loop and leave a small excess of sample in the Needle. This is illustrated in Figure C-4 This provides a contiguous column of sample through the Needle Transfer Line, Needle, Loop Filler Port, Sample Loop and Waste Line. As an example, consider the fixed loop injections made using a 20 µL Sample Loop with a 250 µL syringe. With the use of air segmentation, only a 4 × Loop volume excess of sample is needed to flush the Loop. which for a 20 µL Loop is 80 µL. A 5 µL excess of sample is needed for the Loop Filler Port. This yields a **Withdraw Vol** of 85 µL. The **Injection Vol** will be 80 µL (the 4 × Sample Loop volume).

References

1. Stephen R. Bakalyar and Barbara Spruce, "Achieving Accuracy and Precision with Rheodyne Sample Injectors", **Technical Notes 5**, Rheodyne Corporation, P. O. Box 996, Cotati, CA 94928, USA.

2. Valco Instruments Co. Inc. Catalog, page 8, Valco Instruments Co. Inc., PO Box 55603, Houston, TX 77255

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APPENDIX D

ALCOTT CHROMATOGRAPHY INC
MODEL 718AL CHECKOUT PROCEDURE

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ALCOTT CHROMATOGRAPHY INC. MODEL 718AL CHECKOUT PROCEDURE

The purpose of the Check Out Procedure is to insure that each Model 718AL Autosampler completing the manufacturing process meets the designed performance specifications. The procedure tests the instrument's reproducibility, carry over, injection linearity, and upper pressure limit.

Test Equipment and Procedures

Apparatus

1. One HPLC pump
2. One UV detector (254 nm)
3. One computing integrator or equivalent data collection/reduction device
4. One 50 mL glass beaker for priming syringe
5. A 25 cm X 4.5 mm ID, 5 μm, Spherisorb ODS II Column.
6. Pasteur pipettes and bulbs
7. Methyl and Propyl Paraben test solution--0.010 to 0.030 mg/mL propyl paraben in 70/30 methanol/water.
8. Vial, caps, and septa.
9. HPLC Grade Methanol and Water.
10. One Scientific Calculator with Statistics Functions or a MS-DOS PC and printer running QUATTRO PRO for results analysis.
11. 3 old columns connected in series for back pressure.

Chromatographic Conditions

Column:	25 cm X 4.5 mm ID, Spherisorb ODS II, 5 μm.
Solvent:	70 % Methanol and 30% Water
Flow Rate:	1.0 mL/min.
Detector:	UV at 254 nm.

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Sample:	Propyl Paraben, 0.015 mg/mL in 70% Methanol, 30% Water. 70% Methanol, 30% Water used as a blank
Inj. Sizes:	Sample--5, 10, 20, and 40 µL (The Model 718AL should be equipped with the standard 50 µL syringe / 60 µL loop configuration.)

Procedure

Begin the test by thoroughly checking all screws and nuts to make sure all is secured. Tighten any which are found to be loose. Next, visibly inspect all plumbing connections to make sure all are properly swaged and that all ferrules are set to the proper depth.

To pressure test the instrument, connect the HPLC pump directly to Inlet port of the Autosampler. Connect the three columns in series to the Outlet port of the Model 718AL. Start the flow of Mobile Phase through the system. Adjust the flow rate until the back pressure is approximately 40 MPa (5000 psi). Turn the Autosampler on and do a 1 µL Manual injection. Observe the back pressure on the system as the valve goes to the INJECT position.

Should the pressure be lower while the valve is in the INJECT position than in the LOAD position, and stays there, there is more than likely a leak somewhere in the Sample Loop of the Autosampler. Leave the pump operating to facilitate leak checking and examine the plumbing for leaks. Repair the leak and continue the test. If the pressure is higher in the INJECT position than in the LOAD position, the Sample Loop may either be plugged, or the valve may be misaligned. Stop the pump, check for plugs and valve alignment. Make the necessary adjustments and resume flow.

Assemble the reproducibility test apparatus from the components listed above. The Autosampler should be equipped with a 250 µL syringe and a 300 µL loop. The pump is connected to the Model 718AL's Inlet Port. The column Outlet Port of the Autosampler is connected to the column which is in turn connected to the inlet of the UV detector. The outlet of the detector goes to a suitable waste container. The detector's signal output is connected to the signal input of the Integrator or Data system. Finally, the Model 718AL's injection contact closure (the bottom two pins on the green, 19 pin terminal strip) is connected to the REMOTE START connector of the Integrator to signal the initiation of data collection.

Once the chromatographic system is set up, prepare the Autosampler for evaluation by priming the syringe with Mobile Phase. Make ten RINSES to insure that the syringe and all associated plumbing is air free. Reprime the syringe if air bubbles are present.

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Enter the test Method into the Autosampler. Any of the 10 Method spaces may be used. The Method program used for the test is as follows:

Inject Type	Part	
Withdraw Rate	5	
Loop Fill Rate	5	
Delay Time	5	
Needle Depth	48	
Pre-Injection Vol	5	
Pre-Air Vol	1	
Withdraw Vol	10	(Not Used)
Post-Air Vol	1	
Rinse Strokes	3	
Rinse Rate	100	

Step	1	2	3	4	5
Start Vial	1	2	3	4	5
Stop Vial	1	2	3	4	5
Standard Vial	none	none	none	none	none
Injections	4	4	4	4	4
Inject Vol	5	10	20	40	40
Run Time	5.2	5.2	5.2	5.2	5.2
Event1 Time	0.0	0.0	0.0	0.0	0.0
Event2 Time	0.0	0.0	0.0	0.0	0.0
Event3 Time	0.0	0.0	0.0	0.0	0.0

Prepare four vials to contain the paraben solution and one vial to contain Mobile Phase by filling the vials with the appropriate solution and then capping them. Place the vials containing the paraben solution in vial positions 1 through 4, and place the vial containing pure Mobile Phase in vial position 5.

Set up the integrator to provide the optimum settings for the sample. The peak width and peak threshold values should be selected to provide adequate sensitivity for the test peak, but relative insensitivity to noise peaks. A chart speed of 0.25 cm/min. is sufficient. Select an attenuation value to insure that the largest peak stays on scale. Program the integrator for a 110 minute analysis. Set up in this fashion, the Autosampler will make 20 injections for one integrator run.

Begin the evaluation by running the test Method on the Autosampler. If everything is set up correctly, the Method should begin by injecting the first sample and the integrator should begin data acquisition. Inspect the instrument periodically during the test to insure all is well and that no problems have occurred with the test station.

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Evaluation of Test Data

Data Reduction

The test chromatogram should have 16 peaks. Of the 16 peaks, there will be four peak area values for the 5 µL injections, four for the 10 µL injections, four for the 20 µL injections, and four for the 40 µL injections.

To analyze the injection reproducibility, calculate the average peak area for each injection volume, its standard deviation (σ for n-1) and its relative standard deviation in percent. Most inexpensive, scientific pocket calculators have this capability.

Next plot the average areas for each injection size versus its injection volume. The best way to do this is with a scientific pocket calculator using the Least Squares Straight Line Fitting routine.

A spread sheet program has been prepared to accomplish all calculations and curve fitting. The spread sheet, 718QUICK.WQ1, was developed to work with QUATTRO PRO. Enter the peak areas for each injection volume in its respective column. Once the peak areas have been entered, press the HOME key and type Alt F (or Alt f). The spreadsheet will perform all data analysis and evaluations which may then be printed out.

Data Evaluation

The specification for peak area reproducibility is that it should be better than ± 0.5% RSD (Relative Standard Deviation) for injections sizes from 11 µL to 50 µL with the 50 µL syringe. Injections sizes less than or equal to 10 µL and greater than 5 µL should be better than ± 1.00% RSD. Instruments not meeting these specifications should be checked for leaks, plugged loops, and air in the syringe and/or associated plumbing, an improperly configured syringe, or a faulty syringe drive.

The Least Squares Straight Line Fit of the peak areas versus injection volume analysis should have a goodness of fit (r^2) of 0.990 or higher. Anything less is due to air in the syringe and/or associated plumbing, an improperly configured syringe, or a faulty syringe drive.

The four 40 µL injections of the Mobile Phase blank should ideally be peakless. If propyl paraben peaks are present in the blank injections, their average peak area must be less than 0.01% (> 0.0001 ×) the average peak area of the four 40 µL injections of propyl paraben sample. Blank peak areas higher than 0.01% indicate incomplete rinsing or a leaky Loop Filler Port.

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APPENDIX E

ALCOTT CHROMATOGRAPHY INC MODEL 718AL SOFTWARE LOADING PROCEDURE

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Loading Autosampler Firmware

1. At the instrument Front Panel, go into the System Menu, [FUNCTION] [4].

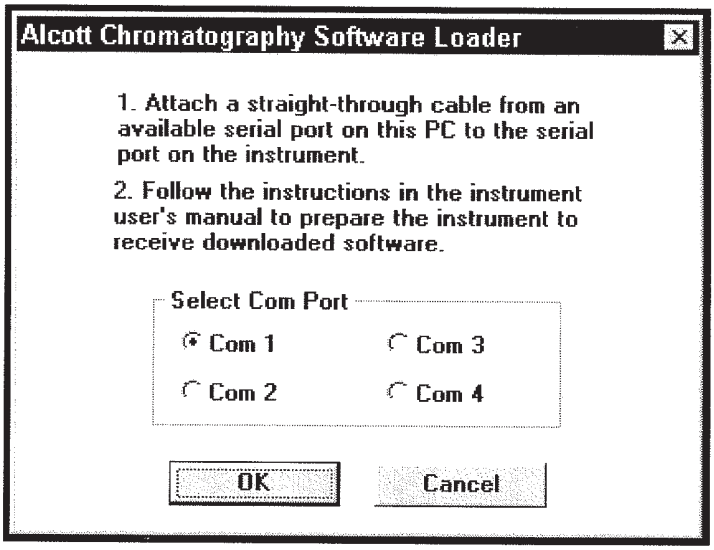
> **NOTE:** **718AL users should note the System Settings values for Needle Depth, Syringe Size and Tray Size. These values will have to be re-entered once the new code is in place.**

2. Select the last menu item STATUS and press [ENTER].

3. Press [ENTER] once more and select the menu item DOWNLOAD SOFTWARE and press [ENTER]

4. Enter the password "5680" that is provided with the software upgrade package and press [ENTER]. The Autosampler is now ready to accept the new software.

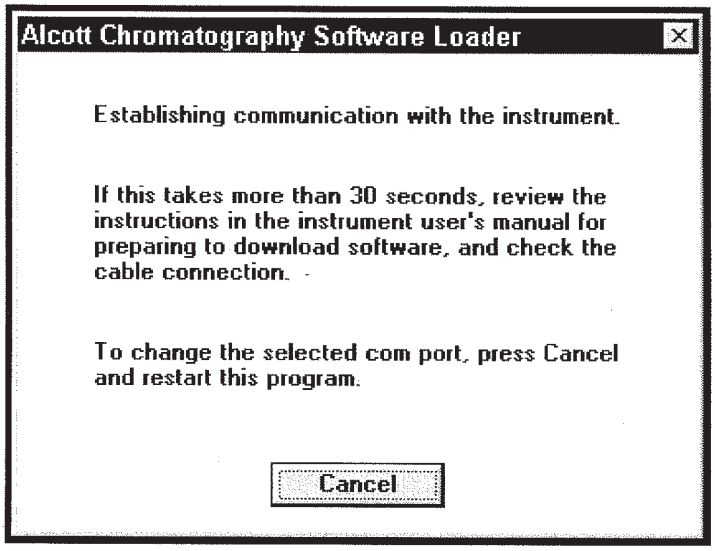
5. Using a PC with Windows 3.X or Windows 95/98/NT, insert the Software diskette and run on the floppy the program 'setup.exe'. It will present the following screen:



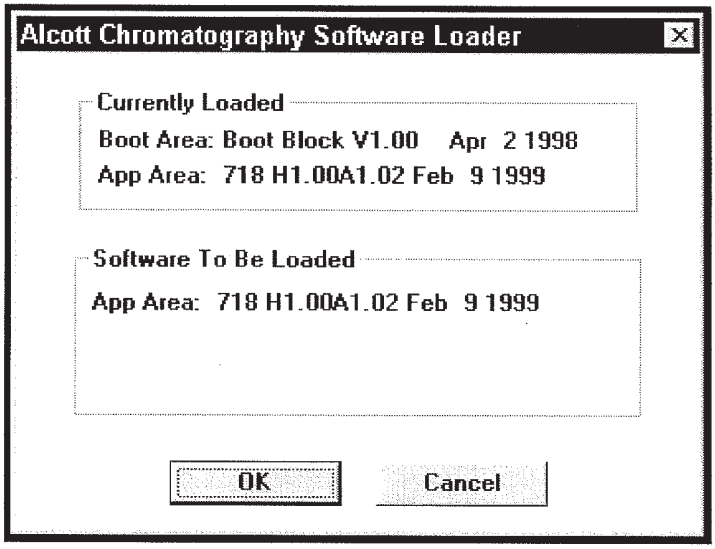
6. Attach a straight-through cable between the serial port on the Alcott instrument and an available serial port on the PC. Select which Com port is being used, then Press OK.

7. The Loader application will establish communication with the instrument. The following screen will be presented, if communications are established additional status screens will be shown as the Loader application connects with the instrument.

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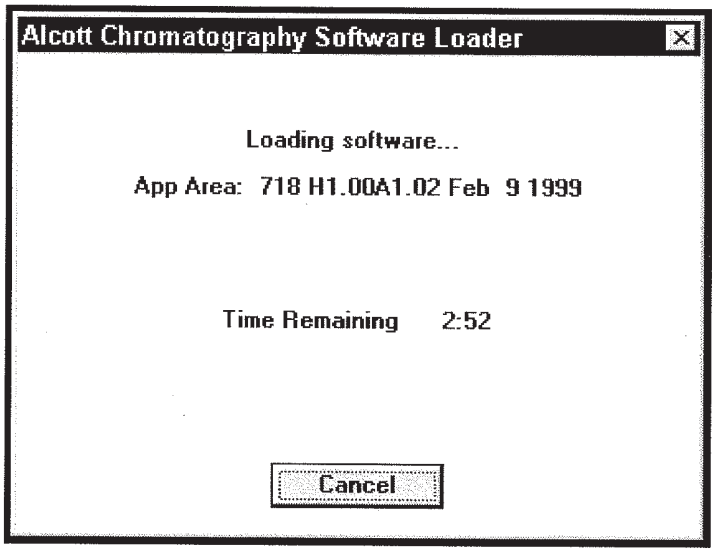


8. At the completion of the connection operation the following screen will be shown. Confirm that the Software to be loaded is as expected.

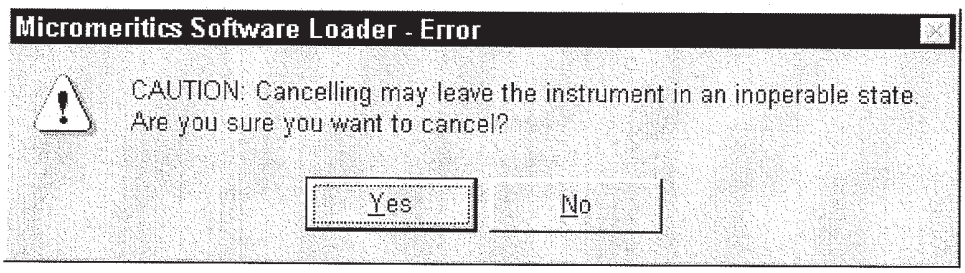


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9. Once OK is pressed the following status screen is shown with the remaining time counted down.

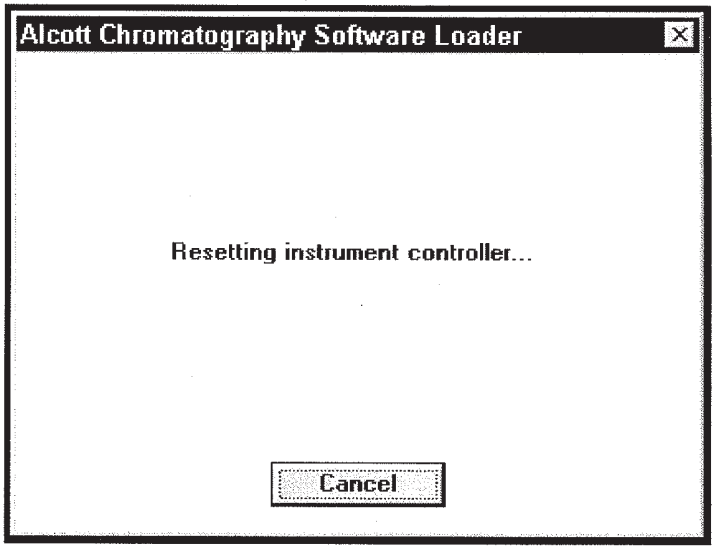


10. If Cancel is selected during the Loading software operation the following warning message is given. Normally canceling before the software load is complete will result in an instrument that cannot function. However, the instrument will still be able to communicate with this software update program, so the update process may be started again.

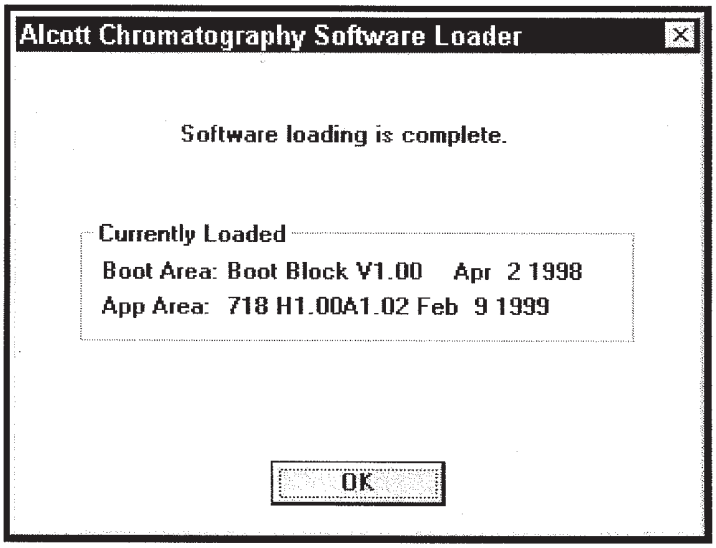


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11. Upon completion of the Load operation, the application is validated. The autosampler will be rebooted. During the rebooting process, the following screen is shown:

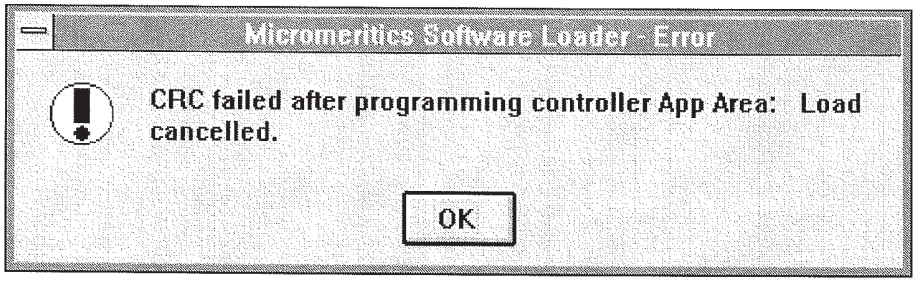


12. A few moments after the instrument has been reset, the following will shown:



Note that a possible error message, as shown below, may occur if the software being loaded was corrupted in some way. Repeat the update procedure and if the problem persists, notify Alcott Service at (800) 229-5052.

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13. Once the autosampler has rebooted with the new code, clear the Methods space by pressing the [F2] key (the [FUNCTION] key followed by the [2] key) then pressing [ENTER]. 718 AL users will need to re-edit their System Setting for syringe size, Tray size and Needle Depth. .

14. Note the Instrument Model Number and Serial Number located on the Rear of the Instrument. These may have to be re-entered into the Autosampler when new code is downloaded into the instrument. To verify, from the Front Panel, press [FNUCTION] then [4]. Press the [↓] key until the cursor points to Status, then press the [ENTER] key. The Screen should display the Model Number and the Serial Number. If these are not present, go on to Step 15. If the correct Model Number and Serial Number are displayed, press the [FUNCTION] followed by the [CLEAR] key.

15. With the Status Screen still displayed, press the [ENTER] key. The following Menu will appear:

```
      Change
→Model and S/N
 Download Software
 Get PIC info       ↓
```

Select "Model and S/N" then press the [ENTER] key. Enter the password 0865 and press [ENTER]. The following screen will appear:

```
   Model and S/N
→      <719/01>
 S/N.   XXXX
 Exit
```

The first line allows selection of the Model Number. The Model Number is selected by pressing either the [←] or [→] keys until the appropriate Model Number is displayed. Once the Model Number is selected, press the [↓] key and enter the Serial Number followed by pressing the [ENTER] key. Once the Model Number and Serial Number have been entered, select Exit and press the [ENTER] key to exit the Menu. The press the [FUNCTION] key followed by the [CLEAR] to return to the main screen.

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