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234 Autoinjector
User's Guide

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234 Autoinjector
User's Guide

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SAFETY

Please read this section carefully before installing and operating the equipment.

For safe and correct use of the instrument, it is essential that both operating and service personnel follow generally accepted safety procedures as well as the safety instructions given in the 234 Autoinjector User's Guide.

The instrument described in the 234 Autoinjector User's Guide is an automatic sample injector and should be used for sample injection operations only. The instrument should only be used in the laboratory or similar indoor environment for analytical purposes, by qualified personnel. If an instrument is used in a manner not specified by Gilson, the protection provided by the instrument may be impaired.

When the vertical arm and needle have been installed, it is necessary to check that nothing hinders the movement of the instrument parts (i.e. needle and arms). Nothing should be placed in or around the field of movement of either the horizontal or vertical arms.

Due to the fact that the needle installed on the vertical arm may contain a dangerous substance, the user is strongly advised against interfering in the work area of the instrument until the instrument has come to a complete stop. The current program that is running must be paused or stopped. Refer to Section 4.9.5 for information about pausing a program, and Section 4.9.6 for information about stopping a program. If you stop the program during a run, ensure that you prime the pump, tubing and needle again before restarting a program; this will flush out any remaining sample liquid from the needle and transfer tubing.

Voltages present inside the instrument are potentially dangerous. If there is a problem with the instrument, the power cable should be removed until qualified service personnel have repaired it. This is to prevent anyone from inadvertently using the instrument, thus causing possible harm to themselves, or damage to the instrument itself.

The leakage current of this instrument is within the limits allowed by international safety standards for laboratory equipment. ***An efficient ground connection is imperative for the physical protection of the user.***

Power supply cord reference 500005 is for use in France and Germany. Power supply cord reference 500006 is for use in the USA and Canada. For other countries contact your local Gilson representative. You must only use the type of fuse described and specified in this document: 3.15 Amp type "T" slow blow.

When installing the instrument, ensure that the ventilation fan on the rear panel functions and is not obstructed.

Adequate protection including clothing and ventilation must be provided if dangerous liquids are used in the analytical work. If incidental spillage occurs, carefully clean-up the spillage, taking into account the nature of the spilled liquid and the necessary safety precautions.

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SAFETY

Cleaning, dismantling, maintenance, adjustment and repair should only be performed by personnel trained in such work, and who are aware of the possible dangers involved.

The instrument must not be sterilized, using an autoclave, or any other mechanical device. When you need to clean the instrument, use one of the following methods:*

1 - A clean dry cloth.
2 - A cloth dampened with water.
3 - A cloth dampened with soapy water.

If a cloth dampened with soapy water is used to clean the instrument, only domestic soap may be used. No other form of detergent or chemical may be used.

☞ ** Individual components on the syringe pump such as the valve, syringe have special autoclaving procedures. These components may be dismounted and cleaned following specific instructions given in the maintenance chapter.*

The following electronic and hazard symbols appear on the Autoinjector:

Symbol	Explanation
~	Alternating current
⏚	PROTECTIVE CONDUCTOR TERMINAL
I	On (Supply switch)
O	Off (Supply switch)
	Caution, risk of electric shock
	Caution (refer to accompanying documents)

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INTRODUCTION

1 NOTATIONAL CONVENTIONS

Typographical conventions used in this *User's Guide* for software options, key touches and on-screen messages are as follows:

- For any soft-key option, that must be pressed the function is represented in bold, for example:

  "Press the **Run** soft-key to run the program"

- Keystrokes relating to specific keys on the Autoinjector keypad are represented in bold capitals, for example...

  "Key in the value and press **ENTER**" Where '**ENTER**' is the Enter key on the keypad.

  and

  "press the **STOP** key to stop a run" Where '**STOP**' is the Stop key on the keypad.

- On-screen messages are represented as normal type-face inside a frame representing the keypad display:

File: 1			Rack: 36	
File	Config	Flow/Vol	Manual	Run

2 DESCRIPTION

The 234 Autoinjector is used to inject ready-prepared samples into an HPLC system for analysis purposes. The instrument can dilute samples and standards and add an internal standard to samples and standards. Vials containing sample liquids are placed in a single rack held at the front of the unit.

The instrument incorporates an integral command keypad for control, an XYZ motion carriage for liquid transfer, a high pressure Rheodyne injection valve to inject samples into an HPLC system, and an integral pump for aspirating and dispensing the samples.

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The instrument can be fully programmed for aspirating, diluting and injecting samples into an HPLC system. The instrument coordinates automatically with other devices; by GSIOC if Gilson devices are connected to the GSIOC channel, or by contact outputs from the rear panel. It is possible to record the events that have occurred during a run using the audit trail facility. The audit trail file can then be transferred to the audit trail diskette via a suitable PC, see Appendix E.

Although the instrument is designed to be user-friendly, you are advised to read this manual carefully before operating the Autoinjector.

3 UNPACKING

The 234 Autoinjector is supplied in a single crate with its various accessories (needles, racks, transfer tubing, etc.). The original crate and packing assembly should be kept in case the equipment has to be returned to the agent.

All parts contained in the crate must be checked off against the packing list supplied. Do this now, even if the product is not to be used immediately. The supplier should be notified immediately of any inconsistencies between the contents of the crate and the packing list or of any damage to parts.

When unpacking the instrument:

- Remove the standard accessories carton from the crate.
- Remove and open the carton containing the instruments standard accessories.
- Slide the instrument and packing assembly out of the crate.
- Remove the packing assembly.

When delivered, the horizontal arm (Fig. 1.1) is secured to the instrument's right-hand side panel by means of a screw - this is the transportation position. The horizontal arm should be secured in the transportation position whenever the Autoinjector is to be moved. Failure to do so could result in severe damage to the instrument. Remove this screw before installing the instrument; it is located behind a plastic plug situated on the right-hand side panel.

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INTRODUCTION

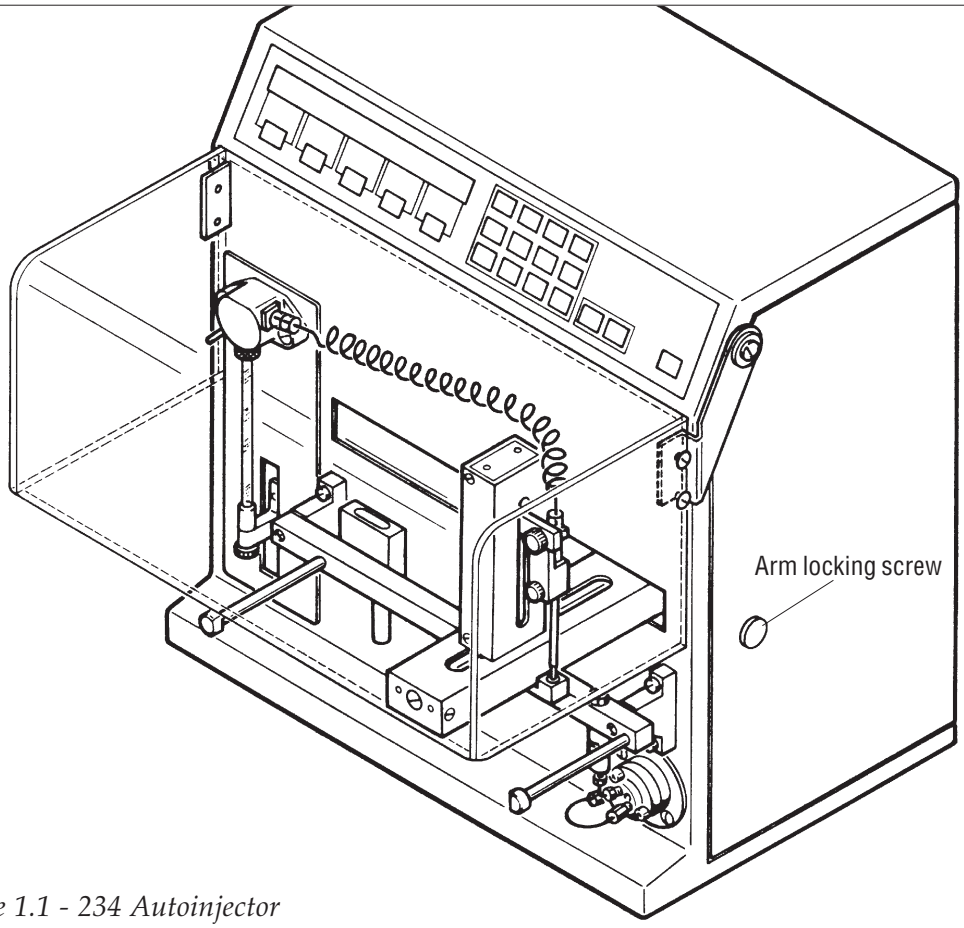


Figure 1.1 - 234 Autoinjector

4 WARRANTY

If the instrument does not appear to function correctly, first verify the electrical connections are correct and that the instruments are switched ON. Contact your Gilson agent for technical advice or an eventual service visit. Any service required will be given within the warranty conditions assured by your Gilson agent.

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5 CUSTOMER SERVICE

Gilson and its worldwide network of authorized representatives provide customers with four types of assistance: sales, technical, applications and service.

The service personnel of Gilson's representatives are able to serve you more efficiently if you provide the following information.

1) The serial number and model number of the equipment involved.
2) The installation procedure you used.
3) A concise list of the symptoms.
4) A list of operating procedures and conditions you were using when the problem arose.
5) A list of other devices connected to the system and a system diagram showing the connections.
6) A list of other electrical connections in the room.

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DESCRIPTION

1 MECHANICAL ASSEMBLY

The front panel of the instrument contains: the integral keypad, pump, injection valve and carriage assembly.

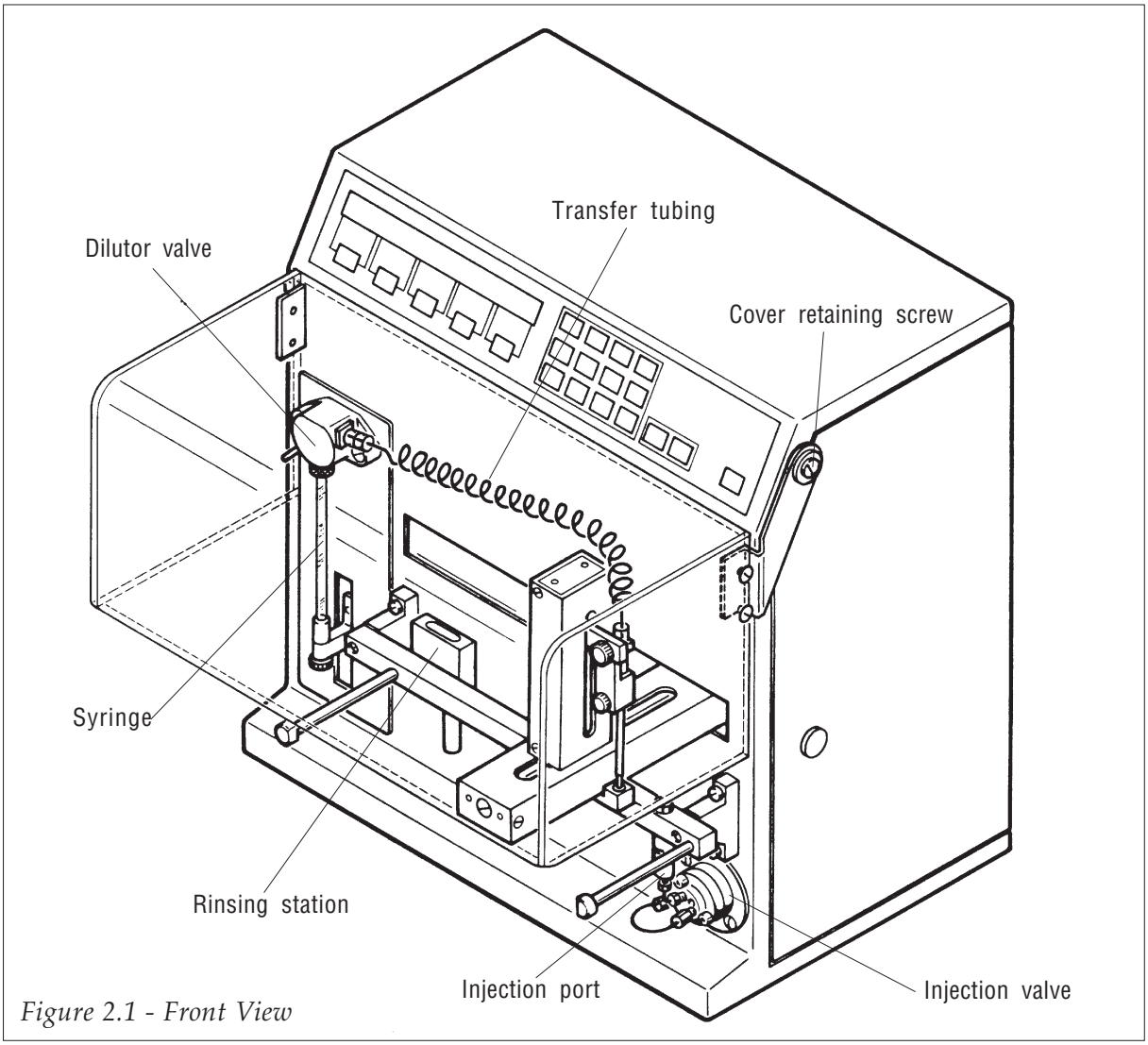


Figure 2.1 - Front View

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The rear panel of the unit contains the input/output connector, GSIOC connector and the sector block (power socket with ON/OFF switch and fuse holder).

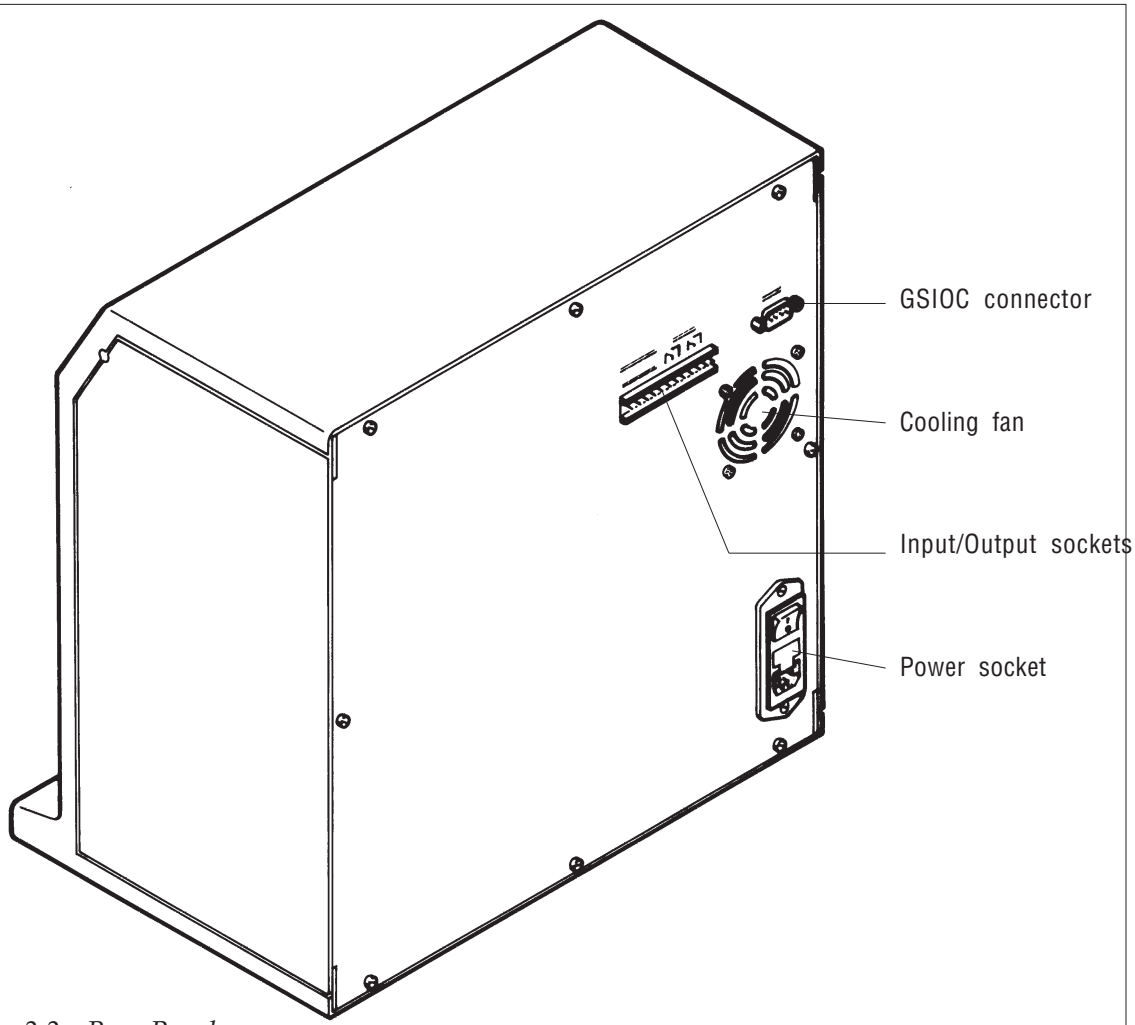


Figure 2.2 - Rear Panel

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DESCRIPTION

1.1 XYZ Motion Carriage Assembly

The purpose of the carriage assembly is to position the needle for the aspirate, dilute, dispense, inject and rinse needle operations. There are three motors that control the position of the needle and carriage, one motor for each axis of motion (X, Y and Z). Vertical movement of the needle is in the Z-axis, movement of the carriage from left to right along the front of the Autoinjector is along the X-axis, and movement of the vertical arm outwards from the Autoinjector is in the Y-axis. The needle position is calculated with reference to the Home Position* for all operations.

☞ ** The Home Position is the front, left-most position with the vertical arm closest to the Autoinjector body, and with the needle raised in the uppermost position.*

1.2 Pump

The pump is a Dilutor liquid transfer station, designed to optimize sample injection applications. It consists of two motors, one that operates the syringe to aspirate and dispense, and a second motor that controls the switching valve. The pump is controlled by programs written using the keypad. To change the syringe, refer to Section 3.3.

1.3 Rheodyne® Valve Assembly

The Rheodyne 7010 injection valve, 20 µl sample loop and injection port are factory fitted to the Autoinjector. The valve is used to inject samples in HPLC applications. The valve has two positions: Load and Inject. Samples are loaded into a sample loop from the needle, then the valve is switched into the inject position and the sample is pumped along with the mobile phase into the column.

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1.4 RINSING STATION

The instrument is equipped with one rinsing station situated at the left of the rack support bar (see Figure 2.3). It is used to clean the needle both internally and externally. The rinsing process is performed as follows:

1) A predetermined volume of rinsing solvent is flushed through the needle, removing bubbles from the FEP tubing and flushing any insoluble matter from the needle. This solvent is dispensed in the rinsing station drain.
2) The needle is then plunged into the rinsing well containing the solvent, which serves to rinse the needle externally. Another volume of rinsing solvent is then dispensed in the rinsing well.

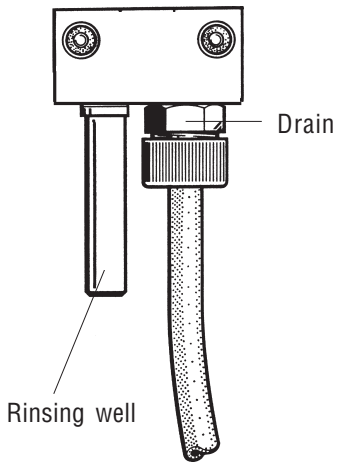


Figure 2.3 - Rinsing Station

Excess solvent in the rinsing well overflows into a tube leading to a waste receptacle.

1.5 GROOVED NEEDLE

The needle used with the 234 Autoinjector has a groove along the outside body. This groove allows a constant pressure equilibrium inside vials fitted with septum covers. When liquid is aspirated from a vial fitted with a septum cover, air enters the vial via the groove. The internal diameter of the needle is 0.4 mm, the external diameter of the needle tip is 0.7 mm.

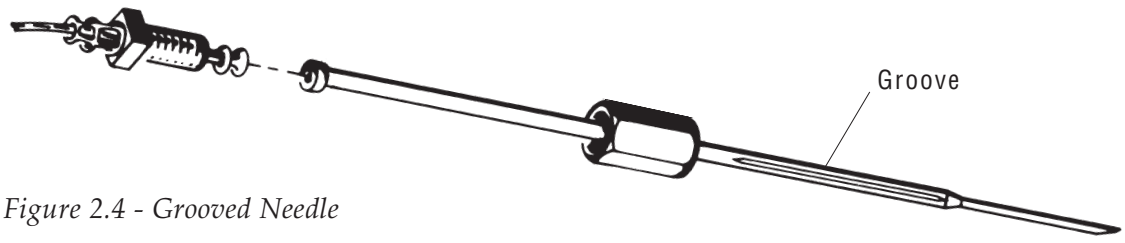


Figure 2.4 - Grooved Needle

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DESCRIPTION

2 ELECTRICAL ASSEMBLY - FRONT PANEL

The integral keypad and display are housed on the front of the instrument.

2.1 KEYPAD AND DISPLAY

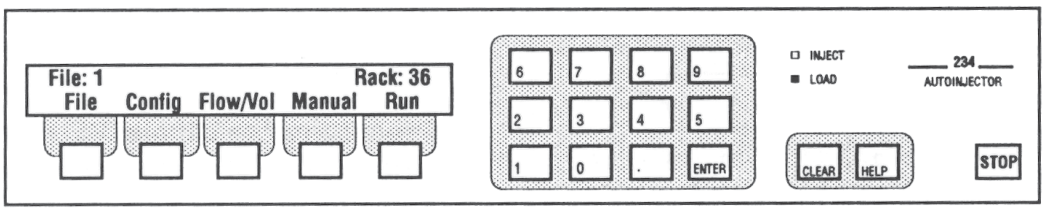


Figure 2.5 - Keypad and Display

The keypad has the following touch-keys:

- Numeric keypad
- Decimal point
- Enter
- Clear
- Help
- Stop
- Five software function keys (Soft-Keys)

The keypad also has two green LEDs indicating the injection valve position (Inject or Load).

The LCD display has two lines, each of 40 characters, and an LED backlight.

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3 ELECTRICAL ASSEMBLY - REAR PANEL

The rear panel of the Autoinjector houses:

- One sector block containing the power socket, fuse drawer and ON/OFF switch.
- One electrical control socket with three inputs, and two relay outputs for coordinating the Autoinjector with other instruments.
- One GSIOC connector through which other Gilson instruments can be linked, including a Gilson 506B or 506C interface for coordinating with Gilson system controller software (712 or 715).

3.1 SECTOR BLOCK

The sector block contains the power socket, fuse holder and ON/OFF switch. The instrument can be connected to 110 V or 220 V.

- When operating from a 110-120 V supply, 3 A type T slow blow fuses must be installed.
- When operating from a 220-240 V supply, 3.15 A type T slow blow fuses must be installed.

Refer to Section 3.1.2. for a description of how to install the fuses.

3.2 ELECTRICAL CONTROL SOCKET

The electrical control socket has two EMC protected relay outputs and three EMC protected inputs. A terminal block connector (reference 506406) is provided with the standard equipment to facilitate wire connections with other devices.

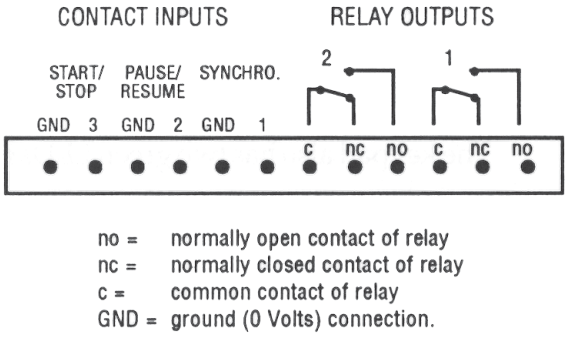


Figure 2.6 - Output Connections

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DESCRIPTION

♦ OUTPUTS

The two relay outputs have three contacts each: common, nc (normally closed) and no (normally open) contacts. These outputs are used for coordination when the injection is achieved.

Output 1 = Priority sample pulse.

Output 2 = When coordination is by chromatography time, this provides the pulse when the sample or standard is injected.
When using coordination by contacts, this provides the handshake signal for integrator when the sample or standard is injected.

♦ USING THE RELAY OUTPUTS

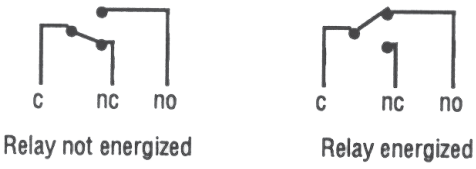


Figure 2.7 - Operation of the Relays (Outputs 1 and

Figure 2.7 and the accompanying text explain how relay contacts are used.

When the relay is energized (ON), as in the example on the right in Figure 2.7, the common (c) contact is connected to the normally open (no) contact. The normally closed (nc) contact is now disconnected.

If you want to connect a device (a bench integrator for example) to an output so that the device operates at the time of injection of a sample, connect two wires from the device start connections (refer to your integrator connections) to the common and normally open contacts of output 2. When a sample is being prepared, the integrator waits for the start signal. On sample injection, the relay is pulsed and the common connection is connected to the normally open contact for 1.8 seconds. This provides the start signal for the integrator.

♦ INPUTS

The three inputs (Figure 2.6) are used for coordinating the following functions:

Input 1 = Coordination input (wait before injection).
Input 2 = Pause/Resume input.
Input 3 = Start/Stop input.

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You use a pair of wires to connect each input to the relevant output of the device that coordinates the injection. (One wire of the pair must be connected to the relevant input, and the second wire of the pair is connected to the associated ground connection.) The connections you make depend on your system. Basically, you must connect the input and its ground connection to a pair of output connections from another device.

3.3 GSIOC CONNECTOR

The GSIOC connector can be used to coordinate with other Gilson devices rather than using the input/output connections, and can be connected to a PC via a 506C interface for communication with a system controller such as 712 or 715. It is also possible to produce an audit trail (a list of events as they happened during the run of a method program) on the PC (refer to Appendix E).

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INSTALLATION

All instruments must be switched off before carrying out any installation procedures!

The 234 Autoinjector is delivered with the support bar, rinsing station and Rheodyne model 7010 injection valve (with sample loop and injection port) factory-fitted. For safety purposes, the fuses are not installed, they are supplied in the standard accessories packing. You must install the fuses before using the instrument.

1 POWER CONNECTION/FUSES

1.1 VOLTAGE

The instrument has no physical voltage selector. The instrument can be connected to 110 or 220 V. The selection is carried out electronically on the power supply board. However, the fuses used for 110 V and 220 V operation are different. Ensure that you install the correct value fuses, detailed in the following section.

1.2 FUSES

The fuse types required are 3.15 Amp type "T" slow blow.

For safety reasons, Autoinjectors are delivered without fuses installed. You must install the fuses on installation.

To install the fuses:

1) The power lead must be disconnected from the instrument.

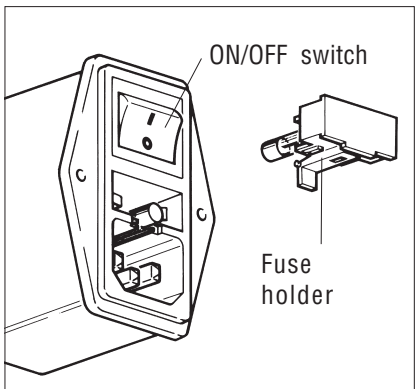


Figure 3.1 - Sector Block

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2) Use a small screwdriver to pry open the fuse holder drawer and slide it out from the power receptacle. The drawer hinges downwards to allow access to the fuse receptacle.
3) Push the two fuses into position.
4) Push the fuse holder back into position in the sector block.

1.3 CONNECTING THE POWER CORD

Before connecting the instrument for the first time (after delivery), check that the arm locking screw that secures the carriage in the transport position has been removed.

Make sure that the ON/OFF switch is in the OFF (down) position. Connect the power cord to the power socket at the rear of the unit and then to the power supply source. When the ON/OFF switch is put in the ON position, the keypad screen illuminates and displays:

```
Model 234 Version X.XX

Initializing...
```

The instrument initializes and carries out the self-calibration automatically.

⚠ ***Do not attempt to impede the functioning of the self-calibration procedure.***

2 INSTALLING THE NEEDLE

To install the needle on the vertical arm:

1) Slide the needle into the tubing fitting, Figure 3.2.
2) Select the FEP transfer tubing for the needle to pump connection.
3) Connect the selected tubing to the tubing fitting.

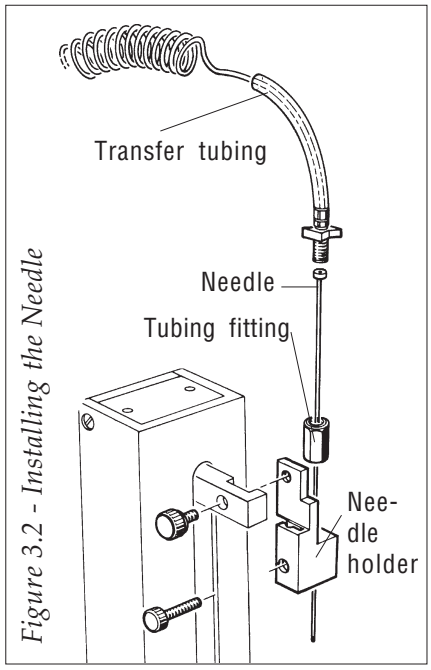


Figure 3.2 - Installing the Needle

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INSTALLATION

4) Slide the needle assembly into the needle holder on the vertical arm so that it passes through the needle guide.
5) Secure the needle in place using the needle retaining screw Figure 3.2.
6) Connect the other end of the FEP transfer tubing to the valve as described in Section 3.4.2.

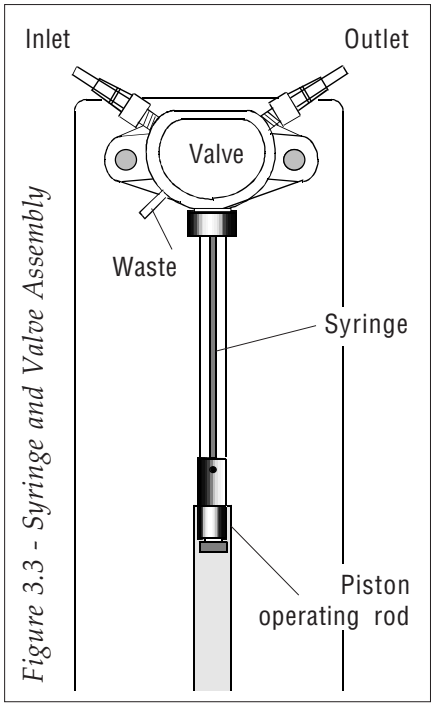


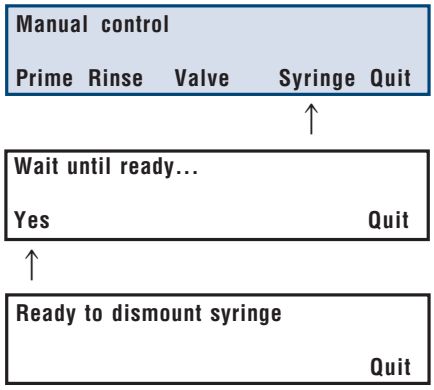
Figure 3.3 - Syringe and Valve Assembly

3 CHANGING THE SYRINGE ASSEMBLY

To optimize the fitting of the 100 µl syringe and 500 µl on the Dilutor valve, the syringes are supplied with a cover seal. The orientation of this seal must be respected to ensure that the connection with the syringe is airtight. (See Figure 3.3b.)

The Autoinjector is supplied with the pump valve and 500 µl syringe fitted. To change the syringe:

1) Undo the screw that secures the syringe piston to the underside of the piston operating rod (Fig. 3.3).
2) Go to the Manual menu.
3) Press the **Syringe** soft-key, a second screen is displayed.
4) The piston operating rod descends. A new screen is displayed:
5) Unscrew the two valve retaining screws (one on each side of the valve) and remove the valve and syringe assembly from the instrument.
6) Unscrew the syringe from the valve.



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- **Before Mounting a 100 µl Syringe**

Reference	Description
F62182	100 µl syringe
425050	Transfer tubing, FEP, 120 µl, 600 x 1.6 x 0.5 mm
501055	Self-adhesive tubing clips.

To mount a 100 µl syringe, you need the following components:

First manually prime the 100 µl syringe by placing the open end of the syringe in the dilutor reservoir and using the piston to aspirate solvent.

Ensure that the syringe is at least half full.

Replace the 550 µl transfer tubing with the 120 µl transfer tubing.

The 120 µl transfer tubing should be coiled and attached to the front face of the vertical arm using the self-adhesive tubing clips in such a way that the tubing does not snag on the instrument when the arm and needle are moving.

- **Before Mounting a 500 µl Syringe**

The piston nose must be wet (with water or liquid from the dilutor reservoir) before it is inserted into the syringe. This helps to ensure the correct operation of the syringe. This is particularly recommended for priming the syringe. If you are pumping viscous liquids it may be necessary to partially fill the syringe with liquid before installing the syringe. Place the open end of the syringe in the pump reservoir and gently pull on the piston button to raise the syringe and aspirate the liquid.

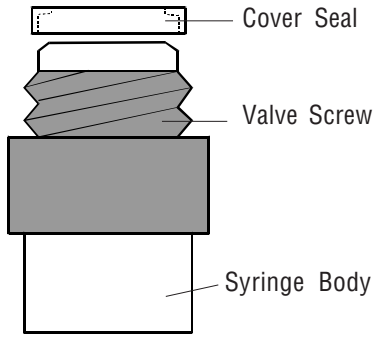


Figure 3.3b - The Cover Seal for the 500 µl Syringe

To mount the new syringe:

7) Ensure that the cover seal is mounted on the valve screw of the syringe. Screw the syringe into the valve unit until tight. Do not over tighten the syringe.

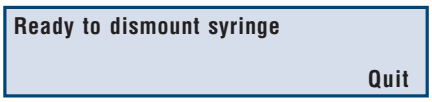
8) Install the valve on the Autoinjector, ensure that you align the valve operating shaft with the recess in the valve motor (visible inside the instrument). Secure the valve in place using the two retaining screws.

9) Move the piston so that it comes into contact with the piston operating rod.

10) Locate the hole at the bottom of the piston on the piston operating rod and tighten the piston holding screw.

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11) Press Quit, the instrument re-initializes.



☞ *If you have installed a new syringe of a different size, don't forget to change the declared syringe volume in the configuration branch of the software.*

4 PUMP CONNECTIONS

The tubing connections to the pump should be made as follows:

4.1 PUMP INLET TUBING

The inlet tubing is connected to the upper left-hand (inlet) port of the valve and leads to the pump's reservoir. Two different lengths of inlet tubing are supplied with the Auto-injector. Select the appropriate length according to the distance between the pump and its reservoir.

The lengths are: E45357 FEP Inlet tubing, 650 x 3 x 2 mm, with a 316L, 20µm filter
425019 FEP Inlet tubing, 350 x 3 x 2 mm*

☞ ** If the shorter length of tubing is used, secure the filter to the non-flanged end.*

Secure the flanged end of the inlet tubing to the inlet port using the PVDF connector. Connect the filter to the free end of the tubing and place this end in the reservoir.

4.2 PUMP OUTLET (TRANSFER) TUBING

The outlet tubing is connected between the right-hand outlet port of the valve and the Auto-injector's needle. This tubing is referred to as FEP transfer tubing (reference B60086, volume 550 µl). Secure the flanged end of the transfer tubing to the outlet port using the PVDF connector, connect the other end of the transfer tubing to the needle on the vertical arm.

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4.3 PUMP WASTE TUBING

Waste tubing push-fits to the lower left-hand (waste) port on the valve. Place the free end of the waste tubing in a suitable receptacle.

The waste port acts as a pressure relief mechanism; if the pressure inside the valve goes above 8 bar, liquid flows out of the waste tubing. Pressure build-up may be the result of: blockage in the tubing, a flow rate set too high for the operating conditions, or the internal diameter of some of the system tubing is too small.

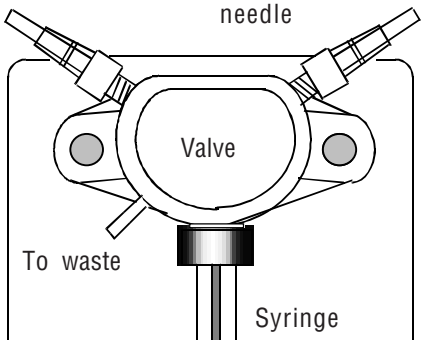


Figure 3.4 - Pump connections

5 INJECTION VALVE CONNECTIONS

When connecting the Rheodyne 7010 injection valve, carefully follow the instructions given below. These instructions are different from those described in the Rheodyne leaflet.

The bushings and ferrules used to make the following connections are shipped in the Rheodyne accessories kit.

The Rheodyne valve is factory-fitted to the Autoinjector, and has a 20 µl sample loop and the injection port fitted. To change the sample loop, follow the instructions in the Rheodyne leaflet.

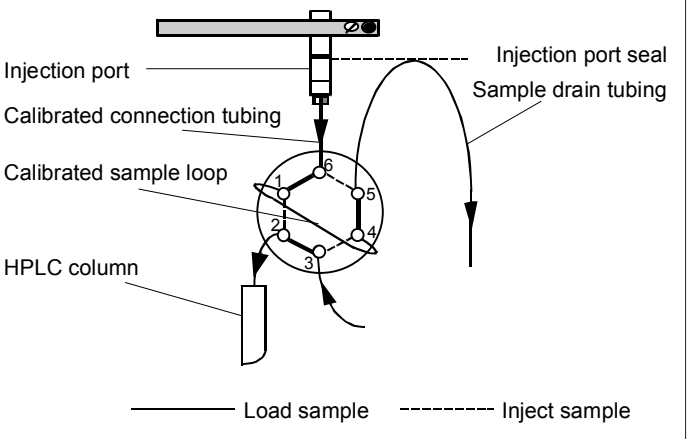


Figure 3.5 - Model 7010 Rheodyne Injection Valve

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INSTALLATION

5.1 Sample Drain Tubing

The sample drain tubing connects to port 5 of the Rheodyne injection valve and leads to a suitable collecting receptacle.

1) Use a long Rheodyne bushing and a ferrule to connect the tubing to port 5.
2) Bend the tubing upwards until it is level with the seal in the injection port. This prevents air bubbles from moving into the loop from the injection port as a result of out-siphoning.
3) Bend the outlet of the tube so that the end of the tubing is 5 mm below the level of the seal in the injection port. This prevents waste liquid from siphoning back into the sample loop when the valve is in the load position.

5.2 Mobile Phase Inlet Tubing

The mobile phase inlet tubing connects from the HPLC system's purge valve to valve port 3.

Use a short Rheodyne bushing and ferrule to connect the tubing to valve port 3.

5.3 Column Inlet Tubing

This tubing connects from valve port 2 to the HPLC column.

For this critical connection, select the tubing length that best suits the physical location of the column in your system (48.5 or 100 mm).

Use a long Rheodyne bushing and a ferrule to connect the tubing to port 2.

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6 ZONE MASKS

There are two zone masks, one for rack code 35 and one for rack code 36. The zone mask indicates the two vial zones when using either dilution or an internal standard and indicates the priority vial zone. The mask helps you identify where the each zone starts. Put the mask on the rack *before* placing the vials in the rack.

- The priority vial zone is colored red
- The raw sample zone is colored yellow
- The prepared sample zone is colored green.

☞*Before installing a new zone mask, remove the thin protective polypropylene film.*

7 THERMOSTATING RACKS

Thermostating racks consist of one aluminum rack and a thermostating cuvette. They are used to regulate the sample temperature, e.g. cooling for the preservation of biological samples or to avoid evaporation and moderate heating for chemical derivatization. There are two different types of thermostating cuvette:

- Hydraulic Thermostating Cuvette,
- Peltier Effect Thermostating Cuvette.

7.1 HYDRAULIC THERMOSTATING CUVETTE

The temperature is controlled by a cryo-thermostat. The possible temperatures range from 4 to 60°C.

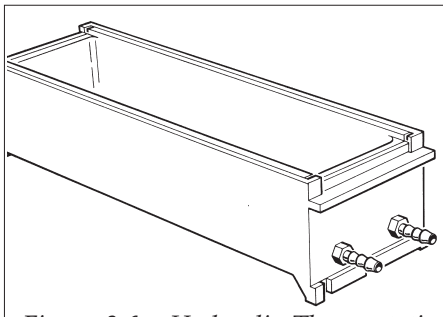


Figure 3.6 - Hydraulic Thermostating

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INSTALLATION

Installation

1) Install the rack in the thermostating cuvette.
2) Position the cuvette and rack on the Autoinjector's rack holding bars.
3) Make the hydraulic connections between the cuvette and the cryothermostat.

7.2 PELTIER EFFECT THERMOSTATING CUVETTE

The thermostating racks can be temperature controlled using a Peltier Effect thermostating cuvette, controlled by the 832 Temperature Regulator. The possible temperatures using a Peltier Effect thermostating cuvette range from 4 to 40°C.

Installation

1) Install the rack in the thermostating cuvette.
2) Position the cuvette and rack on the Auto-injector's rack holding bars.
3) Make the electrical connections between the cuvette and the 832 Temperature Regulator, refer to the *832 Temperature Regulator User's Guide*.

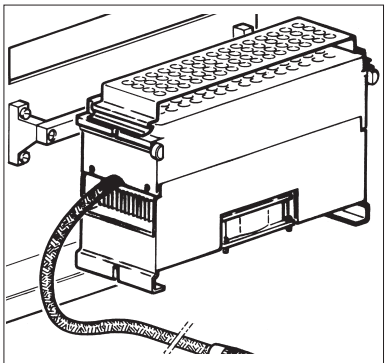


Figure 3.7 - Peltier Effect Thermostating Cuvette

7.3 PERFORATED COVERS FOR THERMOSTATED RACKS

Use the relevant perforated stainless steel cover when cooling samples below the ambient temperature, as condensation forms on the surface of the rack and falls into the rack wells. This water accumulates in the wells and forces the vials in the rack to rise. If this happens, the needle movement may be obstructed, which could lead to needle damage. The stainless steel covers clip onto the rack handles as shown in Figure 3.7.

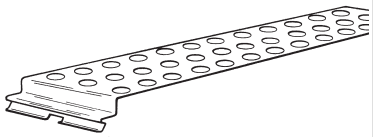


Figure 3.8 - Perforated Cover for Thermostated Rack

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8 REMOVING THE COVER

The plastic cover can be removed from the Autoinjector if you don't want to use it. The cover is secured by two screws, one on either side of the instrument.

To remove the cover:

1) Rotate the cover upwards and backwards until it rests on the top of the instrument.
2) Undo the two retaining screws that secure the cover to the instrument (refer to Fig. 2.1).
3) Remove the screws (with their washers) from the hinges.
4) Lift the cover away from the instrument.

To refit the cover:

1) Place the cover on the top of the instrument.
2) Align the ridge on each hinge with the groove on the side of the Autoinjector.
3) Insert the screws into the hinges and fasten the cover to the instrument using a flat-blade screwdriver.
4) Tighten the screws so that the cover remains in the vertical position when released.

9 FITTING THE RINSING STATION DRAIN TUBING

The instrument is supplied with isoversinic tubing, 1000 x 6.5 x 4 mm (reference B60116) that should be used to connect the rinsing station drain to a suitable waste receptacle.

1) Cut the drain tubing to a suitable length.
2) Slide the securing nut over the end of the drain tubing that you are going to connect to the rinsing station.
3) Slide the drain tubing onto the rinsing station drain outlet.
4) Screw the securing nut onto the rinsing station drain.
5) Place the free end of the drain tubing into the waste receptacle.

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INSTALLATION

10 MODEL 7413 RHEODYNE INJECTION VALVE

For certains applications, i.e. when dealing with smaller volumes, replace the factory installed model 7010 valve with model 7413 valve. The model 7413 Rheodyne injection valve has an internal loop of 1 µl.

The procedure for valve replacement is explained below.

Dismounting the 7010 valve:

- Disconnect all tubing to the valve. Including the injection port calibrated tubing.
- Remove both attachment screws using a screw-driver then slide out the valve assembly.
- Unscrew the Allen screw then slide the pinion assembly off the valve schaft.
- Loosen the two screws and remove the mounting support from the body (refer to Figure 3.9).

Mounting the 7413 Rheodyne injection valve:

- Remove the valve handle.
- Mount the mounting support on the valve body by inserting one of the two screws in the hole marked 1 (or 2 if remounting the 7010 valve).
- Slide the valve through the mounting support. Then slide the pinion assembly onto the valve shaft (refer to Figure 3.10) such that the pinion assembly is against the mounting support. Then insert the fixing screws into their correct holes (1 for 7413 and 2 for 7010). Refer to Figure 3.10. Connect all the tubings as shown in Figure 3.5, Section 3.5.

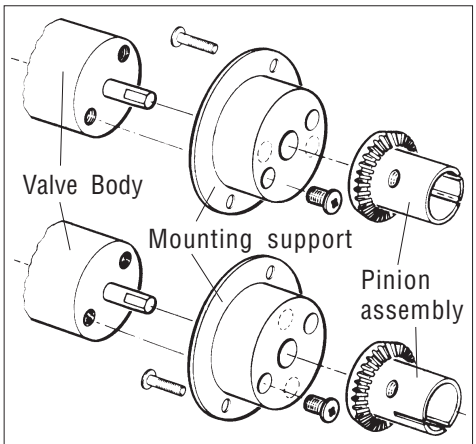


Figure 3.9 - Injection valve assembly

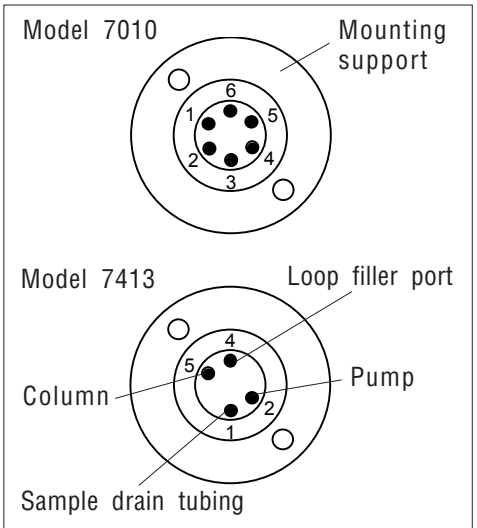


Figure 3.10 - Installed injection valves - front view

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INSTALLATION

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SETTING-UP AND OPERATING

1 INTRODUCTION

The 234 Autoinjector operates in five injection methods:

- Simple injection,
- External standard method,
- External standard method with dilution,
- Internal standard method,
- Internal standard method with dilution.

Each method is described in Section 5.2.

The software offers three loop filling modes:

- Total loop filling,
- Partial loop filling,
- Centered loop filling.

The different loop filling modes are explained in Section 5.3.

The 234 Autoinjector is controlled from its own keypad and can communicate with other devices via input/output contacts on the rear panel, or by GSIOC for communicating with Gilson HPLC System controller software (712 or 715) via a 506 B or C interface. There are two types of file:

- The 'Run file', the file that was last run or is currently running, refer to Section 6.1.1.
- The 'Edit file', the file that was last edited or written, refer to Section 6.1.2.

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2 SWITCHING ON

Use the switch on the line block socket on the rear panel of the 234 Autoinjector to turn it on (I = on, O = off). The LCD displays the following information while the Autoinjector goes through the initialization and self-calibration procedure:

```
Model 234 Version X.XX

Initializing...
```

The carriage, vertical arm and needle are first moved to the home position, then to the extremities of their allowed movements (X, Y and Z movements) as the self-calibration procedure is carried out. This procedure is entirely automatic and only takes a few seconds.

Do not attempt to impede the movement of the arm or needle during the self calibration procedure.

3 USING THE KEYPAD

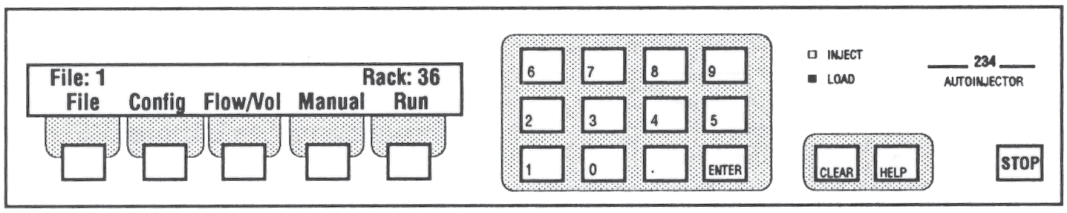


Figure 4.1 - Autoinjector Keypad

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SETTING-UP AND OPERATING

3.1 SOFT-KEYS

There are five white soft-keys situated below the LCD display. The bottom line on the display indicates the function of each soft-key. These functions change according to the screen currently accessed. As you progress through a branch of the software, the available functions change. Pressing a soft-key selects the function displayed directly above that key.

3.2 NUMERIC KEYPAD

The numeric keypad allows you to select file numbers, enter rack code numbers, and values when writing a method file. Values can be entered within the range 0.00 to 999, depending on the parameter you are defining. Key in the desired value using the numeric keypad and press the **ENTER** key. If you do not press the **ENTER** key, the value is not accepted, and the original value displayed is used. For example, if you run a simple injection program with rack code 30, it is possible to process a total of 60 samples, if you want to process only five samples, when you define the number of samples to process (**Nb of samples:** __), you must key in the value and press **ENTER**. If you do not press the **ENTER** key, the Autoinjector will carry out the procedure for 60 samples.

3.3 CLEAR/HELP KEYS

The **CLEAR** key allows you to delete and change a value. Press **CLEAR** and then use the numeric keypad to enter the new value.

The **HELP** key provides you with on-line help messages, when in the ready-to-run mode or in the set-up mode. To access this facility, press the **HELP** key.

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3.4 STOP KEY

Use the **STOP** key, at any time during a run, to abort a method run. The Autoinjector stops immediately and the needle is returned to the home position.

Task	Action after STOP has been pressed
Priming	Pumping stops immediately, the needle returns to the home position.
Rinsing	Pumping stops immediately, the needle returns to the home position.
In run	Pumping stops immediately, the needle returns to the home position. Execution of file ends.
Pause	Execution of file ends.
Other menus	If a file is running or paused, pumping stops immediately, the needle returns to the home position. Execution of file ends.

4 READY-TO-RUN SCREEN

After switch-on and initialization, the Autoinjector displays the Ready-to-Run screen shown below:



File: 1 This indicates the Run file (the file that was last run). On initial installation, no run file exists, the message displayed is: **File: —** and the soft-key option **Run** is not available.

To run an existing file, key in the file number, press **ENTER** and press the **Run** soft-key.

Rack: 36 Indicates the current rack code entered in the configuration branch of the software. Check this code corresponds with the actual rack fitted to the instrument. If the two rack codes are different, re-configure the rack code using the **Config** soft-key. Otherwise you may damage the needle or Z-arm when you run a file.

File This option allows you to carry out file editing operations. Refer to Section 4.5.

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Config The configuration branch of the menu allows you to determine the physical parameters of the system, refer to Section 4.6.

Flow/Vol Allows you to set the flow rates and volumes for the main operations during a run, refer to Section 4.7.

Manual Allows you to dismount the syringe or prepare the system for a run by priming, rinsing or switching the injection valve, refer to Section 4.8.

Run Allows you to run the file that is displayed on the top line of the screen.

5 FILE MENU

Pressing the **File** soft-key on the Ready-to-Run screen puts you in the file editing mode:

You can load an existing file from memory to be copied, deleted or edited, enter a number for a new file to be created, or view the existing files using the **Dir** (directory) soft-key. You can store up to 20 files.



5.1 TOP LINE

File number: This indicates the Edit file. To select a file to edit, key in the file number and press **ENTER**, the file is displayed on the top line of the display. You can write an Edit file while another file is running. You can also edit a file that is currently running, refer to Section 7.7.

(Exists) Tells you that file 2 already contains a method. If the file number entered does not contain a method, the word (New) replaces (Exists) and the soft-key options **Copy** and **Delete** are not available.

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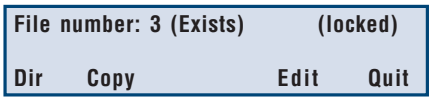
5.2 DIR

This option allows you to scroll through the list of existing files. Press the **Dir** soft-key repeatedly to view all existing file numbers. You will see the following types of screen:

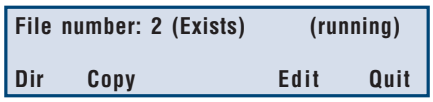
For files that have been written and saved, that can be edited, copied and deleted.



For saved files that have been locked, see Section 4.6.7. They can be reviewed using **Edit**, and copied. You can't delete them or change any parameters. To lock a file, refer to Section 4.6.7.



For a file that is running, the screen displays (running) on the top line. You can edit certain parameters and copy the file while it is running. (Refer to Section 7.7.) You cannot delete a file that is running.



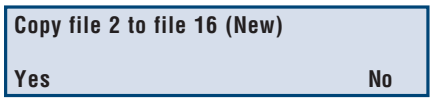
5.3 COPY

The **Copy** soft-key allows you to copy the file displayed on the top line of the display. For example, to make a copy of file 2, press the **Copy** soft-key. The top line of the display now shows:

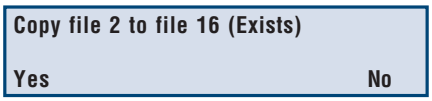


Enter the file number you want to create, for example file 16, and press **ENTER**. Two situations can exist:

- If file 16 is a new file, the top line displays: Press **Yes** to copy the file or **No** to return to the edit menu without copying the file.



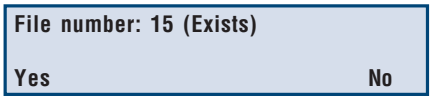
- If file 16 already exists, the top line displays: Press **Yes** to overwrite the existing file or **No** to return to the main menu.



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5.4 DELETE

To delete an existing file, for example file 15, press the **Delete** soft-key:

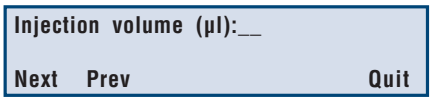


Press the **Yes** soft-key to delete the file or press **No** to return to the File menu without deleting the file. After the file has been deleted, **(New)** replaces **(Exists)**. You cannot delete a locked file or a file that is running.

5.5 EDIT

Use the **Edit** function to write a new file or to edit an existing file. You can also edit a file that is running; it is possible to change certain values, the new values are accepted and used for the next vial to be processed. Writing a file or method program is explained in Chapter 6. To edit the file displayed, press the **Edit** soft-key. This allows you to scroll through the program and modify parameters in the program by pressing the **Next** soft-key.

You cannot change the injection method. To change a value, key in the new value and press the ENTER key. Press the Next soft-key to advance through the program, press the **Prev** soft-key to step backwards through the program. Press **Quit** twice in succession to return to the Ready-to-Run screen.

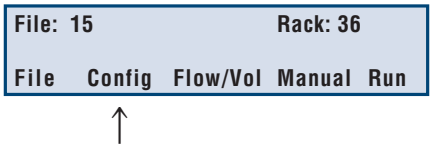


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6 CONFIGURING THE AUTOINJECTOR

Configuring the Autoinjector is the most important part of writing an application. Do this before writing your program. This part determines the movement of the needle, volume limits and available injection methods according to the entered rack code. It also determines the choice of injection method available; the internal standard method is not available for rack codes 30, 31 or 34.

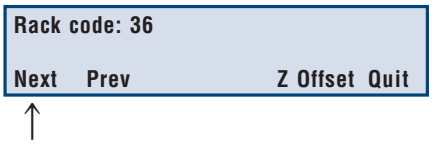
When in the Ready-to-Run screen, press the **Config** soft-key.



Configure the following, in the sequence described:

6.1 RACK CODE AND Z OFFSET

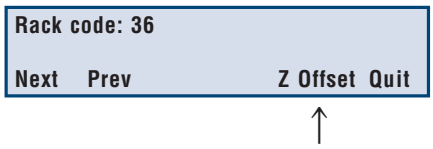
Key in the code that is marked on the rack you have installed on the instrument. Accepted rack codes are: 30, 31, 34, 35, 36, 37 and 38. Press **ENTER**, then press the **Next** soft-key to go on to the next step, or press **Z Offset** to modify the needle height at the vial bottom.



Z Offset

The bottom position of the needle can be offset by as much as ±66.0 mm in steps of 0.1 mm. This allows you to 'fine tune' the bottom position of the needle with respect to the vials that you are using. The default value for Z Offset is zero.

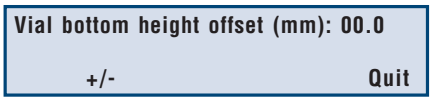
A value of '-2.0' means that the bottom position of the needle is 2.0 mm lower than the default offset position.



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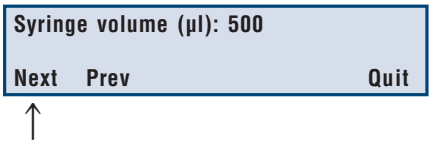
A value of '+2.0' means that the bottom position of the needle is 2.0 mm higher than the default offset position.

Key in the offset value. Press the ENTER key. Press the +/- Soft-key to toggle the sign from + to - or from - to + to obtain the required direction of offset. Press the Quit Soft-key to return to the configuration menu.



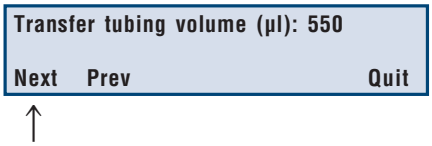
6.2 SYRINGE VOLUME (µl)

There are two sizes of syringe for the 234 Autoinjector pump: 100 µl or 500 µl. The Autoinjector is supplied with the 500 µl syringe fitted. Key in the appropriate value, press **ENTER** and press the **Next** soft-key.



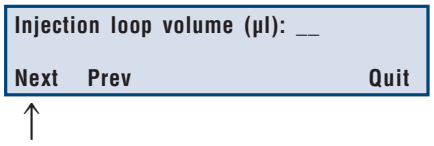
6.3 TRANSFER TUBING VOLUME (µl)

The transfer tubing is fitted between the pump outlet and the needle. The size supplied with the Autoinjector is 550 µl. Key in the appropriate value, press **ENTER**, then press the **Next** soft-key. When you use a 100 µl syringe, use the 120 µl tubing.



6.4 INJECTION LOOP VOLUME (µl)

The Autoinjector is supplied with a 20 µl injection loop. The loop is not calibrated, the value indicated is nominal. Enter the volume of the injection



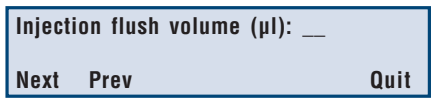
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loop fitted to the Rheodyne valve. There is a small plastic tag attached to the loop with the loop volume indicated. Key in the value, press **ENTER**, then press the **Next** soft-key. You can enter a volume between 1 and 500 µl.

6.5 Injection Flush Volume

This parameter applies to the total and partial loop filling methods. The injection flush volume is the volume of sample liquid required to flush the calibrated tubing between the injection port and the Rheodyne valve in order to inject an accurate volume of sample liquid into the loop. The injection flush volume is explained in more detail in Section 5.3.2.

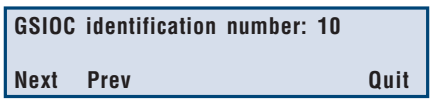
The default value for the injection flush volume is 15 µl, irrespective of syringe size (100 µl or 500 µl).



Key in a different value, if desired, press **ENTER**, then press the **Next** soft-key.

6.6 GSIOC Identification Number

Key in the identification number of the Auto-injector and press **ENTER**. The default value is 10. Press the **Next** soft-key.



6.7 Lock File



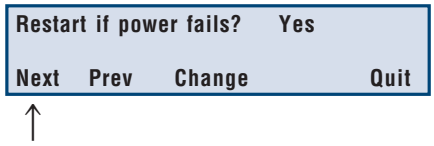
Once you have written and saved a method file, you can lock it to prevent it from being deleted or edited. Locked files can be copied. Use the **Change** soft-key to change the file status from locked to unlocked. Press the **Next** soft-key.



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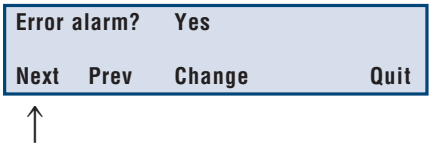
6.8 RESTART AFTER POWER FAILURE

This is a safety feature. If there is a power failure during a run, you select **Yes** for the instrument to automatically continue with the method. After a power failure, on power-up, the instrument initializes, rinses the needle and the injection port and then prepares and injects the next sample in the original sequence. However, the sample that was being prepared when the power failed will not be injected, it is lost. If you don't want the method to automatically restart after a power failure, press the **Change** soft-key so that the screen reads **No**. The default setting is No. Press the **Next** soft-key.

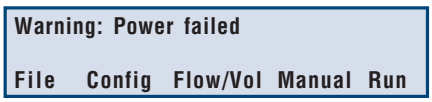


6.9 ERROR ALARM

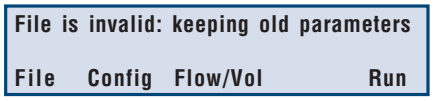
If the error alarm is selected, the Autoinjector will beep at half-second intervals after a power failure has occurred. Press the **Change** soft-key to change the option.



Power failure; after a power failure, the following message 'warning - power failed' appears and the alarm beeps. Press the **CLEAR** key or any key to cancel the warning message.



Invalid file; if you edit a file that is running, all valid parameters are accepted automatically and used for the next sample to be processed. However, if you enter an invalid parameter, the alarm sounds and the following message appears:



The file keeps and uses the original parameter for the method but also saves the invalid parameter. Press the **Next** soft-key.

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6.10 TIME/DATE AND COUNTERS

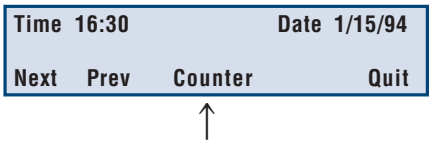
Verify and/or change the date and time shown on the display. This is important if you are going to use the audit trail function (Appendix E).

The time is indicated in Hours:Minutes. Set the time first by keying in the hour, press **ENTER**, the blinking cursor moves to the minutes indicator. Key in the minutes, press **ENTER**. The cursor moves automatically to the date display. The date is displayed in the format Month/Day/Year. Key in the month, press **ENTER**, key in the day, press **ENTER**, then key in the year and press **ENTER**. Press the **Quit** soft-key to return to the Ready-to-Run screen.

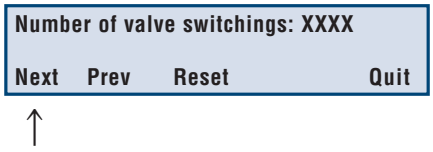


6.11 MAINTENANCE COUNTERS

You can access the maintenance counters from the Time/Date display by pressing the Counter soft-key. These counters indicate how many times the injection valve has switched, the number of XYZ travels, and how many dispenses have been carried out. The values indicated are:



This is the maintenance counter for the injection valve. It is incremented by 1 each time the injection valve is switched. Press the **Reset** soft-key to reset the counter to zero. Press **Next** to go to the XYZ motion counter.

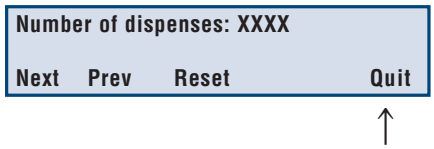


This is for needle and XYZ motion maintenance. The counter is incremented by 1 each time the needle goes into a vial. Press the **Reset** soft-key to reset the counter to zero. Press the **Next** soft-key to go to the pump maintenance counter.



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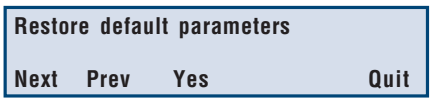
This counter is for pump maintenance. The counter is incremented by 1 for each dispense of the pump, irrespective of the volume dispensed. Press the **Reset** soft-key to reset the counter to zero. Press the **Quit** soft-key to return to the Ready-to-Run screen.



6.12 RESTORING THE ORIGINAL DEFAULT PARAMETERS

At any time, it is possible to reconfigure the Autoinjector with the original (factory set) default parameters. To do this, switch off the instrument, then hold down the **CLEAR** key while switching the instrument on again, hold the key down until the beep sounds.

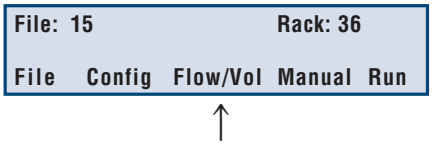
This process allows you to reset the original default parameters and to access the advanced user menus, as described in Section 4.10. When you press the **Quit** soft-key, the instrument initializes with the new settings.



7 FLOW/VOLUME SETTINGS

In this menu you set the values for the needle and valve rinse volumes, the air gap volume and aspiration flow rate and the prime, aspirate, dispense, mixing and diluent aspirate flow rates. The values defined here are used wherever these operations occur in a method run.

Press the **Flow/Vol** soft-key to set the flow and volume values.



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7.1 NEEDLE AND VALVE RINSE VOLUMES

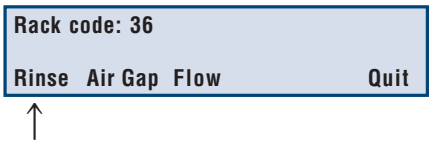
Press the **Rinse** soft-key to set:

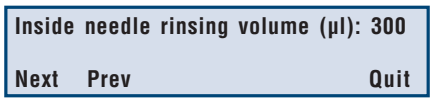
- the inside needle rinsing volume,
- the outside needle rinsing volume,
- the injection valve rinsing volume.

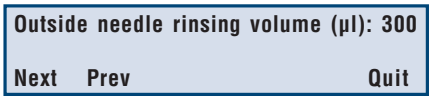
All volumes are in µl. The default values stated here are all for an Autoinjector fitted with a 500 µl syringe. The needle is rinsed in the rinsing well and drain station. The needle is rinsed with diluent from the pump reservoir. Before a run, the needle is rinsed.

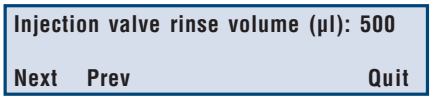
To change the factory set value of 300 µl, for the inside and outside needle rinsing volumes, key in the new value, press **ENTER**, then press the **Next** soft-key.

The injection valve rinse volume is dispensed in the injection port when the injection valve is in the inject position. To change the factory set value of 500 µl, key in the new value, press **ENTER**, then press the **Next** soft-key.





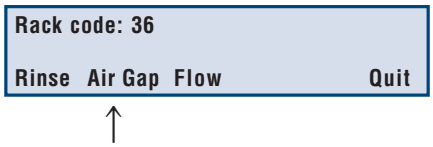




7.2 AIR GAP VALUES

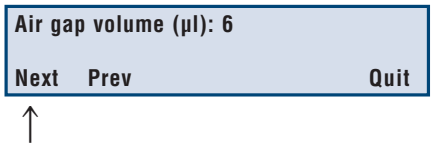
The air gap volume aspirated is the same whenever an air gap is aspirated, except when a mixing is performed. The air gap is fixed as 6 µl.

You can change the volume and aspiration rate of the air gap by pressing the **Air Gap** soft-key.

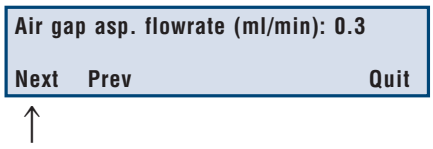


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The default value for the air gap is 6 µl, which gives a column of air 10 mm long in the supplied transfer tubing. To change this value, key in the new value, press **ENTER**, then press the **Next** soft-key. If you use 120 µl transfer tubing, you are recommended to set the air gap as 3 µl.



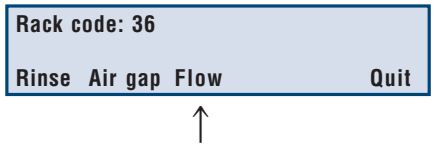
The factory set value for the air gap aspiration flow rate is 0.3 ml/min. To change this value, key in the new value and press **ENTER**, then press the **Next** soft-key.



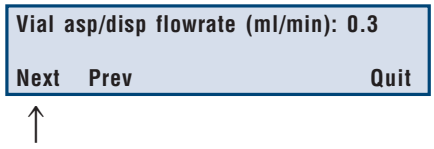
7.3 SETTING THE FLOW RATES

Press the **Flow** soft-key to set the vial aspiration and dispense flow rates, the priming flow rate and the mixing flow rate and the diluent aspirate flow rate (all in ml/min). The ranges are determined by the volume of the syringe;

- 0.1 to 20 ml/min with the 500 µl syringe,
- 0.1 to 4 ml/min with the 100 µl syringe.



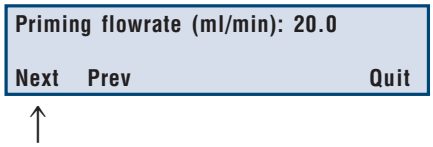
The first screen determines the rate at which liquids are aspirated from and dispensed to vials. The aspirate and dispense rates are the same. You are recommended to use a low flow rate, of around 0.3 ml/min. Key in the new value and press **ENTER**, then press the **Next** soft-key.



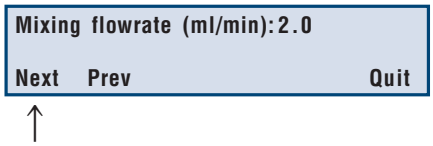
This is the rate at which diluent is pumped through the transfer tubing during the prime operation. This is also the flow rate for the rinsing operations such as rinse needle.

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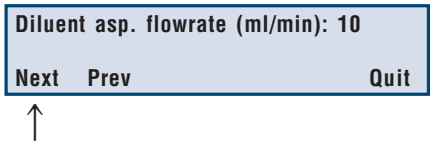
You are recommended to use a high flow rate, although this depends on the nature of the reservoir solvent. Key in the new value, press **ENTER**, then press the **Next** soft-key. The default value for a 100 µl syringe is 4 ml/min.



When you are diluting or adding internal standards to liquids, the liquids can be mixed by aspirating and dispensing several times. Do not use a very high flow rate as there is a tendency for the liquid to pass through the air gap. Key in the new value, press ENTER, then press the **Quit** soft-key to return to the Ready-to-Run screen.

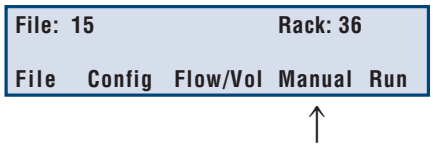


This is the flow rate used to perform dilutions and used for centered loop filling mode to center the injected volume in the injection loop. The default value is 10 ml/min.



8 MANUAL CONTROL MENUS

The manual control menus allow you to prepare the system for a run, or to switch the injection valve as desired. The valve status is indicated by the two green LEDs (INJECT and LOAD) on the front panel keypad.



When in the ready-to-run screen, press the **Manual** soft-key.

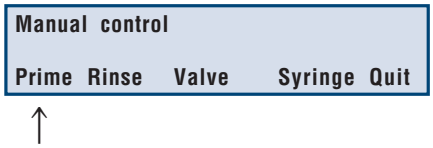
Four options are offered:
Prime, **Rinse, Valve** and **Syringe**.

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SETTING-UP AND OPERATING

8.1 PRIME

Press the **Prime** soft-key to prime the pump, transfer tubing and needle. The needle is moved to the rinsing station drain and the pump begins aspirating from the reservoir and dispensing to the rinsing station drain at the flow rate defined in the **Flow/Vol** branch of the software (normally the maximum possible flow rate).

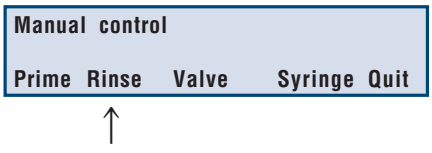


Press the **Stop** soft-key when the tubing has been primed to return to the manual control screen. The instrument finishes the current aspirate/dispense cycle and then stops. The screen displays the message '**Stopping Prime**', ends the current aspirate/dispense cycle and displays the manual control screen.



8.2 RINSE

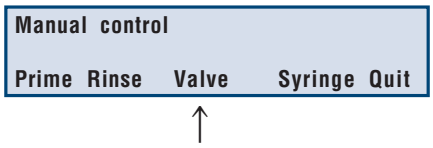
Press the **Rinse** soft-key to rinse the needle and the injection port. The inside and outside of the needle are rinsed in the rinsing station, then the needle is moved to the injection port and the injection port is rinsed. The process stops automatically and the manual control screen is displayed. The process can be interrupted by pressing the dedicated **STOP** key on the keypad. The Autoinjector immediately stops the rinsing process, returns the needle and arm to the home position and displays the manual control screen. The flow rate used is defined in the **Flow/Vol** branch of the software.



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8.3 SWITCH VALVE

Press the **Valve** soft-key to change the status of the injection valve. The valve status (LOAD or INJECT) is indicated by the two green LEDs on the front panel. When you press Valve, the status of the valve is immediately changed.



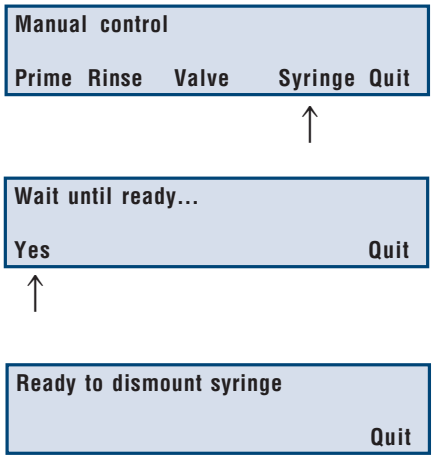
8.4 SYRINGE

Use the **Syringe** function to change the syringe fitted to the Autoinjector.

Press the **Syringe** soft-key, this screen is displayed.

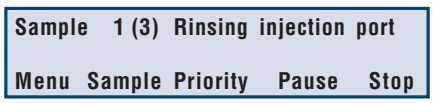
Press Yes, the syringe piston descends as the pump aspirates from the reservoir, then the pump stops. The valve switches to the outlet position. A new screen is displayed:

Changing the syringe is explained in Section 3.3.



9 THE IN-RUN SCREEN

When running a method, the In-Run screen information relating to the task currently being performed, and offers several Soft-key functions.



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SETTING-UP AND OPERATING

9.1 TOP LINE - PROGRAM STATUS

The top line indicates which task is being carried out, the third injection of the first sample vial in the above example. The Autoinjector is rinsing the injection port in preparation for the next injection.

9.2 MENU

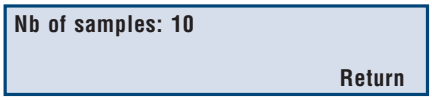
The **Menu** soft-key allows you to return to the Ready-to-Run screen, offering the possibility to edit another file while a (different) method is running. You can also verify or change certain configuration and flow rate/volume parameters using the **Config** and **Flow/Vol** soft-keys.



You cannot change certain parameters in the rack configuration (rack code, syringe volume, transfer tubing volume or injection loop volume), as changing these parameters during a run could result in damage to the instrument, or incorrect volumes being pumped. The Manual option is not available during a run. If you wish to stop a program while viewing the in-run-screen, press the dedicated **STOP** key on the keypad. Press **Quit** to return to the in-run screen.

9.3 SAMPLE

The **Sample** soft-key allows you to verify the total number of samples to be processed during a run.



You can increase or decrease this number. To change the number, key in the new number and press **ENTER**. If you increase the number of samples, further samples are injected without calibration. Press the **Return** soft-key to return to the in-run screen.

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9.4 PRIORITY

The **Priority** soft-key allows you to interrupt a run and process an urgently needed priority sample.

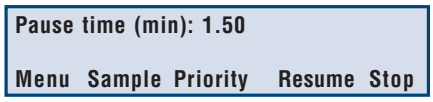
With rack codes 30, 31 and 34, you cannot access the priority function until the first sample has been injected. This is because the priority vial goes into the first sample position.

With rack codes 35, 36, 37 and 38, there is a separate priority vial area, the Priority function is available at all times and you can select the priority vial position within that area according to your selected method.

The full description of how the priority function operates is described in Appendix D.

9.5 PAUSE

The **Pause** Soft-key allows you to pause a run immediately. The Z-arm and carriage are sent to the home position. When you press **Pause**, the screen changes to display the time that the program has been paused.



In the above example, the program has been paused for 1 minute 30 seconds. The time is continually updated as the program waits. Press the **Resume** Soft-key to continue with the method. The message **Resuming...** is displayed and the instrument continues with the method. You can stop the execution of the program by pressing the **Stop** Soft-key, pumping stops immediately and the needle returns to the home position.



The pause is recorded in the audit trail.

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9.6 STOP

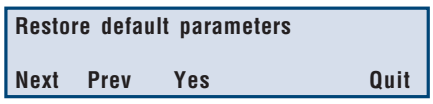
The **Stop** Soft-key stops the program and aborts the run. This works in the same way as the dedicated **STOP** key in section 4.3.4. When pressed, pumping immediately stops and the needle returns to the home position. The execution of the file ends.

10 ADVANCED USER MENUS

It is possible to further develop your methods and the operation of the instrument by modifying certain parameters. This is particularly useful if, for example you change the size of the calibrated tubing. You access these menus by holding the **CLEAR** key pressed down while switching on the instrument. Keep the **CLEAR** key pressed until you hear a beep sound. The menus appear in the order described here. You progress from one menu to the next by pressing the **Next** soft-key. When you press the Quit soft-key, the instrument initializes with the new settings. The available menus are:

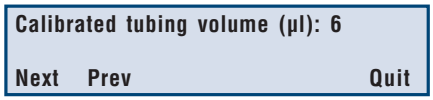
- **Restore default parameters**

  This option allows you to restore the original factory set default values. To restore the parameters, press the Yes soft-key. To ignore this option and go to the other menus, press the Next soft key.



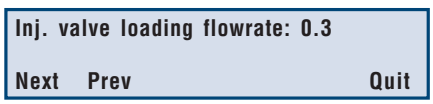
- **Calibrated tubing volume**

  The calibrated tubing connects from the injection port to the load inlet of the injection valve. If you change the size of the calibrated tubing, determine the new volume and enter this value.



- **Injection Valve Loading Flow Rate**

  If you wish to modify the rate at which liquids are loaded into the injection valve, use this menu to enter the new flow rate. You can enter a value

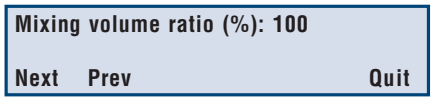


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between:
- 0.1 ml/min and 4 ml/min for a 100 µl syringe
- 0.1 ml/min and 20 ml/min for a 500 µl syringe

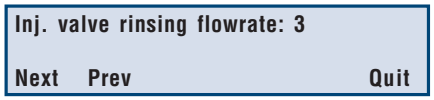
- **Mixing Volume Ratio**

  In order to mix two liquids that have been dispensed into a vial, the Autoinjector aspirates a percentage of the total volume dispensed into the vial. Enter the new value between 0 and 100%.



- **Injection Valve Rinsing Flow Rate**

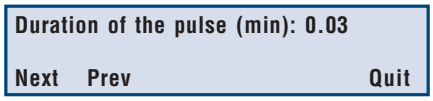
  If you wish to modify the rate at which the injection valve is rinsed, use this menu to enter the new flow rate. You can enter a value between:
  - 0.1 ml/min and 4 ml/min for a 100 µl syringe
  - 0.1 ml/min and 20 ml/min for a 500 µl syringe



- **Duration of the Pulse**

  When the Autoinjector coordinates by contact, an output pulse is sent on output 2 for normal samples, or output 1 for priority samples. The duration of the pulse is 1.8 seconds. If the connected equipment does not recognize the pulse, use this menu to increase the length of the pulse. The maximum pulse duration is 1 minute (1.00).

  Press the **Quit** Soft-key to return to the main menu.



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METHODOLOGY

You must ensure that there is enough liquid in your vials according to the method selected, the number of sample and standard vials to be injected, the number of injections per vial, and the calibration frequency. Remember that a certain volume (about 11 µl for 0.7 ml vials) of liquid remains in the vial and cannot be aspirated.

- *The residual volume for 0.7 ml vials is 11 µl.*
- *The residual volume for 1.7 ml vials is 170 µl.*
- *The residual volume for 4.5 ml vials is 350 µl.*
- *The residual volume for 20 ml vials is 1.4 ml.*

The total volume of internal standard and standard required depends on the total number of sample vials. When the method is initially saved, the total number of vials is not required, so the actual total volume of standard and internal standard for the run cannot be calculated. When you run the method, you enter the number of sample vials to be used.

At this point the total volume of internal standard is calculated according to:

- *The number of sample vials.*
- *The number of standard vials.*
- *The number of calibrations to be carried out.*

The volume of prepared standard (standard + internal standard + diluent) can be calculated according to the number of calibrations required.

If the result volume is insufficient with a single preparation, the volumes of standard, internal standard and diluent are multiplied by a factor of 2, 3, 4 etc. until the total volume is sufficient for the run. If, at this point, the total volume does not exceed the vial volume, the program runs, if the total volume exceeds the vial volume, an error message appears and the original values are retained.

The vial volumes are given in Appendix B.

Appendix F lists the method program used to test the injection precision in the factory. The two injection methods are stored in two locked files; 19 (for the total loop filling method) and 20 (for the partial loop filling method). You can run these files to revalidate your Autoinjector.

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1 INTRODUCTION

♦ METHOD FILES

Writing an application program consists of first configuring the Autoinjector, setting the flow rates and volumes and then writing the method. You can write a total of 20 method files.

♦ RACKS

You can only use the following Gilson coded racks with the 234 Autoinjector: 30, 31, 34, 35, 36, 37, 38. The vial numbering sequence (and needle displacement pattern) is fixed for all racks; it cannot be changed. Each rack has pre-defined zones according to the selected method. The only modification allowed to the pre-defined zones is the number of vials to be used for standard and sample vials (you can use up to nine standard vials in each rack). The zone allocations for each rack type and injection method are detailed in Appendix B.

♦ INJECTION METHODS

There are five possible injection methods you can use with the 234 Autoinjector. The choice of method depends on the rack you are using and your sample injection requirements (if you are using standards and dilution). The different methods, listed below, are explained in the following pages.

- 5.2.1 Simple sample injection,
- 5.2.2 External standard,
- 5.2.3 External standard with dilution,
- 5.2.4 Internal standard,
- 5.2.5 Internal standard with dilution.

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METHODOLOGY

Loop Filling Modes

There are three loop filling modes, described in section 5.3. The choice of loop filling mode depends on your requirements, and the volume of sample liquid available:

- 5.3.1 Total Loop Filling Injection,
- 5.3.2 Partial Loop Filling Injection,
- 5.3.3 Centered Loop Filling Injection.

Diluting the Sample

The sample used by Gilson in evaluation experiments was 0.25% Phenol in Methanol.

Dilution was based on obtaining a result standard volume of 500 µl for the experiment. the dilution ratios were obtained using the following volumes:

- For a dilution of 1/2, 250 µl of sample was added to 250 µl of diluent.
- For a dilution of 1/10, 50 µl of sample was added to 450 µl of diluent.
- For a dilution of 1/25, 20 µl of sample was added to 480 µl of diluent

In all cases, a CV of less than 1.4% can be obtained.

With the above solutions, linearity is assumed up to a dilution ratio of 1/25.

Avoiding Contamination

To avoid contamination of the needle or injection valve, use the following rinsing volumes for the Autoinjector fitted with a 500 µl syringe:

- Inside needle rinsing volume = 300 µl
- Outside needle rinsing volume = 300 µl
- Injection valve rinsing volume = 500 µl

You set these values in the Flow/Vol menu, described in Section 4.7. You can modify these volumes according to your application requirements.

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2 INJECTION METHODS

The choice of injection method depends on your requirements. You can select simple injection method, injection of standards for quantifying results, and dilution of standards or samples to reduce the peak sizes on your chromatograms.

2.1 SIMPLE INJECTION METHOD

You can use all compatible racks (codes 30, 31, 34, 35, 36, 37 and 38) for this method.

♦ METHOD DESCRIPTION

A volume of each sample in the rack is aspirated and then injected, in sequence. You may perform up to 9 injections per sample vial. Ensure you have enough liquid in each vial for the number of injections and loop filling method selected. With small volume vials, rack codes 31, 36 and 38, the maximum volume of liquid the vials can contain is 700 µl.

♦ ZONES

You can use any of the compatible racks (see Section 1.6) for the simple injection method. All the vials in the sample zone are used to contain the sample liquids. The example below shows a rack code 35, for more information about racks and zones, refer to Appendix B.

For racks 35, 36, 37 and 38, the internal standard vial is not used and the vials reserved for priority samples cannot be used for normal samples. The rest of the rack is one zone; the sample zone.

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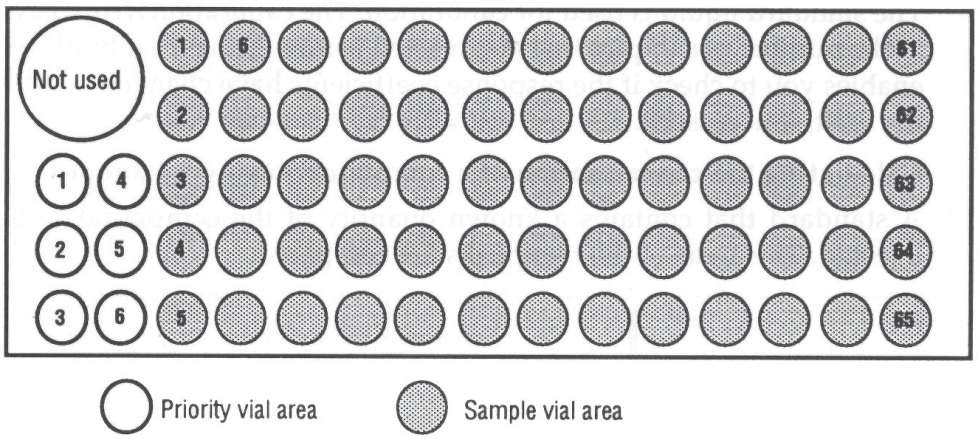


Figure 5.1 - Simple Injection Method Zones (Rack Code 35)

2.2 EXTERNAL STANDARD METHOD

You can use all compatible racks (codes 30, 31, 34, 35, 36, 37 and 38) for this method.

♦ METHOD DESCRIPTION

This method allows you to compare the peak produced by your sample liquid with the peak produced by a reference standard liquid, the chromatographic conditions being identical. By comparing the areas of the peaks obtained, you can carry out calibration between sample batches. If you calibrate several times during a run, then make sure that the standard used is stable, and that there will be no peak variation due to standard evolution.

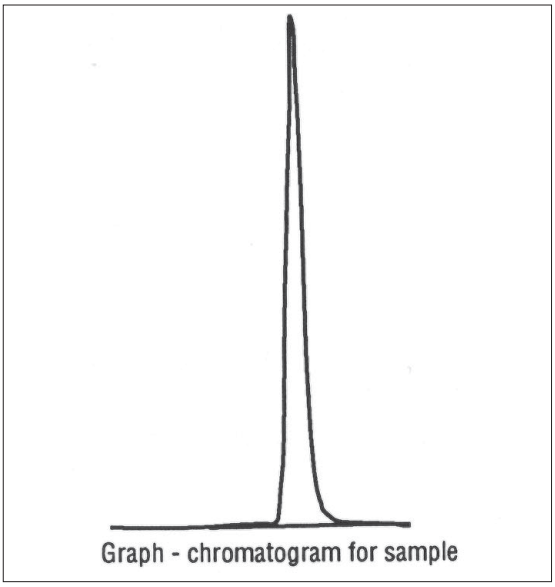
You inject one standard (or more - up to nine standards are allowed) in order to compare your sample liquids with the peak(s) produced by the standard(s). In this way, you can determine the presence and quantity of known substances by comparing the peak areas of the samples with the peak areas given by the standards.

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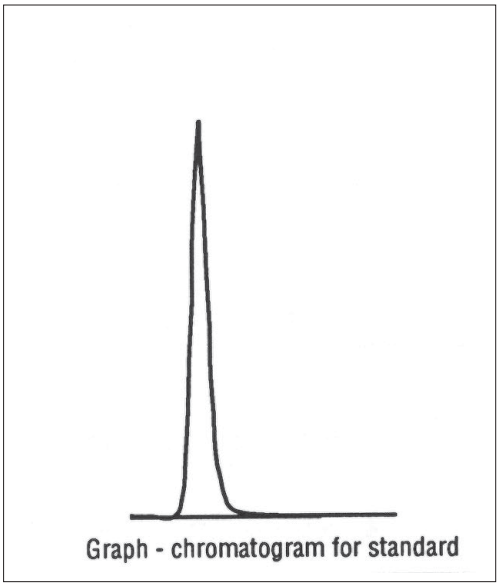
The standard liquid is used for calibration. The calibration frequency (n) corresponds to the number of samples that are injected before the next calibration injection(s). This enables you to check if the response coefficients have changed and to compensate for the drift, if necessary.

Calculating the concentration of a compound - using a standard

A standard that contains a known quantity of the compound to be determined is injected. The areas of the two peaks are compared.



Graph - chromatogram for sample



Graph - chromatogram for standard

The concentration of the compound in the sample can be determined by using the following formula:

$$C_{Sa} = \frac{A_{Sa}}{A_{Std}} \times C_{Std}$$

Where:

C_{Sa} is the concentration of the compound in the sample.
C_{Std} is the known concentration of the compound in the standard.
A_{Sa} is the area of the compound peak in the sample chromatogram.
A_{Std} is the area of the compound peak in the standard chromatogram.

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METHODOLOGY

♦ SEQUENCE OF EVENTS

The first standard is injected.
The second standard is injected.
...
The first sample is injected.
The second sample is injected.
...
The n^{th} sample is injected.
The first standard is injected for calibration.
The second standard is injected.
...
The next sample in the sequence is injected.
And so on.

♦ ZONES

The internal standard vial position (racks 35, 36, 37 and 38) is not used. The main vial area is allocated for standard vials or sample vials. You can have up to nine standard vials. The standard vials are placed in the rack first.

The example in Figure 5.2 shows a code 35 rack with three standard vials. For more information about racks and zones, refer to Appendix B.

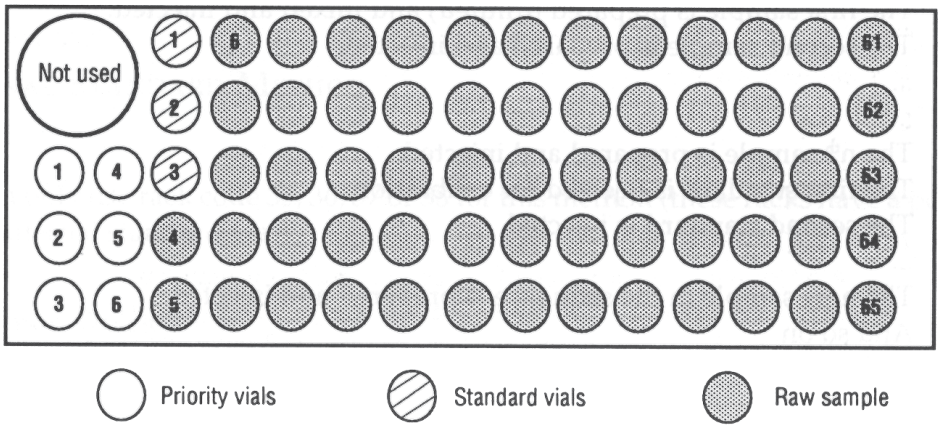


Figure 5.2 - External Standard Method Zones (Rack Code 35)

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2.3 EXTERNAL STANDARD METHOD WITH DILUTION

You can use all compatible racks (codes 30, 31, 34, 35, 36, 37 and 38) for this method.

♦ METHOD DESCRIPTION

In addition to allowing calibration between sample batches, you can dilute the samples and/or the standards in order to reduce the amplitude of the peaks on your chromatogram.

The rack is divided in two halves one half with the raw samples and standards and the second half with empty vials for the dilution and mixing of samples and of standards.

The standard liquid is used for calibration. The calibration frequency (n) corresponds to the number of samples that are injected before the next calibration injection(s).

♦ SEQUENCE OF EVENTS

The first standard is diluted and mixed.
The first standard injection is performed.
The second standard is prepared in the same way as for the first standard.
The second standard is injected.
...
The first sample is prepared (diluted) and mixed and injected.
The second sample is prepared and injected.
...
...
The n^{th} sample is prepared and injected.
The first standard is injected for calibration.
The second standard is injected.
...
The next sample in the sequence is prepared and injected.
And so on.

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METHODOLOGY

♦ ZONES

You can use any rack. The main vial area is divided into two identical halves, each half containing up to nine declared standard vials and the remaining vials in each half being sample vials.

The example in Figure 5.3 shows a code 35 rack with three standard vials. For more information about racks and zones, refer to Appendix B.

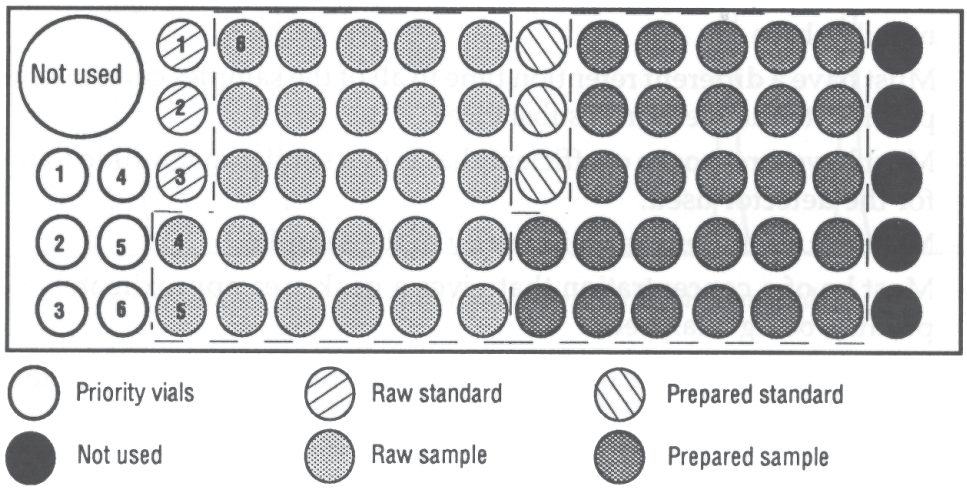


Figure 5.3 - External Standard with Dilution (Rack Code 35)

2.4 INTERNAL STANDARD METHOD

You can only use rack code 35, 36, 37 or 38 for this method (these racks have a position for an internal standard vial).

♦ METHOD DESCRIPTION

You add a fixed volume of internal standard to the standards and samples in order to give a reference peak on your chromatogram.

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The internal standard must not interfere with the sample or standard liquids and must give a separate, clearly identifiable peak, from those produced by your standards and samples. This reference peak allows you to rectify errors in measurements due to possible errors in liquid volumes, instrument errors, or column errors. The internal standard method gives the best quantification of results. You add internal standard liquid to all samples and all standards.

The internal standard:

- Must be of known purity and chemically inert with all liquids used, including the mobile phase.
- Must have a different retention time to all of the samples constituents, but as close as possible to the measured substances.
- Must have a response coefficient that is of a similar magnitude to that of the sample for the detector used.
- Must not be present as an impurity in the sample.
- Must be of a concentration that gives a peak area approximately equivalent to the product being analyzed.

Quite often, homologues of the product being analyzed are used as internal standards.

Calculating the concentration of a compound - Internal Standard method.

The concentration of a compound in the sample can be determined by injecting a standard containing a known quantity of the same compound, plus internal standard. The internal standard allows you to make adjustments for possible volume errors. You obtain chromatograms containing sample peaks and standard peaks (see Figure 5.4).

The concentration of the compound can be calculated using the following formula:

$$(C_c)_{Sa} = (C_c)_{st} \times \frac{(A_c)_{sa}}{(A_c)_{st}} \times \frac{(A_l)_{st}}{(A_l)_{sa}} \times \frac{(C_l)_{sa}}{(C_l)_{st}}$$

Where:

$(C_c)_{Sa}$ = Concentration of the compound to be determined in the sample.
$(C_c)_{st}$ = Known concentration of the compound in the standard.
$(C_l)_{sa}$ = Known concentration of internal standard in the sample.
$(C_l)_{st}$ = Known concentration of internal standard in the standard.
$(A_c)_{sa}$ = Area of the compound peak in the sample.
$(A_l)_{sa}$ = Measured area of the internal standard peak in the sample.
$(A_l)_{st}$ = Measured area of the internal standard peak in the standard.
$(A_c)_{st}$ = Measured area of the compound peak in the standard.

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METHODOLOGY

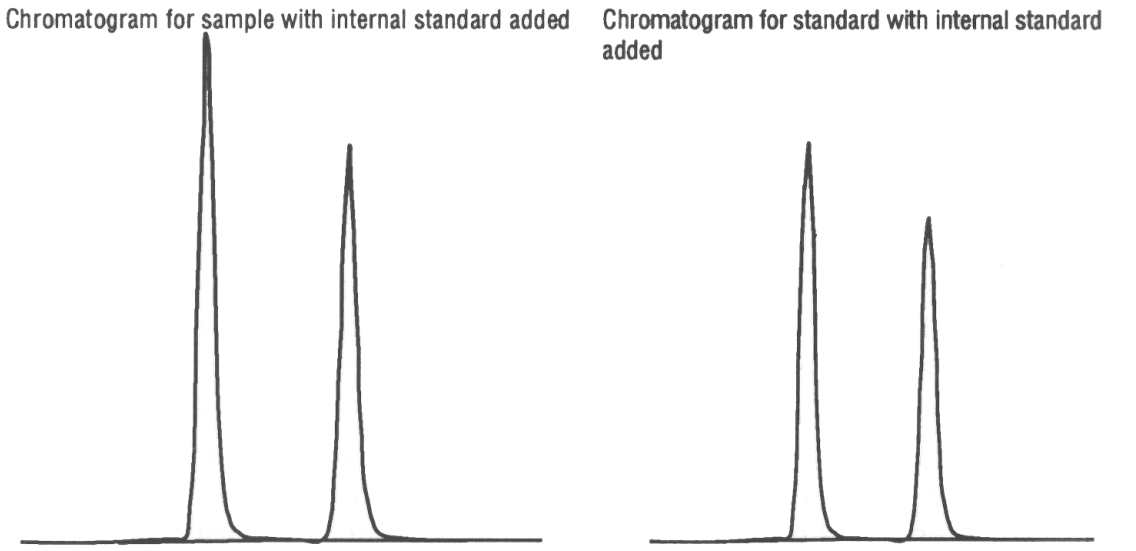


Figure 5.4 - Determination of compound concentration - Internal Standard

The standard liquid is used for calibration. You can inject up to nine different standards. The calibration frequency (n) corresponds to the number of samples that are injected before the next calibration injection(s).

♦ SEQUENCE OF EVENTS

The standard vials are prepared first.
The first standard is prepared, then the first calibration injection is performed.
The second standard is prepared in the same way as the first standard, and injected.
The first sample is prepared and injected.
The second sample is prepared and injected.
...
The n^{th} sample is prepared and injected.
The first standard is injected for calibration.
The second standard is injected.
The next sample in the sequence is prepared and injected.
And so on.

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When internal standard is added to a sample or standard, the result liquid in the result vial is aspirated and dispensed to mix the liquids. You select the number of times the liquid is aspirated and dispensed (number of mixing cycles) when writing the program.

♦ ZONES

You must use rack fitted with the 20 ml vial, code 35, 36, 37 or 38, for this method. The internal standard is located in the 20 ml vial. Apart from the priority vial(s), located in front of the internal standard vial, the rack is divided into two equal halves: the first half containing the raw standard and raw sample vials, and the second half containing empty result vials for accepting the prepared standards and prepared samples.

You declare how many standard vials (up to nine) in the rack you are using. The remaining vials in the first half of the sample vial area are declared automatically as the sample zone. The example in Figure 5.5 shows a code 37 rack using three standard vials. For more information about racks and zones, refer to Appendix B.

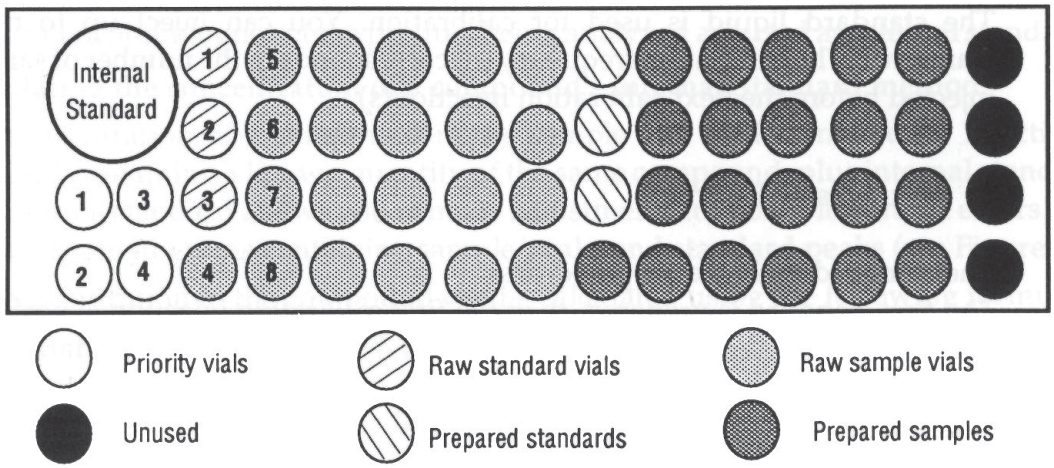


Figure 5.5 - Internal Standard Method Zones (Rack Code 37)

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2.5 INTERNAL STANDARD METHOD WITH DILUTION

You can only use rack code 35, 36, 37 or 38 for this method (these racks contain the internal standard vial).

♦ METHOD DESCRIPTION

You add a fixed volume of internal standard to your samples and to the standards. In addition you can dilute the (prepared) samples and the (prepared) standards.

The internal standard is used to give a separate, identifiable peak on your chromatogram for quantification of results, as described in section 5.2.4.

Dilution is used to ensure that your peaks are quantifiable.

The standard liquid is used for calibration. The calibration frequency (n) is the number of samples that are injected before the next calibration injection(s).

♦ SEQUENCE OF EVENTS

The standard vials are prepared first.
The first standard is prepared.
The first calibration injection for the first standard is performed.
The second standard is prepared in the same way as the first standard, and injected.
The first sample is prepared (diluted, internal standard added, and all liquid mixed) and injected.
The second sample is prepared and injected.
...
The n^{th} sample is prepared and injected.
The first standard is injected for re-calibration.
The second standard is injected for re-calibration.
...
The next sample in the sequence is prepared and injected.
And so on.

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♦ ZONES

The internal standard is located in the first vial, the 20 ml vial. The sample area of the rack is divided into two equal halves: the first half containing the raw standard and raw sample vials, and the second half containing empty result vials for accepting the internal standard, diluent and standards/samples.

The example in Figure 5.6 shows a code 37 rack with three standard vials. For more information about racks and zones, refer to Appendix B.

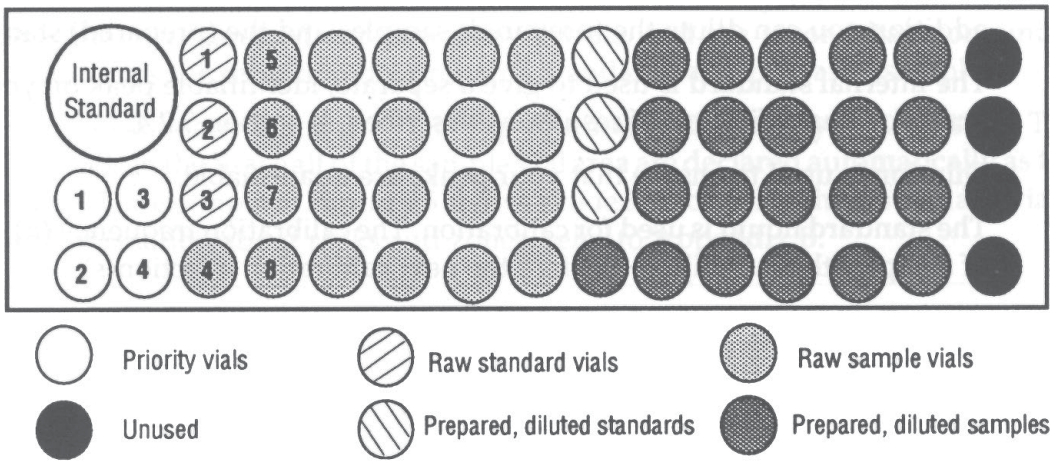


Figure 5.6 - Internal Standard Method with Dilution (Rack Code 37)

3 LOOP FILLING MODES

There are three possible modes:

- 5.3.1 Total Loop Filling,
- 5.3.2 Partial Loop Filling,
- 5.3.3 Centered Loop Filling.

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METHODOLOGY

The loop filling mode you select depends on the amount of sample liquid available and the accuracy you require. If you have a large volume of sample liquid available, use the Total Loop Filling method. If you have a very limited quantity of sample liquid, use Centered Loop Filling.

For partial and total loop filling it is important to note that although an air gap is aspirated before the sample liquid, the air gap remains in the needle after the loop has been loaded. (The air gap does not enter the injection valve components, it will be flushed out when rinsing the needle).

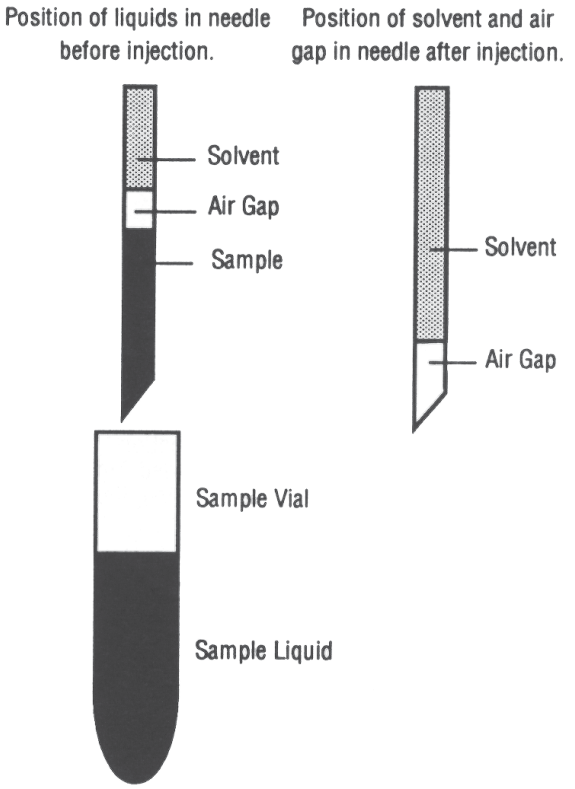


Figure 5.7 - Position of air gap in needle before and after injection

3.1 TOTAL LOOP FILLING INJECTION

The total loop filling method of sample injection gives the best results, CV is typically 0.5% with the instrument in standard configuration, but the volume of sample liquid used is rather large.
For a sample injection of 20 µl, the volume of sample liquid required is around 115 µl (inj. loop vol. x loop filling coefficient + injection flush vol.). The results are less affected by pumping error. However, the accuracy error of the injected volume depends on the accuracy of the loop capacity (about 10% for the 20 µl loops according to Rheodyne).

☞ ** Standard configuration: 500 µl syringe, 550 µl transfer tubing, 0.7 ml vials.*

(A typical value of CV = 0.2% can be obtained for an instrument fitted with a 100 µl syringe and 120 µl transfer tubing).

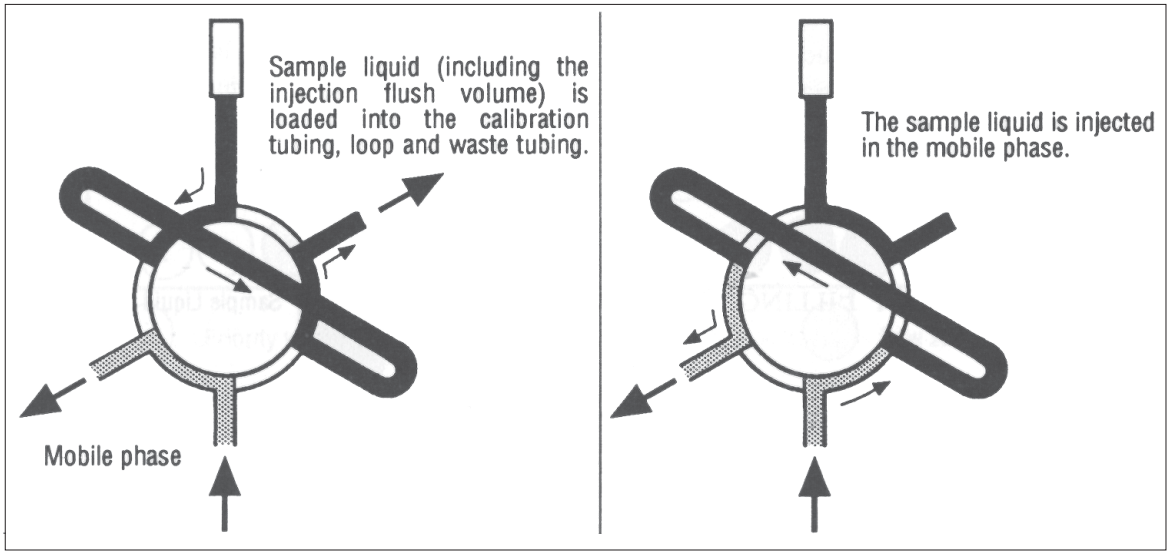
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♦ REPEATABILITY

For total loop filling, a CV of less than 0.5% can always be obtained for an instrument fitted with a 500 µl syringe and 550 µl coiled transfer tubing, or fitted with a 100 µl syringe used with 120 µl non-coiled transfer tubing.

♦ SEQUENCE OF EVENTS

The valve is in the LOAD position. The valve is in the INJECT position.



3.2 PARTIAL LOOP FILLING INJECTION

The partial loop filling method allows you to inject variable volumes of sample or standard. When injecting a sample liquid, a pre-determined "injection flush volume" of sample liquid, typically 15 µl, is flushed through the calibrated tubing to the drain. This ensures that the actual sample injection volume in the loop will be accurate. For a sample injection volume of 5 µl, this corresponds to a total volume requirement of 20 µl.

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♦ INJECTION FLUSH VOLUME

The injection flush volume serves to overcome the effects of laminar flow and sample dilution as the sample liquid passes through the calibrated tubing to the injection loop. If a sample volume smaller than the loop volume is loaded directly into the loop, the front end of the sample volume resembles Figure 5.8, resulting in an inaccurate volume being injected. The smaller the volume injected, the greater the inaccuracy. In addition, the front end of the sample liquid is diluted with the mobile phase, giving inaccurate measurements.

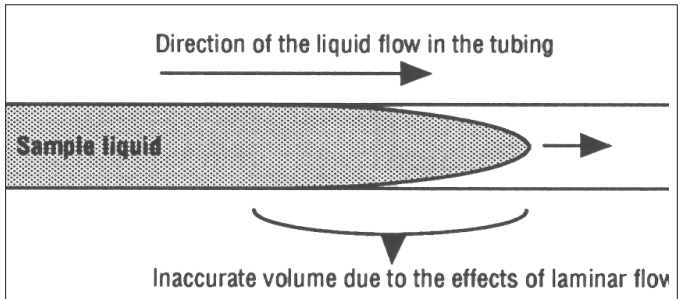


Figure 5.8 - Effect of laminar flow on volume accuracy

To avoid this phenomenon, the Rheodyne valve is first switched to the inject position and sample liquid is flushed through the calibrated tubing to the drain, eliminating the inaccurate volume at the front of the liquid. Thus, when the valve is then switched to the load position, the calibrated tubing is already entirely filled with sample liquid, the actual sample volume can then be loaded. The volume loaded into the loop is precise. You set the injection flush volume in the Configuration branch of the software, Section 4.6.5. The optimum value for the injection flush volume is 15 µl.

♦ REPEATABILITY

Results give a CV of less than or equal to 0.8% for an injection volume of 5 µl for an instrument in standard configuration*. Linearity is assumed for injection volumes of up to half the loop volume.

☞ ** Standard configuration: 500 µl syringe, 550 µl transfer tubing, 0.7 ml vials, injection loop volume 20 µl, air gap = 6 µl.*

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♦ SYRINGE SIZE

Method reproducibility can be improved by using the 100 µl syringe for injecting smaller volumes, giving a CV typically down to 0.4%.

To obtain optimum reproducibility:

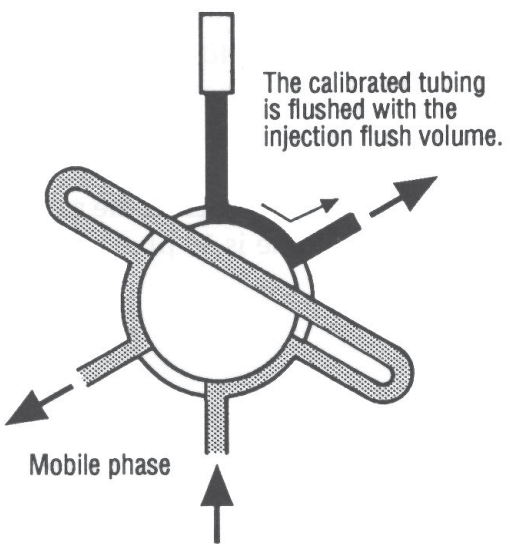
- For volumes from 1 µl to 5 µl, you are advised to use a 100 µl syringe
- For higher volumes, you are advised to use a 500 µl syringe.

♦ LINEARITY

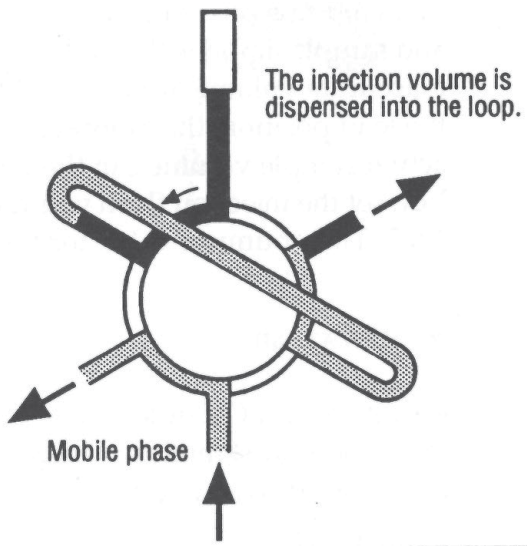
Linearity is assumed for injection volumes of less than 11 µl with a 20 µl injection loop. In general, linearity is assumed for injection volumes up to half of the injection loop size.

♦ SEQUENCE OF EVENTS

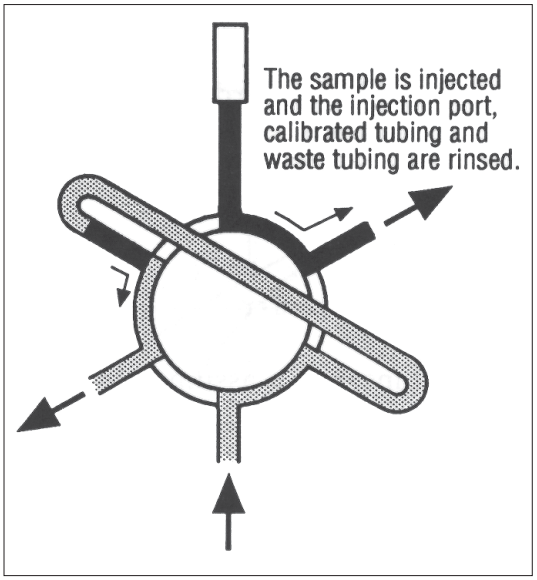
The valve is in the INJECT position.



The valve is in the LOAD position.



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The valve is in the INJECT position.

3.3 CENTERED LOOP FILLING INJECTION

Where sample volumes are small and no sample loss is allowed, this method allows accurate injection volumes with no sample loss. Results give a CV of less than 1.2% for a 5 µl injection with the instrument in the standard configuration*.

The recommended maximum volume to inject is half the loop volume, however, you can inject a greater volume. The total volume (the two air gaps and volume of liquid) must not exceed the loop size.

☞ ** Standard configuration: 500 µl syringe, 550 µl transfer tubing, 0.7 ml vials, using an air gap of 2 µl at each side of the sample, an aspirate/dispense flow rate of 0.3 ml/min and a diluent flow rate of 4 ml/min, injection volume 5 µl.*

♦ REPEATABILITY

Reproducibility does not tend to be affected by the aspirate/dispense flow rate provided that flow rates superior to 0.2 ml/min are used. The recommended aspirate/dispense flow rate for centered loop filling is 0.3 ml/min.

Use the 100 µl syringe for injecting volumes less than 7 µl. The best way to obtain good reproducibility when using centered loop filling is to use a 100 µl syringe. The linearity is conserved for injection volumes of up to 50% of a 20 µl injection loop.

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♦ MINIMUM SAMPLE CONSUMPTION

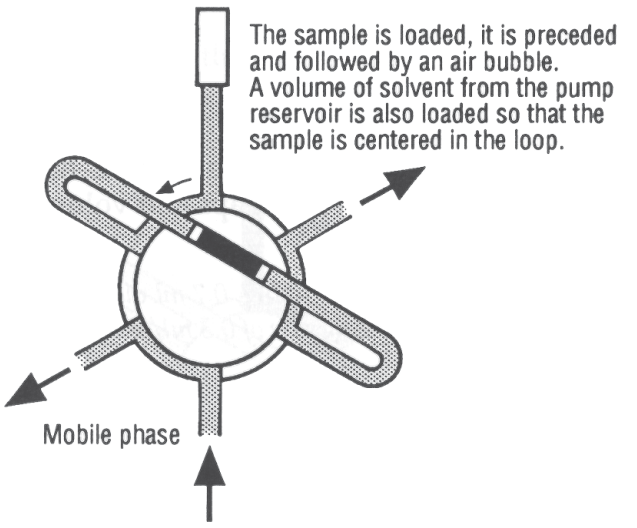
As the needle does not descend until it touches the bottom of a vial, there is a minimum required volume before the instrument can aspirate, independent of the injection mode. The minimum sample volume necessary to permit an injection volume of 1 µl from a 0.7 ml vial is 12 µl.

♦ LINEARITY

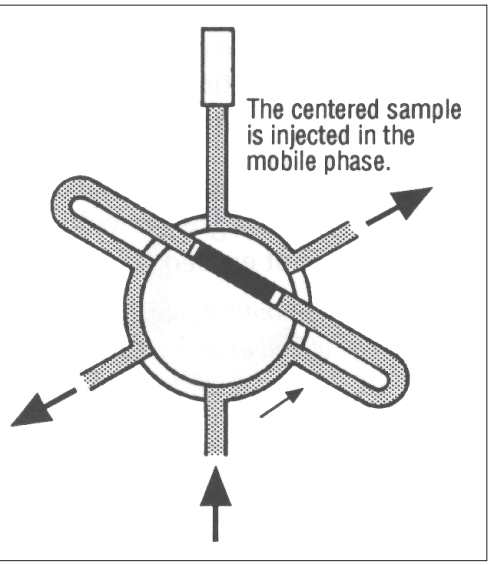
Linearity is assumed for injection volumes up to half of the injection loop size.

♦ SEQUENCE OF EVENTS

The valve is in the LOAD position.



The valve is in the INJECT position.

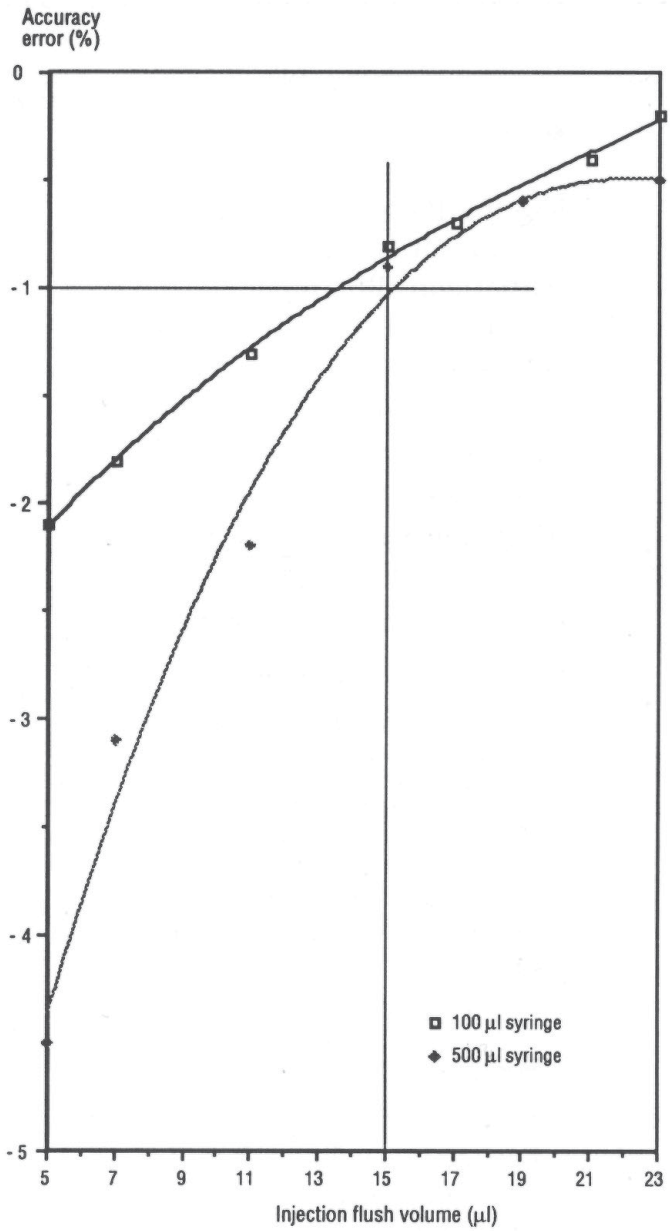


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4 RECOMMENDATIONS

4.1 INJECTION FLUSH VOLUME

Injection flush volume (with either 100 µl or 500 µl syringe) = 15 µl (see Graph 5.1)



Graph 5.1 - Accuracy Vs Injection Flush Volume for 100 and 500 µl syringes (Partial Loop Filling Injection)

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4.2 REPRODUCIBILITY OF INJECTIONS USING 500 µL AND 100 µL SYRINGES

♦ PARTIAL LOOP FILLING MODE

Use the 100 µl syringe for volumes from 1 µl to 5 µl for the partial loop filling mode and the 500 µl syringe for volumes from 5 µl to 10 µl.

You can inject volumes of up to 50% of the injection loop volume and obtain good linearity.

See Graph 5.2, the graph shows the mean CV plotted against injection volume for both syringes.

- **For the 100 µl syringe:**

  Injection volumes of 1 µl gave CV's of higher than 1%.

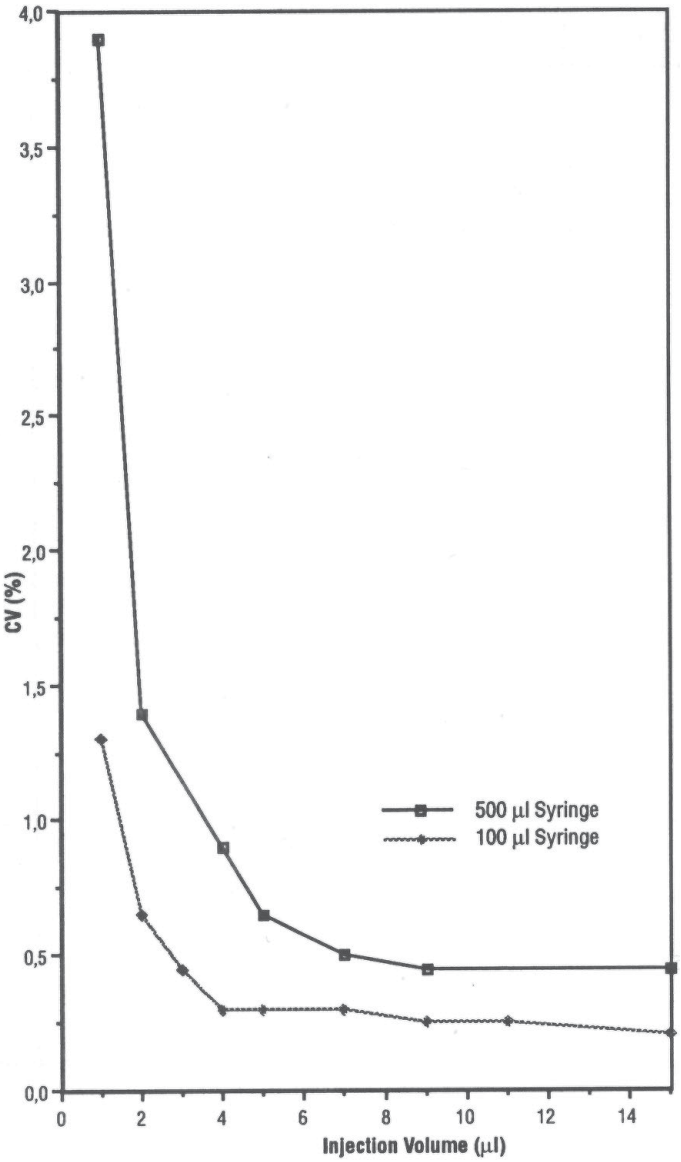
  Injection volumes of 2 µl gave CV's between 0.6 and 0.7%.

  From 4 µl, the CV's obtained were always equal to or less than 0.5%.

- **For the 500 µl syringe:**

  For injection volumes from 4 µl CV's are less than 1%.

  For injection volumes from 7 µl the CV's become constant at around 0.5%.



Graph 5.2 - Reproducibility Vs Injection Volume for 100 and 500 µl syringes (Partial Loop Filling Injection)

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♦ CENTERED LOOP MODE

Use the 100 µl syringe for volumes less than 7 µl. The best acceptable reproducibility is obtained using a 100 µl syringe for centered loop mode.

See Graph 5.3, the graph shows the mean CV plotted against injection volume for both syringes.

- **For the 100 µl syringe:**

  CV's attainable can be grouped as follows:

  Injection volumes of less than 2 µl give a CV of less than 2%.

  Injection volumes of less than 4 µl give a CV of less than 1.2%.

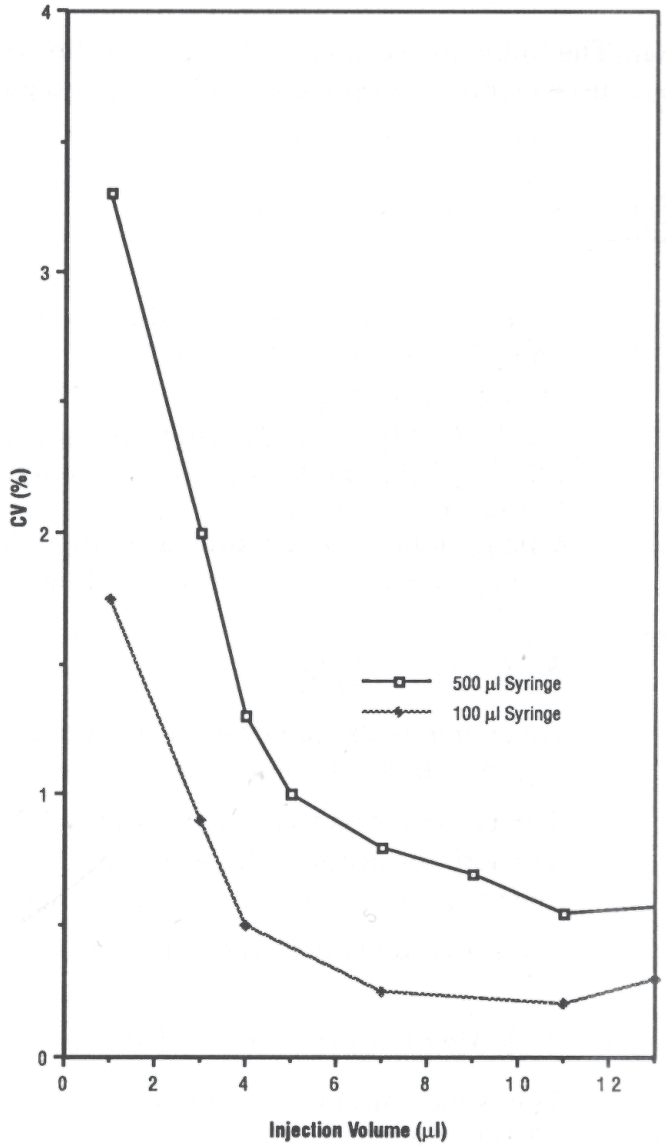
  From 4 µl, the CV's obtained are less than 0.6%.

  Linearity is assumed to be acceptable for injection volumes between 4 and 7 µl.

- **For the 500 µl syringe:**

  CV's attainable can be grouped as follows:

Injection volume	CV
1 µl	< 4 %
< 4 µl	< 2.5 %
< 7 µl	< 1.5 %
> 7 µl	< 0.8 %



Graph 5.3 - Reproducibility Vs Injection Volume for 100 and 500 µl syringes (Centered Loop Injection)

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5 DESCRIPTION OF THE PARAMETERS

The following section explains all the flow rate and volume parameters accessible in the user menus (configuration, advanced user and method writing menus).

5.1 FLOW RATES

You set the flow rates according to your method requirements. The default values supplied are general recommended values for an instrument fitted with the 500 µl syringe (factory fitted). The permitted ranges for flow rates are:

- 0.1 - 4 ml/min for an instrument fitted with a 100 µl syringe.
- 0.1 - 20 ml/min for an instrument fitted with a 500 µl syringe.

Operations such as priming use a high flow rate, and operations such as aspirating an air gap or a liquid use a relatively low flow rate.

♦ AIR GAP ASPIRATE FLOW RATE

An air gap is aspirated before a liquid is aspirated. The air gap separates the aspirated liquid from the diluent in the transfer tubing.

This parameter is accessed in the **Flow/Vol** option of the main menu. Pressing the Flow/Vol Soft-key accesses three options: **Rinse**, **Air Gap** and **Flow**. Press the **Air Gap** Soft-key to access the air gap parameter menus.

Use a low flow rate, the default setting is 0.3 ml/min.

♦ DILUENT ASPIRATE FLOW RATE

This is the rate at which diluent is aspirated from the pump reservoir for the following operations:

- Diluting a sample or a standard.
- Positioning a sample or standard in the center of the injection loop in centered loop filling mode.

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The volume of diluent used for centered loop filling mode is determined by the software. The volume used for diluting samples or standards is declared when writing the method program.

The flow rate is accessed in the **Flow/Vol** option of the main menu. Pressing the **Flow/Vol** Soft-key accesses three options: **Rinse**, **Air Gap** and **Flow**. Press the **Flow** Soft-key to access the diluent aspirate flow rate from the menus available.

Use a medium flow rate, the default setting for a 500 µl syringe is 10 ml/min.

♦ INJECTION VALVE LOADING FLOW RATE

This is the rate at which liquids are loaded into the injection loop. The volumes loaded are determined when you write your method.

This parameter is only available as an advanced user option. To access this parameter, keep the **CLEAR** key pressed down while switching the instrument ON. Release the **CLEAR** key when you hear a beep. The first option is 'restore default values', press the **Next** Soft-key to access the second option; 'Calibrated tubing volume', and press the **Next** Soft-key again to access the 'Inj. valve loading flowrate' option.

The default setting is 0.3 ml/min. Use a low flow rate. The permitted value ranges are:

- 0.1 - 4 ml/min for an instrument fitted with a 100 µl syringe.
- 0.1 - 20 ml/min for an instrument fitted with a 500 µl syringe.

♦ INJECTION VALVE RINSING FLOW RATE

This is the rate at which the injection port and calibrated tubing are rinsed with diluent from the pump reservoir. The injection valve is rinsed in the inject position. This procedure is carried out after the needle has been rinsed; before a run (initialization) and after each injection.

The volume of diluent used is declared in 'Injection valve rinsing volume' option in the **Flow/Vol** menu.

This parameter is only available as an advanced user option. To access this parameter, keep the **CLEAR** key pressed down while switching the instrument ON. Release the **CLEAR** key when you hear a beep. The first option is the 'restore default values', press the **Next** Soft-key four times to pass through other options to access the 'Inj valve rinsing flowrate' option.

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The default setting is 3 ml/min. Use a medium flow rate. The permitted value ranges are:

- 0.1 - 4 ml/min for an instrument fitted with a 100 µl syringe.
- 0.1 - 20 ml/min for an instrument fitted with a 500 µl syringe.

♦ MIXING FLOW RATE

This is the rate at which liquid is aspirated and dispensed in a result vial in order to mix liquids that have been dispensed into the vial, for example, sample and diluent. When you write your method program, you state how many mixing cycles are performed (the number of aspirate/dispense operations). If the mixing volume ratio is too low, or the number of mixing cycles too few, the liquid will not be homogenous, resulting in peak areas changing from one injection to the next.

The volume aspirated is calculated as a percentage of the total volume already dispensed into the result vial. This percentage, the **Mixing Volume Ratio** is declared using the advanced user menus.

The mixing flow rate is accessed in the **Flow/Vol** option of the main menu. Pressing the **Flow/Vol** Soft-key accesses three options: **Rinse**, **Air Gap** and **Flow**. Press the **Flow** Soft-key to access the mixing flow rate option from the menus available.

Use a medium flow rate, the default setting is 2 ml/min.

♦ PRIMING FLOW RATE

This is the rate at which diluent is pumped through the prime operation, and is also used for rinsing the needle (inside and outside) and the injection port.

This parameter is accessed in the **Flow/Vol** option of the main menu. Pressing the **Flow/Vol** Soft-key accesses three options: **Rinse**, **Air Gap** and **Flow**. Press the **Flow** Soft-key to access the priming flow rate option from the menus available.

Use a high flow rate, near the maximum possible value. The default setting is 20 ml/min (the instrument is supplied with a 500 µl syringe installed). For an instrument fitted with a 100 µl syringe, the maximum flow rate is 4 ml/min.

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♦ VIAL ASPIRATE/DISPENSE FLOW RATE

This is the rate at which all liquids are aspirated from vials and dispensed to vials.

Volumes aspirated or dispensed are declared when you write the method.

This parameter is accessed in the **Flow/Vol** option of the main menu. Pressing the **Flow/Vol** Soft-key accesses three options: **Rinse**, **Air Gap** and **Flow**. Press the **Flow** Soft-key to access the 'vial asp/disp flow rate' option.

The rate you select depends on the liquids you are using, you are recommended to use a low flow rate. The default value is 0.3 ml/min.

5.2 VOLUMES

The maximum volume (of liquid plus air gap) that you can aspirate is limited to the volume of the syringe fitted or the volume of the transfer tubing fitted, whichever value is the smaller. This is to prevent the air gap or liquid from entering the pump valve.

♦ MINIMUM SAMPLE CONSUMPTION

When aspirating liquids, the needle does not touch the bottom of a vial. Therefore there is a certain volume of liquid that cannot be aspirated from the vial.

- The residual volume for 0.7 ml vials is 11 µl.
- The residual volume for 1.7 ml vials is 170 µl.
- The residual volume for 4.5 ml vials is 350 µl.
- The residual volume for 20 ml vials is 1.4 ml.

♦ AIR GAP VOLUME

An air gap is aspirated before a liquid is aspirated from a vial, this ensures that there is a column of air inside the transfer tubing that separates the aspirated liquid from the diluent in the tubing. An air gap is aspirated whenever a liquid is aspirated, except when a mixing is performed. The air gap aspiration flow rate is set using the **Flow/Vol** menu.

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This parameter is accessed in the **Flow/Vol** option of the main menu. Pressing the **Flow/Vol** Soft-key accesses three options: **Rinse**, **Air Gap** and **Flow**. Press the **Air Gap** Soft-key to set the air gap volume.

The default setting is 6 µl for an instrument fitted with the standard 550 µl transfer tubing, this gives a column of air around 10 mm long between the aspirated liquid and the diluent in the transfer tubing. If you use 120 µl transfer tubing, you are recommended to set the air gap as 3 µl.

♦ Calibrated Tubing Volume

The calibrated tubing connects from the injection port to the load inlet of the Rheodyne injection valve. If you change the size of the calibrated tubing, determine the volume of the new calibrated tubing and enter this value using the advanced user menus.

This parameter is only available as an advanced user option. To access this parameter, keep the **CLEAR** key pressed down while switching the instrument ON. Release the **CLEAR** key when you hear a beep. The first option is the 'restore default values', press the **Next** Soft-key to access the 'Calibrated tubing volume' option.

This parameter is used in centered loop filling mode to position the sample in the center of the loop.

The default setting for the factory installed calibrated tubing is 6 µl.

♦ Injection Flush Volume

This parameter applies to total loop filling and partial loop filling injection modes. This is the volume of sample liquid required to flush the calibrated tubing between the injection port and the Rheodyne injection valve in order to allow the injection of an accurate volume of sample liquid in the loop.

The reason for using an injection flush volume is explained in detail in Section 5.3.2.

You access this parameter in the **Configuration** menu.

The default value for the injection flush volume is 15 µl for an instrument fitted with 6 µl calibrated tubing, irrespective of the size of the syringe installed.

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♦ INJECTION LOOP VOLUME

The instrument is supplied with a 20 µl injection loop fitted. You enter the volume of the injection loop fitted. Available sizes are: 5, 10, 20, 50, 100 and 500 µl.

You access this parameter in the **Configuration** menu.

♦ MIXING VOLUME RATIO

In order to mix two liquids that have been dispensed into a result vial, a volume of the result liquid is aspirated and dispensed. The actual volume aspirated is determined as a percentage of the total volume dispensed into the vial. The default setting is 100%, i.e. all of the liquid in the vial. The rate at which the mixing volume is aspirated and dispensed is set using the **Flow/Vol** menu. The number of mixing cycles (number of aspirate/dispense operations) is declared when you write the method.

This parameter is only available as an advanced user option. To access this parameter, keep the **CLEAR** key pressed down while switching the instrument ON. Release the **CLEAR** key when you hear a beep. The first option is the 'restore default values', press the **Next** Soft-key until you access the 'Mixing volume ratio' option.

You can enter a value between 0 and 100%.

♦ INSIDE NEEDLE RINSING VOLUME

In order to avoid cross-contamination between liquids, the inside and outside of the needle are rinsed before a run (initialization) and after each injection. The inside needle rinsing volume is the volume of diluent dispensed into the drain station in order to rinse the inside of the needle. The flow rate at which the diluent is dispensed is the same as the priming flow rate, set using the **Flow/Vol** menu.

This parameter is accessed in the **Flow/Vol** option of the main menu. Pressing the **Flow/Vol** Soft-key accesses three options: **Rinse**, **Air Gap** and **Flow**. Press the **Rinse** Soft-key to set the rinse volumes.

The default setting for the instrument supplied with a 500 µl syringe is 300 µl. Use a high value to ensure a thorough rinsing of the inside of the needle.

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♦ OUTSIDE NEEDLE RINSING VOLUME

In order to avoid cross-contamination between liquids, the inside and outside of the needle are rinsed before a run (initialization) and after each inject operation. This is the volume of diluent dispensed into the rinsing well in order to rinse the outside of the needle. The flow rate at which the diluent is dispensed is the same as the priming flow rate, set using the **Flow/Vol** menu.

This parameter is accessed in the **Flow/Vol** option of the main menu. Pressing the **Flow/Vol** Soft-key accesses three options: **Rinse**, **Air Gap** and **Flow**. Press the **Rinse** Soft-key to set the rinse volumes.

The default setting for the instrument supplied with a 500 µl syringe is 300 µl. Use a high value to ensure a thorough rinsing of the outside of the needle.

♦ INJECTION VALVE RINSING VOLUME

This is the volume of diluent dispensed into the injection port and the injection valve (the valve is in the inject position). This procedure is carried out after the needle has been rinsed; before a run (initialization) and after each injection. The rate at which the diluent is dispensed is set using the advanced user menus, accessible by holding the **CLEAR** key down while switching ON the instrument.

This parameter is accessed in the **Flow/Vol** option of the main menu. Pressing the **Flow/Vol** Soft-key accesses three options: **Rinse**, **Air Gap** and **Flow**. Press the **Rinse** Soft-key to set the rinse volumes.

The default setting for the instrument supplied with a 500 µl syringe is 500 µl. Use a high value to ensure a thorough rinsing of the injection valve.

♦ SYRINGE VOLUME

This is the volume of the syringe fitted to the instrument. The instrument has a 500 µl syringe factory fitted.

You access this parameter in the **Configuration** menu.

You can use two possible syringe sizes with the Autoinjector: 100 µl and 500 µl.

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♦ TRANSFER TUBING VOLUME

This is the volume of the transfer tubing fitted between the pump outlet port and the needle on the instrument. The instrument is supplied with two sizes: 550 µl and 120 µl.

You access this parameter in the **Configuration** menu.

Enter the value of the tubing you have installed.

5.3 VOLUMES DECLARED WHEN WRITING THE METHOD

You enter the following volumes when editing (writing) a method file.

♦ INJECTION VOLUME (PARTIAL AND CENTERED LOOP FILLING INJECTION)

This is the volume of sample or standard liquid to be injected for partial loop or centered loop filling methods. The maximum volume depends on the configuration (loop size installed), you are recommended not to exceed 50% the loop volume.

For a 20 µl loop, the recommended ranges are from 1 µl to 10 µl.

♦ SAMPLE DILUENT VOLUME

This is the volume of diluent to be added to the sample liquid in a result vial. Sample dilution is used for external standard and internal standard methods.

The total volume that can be dispensed into a result vial (sample liquid + diluent volume + internal standard) must not exceed the vial volume. The total volume declared should be sufficient for the number of injections that are to be carried out from that vial (Nb. injections per sample) and should take into account the residual volume in the vial.

♦ SAMPLE VOLUME

This is the volume of sample liquid to be dispensed into a result vial. This must be stated when using sample dilution (external standard and internal standard methods) or when adding an internal standard.

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The total volume that can be dispensed into a result vial (sample liquid + diluent volume + internal standard) must not exceed the vial volume. The total volume declared should be sufficient for the number of injections that are to be carried out from that vial (Nb. injections per sample).

♦ STANDARD DILUENT VOLUME

This is the volume of diluent to be added to the standard liquid in a result vial for one calibration (taking into account the 'Nb. injections per standard' value), the total volume of diluent added to the result vial is calculated according to the calibration frequency and the total number of sample vials to be injected. Dilution of the standard is used for external standard and internal standard methods. The total volume of liquid that can be dispensed into a result vial must not exceed the vial volume;

(vol. standard + vol. diluent + vol. internal standard) x total number of calibrations

♦ STANDARD VOLUME

This is the volume of standard liquid to be dispensed into a result vial for one calibration (taking into account the 'Nb. injections per standard' value).

This must be stated when using dilution of the standard or when adding an internal standard.

The total volume required for all the calibrations for a run is compared with the volume dispensed in the result vial. If the volume in the result vial is insufficient for the run, the same volumes of standard, diluent and internal standard are added again to the result vial. The volume in the result vial is increased in this way until there is enough liquid in the result vial for the necessary number of calibrations in the run.

♦ INTERNAL STANDARD VOLUME

This is the volume of internal standard that is to be added to each sample and each standard.

The total volume that can be dispensed into a result vial must not exceed the vial volume.

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WRITING METHOD PROGRAMS

1 FILE STRUCTURE

There are two types of file: the Run file, and the Edit file.

1.1 RUN FILE

The file number indicated in the ready-to-run screen (File 1 in this example) is called the Run file. When in the ready-to-run screen, you key in the number of the file you want to run, press **ENTER**, then press the **Run** soft-key.



1.2 EDIT FILE

When you press the **File** soft-key in the ready-to-run screen (above), you can select a file to edit by keying in the file number, pressing **ENTER** and selecting the **Edit** soft-key option. You can edit one file while running another file, or you can edit a file that is running. To edit a file that is running, refer to Section 7.7.



When you write a method program, the program is saved as two parts: the method program, and the configuration declared at the time the file was written. When the method file is run later, the saved method file configuration is compared with the present configuration. If the two configuration files are different, you have the choice of copying the current configuration file to your method file, or overwriting the current configuration with the configuration file from your method file.

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The configuration parameters are:
- Rack code
- Syringe volume
- Transfer tubing volume
- Injection loop volume
- Injection flush volume
- GSIOC identification number
- File locked/unlocked
- Restart after power failure
- Error alarm
- Time/Date counters

Incomplete Files

When writing a program certain values are proposed by default, others are blank. If you press the **Quit** soft-key during an edit, the program parameters will be lost if the program is incomplete. A warning message will appear allowing you to cancel the Quit operation enabling you to retain the values and complete the program if desired.

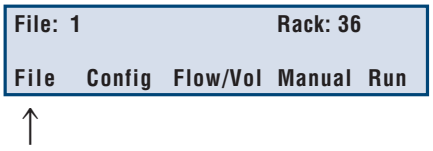
However, if you have not entered all the values, and the remaining values contain proposed default values, the file will be saved.

Parameters that you must enter in order for a file to be saved are:
- Injection method
- Sample and Standard injection volume (partial or centered loop injection)
- Internal standard volume
- Calibration frequency
- Sample diluent volume (for method with dilution)
- Sample volume (for method with dilution)
- Standard diluent volume (for method with dilution)
- Standard volume (for method with dilution)

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2 SIMPLE INJECTION METHOD

In the simple injection method, all the sample vials in the rack contain raw sample liquid only. There is no dilution of the sample, and there is no use of standard liquids or an internal standard. A declared volume of the raw sample liquid is injected using one of three possible loop filling methods. The Simple Injection method is explained in Section 5.2.1.

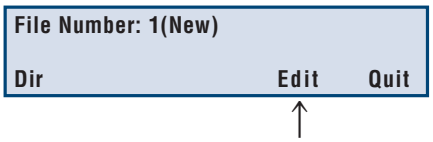


When in the Ready-to-Run screen, press the **File** soft-key to go to the Edit screen.

2.1 WRITING A SIMPLE INJECTION METHOD FILE

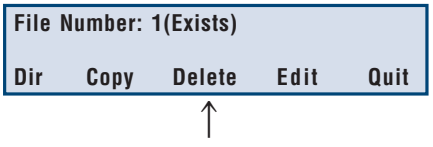
- **Select the File Number**

  The top line of the display indicates the Edit file. Key in the file number you are going to edit or write and press **ENTER**.



  If the file number selected does not already exist, the word **New** appears on the top line of the display.

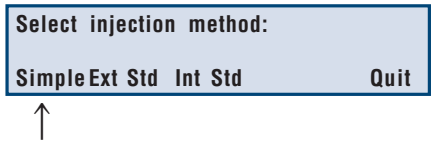
  If the word **Exists** appears, a program already exists in this file. Either delete the existing file using the **Delete** soft-key, edit the file, or enter a new file number to write a new file. Press the **Edit** soft-key to write the program. In the first screen you select the injection method.



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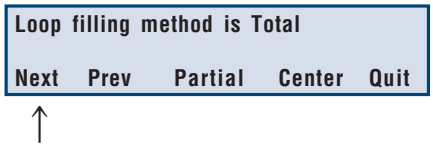
- **Select the Injection Method**

  Choose the injection method by pressing the corresponding Soft-key: Simple injection (**Simple**), external standard (**Ext Std**) or internal standard (**Int Std**). The option **Int Std** only appears if you have selected rack code 35, 36, 37 or 38 in the configuration menu. For a complete explanation of the different injection methods, refer to Section 5.2.



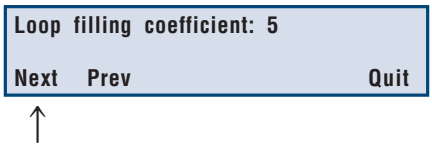
- **Select the Loop Filling Method**

  The loop filling method is set as Total by default, change the method by pressing the corresponding Soft-key: partial loop (**Partial**) or centered loop filling (**Center**). The different methods are explained in Section 5.3.



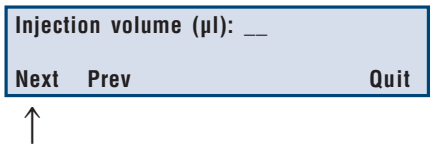
  For Total Loop Filling:

  Key in the loop filling coefficient, if different from the recommended coefficient of 5. Press **ENTER**. Press the **Next** soft-key to define the number of injections.



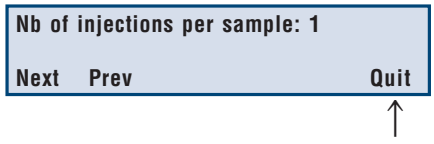
  For Partial or Centered Loop Filling:

  Key in the volume of sample liquid you want to inject (in microliters), press **ENTER** and then press the **Next** soft-key to define the number of injections.



- **Enter the Number of Injections Per Sample**

  Key in the number of injections you want to carry out per sample vial (to a maximum of 9 injections per sample). Press **ENTER** and press the **Quit** soft-key to determine the method of coordination.



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WRITING METHOD PROGRAMS

- **Select the Coordination Method**

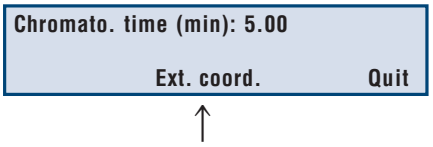
  Select the method of coordination: coordination by chromatography time or external coordination (by contact or GSIOC, explained in Appendix C).

  Key in the chromatography time in minutes, you specify the time to an accuracy of 0.01 min, press **ENTER**. The chromatography time should take into account both the analysis time and the time to print the report. The maximum time allowed for the chromatography time (including for priority samples) is 600.00 minutes.

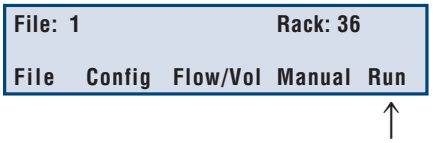
  To set external coordination (either by contacts or GSIOC), press the **Ext. coord.** Soft-key.

  When you press **Quit** the ready-to-run screen displays the last run file, not the file that you have just written.

  Key in the file number, press ENTER. You can now run your written program.





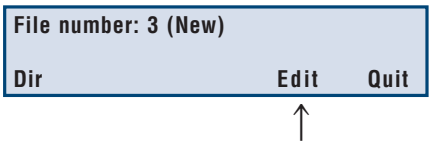


3 EXTERNAL STANDARD METHOD

Sample liquid is injected, with or without dilution, using the selected loop filling method. Standard liquids are used for calibration (up to nine different standards). You can dilute the samples or the standards, or both. The External Standard method is explained in Section 5.2.2.



Press the **File** Soft-key, key in the file number you are going to use. For example 3, and press **ENTER**. If the file is new, the word **New** appears alongside File 3 on the top line of the display:



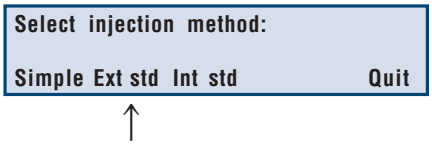
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3.1 WRITING AN EXTERNAL STANDARD INJECTION METHOD FILE

Press the **Edit** Soft-key to write the program. In the first screen you select the injection method.

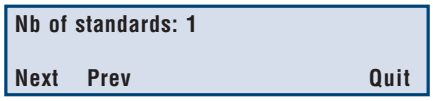
- **Select the Injection Method**

  Choose the injection method by pressing the corresponding Soft-key: **Ext std** for the external standard method. The Internal Standard option (Int Std) only appears for racks 35, 36, 37 and 38. The program automatically advances to the next screen:



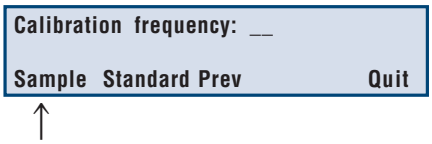
- **Enter the Number of Standards**

  Declare the number of standard vials you are using, the maximum allowed is nine. Key in the number, press **ENTER**, then press the **Next** Soft-key.



- **Declare the Calibration Frequency**

  The calibration frequency corresponds to the number of samples that are injected between two series of calibration injections of the standard(s). A calibration frequency of 4 means that standards are injected, then sample injections are carried out for *four sample vials* before the next series of calibration injections (irrespective of the number of injections per sample vial). Key in the calibration frequency and press **ENTER**, press the **Sample** Soft-key to define the sample injection parameters.

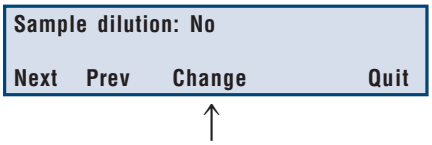


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WRITING METHOD PROGRAMS

- **Diluting the Sample**

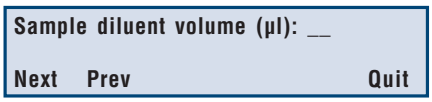
  If you want sample dilution, press the **Change** Soft-key, otherwise press the **Next** Soft-key to select the loop filling method.



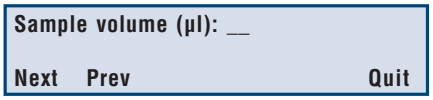
Method with Sample dilution

The total volume dispensed into a result vial must be sufficient for the number of injections to be performed for each sample vial, for the loop filling method selected and must not exceed the vial volume: 700 µl for rack codes 31, 36, and 38; 1700 µl for rack codes 30, 35, 37; 4500 µl for rack code 34.

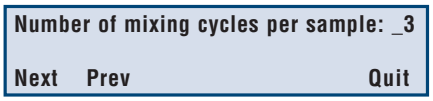
Key in the volume of diluent to be added to the sample, in microliters. Press **ENTER** and press the **Next** Soft-key.



Key in the sample volume you want to dispense into the sample result vial, in microliters, press **ENTER** and press the **Next** Soft-key.

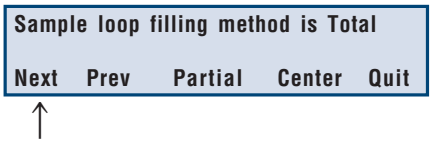


Key in the number of mixing cycles (the number of times the result liquid is aspirated then dispensed), press **ENTER** and press the **Next** Soft-key to select the sample loop filling method.



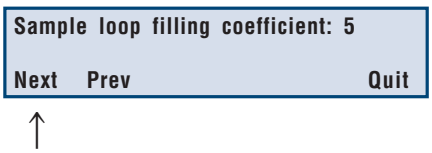
- **Select the Sample Loop Filling Method**

  The loop filling method is set as Total by default, change the method by pressing the corresponding Soft-key: partial loop (**Partial**) or centered loop filling (**Center**). You are recommended to use the same loop filling method for sample and standard injections. The different methods are explained in Section 5.3.



For Total Loop Filling:

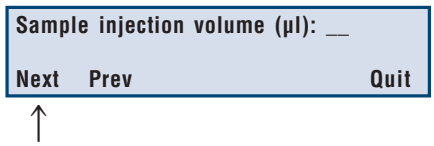
Key in the loop filling coefficient, if different from the recommended coefficient of 5. Press **ENTER**. Press the **Next** Soft-key to define the number of injections.



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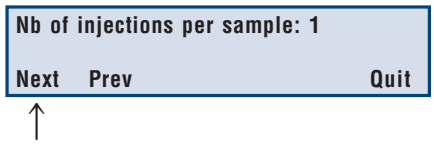
For Partial or Centered Loop Filling:

Key in the volume of sample liquid you want to inject (in microliters), press **ENTER** and then press the **Next** Soft-key to define the number of injections. The volume injected must be smaller than the volume of the injection loop (specified in the configuration menu).

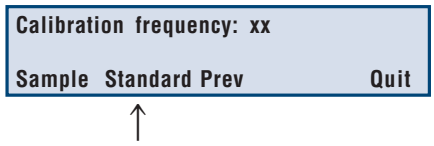


- **Enter the Number of Injections Per Sample**

Key in the number of injections you want to carry out per sample vial, press **ENTER** and then press the **Next** Soft-key to verify the calibration frequency you are using and access the Standard injection method menus.

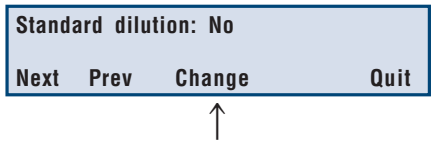


At this point you can modify the calibration frequency by keying in the new frequency and pressing **ENTER**. Press the **Standard** Soft-key to program the standard injection method.



- **Diluting the Standard**

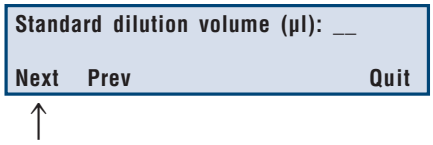
For a method without dilution of the standard(s), press the **Next** Soft-key to go on to loop filling method. To dilute the standard(s), press the **Change** Soft-key, then press the **Next** Soft-key to define the standard dilution parameters.



Method with Standard dilution

The total volume dispensed into a result vial must be sufficient for the loop filling method selected, for the number of injections per vial, calibration frequency and must not exceed the vial volume: 700 µl for rack codes 31, 36, and 38; 1700 µl for rack codes 30, 35, 37; 4500 µl for rack code 34.

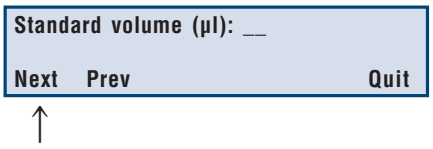
Key in the standard diluent volume, in microliters, press **ENTER** then press the **Next** Soft-key.



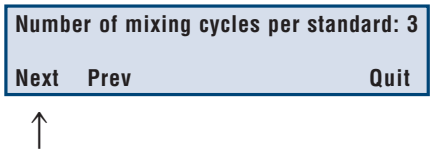
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WRITING METHOD PROGRAMS

Key in the volume of external standard you want to dispense into the result vial, in microliters, press **ENTER** and press the **Next** Soft-key.

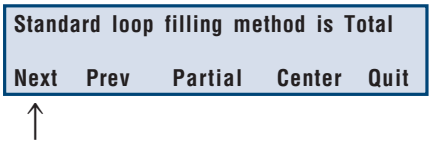


Key in the number of mixing cycles; the number of times the result liquid is aspirated then dispensed to mix the liquids. Press **ENTER**, then press the **Next** Soft-key to select the standard loop filling method.



- **Select the Loop Filling Method for the Standard**

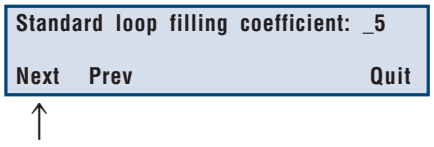
You are recommended to use the same loop filling method for the standards and the samples.



The loop filling method is set as Total by default, change the method by pressing the corresponding Soft-key: partial loop (**Partial**) or centered loop filling (**Center**). The different methods are explained in Section 5.3.

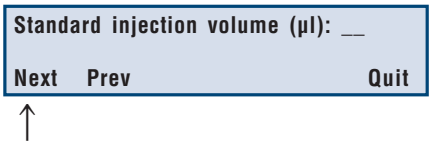
For Total Loop Filling:

Key in the loop filling coefficient, a coefficient of 5 is recommended by Rheodyne. Press **ENTER**. Press the **Next** Soft-key to define the number of injections.



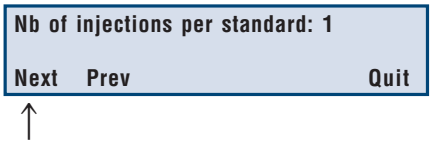
For Partial or Centered Loop Filling:

Key in the volume of standard liquid you want to inject (in microliters), press **ENTER** and then press the **Next** Soft-key to define the number of injections. The volume injected must be smaller than the volume of the injection loop.



- **Enter the Number of Injections Per Standard**

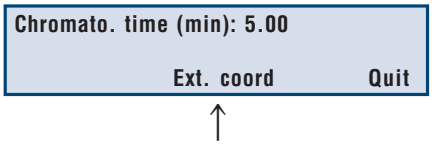
Key in the number of injections you want to carry out per standard vial. Press the **Quit** Soft-key to go to the coordination menu.



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- **Select the Coordination Method**

  Select the method of coordination: coordination by chromatography time or external coordination (by contacts or GSIOC, explained in Appendix C).

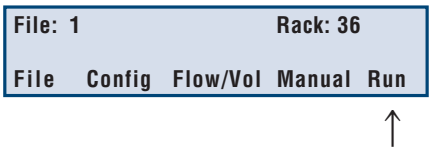


  Key in the chromatography time in minutes, you specify the time to an accuracy of 0.01 min, press **ENTER**. The chromatography time should take into account both the analysis time and the time to print the report. The maximum time allowed for the chromatography time (including for priority samples) is 600.00 minutes.



  To set external coordination (either by contacts or GSIOC), press the **Ext. coord** Soft-key.

  When you press **Quit**, the ready-to-run screen displays the last run file, not the file that you have just written.



  Key in the file number, press **ENTER**. You can now run your written program.

4 INTERNAL STANDARD METHOD

You can add internal standard to samples and standards. You can also dilute samples and standards, if desired. The internal standard method is described in Section 5.2.4. This method is only available for rack codes 35, 36, 37, and 38.



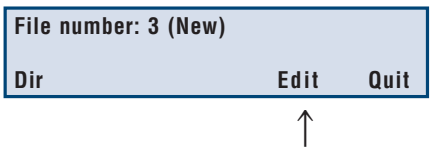
Press the **File** Soft-key. Key in the file number you are going to use, for example 3, and press **ENTER**. If the file is new, the word **New** appears alongside File 3 on the top line of the display.

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WRITING METHOD PROGRAMS

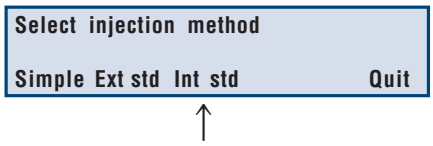
4.1 WRITING AN INTERNAL STANDARD INJECTION METHOD FILE

Press the **Edit** Soft-key to write the program. In the first screen you select the injection method.



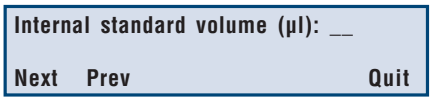
- **Select the Injection Method**

  Choose the injection method by pressing the corresponding Soft-key: **Int. std** for the internal standard method. The program automatically advances to the next screen:



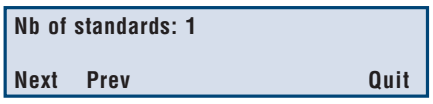
- **Declare the Internal Standard Volume**

  Key in the volume of internal standard liquid you want to add to each sample and standard vial in microliters, press **ENTER**. This volume of internal standard is aspirated and dispensed into each sample and standard result vial. Press the **Next** Soft-key.



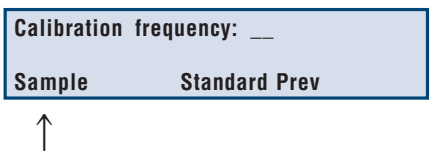
- **Declare the Number of Standards**

  The standard vials occupy the first vial positions in the sample vial area. Key in the number of standard vials you are using (up to nine), press **ENTER**, then press the **Next** Soft-key.



- **Enter the Calibration Frequency**

  The calibration frequency corresponds to the number of samples that are injected before the next series of calibration injections of the standard(s). A calibration frequency of 4 means that standards are injected, then sample injections are carried out for *four sample vials* before the next series of calibration injections (irrespective of the number of injections per sample vial). Key in the calibration frequency and press **ENTER**, press the **Sample** Soft-key to select

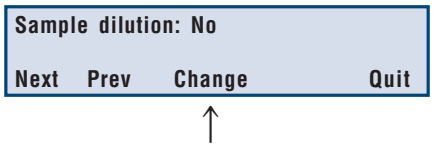


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dilution of the sample and define the sample injection parameters.

- **To Dilute the Sample**

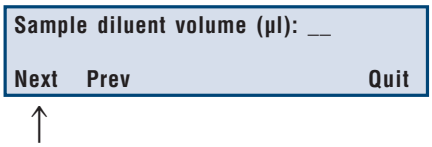
If you want sample dilution, press the **Change** Soft-key, otherwise press the **Next** Soft-key to go on to the loop filling method screen.



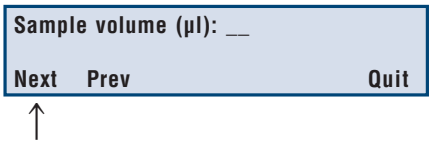
Method with Sample dilution

The total volume dispensed into a result vial must be sufficient for the loop filling mode selected and the number of injections per vial, and must not exceed the vial volume: 700 µl for rack codes 31, 36, and 38; 1700 µl for rack codes 30, 35, 37; 4500 µl for rack code 34.

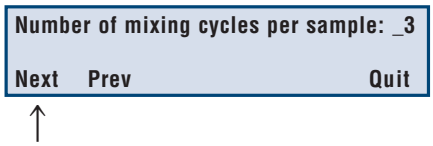
Key in the volume of diluent to be added to the sample, in microliters. Press **ENTER** and press the **Next** Soft-key. The next step is to define the volume of sample liquid to be added to the internal standard liquid and diluent (if used) in the result zone.



Key in the sample volume you want to dispense into the sample result vial, in microliters. Press **ENTER** and press the **Next** Soft-key.

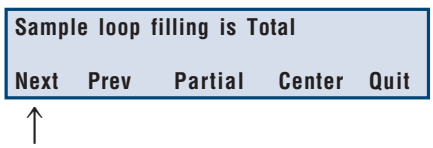


Key in the number of mixing cycles (the number of times the result liquid is aspirated then dispensed), press **ENTER** and press the **Next** Soft-key to select the sample loop filling method.



- **Select the Sample Loop Filling Method**

The loop filling method is set as Total by default, change the method by pressing the corresponding Soft-key: partial loop (**Partial**) or centered loop filling (**Center**). The different methods are explained in Section 5.3.

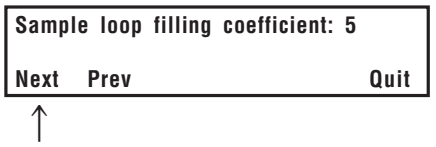


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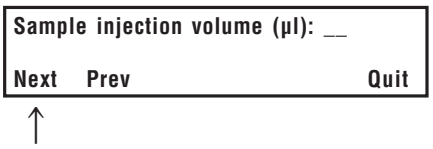
For Total Loop Filling:

Key in the loop filling coefficient, if different from the recommended coefficient of 5. Press **ENTER**. Press the **Next** Soft-key to define the number of injections.



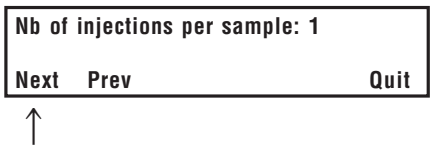
For Partial or Centered Loop Filling:

Key in the volume of sample liquid you want to inject (in microliters), press **ENTER** and then press the **Next** Soft-key to define the number of injections. The volume injected must be smaller than the volume of the injection loop (specified in the configuration menu).

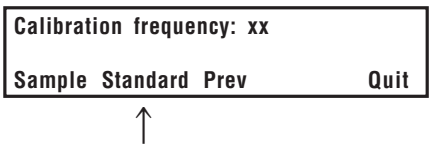


- **Enter the Number of Injections Per Sample**

Key in the number of injections you want to carry out per sample vial. Press **ENTER** and press the **Next** Soft-key to verify the calibration frequency you are using and access the Standard injection method menus.

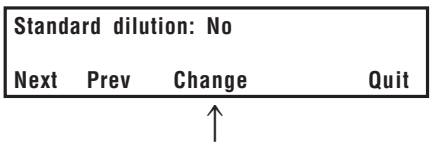


At this point you can modify the calibration frequency by keying in the new frequency and pressing **ENTER**. Press the **Standard** Soft-key to program the standard injection method.



- **Diluting the Standard**

For a method without dilution of the standard(s), press the Next Soft-key to go on to loop filling method. Press the **Change** Soft-key for dilution of the standard, then press the **Next** Soft-key to go on to defining the standard dilution parameters.



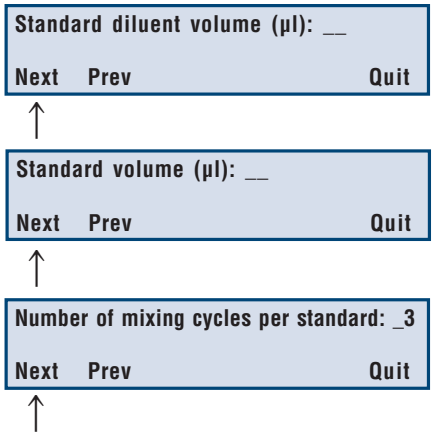
The total volume dispensed into a result vial Internal standard + (diluent + standard) x injection frequency must not exceed the vial volume: 700 µl for rack codes 31, 36, and 38; 1700 µl for rack codes 30, 35, 37; 4500 µl for rack code 34.

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Key in the standard diluent volume, in microliters, press **ENTER** then press the **Next** Soft-key.

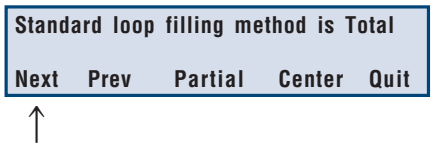
Key in the volume of standard you want to dispense into the result vial, in microliters, press **ENTER** and press the **Next** Soft-key.

Key in the number of mixing cycles; the number of times the result liquid is aspirated then dispensed. Press **ENTER**, then press the **Next** Soft-key to select the standard loop filling method.



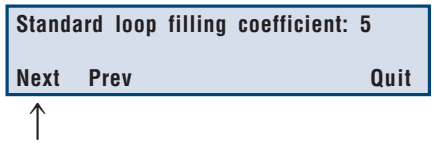
- **Select the Loop Filling Method for the Standard**

The loop filling method is set as Total by default, change the method by pressing the corresponding Soft-key: partial loop (**Partial**) or centered loop filling (**Center**). The different methods are explained in Section 5.3.



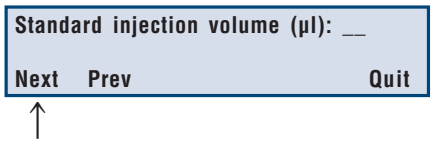
For Total Loop Filling:

Key in the loop filling coefficient, a coefficient of 5 is recommended by Rheodyne. Press **ENTER**. Press the **Next** Soft-key to define the number of injections.



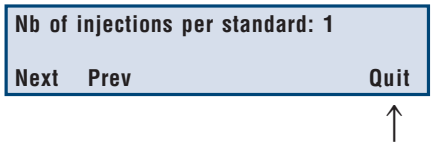
For Partial or Centered Loop Filling:

Key in the volume of standard liquid you want to inject (in microliters), press **ENTER** and then press the **Next** Soft-key to define the number of injections. The volume injected must be smaller than the volume of the injection loop.



- **Select the Number of Injections Per Standard**

Key in the number of injections you want to carry out per sample vial. Press the **Quit** Soft-key to go to the coordination menu.



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WRITING METHOD PROGRAMS

- **Select the Coordination Method**

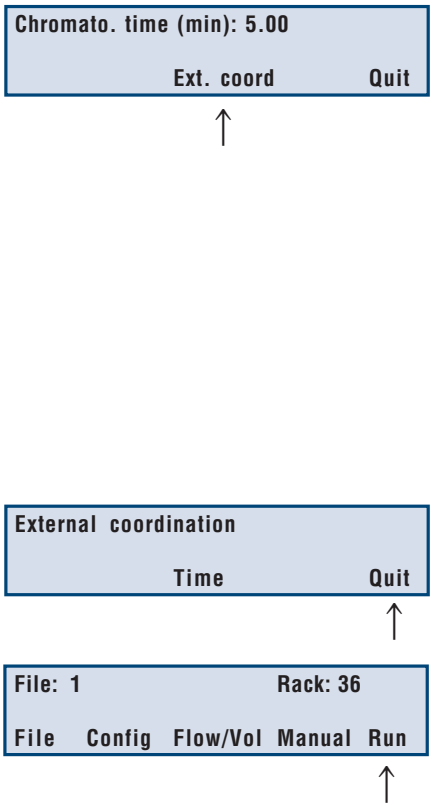
  Select the method of coordination: coordination by chromatography time or external coordination (by contacts or GSIOC, explained in Appendix C).

  Key in the chromatography time in minutes, you specify the time to an accuracy of 0.01 min, press **ENTER**. you specify the time to an accuracy of 0.01 min. The chromatography time should take into account both the analysis time and the time to print the report. The maximum time allowed for the chromatography time (including for priority samples) is 600.00 minutes.

  To set external coordination (either by contacts or GSIOC), press the **Ext. coord** Soft-key.

  When you press **Quit**, the ready-to-run screen displays the last run file, not the file that you have just written.

  Key in the file number, press **ENTER**. You can now run your written program.



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WRITING METHOD PROGRAMS

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RUNNING A METHOD

Before running a method program, ensure that your vials (sample and standard vials) are in the correct positions in the rack according to the written method, and contain sufficient liquid for the declared method. Take into account:

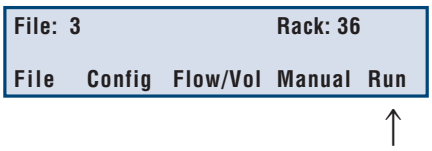
- *The number of injections per sample vial for sample liquid.*
- *The number of injections and calibration frequency for standards,*
- *The total volume added to all standard and sample vials for the internal standard liquid.*
- *The instrument cannot aspirate the last 12 µl of liquid in a 0.7 ml vial - add 12 µl to the total volume of liquid you are using in vials.*

1 PREPARE THE AUTOINJECTOR

Use the **Manual** menu to prepare the system for the run by priming the pump and rinsing the rinsing well and injection port.

2 KEY IN THE FILE TO RUN

To run a file, ensure that the correct file is loaded when in the ready-to-run screen. Key in the file number and press **ENTER** to bring the file into memory. Press the **Run** Soft-key. You are prompted for the vial number for the first sample.



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3 KEY IN THE FIRST SAMPLE POSITION



Key in the vial number of the first sample vial and press **ENTER**. All sample vials must be contiguous and must follow the vial numbering sequence indicated in Appendix B. At this point you can:

- Change the coordination time delay or change the method of coordination by pressing the **Coord**. Soft-key.
- Set the audit trail as ON or OFF. This is the record of events as they occur during the run of your program. To obtain a correct record, ensure that you have set the date and time correctly in the configuration menu (Section 4.6.10). Only one audit trail file can exist. If you use the audit trail function the previous audit trail will be overwritten.

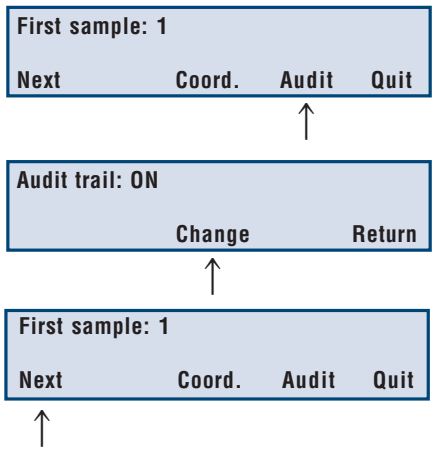
4 SET THE AUDIT TRAIL

You can have only one audit trail file. Each time you run a file with the audit trail function set, the previous audit trail will be deleted. To obtain a printout of the audit trail, you must use the Audit Trail software and a PC. Refer to Appendix E for details.

To set the audit trail, press the **Audit** Soft-key.

To change the audit trail from ON to OFF or from OFF to ON, press the **Change** Soft-key. Return to the menu by pressing the **Return** Soft-key.

Press the **Next** Soft-key to go to the next screen. You normally place the sample vials in the first vial positions in the rack, in a continuous sequence. Key in the position of the first vial and press **ENTER**, then press the **Next** Soft-key.



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RUNNING A METHOD

5 KEY IN THE TOTAL NUMBER OF SAMPLES

The number displayed takes into account the declared number of standard vials and is the maximum number possible for your configuration. Key in the number of vials and press **ENTER**. The **Info** Soft-key option is only available if the Internal Standard or External Standard method has been selected

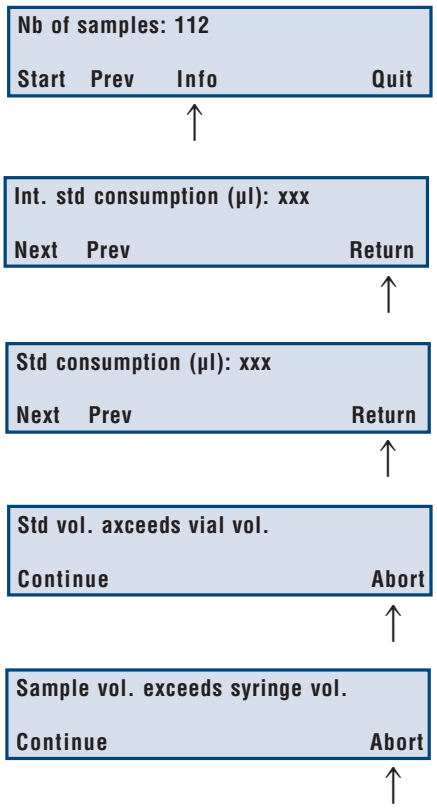
The **Info** Soft-key allows you to view the consumption of standard and internal standard for the declared method and number of sample vials.

Press the **Start** Soft-key to run the program.

At this point, if there are any incompatibilities in the volumes declared in the program, error messages will appear, for example:

Where an error occurs concerning the syringe volume or transfer tubing volume (if these volumes are exceeded), it is not possible to continue with the run, as liquid would enter and contaminate the pump valve. In these instances, the **Continue** Soft-key function is not available.

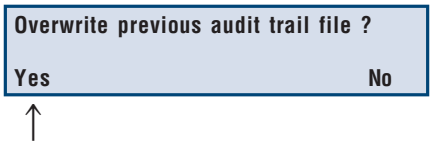
A list of error messages and their meaning is detailed in Chapter 9, Troubleshooting.



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6 OVERWRITE PREVIOUS AUDIT TRAIL

If you are using the audit trail facility, you will be prompted with the following question:

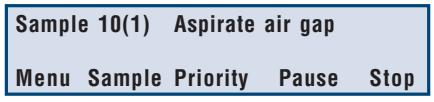


Press the appropriate Soft-key.

Press **Return** to resume entering the run parameters.

In-Run Screens

When the program runs, the Autoinjector first initializes, rinses the needle and the injection port, then carries out the injection program. The display indicates the sample number being processed, how many injections from the sample it has carried out (including the current injection in process), and the task currently taking place. The screen below indicates that the instrument is aspirating the air gap for the first injection of the tenth sample.



7 EDITING A FILE THAT IS RUNNING

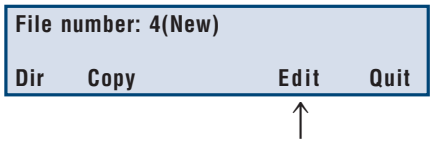
You can edit a file while it is running. You can change many of the file parameters, some of the configuration parameters and also the number of sample vials to be processed. As you enter new values, they are used by the software for the next sample to be processed.

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RUNNING A METHOD

7.1 CHANGING FILE PARAMETERS

Press the **Menu** Soft-key on the In-Run screen, this allows you to access the **File** Soft-key. Press the **File** Soft-key, the following screen is presented:



Press the **Edit** Soft-key.

To progress through the program, press the **Next** Soft-key.

You cannot change all of the parameters of a running file, but you can change:

- The volume of internal standard added to samples and standards
- Sample diluent volume
- Sample volume to be added to diluent
- Number of mixing cycles
- Loop filling coefficient for total loop filling
- Sample injection volume for partial or centered loop filling
- Number of injections per sample
- Standard diluent volume
- Standard volume to be diluted
- Standard injection volume for partial or centered loop filling
- Loop filling coefficient for total loop filling
- Number of injections per standard
- Chromato time (min)
- Mode of coordination

7.2 CHANGING THE CONFIGURATION PARAMETERS

You can change certain configuration parameters while a file is running. Press the **Menu** Soft-key on the In-Run screen, then press the **Config** Soft-key. Proceed through the configuration listing by pressing the **Next** Soft-key.

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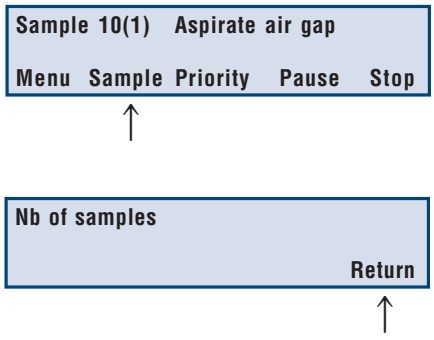
The parameters that you can change are:

- Injection flush volume.
- GSIOC identification number.
- Time and Date.
- File number to be locked or unlocked.

You can also change the Flow/Vol parameters during a run: the rinse, air gap and flow values. The new values are taken into account for the next sample to be processed.

7.3 CHANGING THE NUMBER OF SAMPLE VIALS DURING A RUN

It is possible to increase or decrease the declared number of sample vials during a run. However, if you increase the number, use the **Pause** Soft-key in the program to stop the instrument and add the vials to the rack, then press the **Resume** Soft-key. To change the number of sample vials, press the **Sample** Soft-key visible on the In-Run screen.



Key in the new number of sample vials to be processed, press **ENTER** and then press the **Return** Soft-key.

The Autoinjector will continue with the run, and will inject from the new total of sample vials.

☞*In an external standard or internal standard method, if the number of samples is modified, the samples are still injected with the frequency that was declared for the initial number of samples, after the initial number of samples has been injected, extra samples will be injected* ***without*** *calibration injections.*

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MAINTENANCE

1 MAINTENANCE SCHEDULE

The maintenance schedule assumes that the instrument is used within the set pressure and flow rate limits. These figures represent operation using typical liquids under normal working conditions. However, the nature of the liquids used may have a slight influence on these figures. You can access maintenance counters in the configuration menu in order to determine the remaining lifetime of relevant parts.

Part	Lifetime
Piston nose (PTFE)	> 30 000 cycles* (with water or methanol)
7010 Injection valve Vespel Rotor seal	> 30 000 cycles** at 100 bars

☞ ** accessed from 'number of dispenses' maintenance screen (Config menu).*

***accessed from 'number of switchings' maintenance screen (Config menu), 1 cycle or 'switching' = valve being switched twice (from load to inject then back to load position).*
When you replace one of these parts, remember to reset the relevant maintenance counter to zero.

Maintenance Screens

Three maintenance screens can be accessed in the Time/Date screen of the Configuration menu. Each counter can be reset after a service operation has been carried out.

This is the number of times the injection valve has switched.

Number of valve switchings:

This is the number of times the arm has moved to a vial on the rack.

Number of vials:

This is the number of times the pump has dispensed a liquid. The dispense movement of the syringe is counted; the aspirate movement is not counted.

Number of dispenses:

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2 CLEANING THE INSTRUMENT

The 234 Autoinjector should be cleaned occasionally, using a dry, clean cloth, or if necessary, with a cloth dipped in soapy water. If liquid is accidentally spilled on the instrument, wipe the instrument using a dry, clean cloth.

3 CLEANING THE PUMP VALVE

☞ *The surfaces of the ceramic stator and rotor inside the dilutor valve are treated to give optimum airtight operation of the valve. If the valve has been used with alcohol based substances and then left unused for a while, a grinding noise may be heard during valve switching the next time the dilutor is used. This is due to the friction of the ceramic surfaces. To avoid this problem, you are recommended to disassemble the valve and clean the ceramic surfaces with a damp cloth.*

The pump valve must be cleaned with a non-abrasive cloth after any one of the following situations has occurred:

- Corrosive or hazardous liquids have been pumped.
- The pump has been left unused for a while after pumping alcohol based liquids.
- Possible back flow of liquids into the waste tubing.
- Leakage.

To clean the valve, remove the valve from the pump:

1) Disconnect the inlet, outlet and waste tubing from the valve.
2) Disconnect the syringe from the valve - refer to Section 3.3.
3) Undo the two screws securing the valve to the pump body and remove the valve.

Disassemble the valve body:

1) Hold the valve body firmly in one hand. Using a 17 mm open ended spanner, undo the inner body of the valve from the valve main body. Slide apart the two halves of the valve.
2) Pull the valve axle away from the valve main body.
3) Remove the nylon/ceramic switching plate from the valve axle.

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MAINTENANCE

4) Tap the valve inner body against a solid surface to remove the spring and nylon spring cover.
5) Do not remove the inner ceramic surface from the valve main body.
6) The parts can be cleaned using a non-abrasive cloth dampened with alcohol, or by autoclaving them.
7) Dry the components using a clean lint-free cloth.
8) Check that the parts are dry, then reassemble the valve.
9) Reinstall the valve aligning the valve axle with the motor and mounting the valve on the pump body.
10) Reinstall the syringe by following the instructions in Section 3.3.

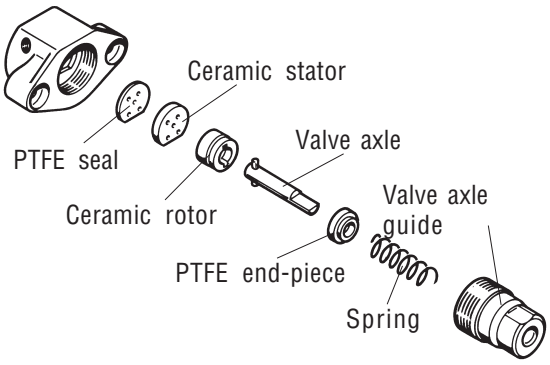


Figure 8.1 - Valve components

4 UNPLUGGING THE INJECTION PORT

If the injection port becomes plugged:

- Disconnect the injection port and the short calibrated tubing from the rack holder and injection valve, and dismantle the injection port.
- Clean the injection port components with acetone.
- Connect the calibrated tubing to the injection port in reverse direction. Hand tighten the fitting.
- Manually dispense the HPLC mobile phase in the injection port, using the female luer needle and a syringe, part number 460082. This should unplug and backflush the calibrated tubing. If the tubing is still plugged, use a capillary stainless steel wire to push out any remaining particles.

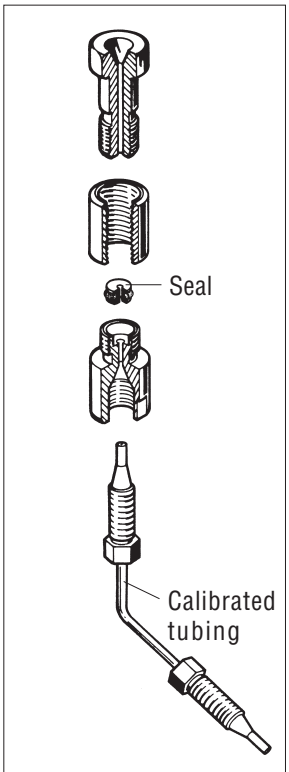


Figure 8.2 - Disassembled injection port

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- Refit the calibrated tubing in the correct orientation.
- Reassemble the components.

If the valve still appears to be plugged, the plug is inside the valve. *Refer to the Rheodyne leaflet.*

5 CHANGING THE CALIBRATED TUBING

To remove the calibrated tubing:

1) Remove the rack holding bar from the front of the instrument (two screws) in order to give good access to the hexagonal nuts securing the calibrated tubing to the Rheodyne valve and the injection port.
2) Unscrew the hexagonal nuts that secure the calibrated tubing to the Rheodyne valve and injection port.
3) Remove the calibrated tubing.

To install the calibrated tubing:

1) The rack holding bar must be removed from the instrument, this enables you to secure the calibrated tubing without twisting it.
2) Secure the tubing to the Rheodyne valve and injection port using a ferrule and hexagonal nut to secure each end of the tubing.
3) Mount the rack holding bar on the instrument.

6 CHANGING THE PISTONS AND PISTON NOSES

The piston nose should be replaced after the pump has performed 30 000 cycles. The support collar should be replaced after around 60 000 cycles. The number of cycles performed can be verified using the 'number of dispenses' screen maintenance counter accessible in the Configuration menu. Go to the Time/Date screen and press the **Counter** Soft-key. The following sections describe how to change the piston and the piston nose for the 100 µl and 500 µl syringes.

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MAINTENANCE

6.1 REPLACING THE PISTON OF A 100 µl SYRINGE

Refer to Figure 8.3:

1) Remove the syringe from the instrument as described in Section 3.3. Remove the syringe cover seal at the same time.
2) Remove the piston and its support collar from the syringe barrel by pulling on the piston button.
3) Remove the piston button from the old piston by undoing the Allen screw.
4) Discard the old piston and support collar, but keep the piston button.
5) Hold the syringe barrel vertically with the valve screw resting on a clean, flat, work surface.
6) Moisten the nose of the new piston with water or diluent from the pump reservoir and gently insert it into the syringe barrel.
7) Push the syringe into the barrel, to about half way down the barrel.

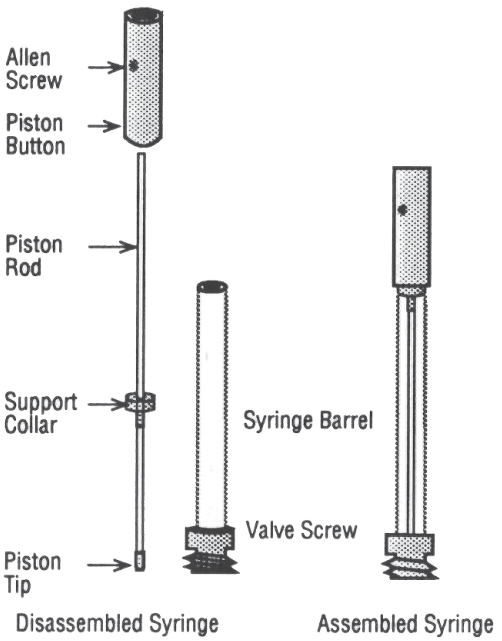


Figure 8.3 - Replacing a 100 µl Syringe

8) Install the support collar by simultaneously pushing and twisting it into the syringe barrel.
9) Push the piston downards until the piston nose reaches the work surface.
10) Fit the piston button by sliding it onto the piston until it rests against the support collar.
11) Tighten the Allen screw on the piston button.
12) Remount the syringe and cover seal as described in Section 3.3.

☞ *When you are pumping viscous liquids, you should prime the syringe by handbefore mounting it on the Autoinjector. This is done by placing the open end of the syringe in the dilutor reservoir liquid and gently pulling the piston button until the syringe is at least half full.*

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6.2 CHANGING THE 100 µL PISTON NOSE

Parts required	
F61162	5 x piston noses, 2 x support collars and mounting tool (for 100 µl pistons).
F62163	5 x piston noses and mounting tool (for 100 µl pistons).

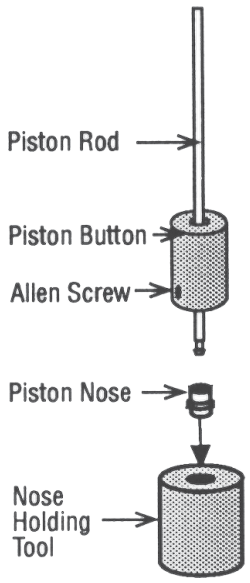


Figure 8.4

1) Remove the piston from the syringe barrel as described in the previous section.
2) Remove the piston nose from the piston rod by carefully pinching the piston nose with a pair of cutters and gently pulling the piston nose off the piston rod. Take care not to damage the end of the piston rod when removing the nose. (It is possible to remove the nose by pinching it between the thumb and forefinger nails and pulling.)
3) Remove any remaining debris of the original piston nose from the end of the rod.
4) The 100 µl pistons have a narrow rod that may be damaged if handled incorrectly. In order to fit a new piston nose without unduly bending the piston rod, slide the button down the piston rod to within 10 mm of the end of the rod where the new piston nose is to be mounted and re-tighten it in position.
5) Put the new piston nose in its recess in the piston nose mounting tool (Figure 8.4). The 100 ml piston noses use a common tool. One face of the tool has a recess for the 100 µl piston nose, the other face has a larger recess that is not used for 234 pistons.
6) Hold the piston button and gently push the end of the piston rod into the new piston nose.
7) Remove the piston assembly from the piston nose holder. Slacken the piston button and slide it away from the piston rod. Reinsert the piston in the syringe barrel as described in Section 8.6.1.
8) Slide the piston up and down in the syringe five or six times to ensure a smooth operation between the piston and syringe.
9) Remount the syringe on the pump as described in Section 3.3.

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MAINTENANCE

6.3 REPLACING THE PISTON FOR A 500 µl SYRINGE

Refer to Figure 8.5:

1) Remove the syringe from the instrument as described in Section 3.3. Remove the cover seal at the same time.
2) Remove the piston from the syringe barrel by pulling on the piston button.
3) Remove the the piston button from the old piston by loosening the Allen screw and pulling on the piston button.
4) Discard the old piston, but keep the piston button.
5) Hold the syringe barrel vertically with the valve screw resting on a clean, flat, work surface.
6) Moisten the nose of the new piston with water or diluent from the pump reservoir and gently insert it into the syringe barrel.
7) Push the piston downwards until the piston nose reaches the work surface.
8) Fit the piston button by sliding it onto the piston until it rests against the syringe barrel.
9) Tighten the Allen screw on the piston button.
10) Remount the syringe as described in Section 3.3, remember to mount the syringe with the cover seal in place.

☞ *When you are pumping viscous liquids, you should prime the syringe by hand before mounting it on the Autoinjector. This is done by placing the open end of the syringe in the dilutor reservoir liquid and gently pulling the piston button until the syringe is at least half full.*

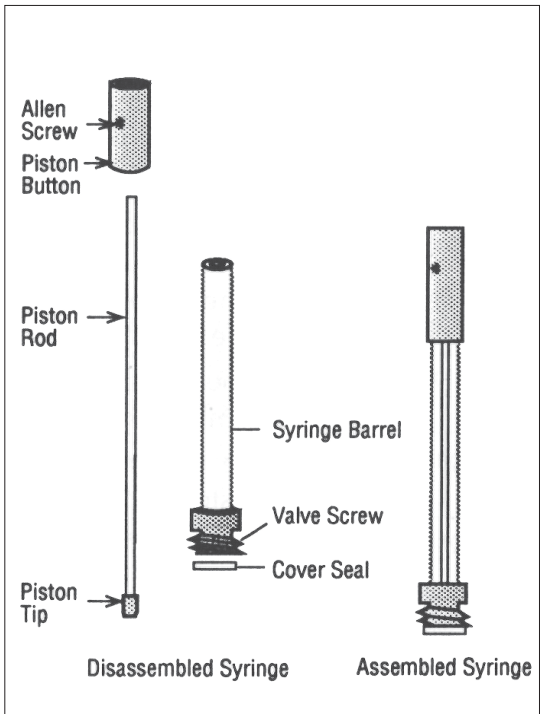


Figure 8.5 - Replacing a 500 µl Syringe

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6.2 CHANGING THE 500 µl PISTON NOSES

Parts required	
F61164	5 x piston noses and mounting tool kit

1) Remove the syringe from the pump as described in Section 3.3.
2) Slide the piston out of the syringe barrel.
3) Remove the piston nose from the piston rod by carefully pinching the piston nose with a pair of cutters and gently pulling the piston nose off the piston rod. Take care not to damage the end of the piston rod when removing the piston nose.
4) Remove any remaining debris of the original nose from the end of the piston rod.
5) Put the new piston nose in its recess in the piston nose holding tool (Figure 8.6).
6) Hold the piston button firmly, gently push the end of the piston rod into the new piston nose until the rod is clipped in the piston nose.
7) Turn the tool upside down and place the piston assembly as in Figure 8.6.
8) Slacken the piston button and remove it from the piston rod. Slide the clamping tool down the piston rod and press the two tools firmly against each other (Figure 8.6).
9) Remove the tools away from the piston assembly.
10) Insert the piston in the syringe barrel as described in Section 8.6.3.
11) Mount the syringe barrel on the valve as described in Section 3.3.

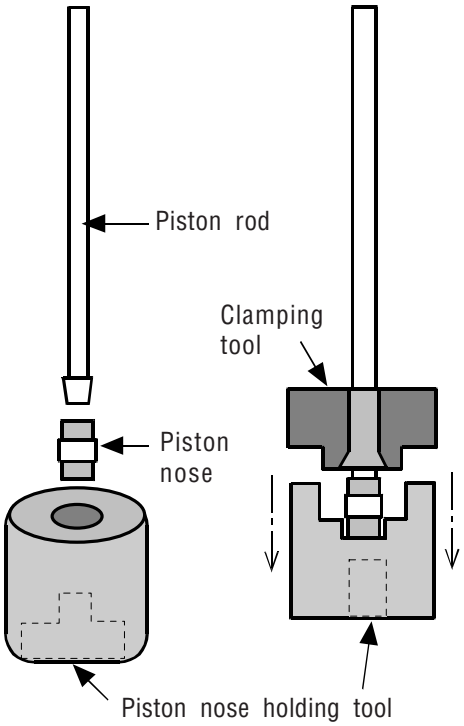


Figure 8.6

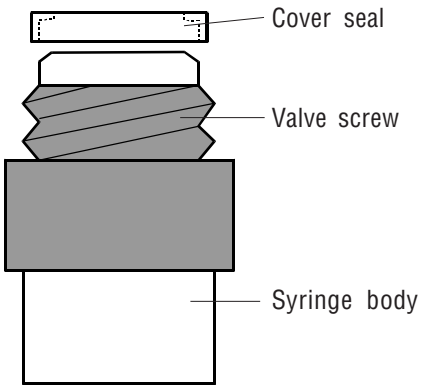


Figure 8.7 - The cover seal for the 500 µl Syringe

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TROUBLESHOOTING

Squeaking or grinding noise in valve. If you hear a grinding noise in the valve, this may be because the ceramic surfaces inside the valve have dried out due to pumping alcohol based substances. Refer to Section 8.3 to clean the valve.

The following error messages may appear when attempting to run a program, when quitting after editing a file, or when a change in the configuration or flow rate settings has been made:

Error Message	Meaning
Flow incompatible with syringe size	One (or more) of the selected flow rates is too great for the configured syringe. The permitted ranges are: • 100 µl syringe 0.1 - 4 ml/min • 500 µl syringe 0.1 - 20 ml/min
Injected vol. exceeds loop vol.	You are not allowed to exceed the loop volume. In centered loop filling mode, the injection volume and 2 x air gap must not exceed the loop volume. In partial loop filling mode, the injection volume must not exceed the loop volume.
Sample vol. exceeds vial vol.	The sample volume used in the method is greater than the vial volume. Refer to table 9.1.
Std. vol. exceeds vial vol.	The standard volume used in the method is greater than the vial volume (used in external and internal standard methods). Refer to table 9.1.
Int. std. vol. exceeds vial vol.	The volume of internal standard to be transferred is greater than the recipient vial volume. Refer to table 9.1.
Sample vol. is too low	The volume of liquid available is less than the volume to be injected.
Std. vol. is too low	The volume of liquid available is less than the volume to be injected. It takes into account the injection volume and number of injections.
Sample vol. exceeds syringe vol.	The maximum volume that can be aspirated is the volume of the syringe, either 100 µl or 500 µl*.
Std. vol. exceeds syringe vol.	The maximum volume that can be aspirated is the volume of the syringe, either 100 µl or 500 µl*.
Sample vol. exceeds tubing vol.	If the syringe volume is greater than the transfer tubing volume (declared in the configuration menu) then the maximum volume that can be aspirated is limited to the tubing volume*.
Std. vol. exceeds tubing vol.	If the syringe volume is greater than the transfer tubing volume (declared in the configuration menu) then the maximum volume that can be aspirated is limited to the tubing volume*.

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Error Message	Meaning
Available Std. vol. is too low	There is insufficient standard liquid for the declared method (volume injected, number of injections per standard, calibration frequency).
Available Int. Std. vol. is too low	There is insufficient internal standard liquid for the declared method (volume added to each vial, number of injections per vial, calibration frequency).
Rack is incompatible with inj. method	This message appears when the method selected is internal standard, and the configuration (rack code) has been changed since the method was written. The present rack code does not allow the original method.
Injected vol exceeds recommended vol.	You are recommended to inject no more than half the actual loop volume for partial or centered loop filling.

☞* *The liquid aspirated from a vial must not reach the pump valve. The volume aspirated (including the air gap) must always be smaller than the syringe or transfer tubing volume, whichever is the smaller.*

The vial volume limits for the sample/standard/priority vials in each rack are:

Table 9.1

The residual volume is the volume of liquid that remains in the vial and cannot be aspirated.

Rack code	Volume limit	Residual volume
30	1700 µl	170 µl
31	700 µl	11 µl
34	4500 µl	350 µl
35	1700 µl	170 µl
36	700 µl	11 µl
37	1700 µl	170 µl
38	700 µl	11 µl

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APPENDIX A - PARTS LISTS

1 STANDARD EQUIPMENT

Reference	Qty	Description
801222	1	234 Autoinjector User's Guide.
Utility Software		
B60105	1	Audit Trail Utility Software.
Pumping module		
E45357	1	FEP™ inlet tubing, 650 x 3 x 2 mm, with a 316L, 20 µm filter.
410153	1	PVDF union connector.
425019	1	FEP inlet tubing, 350 x 3 x 2 mm.
B60115	1	FEP waste tubing, 200 x 4 x 3 mm.
Arm and needle-related items		
501055	3	Self-adhesive tubing clip.
460979	2	Needle, grooved, septum piercing, 316L, 0.4 x 0.7 x 103 mm.
410160	1	Fitting for needle to transfer tubing connection.
B60061	1	Spare injection port seal, PTFE and elastomer.
Sample transfer and rinsing tubing		
B60086	1	Transfer tubing, FEP, coiled, 550 µl (0.8 mm i.d.).
B60116	1	Rinsing station tubing, Isoversinic®, 1000 x 6.5 x 4 mm.
Injection valve tubing and accessories		
430033	1	Tubing, 316L, 48.5 x 1.6 x 0.25 mm, for column to valve port 2 connection.
430021	1	Tubing, 316L, 100 x 1.6 x 0.25 mm, for column to valve port 2 connection.
430035	1	Tubing, 316L, 500 x 1.6 x 0.5 mm, for purge valve to valve port 3 connection.
430019	1	Tubing, 316L, 200 x 1.6 x 0.5 mm, for sample drain tubing to valve port 5 connection.
410184	1	Set of Rheodyne accessories for 7010 valve.
Mechanics		
610101	1	Double-ended wrench, 1/4"-5/16", for tubing to injection valve connection.
610020	2	Double-ended wrench, 10/12 mm for dismounting injection port.
610043	1	Allen wrench, 3 mm, for injection valve removal.
202315	1	Arm fixing screw.
361050	1	Plastic plug to cover arm fixing screw.
Sample rack and vials		
B60103	1	Rack code 36, polypropylene, for 120, 0.7 ml vials, and one 20 ml vial.
450830	1	Box of 1000 sample micro vials, polypropylene, 0.7 ml, 7 x 41 mm without cap.
450831	1	Box of 1000 polyethylene caps for 0.7 ml micro vials.
450922	1	Reagent vials (20 ml) and polyethylene caps, box of 10 of each.
B60113	1	Zone mask for rack code 36, polycarbonate.

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Reference	Qty	Description
Electrical		
500005	1	Power cord for 220-240 V.
502132	5	Fuses 3.15 Amp type “T” slow blow.
500006	1	Power cord for 100-120 V.
506406	1	Terminal block connector 12-pin.
500944	1	Cable, 4 wire, 1.7 m.

2 GILSON RACKS AND RACK ACCESSORIES

Gilson racks				
Reference	Code	Material	Max. nb of samples (including priority)	Capacity and type of vials
B49460 B49465	30 30	Al PP	60 60	2 ml glass or plastic vials, 12 x 32 mm 2 ml glass or plastic vials, 12 x 32 mm
B49461 B49466	31 31	Al PP	108 108	0.7 ml microvial, 7 x 41 mm 0.7 ml microvial, 7 x 41 mm
B49464 B49468	34 34	Al PP	36 36	4 ml vial, 15 x 45 mm 4 ml vial, 15 x 45 mm
B60102	35	PP	71 samples + 1 reagent	2 ml glass or plastic vials, 12 x 32 mm one 20 ml scintillation vial, 28 x 60 mm
B60103	36	PP	120 samples + 1 reagent	0.7 ml microvial, 7 x 40 mm one 20 ml scintillation vial, 28 x 60 mm
B60092	37	Al	56 samples + 1 reagent	2 ml glass or plastic vials, 12 x 32 mm one 20 ml scintillation vial, 28 x 60 mm
B60093	38	Al	96 samples + 1 reagent	0.7 ml microvial, 7 x 40 mm one 20 ml scintillation vial, 28 x 60 mm

☞* *PP = Polypropylene, Al = Aluminum*

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APPENDIX A - PARTS LISTS

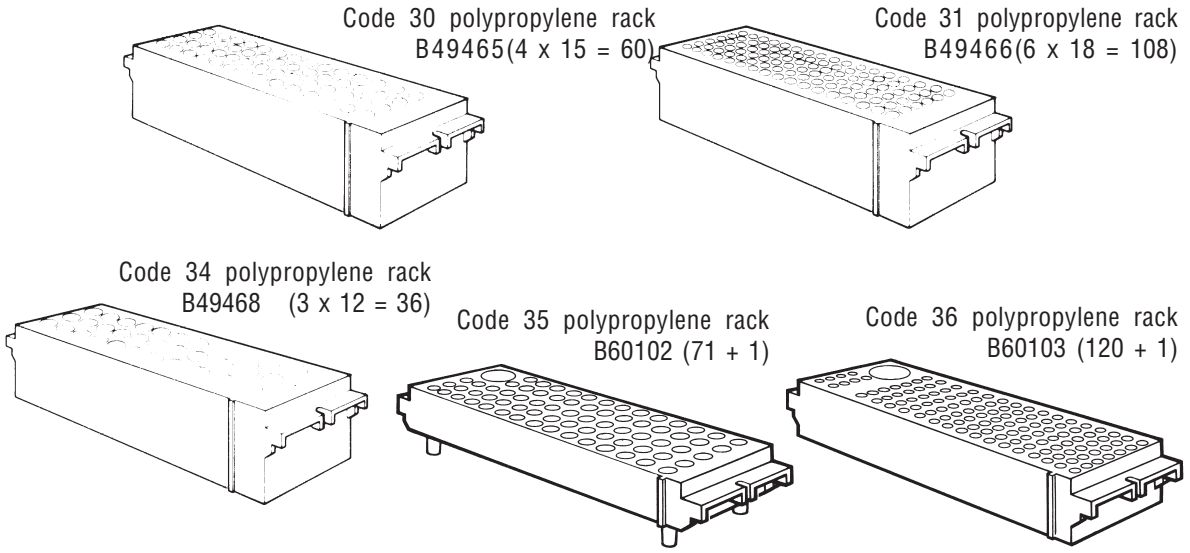


Figure A.1 - Polypropylene racks

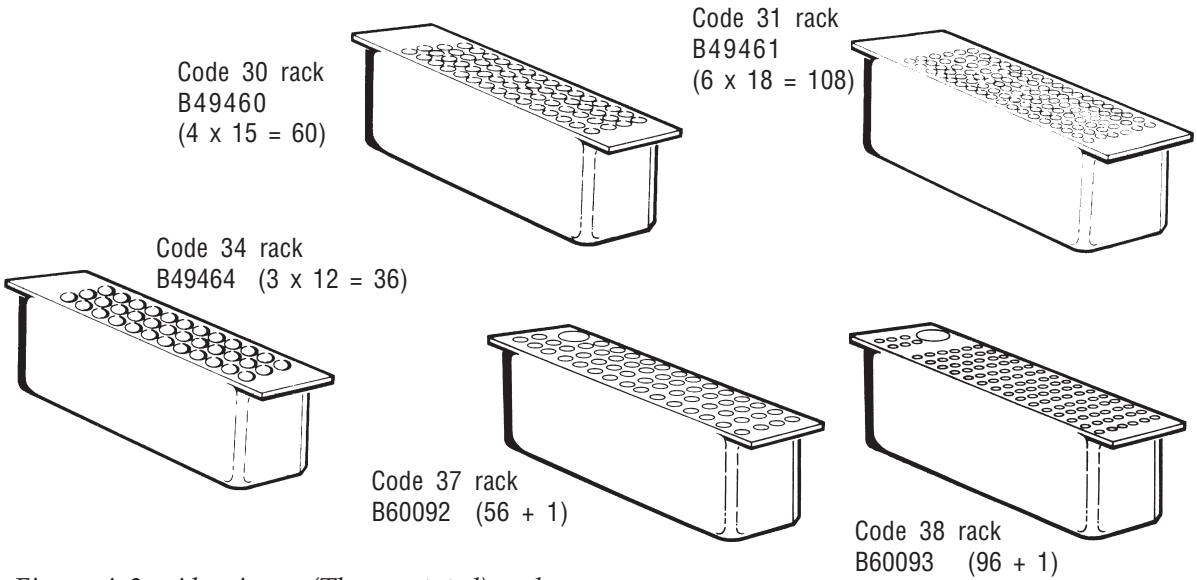


Figure A.2 - Aluminum (Thermostated) racks

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Sample vials, caps and seals	
Reference	Description
450806	Box of 500, 2 ml vials (Chromacol 2-SVA), 12 x 32 mm, amber borosilicate, flat bottom and screw top. To be used in Gilson code 30, 35 and 37 racks.
450807	Box of 500 screw caps (Chromacol 8-SC) for 2 ml vials, black, central hole.
450809	Box of 500, 0.7 ml vials (Chromacol 07-CPVA), 7 x 41 mm, round bottom, crimp top. To be used in Gilson code 31, 36 and 38 racks.
450811	Box of 1000 crimp caps (Chromacol 8-ACB) for 0.7 ml vials, lacquered aluminum, without seal.
450813	Crimper (Chromacol CR-8) for 0.7 ml vials.
450814	De-capper (Chromacol DCR-8) for 0.7 ml vials.
450821	Box of 1000 septum seals (Chromacol 8-TST1) for 2 ml vials, 7 mm diameter, 1 mm thickness, PTFE-silicone rubber-PTFE sandwich.
450822	Box of 1000 non self- sealing PTFE discs (Chromacol 7-T02), for 2 ml vials, 7 mm diameter, 0.2 mm thickness
450823	Box of 100, 20 ml scintillation vials, 28 x 60 mm, flat bottom and screw top.
450824	Box of 100 self-sealing and piercing polypropylene caps for 20 ml scintillation vials.
450830	Box of 1000 sample microvials, polypropylene, 0.8 ml, 7 x 41 mm without cap. To be used in Gilson code 31, 36 and 38 racks.
450831	Box of 1000 polyethylene caps for 0.8 ml microvials.
450832	Pack of 10 boxes of 1000 sample microvials, polypropylene, 0.8 ml, 7 x 41 mm without cap.
450833	Pack of 10 boxes of 1000 polyethylene caps for 0.8 ml microvials.

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3 OTHER ACCESSORIES

Reference	Description
B49445 A59550	Thermostating cuvette for connection with a cryothermostat. Thermostating cuvette for 832 Temperature Regulator.
A59501 A59502	832 Temperature Regulator, 115 V. 832 Temperature Regulator, 220 V.
B49490 B49491 B49493 B60094 B60095	Cover for code 30Al rack, stainless steel. Cover for code 31Al rack, stainless steel. Cover for code 34Al rack, stainless steel. Cover for code 37Al rack, stainless steel. Cover for code 38Al rack, stainless steel.
B60112 B60113	Zone mask for rack code 35, polycarbonate. Zone mask for rack code 36, polycarbonate.
425050	Transfer tubing, FEP, 120 µl, 600 x 1.6 x 0.5 mm.
500933 A47090	GSIOC cable. 605 GSIOC - RS232 Adapter.
F62182 F62184	0.1 ml syringe for dilutor. 0.5 ml syringe for dilutor.
460082	Needle 51 x 0.7 x 0.4 mm, female luer and softened squared tip, for manual sample loading.
B60067 B60062 410257 410258	Calibrated tubing, PEEK, 52 x 1.6 x 0.25 mm. Calibrated tubing, PEEK, 52 x 1.6 x 0.25 mm, fitted with hexagonal nuts and ferrules. Hexagonal nut, PEEK, for standard 1/16", 10/32 fitting. Ferrule, PEEK, 1/16" hole.

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APPENDIX B - RACKS AND ZONES

You can use the following racks with 234: 30, 31, 34, 35, 36, 37 and 38.

The following Sections describe how each rack is divided into sub-zones depending on the method used. The sub-zones can only be modified by stating the number of vials within the sub-zone. For example the external standard sub-zone may contain 1 to 9 vials. Not all methods are allowed for certain racks. Rack codes 30, 31, and 34 can't use the internal standard method.

The number and position of the priority vial(s) depend on the rack used and on the method implied. The priority vials contain the same volume as the sample vials in a rack.

1 RACK CODE 30

Number of sample vials available: 60 maximum
Volume per sample vial: 1700 µl
Number of standard vials: Up to 9
Volume per standard vial: 1700 µl
Number of allowed priority vials: 1
Volume per priority vial: 1700 µl
No internal standard vial.
Methods allowed: Simple injection-External standard with or without dilution

♦ RACK CODE 30 - SIMPLE INJECTION METHOD

Refer to Figure B.1. The entire rack is taken as one zone, the sample zone. When you run a program, you can inject up to 60 samples. If a priority sample is used, it must be placed in vial position 1.

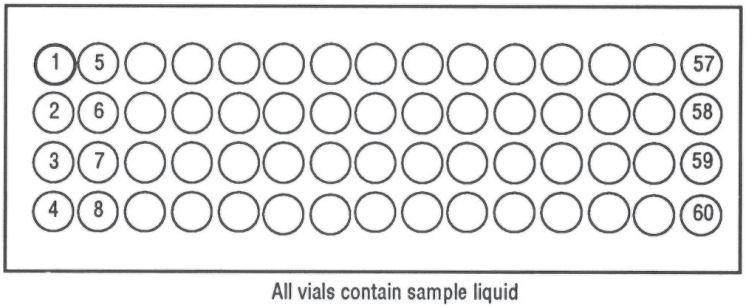


Figure B.1 - Rack code 30, Simple Injection Method

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APPENDIX B - RACKS AND ZONES

RACK CODE 30 - EXTERNAL STANDARD WITHOUT DILUTION

You can allocate up to nine vials to contain standards. The example shown in Fig. B.2 has three standards. These vials occupy the first vial positions in the rack. The remaining vials in the rack (57 in this example) can be used for sample vials. The sample zone and the standard zone must be contiguous. If a priority sample is used, it must be placed in the first sample vial position (in this example, vial position 4).

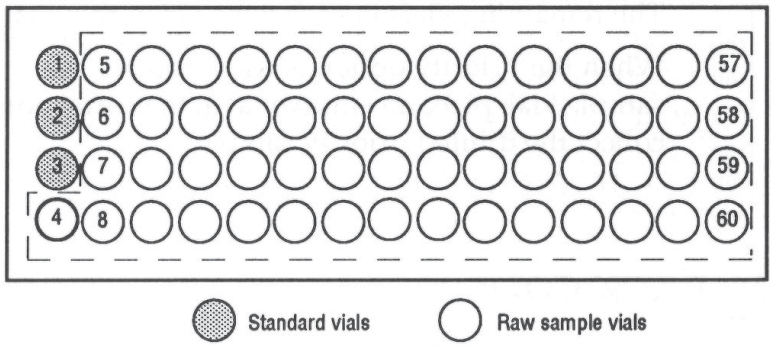


Figure B.2 - Rack Code 30, External Standard Method

RACK CODE 30 - EXTERNAL STANDARD WITH DILUTION

You may dilute samples only, standards only, or both. The rack is divided into two equal zones: the raw standard and sample zone and the diluted standard and sample zone. The last column of vials is unused. The maximum number of vials available in each zone is 28.

You can allocate up to nine standard vials. The example shown in Figure B.3

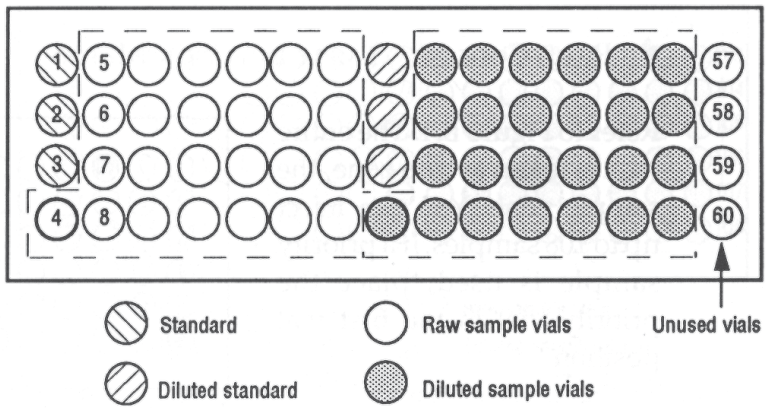


Figure B.3 - Rack Code 30, External Standard with Dilution

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has three standards. These vials occupy the first vial positions in the rack; the vials for the diluted standard occupy the first three vial positions in the second half of the rack. The remaining vials in each zone (25 in this example) are reserved for sample vials.

When the Priority option is used, replace the **first raw sample vial** with the priority sample vial, place an empty vial in the **corresponding diluted sample vial** position to collect the diluted priority sample.

2 RACK CODE 31

Number of sample vials available: 108 maximum
Volume per sample vial: 700 µl
Number of standard vials: Up to 9
Volume per standard vial: 700 µl
Number of allowed priority vials: 1
Volume per priority vial: 700 µl
No internal standard vial.
Methods allowed: Simple injection-External standard with/without dilution

♦ RACK CODE 31 - SIMPLE INJECTION METHOD

Refer to Figure B.4. The entire rack is taken as one zone, the sample zone. You can inject up to 108 samples. If a priority sample is used, place the priority vial in the first vial position.

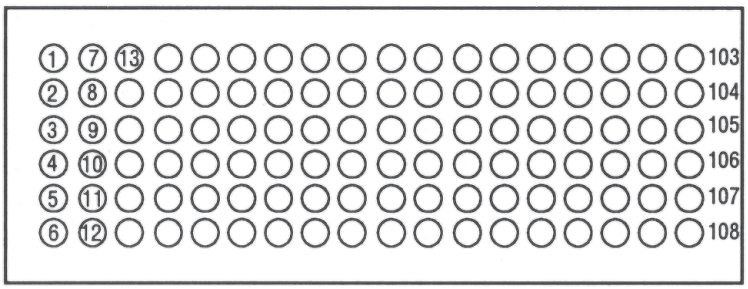


Figure B.4 - Rack code 31

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APPENDIX B - RACKS AND ZONES

♦ RACK CODE 31 - EXTERNAL STANDARD WITHOUT DILUTION

You can allocate up to nine standards. The example shown in Figure B.5 has four standards. These vials occupy the first vial positions in the rack. The remaining vials in the rack (104 in this example) are reserved for sample vials.

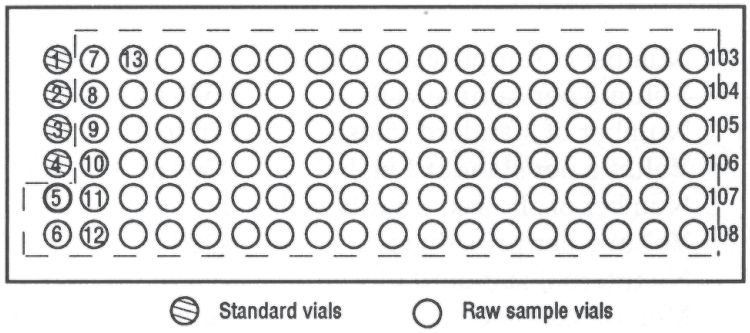


Figure B.5 - Rack Code 31, External Standard Method

When the Priority option is used, replace the **first sample vial** with the priority sample vial.

♦ RACK CODE 31 - EXTERNAL STANDARD WITH DILUTION

The rack is divided into two equal zones; the raw standard and sample zone and the diluted standard and sample zone. The maximum number of vials available in each zone is 54.

You can allocate up to nine standard vials. The example shown in Figure B.6 has four standards. These vials occupy the first vial positions in the rack; the vials for the diluted standard occupy the first four vial positions in the second half of the rack. The remaining vials in each zone (50 in this example) are reserved for sample vials.

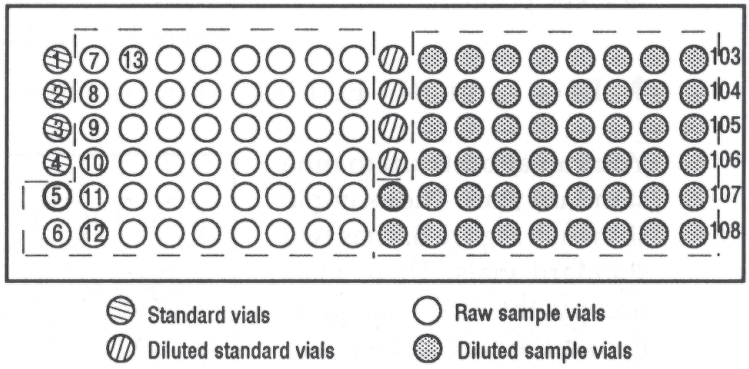


Figure B.6 - Rack Code 31, External Standard with Dilution

When the Priority option is used, replace the **first raw sample vial** with the priority sample vial, place an empty vial in the **corresponding diluted sample vial** position to collect the diluted priority sample.

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3 RACK CODE 34

Number of sample vials available:	36 maximum
Volume per sample vial:	4500 µl
Number of standard vials:	Up to 9
Volume per standard vial:	4500 µl
Number of allowed priority vials:	1
Volume per priority vial:	4500 µl
No internal standard vial.	
Methods allowed:	Simple injection-External standard with/without dilution

♦ RACK CODE 34 - SIMPLE INJECTION METHOD

Refer to Figure B.7. The entire rack is taken as one zone, the sample zone. The maximum number of sample injections available is 36. If a priority sample is used, the priority sample must be placed in the first vial position.

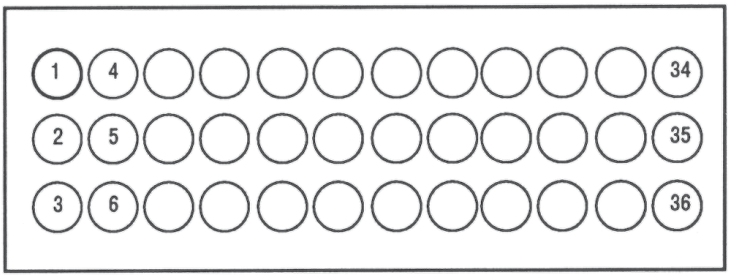


Figure B.7 - Rack Code 34

♦ RACK CODE 34 - EXTERNAL STANDARD WITHOUT DILUTION

You can allocate up to nine standard vials. The example shown in Figure B.8 has two standard vials. These vials occupy the first vial positions in the rack. The remaining vials in the rack (34 in this example) are reserved for sample vials.

When the Priority option is used, replace the **first sample vial** with the priority sample vial.

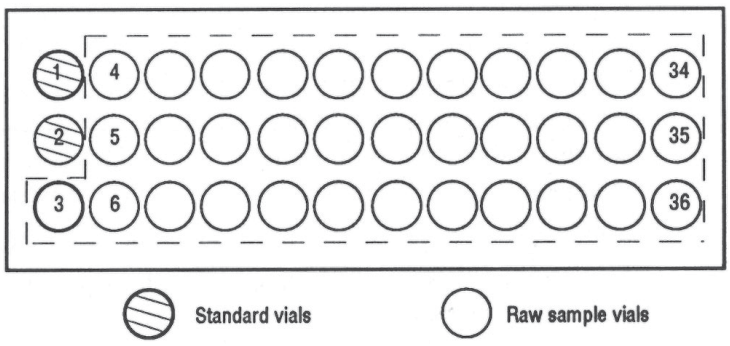


Figure B.8 - Rack Code 34, External Standard Method

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APPENDIX B - RACKS AND ZONES

♦ RACK CODE 34 - EXTERNAL STANDARD WITH DILUTION

The rack is divided into two equal halves; the raw standard and raw sample zone, and the diluted standard and diluted sample zone. The maximum number of vials available in each zone is 18.

You can allocate up to nine standard vials. The example shown in Figure B.9 has two standards. These vials occupy the first vial positions in the rack; the vials for the diluted standard occupy the first two vial positions in the second half of the rack. The remaining vials in each zone (16 in this example) are reserved for sample vials.

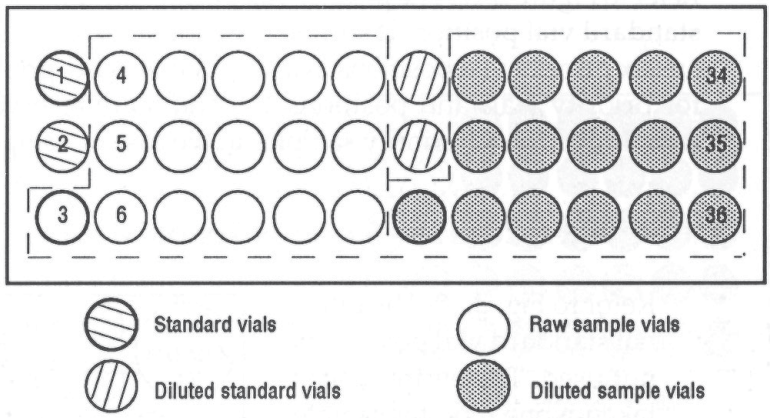


Figure B.9 - Rack Code 34, External Standard with Dilution

When the Priority option is used, replace the **first raw sample vial** with the priority sample vial, place an empty vial in the **corresponding diluted sample vial** position to collect the diluted priority sample.

4 RACK CODE 35

Number of sample vials available:	65 maximum
Volume per sample vial:	1700 µl
Number of standard vials:	Up to 9
Volume per standard vial:	1700 µl
Number of allowed priority vials:	Up to 6 (separate zone from sample vial area)
Volume per priority vial:	1700 µl
Volume of internal standard vial:	20 ml
Methods allowed:	Simple injection External standard with/without dilution Internal standard with/without dilution

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Refer to Figure B.10. The priority vial positions, numbered 1 to 6 are situated in front of the internal standard vial position. If simple injection or external standard without dilution is used, there are six priority vial positions available. Otherwise, priority vial positions 1, 2 and 3 are reserved for priority vials and positions 4, 5 and 6 are reserved for collecting either diluted priority samples, or mixed priority samples in the case of using the internal standard method.

♦ RACK CODE 35 - SIMPLE INJECTION METHOD

Refer to Fig. B.10. The internal standard vial position is not used. The entire rack is taken as one zone, the sample zone. The maximum number of sample injections is 65.

When the Priority option is used, place priority sample vial in any of the six available positions in the priority vial area.

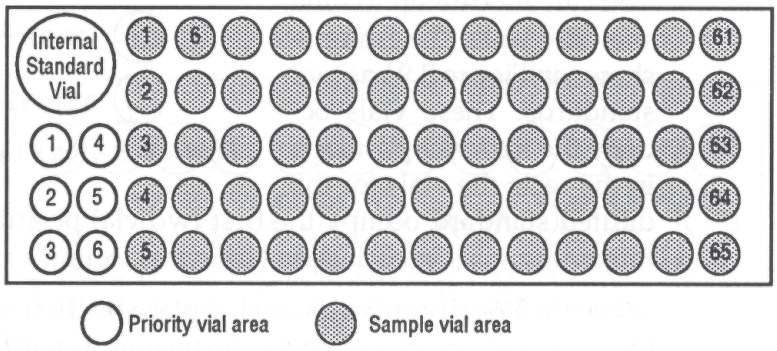


Figure B.10 - Rack Code 35

♦ RACK CODE 35 - EXTERNAL STANDARD WITHOUT DILUTION

You can allocate up to nine vials to contain the standard(s). The example shown in Figure B.11 has three standard vials. These vials occupy the first vial positions in the main vial area. The remaining vials in the rack (62 in this example) are reserved for sample vials.

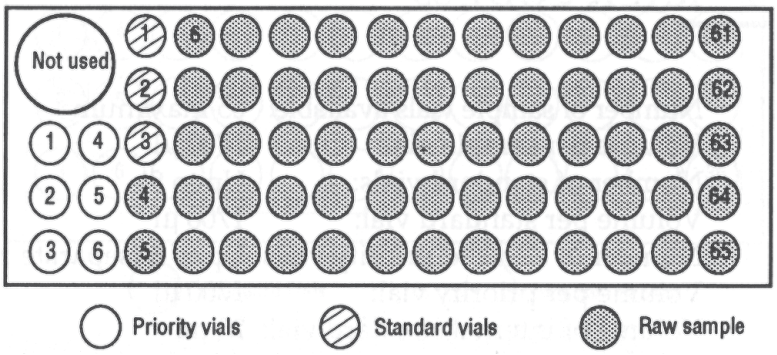


Figure B.11 - Rack Code 35, External Standard Method

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APPENDIX B - RACKS AND ZONES

When the Priority option is used, place priority sample vial in any of the six available positions in the priority vial area.

♦ RACK CODE 35 - EXTERNAL STANDARD WITH DILUTION OR INTERNAL STANDARD WITH OR WITHOUT DILUTION

The rack is divided into two equal main zones; the raw standard and raw sample zone, and the prepared standard and prepared sample zone. The last column of vials is unused. The maximum number of vials available in each zone is 30.

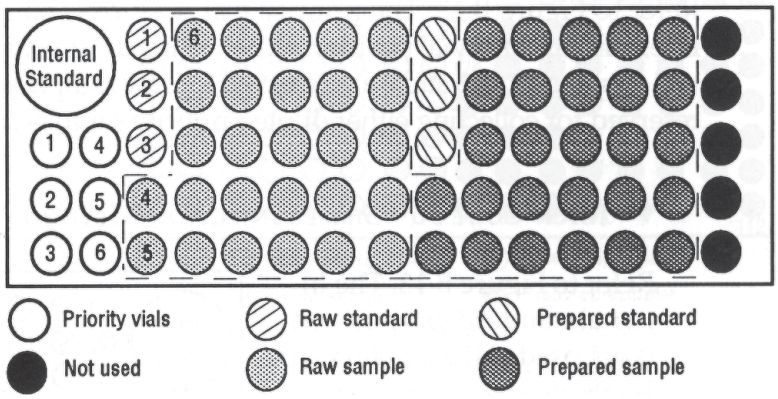


Figure B.12 - Rack Code 35

You can allocate up to nine standard vials. The example shown in Figure B.12 has three standard vials. These vials occupy the first vial positions in the rack; the vials for the prepared standard occupy the first three vial positions in the second half of the rack. The remaining vials in each zone (27 in this example) are reserved for sample vials.

When the Priority option is used, place the priority sample vial in position 1 in the priority zone, place an empty vial in vial position 4 in the priority zone to collect the prepared priority sample. For further priority samples, place the second priority vial in position 2 and the collection vial in position 5, place a third priority vial in position 3 and its collection vial in position 6.

5 RACK CODE 36

Number of sample vials available:	112 maximum
Volume per sample vial:	700 µl
Number of standard vials:	Up to 9
Volume per standard vial:	700 µl

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Number of allowed priority vials:	Up to 8 (separate zone from sample vial area)
Volume per priority vial:	700 µl
Volume of internal standard vial:	20 ml
Methods allowed:	Simple injection
	External standard with/without dilution
	Internal standard with/without dilution

Refer to Figure B.13. The priority vial positions, numbered 1 to 8 are situated in front of the internal standard vial position. When using dilution or internal standard methods, the priority vial positions 1, 2, 3 and 4 are reserved for raw sample priority vials and positions 5, 6, 7 and 8 are reserved for collecting either diluted priority samples, or mixed priority samples.

♦ RACK CODE 36 - SIMPLE INJECTION METHOD

Refer to Figure B.13. The Internal standard vial position is not used. All the main vial area is used for sample vials. The maximum number of sample vials available is 112.

When the Priority option is used, place priority sample vial in any of the eight available positions in the priority vial area.

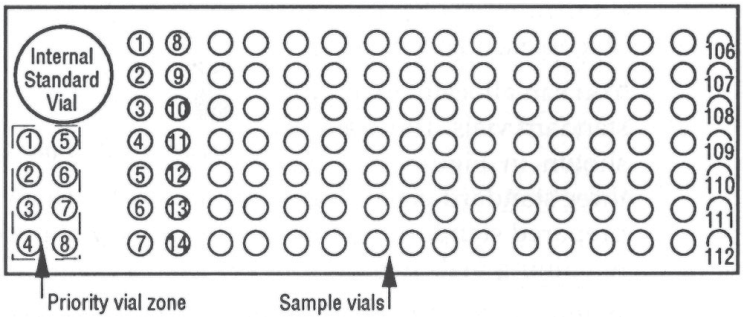


Figure B.13 - Rack Code 36

♦ RACK CODE 36 - EXTERNAL STANDARD WITHOUT DILUTION

You can allocate up to nine standard vials. The example shown in Figure B.14 has three standards. These vials occupy the first vial positions in the main vial area. The remaining vials in the rack (109 in this example) are reserved for sample vials.

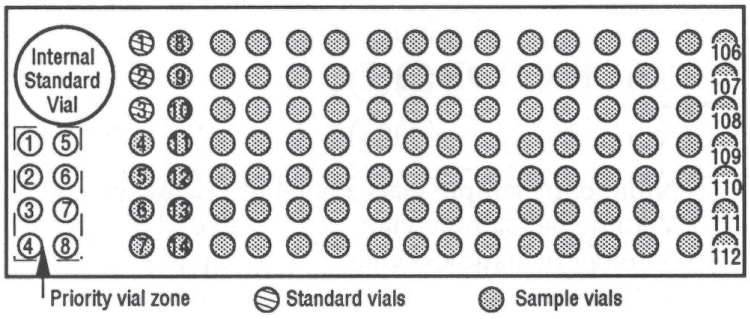


Figure B.14 - Rack Code 36, External Standard Method

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APPENDIX B - RACKS AND ZONES

There are 8 available priority vial positions.

♦ RACK CODE 36 - EXTERNAL STANDARD WITH DILUTION OR INTERNAL STANDARD WITH OR WITHOUT DILUTION

The rack is divided into two equal main zones; the raw standard and raw sample zone, and the prepared standard and prepared sample zone. The maximum number of vials available in each zone is 56.

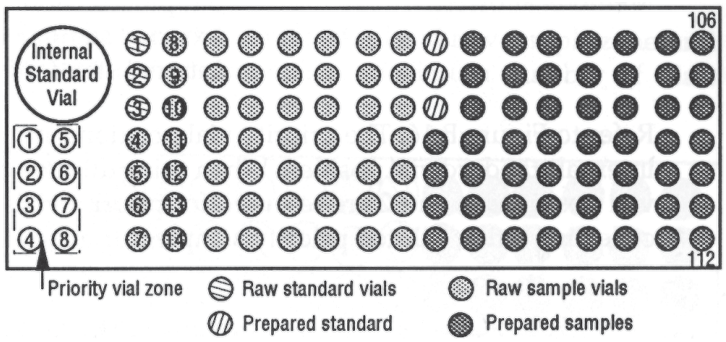


Figure B.15 - Rack Code 36

You can allocate up to nine standard vials. The example shown in Figure B.15 has three standards. These vials occupy the first vial positions in the rack; the vials for the prepared standard occupy the first three vial positions in the second half of the rack. The remaining vials in each zone (53 in this example) are reserved for sample vials.

When the Priority option is used, place the priority sample vial in position 1 in the priority zone, place an empty result vial in vial position 5 in the priority zone to collect the prepared priority sample. For further priority samples, place the second priority vial in position 2 and the result vial in position 6, place a third priority vial in position 3 and its result vial in position 7, place the fourth priority vial in position 4 and its corresponding result vial in position 8.

6 RACK CODE 37

Thermostating rack, can be used with the hydraulic thermostating cuvette or the Peltier Effect thermostating cuvette and the 832 Temperature Regulator.

Number of sample vials available: 52 maximum
Volume per sample vial: 1700 µl

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Number of standard vials:	Up to 9
Volume per standard vial:	1700 µl
Number of allowed priority vials:	Up to 4 (separate zone from sample vial area)
Volume per priority vial:	1700 µl
Volume of internal standard vial:	20 ml
Methods allowed:	Simple injection
	External standard with/without dilution
	Internal standard with/without dilution

Refer to Figure B.16. The priority vial positions, numbered 1 to 4 are situated in front of the internal standard vial position. When using dilution or internal standard method, the priority vial positions 1, and 2 are reserved for priority vials, and positions 3 and 4 are reserved for collecting either diluted priority samples, or mixed priority samples.

♦ RACK CODE 37 - SIMPLE INJECTION METHOD

Refer to Figure B.16. The Internal standard vial position is not used. All the main vial area is used for sample vials. The maximum number of sample vials available is 52.

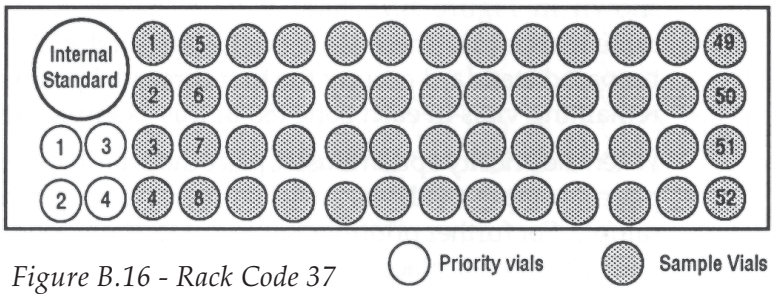


Figure B.16 - Rack Code 37

♦ RACK CODE 37 - EXTERNAL STANDARD WITHOUT DILUTION

You can allocate up to nine standard vials. The example shown in Figure B.17 has three standards. These vials occupy the first vial positions in the main vial area. The remaining vials in the rack (49 in this example) are reserved for sample vials.

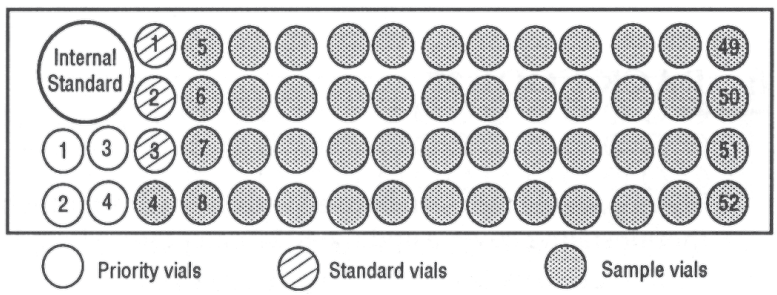


Figure B.17 - Rack Code 37, External Standard Method

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APPENDIX B - RACKS AND ZONES

There are 4 available priority vial positions.

♦ RACK CODE 37 - EXTERNAL STANDARD WITH DILUTION OR INTERNAL STANDARD WITH OR WITHOUT DILUTION

The rack is divided into two equal main zones; the raw standard and raw sample zone, and the prepared standard and prepared sample zone. The maximum number of vials available in each zone is 24.

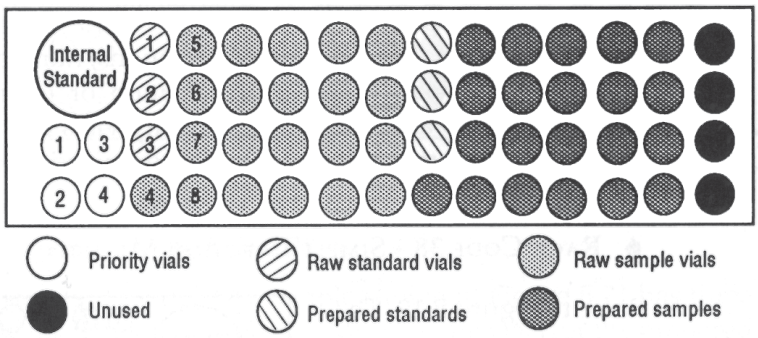


Figure B.18 - Rack Code 37

You can allocate up to nine standard vials. The example shown in Figure B.18 has three standards. These vials occupy the first vial positions in the rack; the vials for the prepared standard occupy the first three vial positions in the second half of the rack. The remaining vials in each zone (21 in this example) are reserved for sample vials.

When the Priority option is used, place the priority sample vial in position 1 in the priority zone, place an empty result vial in vial position 3 in the priority zone to collect the prepared priority sample. For a second priority sample, place the second priority vial in position 2 and the result vial in position 4.

7 RACK CODE 38

Thermostating rack, can be used with a hydraulic thermostating cuvette or with the peltier effect thermostating cuvette and the 832 Temperature Regulator.

Number of sample vials available: 90 maximum
Volume per sample vial: 700 µl

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Number of standard vials:	Up to 9
Volume per standard vial:	700 µl
Number of allowed priority vials:	Up to 6 (separate zone from sample vial area)
Volume per priority vial:	700 µl
Volume of internal standard vial:	20 ml
Methods allowed:	Simple injection
	External standard with/without dilution
	Internal standard with/without dilution

Refer to Figure B.19. The priority vial positions, numbered 1 to 6 are situated in front of the internal standard vial. When using dilution or internal standard method, priority vial positions 1, 2 and 3 are reserved for priority vials and positions 4, 5 and 6 are reserved for collecting either diluted priority samples, or mixed priority samples.

♦ RACK CODE 38 - SIMPLE INJECTION METHOD

Refer to Figure B.19. The Internal standard vial position is not used. All the main vial area is used for sample vials. The maximum number of sample vials available is 90.

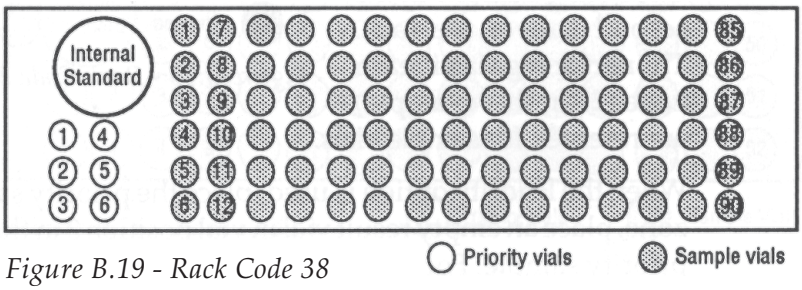


Figure B.19 - Rack Code 38

♦ RACK CODE 38 - EXTERNAL STANDARD WITHOUT DILUTION

You can allocate up to nine standard vials. The example shown in Figure B.20 has three standards. These vials occupy the first vial positions in the main vial area. The remaining vials in the rack (87 in this example)

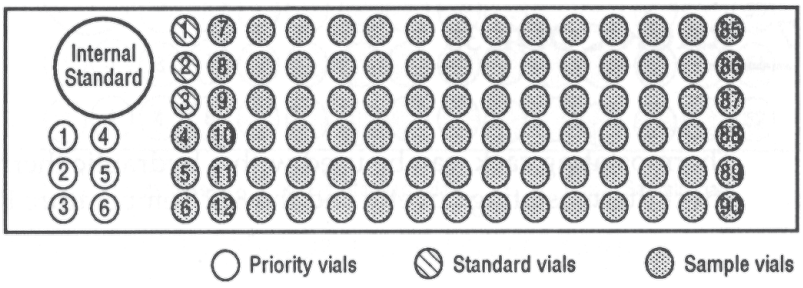


Figure B.20 - Rack Code 38, External Standard Method

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APPENDIX B - RACKS AND ZONES

are reserved for sample vials.

There are 6 available priority vial positions.

♦ RACK CODE 38 - EXTERNAL STANDARD WITH DILUTION OR INTERNAL STANDARD WITH OR WITHOUT DILUTION

The rack is divided into two equal main zones; the raw standard and raw sample zone, and the prepared standard and prepared sample zone. The last row of vials is not used. The maximum number of vials available in each zone is 42.

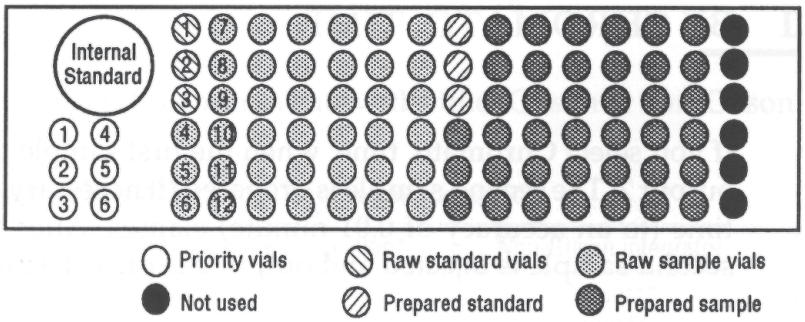


Figure B.21 - Rack Code 38

You can allocate up to nine standard vials. The example shown in Figure B.21 has three standards. These vials occupy the first vial positions in the rack; the vials for the prepared standard occupy the first three vial positions in the second half of the rack. The remaining vials in each zone (39 in this example) are reserved for sample vials.

When the Priority option is used, place the priority sample vial in position 1 in the priority zone, place an empty result vial in vial position 4 in the priority zone to collect the prepared priority sample. For a second priority sample, place the second priority vial in position 2 and the result vial in position 5.

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APPENDIX C - COORDINATION

You can select two methods for coordinating your Autoinjector with other instruments: coordination by chromatogram time or external coordination. The choice of coordination depends on your system requirements. You can also control the Autoinjector using GSIOC commands, explained in Section 3 of this Appendix.

1 BY CHROMATO. TIME

If you select Chromato. time, when the first sample has been injected, a pulse is sent on output 2. The second sample is prepared (if necessary) whilst the specified chromatogram time (to an accuracy of 0.01 minute) elapses. After the specified time has elapsed, the second sample is injected and output 2 is pulsed again, and so on.

The chromatogram time should take into account the analysis time and the time taken to print the report. The maximum time allowed for the chromatography time and priority sample chromatography time is 600.00 minutes.

Output 2 of the Autoinjector is used for coordinating normal sample injections. Refer to Figure C.1. The common (c) contact is connected to the normally open (no) contact for 1.8 seconds. You can change the duration of the output pulse by using the advanced user menus, described in Section 4.10.

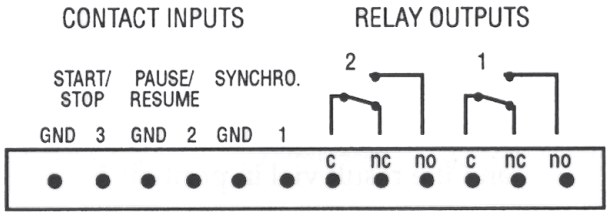


Figure C.1 - Autoinjector Output Connections

Connect output 2 of the Autoinjector to the start input of your integrator.

Priority Samples

For priority samples, output 1 is pulsed.

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APPENDIX C - COORDINATION

2 BY EXTERNAL COORDINATION

You can use 712 or 715 software to coordinate the Autoinjector by sending GSIOC commands or by operating contacts on the 506B or 506C interface. The Autoinjector automatically accepts and recognizes the coordination signal, whether it is a contact event or a GSIOC command.

♦ CONTROL USING CONTACT EVENTS

You can use coordination by contacts to communicate with either Gilson or non-Gilson data collection systems.

When coordinating normal sample/calibration injections, connect:

Autoinjector connection	Gilson 506B/506C interface connection*	Non-Gilson integrator
Input 1 Output 2	Output 2 Input B	Output** Input**

☞ ** The PC should be connected to a Gilson 506B or 506C interface using RS-232C cable.*

***Refer to your integrator User's Guide for details of the output and input connections to make. The output connection from the integrator should give the 'ready to collect data' output signal, and the input should be the 'start data collection' input.*

When running the system controller software, select the Edit menu from the Menu Bar. Click on the **Contact Events** option. You must then write the commands that enable the software to coordinate with the Autoinjector via the interface. The interface must be set as the data collection unit.

♦ WRITING THE 712/715 COORDINATION COMMANDS

In the events table presented, enter the following:

Time	Contact	Action	Description
0.00	2	CLOSE	Authorizes LOAD into the injection loop
0.03	B	WAIT	Wait for 234 to be READY to inject
0.04	2	OPEN	Authorization for 234 to INJECT

Coordination is carried out by a handshake signal on output 2 from the Autoinjector.

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Priority Samples

For priority samples, you need to connect to a second integrator. The Autoinjector gives the output signal on relay output 1.

3 CONTROL USING GSIOC COMMANDS

♦ CONNECTIONS

Connect the PC to a Gilson 506B or 506C interface by RS-232C cable. Connect the Autoinjector to the interface using GSIOC cable.

♦ COORDINATING THE INSTRUMENTS

When running the system controller software, select the Edit menu from the Menu Bar and click on the Gilson Instruments option.

Select the instrument to be controlled as "Other GSIOC Device", enter the Autoinjector ID number as 10 in the ID box.

In the events table presented, enter the following:

Time	Unit ID	Command Type	Command*	Description
0.01	10	I	?R	Waits for the 234 file to start
0.02	10	B	HL	Authorization to transfer and LOAD
0.03	10	B	W0	Read top line message on 234
0.05	10	I	H1	Wait for "ready to inject"
0.06	10	B	HI	Authorization to INJECT
0.07	10	I	W>0	Write the (234) message on the 715 report.

☞ ** Respect the Syntax of the commands (Capital letters, not lowercase).*

If the 234 is stopped while the 715 is waiting for the 234, when the 234 is restarted, both the 234 and the 715 will wait for each other. It is necessary to stop the 715 and restart it.

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APPENDIX C - COORDINATION

TRANSMISSION OF SAMPLE IDENTIFICATION

It is possible to obtain information concerning the injections that have taken place.

When the 234 Autoinjector is part of a non-Gilson HPLC system, a Gilson 605 interface is used to transmit sample identification information to the computer doing the data handling, in order to incorporate Sample ID information in the chromatographic report.

The transmitted sample ID information consists of GSIOC responses that indicate:

- Number of injections performed (since start of application).
- Liquid type (sample/standard/priority) and sample tube number of the latest injection.
- Actual volume injected (for the latest injection).

When the next injection takes place, the information is updated.

These data are sent out of the GSIOC port of the Autoinjector.

Three GSIOC immediate commands have been created so that this information can be obtained.

Refer to the following GSIOC description for full details of these and all GSIOC commands for the Autoinjector.

Command	Type	Description
a	I	Read injection number
b	I	Read last injection status
v	I	Read last injected

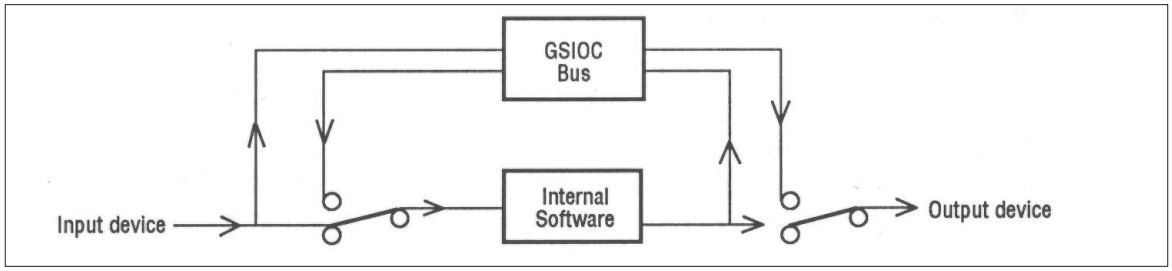
GSIOC COMMANDS

All M234 GSIOC commands are detailed below with their type and function. Immediate commands have their response format described. Buffered command parameters are documented.

M234 peripheral devices can be either processed by the internal software or by the GSIOC bus. There are two devices types: input devices and output devices.

A kind of software switch (actually a dedicated GSIOC command) disconnects and reconnects each input/output device from the internal software, as illustrated below:

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Commands summary

Command	Type*	Description
%	I	Request module identification
$	I	Master reset
?	I	M234 status
a	I	Read injection number
A	B	Syringe motor down stroke
b	I	Read last injection status
B	I	Read file directory
C	I	Syringe motor status
D	B	Syringe motor up stroke
E	B	Assigns a value to a parameter
E	I	Read value of parameter
F	B	Set syringe flow rate
F	I	Read syringe flow rate
G	B	Pump valve control
G	I	Pump valve status
H	B	Coordination flag
H	I	Injection Flag
I	B	Write contact inputs
I	I	Read contact inputs
i	I	Read contact input buffer
J	B	Write contact outputs
J	I	Read contact outputs
j	I	Read contact output buffers
K	B	Input remote keystrokes

Command	Type*	Description
K	I	Read key entry
L	B	Lock/Unlock keypad command
M	B	Set injection valve maintenance counter
M	I	Read injection valve maintenance counter
N	B	Start file
Q	B	Injection valve control
Q	I	Injection valve state
S	B	Stop running file
S	I	Read audit trail file event
T	I	Reset audit trail event pointer
U	B	Delete audit trail file
v	I	Read last injected volume
V	B	Sets the XYZ motor speed
V	I	Read the XYZ motor speed
W	B	Write display line
W	I	Read display line
w	I	Read display buffer
X	B	X axis control
Y	B	Y axis control
Z	B	Z axis control
X	I	X axis status
Y	I	Y axis status
Z	I	Z axis status

☞ ** I = Immediate command, B = Buffered command*

☞ *After turning on the machine or GSIOC reset and during M234 initialization, the response for all immediate commands is '~'.The maximum number of characters acceptable for a line of commands is 63 + the carriage return. All buffered commands are ignored.*

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APPENDIX C - COORDINATION

Commands description

Command Type Function Response format	**%** I Request module identification. 234V*a.bc* where V*a.bc* is the software version.
Command Type Function	**$** I Master reset. Returns $ and resets the machine in its power-up state.
Command Type Function	**?** I M234 status. Returns: 'S' for stop. 'R' for running. 'P' for pause.
Command Type Function	**a** I Read Injection Number. Returns: *nnnn* where *nnnn* is the number of injections (either sample or standard, but not priority) since starting the application. If there is no application running or no injection has yet taken place, returns '0'. ☞ *This is the value for the last completed injection and does not take into account an injection that is not yet completed.*
Command Type Function Syntax Comment	**A** B Syringe motor down stroke A*nnn* where *nnn* is the volume in µl The syringe aspirates *nnn* µl according to the syringe volume. If *nnn* is greater than the syringe volume, the command is ignored. This command disconnects the motor syringe from the internal software. The motor may be reconnected by sending the command 'A-'.
Command Type Function Response format	**b** I Read last injection status returns a*nnn* where a is the zone type of the latest injection E = sample, S = standard, P = priority *nnn* is the sample vial number from 0 to 108, or the standard vial number if the standard zone is used, or the priority vial number if the priority zone is used. If there is no application running, this command returns '™'. If no injection has yet taken place, this command returns 'E0'. ☞ *This is the value for the last completed injection and does not take into account an injection that is not yet completed.*

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Command Type Function Response format	**B** I Read file directory returns all existing file numbers separated by a comma. If there is no existing file, returns 'N'.
Command Type Function Response format	**C** I Syringe motor status ab*nnn* where a is: 'S' if syringe motor stopped. 'R' if syringe motor is running. 'E' if syringe motor error. b is: 'C' if syringe motor is connected to internal software 'D' if syringe motor is connected to GSIOC channel *nnn* is the volume contained in the syringe in µl. If the syringe motor is running or in error, *nnn* is replaced by "?????".
Command Type Function Syntax Comment	**D** B Syringe motor up stroke D*nnn* where *nnn* is the volume in µl The syringe dispenses *nnn* µl according to the syringe volume. If *nnn* is greater than the syringe volume, the command is ignored. If *nnn* is greater than the volume contained in the syringe, the syringe goes to the top position and stops. This command disconnects the motor syringe from the internal software. The motor may be reconnected by sending the command 'D-'.
Command Type Function Syntax Comment	**E** B Assigns a value to a parameter. E*nnn=mmm* where *nnn* is the parameter code (see table) *mmm* is the value assigned to the parameter. If the value *mmm* and the '=' sign are omitted, the value is unchanged, but the command sets the parameter pointer to the specified parameter (see table). To define a file, first send the editing file number, then injection method and loop filling method and after all parameters defining this method. Finally set 'File used' flag to create file. Parameters have to be consistent with the injection method and loop filling method selected. The following table identifies the code assigned to each parameter and the allowed ranges of values.

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	Code	Parameter	Unit	Range
	0	Rack code		30, 31, 34, 35, 36, 37, 38
	1	Syringe volume	µl	100 or 500
	2	Transfer tubing volume	µl	100 - 999
	3	Injection loop volume	µl	1 - 500
	4	Injection flush volume	µl	6 - 99
	5	Inside needle rinsing volume	µl	0 - 1000
	6	Outside needle rinsing volume	µl	0 - 1000
	7	Injection valve rinse volume	µl	0 - 500
	8	Air gap volume	µl	0 - 99
	9	Air gap asp. flow rate	µl/min	note 1
	10	Vial Asp/disp flow rate	µl/min	note 1
	11	Priming flow rate	µl/min	note 1
	12	Mixing flow rate	µl/min	note 1
	13	Calibrated tubing volume	µl	0 - 20
	14	Injection valve flow	µl/min	note 1
	15	Injection valve rinsing flow	µl/min	note 1
	16	Mixing ratio	%	0 - 100
	17	Audit trail file request		0=No , else Yes
	18	Running file number		1 - 20
	19	Editing file number		1 - 20
	20	Reservoir aspirate flow rate	µl/min	note 1
	21	Duration of pulse	1/100 min	1 - 100
	22	Injection method		1 for Simple 2 for External standard 3 for Internal standard
	23	Sample Loop filling method		1 for Total - 2 for Partial 3 for Centered
	24	Sample Loop filling coefficient		1 - 10
	25	Sample Injection volume	µl	note 2
	26	Nb of injections per sample		1 - 9
	27	Nb of standard		1 - 9
	28	Calibration frequency		1..max nb vial
	29	Sample dilution volume	µl	note 2
	30	Sample volume	µl	note 2
	31	Nb of mixing cycle per sample		0 - 10
	32	Standard dilution volume	µl	note 2
	33	Standard volume	µl	note 2
	34	Nb of mixing cycle per standard		0 - 10
	35	Standard loop filling method		1 for Total - 2 for Partial 3 for Centered
	36	Standard loop filling coefficient		1 - 10
	37	Standard injection volume	µl	note 2

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	Code	Parameter	Unit	Range
	38	Nb of injections per standard		1 - 9
	39	Internal standard volume	µl	note 2
	40	External synchronization		0=TIME else EXTERNAL
	41	Chromato time	1/100th min	0 - 99.99
	42	File locked		0=unlock else lock
	43	File used		0= file does not exist.
	☞ *1: Flow range depends on the syringe volume:* *Syringe = 100 µl -> 100 - 4000 µl/min* *Syringe = 500 µl -> 100 - 20000 µl/min.* *2: These volumes must be consistent with rack, syringe, tubing, injection and loop filling method. There is no test during entry. Unpredictable effects will result if parameters are inconsistent.*			

Command	**E**
Type	I
Function	Read value of parameter.
Response format:	*nnn = mmm* where *nnn* identifies the parameter (see table above) *mmm* is the value.
Comment	If the M234 data base is busy, the response is '!'. In this case, send the command again. Use the buffered 'E*nnn*' command to indicate the parameter to be reviewed. To read a file, first send the editing file number and then read all parameters.

Command	**F**
Type	B
Function	Set syringe flow rate.
Syntax	F*nnnn* where *nnnn* is the syringe flow rate in µl/min.
Comment	The flow rate range is 100 to 4000 µl/min for the 100 µl syringe, 100 to 20000 µl/min for the 500 µl syringe. The default value is 2500 µl/min for the 100 µl syringe, 12500 µl/min for the 500 µl syringe. When a new value is defined with this command, it used for the next syringe motion if the syringe motor is connected to the GSIOC channel.

Command	**F**
Type	I
Function	Read syringe flow rate.
Response format	*nnnn* in µl/min.

Command	**G**
Type	B
Function	Pump valve control.
Syntax	Ga where a is '0' means Valve to needle.'1' means Valve to reservoir. '-' means Reconnected to internal software.

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Command Type Function Response format	**G** I Read pump valve status. ab where a is 'C' if valve is connected to internal software. 'D' if valve is connected to GSIOC channel. b is '0' if valve to needle. '1' if valve to reservoir. 'R' if valve running. 'E' if valve jammed.
Command Type Function Syntax	**H** B Coordination flag Hx This command allows the M234 to coordinate with a master software. M234 needs two synchronization flags to perform complete injection cycle when external coordination is selected by the user. These flags may be carried out by contact or by GSIOC. It waits for the first flag before loading the injection loop. It waits for the second flag before turning the injection valve in <Inject> position. The flag is differentiated by x. For the first flag: x = 'L' For the second flag: x = 'I'. When the M234 uses one flag to go through a synchronization step, the flag is cleared. Only one GSIOC flag is stored at a time in the M234.
Command Type Function Response format	**H** I Read injection flag *n* where *n* = '0' when the flag is cleared. *n* = '1' when the flag is set. When the M234 is ready to inject (injection loop loaded), it sets this flag to request the coordination flag. This injection flag is cleared by reading it.
Command Type Function Syntax Parameters Comment	**I** B Set contact inputs. Iabc where 'a' is the coordination input. 'b' is the Pause/Continue input. 'c' is the Run/Stop input. 'C' for closed. 'D' for open. 'X' for state unchanged. '-' for reconnect to internal software. This command disconnects the input from the internal software if the corresponding parameter is 'C' or 'D'.
Command Type Function Response format	**I** I Read contact inputs. 'abc' as above. For each input: 'C' if closed and connect to internal software. 'c' if closed and disconnect from internal software. 'D' if open and connect to internal software. 'd' if open and disconnect from internal software.

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Command	**i**
Type	I
Function	Read contact input buffer
Response format	Same as I immediate command.
Comment	This command reads the input buffer of the internal software instead of the physical input.
Command	**J**
Type	B
Function	Set contact outputs.
Syntax	Jab where 'a' is the Out1 relay (sample and standard coordination). 'b' is the Out2 relay (priority sample coordination).
Parameters	'C' for Close. 'D' for Open. 'P' for Pulse. 'X' for state unchanged. '-' for reconnect to internal software.
Command	**J**
Type	I
Function	Read contact outputs.
Response format	'ab' where 'a' is the Out1 relay (sample and standard coordination). 'b' is the Out2 relay (priority sample coordination). For each input 'C' if closed and connect to internal software. 'c' if closed and disconnect from internal software. 'D' if open and connect to internal software. 'd' if open and disconnect from internal software.
Command	**j**
Type	I
Function	Read contact output buffers.
Response format	Same as immediate J command.
Parameters	Same as immediate J command.
Command	**K**
Type	B
Function	Input remote keystrokes.
Syntax	K+key codes+'|'.
Parameters	ASCII codes, as follows: The ASCII codes string must be terminated by the '|' ASCII character.
Comment	When this command has been sent, the keyboard is locked, but it can memorize up to 8 key strokes (beep can be heard). Key codes are stored in a buffer. When the keyboard is reconnected to the software, the buffer is cleared. The command 'K' without any codes reconnects the keypad to the internal software and clears the buffer.
Comment	'Keypad locked' is shown on the lower line of the display instead of the Soft-key label.

Code	Key name	Code	Key name
'a'	Softkey F1	'0'	Zero
'b'	Softkey F2	'1'	One
'c'	Softkey F3	'2'	Two
'd'	Softkey F4	'3'	Three
'e'	Softkey F5	'4'	Four
'C'	Clear	'5'	Five
'E'	Enter	'6'	Six
'.'	Dec. point	'7'	Seven
'S'	Stop	'8'	Height
'H'	Help	'9'	Nine

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Command Type Function Response format Comment	**K** I Read key entry. 'x..xx', where 'x..xx' is an ASCII string, 1 to 8 characters long, encoding the last keys pressed. See table above. This command clears the buffer. If no key pressed, or if the keyboard has not been disconnected by a buffered command 'K' or 'L', the '|' ASCII character is returned.
Command Type Function Syntax Comment	**L** B Lock/Unlock keypad command 'L*n*' where *n*=0 means Unlock keypad (and clear key buffer). *n* = 1 means Lock keypad. The keypad is locked but the beeper is still active. 'Keypad locked' is shown on the lower line of the display instead of the softkey labels. At the time the keyboard is reconnected to the software, the buffer is cleared.
Command Type Function Syntax	**M** B Set injection valve maintenance counter. M*nnnnnnnn* where *nnnnnnnn* is the value to load into the counter from 0 up to 99.000.000
Command Type Function Response format	**M** I Read injection valve maintenance counter. *nnnnnnnn* where *nnnnnnnn* is the number of injection valve movements
Command Type Function Syntax	**N** B Start file. N*nn,ff,ll* where *nn* is the file number. *ff* is the first sample number. *ll* is the number of samples.
Command Type Function Syntax Comment	**Q** B Injection valve control. Q*a* where a is '0' to send valve to inject position. '1' to send valve to load position. '-' to reconnect valve to internal software. This command disconnects the input from the internal software if the parameter is '0' or '1'.
Command Type Function Response format	**Q** I Read injection valve state. 'ab' where a is 'C' if valve is connected to internal software. 'D' if valve is connected to GSIOC channel. b is '0' if valve is in inject position. '1' if valve is in load position. 'R' if valve motor is running. 'E' if injection valve is jammed.

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Command Type Function	**S** B Stop running file. If a file is currently running, this command stops the run.
Command Type Function Response format	**S** I Read audit trail file event. Event code,par1,par2,par3. where: Event code is the event designation. See table below. Par1 is the first additional value (0 - 255) Par2 is the second additional value (0 - 255) Par3 is the third additional value (0 - 32767) Certain parameters are not required for all events. In this case, the parameter exists in the response but has no meaning. When the M234 data base is busy, the response is '!'. So try again. If there is no event available, the response is '@'. This command automatically increments the audit trail line pointer. Use immediate T command to set pointer at the beginning of the file.

Event Code	Event designation	Par1	Par2	Par3
'a'	Loading with xxxx µl	Hour	Minute	Volume
'b'	Aspirate xxxx µl	Hour	Minute	Volume
'c'	Dispense xxxx µl	Hour	Minute	Volume
'd'	Flushing	Hour	Minute	
'e'	Adding xxxx µl of diluent	Hour	Minute	Volume
'f'	Aspirate air gap	Hour	Minute	
'g'	Rinsing needle	Hour	Minute	
'h'	Injecting	Hour	Minute	Inj Nb
'i'	Rinsing injection port	Hour	Minute	
'j'	Mixing	Hour	Minute	
'k'	Initialization	Hour	Minute	
'l'	End of running	Hour	Minute	
'm'	Waiting	Hour	Minute	
'n'	Wait external coordination	Hour	Minute	
'r'	Aspirate xxxx µl from sample	Hour	Minute	Volume
's'	Aspirate xxxx µl from standard	Hour	Minute	Volume
't'	Aspirate xxxx µl from priority vial	Hour	Minute	Volume
'u'	Aspirate xxxx µl from result	Hour	Minute	Volume
'v'	Aspirate xxxx µl from internal standard	Hour	Minute	Volume
'w'	Dispense xxxx µl to result	Hour	Minute	Volume
'x'	Dispense xxxx µl to injection port	Hour	Minute	Volume
'A'	Date	Day	Month	Year
'B'	New tube*	Type**		Vial Nb
'C'	File	Inj Method***		File Nb
'D'	Stop	Hour	Minute	
'E'	Pause	Hour	Minute	
'F'	Resume	Hour	Minute	
'G'	Power failed restart	Hour	Minute	

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	☞ * *M234 sends this event for each new vial.* ** *Type = 'a' for sample, 'b' for standard, 'd' for priority vial.* *** *Inj Method = 1 for simple, 2 for Ext Std, 3 for Int Std.*
Command Type Function Response format Comment	**T** I Reset audit trail event pointer. 'T' if file exists. 'N' if file does not exist. The audit trail file is an event file. Each line stores one event. This file is read via GSIOC, event by event. See 'immediate S' command. This command sets the pointer to the first event.
Command Type Function Syntax	**U** B Delete audit trail file. 'U'
Command Type Function Response format	**v** I Read last injected volume Returns *nnnn* where *nnnn* is the last injected volume in µl ranging from 0 to the injection loop volume. This value is the volume of liquid actually injected, not the loaded volume. This volume is the injection loop volume if the loop filling method is TOTAL or the 'injection volume' for other loop filling methods. If there is no application running, this command returns '0'. If no injection has yet taken place, this command returns '0'. ☞ *This is the value for the last completed injection and does not take into account an injection that is not yet completed.*
Command Type Function Syntax	**V** B Sets the XYZ motor speed. Va*xxxx* where a is the axis name X,Y or Z *xxxx* is the speed in tenths of mm per second. Maximum value is 5000 tenths of mm:second for X and Y axes, 3000 tenths of mm/second for the Z axis.
Command Type Function Response format	**V** I Read the XYZ motor speed. a/*xxxx*/*yyyy*/*zzzz* a is the axis name: X, Y or Z *xxxx* is the speed in tenths of mm per second for X axis *yyyy* is the speed in tenths of mm per second for Y axis *zzzz* is the speed in tenths of mm per second for Z axis

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Command Type Function Syntax Parameter	**W** B Write display line. 'W0=x..xx' for upper line, 'W1= x.xx' for lower line. x.xx is a 40-character long alphanumeric string. All 7-bit ASCII characters are valid. ☞ *Characters shown on the Japanese-made display unit may differ from characters sent by a computer. If the string is less than 43 characters (3+40), the remaining rightmost characters of the display are blanked. If it is longer, extra characters are ignored. This command disconnects the corresponding line from the internal software. Lines may be reconnected to the software by sending W0 or W1. Sending W alone reconnects all the display.*
Command Type Function Response format Comment	**W** I Read display line. 'W0='(upper line) or 'W1='(lower line) plus a 40-character alphanumeric string. If the display line is connected to GSIOC channel, the response is wn='line string' The first line to be returned is the last entered by the buffer 'W' write command. If the write command has not been used, the upper line is read at the first occurrence of the read command. The other line is read at the second one.
Command Type Function Response format Comment	**w** I Read display buffer. As for immediate W command. Reading mechanism same as immediate W command. The response is the image of the display line if it is connected to the internal software. This is also the message which will be restored to the display after a reconnect command.
Commands Type Function Format Comment	**X, Y, Z** B Individual axis control. I*nnnn* where I is the axis name X, Y or Z *nnnn* is the coordinate in tenths of mm. 0 is at home for X and Y and at the top for Z. This command disconnects axis motor from the internal software. Motors may be reconnected by replacing *nnnn* by '-'. When the axis is reconnected, the axis goes to the coordinate given by the internal software. The coordinate of: - The injection port is X = 194 mm, Y = 11.7 mm, Z = 57 mm. - The inside needle rinsing station is X = 0 mm, Y = 0 mm, Z = 41 mm. - The outside needle rinsing station is X =14 mm, Y = 0 mm, Z = 66 mm.

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APPENDIX C - COORDINATION

Commands	**X, Y, Z**
Type	I
Function	Read individual axis status
Response format	ab*nnnn*. where a is: 'S' if the axis is stopped. 'R' if the axis is running. 'E' if the axis is jammed. b is: 'C' if the axis is connected to the internal software. 'D' if it disconnected. *nnnn* is the coordinate in tenths of mm if the axis is at rest. If it is running or jammed, coordinate *nnnn* is replaced by "????".

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APPENDIX D - PRIORITY SAMPLES

It is possible to interrupt a method run to process an urgently required priority sample. The operation of the priority function depends on the rack and injection method being used.

1 VIAL POSITIONING

For rack codes 35, 36, 37 and 38, there is a specific priority vial area available. If you are using simple injection, or external standard without dilution, all of the available priority vial positions can be used, you must indicate which vial position you are using. If you are using dilution or the internal standard method, you can only use the first half of the vial positions for the priority sample as the remaining vials must be available for the corresponding result liquid. Place a result vial in the corresponding result vial position (refer to Appendix B - Zones for precise details on vial positioning).

Rack Code	Available vial positions
35	6 for injection method without dilution or internal standard. 3 for injection method with dilution or internal standard. (Vials 1-3 reserved for priority sample, vials 4-6 for result)
36	8 for injection method without dilution or internal standard. 4 for injection method with dilution or internal standard. (Vials 1-4 reserved for priority sample, vials 5-8 for result)
37	4 for injection method without dilution or internal standard. 2 for injection method with dilution or internal standard. (Vials 1-2 reserved for priority sample, vials 3-4 for result)
38	6 for injection method without dilution or internal standard. 3 for injection method with dilution or internal standard. (Vials 1-3 reserved for priority sample, vials 4-6 for result)

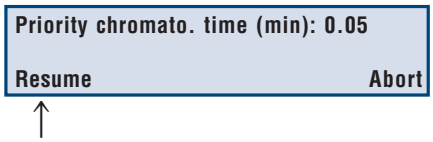
For rack codes 30, 31, and 34, you use the first sample vial position for the priority vial. If you are using dilution or internal standard method, you must place a result vial in the corresponding position in the result vial area.

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APPENDIX D - PRIORITY SAMPLES

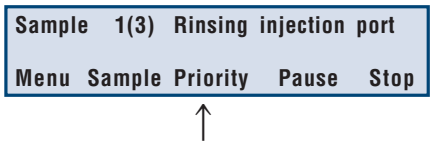
2 COORDINATION

When processing a priority sample, the instrument does not wait for a coordination signal. You must specify the chromatography time. The maximum allowed time is 600.00 minutes. The instrument waits for the specified priority chromatography time before injecting the next standard/sample. Key in the priority chromatography time and press **ENTER**. A pulse is output at output contact 1.



3 USING THE PRIORITY FUNCTION

To process a priority vial when a method is running, press the **Priority** soft-key, available on the bottom line of the in-run screen.



When using racks 30, 31 or 34, the priority function is not available until after the first sample injection has been performed. This is because the priority sample must be situated in the first sample vial position. If you are using a method with dilution or with an external standard, you must replace the first raw sample vial with the priority sample, and replace the first prepared sample vial with an empty vial.

When using racks 35, 36, 37 or 38, the priority function is always available, there is a separate priority vial area.

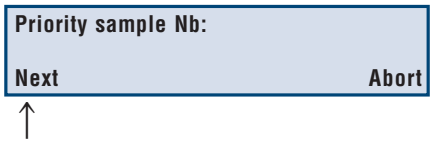
After pressing the **Priority** soft-key, the program pauses immediately. At this point, the Z-arm and carriage move to the home position, allowing you to gain access to the rack to insert the priority vial in the rack.

Replace the relevant sample vial with the priority vial. Ensure that there is sufficient liquid in the priority sample vial to carry out the injection according to your defined method.

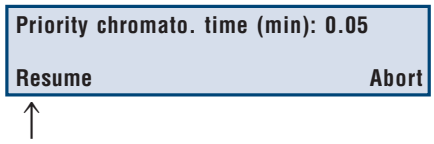
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If you are using dilution or internal standard method, remember to replace the corresponding result vial with an empty priority result vial.

Key in the position number of priority vial and press **ENTER**.

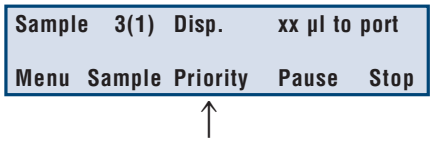


Enter the chromatography time necessary for the priority sample. The instrument prepares the priority sample, injects it and then waits for the specified priority chromatography time before injecting the next sample in the original sequence.

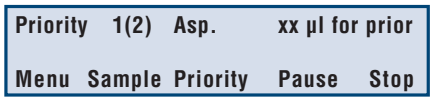


If you press the **Abort** Soft-key, the priority function aborts and the original injection sequence resumes.

Press the **Resume** soft-key and the paused program finishes preparing and injecting the current sample. The word priority blinks on and off to indicate that the next sample to be injected is the priority sample. At this point it is possible to cancel the priority sample by pressing the **Priority** soft-key. The cancel function only works while **Priority** blinks.



After the priority sample has been injected, the program continues with the original sample sequence.



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APPENDIX F - VERIFYING THE CV OF YOUR INSTRUMENT

Two pre-stored programs can be used to measure the CV of your instrument for the configuration that you are using. Use File 19 for total loop filling method injection, use File 20 for partial loop filling method.

*Prepare your system as described below, key in the relevant file number (19 or 20) and press the **Run** Soft-key.*

One sample vial is used (Chromacol 1.8 ml vial filled with 1.6 ml of sample for all three modes). Nine injections per injection mode are performed for the sample liquid.

Use total loop filling injection for a first run, and partial loop filling injection for a second run.

For verification at the Gilson factory, the sample liquid is 0.05% phenol in methanol. The mobile phase is methanol, the reservoir solvent is methanol.

The system components are:

- The pump for the mobile phase is a Gilson 307 pump with a pump head type 5SC, with the flow rate set at 2 ml/min.
- Gilson 117 detector, Wavelength = 254 nm, sensitivity = 0.2 AUFS, output 10 mV.
- System controller software: Gilson 715 software.
  Peak sensitivity is set at 2% and peak width = 0.05 min.
- Column: Spherisorb™ ODS2, 5µm, 150x4.6 mm.

Set your Autoinjector parameters as follows:

- **Configuration parameters:**

Rack code:	30
Syringe volume:	500 µl
Transfer tubing volume:	550 µl
Injection loop volume:	20 µl
Injection flush volume:	15 µl
GSIOC ID number:	10

- **Flow/Vol parameters:**

Inside needle rinsing volume:	300 µl
Outside needle rinsing volume:	300 µl
Injection valve rinse volume:	500 µl

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Air gap volume:	6 µl
Air gap aspirate flow rate:	0.3 ml/min
Vial asp/disp flow rate:	0.3 ml/min
Priming flow rate:	20 ml/min
Mixing flow rate:	2.0 ml/min
Diluent asp. flow rate:	10 ml/min

- **Advanced User Menus:**

Calibrated tubing volume:	6 µl
Injection valve loading flow rate:	0.3 ml/min
Mixing volume ratio:	100 %
Injection valve rinsing flow rate:	3 ml/min

- **Method parameters:**

Total loop filling injection:

Loop filling coefficient:	5
Number of injections	9
Number of sample vials	1

Partial loop filling injection:

Injection volume:	5 µl
Number of injections	9
Number of sample vials	1

Before running the method program:

The column must be replaced by tubing, then purge the system for two minutes. Stop the purge and reinstall the column.

Prime the 234 dilutor.

Run the program.

Calculate the CV of peak areas and compare them with the following value:

CV ≤ 0.5 % for total loop filling (specified)
CV ≤ 0.8 % for partial loop filling (typical).

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APPENDIX G - TECHNICAL DATA

All technical data, unless specified otherwise is with the instrument in the following configuration:

Syringe volume: 0.5 ml
Transfer tubing: 550 µl, FEP
Injection valve: Rheodyne 7010 valve
Injection loop: 20 µl

TYPE

The instrument is an Autoinjector with basic sample handling capabilities: dilution, addition of an internal standard, automatic and periodic re-injection of standards for external standard and internal standard methods.

HYDRAULICS

- **Vial Capacity:** 120 microvials of 0.7 ml including up to 8 priority vial positions and one 20 ml vial. Choice of 10 Gilson racks, including 5 thermostated racks connected to a 832 Peltier Unit or to a cryothermostat.
- **Syringe capacity:** 0.5 ml (standard); and 0.1 ml.
- **Injection loop:** 20 µl (standard); additional choice: 5, 10, 50, 100 and 500 µl fitted on a Rheodyne 7010 valve.
- **Injection volume:** 1 to 500 µl selectable in 1 µl steps.
- **Number of injections per vial:** 1 to 9.
- **Precision:**

Specified reproducibility* with a 0.5 ml syringe

Total loop filling method		CV < 0.5 %
Typical values	*with a 0.5 ml syringe*	
	Partial loop filling injection of 5 µl	CV < 0.8 %
	with a 0.1 ml syringe	
	Total loop filling method	CV ³ 0.2 %
	Partial loop filling injection of 2 µl*	CV ³ 0.7 %
	Partial loop filling injection of volume > 4 µl	CV < 0.5 %

☞ ** Values obtained with the test procedure described in Appendix F.*

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- **Liquid flow rate:** 0.1 to 20 ml/min with a 0.5 ml syringe,
  0.1 to 4 ml/min with a 0.1 ml syringe.
- **Minimum sample volume:** 1 µl can be injected from 12 µl sample in a 0.7 ml vial.
- **Sample cross-contamination:** lower than 50 ppm with recommended values for rinsing parameters:
  Valve rinsing volume: 500 µl
  Inside needle rinsing volume: 300 µl
  Outside needle rinsing volume: 300 µl
- **Liquid contact materials:** 316L stainless steel, FEP, PEEK, PP and PI.

MECHANICS

- **Arm motion speed:** 250 mm/s horizontal, 150 mm/s for the Z axis.
- **Injection valve switching time:** less than 0.5 seconds.

ELECTRICAL

- **Remote control:** GSIOC (Gilson Serial Input Output Channel).
- **Inputs:** 3 electrical contacts dedicated as follows: Start/Stop, Pause/Resume and coordination function (wait before injection).
- **Outputs:** 2 relays used to send a signal at the injection time: one for regular samples and standards and the other one for priority samples.
- **Conformity to norms** *(From Serial Number 168001)*:
  Gilson's 234 Autoinjector conforms to EEC Directive 89/336 for electromagnetic compatibility: Standard EN 50081-1 for emission, Standard EN 50082-1 for immunity.

  Gilson's 234 Autoinjector conforms to EEC Directive 73/23/EEC for safety: Standards EN 61010-1, UL 3101-1, and CAN/CSA 22.2 N°1010-1.

  Each 234 Autoinjector is subjected to a Dielectric Test (1500 V between live/neutral and earth) and an Earth Bonding Impedance Test (- 0.1 Ω).

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APPENDIX G - TECHNICAL DATA

CONTROL

- **Display:** 2-line, 40 character, 20 keys.
- **Software:** 3 built-in methods.
  Simple injection, external standard and internal standard with 3 possible injection modes: total loop, partial loop and centered loop (no loss of sample).
- **Storage:** Permanent storage for up to 20 files.

ENVIRONMENT

- **Dimensions (W x D x H):** 380 x 380 x 385 mm.
- **Temperature range:** 4 to 40°C.
- **Humidity:** Up to 80 %.
- **Altitude:** Up to 2000 m.
- **Voltage range:** 100 - 240 V, 50 - 60 Hz.
- **Electrical power:** 100 VA.
- **Weight:** 18 kg.
- **Pollution:** Pollution degree 2.
- **Installation:** Category II.

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