SERIES 200 AUTOSAMPLER



User's Manual



Series 200 Autosampler User's Manual

Release History

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Safety and Regulatory Information

Electromagnetic Compatibility (EMC)

United States

This product is classified as a digital device used exclusively as industrial, commercial, or medical test equipment. It is exempt from the technical standards specified in Part 15 of the FCC Rules and Regulations, based on Section 15.103 (c).

Symbols and Conventions used in this Manual

In this manual, the following graphic symbols and special text formats are used to set apart important safety information.



A warning indicates an operation that could cause *personal injury* if precautions are not followed.



A caution indicates an operation that could cause *instrument damage* if precautions are not followed.

Symbols used on the Instrument

There are two different types of warning symbols that appear on the instrument.



This symbol indicates an operation (or condition on the instrument) that could cause *personal injury* if precautions are not followed. This can be any type of hazard.

When you see this symbol, refer to the safety pages in the manual for further information.

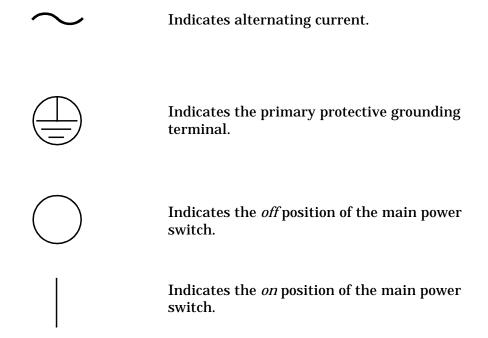
Consulter les documents d'accompagnement.



This symbol indicates the danger of electric shock, if precautions are not followed, due to the presence of high voltage.

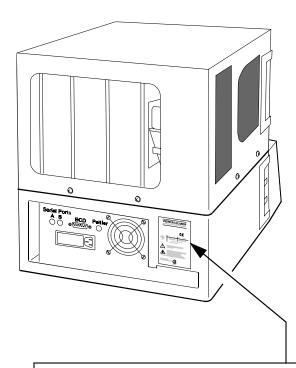
Attention. Risque de choc électrique.

Additional graphic symbols used on the instrument are the following:



Warnings on the Instrument

The following warning labels are affixed to the rear panel of the instrument for all models as shown in the figure below.





FOR PROTECTION AGAINST FIRE HAZARD, REPLACE ONLY WITH SAME TYPE AND RATING OF FUSE.

AFIN D'ASSURER LA PROTECTION CONTRE LES RISQUES D'INCENDIE, REMPLACER UNIQUEMENT PAR UN FUSIBLE DE MÊME TYPE ET DE MÊME COURANT NOMINAL.



DISCONNECT SUPPLY CORD BEFORE OPENING. GROUNDING CIRCUIT CONTINUITY IS VITAL FOR SAFE OPERATION OF EQUIPMENT. NEVER OPERATE EQUIPMENT WITH GROUNDING CONDUCTOR DISCONNECTED.

DÉBRANCHER LE CORDON D'ALIMENTATION AVANT D'OUVRIR DA CONTINUITÉ DES MASSES EST ESSENTIELLE. POUR UN FONCTIONNEMENT SANS DANGER. NE JAMAIS UTILISER L'ÉQUIP-MENT SI LE FIL DE TERRE N'EST PAS RACCORDÉ.

Electrical Warnings



Connect the instrument to an AC line power outlet that has a protective ground connection. To ensure satisfactory and safe operation of the instrument, it is essential that the protective ground conductor (the green /yellow lead) of the line power cord is connected to true electrical ground. Any interruption of the protective ground conductor, inside or outside the instrument, or disconnection of the protective ground terminal may impair the protection provided by the instrument.



Do not operate the instrument with any covers or parts removed.



Do not attempt to make adjustments, replacements or repairs to this instrument except as described in the accompanying User Documentation. Only a Perkin-Elmer service representative or similarly trained and authorized person should be permitted to service the instrument.



Use only fuses with the required current rating and of the specified type for replacement.

Quality Control/Good Laboratory Practices

Quality Control

The user should develop appropriate quality control procedures for the LC Autosampler (and the entire LC system) to ensure suitability for its intended use. These procedures typically consist of periodic performance verifications and routine inspections and suitability tests.

Certificate of System Conformity

Each Series 200 LC Autosampler is carefully built and tested in a controlled system in accordance with the requirements specified in its applicable PerkinElmer Final Assembly and Test Specification.

Each instrument is certified to meet its functional and performance specification upon release to shipment. The integrity of this quality system is routinely audited and is certified by the British Standards Institution as meeting all requirements of ISO 9001:1987 (Certification No. FM 22179).

Instrument Performance Verification (IPV)

To maintain functional performance, PerkinElmer recommends a yearly Instrument Performance Verification (IPV) of the Series 200 Autosampler by a PerkinElmer Service Engineer to ensure its operation within published specifications. These tests consist of measuring the most important autosampler characteristics such as % Relative Standard Deviation (of peak area) and sample carryover. Certification is available for regulatory compliance. Contact your local PerkinElmer Sales and Service office.

Routine Inspection and Suitability Test

The Series 200 Autosampler housing should also be inspected weekly through the injection valve access door and the top sample access door for signs of liquid leaks. Prior to any sample analysis, a system suitability test, which closely resembles the intended assay, should be performed to ensure that the LC system is operating within established criteria (e.g., peak resolution, peak asymmetry, precision, retention time, column plate count, pressure limits, signal/noise ratio, etc.)

While the Universal Test Mix (UTM) Part No. 0089-0893, can be used for a system check, we recommend that you develop a separate system suitability test and acceptance criteria for each of your assays.

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Hazardous Chemicals

Before using mobile phase solvents, you should be thoroughly familiar with all hazards and safe handling practices. Observe the manufacturer's recommendations for use, storage and disposal. These recommendations are normally provided in the material safety data sheets (MSDS) supplied with the solvents.



Some chemicals used with this instrument may be hazardous or may become hazardous after completion of an analysis. The responsible body (e.g. Lab Manager) must take the necessary precautions to ensure that the surrounding workplace and that the instrument operators are not exposed to hazardous levels of toxic substances (chemical or biological) as defined in the applicable Material Safety Data Sheets (MSDS) or OSHA, ACGIH, or COSHH documents. Venting for fumes and disposal of waste must be in accordance with all national, state and local health and safety regulations and laws.

Definitions in Warning for Hazardous Chemicals

Responsible body. "Individual or group responsible for the use and maintenance of equipment, and for ensuring that operators are adequately trained." [per IEC 1010-1, Amendment 2 (draft)].

Operator. "Person operating equipment for its intended purpose." [per IEC 1010-1, Amendment 2 (draft)].

OSHA: Occupational Safety and Health Administration (United States)

ACGIH: American Conference of Governmental Industrial Hygienists

COSHH: Control of Substances Hazardous to Health (United Kingdom)

Temperature, Humidity and Environment

Caution This instrument is designed for indoor use.

Caution

Do not operate in a Cold Room or a refrigerated area. The Series 200 LC Autosampler operates most efficiently under the following conditions:

- ambient temperature is 10 to 30°C
- ambient relative humidity is 20 to 80% (without condensation)
- altitude is in the range 0 to 2000 meters.



This instrument is not designed for operation in an explosive environment.

Installation Category

This instrument is able to withstand transient overvoltage according to Installation Category II as defined in IEC 1010-1.

Pollution Degree

This equipment will operate safely in environments that contain nonconductive foreign matter up to Pollution Degree 2 in IEC 1010-1.

Storage Conditions

The Series 200 LC Autosampler may be stored under the following conditions:

- ambient temperature is -20 to +60°C,
- ambient relative humidity is 20 to 80% (without condensation),
- altitude is in the range 0 to 12,000 meters.

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Preface: Using the Manual

The PerkinElmer Series 200 LC Autosampler is an advanced sample processor that can be used with virtually every liquid chromatography system or application. If you use the PerkinElmer Series 200 LC Pump, you will find that the Autosampler front panel is almost identical to the one on the Pump. In addition, the Pump and the Autosampler share many common operating features that will allow you to get your system up and running quickly.

Using the Manual

The main function of this manual is to give you all the information you need to operate the Series 200 LC Autosampler as productively as possible, using all of the instrument features.

Caution

The instrument should be used according to the instructions provided in this manual. If used otherwise, the protection provided by the instrument may be impaired.

Note The unpacking and setup procedures for the Series 200 LC Autosampler are included in Chapter 2 of this manual. If you have not installed the instrument, you should first refer to the instructions given in Chapter 2.

Preface: Using the Manual Definitions

Definitions

The following definitions of key terms will help you in using this manual to operate the Series 200 LC Autosampler.

Method

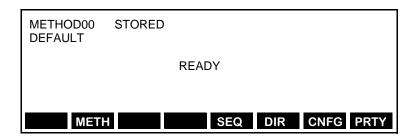
A method is a procedure that may consist of a single injection or a series of injections of several samples and/or calibrants. The method also defines the location of each sample or calibrant vial, as well as the injection volume and the number of replicates for each sample/calibrant. There is a default method, method00, provided with the Autosampler. You can make changes to this default method and save those changes by saving them under a new method number (from 1 - 19). If you choose not to save the changes in this way, the changes will be lost when you turn the power off on the Autosampler.

Screens and Pages

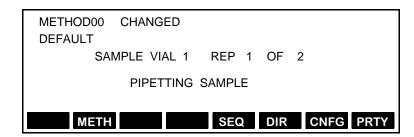
Screens display the information for defining the operation or configuration of the Series 200 LC Autosampler. If all of the information for a particular operation set cannot be displayed on a single screen, then the screen is divided into pages.

Main Screen

The Main Screen is the top-level screen that provides you with access to all of the Autosampler's functions. All of the functions are displayed along the bottom of the screen. The Main Screen has two display formats. The first format shown here is the one displayed when the Autosampler is idle.



The second format is displayed when the Autosampler is performing an operation. It provides status information about the operation. A typical example of status information is shown here for an active method00 currently being run.



Definitions Preface: Using the Manual

Active Method

The active method is the method that is currently running on the Autosampler, or the method currently shown on the display.

Sequence

A sequence is a series of stored methods that are linked together. When you start a sequence, each method in the sequence is run in the order defined during the setup of the sequence.

Priority Method

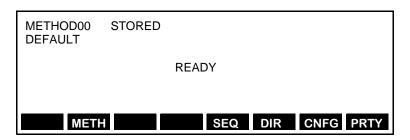
The priority method is similar to a method in that it may also consist of a single injection or series of injections of several samples and/or calibrants. It differs from the active method, however, in that there is a only a single priority method and you can interrupt the active method (or sequence) with the priority method (the active method resumes as soon as the priority method is completed). In addition, any changes you make to the priority method are automatically saved so that if you turn the power off on the Autosampler you will not lose the changes.

Preface: Using the Manual Conventions

Conventions

The following conventions are used in this manual.

• An instrument screen display is pictured as a single-lined box:



- Instructions for using the softkey functions are displayed in the text as: "press F2 METH to ..."
- Instructions for using hard keys on the instrument keyboard are displayed in text as: "press the return key ..."

Customer Service Preface: Using the Manual

Customer Service

Company Name and Address:

PerkinElmer Instruments 761 Main Avenue. Norwalk, CT 06859 USA

For Sales Assistance and Information: 1-800-762-4000

Supplies, accessories, and replacement parts can be ordered directly from PerkinElmer using the eight-digit part numbers provided in this manual.

PerkinElmer's catalog service offers a full selection of high quality intrumentation, reagents and consumables through the HPLC and CE Supplies Catalog. For information, to place an order, or request a free catalog:

If you are located within the U.S., call toll-free: 1-800-762-400, 8:30 a.m. to 7 p.m. EST.

Your order will be shipped promptly, usually within 24 hours.

If you are located outside the U.S., call your PerkinElmer Sales Representative.

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Introduction

This chapter introduces you to the Series 200 LC Autosampler and includes the following information.

- A Description of the Autosampler
- · The Autosampler's Features and Benefits
- The Major Components of the Autosampler
- Using the Keyboard and Display
- Instrument Specifications

A Description of the Autosampler

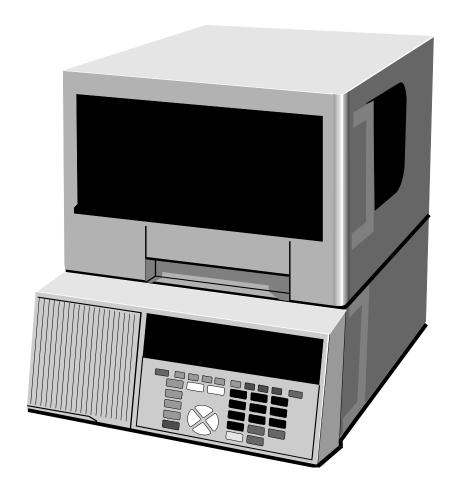


Figure 1-1. The Series 200 LC Autosampler.

The PerkinElmer Series 200 LC Autosampler is an advanced sample processor that can be used with virtually every liquid chromatography system or application. You configure the Autosampler and define all of your methods and operations using function keys on a keyboard located on the front panel of the instrument. A backlit Liquid Crystal Display (LCD) located directly above the keyboard shows both status information about the instrument and provides access to the screens you use to define methods and set configuration parameters. If you use the PerkinElmer Series 200 LC Pump, you will find that the Autosampler front panel is almost identical to the one on the Pump. In addition, the Pump and the Autosampler share many common operating features that will allow you to get your system up and running quickly. The Series 200 LC Autosampler provides you with interchangeable technology so that you do not have to buy a new Autosampler and learn new user interfaces every time your needs change.

Series 200 LC Autosampler Features and Benefits

- Upgradable to biocompatible versions for maximum flexibility.
- Small, compact design (12.5 inches (31.8 cm) wide) saves valuable bench space.
- Directory of up to 20 methods.
- Ability to link up to 10 methods together in a sequence.
- Priority method which allows you to interrupt a method or sequence to run critical samples. The method or sequence resumes once the priority samples are analyzed.
- Edit the active method or sequence in real-time with easy access function keys.
- Large backlit liquid crystal display allows clear viewing of autosampler method and operating parameters at all times.
- Tactile keyboard with unique "key click" so that you know your entry has been made.
- Phone type contact closure connections and finger tight plumbing fittings make it simple to connect or disconnect your system.
- Full method documentation including modification with method name, number, date and time stamp.
- · Visual access front-door panel allows quick plumbing inspection and easy servicing.
- Maintenance log coded by the serial number of the autosampler, including autosampler injection cycles.
- Instrument log records history of operation for quick diagnosis and servicing to get you up and running quickly.
- Extensive service diagnostics.
- Sampler module can be completely removed to lab bench for maintenance without having to unstack modules.

Main Components of the Series 200 LC Autosampler

The Series 200 LC Autosampler consists of the following main components. A brief description of each of the components follows.

Refer to Figure 1-2 for the location of the following components.

Keyboard and Display

All instrument functions are accessed using the keyboard. Status information is shown on the screens appearing in the display. An overview describing how to perform some basic operations using keyboard and display is provided in "Using the Keyboard and Display" section of this chapter.

Input/Output Connector Panel

The connector panel consists of six contact closure connectors for automated instrument control of devices like an LC pump, LC detector, and a data handling system.

Sample Tray

The Autosampler accepts eight different types of sample trays. The trays hold sample and calibrant vials, as well as dilution and derivatization vials or tanks. For a complete list of available tray types, refer to Appendix A in this manual. The Autosampler identifies the tray type by reading the pattern of fingers on the back of the tray. When you load a tray, these fingers slide into a sensor which is recessed in the back wall of the tray well.

Sample Needle Assembly

This assembly consists of a sample needle that moves up and down (Z direction) and is mounted in a transport mechanism that moves backwards and forwards and left to right (XY direction). The sample needle picks up sample or calibrant from the vial and, depending on the method, performs dilutions and/or derivatizations and injects the sample onto the column. It is connected to and works in conjunction with the Injection Valve Assembly and the Dual Syringe System. The Injection Valve Assembly is accessed through the Injection Valve Access Door. (The location of the Dual Syringe System is shown in Figure 1-3.)

During the sampling operation:

- The transport mechanism positions the sample needle above the sample vial.
- A cushion of air is drawn up into the needle.
- The needle is lowered to just pierce the vial septum. At this point, the system verifies that the vial is present.
- The needle is fully lowered into the vial and the required sample volume is drawn up.
- The needle is raised clear of the vial and another cushion of air is drawn up.
- The transport mechanism moves the needle to the Injection Valve Port.
- The needle is lowered into the port and the sample is injected onto the column.
- The transport mechanism moves the needle to the flush port and a flush cycle is performed.

The sampling modes and the sampling volumes available on the Autosampler are described in detail in the section, "Reviewing the Default Performance Parameters," in Chapter 3 of this manual.

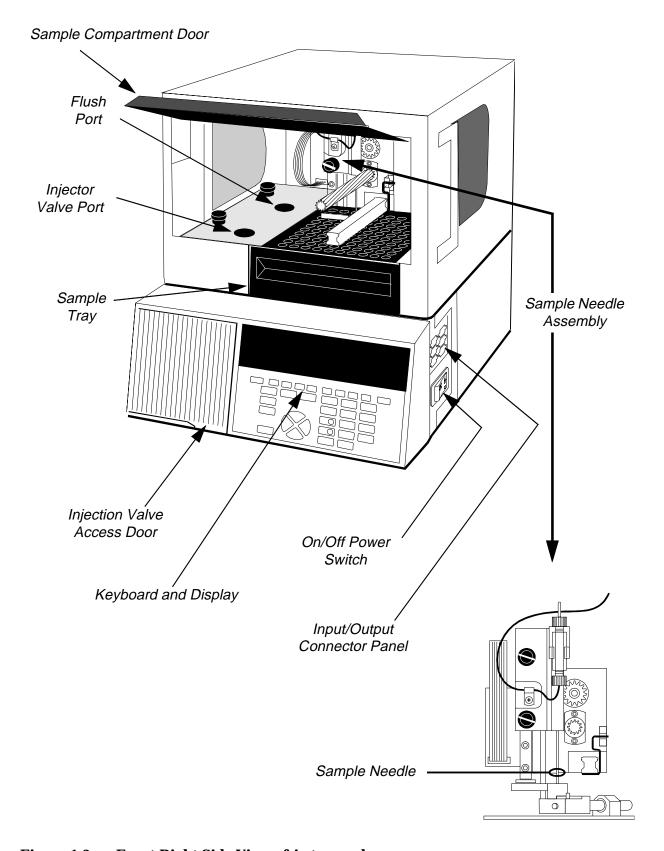


Figure 1-2. Front Right Side View of Autosampler.

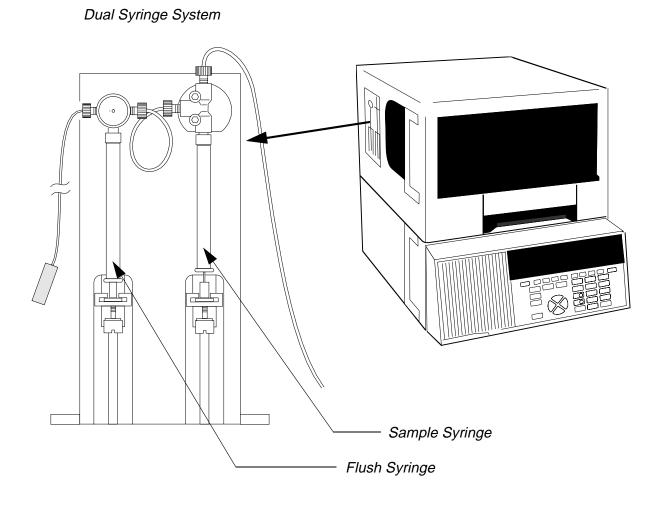


Figure 1-3. Front Left Side View of the Autosampler showing the Dual Syringe System.

Dual Syringe System

This system, shown in Figure 1-3, contains the Sample syringe and the Flush syringe. The Sample syringe actually draws up the sample through the sample needle into the sample loop and ejects it into the injection valve port. The Flush syringe flushes the sample loop, needle and sample tubing between injections.

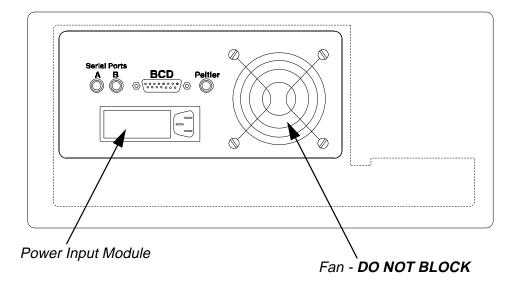


Figure 1-4. Rear Panel of the Autosampler.

Rear Panel

The rear panel contains the Power Input Module and the communication ports for the Autosampler. The power module accepts the AC line cord and contains the system fuses and voltage selector card. The function and procedures required to set up the various communication ports are described in the "Defining the Communications Options" section in Chapter 3 of this manual.

Using the Keyboard and Display

The Autosampler keyboard and Liquid Crystal Display (LCD) are located on the front panel of the instrument as shown in Figure 1-2. A closer view of the keyboard and display is provided here in Figure 1-5. You configure the Autosampler and define all of your methods by filling in information on the screens that appear in the LCD. You use the keys on the keyboard to enter the information on the screens and also to start or stop certain operations on the Autosampler. As you move from one screen to another, the screen defined function keys change. The functions of the various keys are summarized in Table 1-1 on the next page.

You access all of the Autosampler screens starting at the top level Main Screen which is shown in the LCD in Figure 1-5. All of the Autosampler screens are shown in the screen hierarchy diagram in Figure 1-6.

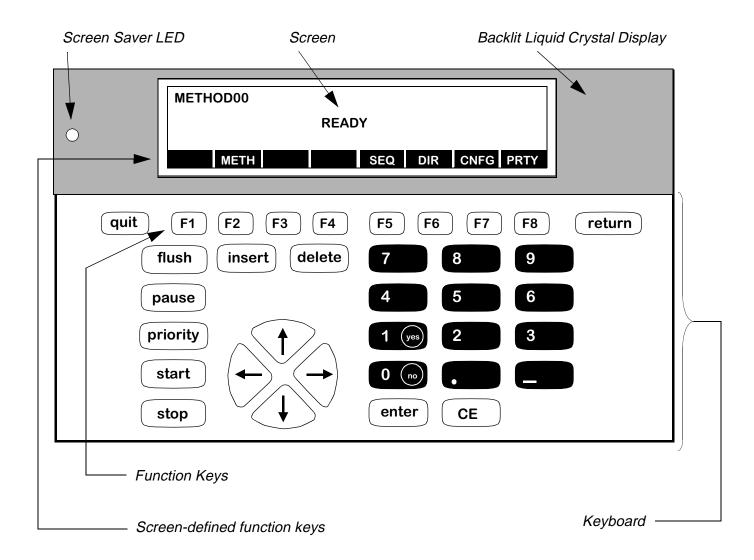


Figure 1-5. Keyboard and Display.

Summary of the Keyboard Functions

Table 1-1. Summary of Keyboard Functions.

| Key | Function | |
|--------------|--|--|
| F1 To F8 | Press a function key to select the corresponding screen-defined soft key label displayed above it. For example, press F7 CNFG from the Main Screen to go to the Configure Screen. | |
| flush | Press to flush and clean the sample needle and transfer line. This is active only when a method is not controlling the instrument. | |
| pause | Press pause if you want to add samples to the sample tray during a run. This prevents the needle, arm and sample pump from moving until you press pause again to resume operation. | |
| priority | Press priority to start a priority method. The priority method will start after the current injection completes. | |
| start | Press start to initiate a sequence or the active method. The sequence will start if the methods within a sequence are linked; otherwise, the active method will start. | |
| stop | Press to stop the autosampler. Pressing stop raises and then flushes the sample needle. | |
| quit | Press quit to cancel any changes you have made to the screen (or one of the pages within a screen) and return to the previous screen. | |
| return | Press return to save changes you have made to the current screen (or one of the pages within the screen) and return to the previous screen. | |
| enter | Press enter to accept a typed value and move to the next position or field. | |
| CE | Press CE to clear a typed value and redisplay the original value. | |
| insert | Press to insert a duplicate of the current step below the current step and increment the steps following it by one (for example, when adding methods in a sequence). | |
| delete | Press to delete the current step and decrement the steps following it by one. | |
| Numeric Keys | Use numeric keys 1 - 9 for numeric entry. Use numeric keys 0 and 1 (no and yes) to answer to yes/no prompts. | |
| | Use the cursor arrow keys to accept a typed value and to move between fields within a screen or page of a screen. | |

Include Hierarchy Diagram here as

Figure 1-6. Screen Hierarchy Diagram

Keyboard Operations

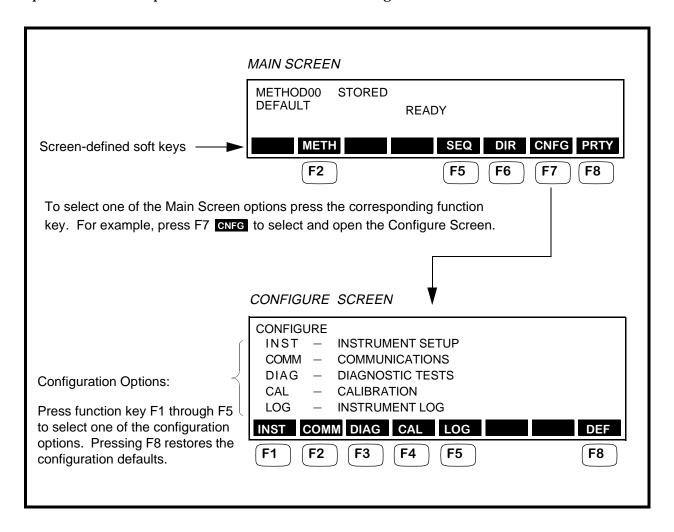
This section provides an overview of how to perform the following basic operations using the Autosampler keyboard:

- Selecting screen options
- Selecting data fields and entering numeric information
- Entering non-numeric information into data fields
- Scrolling through screen lists
- Responding to screen prompts
- Saving changes to screens

Selecting Screen Options

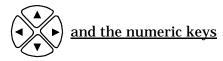
<u>Use the</u> $(F1) T_0 (F8)$ <u>Function Keys</u>:

To select options displayed on a screen, either as screen-defined soft keys, or as a list of items, use the appropriate function key. Once you press the appropriate function key, the screen corresponding to the selected option appears. Typical examples include selecting options from the top level Main Screen and the Configure Screen as shown here.



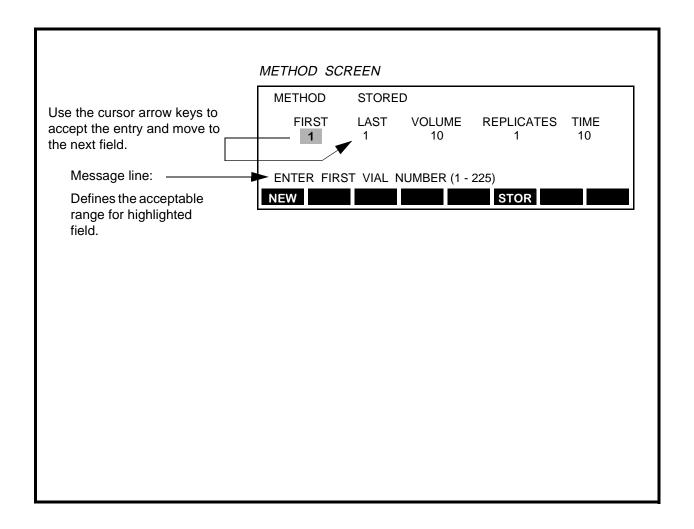
Selecting Data Fields and Entering Numeric Information

<u>Use the cursor arrow keys</u>,



To accept the entry in a highlighted data field and move to the next field, use the cursor arrow keys. For example, when you first enter the top level Method Screen shown below, the data field for the FIRST sample vial is highlighted. To accept the current entry and move to and highlight the LAST sample vial, press the right cursor arrow key.

To change a numeric entry use the numeric keys on the keypad. The acceptable range for each highlighted data field is displayed in the message line.



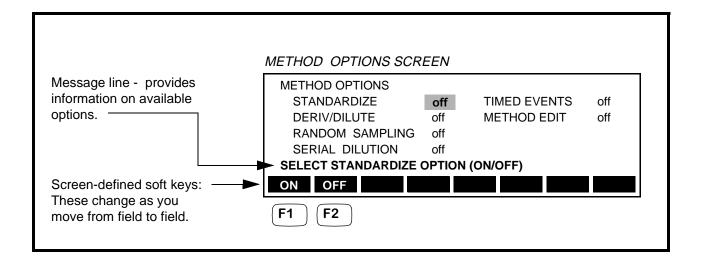
Entering Non-numeric Information into Data Fields within a Screen

<u>Use the</u> (F1) To (F8) <u>Function Keys</u>:

If a highlighted data field requires a non-numeric entry, the options for that field appear as screen-defined soft keys.

The message line also provides information on the options available for the highlighted field.

To change the current entry in the highlighted field, press the corresponding function key (F1 through F8). For example, in the following Method Options Screen, in order to change the OFF entry to ON (and thereby enabling the STANDARDIZE option), press F1 ON.

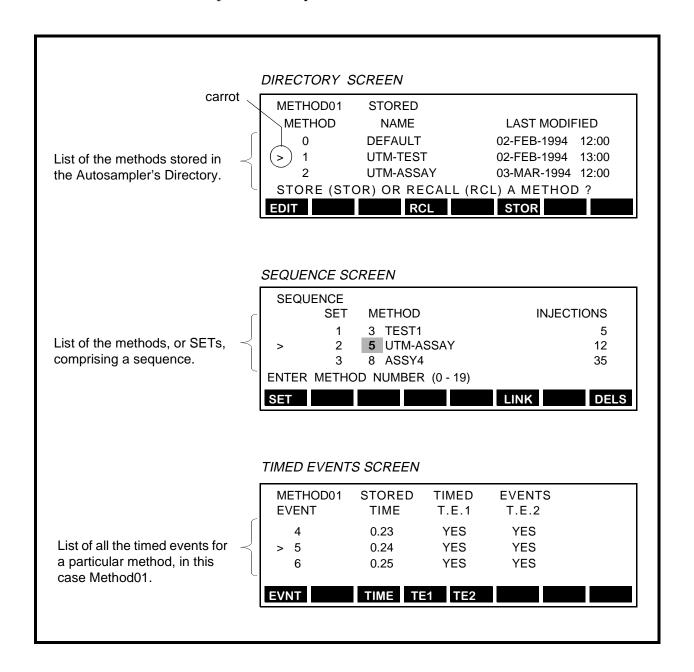


Scrolling Through Screen Lists

Use the cursor arrow keys,

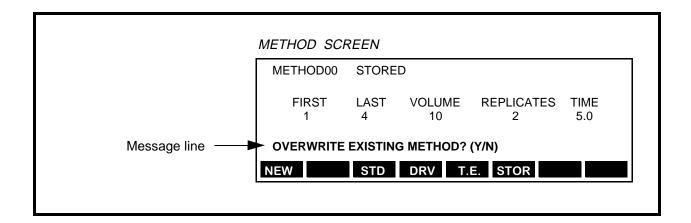


If a list of items is displayed on a screen, use the cursor arrow keys to move the carrot up and down through the list of entries. The following example shows the three most common types of screen lists, the Directory Screen, Sequence Screen and the Timed Events Screen.



Responding to Screen Prompts

To respond to a Y/N prompt appearing in the Message line of a screen, such as the one shown in the following Method Screen, press the $\boxed{1 \text{ yes}}$ numeric key to select Y and the $\boxed{0 \text{ no}}$ to select N.



Saving Changes to Screens

When you finish making changes to a screen, press the return key to save those changes. Changes you make to the default active method screens will remain in effect only until you turn off the power to the Autosampler. To permanently store changes to the default method, refer to Chapter 4 in this manual.

Instrument Specifications

Table 1-2. Specifications.

| Specification | Description |
|--|--|
| Injection range | Programmable from 0.1 - 1.0 μL at 0.1 μL increments, 1 μL to 2.5 mL at 1 μL increments |
| Replicate injections | 1 - 99 from one vial |
| Precision | Typically <0.5% RSD of peak areas from 3 μL |
| Linerarity | >0.999 |
| Minimum sample volume | 1 μL in 7 μL |
| Sample syringe sizes | 50, 100, 250, 500, 1000, 2500 μL |
| Sample waste | None (or user selectable) |
| Carryover | <0.02% |
| Flush cycles | User selectable, 1 - 9 |
| Flush volume | 0 - 2500 μL |
| Sample capacity | up to 225 vials with optional tray |
| Method parameters | Injection volume, sample draw speed, flush volume, flush cycle, 2 external contacts, start and stop time |
| Methods | Battery-backed storage of up to 20 methods including time programming. Editing of stored methods possible during runs. |
| Injection cycle time | 2 - 3 samples per minute |
| Valve materials in contact with sample | With Rheodyne 7725 for Analytical - stainless steel, Vespel, ceramic, PEEK With Rheodyne 9725 for Bio Applications - PEEK, Tefzel, ceramic With Rheodyne 8125 for Microbore - stainless steel, Vespel, ceramic, PEEK |
| Display | 7-line by 40-character backlit liquid crystal display |
| Communications | Integrated communications for derivatization and dilution options Serial communications - standard BCD interface - standard 5 relay outputs; 2 timed events, 2 inject, 1 start |
| AC Power Requirements: | |
| AC power | 70 Watts |

| Specification | Description |
|--|--|
| AC voltage range | Grounded 100, 120, 220, 230, VAC (±10%) Grounded 240 VAC (+6%/-10%) |
| Frequency | 50/60 Hz (<u>+</u> 1%) |
| Fuse rating characteristics: | |
| 100/120 VAC Single Fuse | 1.6 A 3 AG Slow Blow, 250 V |
| 220-240 VAC Single/Dual Fuse | 0.8 A TT (5x20 mm), 250 V |
| Line cord | See "Installing the AC Line Cord " section in Chapter 2 of this manual. |
| Operating Conditions : | |
| Environment | This instrument is designed for indoor use. It is not designed to operate in an explosive environment. |
| Ambient temperature | 10 - 30°C |
| Ambient relative humidity | 10 - 80% (without condensation) |
| Altitude | 0 to 2000 meters |
| Installation Category | This instrument is able to withstand transient overvoltage according to Installation Category II as defined in IEC 1010-1. |
| Pollution Degree | This instrument will operate safely in environments that contain nonconductive foreign matter to Pollution Degree 2 in IEC 1010-1. |
| Peltier sample cooling temperature range | 4 - 50°C, <u>+</u> 2°C |
| Storage Conditions: | |
| Ambient temperature | -20 to 60°C (-4 to 140°F) |
| Ambient relative humidity | 10 - 80% (without condensation) |
| Altitude | 0 to 12,000 meters |
| Dimensions | Height: 400 mm (15.8 in) Width: 318 mm (12.5 in) Depth: 483 mm (19 in) |
| Weight | 21 kg (46 lbs) |

Installation 2

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   Solvents with Low Boiling Points 2-2
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```

This chapter describes how to install your Series 200 LC Autosampler. It includes the following information: $\frac{1}{2}$

- Warnings and Precautions
- Preparing your laboratory
- Unpacking your Series 200 LC Autosampler
- Making the Electrical Connections
- Making the External Tubing Connections
- Making Input/Output Terminal Connections

Warnings and Precautions

Before installing the Series 200 LC Autosampler, read the following warnings and precautions.

Solvents

- Always use clean solvents. Solvents which have been distilled in glass (HPLC Grade) are recommended.
- Filter the solvents and buffers through a 0.5-micron medium as an additional precaution.
- Degas all aqueous and most organic solvents prior to use.

Note Mixtures of organics and water, once degassed, remain in a degassed state for longer periods of time than pure organics. This minimizes bubble formation in the transfer lines. In general, depending on your mobile phase, strengths are: 3:1 methanol:water, 1:1 methanol:water, or your specific mobile phase without buffer.

- Store flammable solvents or solvents which may form hazardous by-products when the instrument is shut down, by following the recommended shutdown procedure.
- Check compatibility of solvent(s) with the type of column(s) being used.
- Know the relative polarity and miscibility of the solvents being used.

Solvents with Low Boiling Points

Do not use liquids that have a boiling point less than 30°C.



Warning

Do not use carbon disulfide or other solvents which have an auto-ignition temperature below 110°C.

Buffers

Exercise care when using buffers in conjunction with organic solvents. NEVER LEAVE BUFFERS IN THE SYSTEM OVERNIGHT. Buffers left in the system can form salt crystals which may cause premature injector valve failure and plug the transfer tubing and sample needle. To remove buffers, flush the system with water followed by methanol or isopropanol. Remember to change the flush solvent from methanol or isopropanol to water before using buffers.

Corrosion

The following corrosion precautions apply to the standard (stainless steel) autosampler and the biocompatible (titanium) autosampler.

Standard Autosampler

All parts of the autosampler that contact mobile phase (from the pump) and flush solvent are made of stainless steel, Teflon, and glass. Some of these materials are extremely sensitive to acid chlorides. If you have questions about your mobile phase or flush solvent, contact a PerkinElmer representative. Refer to Table 2-1.

Table 2-1. Solvents Which May Corrode the Series 200 LC Autosampler.

| Aqua Regia (80% HCl, 20% HNO_3) | Bromine |
|---|-------------------------------|
| Hydrogen Peroxide | Freon 12 (wet) |
| Anhydrous Chloride | Chlorinated solvents |
| Dichloromethane | Hydrofluorosilicic Acid (20%) |
| Sulfuric Acid (Conc.) | Hydrobromic Acid (20%) |
| Hydrochloric Acid (20% and 37%) | Ferric Chloride |
| Hydrofluoric Acid (20%, 50%, and 75%) | Ferrous Chloride |
| Copper Chloride | Mercuric Chloride (Dilute) |
| | |

Biocompatible Autosampler

All parts of the Series 200 BIO LC Autosampler that contact the mobile phase (from the pump) and flush solvent are made of titanium, fluoropolymers, or glass. This makes the Series 200 BIO LC Autosampler inert to the most common mobile phases that are used for biological assays. If you have questions about your mobile phase, contact a PerkinElmer representative. Refer to Table 2-2.

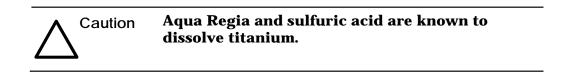


Table 2-2. Solvents Which May Corrode the Series 200 Bio LC Autosampler.

| Aqua Regia (80% HCl, 20% HNO ₃) | Anhydrous Methanol |
|---|-----------------------|
| Hydrogen Peroxide | Chlorinated solvents |
| Dichloromethane | Sulfuric Acid (Conc.) |

Solvents with Auto-Ignition Temperature Below 110°C

Certain solvents have a temperature at which they are combustible upon contact with a heated surface, even in the absence of a spark or other source of ignition. A list of common solvents with their auto-ignition temperatures are in Table 2-3.



Warning

Do not use carbon disulfide or other solvents which have an auto-ignition temperature below 110°C.

Table 2-3. Auto-Ignition Temperatures of Common LC Solvents.

| Solvent | Auto-Ignition Temp°C |
|---------------------------|----------------------|
| carbon disulfide | 100 |
| diethyl ether | 180 |
| cyclohexane | 260 |
| hexane | 261 |
| petroleum ether (naphtha) | 288 |
| pentane | 309 |
| THF | 321 |
| dioxane | 366 |
| propanol | 404 |
| iso-octane | 418 |
| ethanol | 423 |
| isopropyl ether | 443 |
| dimethylformamide | 445 |
| methanol | 446 |
| isopropanol | 455 |
| methylethylketone | 474 |
| acetonitrile | 524 |
| ethyl acetate | 524 |
| toluene | 536 |
| acetone | 538 |
| benzene | 562 |

Preparing Your Laboratory

Before installing your Series 200 LC Autosampler, prepare your laboratory according to the following guidelines:

Required Air Quality

To minimize contamination problems in your laboratory, provide a relatively dust-free environment. Make sure that the following gases or vapors are not present at levels exceeding federal, state, and local ordinances for continuous human exposure:

- Flammable
- Explosive
- Toxic
- Caustic
- Corrosive

Make sure that your laboratory environment consists of the following temperature and humidity levels:

- Ambient temperature between 10°C and 30°C
- Constant relative humidity between 20% and 80%, without condensation.

Use care when working with hazardous solvents or solvents that produce hazardous by-products.



Solvent vapor levels that are high enough to interfere with the detector performance should be considered hazardous to someone who is continuously exposed to the same vapors.

Adequate Bench Space

Provide bench or table space to accommodate the dimensions of the autosampler listed in the specification section. The weight of the Series 200 LC Autosampler is 21 kg (46 lbs). The bench must also support the weight of the detector and other instruments in the system. Provide space at the rear of the autosampler for air circulation, and provide access space on both sides of the autosampler.

Waste/Solvent Disposal

Provide a properly labeled chemical waste container in a safe and vented place. Make sure that it is within all of the specified safety requirements for your location. Wear gloves, eye protection, and a laboratory coat when handling or disposing of chemical waste.

Unpacking Your Series 200 LC Autosampler

Removing the Instrument from the Shipping Container

- 1. The weight of the autosampler is approximately 21 kg. For convenience, lifting points are provided in the packing foam at the front and rear of the autosampler.
- 2. Grasp the autosampler at the lift points and carefully lift it so that it clears the packing foam.
- 3. Check for obvious signs of damage that may have occurred during shipment. Immediately report damaged or missing items to the shipping carrier and PerkinElmer.
- 4. Save the original packing in case you need to return the instrument.

Items Included

When you open the shipping container, the first box you will see is the Startup Kit. Depending on your application, you will receive either the Start-Up Kit for the Series 200 LC Autosampler, Part No. N293-0305, or the Start-Up Kit for the Series 200 BIO Compatible LC Autosampler, Part No. N293-0306. For N293-0305, refer to the Start-Up Kit Packing List, Part No. 0993-6176; for N293-0306, refer to the Start-Up Kit Packing List, Part No. 0993-6177. The part numbers in these lists should help you to identify items in the kits, but do not use these part numbers to order replacements. To order replacement parts, refer to the PerkinElmer HPLC and CE Supplies Catalog or to a PerkinElmer Product Description List.

Removing the Packing Foam

- 1. Cut the shipping strap securing the needle assembly.
- 2. Hold the needle assembly in the most upright position.
- 3. Slide the packing material forward to remove as shown in Figure 2-1.

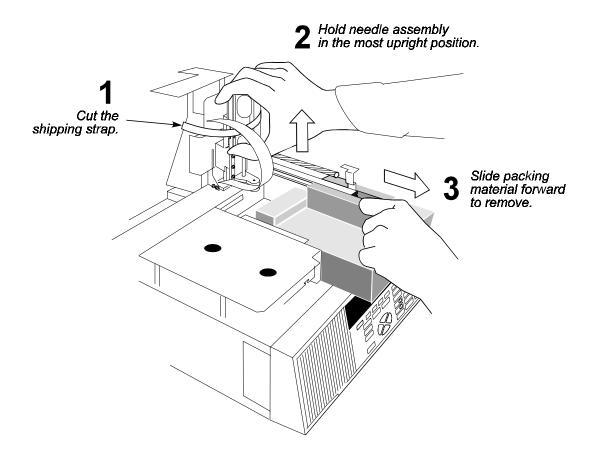


Figure 2-1. Removing the Packing Foam.

Making the Electrical Connections

Caution

The Series 200 LC Autosampler is shipped from the factory with:

- The operating voltage set to 120 VAC.
- The main AC power switch set to the OFF position.
- No fuses installed.

You need to select the appropriate voltage setting and install the proper fuse(s) before installing the line cord and applying AC power.



Checking Electrical Power Requirements

The Series 200 LC Autosampler requires a grounded, nominal 100-, 120-, 220-, 230-, or 240 VAC source. The Series 200 LC Autosampler uses 70 Watts maximum power.

The AC line voltage must remain within $\pm 10\%$ of the nominal specified voltage (+6%/-10% for 240 VAC). If the electrical supply voltage produces large AC line voltage fluctuations, install a voltage regulator between the AC line voltage outlet and the Series 200 LC Autosampler.

Instrument grounding is required and is accomplished by a third wire in the line voltage box. If your electrical outlet does not contain a ground, then contact your local electrical contractor.



Setting the Operating Voltage

The power input module (located on the rear panel) contains an outlet for the AC line cord, the voltage selector card, and the system fuse(s). Inside the power input module is a small voltage selector card that you need to set to the local line voltage. The selected voltage is indicated by a white dot in the power input module cover next to the displayed voltage. Table 2-4 shows the setting to use for various line voltages.

| Table 2-4. | Voltago | Salactor | Settings. |
|-------------|---------|----------|-----------|
| 1 anie 2-4. | vonage | Selector | Settings. |

| AC Voltage Range | AC Voltage Tap | Set Voltage Selector To: |
|------------------|----------------|--------------------------|
| 90-110 VAC | 100 | 100 |
| 108-132 | 120 | 120 |
| 198-242 | 220 | 220 |
| 207-253 | 230 | 240 |
| 216-264 | 240 | 240 |

To set the Series 200 LC Autosampler operating voltage:

- 1. Make sure the main power switch is in the OFF position.
- 2. Refer to Figure 2-2. Remove the power input module cover by inserting a small flat blade screwdriver into the notch and carefully pry off the cover.
- 3. Remove the voltage selector card from the power input module.

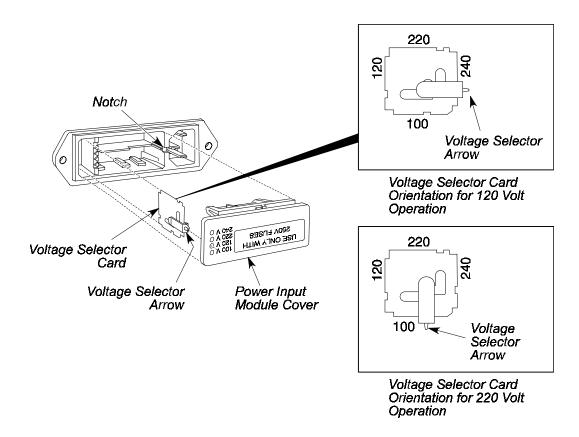


Figure 2-2. Power Input Module (exploded view).

- 4. Position the number (100, 120, 220, 240) on the side of the voltage selector card so that it is **opposite** the voltage selector arrow. THIS NUMBER MUST CORRESPOND TO THE VOLTAGE FOR YOUR LOCATION. Figure 2-2 shows the voltage selector card settings for both the 120 V and the 220 V position.
- 5. Reinstall the voltage selector card into the power input module.

3

Installing the Primary Fuse(s)

After setting the voltage range, select and install the proper fuse(s). The fuse block is located on the power input module cover. See Figure 2-3 for the location of the fuse block.

Single Fuse Configuration

1. Select the required fuse from Table 2-5 below.

Table 2-5. Single Fuse Configuration Table (Use only 3AG 1/4 x 1-1/4" 250 V Fuses).

| Voltage Selector | Insert the following fuse: | P/N |
|------------------|----------------------------|-----------|
| 100 | 1.6 amp Slow-Blow | 0998-1613 |
| 120 | 1.6 amp Slow-Blow | 0998-1613 |
| 220 | 0.8 amp Slow-Blow | 0999-1671 |
| 240 | 0.8 amp Slow-Blow | 0999-1671 |

- 2. Slide the fuse into the fuse holder. See Figure 2-3.
- 3. Reinsert power input module cover back into power input module.

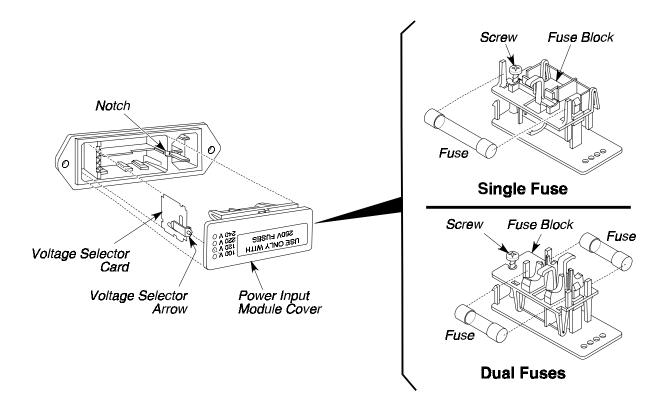


Figure 2-3. Fuse Location.

Dual Fuse Configuration

1. Select the required fuse from Table 2-6 below.

Table 2-6. Dual Fuse Configuration Table (Use only 5 x 20 mm 250V Rated Fuses).

| Voltage Selector | Insert the following fuses: | P/N |
|------------------|-----------------------------|-----------|
| 220 | 0.8 A Type TT | 0999-1670 |
| 240 | 0.8 A Type TT | 0999-1670 |

- 2. Loosen the screw on the fuse block and remove the fuse block from the power input module cover. See Figure 2-3.
- 3. Invert the fuse block and reinstall it on the power input module cover. Tighten the screw.
- 4. Slide two fuses into the fuse block.
- 5. Reinsert the power module cover back into the power input module.

4

Installing the AC Line Cord



Warning

Ground circuit continuity is vital for safe operation of equipment.

Never operate equipment with grounding conductor disconnected. Do not remove or alter the ground pin on a three-prong line cord.

The line cord should have the electrical rating and plug appropriate for your location. See Figure 2-4 for the correct cord.

- 1. Select the proper line cord for your location.
- 2. Insure that the power switch is off and insert the line cord plug into the AC supply.

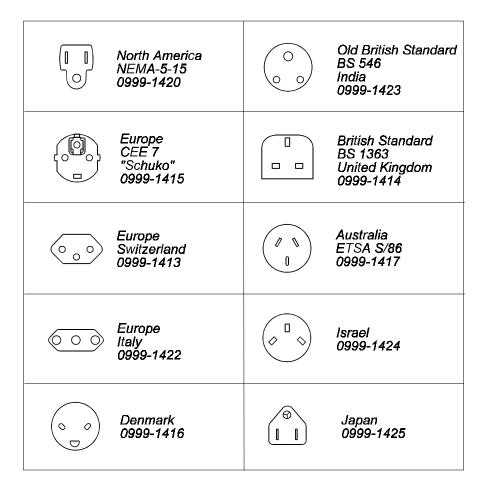


Figure 2-4. AC Line Cord Selections.

Making the External Tubing Connections

This section contains procedures describing how to make all the necessary external tubing connections and includes:

- 1. Connecting the drain hoses to the solvent waste container
- 2. Connecting the Autosampler to the column
- 3. Connecting the Autosampler to the pump
- 4. Installing and connecting the sample needle
- 5. Connecting the flush pump syringe tubing to the solvent container

For your convenience, all of the required internal tubing connections <u>to</u> the injector valve of the Series 200 LC Autosampler are made at the factory. If you need to change the sample loop, replace tubing or connectors, refer to Chapter 7 in this manual.

Figure 2-5 provides an overview of typical tubing connections between the Autosampler and other instruments making up an LC system.

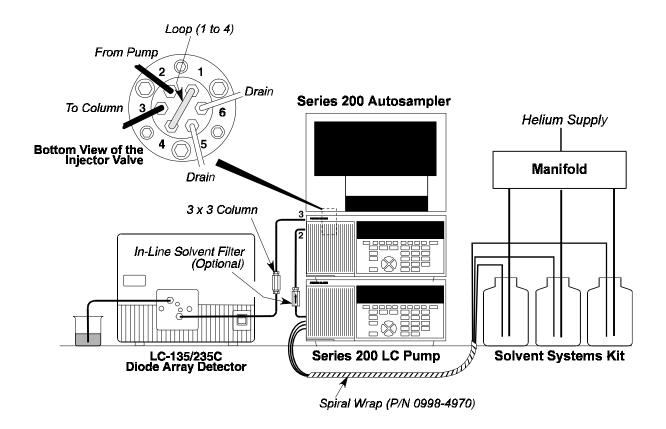


Figure 2-5. Typical Tubing Connections in an LC System.

1 Connecting the Drain Hoses to the Solvent Waste Container

- 1. Refer to Figure 2-6 which shows the location of the drain hoses on the left side of the Autosampler.
- 2. Place the free ends of both drain hoses into the solvent waste container.

Important! Both drain hoses rely on gravity to carry away waste solvent. Make sure to place the solvent waste container lower than the Autosampler.

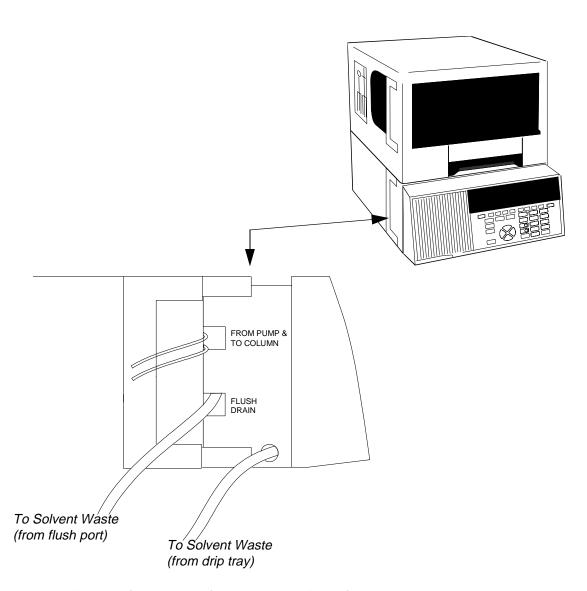


Figure 2-6. Connecting Waste Lines to Waste Container.

2 Connecting the Autosampler to the Column

- 1. Refer to Figure 2-7. Using nuts and ferrules recommended by the column manufacturer, add a nut and ferrule to the end of the top section of tubing coming from the top cutout. Note that the other section of tubing coming from the top cutout is labelled "Pump".
- 2. Thread the nut and ferrule into the column.

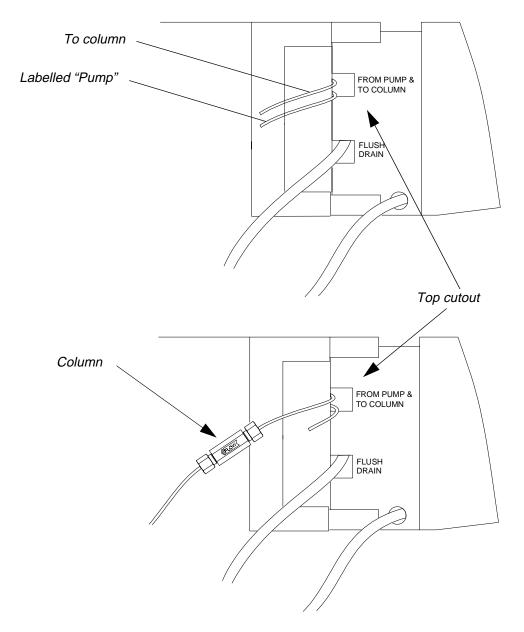


Figure 2-7. Connecting the Autosampler to a Column.

3 Connecting the Autosampler to the Pump

- 1. You can connect the other section of tubing coming from the top cutout, labelled "Pump," directly to the pump, or you can insert an in-line device between the pump and the Autosampler. Table 2-7 lists some common types of in-line devices. Figure 2-8 shows two types of in-line devices.
- 2. Add a nut and ferrule to the end of the tubing going to the pump or to the in-line device. Use a nut and ferrule recommended by the pump manufacturer, or one that is suitable for the in-line device.

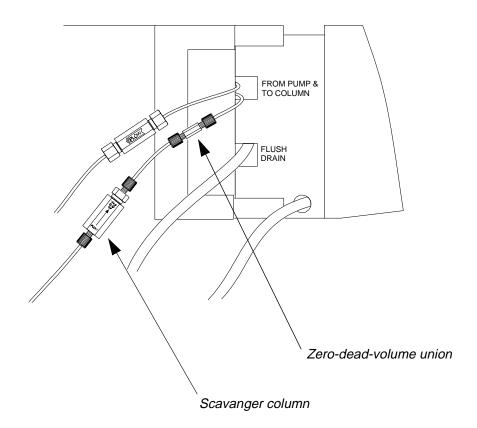


Figure 2-8. Connecting the Autosampler to a Pump

Table 2-7. Types of In-Line Devices Between Pump and Autosampler.

| In-Line Device | Part No. | Description |
|---------------------------|--|---|
| Zero-dead-volume union | Parker-Hannifin Zero- Dead-Volume Union, PerkinElmer Part No. 0990-3289 | This union combines two pieces of 1/16-inch tubing for a zero-dead-volume union. This type of union makes it easy for you to disconnect the Autosampler from the pump. |
| Scavanger Column | PerkinElmer Scavanger Column Kit, Part No. 0258-0204 | Scavanger columns are placed between the pump and the Autosampler to protect the analytical column from particulate matter and any absorbed contaminants in the mobile phase. |
| In-line solvent filter | PerkinElmer In-Line Solvent Filter System, Part No. 0990-3606 | This filter is similar to the Scavanger Column. |

4

Installing and Connecting the Sample Needle

1. Locate the following items in the appropriate startup kit. The part numbers for each of the available start up kits are listed in Table 2-8 for your convenience.

 Table 2-8.
 Parts Needed for Installing and Connecting the Sample Needle.

| Qty | Description | SS Startup Kit N293-0305 Part Numbers | Bio Startup Kit N293-0306 Part Numbers |
|-----|------------------------------|---|--|
| 1 | Sample Needle | N293-0023 | N293-0024 |
| 1 | Needle Cartridge | N293-1012 | N293-1012 |
| 2 | Needle Cartridge Thumbscrews | 0992-0672 | 0992-0672 |
| 1 | Slotted Needle Nut | N293-1064 | N293-1064 |
| 1 | Needle Spring | 0992-0429 | 0992-0429 |
| 1 | Union | 0990-3289 | 0990-3289 |
| 1 | Union Holder | N293-1081 | N293-1081 |
| 1 | Screw M3 X 6 | 0991-9200 | 0991-9200 |
| 1 | Clamp | 0998-4738 | 0998-4738 |
| 2 | PEEK Nut | 0992-0513 | 0992-0513 |

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2. Lift the needle assembly up and move it over the injector valve port. The location of the injector valve port is shown in Figure 2-9.

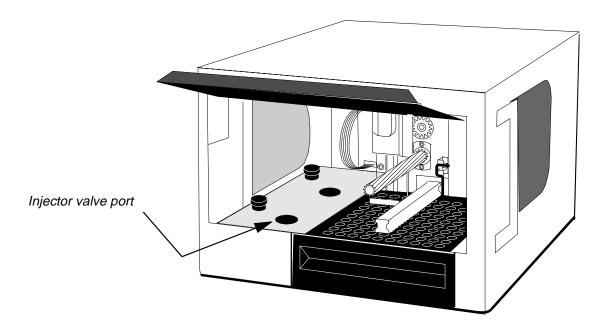


Figure 2-9. Location of the Injector Valve Port.

The following steps are keyed to Figure 2-10.

- 3. Slide the needle spring over the curved top portion of the sample needle as shown in Figure 2-10.
- 4. Slide one of the PEEK nuts over the end of the sample needle as shown.
- 5. Thread the PEEK nut on the sample needle into the top portion of the union.
- 6. Slide the union holder up over the sample needle and union. Make sure the union holder is oriented as shown, facing forward.
- 7. Insert the sample needle/union holder assembly into the needle cartridge.
- 8. To hold the sample needle in place, thread and tighten the slotted needle nut into the top of the needle cartridge as shown.
- 9. Press and <u>hold the needle cartridge up against the face plate so that the lower surfaces of the two plates are flush</u>. While holding the needle cartridge securely in place, tighten the two thumbscrews.
- 10. Locate the sample tubing coming from the sample syringe. Slide the sample tubing under the curved top portion of the sample needle as indicated in the lower portion of Figure 2-10.
- 11. Slide the remaining PEEK nut over the end of the sample tubing and thread it into the bottom of the union.
- 12. Secure the sample tubing to the needle cartridge using the clamp and screw.

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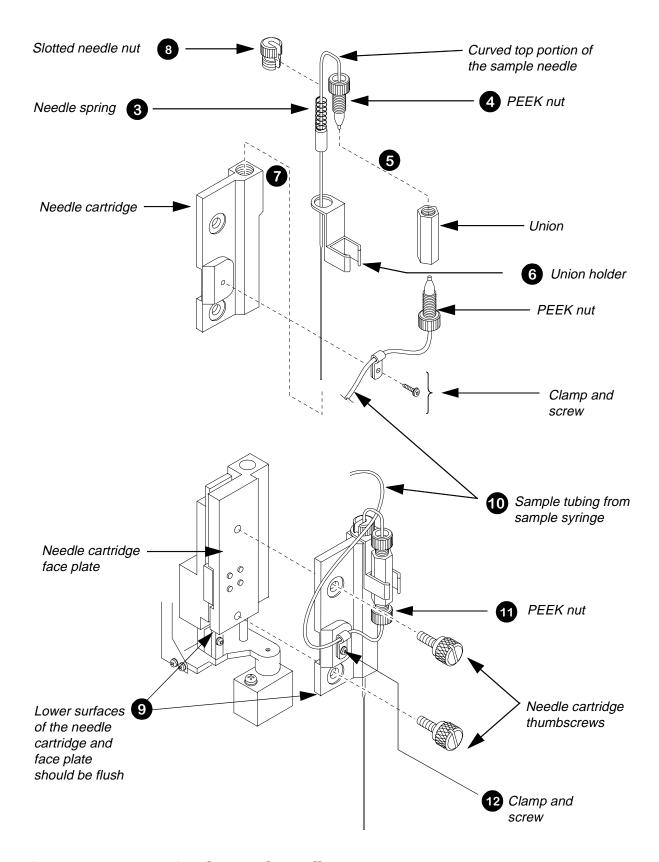


Figure 2-10. Connecting the Sample Needle.

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Connecting the Flush Syringe Tubing to the Solvent Container

- 1. Refer to Figure 2-11. Locate the flush line tubing with the solvent filter (Part No. 0990-3615) attached to the free end. The other end of the flush line tubing is already connected to the flush pump syringe.
- 2. Place the solvent filter into the solvent reservoir.

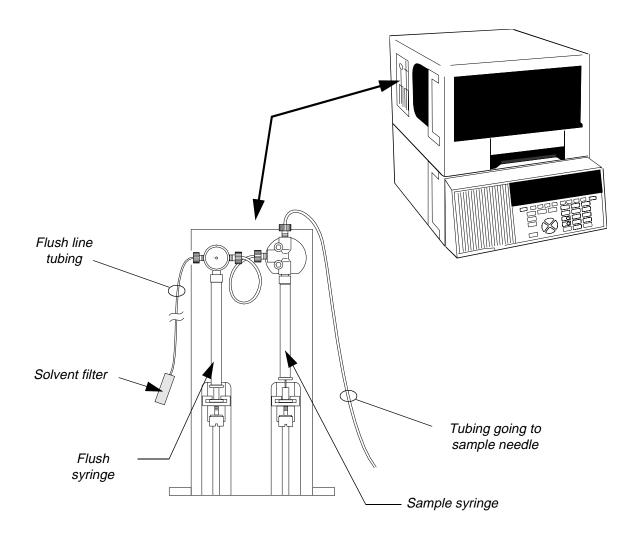


Figure 2-11. Connecting the Flush Line Tubing to the Solvent Container.

- 3. Apply power to the Autosampler.
- 4. Press the flush key on the keyboard and flush solvent through the system until there are no bubbles in the sample tubing. To insure that there are no bubbles, inspect closely the tubing near the point where it enters the Tefzel union.

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Making Input/Output Terminal Connections

The input/output terminal on the Series 200 LC Autosampler uses phone jack connectors for quick contact closure connections between instruments. Figure 2-12 shows the input/output terminal connectors on the right-side panel of the Series 200 Autosampler. Table 2-9 provides a description of each input/output connector.

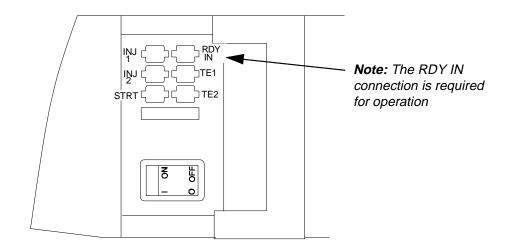


Figure 2-12. The Series 200 LC Autosampler Terminal Connectors.

Table 2-9. Input/Output Terminal Connectors.

| Input\Output Connector | Description |
|--------------------------------------|---|
| INJ1 | This contact closure activates (for a duration of 2 seconds) when the injection value rotates from load to inject. This is typically used to start an external device (typically a pump). |
| INJ2 | Identical to INJ1 except this is typically used to start a data handling system. |
| START | This contact closure activates at the beginning of the sampling operation and is typically used to autozero the detector. |
| READY IN (Required for operation) | This contact closure is used when the Autosampler is controlled by an external device, typically a pump. The Autosampler waits to start sampling until this contact closes, thereby synchronizing the timing between the Autosampler and the controlling external device. |
| TE1 and TE2 | These contact closures actuate at a time specified in the Method and control an external device. When activated, the TE1 and TE2 contacts are switched and held for 2 seconds. |

Making a Connection between Modular Cable and Twisted Pair Wires

You will need to make this connection when you are connecting the Autosampler to accessories like the LC Series 200 Pump, the LC-135/235C Diode Array Detector, or Turbochrom. The proper way to connect the modular cable and the two wires from one of these accessories is to use the modular connector (Part No. 0999-6640) that is supplied in the Start Up Kit. To make a connection:

- 1. Refer to Figure 2-13. Cut off about 10 mm of plastic sleeve from each of the two wires. Insert the two wires into the two center positions (labelled "R" and "G") in the inverted top piece.
- 2. Combine the top and bottom pieces. Secure them with the Phillips screw.
- 3. Plug the modular connector into the I/O panel connector on the Series 200 LC Autosampler. Figure 2-13 shows a typical connection between the Autosampler and the Series 200 Pump.
- 4. Refer to Figures 2-14 and 2-15 for examples of typical I/O connections between the Autosampler and other instruments making up an LC system.

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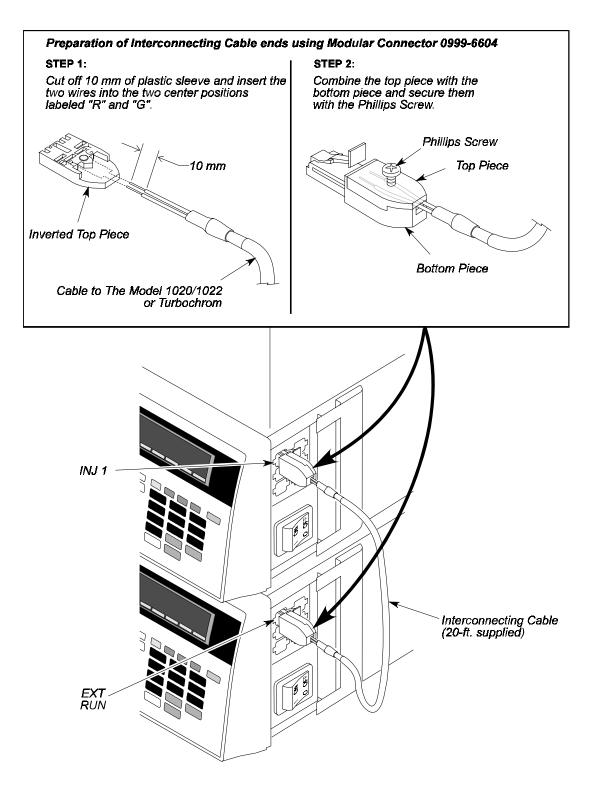


Figure 2-13. Connecting a Wire to the Modular Connector.

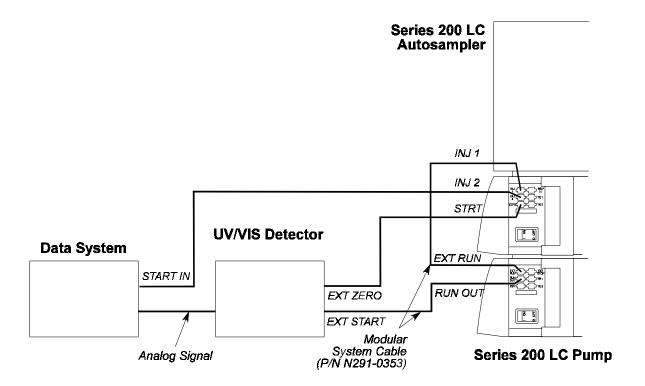


Figure 2-14. System Connection Diagram: Series 200 LC Autosampler, Series 200 LC Pump, LC UV/VIS Detector, and a Data System.

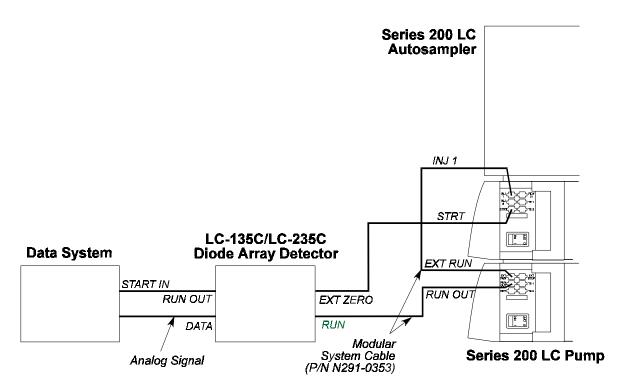


Figure 2-15. System Connection Diagram: Series 200 LC Autosampler, Series 200 LC Pump, LC-235C Diode Array Detector, and a Data System.

Configuring the Autosampler 3

Powering Up the Autosampler .. 3-2 The Default Top Level Main Screen .. 3-2 Reviewing the Default Performance Parameters .. 3-2 Defining System Parameters .. 3-5 Adding Method Options .. 3-6 Setting the Screen Contrast and Screen Saver .. 3-12 Setting the System Clock .. 3-13 Checking the Hardware Options (tray type) .. 3-14 Setting the Error Recovery Mode .. 3-15 Defining the Communications Options .. 3-16 Serial Ports .. 3-16 BCD Port Output .. 3-16 Accessing the Communication Screen .. 3-18 Selecting the Communication Option .. 3-19 Setting the Temperature of the Optional Peltier Accessory .. 3-20 Accessing the Peltier Accessory Screen .. 3-20

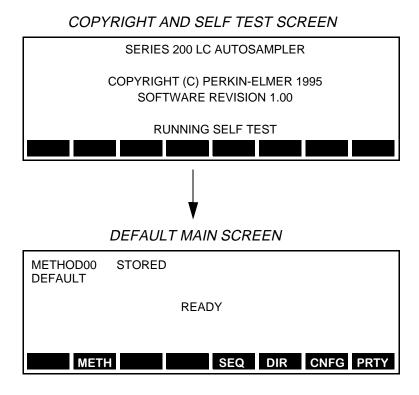
Configuring the Autosampler 3

This chapter describes how to configure your Series 200 LC Autosampler. It includes the following information:

- Powering up the Autosampler
- Reviewing the Default Injection Performance Parameters
- Defining System Parameters
 - Adding Method Options
 - Setting the Screen Contrast
 - Setting the System Clock
 - Checking the Hardware Options
 - Defining the Error Recovery Mode
- Defining Communications Options
- Setting the Temperature of the Optional Peltier Accessory

Powering Up the Autosampler

Press the power switch located on the lower right side of the Autosampler to the ON position. The Copyright and Self Test screen first appears on the Liquid Crystal Display. The display is located directly above the keyboard on the front panel. As soon the self test is completed, the Main Screen appears.



The Default Top Level Main Screen

The Main Screen provides access to all of the Autosampler's functions. The soft keys displayed along the bottom of the default Main Screen correspond to the functions that are available when you first power up the Autosampler. Later in this chapter you will see how to add other optional functions to the Main Screen.

Reviewing the Default Performance Parameters

Table 3-1 lists the default sample injection and flush pump parameters for the Autosampler. These parameters have been set to insure high precision, low carryover, high throughput and economical use of sample. If you need to change any of these settings, due to a special requirement of your analysis, you can do so from the Default Main Screen as described in Appendix B, "Changing the Performance Parameters".

 Table 3-1.
 Default Performance Parameters

| | Parameter | Description | Default Value | Acceptable Value or Range |
|----|------------------------------|--|-------------------------------------|--------------------------------------|
| 1. | Air Cushion | Volume of air drawn up before sample. Air cushion is used to prevent mixing of the flush buffer and the sample. | 10 μL | 1 - 99 μL |
| 2. | Loop Mode | Partial = loop is partially filled with the volume specified during method setup. Fixed = loop fills up completely with sample. If you change the default from partial to fixed, refer to Appendix D in this manual for the recommended sample loop and sample syringe sizes. | Partial | Partial/Fixed |
| 3. | Excess Volume (Sample) | Extra volume of sample drawn up during sampling operation. Note: in the Fixed Loop Mode, the sample volume drawn up is already ten times the loop volume. The excess volume is then added to that volume. | 10 μL | 0 - 100 μL |
| 4. | Sample Pump Syringe Size | The size of the syringe used to draw up the sample. | 250 μL | 50, 100, 250, 500, 1000, 2500 μL |
| 5. | Sample Pump Syringe Speed | The speed at which the sample syringe picks up and dispenses sample. The speed varies with the size of the sample pump syringe. Note that for viscous samples, you should set the speed to VSLO (very slow) or SLOW. | MEDM for all syringe sizes | VSLO SLOW MEDM FAST VFST |
| 6. | Sample Pump Syringe Pause | The pause time following each sample syringe movement. This allows the air segment between the sample and the flush buffer to equilibrate. | 2 seconds | 0 - 10 seconds |
| 7. | Delay Time | This is used for gradient methods. It allows you to delay the sample injection by this amount of time to compensate for the dead volume between the pump and the Autosampler. | 0.00 minute | 0.00 - 999 minutes |
| 8. | Time Saver | If this feature is enabled (On), it allows the Autosampler to get the next sample and perform sample preparation before the other modules (pump and detector) are ready. | Off | On/Off |
| 9. | Needle Level | This determines how far the sample needle goes into the sample vial. The value corresponds to a percentage of the total distance from the shoulder (top) of the sample vial to the bottom of the sample holder. For example, 0% is at the bottom of the sample holder; 99% is at the shoulder of the vial. | 10% | 1 - 99 % |

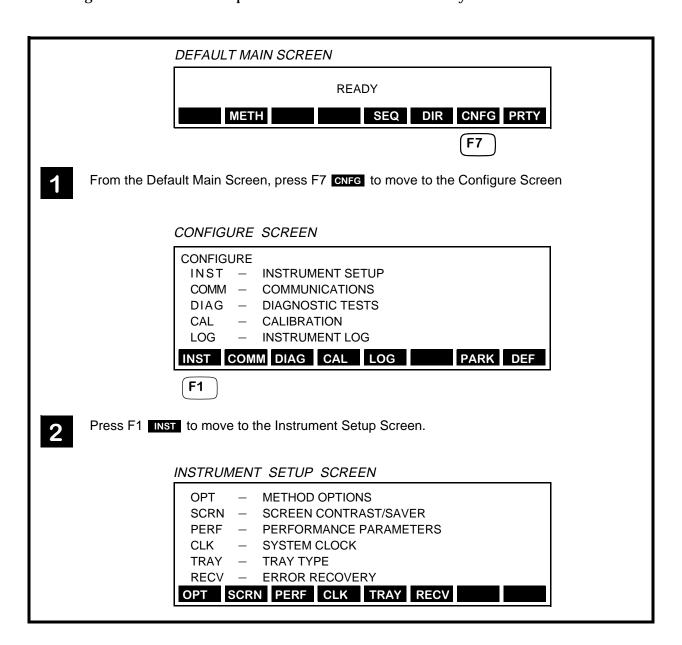
| Parameter | Description | Default Value | Acceptable Value or Range |
|------------------------------------|---|------------------|--------------------------------------|
| 10. Vent Mode | When this feature is enabled (On), a volume of air, equal to the volume of sample and buffer to be withdrawn from the vial, is first injected into the sample vial. This prevents a vacuum from developing when multiple samples are withdrawn from the vial. | On | Off/On |
| 11. Flush Volume | The volume of flush liquid used for each cycle during the pre- and post-injection flush operation. | 700 μL | 0 - 2500 μL |
| 12. Speed of the Flush Syringe | The speed at which the flush syringe picks up buffer from the buffer reservoir and ejects it through the sample needle. | MEDM | VSLO SLOW MEDM FAST VFST |
| 13. Pre-injection Flush Cycles | The number of flush cycles before sample is injected. | 1 | 0 - 9 |
| 14. Post-injection Flush Cycles | The number of flush cycles after sample is injected. | 1 | 0 - 9 |
| 15. End of Method Flush Cycles | The number of flush cycles at the end of a method. | 0 | 0 - 9 |
| 16. Hard Key Cycles | The number of times the flush syringe will cycle when you press the flush key. | 2 | 1 - 9 |
| 17. Reagent Mixing Speed - In | The speed of the flush syringe when it picks up sample plus reagent from the vial through the sample needle for mixing. Note that for viscous samples, you should set the speed to VSLO (very slow) or SLOW. | SLOW | VSLO SLOW MEDM FAST VFST |
| 18. Reagent Mixing Speed - Out | The speed of the flush syringe when it ejects sample plus reagent through the sample needle back into the vial for mixing. | FAST | VSLO SLOW MEDM FAST VFST |

Defining System Parameters

This section describes how to make changes to the Autosampler's system parameters and includes:

- Adding Method Options
- Setting the Screen Contrast and Screen Saver
- Setting the System Clock
- Checking the Hardware Options (tray type)
- Setting the Error Recovery Mode

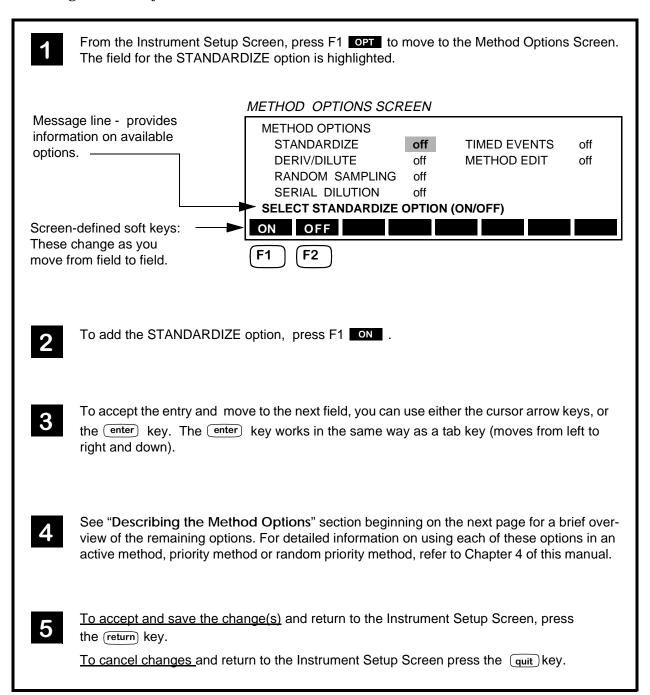
<u>You can perform all of these functions from the Instrument Setup Screen</u>. The procedure for accessing the Instrument Setup Screen is summarized here for your convenience.



Adding Method Options

The Method Options Screen allows you to customize the Series 200 LC Autosampler so that only those operations which you commonly perform are presented as selections in the Autosampler screens. You may find it convenient to use the Autosampler's default configuration if you inject primarily samples and do not vary their injection volumes.

If you need to use calibrants, or perform dilutions or derivatizations, the steps in this section show you how to add those options. Note that <u>the options you add here apply to all methods</u>, including the Priority Method.



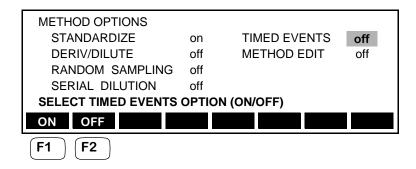
Describing the Method Options

This section gives a brief description of the options provided by the Method Options Screen. As you move to and highlight each option field, directions for selecting the option appear in the message line and the soft keys change to reflect the choices available for that option. For detailed information on using each of these options in a method, refer to "Defining the Active Method" in Chapter 4 of this manual.

STANDARDIZE Option

The STANDARDIZE Option allows you to add up to 7 calibrants to a method. Directions for enabling this option are on the previous page.

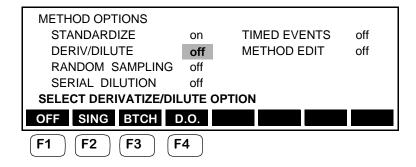
TIMED EVENTS Option



The TIMED EVENTS option allows you to control relay contact closures which in turn control auxiliary instruments, like a column switching valve or a fraction collector. When actuated, the relays close for two seconds, then reopen. This option allows you to activate two timed events (TE1 and TE2) up to 20 times during a method. The relay contact closures, TE1 and TE2, are located on the Input/Output Terminal (on the right side panel of the Autosampler) and are described in Chapter 2 of this manual.

To add the TIMED EVENTS option, press F1 ON .

DERIV/DILUTE Option



The DERIV/DILUTE option allows you to:

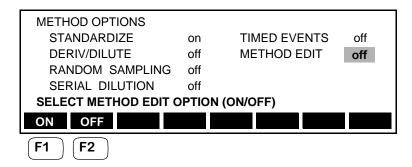
- Perform derivatizations by adding up to 5 different reagents to the sample vials.
- Perform dilutions by adding diluent to the sample vials.

| Note 1 | Reagents and diluent are only added to sample vials and not to vials designated as containing calibrants. |
|--------|--|
| Note 2 | In order to perform dilutions, you need to insure that the dilution sample tray is installed in the Autosampler. |

To add the DERIV/DILUTE option, press F2, F3 or F4:

- F2 If you select the SING (single) option, the Autosampler will perform the derivatization or dilution operation on the first sample in the method and then inject that sample.
- F3 If you select the BTCH (batch) option, the Autosampler will first perform the derivatization or dilution operation on all of the samples in a method; it will then return to the first sample and begin the injections.
- F4 If you select the D.O. (derivatization/dilution only) option, the Autosampler will only perform the dilution or derivatization on the samples. It will not inject the samples.

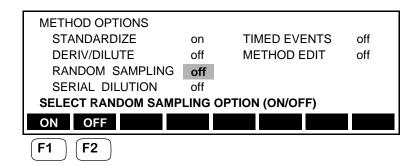
METHOD EDIT Option



The METHOD EDIT option allows you to retrieve a stored method from the directory, modify it, and save it while a different (active) method is running. You can also leave the edit session to perform other operations on the Autosampler and then return and continue editing without losing any of your changes.

To add the METHOD EDIT option, press F1 ON.

RANDOM SAMPLING Option



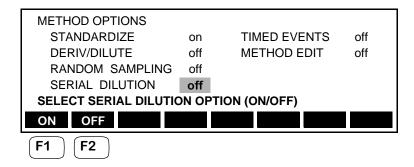
The RANDOM SAMPLING option allows you to sample vials in a nonconsecutive or random manner. For example, by enabling this option you can program the Autosampler to pick up sample from vial 1, then move to and pick up sample from vials 12 through 18, then go back and pick up sample from vial 6.

By adding the RANDOM SAMPLING option, you replace the Priority Method with the Random Priority Method.

| Note 1 | You cannot include calibrants, perform dilutions or derivatizations, or program timed events in a Random Priority Method. |
|--------|--|
| Note 2 | Unlike methods, you can only have one Random Priority Method. |
| Note 3 | Enabling RANDOM SAMPLING <u>does not</u> affect your ability to add calibrants or perform dilutions, derivatizations, or include timed events in the active (or stored) methods. |

To add the RANDOM SAMPLING option, press F1 ON.

SERIAL DILUTION Option



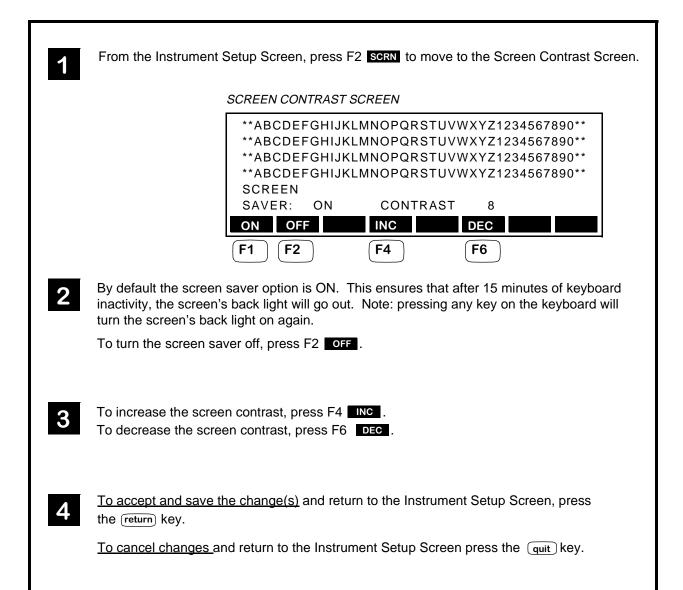
The SERIAL DILUTION option allows you to perform a serial dilution starting from a single calibrant and then making a series of vial to vial transfers. No injections are performed between the vial to vial transfers.

| Note 1 | Unlike methods, you can only have a single serial dilution program. |
|--------|---|
| Note 2 | The start key and the priority key are disabled in the serial dilution. |

To add the SERIAL DILUTION option, press F1.

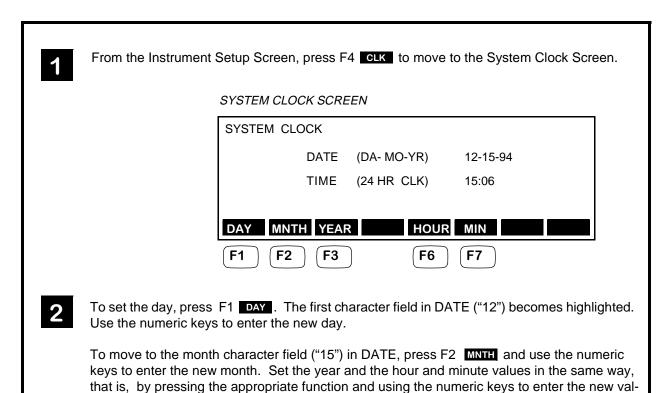
For more information, refer to Appendix C, "Setting up Serial Dilutions" in this manual.

Setting the Screen Contrast and Screen Saver



ues.

Setting the System Clock

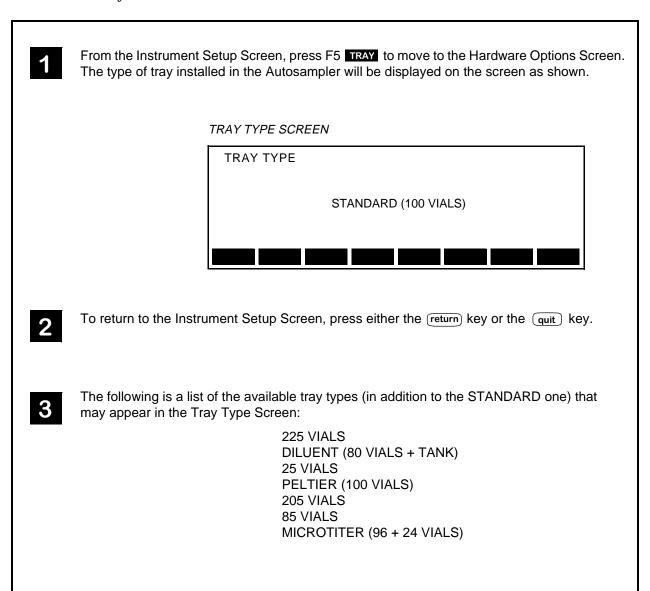


To accept and save the change(s) and return to the Instrument Setup Screen, press the return key.

<u>To cancel changes</u> and return to the Instrument Setup Screen press the <u>quit</u> key.

Checking the Hardware Options (tray type)

The Tray Type screen provides information about the type of sample tray that is installed in the Series 200 LC Autosampler. This screen is for information only and does not contain any soft function keys.



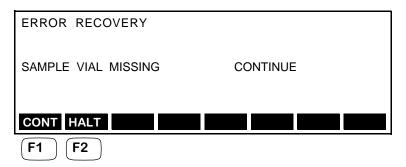
Setting the Error Recovery Mode

This Error Recovery Screen allows you to specify how the Series 200 LC Autosampler responds to a missing sample vial.

1

From the Instrument Setup Screen, press F6 RECV to move to the Error Recovery Screen.

ERROR RECOVERY SCREEN.



Press F1 CONT if you want the Autosampler to resume and continue processing sample vials if it encounters a missing sample vial.

Press F2 HALT if you want the Autosampler to stop processing any additional sample vials and terminate the analysis if it encounters a missing sample vial.

Note If a <u>reagent</u> or <u>calibrant</u> vial is missing, the method or sequence will terminate.

To accept and save the change(s) and return to the Instrument Setup Screen, press the return key.

To cancel changes and return to the Instrument Setup Screen, press the quit key.

Defining the Communications Options

This section describes how to select either the seriel communication ports or the Binary Coded communication port on the rear panel of the Autosampler. It also describes how to specify the output format for the BCD port. The location of these ports on the rear panel of the Autosampler is shown here for your convenience.

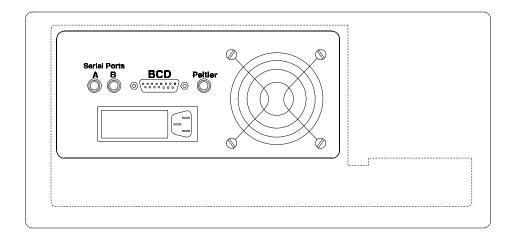


Figure 3-1. Location of the Communication Ports on the Rear Panel of the Autosampler.

Serial Ports

<u>If you intend to control the Autosampler using Turbochrom</u> through a 600 Series Link box, you will need PerkinElmer cable Part No. N293-0343.

<u>If you intend to control the Autosampler using a 1022 Integrator,</u> you will need Perkin Elmer cable Part No. N293-0344.

If you are connecting through the serial ports using either one of the above cables, you will need to select the EXTERNAL CONTROL option on the Communication Screen.

BCD Port Output

The BCD port supports two output formats, the BCD format and the Binary Data format. The BCD format is a two digit format (00 ... 99), and the Binary Data format is a three-digit format (000 ... 255).

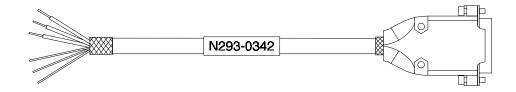
If you intend to program the Autosampler so that all control is done at the instrument and you only need to output vial numbers, use:

- PerkinElmer cable Part No. N293-0340 when connecting to Turbochrom or,
- PerkinElmer cable Part No. N293-0341 when connecting to the 1022 Integrator or,
- PerkinElmer generic cable Part No. N293-0342 when connecting to some other device (the pin-outs for this cable are provided on the next page).

If you are connecting to the BCD port using one of these cables, you need to select the BCD NEGATIVE option on the Communication Screen.

Pin assigments for PerkinElmer Generic BCD Cable, Part No. N293-0342

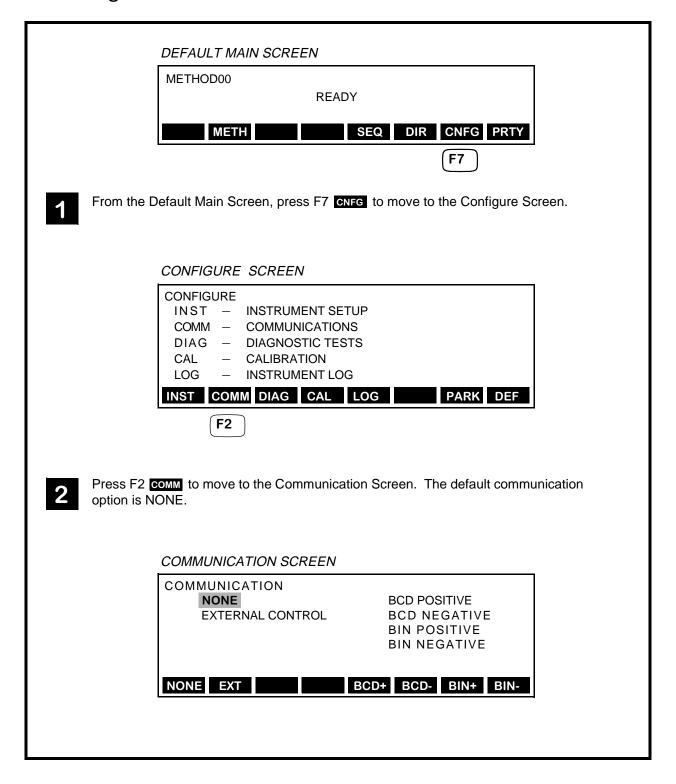
Figure 3-2 shows the PerkinElmer Generic BCD cable and the pin assignments.



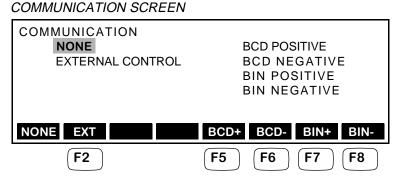
| PIN | Color | BCD | BIN |
|-----|-------------|----------------|----------------|
| 1 | Black | BCD-01 | 1 |
| 2 | Brown | BCD-02 | 2 |
| 3 | Red | BCD-04 | 4 |
| 4 | Orange | BCD-08 | 8 |
| 5 | Yellow | BCD-10 | 16 |
| 6 | Green | BCD-20 | 32 |
| 7 | Blue | BCD-40 | 64 |
| 8 | Violet | BCD-80 | 128 |
| 10 | Gray | Tray - Present | Tray - Present |
| 11 | White | Vial - Present | Vial - Present |
| 12 | White/Black | Data - Valid | Data - Valid |
| 14 | Shield | GND | GND |
| 15 | Shield | GND | GND |

Figure 3-2. Pin Assignments for Generic BCD Cable.

Accessing the Communication Screen



Selecting the Communication Option



To select a communication option, press the corresponding soft function key. For example, to select BCD POSITIVE, press F5 BIN+ . The available options are summarized below.

BCD POSITIVE: Binary Coded Decimal - positive polarity. Range of values for output: 0 - 99. BCD NEGATIVE: Binary Coded Decimal - negative polarity. Range of values for output: 0 - 99.

BIN POSITIVE: Binary Data - positive polarity. Range of values for output: 0 - 255. BIN NEGATIVE: Binary Data - negative polarity. Range of values for output: 0 - 255.

EXTERNAL: Choosing this selection simultaneously enables the serial ports A and B and

disables the BCD port.

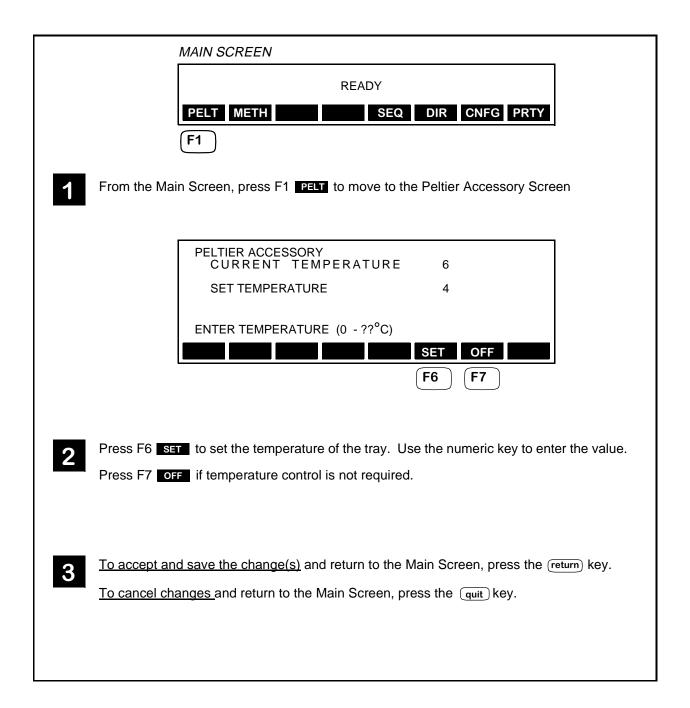
To accept and save the change(s) and return to the Configure Screen, press the return key.

To cancel changes and return to the Configure Screen press the quit key.

Setting the Temperature of the Optional Peltier Accessory

This section describes how to set the temperature of the Peltier Accessory. Make sure the Peltier tray is in the Autosampler and the cable coming from the tray is plugged into the Peltier connector on the rear panel of the Autosampler.

Accessing the Peltier Accessory Screen



Creating Methods and Sequences

Defining the Active Method - Samples Only .. 4-2 Procedure for Modifying the Default Active Method - Samples Only .. 4-3 Defining the Active Method - All Options Enabled .. 4-5 Adding Calibrants .. 4-6 Setting Up Timed Events .. 4-9 Setting Up a Derivatization .. 4-11 Setting Up a Dilution .. 4-13 Creating New Methods .. 4-18 Creating a New Method from within the Active Method .. 4-19 Creating a New Method by Recalling a Method from the Directory .. 4-21 Editing a Stored Method .. 4-24 Defining a Sequence .. 4-26 Rules for Defining a Sequence .. 4-26 Identifying the Methods to Include in a Sequence .. 4-26 Procedure for Defining a Sequence .. 4-28 Defining a Priority Method .. 4-30 Defining a Random Priority Method .. 4-32

Creating Methods and Sequences

This chapter describes how to create Methods and Sequences on your Series 200 LC Autosampler. It includes the following information:

- Defining the Active Method Samples Only
- Defining the Active Method All Method Options Enabled
- Creating New Methods
- Editing a Stored Method
- Defining a Sequence
- · Defining a Priority Method
- · Defining a Random Priority Method

Defining the Active Method - Samples Only

The active method is the method that is currently running on the Autosampler, or the method currently shown on the display.

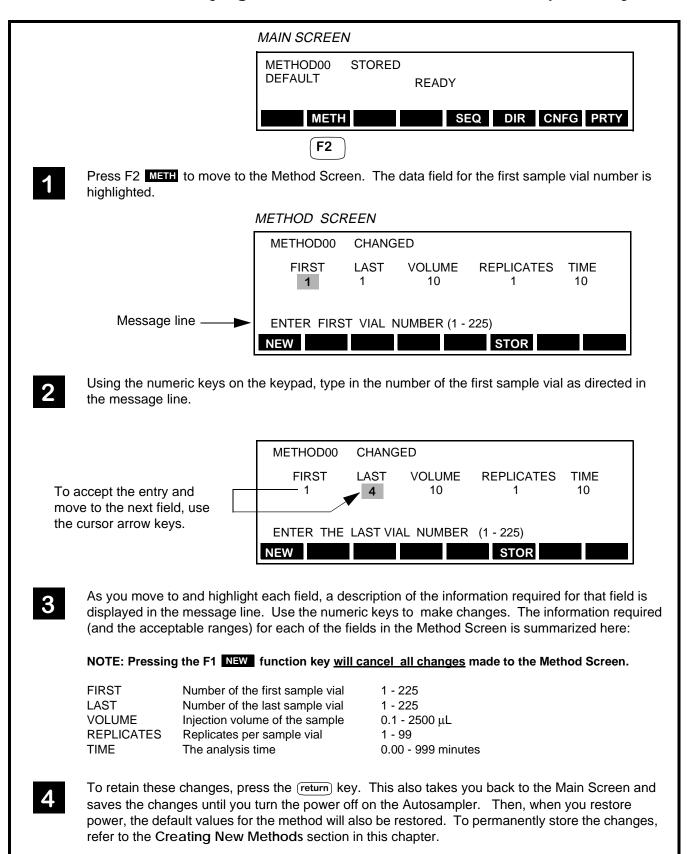
A method is a procedure that may consist of a single injection or a series of injections of several samples, calibrants, or a combination of samples and calibrants. The method also defines the location of each sample or calibrant vial, as well as the injection volume and the number of replicates for each sample or calibrant.

The default method, method00, provided with the Autosampler is set up to perform a single sample injection. This section describes how to make some simple changes to this default method. The procedure is shown on the facing page.

To permanently store changes to the default active method, refer to the "Creating New Methods" section in this chapter.

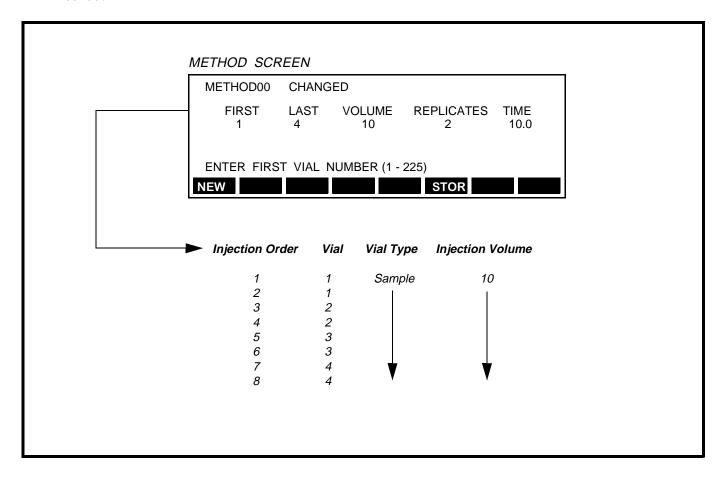
4 - 2

Procedure for Modifying the Default Active Method - Samples Only



Example of Sample Injection Order

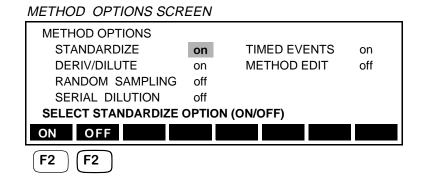
The following example shows the order in which the samples are injected for the previous method.



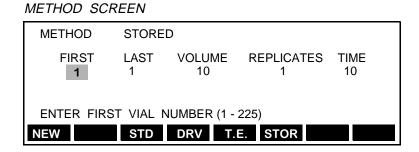
Defining the Active Method - All Options Enabled

This section shows how to change the default method so that you can add standards, perform dilutions or derivatizations, and set up timed events. The procedures in this section assume that you have enabled all of the appropriate Method Options as defined in the "Adding Method Options" section of Chapter 3 in this manual.

The Method Options Screen, with the appropriate options enabled, is repeated here for your convenience. (The RANDOM SAMPLING and METHOD EDIT options are special procedures described in later sections in this chapter; the SERIAL DILUTION option is described in Appendix C.)



When the above method options are enabled, the top level Method Screen displays them as the STD, DRV, and T.E. soft function keys as shown here.



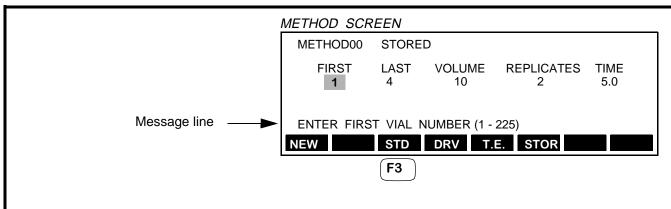
Each of these method options is described in a separate procedure within this section as indicated here.

| Method Option | Method Screen Function Key | Procedure |
|---------------|----------------------------|-----------------------------|
| STANDARDIZE | STD | Adding Calibrants |
| TIMED EVENTS | T.E. | Setting up Timed Events |
| DERIV/DILUT | DRV | Setting up a Derivatization |
| DERIV/DILUT | DIL* | Setting up a Dilution |

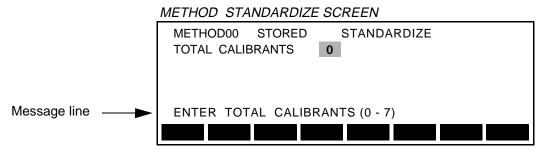
• The DIL function key is displayed when the special dilution tray is inserted in the Autosampler.

Adding Calibrants

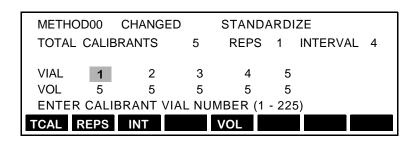
This section describes how to add calibrants (STANDARDIZE option enabled) to the method.



From the Method Screen, press F3 **STD** to move to the Method Standardize Screen. The data field for the Total Calibrants is highlighed.

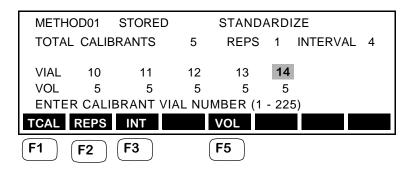


- Using the numeric keys on the keypad, type in the total number of calibrants as directed by the message line. In this example, 5 is used.
- Press the down cursor arrow key to accept the number of calibrants. The screen will change to display the correct number of VIAL and VOL columns (5), and the available soft function keys as shown here. Note that the data field for the first VIAL is highlighted.

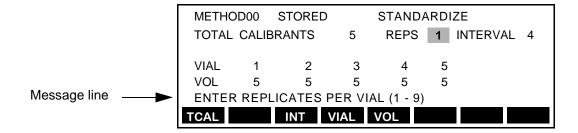


Use the numeric keys to change the vial number and the cursor arrow keys to move between vial numbers. In this example, the vial numbers have been changed as shown on the next page.

Adding Calibrants (continued)



To select the next field, press the corresponding soft function key. For example, to change the number of replicates, press F2 REPS and the data field for Reps becomes highlighted. The message line changes instructing you to enter the number of replicates.



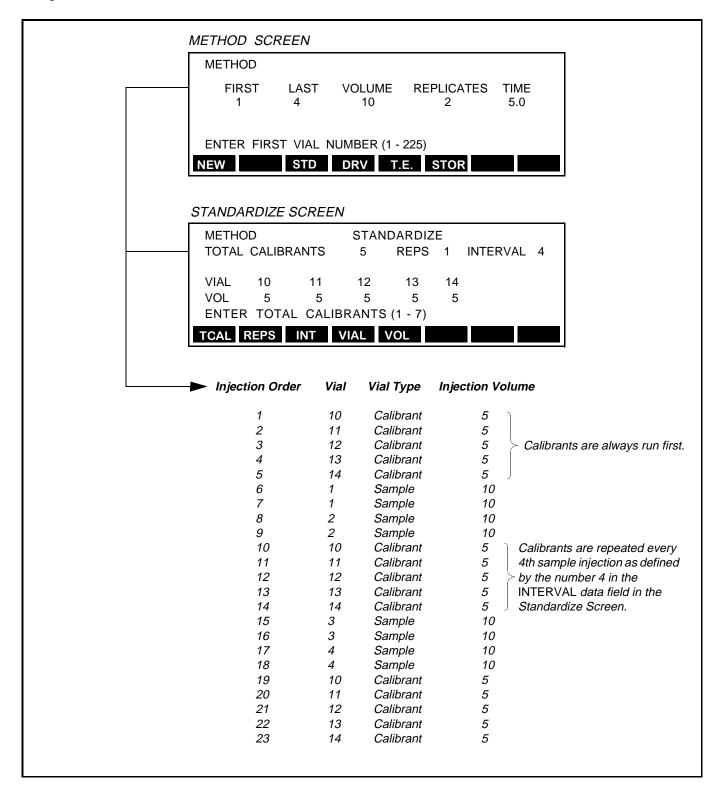
To move to other fields, use the cursor arrow keys. As you move to and highlight each field, a description of the information required for that field is displayed in the message line. Use the numeric keys to make changes. The information required (and the acceptable ranges) for each of the fields in the Method Standardize Screen is summarized here:

| TOTAL CALIBRANTS | Total number of calibrants | 1 - 7 |
|------------------|---|---------------|
| REPS | Number replicates per calibrant vial | 1 - 9 |
| INTERVAL | Defines how often the calibrants are run | 1 - 99 |
| VIAL | The vial number for each calibrant | 1 - 225 |
| VOL | The calibrant injection volume for the corresponding vial | 0.1 - 2500 μL |

To save these changes, press the return key. This also takes you back to the Method Screen and saves the changes until you turn the power off on the Autosampler. When you restore power, the default values for the method will also be restored. To permanently store the changes, refer to the Creating New Methods section in this chapter.

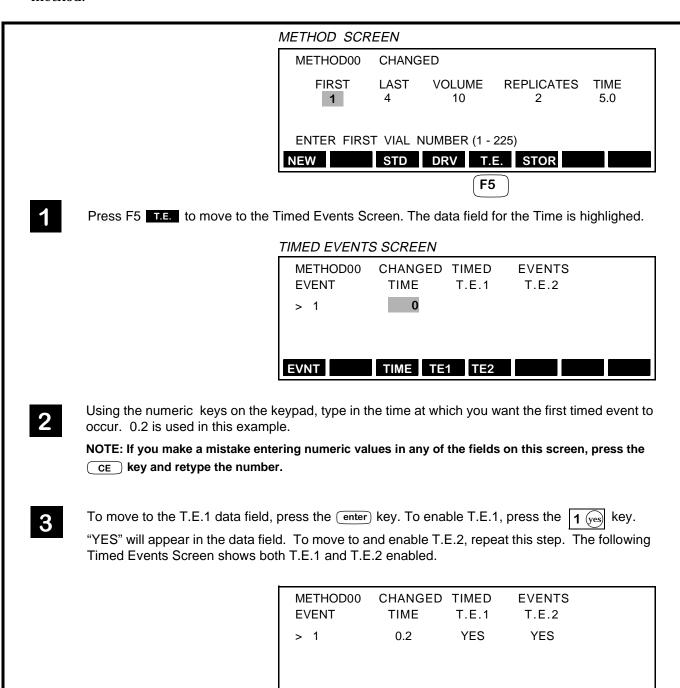
Example of Sample and Calibrant Injection Order

The following example shows the order in which samples and calibrants are injected for the previous method.



Setting Up Timed Events

This section describes how to set up timed events (TIMED EVENTS option enabled) within a method.



To add another event, press either the enter key or the insert key. A second event will be added and the Time field for the next event will increment by 0.01 minute. You can add up to 20 events using this process. Go to the next page to continue.

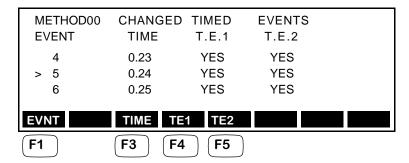
TIME TE1

TE2

EVNT

Setting Up Timed Events (continued)

The next screen shows what the Timed Events Screen looks like after you add several more events using the process described in step 4.



- To save these entries, press the return key. This also takes you back to the Method Screen and saves the changes until you turn the power off on the Autosampler. When you restore power, the default values for the method will also be restored. To permanently store the changes, refer to the "Creating New Methods" section in this chapter.
- To make changes to a method containing timed events, press F5 T.E. from the Method Screen and use the cursor arrow keys to scroll through the list of events.

To turn off T.E.1 or T.E.2, use the cursor arrow to move to T.E.1 or T.E.2 and press the numeric key. The "YES" will be deleted from the field.

To change the time of an event, select the event and press the F3 TIME key. Then use the numeric keys to enter a new time.

To delete an event, select the event and press the (delete) key.

<u>To add an event</u>, use the cursor arrow keys to scroll down to the location in the event list where you want to add the event and press the <u>insert</u> key. If there is a time slot available between the two successive events, an event will be added immediately after the first event; otherwise, the system will ignore the <u>insert</u> key. For example, if the time for event 5 is 0.30 and the time for event 6 is 0.31, you will not be able to add an event between 5 and 6. The time difference between the two events must be at least 0.02 minutes.

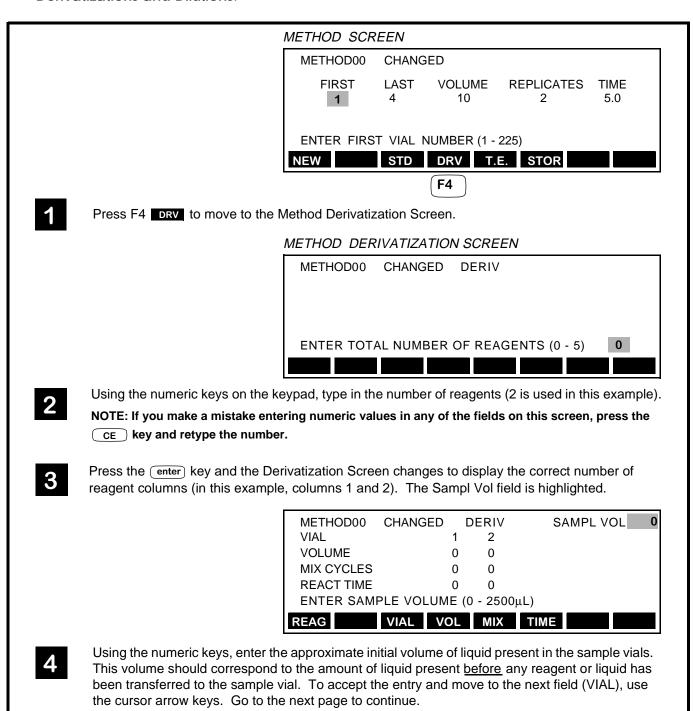
Save the changes as described in step 6.

<u>To permanently store the changes</u>, refer to the "Creating New Methods" section in this chapter.

Setting Up a Derivatization

This section describes how to set up a derivatization (DERIV/DILUT option enabled) within a method.

NOTE: To achieve the best performance, use the default Performance Parameter settings. To review or change the Performance Parameters, refer to Appendix B, "Changing the Performance Parameters." Depending on the types of vial caps you are using, you may need to change the Vent Mode parameter - refer to Appendix E, "Operating Guidelines for Derivatizations and Dilutions."



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Setting Up a Derivatization (continued)

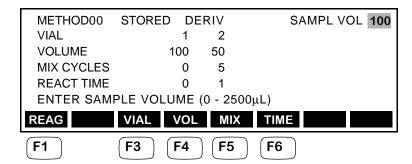
As you move to and highlight each field, a description of the information required for that field is displayed in the message line. Use the numeric keys to enter the information for each of the reagents. The information required (and the acceptable range) for each of the fields in the Method Derivatization Screen is summarized here:

and reagent) will be mixed after reagent transfer.
Unless a specific chemistry requires it, best results
are obtained by adding reagents and performing two

mixing series.

REACT TIME Reaction time for the mixture 0 - 99 Min

The following example shows a completed Method Derivatization Screen with some typical entries.



- To save these entries, press the return key. This also takes you back to the Method Screen and saves the changes until you turn the power off on the Autosampler. When you restore power, the default values for the method will also be restored. To permanently store the changes, refer to the "Creating New Methods" section in this chapter.
- To modify the entries, press F4 **DRV** from the Method Screen. A screen, similar to the one in step 7 will appear with the Sampl Vol field highlighted. You can use cursor arrow keys to move to the appropriate field, or press the corresponding function key.

Save the changes as described in step 7.

To permanently store the changes, refer to the "Creating New Methods" section in this chap-

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Setting Up a Dilution

In order to have access to the **DIL** function from the Method Screen, you need to make sure that you have inserted the dilution tray, Part No. N293-0314, into the Autosampler.

NOTE: To achieve the best performance, use the default Performance Parameter settings. To review or change the Performance Parameters, refer to Appendix B, "Changing the Performance Parameters." Depending on the types of vial caps you are using, you may need to change the Vent Mode parameter - refer to Appendix E, "Operating Guidelines for Derivatizations and Dilutions."

Two examples are presented here to show how to use the dilution feature on the Autosampler.

<u>In the first example</u>, there are 40 sample vials and 40 empty target vials. Each sample vial contains a different volume of liquid. You will program the Autosampler to:

- Add 500 μL of diluent to each of the 40 target vials, then;
- Pipet $100~\mu L$ of liquid from each of the 40 sample (source) vials into its corresponding target vial.

<u>In the second example</u>, there are 20 sample vials, 20 vials containing an internal standard, and 20 target vials. As in the first example, each sample vial contains a different volume of liquid. You will program the Autosampler to:

- Add 100 µL of diluent to each of the 20 target vials, then;
- Pipet 50 μ L of liquid from each of the 20 sample (source) vials into its corresponding target vial, then;
- Pipet 25 μL of internal standard from each of the 20 standard vials into its corresponding target vial.

Each example will show the dilution tray setup and the corresponding screen entries.

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Example 1 Dilution

Refer to Figure 4-1. The dilution tray is set up so that the first 40 vial positions are reserved for sample (source) vials, and the second 40 vial positions (41 - 80) are reserved for target vials. The screen entries and the procedure for this dilution scheme are on the facing page.

- 1 500 μL of diluent is transferred from the Diluent Tank to target vial 41.
- 2 100 μL of sample in sample vial 1 is transferred to target vial 41.

 For the second dilution, 500 μL of diluent is transferred from the Diluent Tank to target vial 42; then 100 μL of sample in sample vial 2 is transferred to target vial 42. This process will continue until diluent and sample have been added to the remaining vials, 43 through 80.
- A portion of the mixture, specified in the VOLUME field in the Method Screen (and shown on the facing page as 20 μL), may be injected. Whether or not the final dilution is injected, and when the injection occurs, depends on the DERIV/DILUTE option selected from the Method Options Screen described in the "Adding Method Options" section in Chapter 3 of this manual.

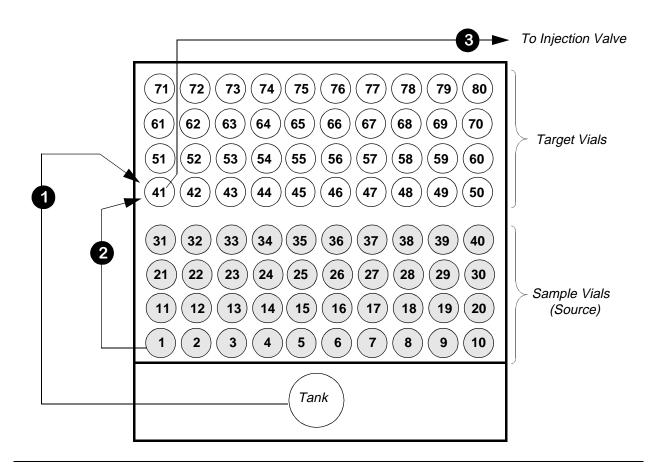
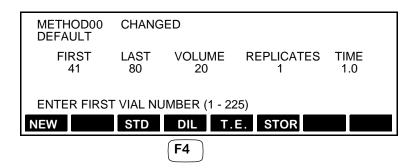


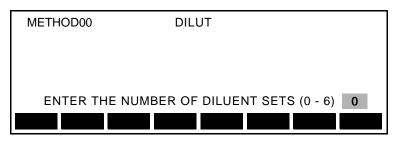
Figure 4-1. Dilution Tray Setup for Pipetting 500 μ L of Diluent and 100 μ L of Sample.

Procedure and Screen Entries for Example 1

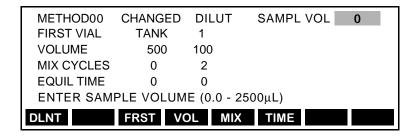
From the Main Screen, press F2 METH to move to the Method Screen. From the Method Screen, press F1 NEW to create a new active method. Fill in the fields with the entries shown here. Note that the VOLUME specified in this screen refers to the portion of the final dilution in the target vial that is injected.



Press F4 DIL to move to the Method Dilution Screen.



Enter 2 for the number of DILUENTS and press the (enter) key. The following screen 3



Fill in the fields with the entries shown in the above screen. As you move to and highlight each field, a description of the information required for that field is displayed in the message line. The information required for each data field is summarized here.

SAMPL VOL The volume of sample in each of the target vials (in this example, 0) FIRST VIAL First vial of a source set (Diluent from the TANK is dispensed first. Sample from the vial beginning the 2nd set, in this example, 1, is dispensed next) **VOLUME** Volume of diluent (1st set), or sample (2nd set)

Number of times the mixture in the target vials is mixed. For best results, MIX CYCLES

set this value to 2 as shown in the screen above.

EQUIL TIME The amount of time mixture in target vial is allowed to equilibrate between

vial to vial transfers of liquid

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Example 2 Dilution

Refer to Figure 4-2. The dilution tray is set up so that the first 20 vial positions are reserved for sample (source) vials, the second 20 vial positions (21 - 40) for internal standards and the third set of 20 vial positions (41 - 60) for target vials. The screen entries and the procedure for this dilution scheme are on the facing page.

- 100 μL of diluent is transferred from the Diluent Tank to target vial 41.
- 2 50 μL of sample in sample vial 1 is transferred to target vial 41.
- 3 25 μL of internal standard is transferred from vial 21 to target vial 41. For the second dilution, 100 μL of diluent is transferred from the Diluent Tank to target vial 42, followed by 50 μL of sample from sample vial 2. Then, 25 μL of internal standard is transferred from vial 22 to target vial 42. This process will continue until diluent, sample, and internal standard have been added to the remaining target vials, 43 through 60.
- A portion of the mixture, specified in the VOLUME field in the Method Screen (and shown on the facing page as 20 μL), may be injected. Whether or not the final dilution is injected, and when the injection occurs, depends on the DERIV/DILUTE option selected from the Method Options Screen described in the "Adding Method Options" section in Chapter 3 of this manual.

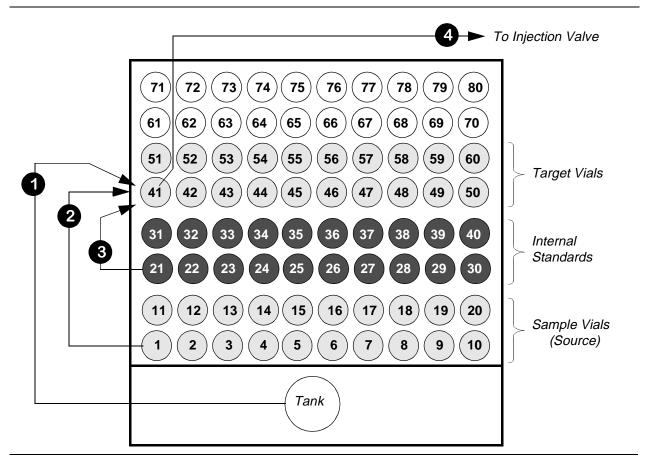
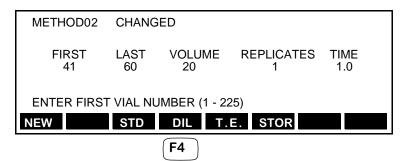


Figure 4-2. Dilution Tray Setup for Pipetting 100 μ L of Diluent, 50 μ L of Sample, and 25 μ L of Internal Standard.

Procedure and Screen Entries for Example 2

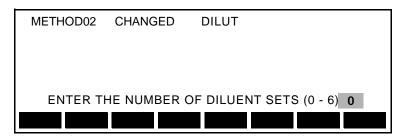
1

From the Main Screen, press F2 METH to move to the Method Screen. From the Method Screen, press F1 NEW to create a new active method. Fill in the fields with the entries shown here. Note that the VOLUME specified in this screen refers to the portion of the mixture in the target vial that is injected.

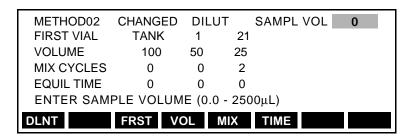


2

Press F4 DIL to move to the Method Dilution Screen.



Enter 3 for the number of DILUENTS and press the enter key. The following screen appears.



Fill in the fields with the entries shown in the above screen. As you move to and highlight each field, a description of the information required for that field is displayed in the message line. The information required for each data field is summarized here.

SAMPL VOL The volume of sample in the target vial (in this example, 0) before pressing start

FIRST VIAL First vial of a source set (Diluent from the TANK is dispensed first.

Sample from the vial beginning the 2nd set, in this example, 1, is dispensed next. Internal standard from the vial beginning the 3rd set, in

this example, 21 is dispensed next)

VOLUME Volume of diluent (1st set), or sample (2nd set), or internal standard (3rd set)
MIX CYCLES Number of times the mixture in the target vials is mixed. For best results, set this

value to 2 as shown in the screen above.

EQUIL TIME The amount of time mixture in target vial is allowed to equilibrate

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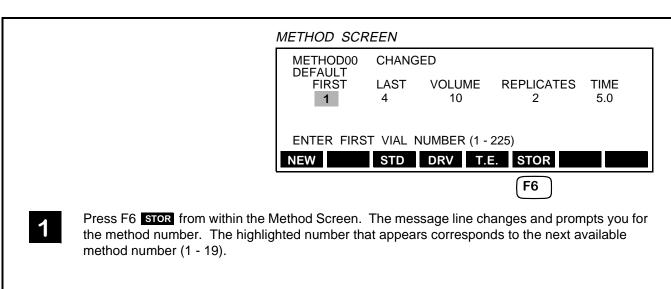
Creating New Methods

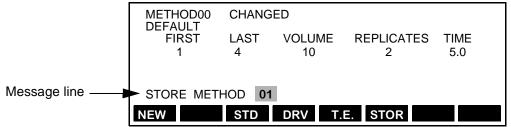
This section describes how to create new methods by permanently storing changes to the active method. You can do this either from within the active Method Screen or by recalling an existing method from the Autosampler directory.

| Note | When you recall a method from the Autosam- pler directory, that method replaces or over- writes the current active method. |
|------|--|
| | |

If you need to create a new method while the Autosampler is running the active method, you can do so by copying an existing method stored in the Autosampler's directory. This procedure is described in the "Editing a Stored Method" section of this chapter.

Creating a New Method from within the Active Method

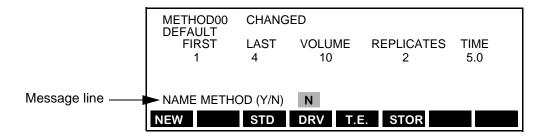




Press the <a>enter key to accept the method number shown, or use the numeric keys to type in a new value. If the method already exists, the message line will display the following prompt:

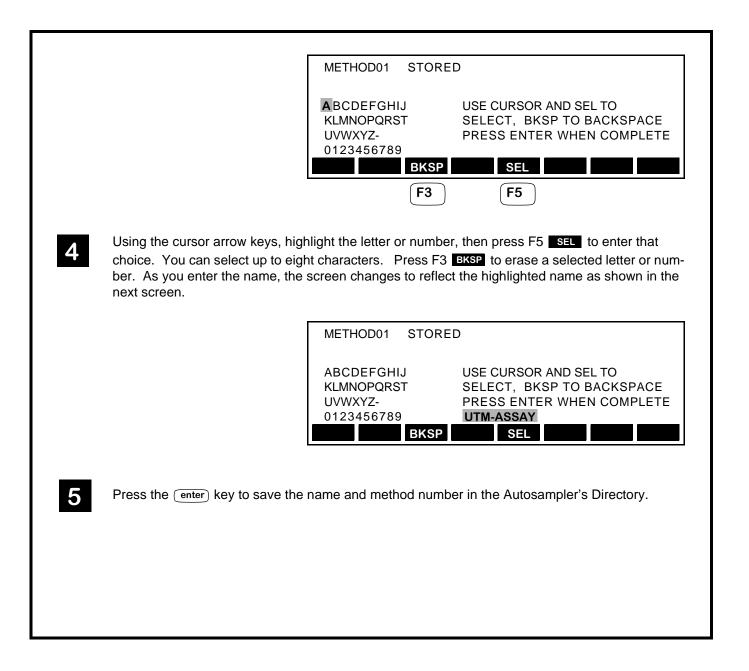
OVERWRITE EXISTING METHOD? (Y/N)

Use the 1 (yes) 0 (no) numeric keys to respond. The message line changes and asks you if you want to name the method as shown in the next screen.



To name the method press the numeric key. The screen shown on the next page appears.

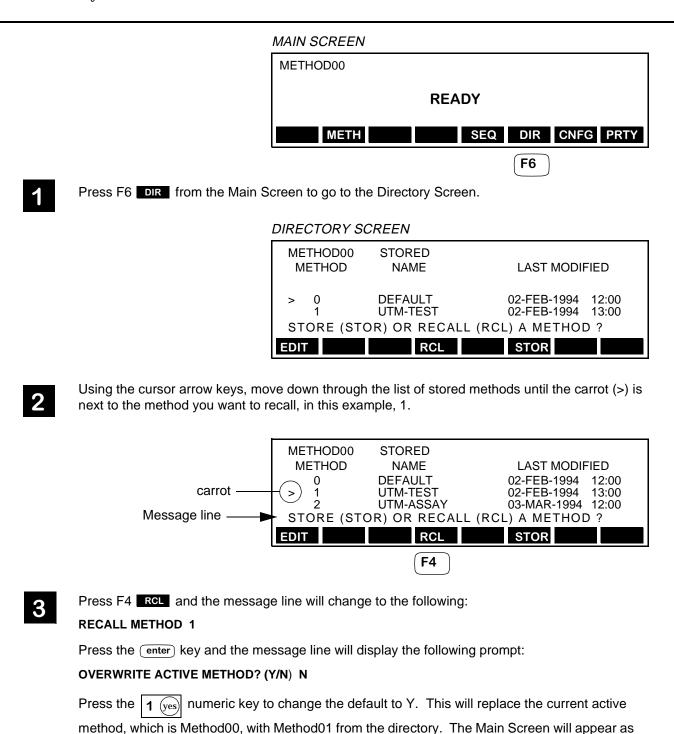
Creating a New Method from within the Active Method (continued)



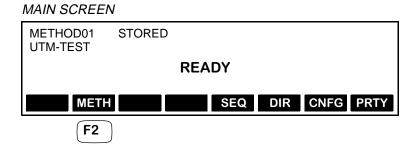
shown on the next page.

Creating a New Method by Recalling a Method from the Directory

This procedure describes how to recall a method from the Autosampler directory so that you can modify it and save it under a new name and number.



Recalling a Method from the Directory (continued)



- At this point, press F2 METH from the Main Screen to access the Method Screen. You can modify the method information by changing the fields directly on the Method Screen, or by changing the fields on the screens which can be accessed from the Method Screen (Standardize, Derivatization, Dilution, or Timed Events). Refer to Figure 4-3 on the facing page for a summary of how to access these screens.
- Store the method under a new name and method number as described in the previous section, "Creating a New Method within the Active Method."

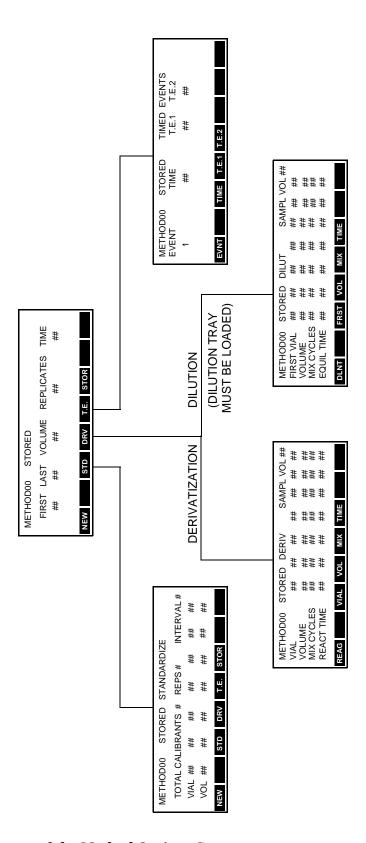
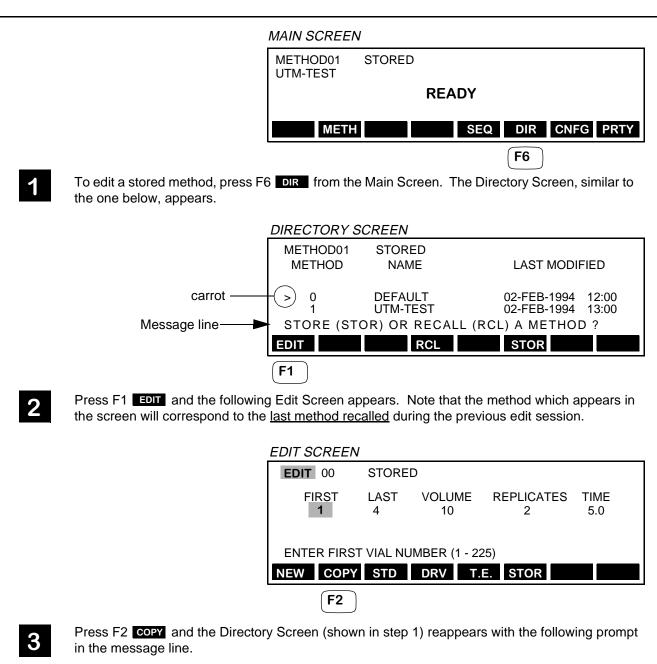


Figure 4-3. Summary of the Method Options Screens.

Editing a Stored Method

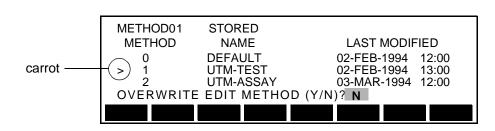
This section describes how to retrieve a stored method from the Autosampler Directory, modify it, and save it while a different (active) method is running. When editing a method, you can leave the edit session to perform other operations on the Autosampler and then return and continue editing without losing any of your changes.



COPY METHOD 0 FOR EDITING

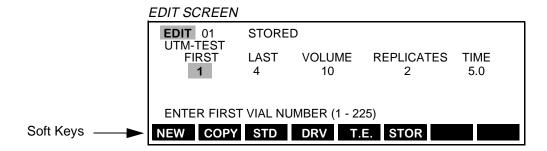
Using the cursor arrow keys, move down through the list of stored methods until the carrot (>) is next to the method you want to copy (this example will use Method 1). To accept, press the (enter) key. The Directory Screen then prompts you to overwrite the method.

Editing a Stored Method (continued)



To overwrite the selected method, press the 1 (yes) numeric key.

As soon as you press this key, the following Edit Screen will appear. You will note that except for the COPY function, the Edit Screen is identical to the Method Screen



- Use the same procedures described in the "Defining the Active Method All Options Enabled" section in this chapter to make changes to the method.
 - **Note 1:** If you are making changes to one of the Standardize, Derivatization, Dilution, or Timed Events Screens (that you access using one of the soft keys), you need to press the return key to save those changes and return to the Edit Screen above.
 - Note 2: To leave the Edit mode to view, for example, the status of the current method or sequence, press the return key. To return to the Edit mode again, press F6 DIR from the Main Screen, then press F1 EDIT
 - **Note 3:** If you make a mistake entering numeric values in any of the fields on the screen(s), press the ce key and retype the number.
- To store the changes under a new method number and name, press F6 STOR from within the Edit Screen and follow the procedure described in the "Creating a New Method from within the Active Method" section in this chapter.

Defining a Sequence

A sequence is a series of stored methods that are linked together. This section contains the information you need to define a sequence. Before you begin, you need to know the rules for defining a sequence and how to identify the methods that comprise the sequence.

Rules for Defining a Sequence

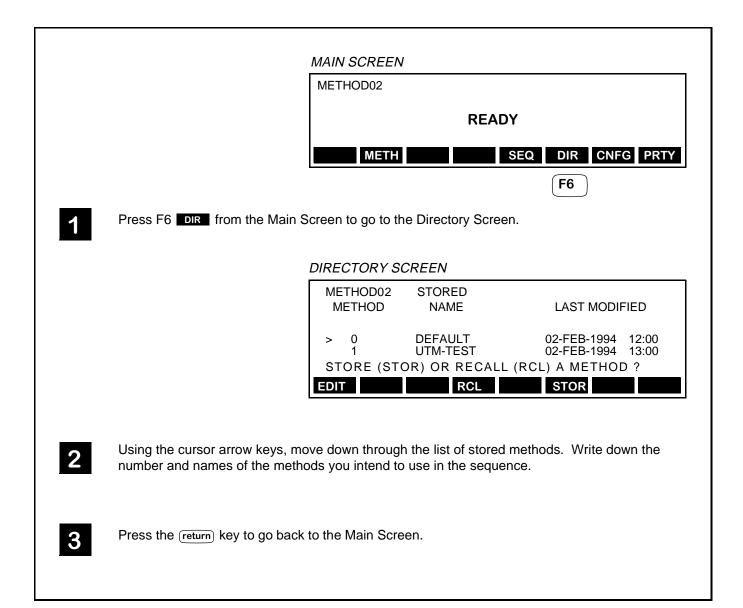
- 1. If you have not already done so, you need to create and store in the Autosampler's Directory all of the methods that you intend to include in the sequence. Procedures for creating and storing methods are contained in the "Creating New Methods" section in this chapter.
- 2. The maximum number of methods comprising a sequence is 10.
- 3. If you have previously defined a sequence, you will be able to overwrite (but not delete) methods that you have stored and assigned to the sequence, as long as you have not linked the sequence. Once you link the sequence, you cannot overwrite or delete any of the methods in the sequence.

Identifying the Methods to Include in a Sequence

Before defining the sequence, you need to view the methods stored in the Autosampler's Directory so that you can identify the numbers and names of the methods you want to include in the Sequence. To view the methods, follow this procedure on the facing page.

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Viewing the Methods in the Directory

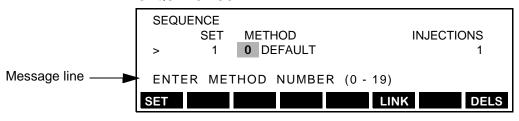


Procedure for Defining a Sequence

MAIN SCREEN METHODO0 READY METH SEQ DIR CNFG PRTY F5

Press F5 SEQ from the Main Screen to go to the Sequence Screen. The Method Number for SET 1 is highlighted.

SEQUENCE SCREEN



To change the default method for SET 1, use the numeric keys to type in a new method number.

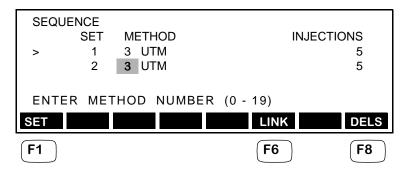
If you do not want to create another SET, press the return key and the number and name of the method you typed in will be accepted and you will return to the Main Screen.

To create another SET, press either the enter key or the insert key.

The screen changes to reflect the new method in SET 1 and adds an entry for SET 2 as shown in the next Sequence Screen on the facing page.

Procedure for Defining a Sequence (continued)

When you press the <a>enter key or the <a>(insert) key to add another set, the system uses the same method as in the previous set. Use the numeric keys to type in a new method number for the new set.



Continue to add sets until you have entered all of the methods for the Sequence. Remember, the maximum number of methods in a Sequence is 10.

Note: If you make a mistake and add the wrong method, remove it by pressing the delete key.

To link the sequence to the method in Set 1, press F6 LINK.

To select the SET position in the Sequence Screen, press F1 SET .

To delete the entire sequence so that you can create a new sequence, press F8 DELS.

Defining a Priority Method

A priority method is similar to an active method in that it may also consist of a single injection or series of injections of several samples and/or calibrants. It differs from the active method, however, in several important ways.

How is the Priority Method different from an Active Method?

- There is a single priority method. For the active method, you can store up to 19 different methods.
- You can interrupt the active method or sequence and run the priority method. As soon as the priority method is completed, the active method or sequence will resume.
- Any changes you make to the priority method are automatically saved so that if you turn
 the power off on the Autosampler you will not lose the changes.

When do you run the Priority Method?

While you can run a priority method any time, it is usually reserved for those occasions when you need to interrupt an active method or sequence that is already in progress to insert priority sample(s).

How do you define a Priority Method?

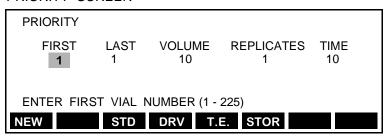
The procedures and screens used to define the priority method are virtually identical to the ones used for defining the active method, described in the first two sections of this chapter, "Defining the Active Method - Samples Only" and "Defining the Active Method - All Options Enabled." The only difference is in how you access the Priority Screen and the titles of the screens. Both of these differences are shown in the diagram on the facing page.

4 - 30

MAIN SCREEN METHOD00 READY METH SEQ DIR CNFG PRTY F8

Press F8 PRTY to go to the Priority screen.

PRIORITY SCREEN



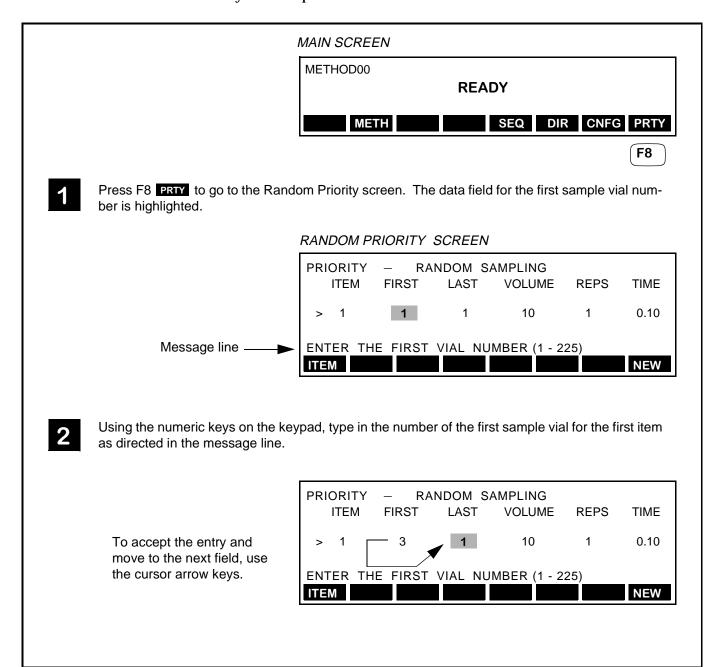
Note: Any method options you enabled for active method (and stored methods) in "Adding Method Options" in Chapter 3, also apply to the Priority Method. The top level Priority Screen shown here assumes that you have enabled all of the method options.

At this point, you can modify the priority method information by changing the fields directly on the top level screen, or by changing the fields on the screens which are accessed from the Priority Screen (Standardize, Derivatization, Dilution or Timed Events).

Defining a Random Priority Method

This section shows how to define a random priority method. It assumes that you have enabled the Random Sampling option as described in the "Adding Method Options" section in Chapter 3 of this manual.

The random priority method allows you to sample vials in a non-consecutive or random manner. Each item in a random priority method represents a single tube or multiple tubes in a consecutive series. You may define up to 99 items.



3

As you move to and highlight each field, a description of the information required for that field is displayed in the message line. Use the numeric keys to make changes. The information required (and the acceptable ranges) for each of the fields in the Random Priority Screen is summarized here:

NOTE: Pressing the F8 NEW function key will cancel all of the changes made to the Random Priority Screen.

FIRST Number of the first sample vial 1 - 225 LAST Number of the last sample vial 1 - 2250.1 - 2500 μL VOLUME Injection volume of the sample REPLICATES Replicates per sample vial 1 - 99

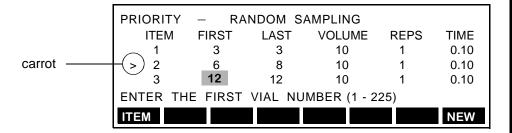
TIME The analysis time 0.00 - 999 minutes

To add another item, press either the (enter) key at the last data field or the (insert) key.

The system adds item 2 and uses the same settings as item 1 as shown in the next screen. To change the settings, use the cursor arrow and numeric keys.

| PRIORITY - RANDOM SAMPLING | | | | | |
|---------------------------------------|-------|------|--------|------|------|
| ITEM | FIRST | LAST | VOLUME | REPS | TIME |
| > 1 | _3_ | 3 | 10 | 1 | 0.10 |
| 2 | 3 | 3 | 10 | 1 | 0.10 |
| ENTER THE FIRST VIAL NUMBER (1 - 225) | | | | | |
| ITEM | | | | | NEW |

You can continue to add and modify items in this manner, up to a maximum of 99. The next 5 screen shows the first three items in a completed Random Priority screen. In the items shown here, the Autosampler would sample from vials 3, then 6 - 8, and then 12.



To select an item from the list, press F1 TEM, and enter the item number when prompted, then press the enter key. The carrot will move to the selected item. Alternatively, you can use the cursor arrow keys to scroll up and down the list.

To delete an item, select the item and press the delete key.

To permanently save these changes, press the (return) key.

NOTES

Running Methods and Sequences

5

Getting Ready - A Checklist .. 5-2
Powering Up the Autosampler .. 5-3
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Starting a Method .. 5-4
Starting the Active Method .. 5-4
Recalling and Starting a Stored Method .. 5-5
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Starting a Priority Method (or Random Priority Method).. 5-8
Starting a Priority Method - No Active Method or Sequence
Running .. 5-8
Interrupting the Active Method or Sequence and Running the
Priority Method .. 5-9

Running Methods and Sequences 5

This chapter describes how to run Methods and Sequences on your Series 200 LC Autosampler. It includes the following information:

- · Getting Ready a Checklist
- Powering up the Autosampler
- Starting a Method
- Starting a Sequence
- · Starting a Priority Method

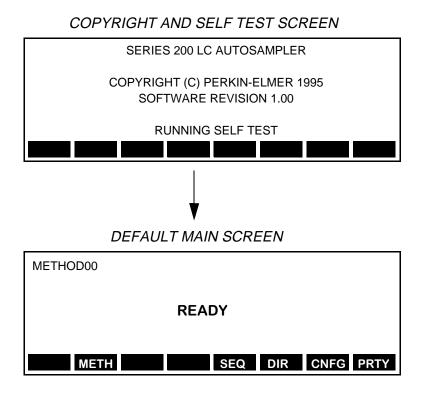
Getting Ready - A Checklist

ready to begin running samples. Review the safety and warning notes at the beginning of this manual (Safety and ____ 1. Regulatory Information). Verify that the tubing to your pump, column, and detector is properly connected. 2. ____ 3. Verify that the electrical connections to relays and timed events are properly connected. Start the pump and detector. Flush and equilibrate both with the mobile phase required for your analysis conditions. To set the pump and detector conditions, refer to the user's manual provided with the instruments. ____ 5. Start the Autosampler. Flush the Autosampler by pressing the (flush) key on the keyboard. The default 6. number of flush cycles per key press is set at 2. Each cycle flushes 500 µL of solvent through the system for a total of 1.0 mL which corresponds to the amount of solvent held in the system tubing. To change this value, refer to Appendix B in this manual. Note If you have not run solvent through the Autosampler within the last 24 hours, flush the system with isopropanol. Check the syringes and tubing for air bubbles. If bubbles are present, flush the system with isopropanol, then again with the appropriate degassed flush solvent. Select the proper tray for analysis. Appendix A lists all of the available tray ___ 8. types. Select or create the method or sequence. Creating methods and sequences is described in Chapter 4 of this manual.

Before you start a method or sequence, use the following checklist to insure that you are

Powering Up the Autosampler

Press the power switch located on the lower right side of the Autosampler to the ON position. The Copyright and Self Test screen first appears on the Liquid Crystal Display. The display is located directly above the keyboard on the front panel. As soon as the self test is completed, the Main Screen appears.



The Default Top Level Main Screen

The Main Screen provides access to all of the Autosampler's functions. The soft keys displayed along the bottom of the default Main Screen shown here correspond to the functions that are available when you first power-up the Autosampler. Chapter 3 in this manual describes how to add other optional functions to the Main Screen.

Starting a Method

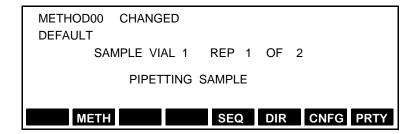
This section describes the procedures for starting a method and includes:

- Starting the Active Method
- Recalling and Starting a Stored Method

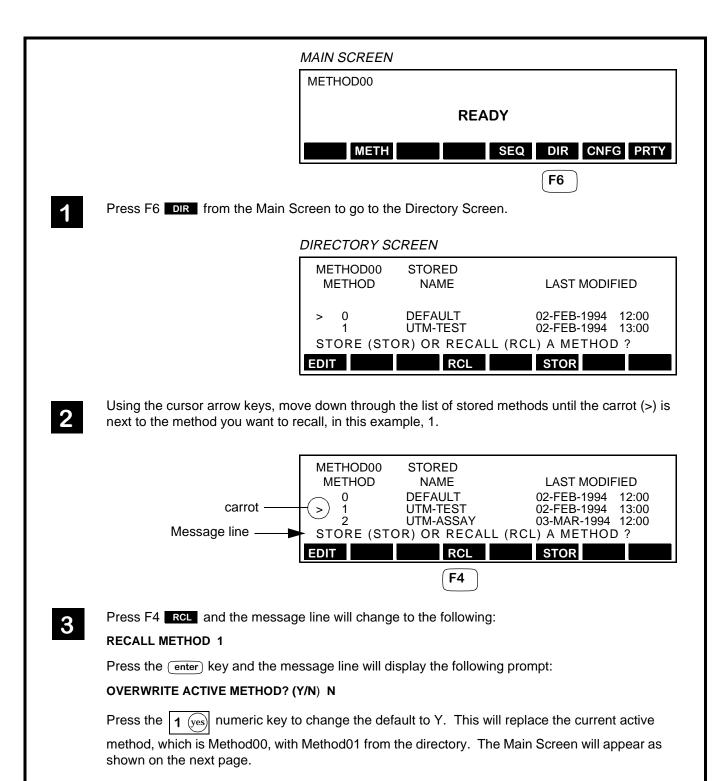
Starting the Active Method

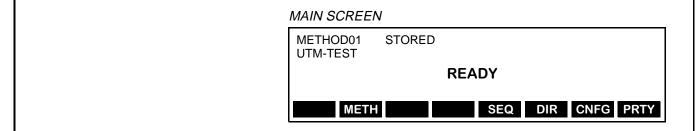
You can start the active method from any screen by pressing the <code>start</code> key on the keyboard. As soon as you start the active method, the Main Screen appears and displays information about the status of the active method.

The following screen shows typical status information for the default method, Method00, which is now being run.

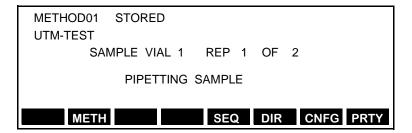


Recalling and Starting a Stored Method



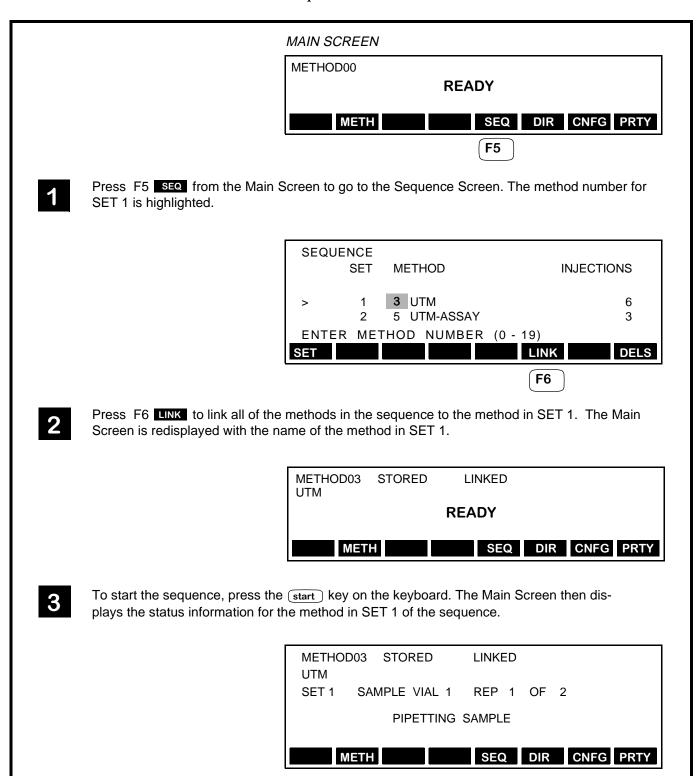


At this point, press the <u>start</u> key on the keyboard and the screen will change to display status information about the new current active method, method01.



Starting a Sequence

This section describes how to start a sequence.



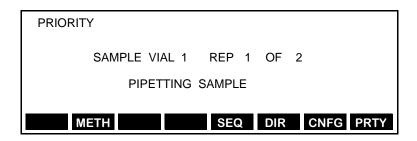
Starting a Priority Method (or Random Priority Method)

This section describes how to start a priority method. Note that the operations and the screens are identical for starting a random priority method.

Starting a Priority Method - No Active Method or Sequence Running



You can start the priority method from any screen by pressing the priority key on the keyboard. As soon as you start the priority method, the Main Screen appears and displays information about the status of the priority method, similar to the screen shown here.

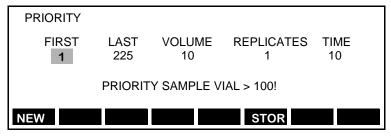


2

Note: if the priority method you defined and the sample tray are incompatible, the Priority Screen will appear with an error message as soon as you press the priority key. For example, if the last vial in the Priority Method is set at 225, and you have a standard 100 vial sample tray in the Autosampler, the Priority Screen will appear as shown here.

To correct the error and clear the message, use the cursor arrow keys to move to the LAST field and enter the correct number.

PRIORITY SCREEN



Interrupting the Active Method or Sequence and Running the Priority Method

1

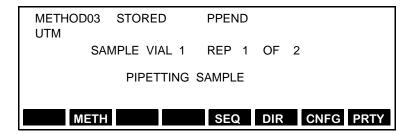
To insert the priority method into the active method or sequence, press the priority key on the keyboard.

Refer to item 2 if you are interrupting the active method. If you are interrupting a sequence, refer to item 3.

2

Interrupting the Active Method

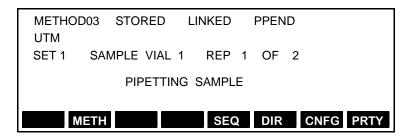
If the Autosampler is performing an operation on a method (like sampling) when you press the priority key, the following Main Screen display appears. The PPEND in the top line denotes that a priority method is pending and will start as soon as the Autosampler completes its current operation. The method will resume once the priority method is completed.



3

Interrupting a Sequence

If the Autosampler is performing an operation on a method (like sampling) within a sequence, when you press the priority key the following Main Screen display appears. The PPEND in the top line denotes that a priority method is pending and will start as soon as the Autosampler completes its current operation. The sequence will resume once the priority method is completed.



Notes

Calibration and Diagnostics

Calibrating the Sample Needle .. 6-3 Accessing the Diagnostics Screen .. 6-6 Moving Between Pages of the Diagnostics Screen .. 6-7 Running the Diagnostic Tests .. 6-8 Keypad Test 1 .. 6-8 Keypad Test 2 .. 6-9 Flash Test .. **6-10** RAM Test .. 6-11 Battery Backup Test .. 6-12 Peltier Communications Test .. 6-14 DUART Test Internal .. 6-16 DUART Test External .. 6-17 Remote Input Test .. 6-19 Output Relays Test .. 6-20 Read Firmware Part Number .. 6-21 Clear All Methods .. 6-22 Clear Battery RAM .. 6-23 BCD Outputs Test .. 6-24 Viewing the Instrument Log .. 6-26

Calibration and Diagnostics

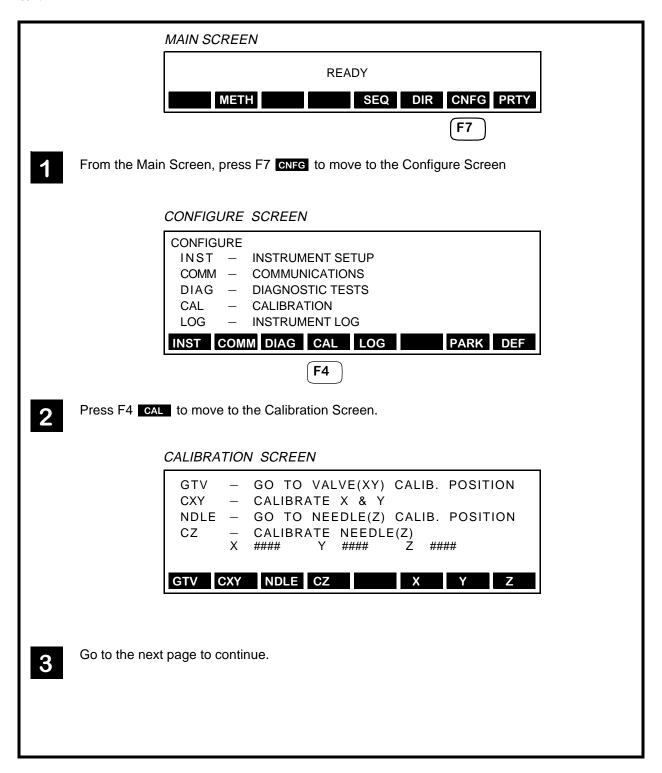
This chapter contains procedures for calibrating and running diagnostics on your Series 200 LC Autosampler. It includes the following information:

- Calibrating the Sample Needle
- Accessing the Diagnostics Screen
- Running the Diagnostics Tests
 - Keypad Display Tests
 - FLASH Test
 - RAM Test
 - Battery Backup Test
 - Peltier Communications Test
 - DUART Test Internal
 - DUART Test External
 - Remote Input Test
 - Output Relays Test
 - Read Firmware Part Number
 - Clear All Methods
 - Clear Battery RAM
 - BCD Outputs Test
- Viewing the Instrument Log

Notes

Calibrating the Sample Needle

You need to run the following calibration procedure whenever you change the <u>type</u> of injector.



Calibrating the Sample Needle (continued)

CALIBRATION SCREEN GTV GO TO VALVE(XY) CALIB. POSITION CXY CALIBRATE X & Y NDLE -GO TO NEEDLE(Z) CALIB. POSITION CALIBRATE NEEDLE(Z) Χ #### Υ #### #### CXY NDLE CZ F2 F3 F6 F1 F4 F7 F8 Press F1 GTV. This positions the needle over the injection valve. Push the needle all the way down into the valve. Being careful to keep your fingers clear of the Sample Needle Assembly, press F4 CZ. 6 This sets the z-coordinate for the needle and then raises it up out of the injector valve. The new "Z" value appears in the Calibration Screen. Check the calibration by pressing F3 NDLE. This positions the needle over the injection valve and then lowers it all the way into the valve. Verify that the upper surface of the vial probe firmly contacts the lower surface of the needle cartridge.

Record the new value that appears in the Z data field of the Calibration Screen. You can

overwrite the old Z value which appears on the Calibration Label on the electronic cover in

the Autosampler. The location of the Calibration Label is shown in Figure 6-1.

8

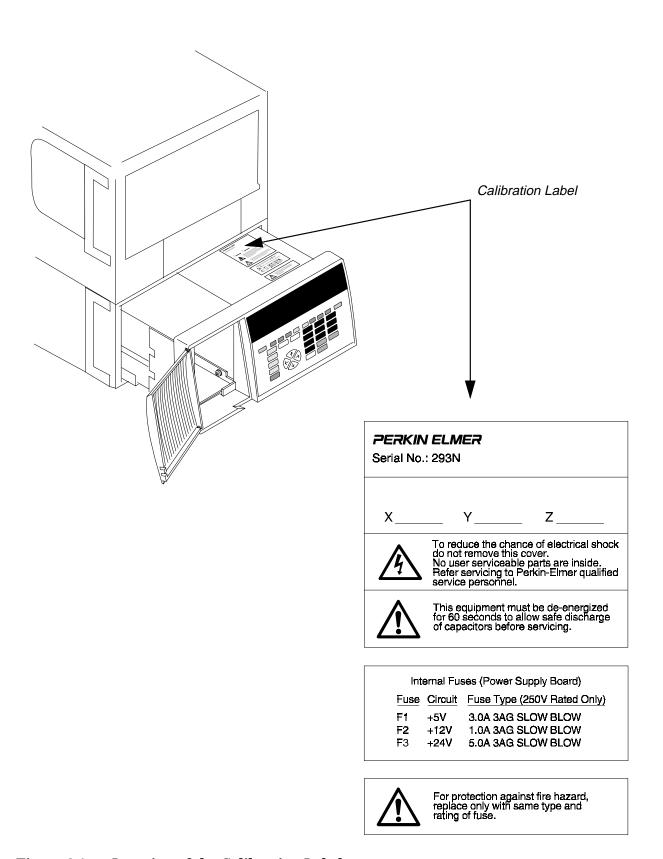
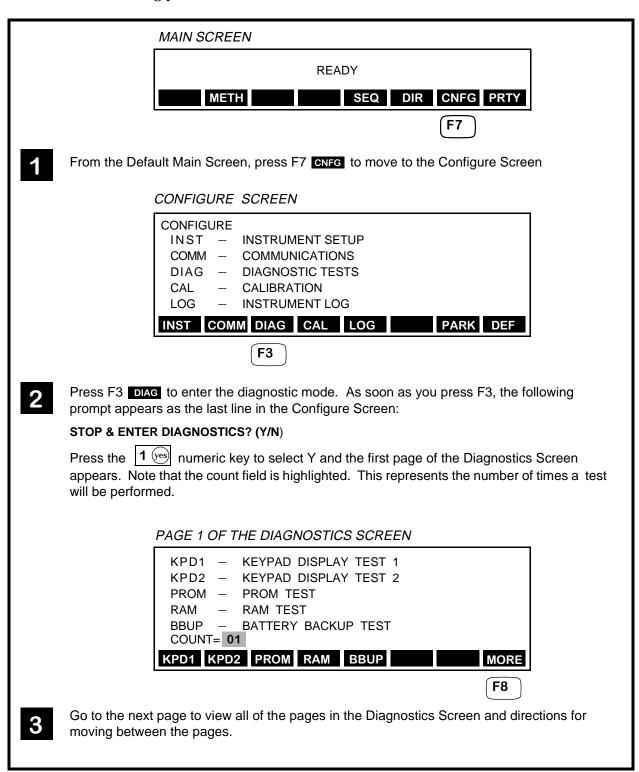


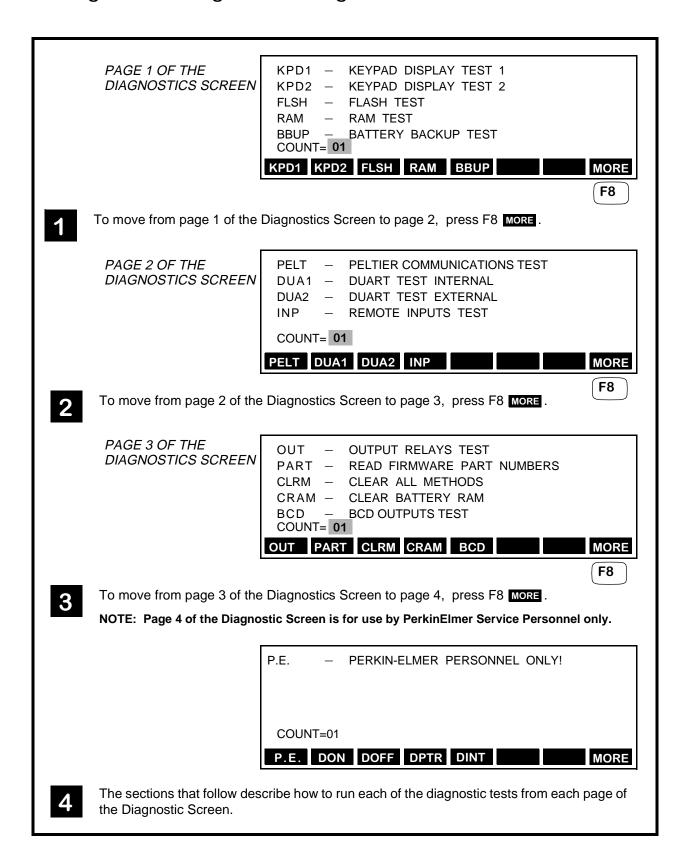
Figure 6-1. Location of the Calibration Label.

Accessing the Diagnostics Screen

The Diagnostics tests are used to check the condition of the Autosampler hardware. They are performed from the Diagnostics Screen which is comprised of four pages and is accessed as shown in the following procedure.



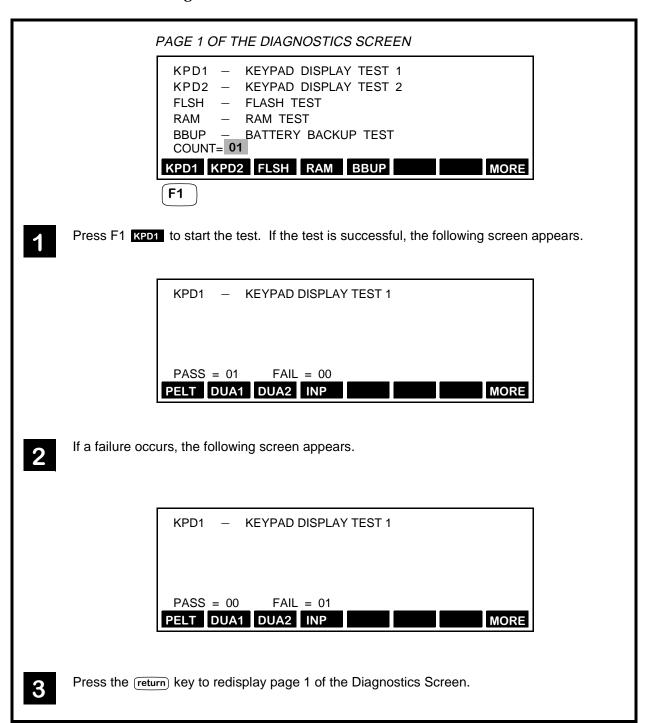
Moving Between Pages of the Diagnostics Screen



Running the Diagnostic Tests

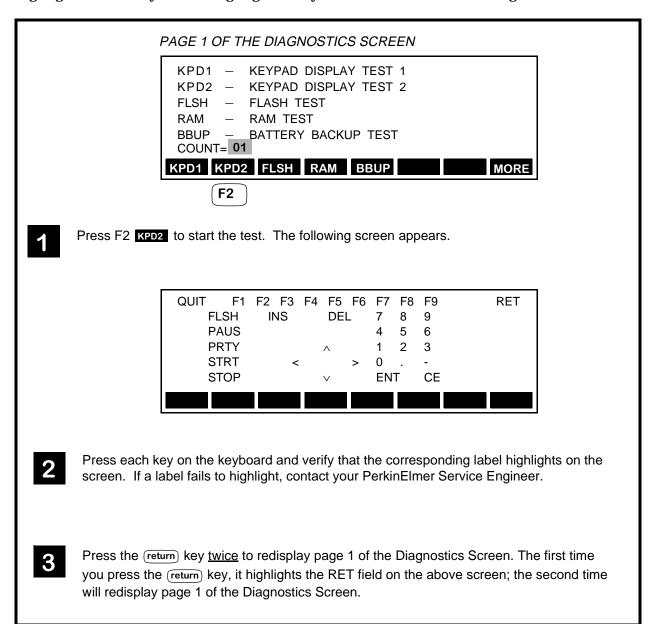
Keypad Test 1

This test is designed to test the general functionality of the keypad. If a number is displayed next to pass, the keypad passed the test. If a number is displayed next to fail, call your Perkin Elmer Service Engineer.



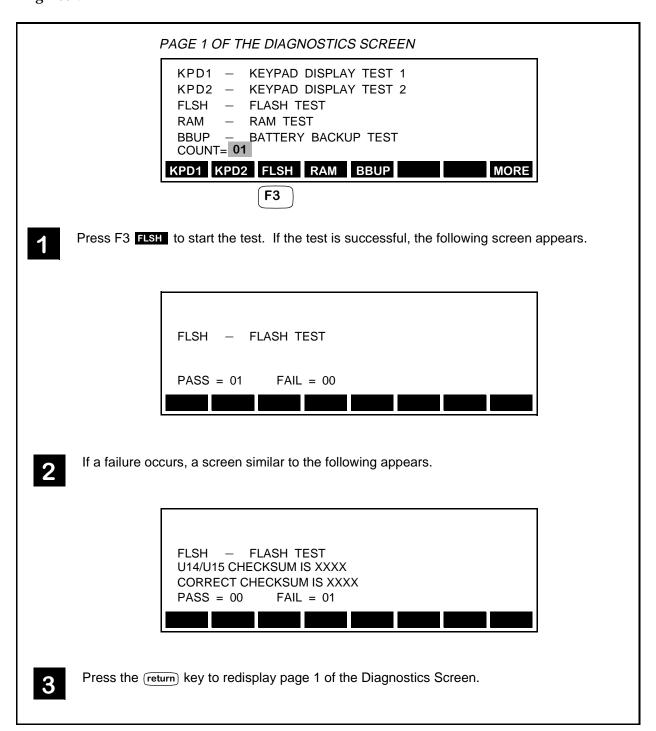
Keypad Test 2

This test is designed to test the operation of each of the keypad keys. If a key works, it is highlighted. If a key fails to highlight, call your PerkinElmer Service Engineer.



Flash Test

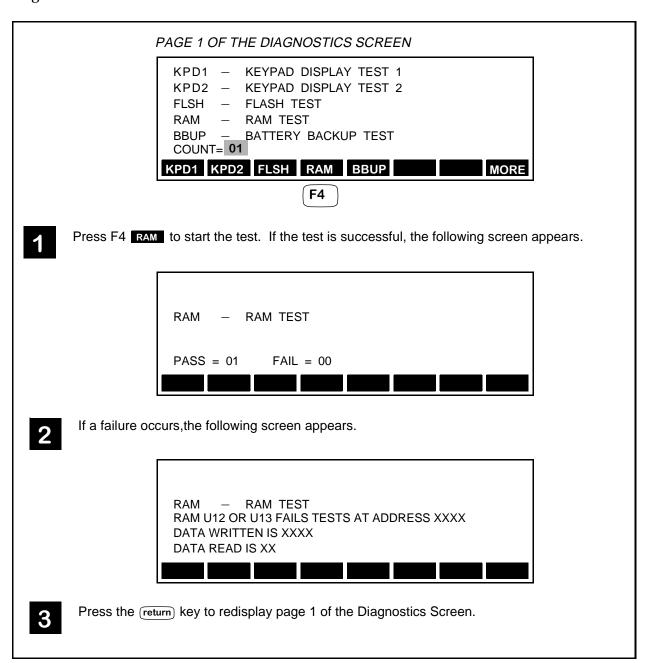
This test calculates a checksum for the flash memory parts which is then compared with a checksum stored on the flash memory. If a failure occurs, call your PerkinElmer Service Engineer.



RAM Test

This diagnostic writes a number of test patterns into RAM and then reads them to verify that they were written correctly. Each RAM is tested with three patterns. The first is module 256 of each byte address. The second is the hexadecimal value 55 and AA written into the same part of every other byte.

The system RAM is tested first, then the battery backup RAM is tested. The contents of the battery backup RAM are stored in the system during testing. The battery backup RAM will not be tested if the system RAM fails. If a failure occurs, call your PerkinElmer Service Engineer.

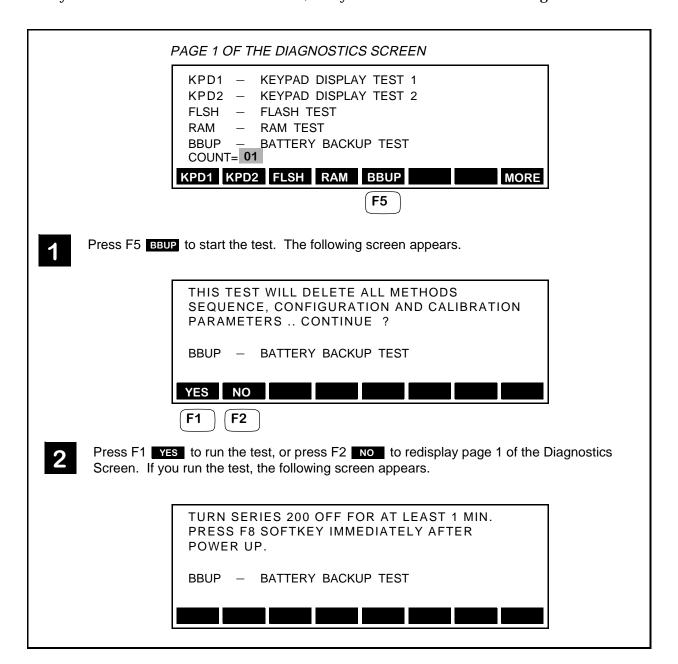


Battery Backup Test

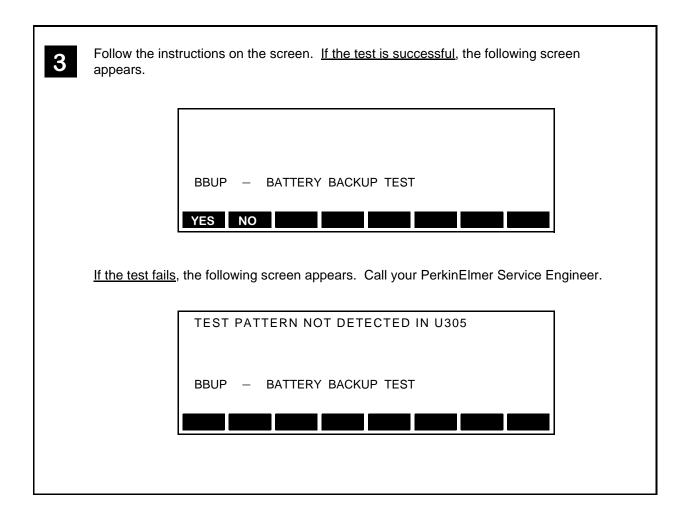


This test will delete all methods, the sequence, configuration and calibration parameters.

This test is designed to test the battery backup RAM. The contents for the battery backup RAM are stored in the system during testing. The battery backup RAM will not be tested if the system RAM fails. If a failure occurs, call your PerkinElmer Service Engineer.



Backup Battery Test (continued)



Peltier Communications Test

Note

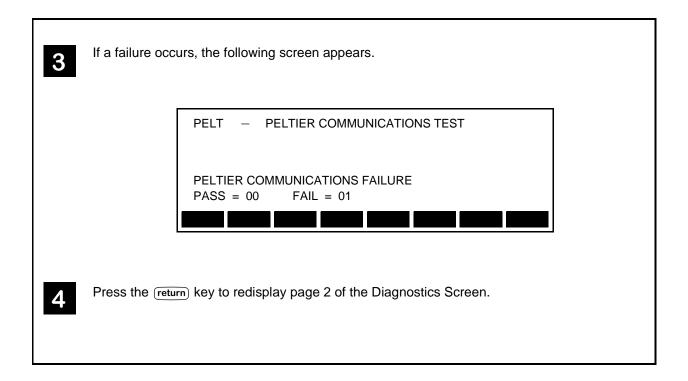
This test is designed to test communication between the optional Peltier Accessory and the Autosampler through the Peltier Connector on the rear panel of the Autosampler. See Figure 6-2 for the location of the connector. If a failure occurs, call your PerkinElmer Service Engineer.

You cannot run this test without a special test

fixture (loopback connector).

PAGE 2 OF THE DIAGNOSTICS SCREEN PELT PELTIER COMMUNICATIONS TEST DUA1 -DUART TEST INTERNAL DUA2 DUART TEST EXTERNAL INP REMOTE INPUTS TEST COUNT= 01 PELT DUA1 DUA2 INP MORE F1 Press F1 PELT to start the test. The following screen appears. PELT - PELTIER COMMUNICATIONS TEST PLACE TEST CONNECTOR ON J4 (PELTIER) PRESS PELT **PELT** F1 Attach the loopback connector to the Peltier (J4) connector on the rear panel of the 2 Autosampler. See Figure 6-2. Press F1 PELT to continue. If the test is successful, the following screen appears. PELT - PELTIER COMMUNICATIONS TEST PASS = 01FAIL = 00

Peltier Communications Test (continued)



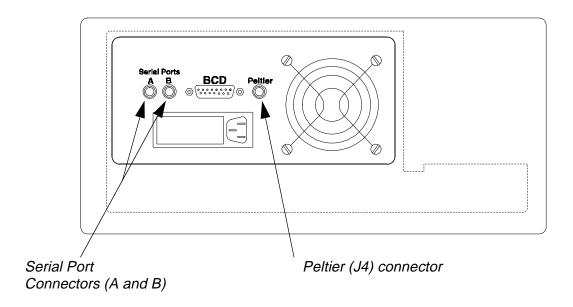
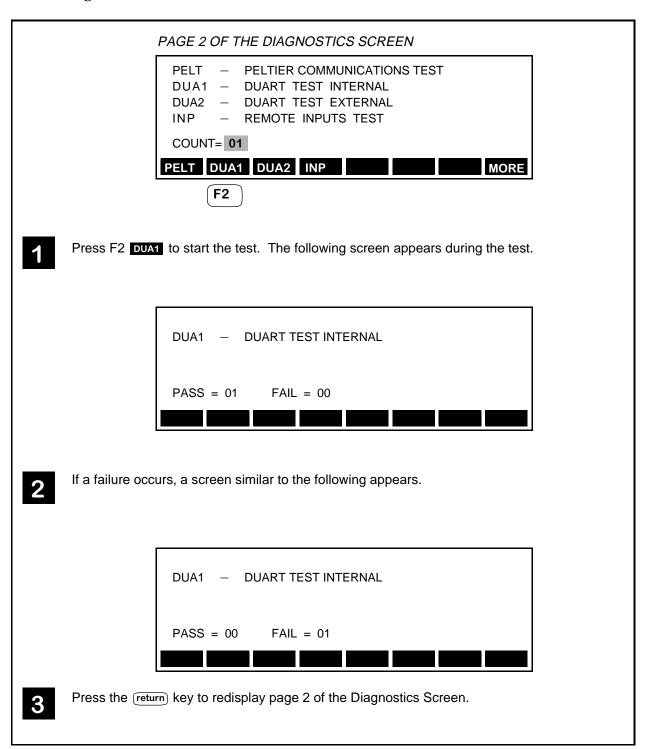


Figure 6-2. Rear Panel of the Autosampler.

DUART Test Internal

This test is designed to test the internal DUART which controls serial communications. The DUART is configured for local loop-back mode. Data bytes 55 and AA are transmitted. This test verifies that bytes 55 and AA are received. If a failure occurs, call your PerkinElmer Service Engineer.



DUART Test External

Note

This test is designed to test DUART and connections between the Serial A and Serial B ports on the rear panel of the Autosampler. See Figure 6-2 for the location of the connectors. If a failure occurs, call your PerkinElmer Service Engineer.

You cannot run this test without a special test

fixture (loopback connector). PAGE 2 OF THE DIAGNOSTICS SCREEN PELTIER COMMUNICATIONS TEST* PELT DUART TEST INTERNAL DUA1 -DUA2 — DUART TEST EXTERNAL INP - REMOTE INPUTS TEST COUNT= 01 PELT DUA1 DUA2 INP **MORE** F3 Press F3 DUA2 to start the test. The following screen appears. PLACE TEST CONNECTOR ON J8/J15 (SERIAL) PRESS DUA2 DUA2 - DUART TEST EXTERNAL DUA2 F3 Attach the loopback connector to the Serial A connector on the rear panel of the Autosampler. See Figure 6-2. Press F3 DUA2 to continue. If the test is successful, the following screen appears. DUA2 - DUART TEST EXTERNAL PASS = 01FAIL = 00

DUART Test External (continued)

If a failure occurs, the following screen appears.

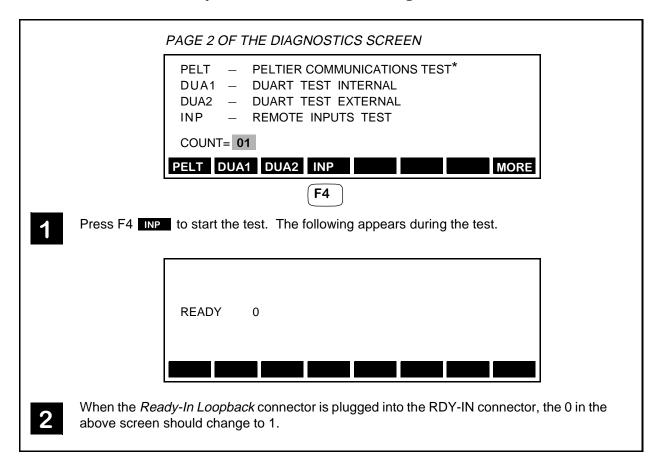
DUA2 — DUART TEST EXTERNAL

U23 FAILURE
PASS = 00 FAIL = 01

- Repeat steps 1 3 above for Serial B connector.
- Press the return key to redisplay page 2 of the Diagnostics Screen.

Remote Input Test

This test is designed to test the remote input. In order to perform this test, you need the *Ready-In Loopback* connector, Part No. N293-0345. Plug the connector into the RDY-IN terminal connector on the Autosampler's I/O Terminal Connector panel. This panel is located on the right side of the Autosampler. Refer to Figure 6-3 for location of the RDY-IN connector. If a failure occurs, call your PerkinElmer Service Engineer.



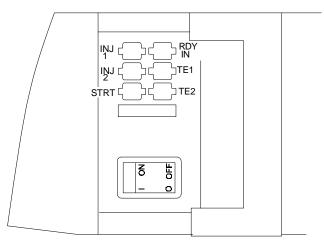
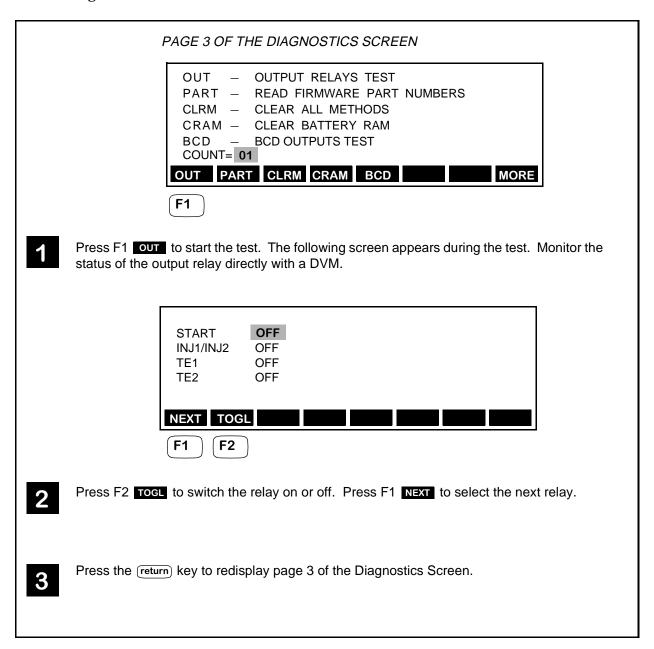


Figure 6-3. Location of the RDY-IN Connector.

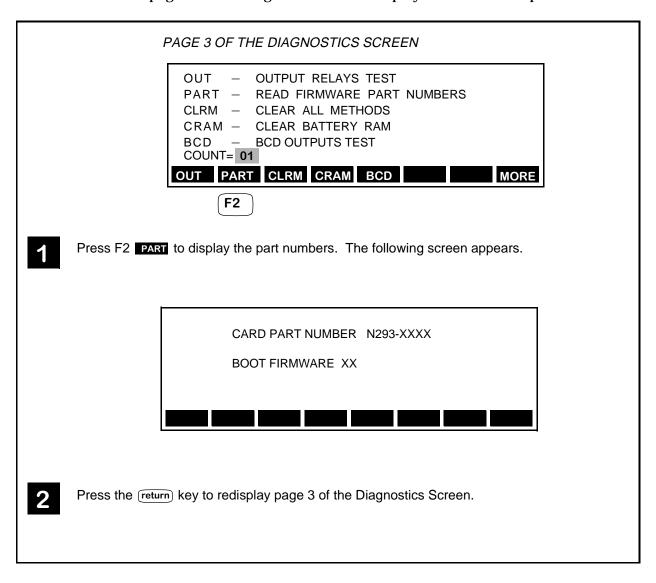
Output Relays Test

This test is designed to test the output relays. If a failure occurs, call your PerkinElmer Service Engineer.



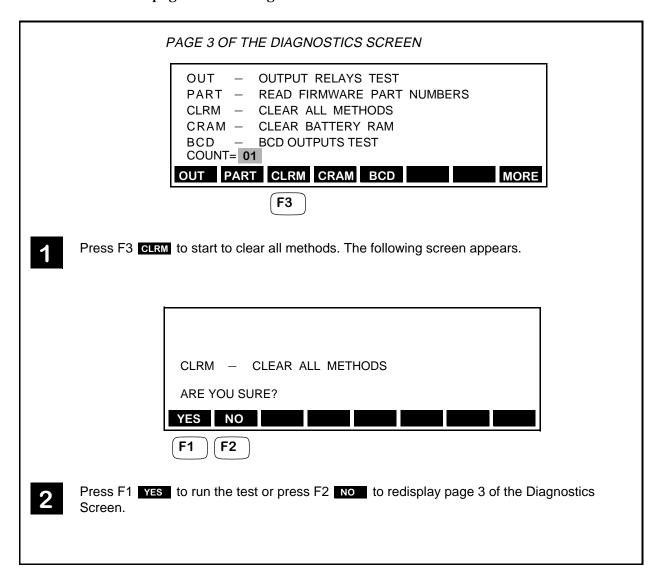
Read Firmware Part Number

This selection from page 3 of the Diagnostics Screen displays the Firmware part number.



Clear All Methods

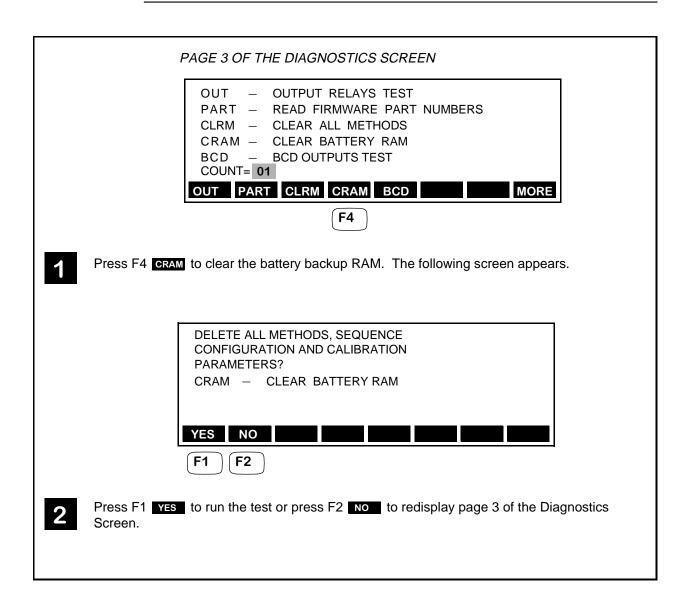
This selection from page 3 of the Diagnostics Screen clears all stored methods.



Clear Battery RAM

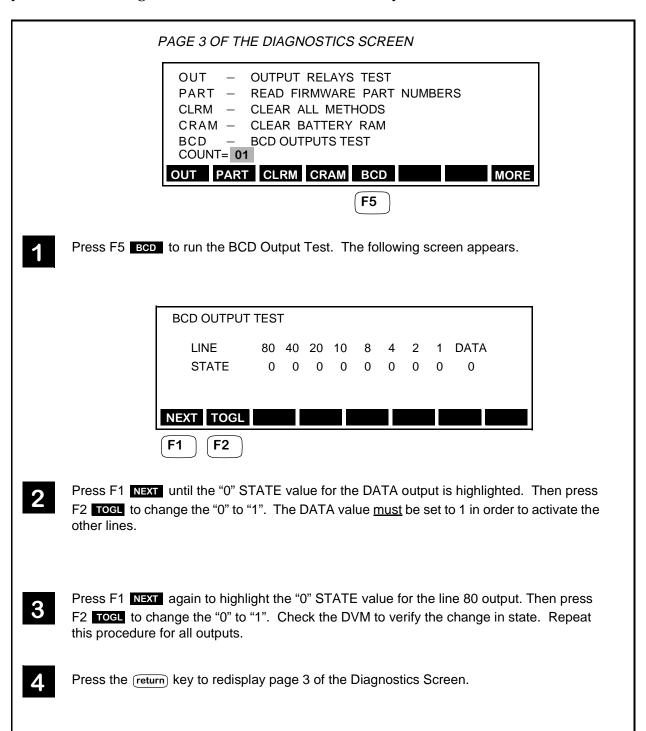


This test will delete all methods, the sequence, configuration, and calibration parameters.



BCD Outputs Test

To perform the BCD Output Tests, first connect the PerkinElmer cable, Part No. N293-0340 (or similar), to the BCD port on the rear panel of the Autosampler. The location of the BDC port is shown in Figure 6-4. Use a DVM to check the outputs.



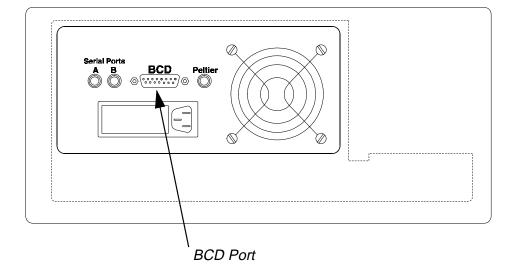
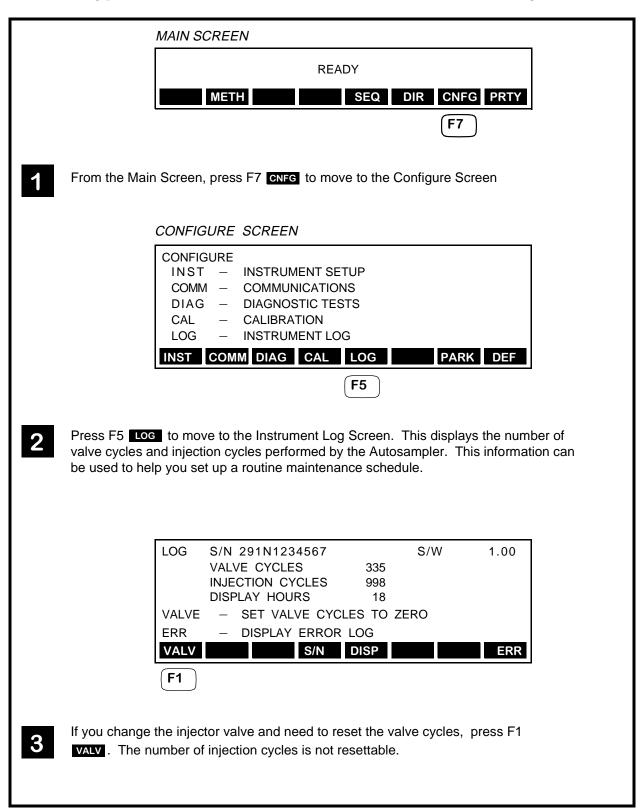


Figure 6-4. Rear Panel of the Autosampler.

Viewing the Instrument Log

The following procedure describes how to access and view the Instrument Log.



Viewing the Instrument Log (continued)

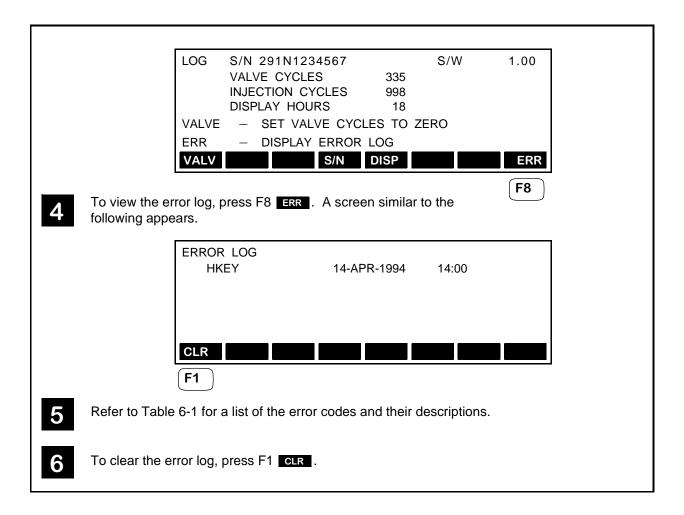


Table 6-1. Error Codes.

| Error | Description | Error | Description | | |
|--|---------------------------------------|-------|------------------------|--|--|
| ♦HKEY | Hard key stuck | XMTR | X Motor | | |
| ♦KPD1 | Keypad display test 1 | YMTR | Y Motor | | |
| ♦FLSH | Flash test | ZMTR | Z Motor | | |
| ♦RAM | RAM test | FMTR | Flush Motor | | |
| ♦BRAM | Battery RAM test | SMTR | Sample Motor | | |
| TEMP | Failure to control to set temperature | IVLD | Injection valve load | | |
| ♦DUA1 | DUART test internal | IVIN | Injection valve inject | | |
| ♦ These occur only on start-up. Contact PerkinElmer Service. | | | | | |

Notes

Maintenance 7

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|---|
| Preventative Measures to Avoid Problems 7-3 |
| Routine Maintenance and Inspection 7-4 |
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| Replacing a Fuse 7-5 |
| Locating and Removing the Power Input Module 7-5 |
| Changing the Fuse(s) 7-6 |
| Changing the Sample and Flush Syringes 7-8 |
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| Replacing the Tubing to the Pump 7-33 |
| Replacing the Waste Tubes 7-35 |

Maintenance 7

This chapter describes how to perform routine and preventive maintenance procedures on your Series 200 LC Autosampler. It includes the following information:

- Guidelines for Maintaining the Autosampler
- Replacing a Fuse
- Changing the Sample and Flush Pump Syringes
- Replacing the Sample Needle
- · Changing the Transfer Tubing
- Removing the Injector Valve
- Dismantling the Injector Valve
- Changing the Sample Loop
- · Replacing the Tubing to the Column
- Replacing the Tubing to the Pump
- Replacing the Waste Tubes

Maintenance

Notes

Guidelines for Maintaining the Autosampler

The Autosampler is constructed with high quality components and requires little maintenance other than to keep it clean. The following guidelines are offered to keep your Autosampler up and running.

- Wipe up spilled liquids immediately with a soft lint-free cloth or absorbant paper to prevent damage to the instrument.
- Carry out the maintenance tasks described in this chapter.
- If you have any problems or inquires, contact your nearest PerkinElmer office.
- If you need to return the instrument for maintenance, send it in the original packing.

Preventative Measures to Avoid Problems

- Follow good laboratory practice.
- Use only high quality purity HPLC grade solvents.

| Note | Mixtures of organics and water, once degassed, remain in a degassed state for longer periods of time than pure organics. This minimizes bubble formation in the transfer lines. In general, depending on your mobile phase, strengths are: |
|------|--|
| | 3:1 methanol:water, 1:1 methanol:water, or |
| | your specific mobile phase without buffer. |

- Use filtered solvents to prevent the tubing in the LC system from becoming clogged.
- Use an in-line filter (for example, PerkinElmer Part No. 0990-3606) between the pump and the injector valve.
- Use only clean, passivated tubing for the solvents.
- Follow the operating and warning instructions in this manual.

7 - 3

Routine Maintenance and Inspection

Carry out the following tasks regularly to avoid problems.

- Use only degassed and filtered HPLC grade solvents for the flushing solution.
- Make sure the flushing solution container is clean and rinse it out with fresh solvent before filling.
- Do not let the waste container overfill. Empty it regularly, ensuring the solvent is disposed of correctly.
- Check the filters regularly, and clean or replace them as required.
- Check the system regularly for leaks. Where necessary, tighten joints or replace damaged parts.
- If you do not run solvent through the Autosampler for a period of 24 hours, flush the sample and flush pump syringes, and the transer tubing, with isopropanol before running any samples.

Viewing the Instrument Log

Periodically check the Instrument Log as described in section "Viewing the Instrument Log" in Chapter 6 of this manual. This lists the number of valve and injection cycles performed by the Autosampler. You can use this information to help set up a maintenance schedule.



Before starting any maintenance work:

- 1. Switch off all instruments in the system.
- 2. Disconnect the Autosampler from the electrical supply.
- 3. Follow the maintenance instructions exactly as described in this chapter.

Replacing a Fuse Maintenance

Replacing a Fuse

1 Locating and Removing the Power Input Module

1. Switch off the power to the Autosampler and disconnect it from the electrical supply.

2. Refer to Figure 7-1. The fuse is contained in the Power Input Module located on the rear of the Autosampler.

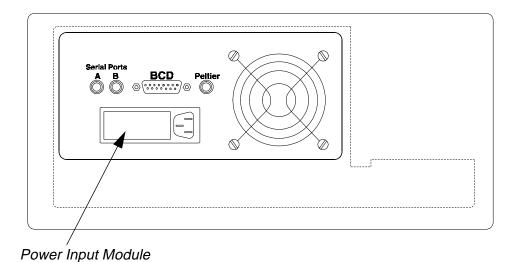


Figure 7-1. Power Input Module.

- 3. Refer to Figure 7-2. Remove the Power Input Module cover by inserting a small, flat blade screwdriver into the notch and carefully prying off the cover.
- 4. Remove the voltage selector card from the Power Input Module and store in a safe place. Be careful not to change the voltage selector setting.

Maintenance Replacing a Fuse

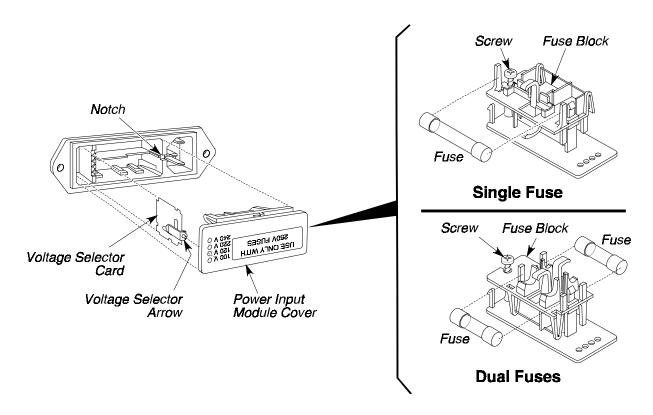


Figure 7-2. Fuse Location.

2 Changing the Fuse(s)

1. Remove the old fuse(s). For a single fuse configuration, select the replacement fuse from Table 7-1. For a dual fuse configuration, select the replacement fuses from Table 7-2.

Table 7-1. Single Fuse Configuration Table (Use only 3AG 1/4 x 1-1/4" 250V Rated Fuses)

| Voltage Selector | Replacement fuse: | P/N |
|------------------|-------------------|-----------|
| 100 | 1.6 amp Slow-Blow | 0998-1613 |
| 120 | 1.6 amp Slow-Blow | 0998-1613 |
| 220 | 0.8 amp Slow-Blow | 0999-1671 |
| 240 | 0.8 amp Slow-Blow | 0999-1671 |

Replacing a Fuse Maintenance

Table 7-2. Dual Fuse Configuration Table (Use only 5 x 20 mm 250V Rated Fuses)

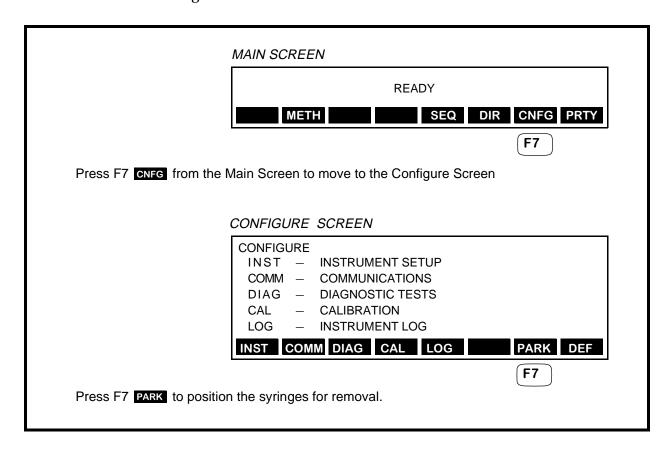
| Voltage Selector | Replacement fuse: | P/N |
|------------------|-------------------|-----------|
| 220 | 0.8 A Type TT | 0999-1670 |
| 240 | 0.8 A Type TT | 0999-1670 |

- 2. Slide the fuse(s) into the fuse holder. See Figure 7-2.
- 3. Replace the voltage selector card.
- 4. Reinsert the power input module cover back into the power input module.

Changing the Sample and Flush Syringes

1 Removing the Old Syringes

1. Position the syringe plungers in the Dual Pump System for removal by using the PARK function on the Configure Screen as shown here.



- 2. Switch off the power to the Autosampler and disconnect it from the electrical supply.
- 3. Refer to Figure 7-3. The Dual Pump System (consisting of the Sample and Flush Syringes) is located on the left side of the Autosampler.
- 4. Pull the solvent filter (at the end of the flush line tubing) out of the solvent reservoir.
- 5. Loosen the thumbscrew on the bottom of each syringe by turning it clockwise.
- 6. Grasp each syringe barrel and loosen it by turning it clockwise.
- 7. For the flush syringe, unscrew the top of the syringe from the flush solenoid.
- 8. For the sample syringe, unscrew the top of the syringe from the "T".

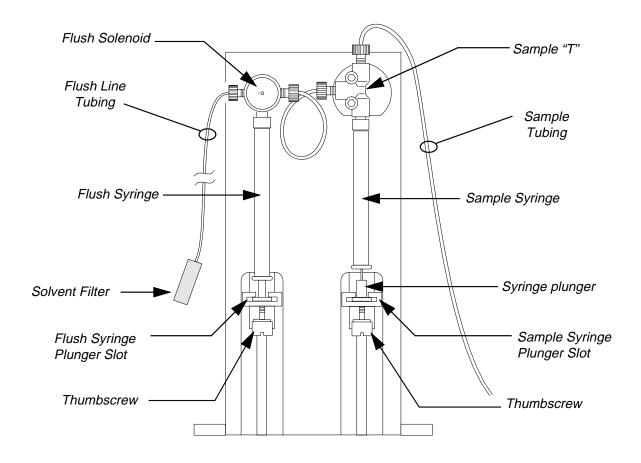


Figure 7-3. Dual Pump System - Sample and Flush Syringes.

2 Loading the New Syringes

- 1. Select a new sample and flush syringe. See Table 7-3 for a list of available replacement sample syringes and their part numbers. There is a single 2500 μ L replacement flush syringe.
 - For the flush syringe, you can order the syringe, Part No. 0992-3219, or the 2500 μL Syringe Startup Kit, Part No. N293-0213. The Startup Kit includes the syringe, union, and the tubing needed to accommodate the 2500 μL volume.
- 2. Fill the inside of each new syringe with isopropanol, moving the syringe plunger up and down the entire inside surface.
- 3. Remove all of the isopropanol bubbles from each syringe. Leave enough volume of isopropanol in each syringe so that when you thread it into the Dual Pump system, there are no air bubbles in the top of the syringe.
- 4. For the flush syringe, slide the plunger into the flush syringe plunger slot. Thread the top of the flush syringe into the flush solenoid.

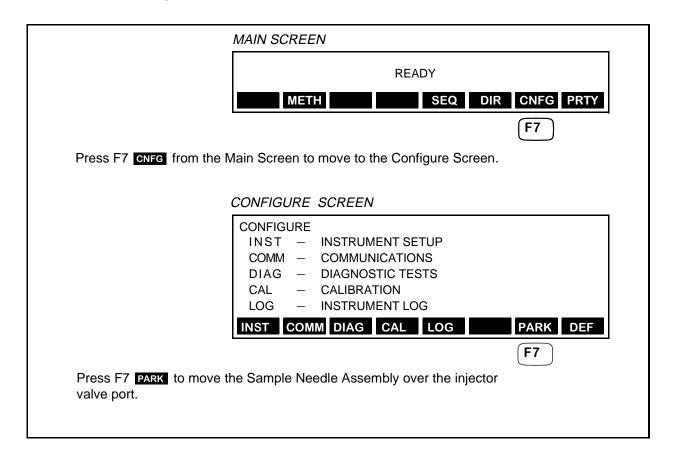
- 5. For the sample syringe, slide the plunger into the sample syringe plunger slot. Thread the top of the sample syringe into the sample "T".
- 6. Replace the solvent filter in the solvent reservoir.
- 7. Reconnect the Autosampler to the electrical supply.
- 8. Switch on the power to the Autosampler.
- 9. Press the flush key on the keyboard and flush solvent through the system until there are no bubbles in the syringes or transfer tubing. To ensure that there are no bubbles in the transfer tubing, inspect closely the tubing near the top of the sample needle.

Table 7-3. Replacement Sample Syringes.

| Sample Syringe | Injection Range | Replacement Part No. |
|----------------|-----------------|----------------------|
| 50 μL | 0.1 - 20 μL | 0992-3304 |
| 100 μL | 0.1 - 65 μL | 0992-3305 |
| 250 μL | 0.1 - 195 μL | 0992-3270 |
| 500 μL | 0.1 - 430 μL | 0992-3306 |
| 1000 μL | 1.0 - 880 μL | 0992-3307 |
| 2500 μL | 1.0 - 2475 μL | 0992-3219 |

Replacing the Sample Needle

1. Position the sample needle assembly over the injector valve port using the PARK function on the Configure Screen as shown here.



2. Switch off the power to the Autosampler and disconnect it from the electrical supply.

3. Refer to Figure 7-4. Remove the needle cartridge from the needle cartridge face plate by removing the two thumbscrews.

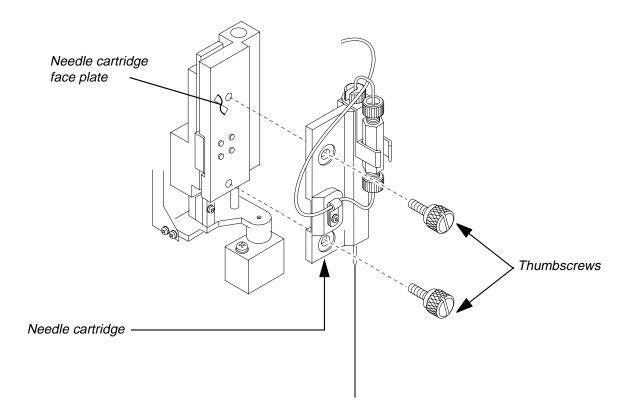


Figure 7-4. Removing the Needle Cartridge.

Steps 4 - 9 below are keyed to Figure 7-5.

- 4. Remove the PEEK nut connecting the sample tubing to the union.
- 5. Remove the slotted needle nut holding the sample needle and union holder in the needle cartridge.
- 6. Remove the sample needle, union, and union holder from the cartridge; slide the union holder off the sample needle.
- 7. Unscrew the PEEK nut connecting the sample needle to the union and remove the PEEK nut from the needle.
- 8. Slide the needle spring over the curved top portion of the sample needle and set aside.
- 9. Replace the sample needle. Reverse the above steps to install the new needle.

Note: When reattaching the needle cartridge to the faceplate, as shown in the lower part of Figure 7-5, press and <u>hold the needle cartridge up against the face plate so that the lower surfaces of the two plates are flush</u>. While holding the needle cartridge securely in place, tighten the two thumbscrews.

- 10. Switch on the power to the Autosampler.
- 11. Press the (flush) key on the keyboard and flush solvent through the system until there are no bubbles in the sample tubing. To ensure that there are no bubbles, inspect closely the tubing near the point where it enters the union.

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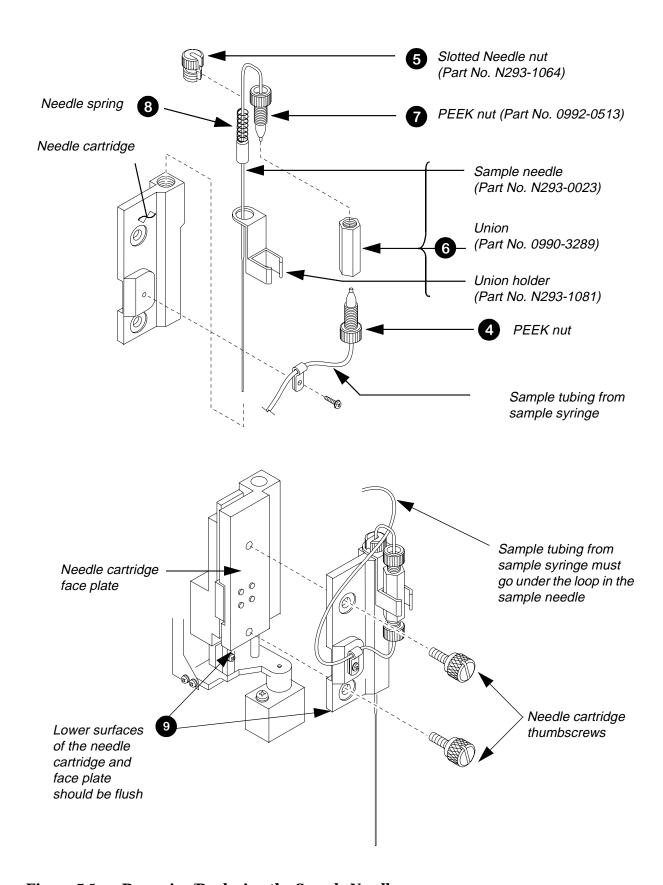


Figure 7-5. Removing/Replacing the Sample Needle.

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Maintenance

Notes

Changing the Transfer Tubing

1 Removing the Injector Valve Cover

- 1. Switch off the power to the Autosampler and disconnect it from the electrical supply.
- 2. Refer to Figure 7-6. Open the sample compartment door (sampling section) of the Autosampler.
- 3. Grasp the thumbscrews on the front of the needle cartridge and press up and slide the assembly to the right until it is over the sample tray as shown in Figure 7-6.
- 4. Loosen the two thumbscrews holding the top cover over the injector valve assembly. Slide the cover out from under the thumbscrews and lift the injector cover off the assembly.

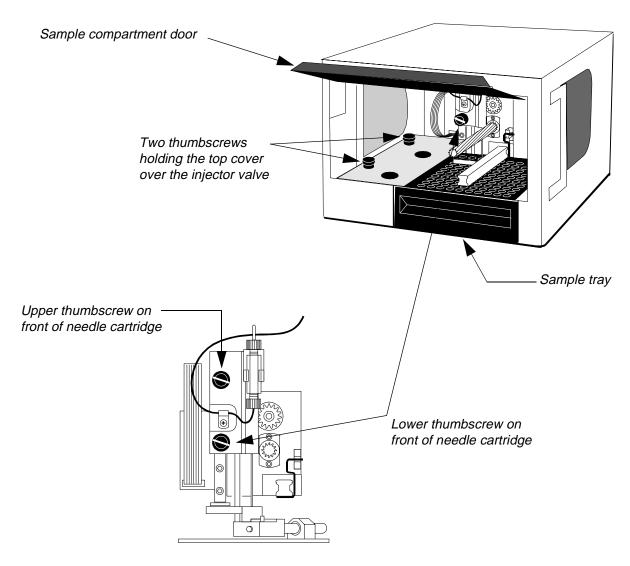


Figure 7-6. Removing the Injector Valve Cover.

2 Removing the Autosampler Cover

Refer to Figure 7-7. Remove the three pairs of screws holding the cover on the Autosampler. There are a pair of screws located on the left and right sides as well as the back.

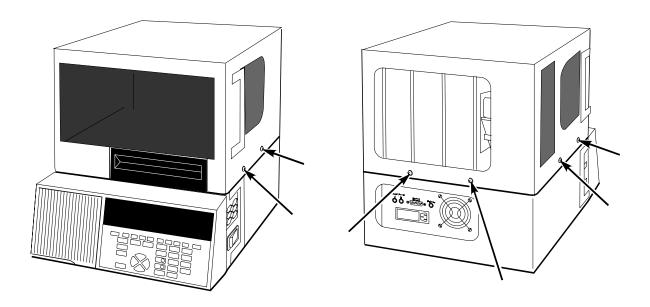


Figure 7-7. Removing the Autosampler Cover.

Replacing the Tubing

There are three main sections of transfer tubing in the Autosampler:

- Solvent reservoir to flush solenoid, length = 4 ft.
- Flush solenoid to sample syringe "T", length = 150 mm.
- Sample syringe "T" to sample needle, length = 5 ft.

When replacing the tubing, cut the length of tubing you need from the Teflon Transfer line, Part No. N293-1139, which is supplied in a single 10-ft. long section.

Replacing the Solvent Reservoir to Flush Solenoid Tubing

When you replace this section of tubing, you should also replace the solvent filter. Refer to Figure 7-8.

- 1. Cut a new 4 ft. section of tubing from the Transfer Line (N293-1139).
- 2. Add a nut (Part No. 0992-0381) and reverse ferrule (Part No. 0992-0382) to the end of the tubing going to the flush solenoid inlet as shown in Figure 7-8.

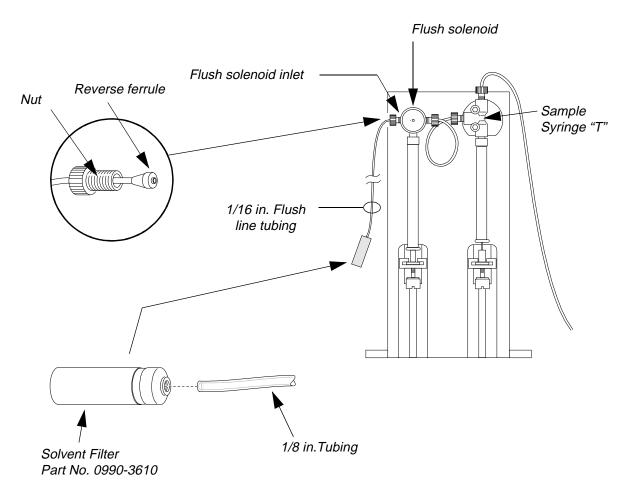


Figure 7-8. Replacing the Flush Line Tubing, Solvent Filter, and Flush Solenoid to Sample "T" Tubing.

- 3. To attach the new solvent filter to the other end of the flush line tubing, first insert a section of 1/8 in. tubing into the solvent filter. Then slide the 1/16 in. flush line tubing into the 1/8 in. tubing.
- 4. Firmly screw the solvent filter onto the end of the tubing.
- 5. Thread the nut and ferrule end of the tubing into the flush solenoid inlet.
- 6. Place the end with the solvent filter into the solvent reservoir.

Replacing the Flush solenoid to Sample Syringe "T" Tubing

- 1. Cut a new 150-mm section of tubing from the Transfer Line (N293-1139).
- 2. Add a nut (Part No. 0992-0381) and reverse ferrule (Part No. 0992-0382) to each end of the 150-mm tubing section. Orient the each nut and ferrule as shown in Figure 7-8.
- 3. Thread one end into the flush solenoid and the other into the sample syringe "T" as shown in Figure 7-8.

Replacing the Tubing from Sample "T" to Needle

- 1. Refer to Figure 7-9 on the facing page which shows the path of the transfer tubing from the sample syringe "T" to the union on the sample needle.
- 2. Cut a new 5 ft. section of tubing from the Transfer Line (N293-1139).
- 3. Add a PEEK nut (0992-0513) to one end of the 5 ft. tubing section.
- 4. Unscrew the nut and ferrule connecting the old section of tubing to the sample syringe "T". Remove the nut and ferrule from the end of the tubing.
- 5. Thread the tubing back through the path shown in Figure 7-9 all the way to the point where it connects to the union on the sample needle.
- 6. Unscrew the PEEK nut connecting the old sample tubing to the union.
- 7. Connect the end of the replacement tubing with the PEEK nut to the union.
- 8. Thread the replacement tubing back through all of the clamps identified in Figure 7-9, to the sample syringe "T". When you reach the two adjacent clamps on the top of the PC board, be sure and loop the transfer tubing three times through the clamps as shown.
- 9. Add a nut and reverse ferrule to the free end of the tubing and thread it into the sample syringe "T".

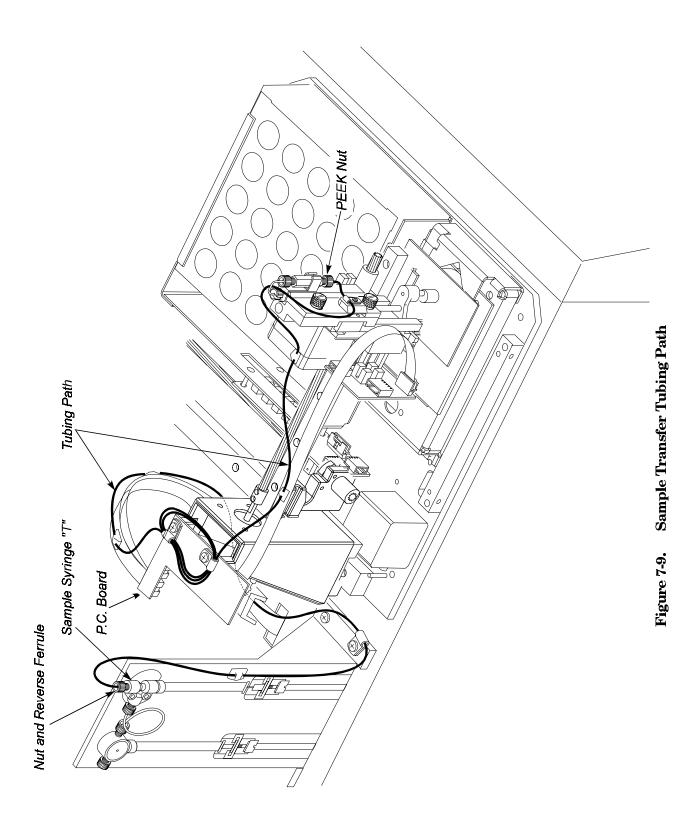
Replacing Part of the Sample "T" to Needle Tubing

If you only need to replace a portion of the tubing at or near the point where it enters the union on the sample needle, do the following:

- 1. Unscrew the PEEK nut connecting the sample tubing to the union.
- 2. Remove the PEEK nut from the end of the tubing and cut off the old section.
- 3. Thread the tubing back through the 2 adjacent clamps on top of the PC board (see Figure 7-9) to free up an additional length of tubing. Each loop yields about 5 inches of tubing.
- 4. Rethread the tubing back through the clamps to the union.
- 5. Replace the PEEK nut on the free end of the tubing and rethread it into the union.

4 Flushing the System/Replacing the Cover

- 1. When you are done replacing the tubing, reconnect the Autosampler to the electrical supply and switch on the power.
- 2. Press the flush key on the keyboard and flush solvent through the system until there are no bubbles in the sample tubing. To ensure that there are no bubbles, inspect closely the tubing near the point where it enters the union on the sample needle.
- 3. Replace the injector valve cover by reversing the procedure in item 1 in this section.
- 4. Replace the Autosampler cover by reversing the procedure in item 2 in this section.



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Removing the Injector Valve

1 Removing the Injector Valve Cover

- 1. Switch off the power to the Autosampler and disconnect it from the electrical supply.
- 2. Follow the same procedure for removing the injector valve cover as described in "Changing the Transfer Tubing" section in this chapter.

2 Disconnecting the External Tubing Connections

1. Disconnect the Autosampler from the pump and the column. The pump and column tubes exit the top cutout on the left side of the Autosampler and are marked as shown in Figure 7-10.

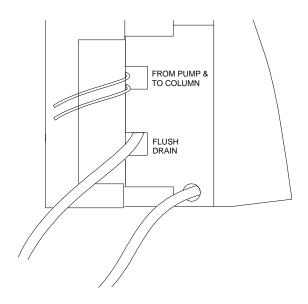


Figure 7-10. Pump and Column Connections to the Autosampler.

2. Open the front access door on the Autosampler and pull the drain tubes extending from ports 5 and 6 on the injector valve out of the "Y" drain connector as shown in Figure 7-11.

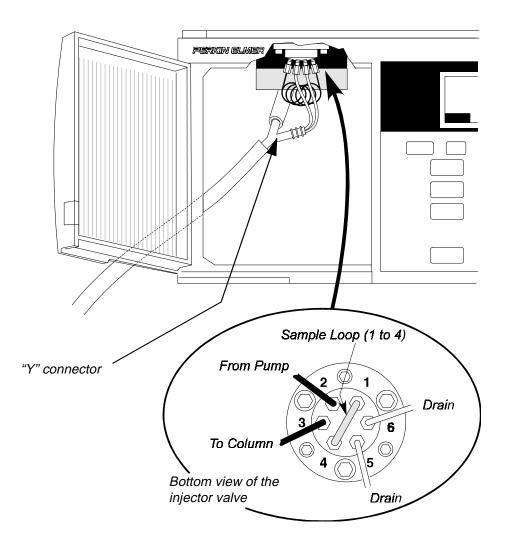


Figure 7-11. Removing the Drain Tubes from the Waste Drain.

Removing the Injector Valve Assembly

- 1. Refer to Figure 7-12. Remove the stainless steel needle guide, Part No.N293-1054. **Store in a safe place**.
- 2. Using the allen wrench supplied, loosen the two allen screws on the valve actuator arm. Lift the valve actuator arm straight up and off the injector valve stem.

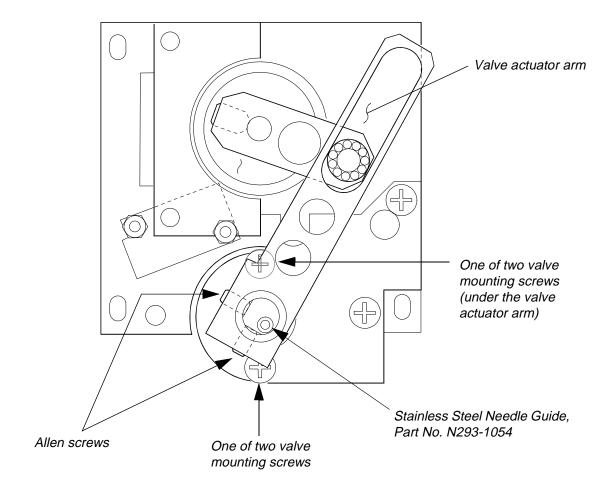


Figure 7-12. Top of the Injector Valve Assembly.

3. Refer to Figure 7-13. With one hand, grasp the injector valve just above the sample loop. With the other hand, use a phillips head screwdriver (not supplied) to loosen the valve mounting screws on top of the valve injector assembly. The mounting screws are located directly in front of and behind the injector valve stem (see Figure 7-12).

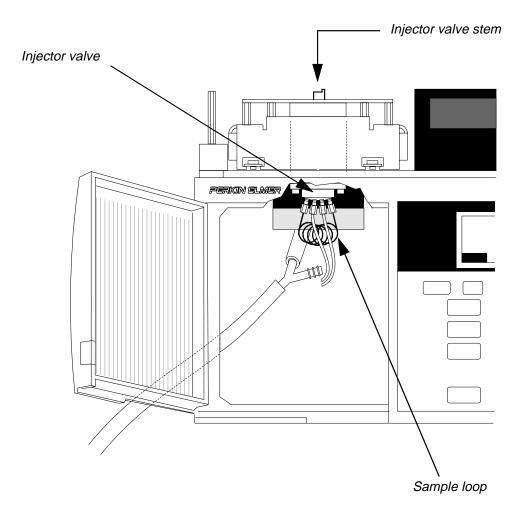
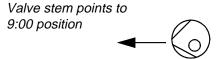


Figure 7-13. Front View of Injector Assembly

4. Before you remove the injector valve, note that the valve stem points to the 9:00 position as shown here:



When you replace the injector, it \underline{must} be replaced so that the valve stem points in this direction.

5. Remove the valve mounting screws and **store them in a safe place**.

Dismantling the Injector Valve

Under normal conditions, the injector valve will perform thousands of injections before requiring any maintenance. The two components of the injector valve most likely to require maintenance are the rotor seal and the stator face assembly, respectively.

1

Replacing the Rotor Seal and Stator Face Assembly

- 1. Refer to Figure 7-14. Remove the three stator screws.
- 2. Remove the stator. Note that the stator face assembly usually comes off with the stator.
- 3. Remove the stator ring (2-piece assembly).
- 4. Pull the rotor seal off the pins.

| Note | Leave the isolation seal and bearing ring in place. |
|------|---|
| | |

5. See item **2** in this section for reassembly.

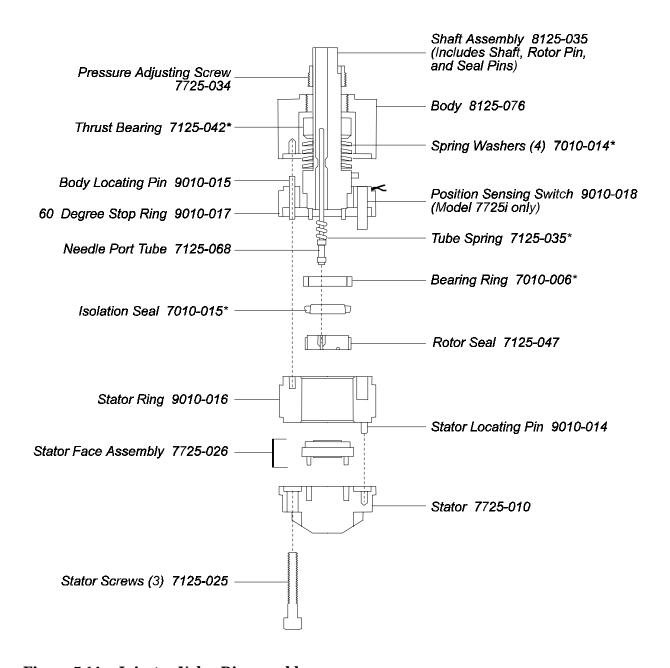


Figure 7-14. Injector Valve Disassembly.

Note All part numbers in Figure 7-14 are Rheodyne.

2 Reassembling the Injector Valve

- 1. Line up the rotor seal as shown in Figure 7-15. The rotor seal slots face the stator.
- 2. Replace the stator ring so that the pin in the 60° stop ring enters the mating hole in the stator ring.

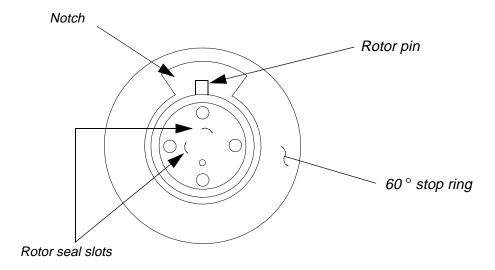


Figure 7-15. Correct Position of Rotor Seal.

3. Loosen the pressure adjusting screw about 1/2 turn. Note the original position of the two red dots as shown in Figure 7-16.

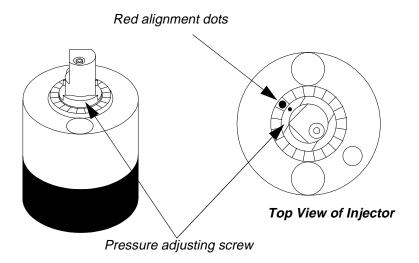


Figure 7-16. Guides on Adjusting Screw and Body of Injector.

- 4. Put the stator face assembly on the stator. The three pins on the assembly fit into the mating holes in the stator only one way.
- 5. Replace the stator and stator face assembly on the valve so that the pin in the stator ring enters the mating hole in the stator.
- 6. Add the three stator screws. Tighten each an equal amount until the screws are tight.

3

Reinstalling the Injector Valve in the Autosampler

Note

If you need to change the sample loop, drain tubes, pump or column tubes, do so before reinstalling the Injector Valve. Refer to the appropriate sections in this chapter.

1. Refer to Figure 7-17.

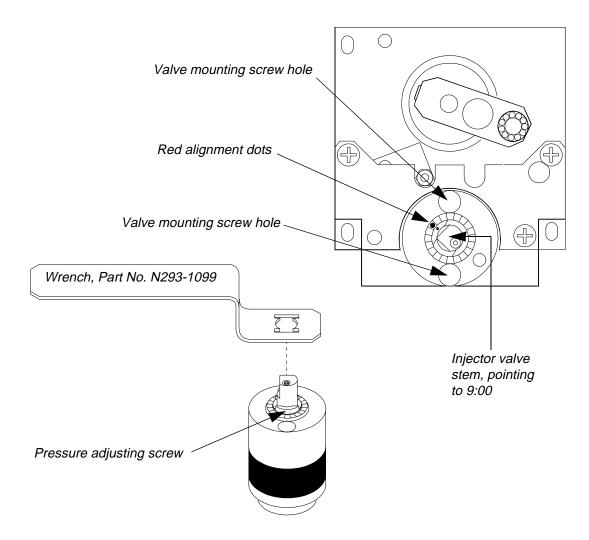


Figure 7-17. Orientation of Injector Valve Stem in the Injector Valve Assembly.

2. Reattach the injector valve to the injector assembly using the two phillips head valve mounting screws you set aside in the procedure "Removing the Injector Valve" in this section.

- 3. Using the Valve Wrench, Part No. N293-1099, realign the red dot on the outer (lower) ring with the red dot on the pressure adjusting screw as shown in Figure 7-17. Note that the wrench fits over the pressure adjusting screw. This action sets the pressure rating of the injector valve.
- 4. Reinstall the valve actuator arm over the valve stem and lock it in place by tightening the two allen screws on the arm. Use the allen wrench provided.
- 5. Reinsert the stainless steel needle guide you set aside earlier in the procedure "Removing the Injector Valve" in this section.
- 6. Slide the top cover over the injector valve assembly and under the thumbscrews on the left side of the sample compartment. Tighten the thumbscrews.
- 7. Reconnect all the external tubing connections.
- 8. Reconnect the Autosampler to the electrical supply and switch on the power.
- 9. Press the flush key on the keyboard and flush solvent through the system until there are no bubbles in the sample tubing. To ensure that there are no bubbles, inspect closely the tubing near the point where it enters the Teflon union on the sample needle.

Important

If you have changed the <u>type</u> of injector valve, or if you have rebuilt the injector valve, you need to do the following:

- 1. Recalibrate the sample needle as described in the "Calibrating the Sample Needle" section in Chapter 6 of this manual.
- 2. Reset the valve cycles to zero as described in the "Viewing the Instrument Log" section in Chapter 6 of this manual.

Changing the Sample Loop

If you have not already done so, remove the injector valve from the Autosampler as described in "Removing the Injector Valve" section in this chapter.

If you have also changed the loop mode performance parameter from partial to fixed, refer to Appendix D for the recommended sample loop and sample syringe sizes.

Appendix B provides a list of all the performance parameters and describes how to change them.

1. Refer to Figure 7-18 which shows the location of all the ports on the bottom of the injector valve and their connections.

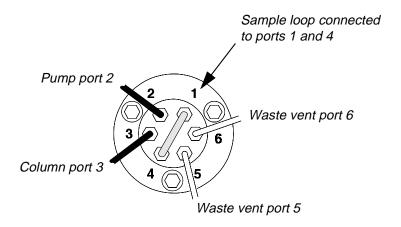


Figure 7-18. Bottom View of the Injector Valve.

- 2. Using a standard 1/4" wrench, remove the sample loop attached to ports 1 and 4.
- 3. Select the appropriate replacement sample loop, nuts and ferrules.

For sample loop types and their part numbers, refer to the PerkinElmer HPLC and CE Supplies Catalog or to a PerkinElmer Product Description List.

4. Thread each nut and ferrule into ports 1 and 4. Finger tighten each nut as far as it will go. Take the 1/4" wrench and tighten the nut an additional 1/8 turn.

Replacing the Tubing to the Column

If you have not already done so, remove the injector valve from the Autosampler as described in "Removing the Injector Valve" section in this chapter.

1. Refer to Figure 7-19 which shows the location of all the ports on the bottom of the injector valve and their connections.

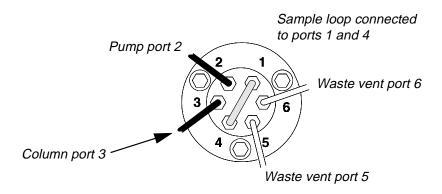


Figure 7-19. Bottom View of the Injector Valve.

2. Select the appropriate replacement tubes, nuts and ferrules from Table 7-4.

Table 7-4. Replacement Tubing, Nuts, and Ferrules.

| Replacement Part | Stainless Steel Part No. | Bio Applications Part No. | |
|--------------------|--|--|--|
| Tubing sections | 0271-1441 [20 cm (1/16-in. O.D., 0.007-in. I.D.)] N293-1089 [30 cm (1/16-in. O.D., 0.007-in. I.D.) 0089-1480 [50 cm (1/16-in. O.D., 0.007-in. I.D.) | N930-2678 [5 ft.(1/16-in. O.D., 0.007-in. I.D.)] N930-2650 [5 ft.(1/16-in. O.D., 0.010-in. I.D.)] | |
| Rheodyne short nut | 0990-4956 | | |
| Rheodyne long nut | 0990-4974 | N930-2676 Pkg. of 5 Rheflex Nuts and Ferrules. | |
| Rheodyne ferrule | 0990-4947 | | |

- 3. Using a standard 1/4'' wrench, remove the nut and ferrule connecting the column tube to port 3.
- 4. Slide the nut and ferrule over the tube. Orient the nut and ferrule as shown in Figure 7-20.

- 5. Insert the tube into port 3 on the bottom of the injector value. Push the tubing into the port until it stops. Finger tighten the nut as far as it will go. Take the 1/4" wrench and tighten the nut an additional 1/2 turn.
- 6. Completely remove the nut and ferrule. The leading edge of the tube should be tapered towards the tubing. There should also be about 1/8 inch (3 mm) of tubing that protrudes beyond the ferrule.
- 7. Add the nut and ferrule to the other end of the tubing (the end going to the column). Use nuts and ferrules recommended by the column manufacturer, or, if you are inserting an in-line device between the Autosampler and the column, use nuts and ferrules compatible with the device.
- 8. Rethread the nut and ferrule going to the injector into port 3 on the injector valve. Finger tighten the nut as far as it will go. Take the 1/4" wrench and tighten the nut an additional 1/8 turn.
- 9. Do not connect the other end to the column or in-line device until after you reinstall the injector valve.

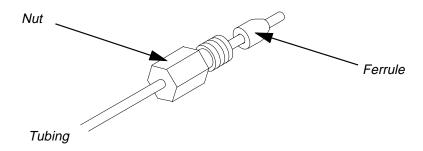


Figure 7-20. Correct Orientation of Nut and Ferrule on Tubing.

Replacing the Tubing to the Pump

If you have not already done so, remove the injector valve from the Autosampler as described in "Removing the Injector Valve" section in this chapter.

1. Refer to Figure 7-21 which shows the location of all the ports on the bottom of the injector valve and their connections.

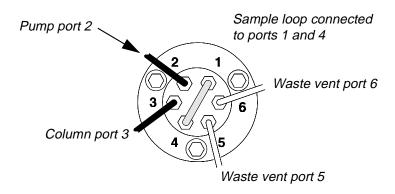


Figure 7-21. Bottom View of the Injector Valve.

2. Select the appropriate replacement tubes, nuts and ferrules from Table 7-5.

Table 7-5. Replacement Tubing, Nuts, and Ferrules.

| Replacement Part | Stainless Steel Part No. | Bio Applications Part No. |
|--------------------|--|--|
| Tubing sections | 0271-1441 [20 cm (1/16-in. O.D., 0.007-in. I.D.)] N293-1089 [30 cm (1/16-in. O.D., 0.007-in. I.D.) 0089-1480 [50 cm (1/16-in. O.D., 0.007-in. I.D.) | N930-2678 [5 ft.(1/16-in. O.D., 0.007-in. I.D.)] N930-2650 [5 ft.(1/16-in. O.D., 0.010-in. I.D.)] |
| Rheodyne short nut | 0990-4956 | None come Di an Di di |
| Rheodyne long nut | 0990-4974 | N930-2676 Pkg. of 5 Rheflex Nuts and Ferrules. |
| Rheodyne ferrule | 0990-4947 | |

- 3. Using a standard 1/4" wrench, remove the nut and ferrule connecting the pump tube to port 2.
- 4. Slide the nut and ferrule over the tube. Orient the nut and ferrule as shown in Figure 7-22.

- 5. Insert the tube into port 2 on the bottom of the injector value. Push the tubing into the port until it stops. Finger tighten the nut as far as it will go. Take the 1/4" wrench and tighten the nut an additional 1/2 turn.
- 6. Completely remove the nut and ferrule. The leading edge of the tube should be tapered towards the tubing. There should also be about 1/8 inch (3 mm) of tubing that protrudes beyond the ferrule.
- 7. Add the nut and ferrule to the other end of the tubing (the end going to the pump). Use nuts and ferrules recommended by the pump manufacturer, or, if you are inserting an inline device between the Autosampler and the pump, use nuts and ferrules compatible with the device.
- 8. Rethread the nut and ferrule going to the injector into port 2 on the injector valve. Finger tighten the nut as far as it will go. Take the 1/4" wrench and tighten the nut an additional 1/8 turn.
- 9. Do not connect the other end to the pump or in-line device until after you reinstall the injector valve.

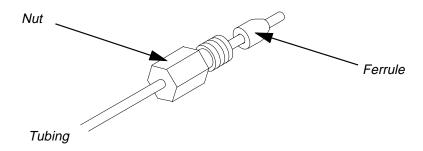


Figure 7-22. Correct Orientation of Nut and Ferrule on Tubing.

Replacing the Waste Tubes

If you have not already done so, remove the injector valve from the Autosampler as described in "Removing the Injector Valve" section in this chapter.

1. Refer to Figure 7-23 which shows the location of all the ports on the bottom of the injector valve and their connections.

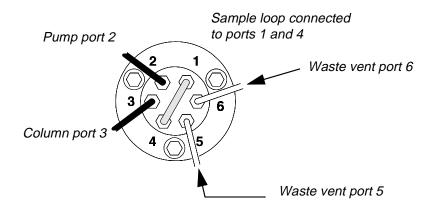


Figure 7-23. Bottom View of the Injector Valve.

2. Select the appropriate replacement waste capilliary vent tubes, nuts and ferrules from Table 7-6.

| Table 7-6. | Replacement ' | Vent Capilliar | ry Tubes, Nuts, a | and Ferrules. |
|------------|---------------|----------------|-------------------|---------------|
|------------|---------------|----------------|-------------------|---------------|

| Replacement Part | Stainless Steel Part No. | Bio Applications Part No. |
|---------------------------------|--------------------------|---|
| (2) 4-in. Vent capilliary tubes | N293-1085 | N293-1137 |
| Rheodyne short nut | 0990-4956 | NIOOO OOTO DI CEDI CI |
| Rheodyne long nut | 0990-4974 | N930-2676 Pkg. of 5 Rheflex Nuts and Ferrules. |
| Rheodyne ferrule | 0990-4947 | |

- 3. Using a standard 1/4" wrench, remove each nut and ferrule connecting the waste vent tubes to ports 5 and 6.
- 4. Slide the one nut and one ferrule over each tube. Orient each nut and ferrule as shown in Figure 7-24.
- 5. One at a time, insert the tube into port 5 or 6 (whichever is appropriate) on the bottom of

the injector valve. Push the tubing into the port until it stops. Finger tighten the male nut as far as it will go. Take the wrench supplied and tighten the nut an additional 1/2 turn.

- 6. Completely remove the nut and ferrule. Compare the tube with the attached ferrule to the tube without the ferrule. The leading edge of the tube with the ferrule should be tapered towards the tubing. There should also be about 1/8 inch (3 mm) of tubing that protrudes beyond the ferrule.
- 7. Repeat steps 4 and 5 for the remaining waste tube.
- 8. Rethread the nut and ferrule on each tube into its corresponding port. Finger tighten the nut as far as it will go. Take the wrench supplied and tighten the nut an additional 1/8 turn.

You will insert the free ends of the waste tubes from ports 5 and 6 into the remaining free top arm of the Y-shaped drain tube (coming from the flush port) after you reinstall the injector valve.

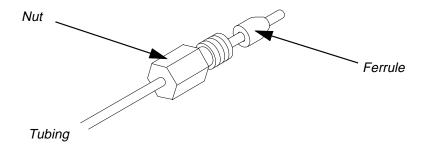


Figure 7-24. Correct Orientation of Nut and Ferrule on Tubing.

Troubleshooting 8

Troubleshooting Table 1 - Performance Problems .. 8-2
Troubleshooting Table 2 - General Instrument Problems .. 8-8
Troubleshooting Table 3 - Display Error Messages .. 8-10

Troubleshooting 8

This chapter describes how to solve some basic performance and instrument problems.

Table 1 lists the most frequently encountered performance problems and what you can do to correct them. In addition, illustrations are provided at the end of the Table 1 to assist you in troubleshooting.

Table 2 lists general instrument problems.

Table 3 lists display error messages, their causes and solutions.

Troubleshooting Table 1 - Performance Problems

| Performance Problems | Possible Cause(s) | Check and/or remedy |
|---|---|---|
| Poor Area and Height Reproducibility | Leaks | Check for leaks in the Dual Syringe System (refer to Figure 8-1), specifi- cally: |
| Retention Time Good | | Check connection between flush line tubing and flush solenoid. |
| | | Check connection between sample line and the Sample "T". Check connections to needle. |
| | Bubbles forming in flush pump | Degass the flush solvent. Slow down the flush pump speed. Check inlet solvent filter. |
| | Bubbles trapped in flush syringe or transfer tubing | Flush the system with isopropanol. Remove the syringe and flush it manually with isopropanol. |
| | Improperly degassed solvent | Use only degassed and filtered HPLC grade solvents for flushing solution and mobile phase. |
| | Blocked sample needle | Remove needle, clean, flush and check stream. If necessary, replace needle as described in Chapter 7 of this manual. |

| Performance Problems | Possible Cause(s) | Check and/or remedy |
|--|---|--|
| Poor Area and Height Reproducibility Retention Time Good | Incorrect sample syringe size | Make sure sample syringe size and sample volume requirements are compatible (for example, do not use a large syringe for injecting small sample volumes). |
| | Injector valve waste lines par- tially plugged, or injector valve needs to be rebuilt | Check the waste vent lines 5 and 6 for reduced flow when doing an injection, or check to see if liquid is coming out of the needle guide on top of the injector valve. Remove the vent waste tubes from ports 5 and 6: |
| | | 1. If liquid flows out of ports 5 and 6, and no liquid comes out of the needle guide, replace the waste line tubes. |
| | | 2. If liquid still flows out of the needle guide, rebuild the injector valve. |
| | | Refer to Chapter 7 in this manual for directions on replacing the waste vent tubes and rebuilding the injector valve. |
| | Worn injector valve seals | Check the instrument log (see "Viewing the Instrument Log" in Chapter 6 of this manual) to determine if the number of injection cycles > 5000. If the number is > 5000, replace the injector valve rotor seal and stator face assembly as described in Chapter 7 of this manual. |
| | Incorrect vial/closure | Check vial caps used on sample vials, especially for large samples. Use only recommended vials. |
| | | 2. Check the needle height and be sure the needle is not hitting the bottom of the vial. If necessary, set a new needle level in the vial. Refer to "Table 3-1, Default Performance Parameters" in Chapter 3 of this manual. |

| Performance Problems | Possible Cause(s) | Check and/or remedy |
|----------------------------|---------------------------------|--|
| | Improper performance parameters | Verify that the performance parameters are set properly for your application: |
| | | 1. Is the autosampler set for the correct mode, fixed or loop? |
| | | NOTE : If your autosampler is set for fixed loop mode, the poor reproducibility is not due to the autosampler. If this is the case, check other components (pump, detector etc.) in your LC system. |
| | | 2. Check excess volume and air cushion settings and try reverting to the default values. |
| | | 3. Check the sample syringe speed setting. If this is set too high it can cause cavitation, especially for viscous samples. |
| | | 4. For mixed samples, check the "reagent mixing - out of vial" parameter. For viscous samples this should be set to the slowest speed. |
| | | 5. For mixed samples, check the "reagent mixing - into vial" parameter and make sure it is adequate for your sample size. |
| | | To change the parameter values, refer to Appendix B in this manual. |
| | | Restore factory settings by pressing the F8 DEF key from the Configure Screen (see "Defining System Parameters" in Chapter 3 of this manual). |
| | Chemical interaction | See Ghost Peaks/carryover below. |
| Peak doubling or splitting | Sample volume too large | Inject sample prepared in mobile phase with a total volume <15% of volume of first peak. |
| | | Check or replace column. Replace injector valve rotor seal as in Chapter 7 of this man- ual. |

| Performance Problems | Possible Cause(s) | Check and/or remedy |
|---|--|---|
| Peak tailing | Column overload | Decrease the sample size. Increase column diameter. Use higher capacity stationary phase. |
| Peaks are broad | Injection volume too large | Inject sample prepared in mobile phase with total sample volume <15% of volume of first peak. |
| | Peak dispersion in injector valve | Load air bubble before and after sample to decrease dispersion. |
| | Sample overload | Dilute sample 1:10 and reinject. Inject a smaller sample volume. |
| Ghost peaks/carryover | Peaks from previous injection | Use strong solvent after each run. |
| | Column contamination | Improve sample cleanup. Wash sample needle guide. |
| | Chemical interaction - carryover or poor reproducibility may be due to certain compounds adsorbing to the transfer tubing or valve rotor seal. | Adsorption to tranfer tubing: Perform injections in the 10 to 20 μL range to ensure that excess sample and excess volume remain entirely in the sampler needle. Adsorption typically occurs with sample volumes >25 μL. If this eliminates the problem, you can add the stainless steel holding loop. Adsorption to valve rotor seal: If item 1 fails to eliminate problem, try switching to PEEK rotor seal instead of VespelTM. Polynuclear aromatics are known to adsorb to Vespel. |
| Poor area reproducibility | Data handling system | Check data handling component of LC system. |
| Good height reproducibilityGood retention time | Pump | Check short term pump flow stability. |
| Poor reproducibilityPoor retention time | Pump | Note: Retention performance on the pump should be 0.1% RSD or better to eliminate the pump as a source of problems. Refer to pump manufacturer's guide. |
| | Column | Refer to column manufacturer's guide. |

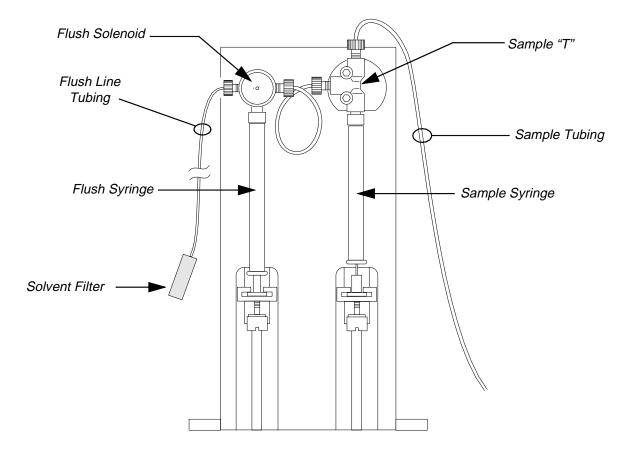


Figure 8-1. Dual Syringe System.

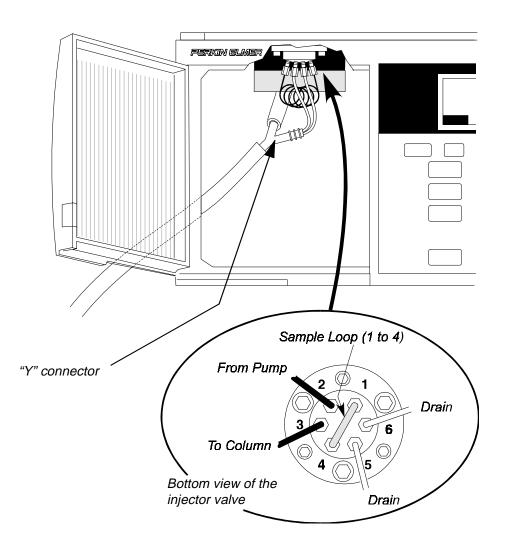


Figure 8-2. Location of Waste Lines on Injector Valve.

Troubleshooting Table 2 - General Instrument Problems

| General Instrument Problems | Possible Cause(s) | Check and/or remedy | |
|--|--|--|--|
| Autosampler will not start | Series 200 LC Autosampler line cord is not plugged into AC outlet | Plug Series 200 LC Autosampler line cord into the AC outlet. | |
| | Power switch is off | Turn on the power switch. | |
| | Blown fuse | Replace the fuse as described in Chapter 7 of this manual. | |
| No display. Power light is on. | Screen saver enabled | Press any key. | |
| Autosampler runs slow | Sample compartment door is open | Close sample door. | |
| Autosampler will not inject. | No ready-in connection | Connect controlling external device (pump) to ready-in. Refer to "Making Input/Output Terminal Connections" section in Chapter 2 of this manual. | |
| Autosampler performs injection but external LC devices do not function. | Improper external connections Not connected as serial port A or B | Check I/O connections. Refer to "Making Input/Output Terminal Connections" section in Chapter 2 of this manual. | |
| Autosampler will not communicate with external controller (TC4 or 1022). | Not configured for external control | Refer to "Defining the Communications Options" section in Chapter 3 of this manual. | |
| Autosampler will not | No flush solvent | Fill solvent reservoir. | |
| prime (syringes will not fill). | Flush syringe has been allowed to dry out | Hold solvent reservoir above the level of the flush syringe to increase head pressure. | |
| | Solenoid valve fails | Call PerkinElmer service. | |
| | Worn or defective flush syringe | Replace flush syringe. | |

| General Instrument Problems | Possible Cause(s) | Check and/or remedy | |
|---|-------------------------|--|--|
| Short injector valve rotor seal life | Dirty mobile phase | Replace inlet solvent filter with recommended filter. Use only degassed and filtered HPLC grade solvents for flushing solution and mobile phase. | |
| | Dirty sample | Improve sample cleanup. | |
| | Incorrect vial caps | Use recommended vial caps. | |
| Short sample or flush syringe life | Dirty flush solvent | Replace inlet solvent filter with recommended filter. Use only degassed and filtered HPLC grade solvents for flushing solution and mobile phase. | |
| Injection valve mechanism is noisy or injection valve error is displayed. | Injection valve is worn | Rebuild injection valve as described in Chapter 7 of this manual. If the valve appears to be working properly, call Perkin Elmer service. | |

Troubleshooting Table 3 - Display Error Messages

| Error Message | Possible Cause | Check and/or remedy |
|------------------------------------|-------------------------------------|---|
| Flush pump failed. | Needle blocked | Remove needle and press flush and check for steady stream of solvent. If there is no solvent flow, replace the needle as described in Chapter 7 of this manual. |
| X motor failed, or Y motor failed. | Inappropriate vial or cap size | Use correct recommended vials and caps. |
| | Obstruction in sample tray | Clear obstruction. If there is no obstruction, call PerkinElmer service. |
| Z motor failed. | Inappropriate vial or cap size | Use correct recommended vials and caps. If this fails, call PerkinElmer service. |
| Vial missing | Incorrect vial size or missing vial | Use correct recommended vials and caps. |

Appendixes

Appendix A - Sample Trays

Available Tray Types

You can select from one of the following eight different sample trays:

| Part No. |
|-----------|
| N293-1007 |
| N293-0043 |
| N293-0042 |
| N293-0041 |
| N293-0314 |
| N293-0310 |
| N293-0045 |
| |

Each of the following sections contains a description and diagram of the available sample trays.

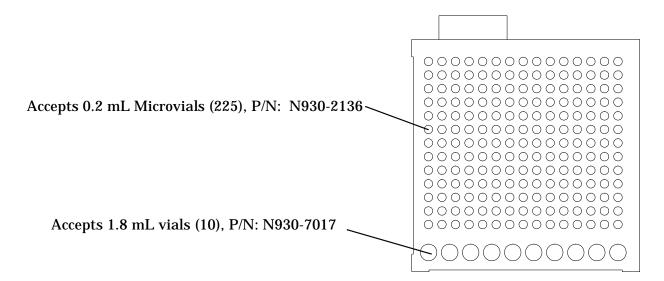
Standard (100), Part Number N293-1007

| Accepts: 1. 1.8 mL vials (80), P/N: N930-7017 2. 0.2 mL Microvials (80), P/N: N930-2136 Requires Glass Sleeve, P/N: N930-7027 | 000000000 000000000 000000000 00000000 |
|---|---|

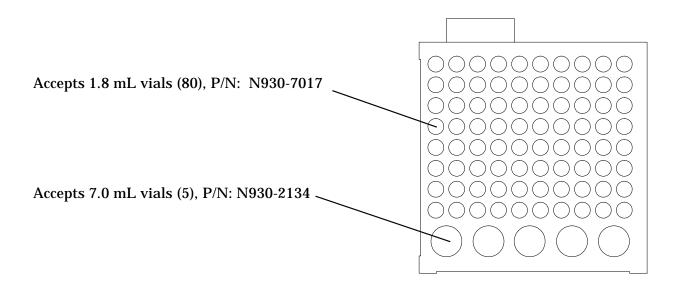
225 Vial, Part Number N293-0043

Accepts 0.2 mL Microvials (225), P/N: N930-2136

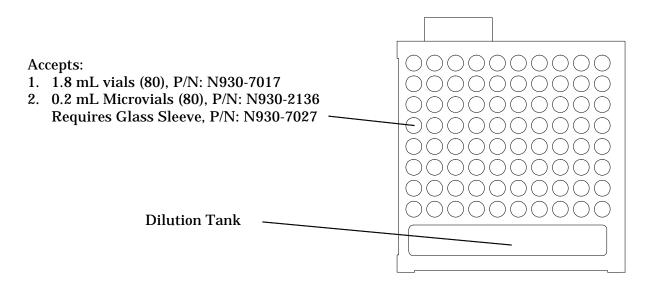
205 Vial, Part Number N293-0042



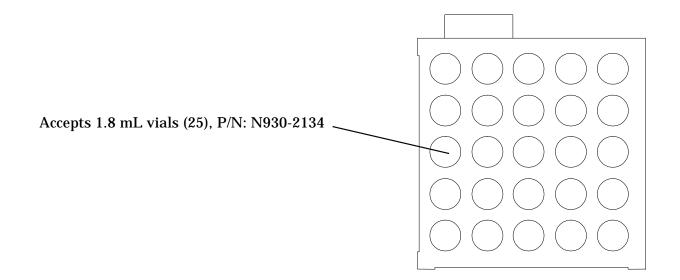
85, Part Number N294-0041



Dilution (80 Vials + Tank), Part Number N294-0044



25, Part Number N294-0040



Appendix B - Changing the Performance Parameters

This appendix describes how to change the default injection performance parameters listed in Chapter 3, "Configuring the Autosampler." In order to change the parameters you need to do the following.

- Access the Performance Parameter Screen (this screen is comprised of three pages)
- Move within and between the pages of the Performance Parameter Screen
- Change the necessary parameters

For your convenience, the default performance parameters are repeated again here in Table B-1.

The Default Performance Parameters

Table B-1. Default Performance Parameters

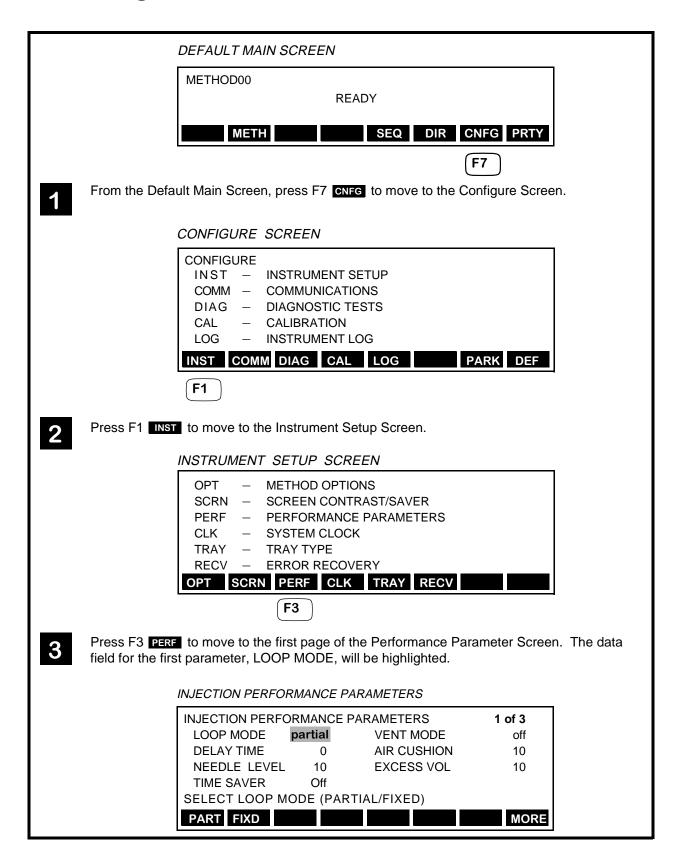
| | Parameter | Description Default Value | | Acceptable Value or Range |
|----|-----------------------------|---|---------------------------------------|-------------------------------------|
| 1. | Air Cushion | $\begin{tabular}{lll} Volume of air drawn up before sample. & 10 μL\\ Air cushion is used to prevent mixing of the flush buffer and the sample. & \end{tabular}$ | | 1 - 99 μL |
| 2. | Loop Mode | Partial = loop is partially filled with the volume specified during method setup. Fixed = loop fills up completely with sample. If you change the default from partial to fixed, refer to Appendix D in this manual for the recommended sample loop and sample syringe sizes. | l setup. with sam- rom par- D in this | |
| 3. | Excess Volume (Sample) | Extra volume of sample drawn up during sampling operation. Note: in the Fixed Loop Mode, the sample volume drawn up is already ten times the loop volume. The excess volume is then added to that volume. | | 0 - 100 μL |
| 4. | Sample Pump Syringe Size | The size of the syringe used to draw up the sample. | 250 μL | 50, 100, 250, 500, 1000, 2500 μL |

| Parameter | Parameter Description | | Acceptable Value or Range | |
|------------------------------------|---|-------------------------------------|--------------------------------------|--|
| 5. Sample Pump Syringe Speed | The speed at which the sample syringe picks up and dispenses sample. The speed varies with the size of the sample syringe. Note that for viscous samples, you should set the speed to VSLO (very slow) or SLOW. | MEDM for all syringe sizes | VSLO SLOW MEDM FAST VFST | |
| 6. Sample Pump Syringe Pause | The pause time following each sample syringe movement. This allows the air segment between the sample and the flush buffer to equilibrate. | 2 seconds | 0 - 10 seconds | |
| 7. Delay Time | This is used for gradient methods. It allows you to delay the sample injection by this amount of time to compensate for the dead volume between the pump and the Autosampler. | 0.00 minute | 0.00 - 999 minutes | |
| 8. Time Saver | If this feature is enabled (On), it allows the Autosampler to get the next sample and perform sample preparation before the other modules (pump and detector) are ready. | Off | On/Off | |
| 9. Needle Level | This determines how far the sample needle goes into the sample vial. The value corresponds to a percentage of the total distance from the shoulder (top) of the sample <u>vial</u> to the bottom of the sample <u>holder</u> . For example, 0% is at the bottom of the sample holder; 99% is at the shoulder of the vial. | 10% | 1 - 99 % | |
| 10. Vent Mode | If this feature is enabled (On) a volume of air, equal to the volume of sample and buffer to be withdrawn from the vial, is first injected into the sample vial. This prevents a vacuum from developing when multiple samples are withdrawn from the vial. | On | Off/On | |
| 11. Flush Volume | The volume of flush liquid used for each cycle during the pre- and postinjection flush operation. | 700 μL | 0 - 2500 μL | |
| 12. Speed of the Flush Syringe | The speed at which the flush syringe picks up buffer from the buffer reservoir and ejects it through the sample needle. | MEDM | VSLO SLOW MEDM FAST VFST | |
| 13. Pre-injection Flush Cycles | The number of flush cycles before sample is injected. | 1 | 0 -9 | |
| 14. Post-injection Flush Cycles | The number of flush cycles after sample is injected. | 1 | 0 - 9 | |

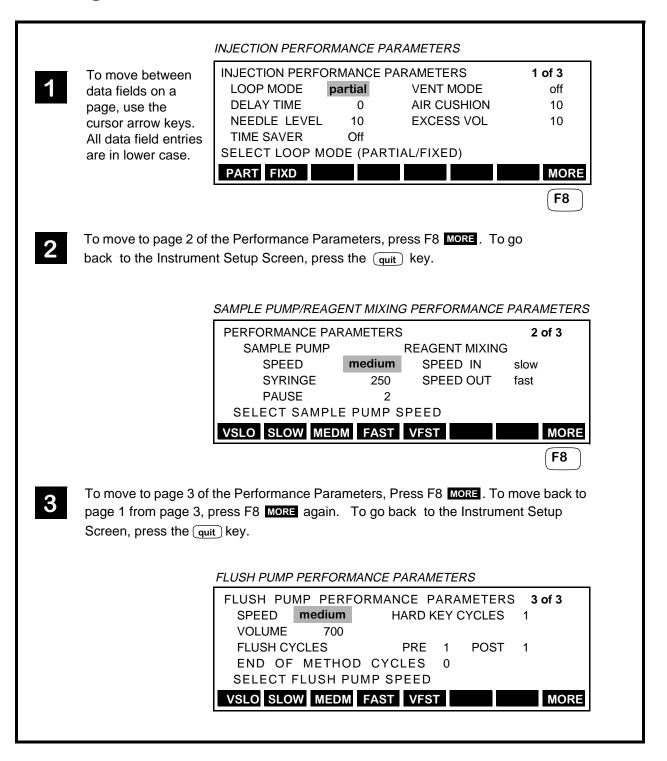
| Parameter | Description | Default Value | Acceptable Value or Range |
|-----------------------------------|--|------------------|--------------------------------------|
| 15. End of Method Flush Cycles | The number of flush cycles at the end of a method. | 0 | 0 - 9 |
| 16. Hard Key Cycles | The number of times the flush syringe 2 will cycle when you press the flush key. | | 1 -9 |
| 17. Reagent Mixing Speed - In | The speed of the flush syringe when it picks up sample plus reagent from the vial through the sample needle for mixing. Note that for viscous samples, you should set the speed to VSLO (very slow) or SLOW. | SLOW | VSLO SLOW MEDM FAST VFST |
| 18. Reagent Mixing Speed - Out | The speed of the flush syringe when it ejects sample plus reagent through the sample needle back into the vial for mixing. | FAST | VSLO SLOW MEDM FAST VFST |

B - 3

Accessing the Performance Parameter Screen



Moving in the Performance Parameter Screen



Changing Information on the Screen

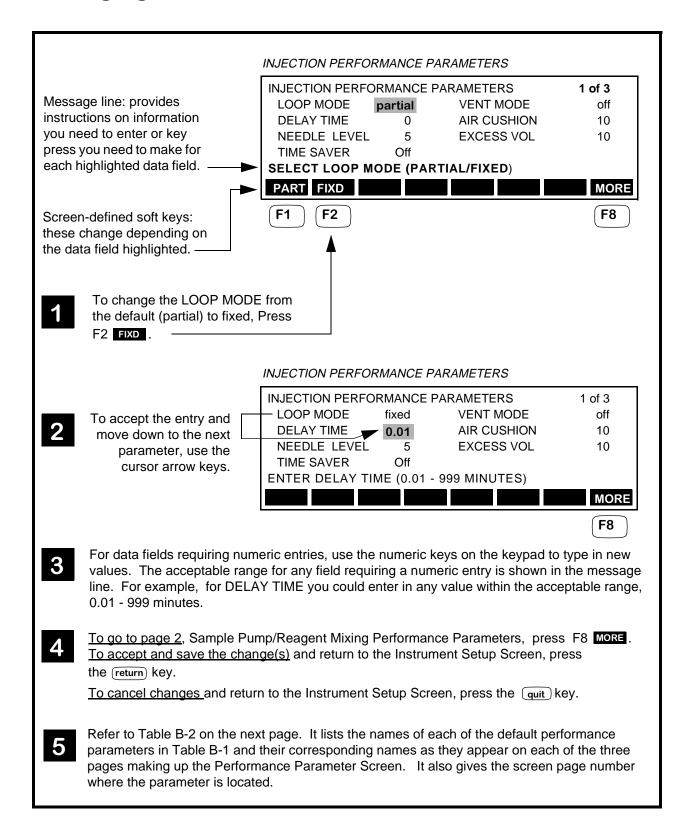


 Table B-2.
 Performance Parameters/Page Number

| Default Parameters | Parameter Name on Screen Page/Page # of 3 |
|---------------------------------|--|
| 1. Air Cushion | AIR CUSHION/Page 1 of 3 |
| 2. Loop Mode | LOOP MODE/Page 1 of 3 |
| 3. Excess Volume (Sample) | EXCESS VOL/Page 1 of 3 |
| 4. Sample Pump Syringe Size | SAMPLE PUMP: SYRINGE/Page 2 of 3 |
| 5. Sample Pump Syringe Speed | SAMPLE PUMP: SPEED/Page 2 of 3 |
| 6. Sample Pump Syringe Pause | SAMPLE PUMP: PAUSE/Page 2 of 3 |
| 7. Delay Time | DELAY TIME/Page 1 of 3 |
| 8. Time Saver | TIME SAVER/Page 1 of 3 |
| 9. Needle Level | NEEDLE LEVEL/Page 1 of 3 |
| 10. Vent Mode | VENT MODE/Page 1 of 3 |
| 11. Flush Volume | FLUSH PUMP PERFORMANCE PARAMETERS: VOLUME/Page 3 of 3 |
| 12. Speed of the Flush Pump | FLUSH PUMP PERFORMANCE PARAMETERS: SPEED/Page 3 of 3 |
| 13. Pre-injection Flush Cycles | FLUSH PUMP PERFORMANCE PARAMETERS: FLUSH CYCLES PRE/Page 3 of 3 |
| 14. Post-injection Flush Cycles | FLUSH PUMP PERFORMANCE PARAMETERS: FLUSH CYCLES POST/Page 3 of 3 |
| 15. End of Method Flush Cycles | FLUSH PUMP PERFORMANCE PARAMETERS: END OF METHOD CYCLES/Page 3 of 3 |
| 16. Hard Key Cycles | FLUSH PUMP PERFORMANCE PARAMETERS: HARD KEY CYCLES/Page 3 of 3 |
| 17. Reagent Mixing Speed - In | REAGENT MIXING: SPEED IN/Page 2 of 3 |
| 18. Reagent Mixing Speed - Out | REAGENT MIXING: SPEED OUT/Page 2 of 3 |

Appendix C - Serial Dilutions

This appendix describes how to define a serial dilution. It assumes that you have enabled the Serial Dilution option as described in the "Adding Method Options" section in Chapter 3 of this manual.

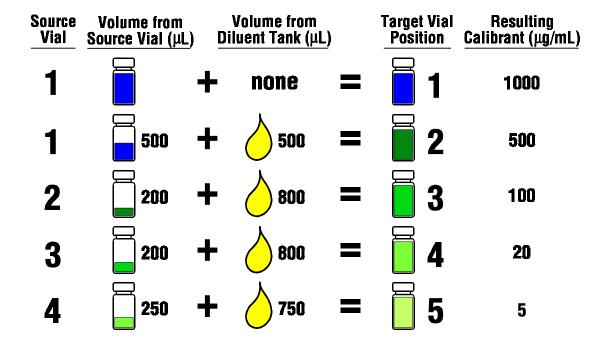
The Serial Dilution option allows you to perform a serial dilution starting from a single calibrant and then making a series of vial to vial transfers. No injections are performed between the vial to vial transfers.

An example is presented here to show you how to set up the Serial Dilution screen to perform a serial dilution starting with a single 1000 $\mu g/mL$ standard. The dilution sequence is shown in Figure C-1, Serial Dilution Example.

In addition to the example, there are two sections, "Operating Notes" and "Summary of Data Field Requirements," to provide you with the information you need to customize your own serial dilutions.

To achieve the best performance, use the default Performance Parameter settings. To review or change the Performance Parameters, refer to Appendix B, "Changing the Performance Parameters." Depending on the types of vial caps you are using, you may need to change the Vent Mode parameter - refer to Appendix E, "Operating Guidelines for Derivatizations and Dilutions."

Serial Dilution Example



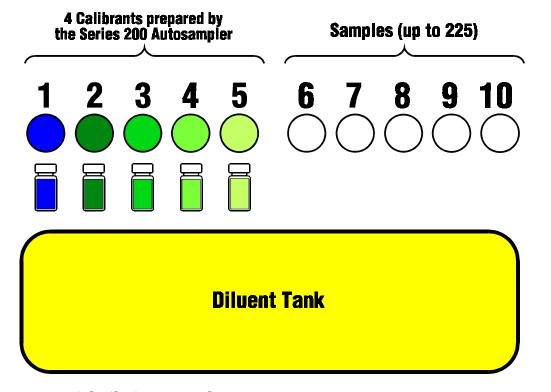
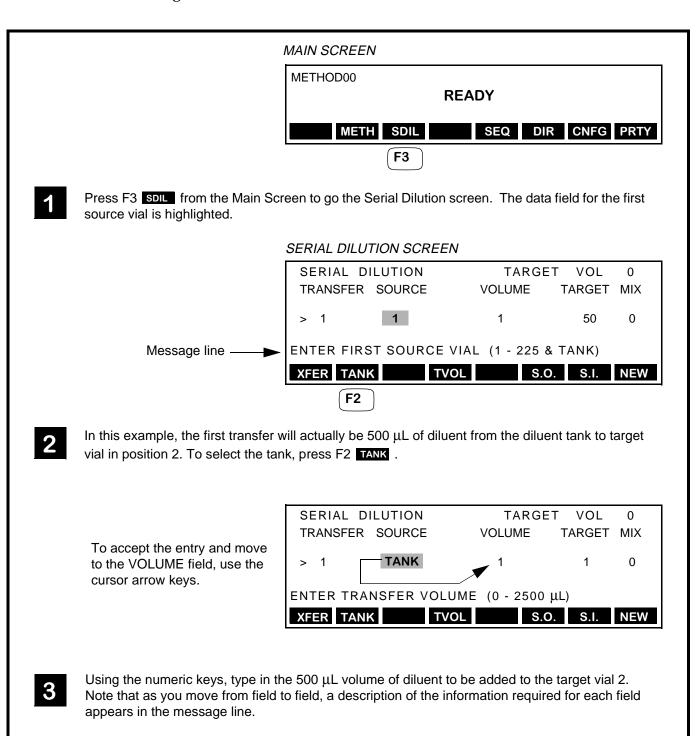
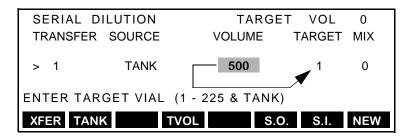


Figure C-1. Serial Dilution Example.

Setting up the Serial Dilution Screen

The following procedure describes how to set up the Serial Dilution screen to perform the dilution shown in Figure C-1.





Using the numeric keys, type in target vial 2 for the first transfer. The entry for the Mix data field should be left as 0 for the first transfer entry. The completed screen for the first transfer is shown next.

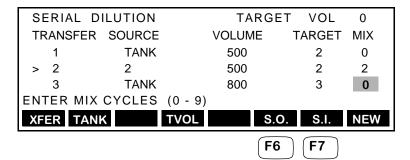
| SERIAL D | ILUTION | TARGE | T VOL 0 |
|-----------|-----------|--------|-------------|
| TRANSFER | SOURCE | VOLUME | TARGET MIX |
| > 1 | TANK | 500 | 2 0 |
| ENTER MIX | CYCLES (0 | - 9) | |
| XFER TANK | K TV | OL S.O | O. S.I. NEW |

- To add the next transfer entry, press either the enter key (at the last data field) or the insert key.
- Modify transfer entry 2 and then add and modify the remaining transfer entries required to accomplish the serial dilution shown in Figure C-1. Since only three transfer entries can be displayed on the screen at one time, the eight transfer entries you need to make are listed inTable C-1 below for your convenience.

Table C-1. Sample Dilution Screen Entries.

| SERIAL D TRANSFER | | TARGET VOLUME | VOL TARGET | 0 MIX |
|----------------------|------|------------------|---------------|----------|
| 1 | TANK | 500 | 2 | 0 |
| 2 | 2 | 500 | 2 | 2 |
| 3 | TANK | 800 | 3 | 0 |
| 4 | 2 | 200 | 3 | 2 |
| 5 | TANK | 800 | 4 | 0 |
| 6 | 3 | 200 | 4 | 2 |
| 7 | TANK | 750 | 5 | 0 |
| 8 | 4 | 250 | 5 | 2 |
| | | | | |

IMPORTANT: Make sure and complete all additions to each vial, in the order shown in Table C-1, before proceeding to the next vial.



Once you complete the entries in Table C-1, you can start the serial dilution by pressing either F6 s.o. or F7 s.i.:

NOTE: The start key and the priority key are disabled when the Serial Dilution screen is open.

 $\label{eq:F6-F6} \textbf{F6} \ \ \textbf{-} \quad \ \ \text{If you press F6, the following prompt will appear in the message line:}$

PERFORM SERIAL DIL. ONLY? (Y/N) N

If you want the autosampler to perform the dilutions and stop, press the very likely to change the default to Y.

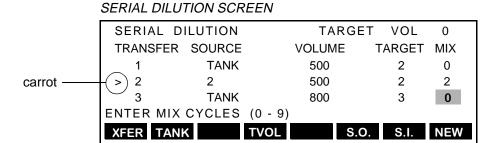
F7 - If you press F7, the following prompt will appear in the message line:

PERFORM SERIAL DIL. AND START? (Y/N) N

If you want the autosampler to perform the dilutions and then start the active method or sequence, press the 1 yes numeric key to change the default to Y.

Refer to "Operating Notes" and "Summary of Data Field Requirements and Ranges" for more information on the Serial Dilution option.

Operating Notes



To select a transfer entry from the list in the Serial Dilution screen, press F1 XFER, and enter the transfer entry number when prompted, then press the enter key. The carrot will move to the selected item. Alternatively, you can use the cursor arrow keys to scroll up and down the list.

To delete a tranfer entry, select the item and press the delete key.

To permanently save changes to the screen without running the dilution, press the return key. To run the dilution later, press the F3 SDIL from the Main Screen to enter the Serial Dilution screen; then start the dilution by pressing either F6 S.O. or F7 S.I. .

To clear all the changes made to the Serial Dilution screen, press the F8 NEW function key.

Summary of the Serial Dilution Data Field Requirements and Ranges

The following is a summary of the data fields and their acceptable range of entries.

| Data Field: | Description: |
|-------------|--|
| TARGET VOL | The volume of sample in the first target vial (0 - 2500 $\mu L). \ $ To access |
| | this data field, press F4 TVOL from the Serial Dilution screen. |
| SOURCE | The first source vial (1 - 225 & Tank). To specify the diluent tank, press |
| | F2 TANK from the Serial Dilution screen. |
| VOLUME | The volume to be tranferred from the source vial or diluent tank |
| | $(0 - 2500 \ \mu L)$. |
| TARGET | The target vial to receive the volume of material from the source vial or |
| | the diluent tank (0 - 2500 μL). |
| MIX | The number of mix cycles or times the transferred material from the |
| | source vial or diluent tank will be mixed with the sample in the target |
| | vial (0 - 9). If there is $0 \mu L$ of sample in the target vial, this should be |
| | set to 0. For best results, set this value to 2 as shown in the Serial |
| | Dilution Screen at the top of this page. |

Appendix D - Fixed Loop Mode

This appendix contains the recommended sample loop and sample syringe sizes when operating the Series 200 LC Autosampler using the fixed loop mode.

In the fixed loop mode, the volume of sample injected onto the column is defined by the size of the sample loop. Since the volume of the sample loop is exact to within very close tolerances, this mode is suitable for applications requiring high precision.

In the fixed loop mode, the autosampler withdraws 10 times the amount specified in the injection VOLUME field on the method screen. The loop is rinsed with the sample and the excess is flushed to waste. A typical method screen is shown in Figure D-1. In this method, the injection volume is 5 μL . Therefore, 50 μL of sample will actually be withdrawn from vial 1 and rinsed through the loop.

METHOD SCREEN METHOD00 CHANGED FIRST LAST VOLUME REPLICATES TIME 1 1 1 10 ENTER INJECTION VOLUME (0.1 - 2500 μL) NEW STOR

Figure D-1. Method Screen.

<u>Make sure that the injection VOLUME corresponds as closely as possible to the size of the sample loop</u>. This ensures that the system is well flushed with sample and the sample loop is overfilled so that the sample concentration will be correct.

To ensure adequate excess sample volume in the fixed mode, refer to Table D-1 for the recommended sample syringe and sample loop size combinations.

| Sample Loop Size (μL) | Sample Syringe Size (μL) | |
|-----------------------|--------------------------|--|
| 5, 10, 20 | 250 | |
| 50, 100 | 1000 | |
| 200 | 2500 | |

Table D-1. Sample Syringe vs. Sample Loop Size.

Appendix E - Operating Guidelines for Derivatizations and Dilutions

When performing derivatizations, dilutions or serial dilutions on the Series 200 LC Autosampler using the vial caps listed in this appendix, use the default settings for the Performance Parameters.

In addition, for derivatizations, dilutions or serial dilutions, set the number of mix cycles to 2 when you set up the method.

For directions on reviewing and changing the Performance Parameters, refer to Appendix B, "Changing the Performance Parameters."

For directions on setting up dilutions or derivatizations, refer to Chapter 4, "Creating Methods and Sequences." For setting up serial dilutions, refer to Appendix C, "Serial Dilutions."

When using the following vial caps:

<u>When using vial caps with resealing septa</u>, such as rubber or silicone, ensure that the Vent Mode Performance Parameter is set to **ON**. Examples of these types of vial caps include:

| Description | Part No. |
|---|-----------|
| Blue 11 mm Silicone/Teflon vial septa | N930-7022 |
| Blue 11 mm Silicone/Teflon vial with cross-cut septa | N930-7021 |
| Red 11 mm Screw Cap Silicone/Teflon vial with cross-cut septa | N930-7019 |

When using Polyethylene Snap Caps, Part No. N930-7023, set the Vent Mode to **OFF**.

When performing dilutions, derivatizations or serial dilutions:

Verify or set the following Performance Parameters to the values/modes listed here:

| Value or Mode | | |
|---------------|--|--|
| 10 μL | | |
| SLOW | | |
| FAST | | |
| | | |

Also, remember to set the number of mix cycles to 2 for your derivatization or dilution method.

Using these conditions, you can expect a vial-to-vial reproducibility of 1 - 2% RSD for dilutions in the 1:2 through 1:50 range.

Appendix E - Operating Guidelines for Derivatizations and Dilutions

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