Finnigan™ SpectraSYSTEM™

Autosamplers
Reference Manual

A0099-587 Revision E

Thermo
ELECTRON CORPORATION

Thermo
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Contents

List of Tables

Technical and Customer Support

This manual contains procedures for installing your equipment and verifying that it is operating within specifications. It will also help you understand how to use and care for your equipment. For additional support, contact one of the customer service offices listed below.

In North America

In North America, Thermo Electron San Jose Customer Service Engineers are available from the following offices:

Northeastern Region
Phone   [1] (732) 627-0220
Fax   [1] (732) 627-0260

Southern Region
Phone   [1] (770) 516-5589
Fax   [1] (770) 516-6916

Central Region
Phone   [1] (847) 310-0140
Fax   [1] (847) 310-0145

Western Region
Phone   [1] (408) 965-6000
Fax   [1] (408) 965-6123

Canada
Phone   [1] (905) 712-2258
Fax   [1] (905) 712-4203

REPLACEABLE PARTS

Contact Customer Service Operations to order replaceable parts. The location and telephone and fax numbers for North America are as follows:

North America Customer Service Operations
1400 Northpoint Parkway, Suite 10
West Palm Beach, FL 33407

Phone: [1] (800) 532-4752
Fax: [1] (561) 688-8731

**TECHNICAL
SUPPORT**

You can contact Technical Support at the following location, telephone and fax numbers, and e-mail address:

North America Technical Support Operations
1400 Northpoint Parkway, Suite 10
West Palm Beach, FL 33407

Phone: [1] (800) 685-9535
Fax: [1] (561) 688-8736

E-mail: techsupport.finnigan@thermo.com

In Europe

In Europe, customer support, replaceable parts, and technical support are available from each of the following offices.

Technical support is also available from North America Technical Support Operations at the following phone number and e-mail address:

Phone   [1] (561) 688-8700
E-mail   techsupport.finnigan@thermo.com

Wien (Vienna), Austria
Phone   [43] (01) 333 50 34-0
Fax   [43] (01) 333 50 34-26

Brussels, Belgium
Phone   [32] (02) 482 30 30
Fax   [32] (02) 482 30 31

Les Ulis, France
Phone   [33] (01) 60 92 48 00
Fax   [33] (01) 60 92 49 00

Dreieich, Germany
Phone   [49] (06103) 408 0
Fax   [49] (06103) 408 1222

Milano, Italy
Phone   [39] (02) 95059 226
Fax   [39] (02) 95320 370

Breda, Netherlands
Phone   [31] (076) 587 8722
Fax   [31] (076) 571 4171

Madrid, Spain
Phone   [34] (091) 657 4930
Fax   [34] (091) 657 4937

Barcelona, Spain
Phone   [34] (093) 223 0918
Fax   [34] (093) 223 0982

Stockholm, Sweden
Phone   [46] (08) 556 468 00
Fax   [46] (08) 556 468 08

Hemel Hempstead, United Kingdom
Phone   [44] (01442) 233 555
Fax   [44] (01442) 233 667

For all other countries, contact your local Thermo Electron San Jose products dealer.

In Australasia and Asia

In Australasia and Asia, customer support, replaceable parts, and technical support are available from each of the following offices.

Technical support is also available from North America Technical Support Operations at the following phone number and E-mail address:

Phone   [1] (561) 688-8700
E-mail   techsupport.finnigan@thermo.com

Rydalmere, N.S.W., Australia
Phone   [61] (02) 9898-9000
Fax   [61] (02) 9898-9800

Yokohama, Japan
Phone   [81] (45) 453-9100
Fax   [81] (45) 453-9110

Osaka, Japan
Phone   [81] (06) 6387-6681
Fax   [81] (06) 6387-6641

Beijing, P.R. China
Phone   [86] (010) 6621 0839
Fax   [86] (010) 6621 0851

For all other countries, contact your local Thermo Electron San Jose products dealer.

Safety and EMC Information

In accordance with Thermo Finnigan's commitment to customer service and safety, these instruments have satisfied the requirements for the FCC and the European CE Mark including the Low Voltage Directive.

Designed, manufactured and tested in an ISO9001 Registered facility, this system has been shipped to you from our manufacturing facility in a safe condition.

IDENTIFYING SAFETY INFORMATION

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

CAUTION!   HOT SURFACE!   HIGH VOLTAGE!

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

1. Before plugging in any of the instrument modules or turning on the power, always make sure that the voltage and fuses are set appropriately for your local power supply.

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

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

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

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

5. Never run the system without the top cover on. Permanent damage can occur.

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

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

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

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

10. Never try to repair or replace any component of the system that is not described in this manual without the assistance of Thermo Finnigan.

GOOD LABORATORY PRACTICES

Keep Good Records

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

Chemical Toxicity

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

Sample Preparation

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

Solvent Requirements

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

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

Degas the Eluants

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

Solvent Disposal

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

High-pressure Systems and Leaks

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

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

Information sur la sécurité et la compatibilité électromagnétique (CEM)

Selon notre engagement à assurer à nos clients service et sécurité, ces instruments sont déclarés conformes aux normes de la FCC et à la réglementation européenne (CE), y compris à la directive sur les basses tensions.

Conçu, fabriqué et testé dans une installation homologuée ISO9001, cet instrument a été livré à partir de notre usine de fabrication dans le respect des règles de sécurité.

MISE EN GARDE ! Cet instrument doit être utilisé selon les instructions figurant dans ce manuel. Le non respect des consignes d'utilisation de cet instrument décrites dans le présent manuel risque d'endommager l'instrument et/ou d'infliger des blessures à l'opérateur.

IDENTIFICATION DES INFORMATIONS SUR LA SÉCURITÉ

Ce manuel de référence contient des précautions d'usage afin de prévenir tout dommage corporel ou matériel ainsi que toute perte de données lorsque l'opérateur se conforme aux instructions indiquées. Ces instructions sont accompagnées des icônes suivantes et sont affichées en caractères gras pour attirer l'attention de l'opérateur :

MISE EN GARDE !   SURFACE BRÛLANTE !   HAUTE TENSION !

Chaque instrument présentant des dangers spécifiques, il incombe à l'opérateur de lire les précautions suivantes et de s'y conformer, afin de maintenir la durée de vie et la sécurité du système.

1. Avant de brancher un module d'instruments ou de le mettre sous tension, toujours s'assurer que la tension et les fusibles sont réglés de façon à correspondre à la tension locale du secteur.

2. N'utiliser que des fusibles du type et du courant nominal spécifiés. Ne pas utiliser de fusibles réparés et ne pas court-circuiter le porte-fusible.

3. Le cordon d'alimentation accompagnant l'instrument doit être branché à une prise de courant avec mise à la terre. En cas d'utilisation d'une rallonge électrique, s'assurer que celle-ci comporte également une mise à la terre.

4. Ne pas modifier les connexions de mise à la terre internes ou externes. La modification ou le débranchement de ces connexions représente un danger pour l'opérateur et/ou risque d'endommager le système.

⚠ **MISE EN GARDE ! Cet instrument est mis à la terre conformément aux règlements applicables lors de son expédition. Ne pas modifier les branchements électriques ou le châssis de l'instrument afin d'assurer un fonctionnement en toute sécurité.**

5. Ne jamais faire fonctionner le système sans son boîtier. Des dommages permanents pourraient en résulter.

6. Ne pas mettre l'instrument sous tension si celui-ci a subi des dommages électriques. Débrancher le cordon d'alimentation de l'appareil et consulter un représentant du service technique pour procéder à un examen du produit. Ne pas essayer d'utiliser l'instrument avant qu'il n'ait été examiné. (Des dommages électriques peuvent s'être produits si le système montre des signes visibles d'endommagement ou si les conditions de transport ont été extrêmement difficiles.)

7. L'instrument peut également être endommagé s'il est entreposé pendant une période de temps prolongée, dans de mauvaises conditions (par exemple, s'il est exposé à la chaleur, à l'humidité, etc.).

8. Toujours débrancher le cordon d'alimentation avant d'effectuer n'importe quel type d'entretien.

9. Les condensateurs présents à l'intérieur de l'instrument peuvent toujours être chargés, même si l'instrument est hors tension.

10. Ne jamais tenter de réparer ou de remplacer un composant du système non décrit dans ce manuel sans obtenir de l'aide auprès d'un représentant du service technique.

BONNES PRATIQUES DE LABORATOIRE

Bonne tenue des dossiers

Pour permettre d'identifier et d'isoler les problèmes pouvant survenir avec l'équipement ou la méthodologie utilisés, il est recommandé de tenir correctement des dossiers de toutes les conditions du système (*p. ex.*, % CV sur les temps de rétention et les zones de pics, la forme et la résolution des pics). Il est recommandé tout au moins de conserver pour référence future un chromatogramme d'un échantillon type et d'un mélange standard, bien documenté et accompagné des conditions du système. Une comparaison précise des temps de rétention, des formes et de la sensibilité des pics ainsi que des bruits

de référence peuvent fournir des indices précieux pour l'identification et la résolution de problèmes futurs.

Toxicité chimique

Bien que l'utilisation et l'entreposage dans les laboratoires de grandes quantités de solvants inflammables et toxiques puissent représenter un danger, ne pas négliger les dangers potentiels posés par les échantillons. Veiller particulièrement à lire et à suivre toutes les précautions indiquées pour assurer la ventilation, le stockage, la manutention et l'élimination des solvants et des échantillons. Se familiariser avec les données sur la toxicité et les dangers potentiels associés à tous les produits chimiques en consultant les fiches techniques sur la sécurité des substances (FTSS) du fabricant.

Préparation des échantillons

Toujours considérer la solubilité de l'échantillon dans la phase mobile. La précipitation des échantillons peut boucher la colonne, les tubes et/ou la cellule de dilution, et en limiter le débit. Cette obstruction peut endommager le système de façon irréparable. L'accumulation de particules peut être évitée par la filtration des échantillons à travers des filtres de 0,45 ou 0,2 µm (ou moins).

Caractéristiques des solvants

Un grand nombre de fabricants de produits chimiques fournissent des réactifs de pureté élevée ou de qualité spectrographique dépourvue de toute impureté chimique. La filtration systématique de tous les solvants ou éluants à travers un filtre fluorocarboné de 0,45 ou 0,2 µm (ou moins) avant de les placer dans le réservoir de solvants prolonge de façon significative la durée de vie et l'efficacité des filtres d'entrée, des clapets et des joints d'étanchéité, de l'injecteur et de la colonne. De façon générale, les solvants pour chromatographie liquide sous haute pression ne nécessitent pas de filtration.

Choisir une phase mobile qui est compatible avec l'échantillon et la colonne sélectionnés pour la séparation. Noter que certains solvants sont corrosifs pour l'acier inoxydable. Des versions inertes et biocompatibles des instruments sont disponibles auprès de Thermo Finnigan.

Dégazage des éluants

Effectuer le dégazage des éluants selon la méthode de dégazage par le vide ou à l'hélium. Une description complète de ces méthodes est disponible dans la documentation fournie séparément avec les accessoires de dégazage.

Élimination des solvants

S'assurer qu'il existe un conteneur pour solvants à éliminer ou tout autre système de vidange au niveau de la table de travail ou au-dessous de celle-ci. La plupart des solvants doivent être éliminés dans des conditions particulières et ne doivent pas être évacués directement

par les canalisations. Respecter la réglementation en vigueur concernant l'évacuation des produits chimiques.

Systèmes à haute pression et fuites

Les systèmes de chromatographie liquide (CL) fonctionnent à des pressions élevées. Les liquides n'accumulent pas de grandes quantités d'énergie car ils ne sont pas hautement compressibles. Par conséquent, le risque d'un danger immédiat causé par les pressions élevées dans un système CL est faible. En revanche, si une fuite survient, il est nécessaire de la réparer le plus rapidement possible. Enfin, il est recommandé à l'opérateur de se protéger en permanence les yeux et la peau lorsqu'il travaille sur un système CL. De plus, il doit toujours mettre le système hors tension et le ramener à la pression atmosphérique avant de procéder à tout entretien.

Informationen zu Sicherheit und Funkentstörung

Wir sind dem Dienst am Kunden und der Sicherheit des Kunden verpflichtet. Diese Geräte entsprechen den Anforderungen für die FCC-Zulassung und für das CE-Zeichen sowie den Bestimmungen der Richtlinie für Niederspannungsgeräte.

Dieses Gerät wurde in einer nach ISO 9001 zertifizierten Fertigungsstätte entwickelt, hergestellt und getestet und hat unser Werk in sicherem Zustand verlassen.

VORSICHT! Dieses Gerät darf nur nach den Vorschriften dieser Bedienungsanleitung benutzt werden. Wenn dieses Gerät auf andere Weise als hier beschrieben benutzt wird, kann dies zu Schäden am Gerät oder zur Verletzung des Bedieners führen.

ERKENNEN VON SICHERHEITS-INFORMATIONEN

Dieses Handbuch enthält Warnhinweise, deren genaue Befolgung Personenschäden, Schäden am Gerät oder Datenverluste verhindern kann. Auf alle derartigen Warnhinweise wird durch Fettschrift und durch
Verwendung der nachfolgenden Symbole gesondert aufmerksam gemacht:

VORSICHT! OBERFLÄCHE HEISS! HOCHSPAN-NUNG

Jedes Gerät kann unter bestimmten Umständen gefährlich sein. Lesen Sie daher in jedem Fall die nachstehenden Sicherheitshinweise, und ergreifen Sie die entsprechenden Maßnahmen. Auf dieses Weise sorgen Sie für einen sicheren Betrieb und eine lange Lebensdauer des Geräts.

1. Bevor Sie eines der Gerätemodule einstecken oder das Gerät einschalten, überprüfen Sie in jedem Fall, ob die Nennspannung und die Sicherungen der Netzspannung der örtlichen Stromversorgung entsprechen.

2. Verwenden Sie nur Sicherungen des angegebenen Typs und der angegebenen Amperezahl. Verwenden Sie keine reparierten Sicherungen, und überbrücken Sie die Sicherung nicht.

3. Das mitgelieferte Netzkabel muß in eine Steckdose mit Schutzleiter eingesteckt werden. Wird ein Verlängerungskabel verwendet, muß auch hier der Schutzleiter durchgeführt sein.

4. Verändern Sie nichts an den externen oder internen Schutz- bzw. Erdungskontakten. Wenn Sie sich an diesen zu schaffen machen oder sie unterbrechen, können Sie sich selbst und andere gefährden, oder das Gerät könnte beschädigt werden.

VORSICHT! Das Gerät ist bei der Auslieferung vorschriftsmäßig geerdet. Es brauchen keine Veränderungen an der elektrischen Verkabelung oder am Gerätechassis vorgenommen werden, um einen sicheren Betrieb zu gewährleisten.

5. Nehmen Sie das Gerät nie mit geöffnetem Gehäuse in Betrieb, da dies zu irreparablen Schäden führen kann.

6. Schalten Sie das Gerät nicht ein, wenn Sie den Verdacht haben, daß an der Elektrik möglicherweise Schäden eingetreten sind. Ziehen Sie in diesem Fall den Netzstecker heraus, und lassen Sie das Gerät von einem Kundendiensttechniker untersuchen. Versuchen Sie bis zu dieser Untersuchung keinesfalls, das Gerät in Betrieb zu nehmen. (Eine Beschädigung der Elektrik kann z.B. eingetreten sein, wenn das Gerät äußere Schäden aufweist oder unter problematischen Umständen transportiert wurde.)

7. Schäden können auch eintreten, wenn das Gerät längere Zeit unter ungünstigen Umständen gelagert wurde (*z.B.* unter der Einwirkung von Hitze oder Wasser).

8. Ziehen Sie vor allen Wartungsmaßnahmen immer zuerst den Netzstecker aus der Steckdose.

9. Auch wenn das Gerät abgeschaltet ist, können die im Inneren befindlichen Kondensatoren nach wie vor unter Spannung stehen.

10. Versuchen Sie niemals, Gerätekomponenten zu reparieren oder auszutauschen, die nicht in diesem Handbuch beschrieben sind, ohne einen Kundendiensttechniker zu Rate zu ziehen.

GLP-VORSCHRIFTEN (GOOD LABORATORY PRACTICES)

Ordnungsgemäße Aufzeichnungen

Damit Probleme mit Geräten oder Methoden erkannt und eingegrenzt werden können, empfehlen wir Ihnen, ordnungsgemäße Aufzeichnungen sämtlicher Gerätezustände (*z.B.* % RSDs zu Retentionszeiten, Kurvenflächen, Kurvenformen und Auflösung). Archivieren Sie als Minimum ein Chromatogramm einer typischen Probe und

einer Standardmixtur mit umfassender Dokumentation der Systembedingungen zum späteren Vergleich. Ein sorgfältiger Vergleich von Retentionszeiten, Kurvenformen, Empfindlichkeitswerten und Hintergrundrauschen liefert wertvolle Hinweise für den Fall, daß zu einem späteren Zeitpunkt Probleme auftreten und eingegrenzt und behoben werden müssen.

Chemische Toxizität

Die großen Mengen an toxischen oder brennbaren Lösungsmitteln, die im Labor verwendet und aufbewahrt werden, können ein erhebliches Gefahrenpotential darstellen, doch darf man hierüber nicht die mögliche Gefährdung durch die Proben selbst vergessen. Achten Sie insbesondere darauf, sämtliche Warnhinweise hinsichtlich ausreichender Belüftung, Lagerung, Handhabung und Entsorgung von Lösungsmitteln ebenso wie von Proben sorgfältig zu lesen und zu befolgen. Machen Sie sich mit den Toxizitätsdaten und den möglichen Gefahren sämtlicher verwendeter Chemikalien anhand der betreffenden Sicherheitsdatenblätter vertraut, die von den Produktherstellern zur Verfügung gestellt werden.

Probenvorbereitung

Überprüfen Sie stets die Löslichkeit der Probe in der mobilen Phase. Durch das Ausfällen von Feststoffen können die Säule, die Leitungen oder die Durchflußzelle verstopfen und damit den Durchfluß hemmen. Durch eine solche Verstopfung können irreparable Schäden am System entstehen. Die Ablagerung von Partikeln läßt sich durch Filtrieren der Proben durch ein Filter mit einer Porengröße von 0,45 oder 0,2 µm (oder weniger) vermeiden.

Anforderungen an das Lösungsmittel

Viele chemische Hersteller bieten eine Produktserie hochreiner Reagenzien in spektroskopisch reiner Qualität an, die frei von chemischen Unreinheiten sind. Die routinemäßige Filtrierung aller Lösungs- und Extraktionsmittel durch ein Fluorkohlenwasserstoff-Filter mit einer Porengröße von 0,45 oder 0,2 µm (oder weniger) vor dem Einfüllen in den Lösungsmittelbehälter verlängert die Lebensdauer der Einlaßfilter, der Ventile und Dichtungen, des Injektors und der Säule beträchtlich. Spezielle HPLC-Lösungsmittel brauchen normalerweise nicht filtriert zu werden.

Wählen Sie eine mobile Phase, die zur Probe und zur für die Separation verwendete Säule kompatibel ist. Dabei ist darauf zu achten, daß Edelstahl durch bestimmte Lösungsmittel korrodiert wird. Reaktionsträge, biokompatible Geräteausführungen werden ebenfalls von Thermo Separation Instruments angeboten.

Entgasen des Lösungsmittels

Lösungs- und Extraktionsmittel sollten entgast werden, und zwar entweder durch Vakuum oder Heliumdurchperlung. Eine umfassende

Beschreibung dieser Techniken finden Sie in dem separaten
Handbuch, das dem Entgasungszubehör beiliegt.

Entsorgung von Lösungsmitteln

Sorgen Sie dafür, daß ein Auffangbehälter für Lösungsmittel oder
eine andere Auffangvorrichtung in Höhe des Arbeitstisches oder
darunter zur Verfügung steht. Für die meisten Lösungsmittel gelten
besondere Entsorgungsvorschriften; eine Entsorgung über die
Abwasserleitung ist hier nicht zulässig.
Bei der Entsorgung von Chemikalien gleich welcher Art sind
die einschlägigen Vorschriften streng zu beachten.

Hochdrucksysteme und Undichtigkeiten

Flüssigchromatographen arbeiten unter hohem Druck. Da
Flüssigkeiten kaum komprimierbar sind, können sie nicht viel Energie
speichern. Dementsprechend stellt der hohe Druck in einem
Flüssigchromatographen auch kaum eine unmittelbare Gefahr dar.
Jedoch sollten auftretende Undichtigkeiten umgehend beseitigt
werden. Schließlich ist noch zu empfehlen, bei der Arbeit mit einem
Flüssigchromatographen stets Augen und Haut zu schützen und vor
allen Wartungsarbeiten darauf zu achten, daß das Gerät abgeschaltet
und druckfrei gemacht wurde.

Manual Conventions

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

DISPLAYS

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

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

A two-line menu display

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

```
   Injection Volume          1.0 (AS3000)
   Injections/Sample                  1
--------------------------------------------------------
   Cycle Time                        2.5
   Col Oven:  OFF_           Temp   18
   TrayTemp:   OFF_          Temp   20
```

A menu longer than two lines

COMMENTS

Fields that appear only on some SpectraSYSTEM™ and SpectraSERIES autosampler models or only when certain options are installed are documented with a comment to the right of the field. For example, in the menu shown above, the Injection Volume field appears only on the variable-injection volume (VIV) AS3000/AS3500 or AS300 autosamplers.

TEXT

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

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

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

Capitalization

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

STANDARD WORDS

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

select

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

enter

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

ICONS

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

Caution!
A caution alerts you to situations that could result in personal injury. It also tells you how to avoid them.

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

Hot Surface!
This icon alerts you to potential injury that could occur from coming in contact with a heated surface or area on or in an instrument. It also tells you how to avoid contact with the heated surfaces in your instrument.

Note
Notes alert you to the correct operating or maintenance procedures needed to prevent equipment or data damage. They also alert you to important exceptions, side effects, or unexpected occurrences that may result from certain action(s).

Hint
Hints call out general rules or shortcuts. They specify ways to obtain the best performance and results from your instrument.

1

Installation and Specifications

Introduction

This chapter describes the site requirements, installation procedures, and performance testing for the Thermo Electron, Finnigan SpectraSYSTEM and SpectraSERIES autosamplers. It covers the AS1000, AS3000, and AS3500 SpectraSYSTEM autosamplers, and the AS100 and AS300 SpectraSERIES autosamplers. The following topics are included in this chapter:

- Installation
- Instrument Startup and Priming Procedures
- System Communication
- Communication
- Ready Participation
- Instrument Configurations and Other Features
- Performance Verification

BEFORE YOU START

Before you start, review the site requirements and assemble the tools you'll need to install your instrument:

- One Phillips screwdriver
- Two 1/4" open-end wrenches
- One small, standard screwdriver

Startup Checklist

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

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

UNPACKING

☐ Unpack and inspect your instrument

☐ Remove cardboard/foam packing insert from sample tub

☐ Check for parts shortages

☐ Read the Safety Information Section on page ix

REAR PANEL

☐ Place the autosampler with rear panel accessible

☐ Install flush bottle/bracket

☐ Install filter/solvent line and flush bottle

☐ Connect the external solvent tubing to tubing extensions (sample preparation only)*

☐ Connect heater/mixer to an air supply*

☐ Connect the power cord

☐ Hardwire external events, make connection to other SpectraSYSTEM instruments**

FRONT PANEL

☐ Install solvent tray/waste container

☐ Connect autosampler to pump
On instruments without the column oven or
inert/biocompatible versions, connect pump outlet line to port
6 on injector valve
Instruments with column oven installed, connect pump outlet
line to solvent preheat tube

☐ Mount column
On instruments without the column oven, connect inlet end to
injector valve port 5

On instruments with the column oven installed, install the
column between the column mounts, resecure column mounts
to oven, and connect inlet end to injector valve port 5.

☐ Connect autosampler to detector
(Connect 12-inch 0.010 ID tubing to inlet side of flowcell)

STARTUP/PRIMING

☐ Turn on, check initial response to power-on

☐ Using the 2.5 mL syringe, flush lines with 5000 µL from the
Btl reservoir.*

Using the 250 µL syringe, flush lines with 500 µL

**PERFORMANCE
VERIFICATION**

☐ Run a standard sample (a test mix is shipped with the
instrument)

☐ Date and retain run results with instrument documentation

☐ Customize READY participation**

☐ Run an unretained sample to determine gradient delay time**

☐ Optimize needle height**

Installed by:_____ Date:_____

Site Requirements

SPACE

The standard autosampler weighs 16 kg. with all options installed, the autosampler weighs 24 kg. The instrument requires a bench space at least 37 cm by 41 cm by 51 cm. Be sure to allow adequate ventilation away from heating or air conditioning ducts. Avoid exposure to direct sunlight. If used with the SpectraSYSTEM pump and detector, place the autosampler in the middle (Figure 1.1).

HINT: You may wish to set your instrument on the bench such that you have easy access to the rear panel.
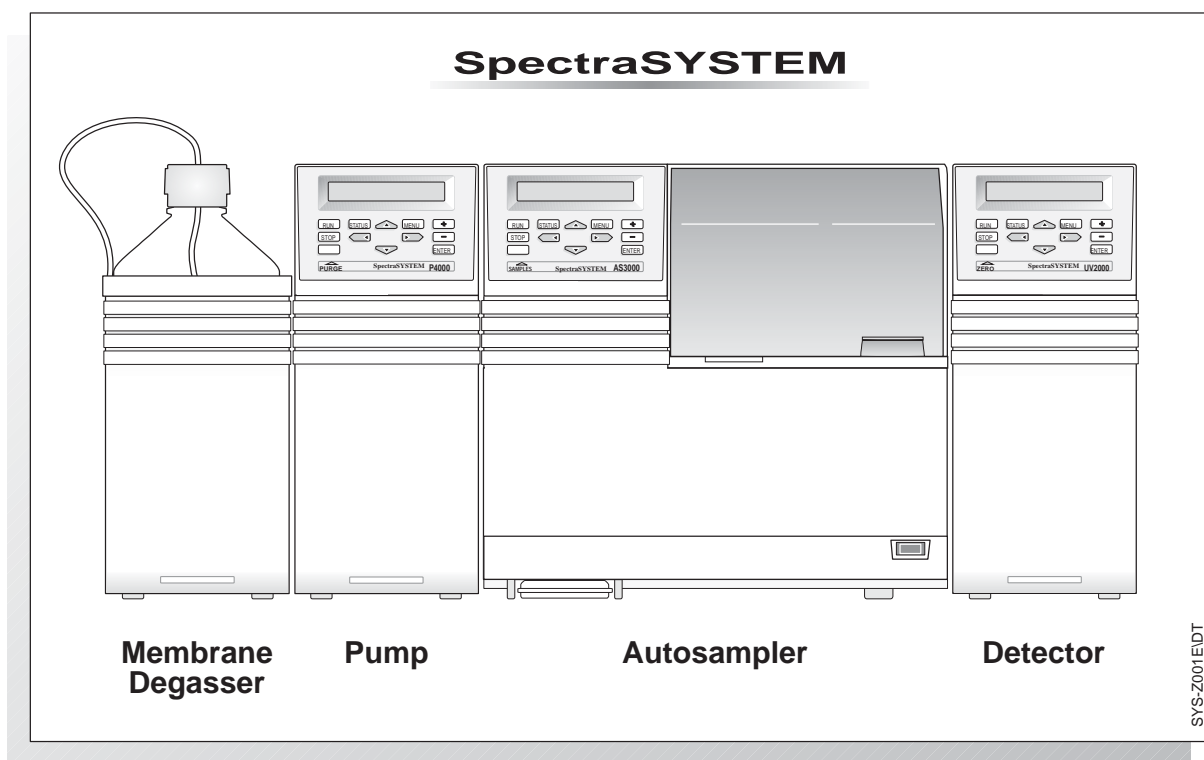
Figure 1.1 The SpectraSYSTEM or SpectraSERIES configuration

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

ELECTRICAL

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

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

Unpacking

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

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

After unpacking, inspect your autosampler and its accessories for missing parts and/or physical damage. If damage is found, notify both the carrier and your Thermo Finnigan representative. Please do not return goods without prior authorization from Thermo Finnigan.

The shipping kit contains the following:

- Autosampler
- Accessory Kit (see Appendix C)
- Vial Kit (see Appendix C)
- Declaration of Conformity (p/n A0099-813)
- *SpectraSYSTEM and SpectraSERIES Autosamplers Reference Manual* (p/n A0099-587)

Installation

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

The easiest way to install your SpectraSYSTEM or SpectraSERIES autosampler is to unpack it and set it on the bench, and then complete the plumbing, electrical, priming, and communication procedures. Additional or modified procedures required for instruments with one or more of the integrated options are incorporated into this section.

NOTE: For a list of all available options, upgrades, and accessories for your autosampler, see Appendix C or contact your Thermo Finnigan sales representative. Note that all upgrades require specialized installation. Contact your Thermo Finnigan field service representative for further information.

PLUMBING

Use the procedures below to install your bottles, solvent tray and solvent lines. Then connect your autosampler to the pump and detector and install your column and sample trays. Specific plumbing procedures include:

- Flush solvent and bottle bracket
- External solvents (with Sample Preparation Option only)
- Heater/mixer air supply (with Sample Preparation Option only)
- Solvent tray/waste container installation
- Pump-autosampler connection
- Column installation
- Autosampler-detector connection

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

NOTE: The AS1000 and AS100 come with the 20 µL sample loop installed.

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

Bottles

Flush Solvent Bottle Bracket

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

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

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

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

1. Align the bracket's mounting holes with the corresponding screw holes on the autosampler's rear panel (Figure 1.2).

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

COMM Port   6-Port Solvent-Selection   12-Pin Terminal   Bottle-Bracket
                    Valve   Screws

1 6
2 5
3 4

WARNING

TERMINAL CONFIGURATION

AS-Z004EIDT

Fuse Compartment   AC Power   Air-Supply Port
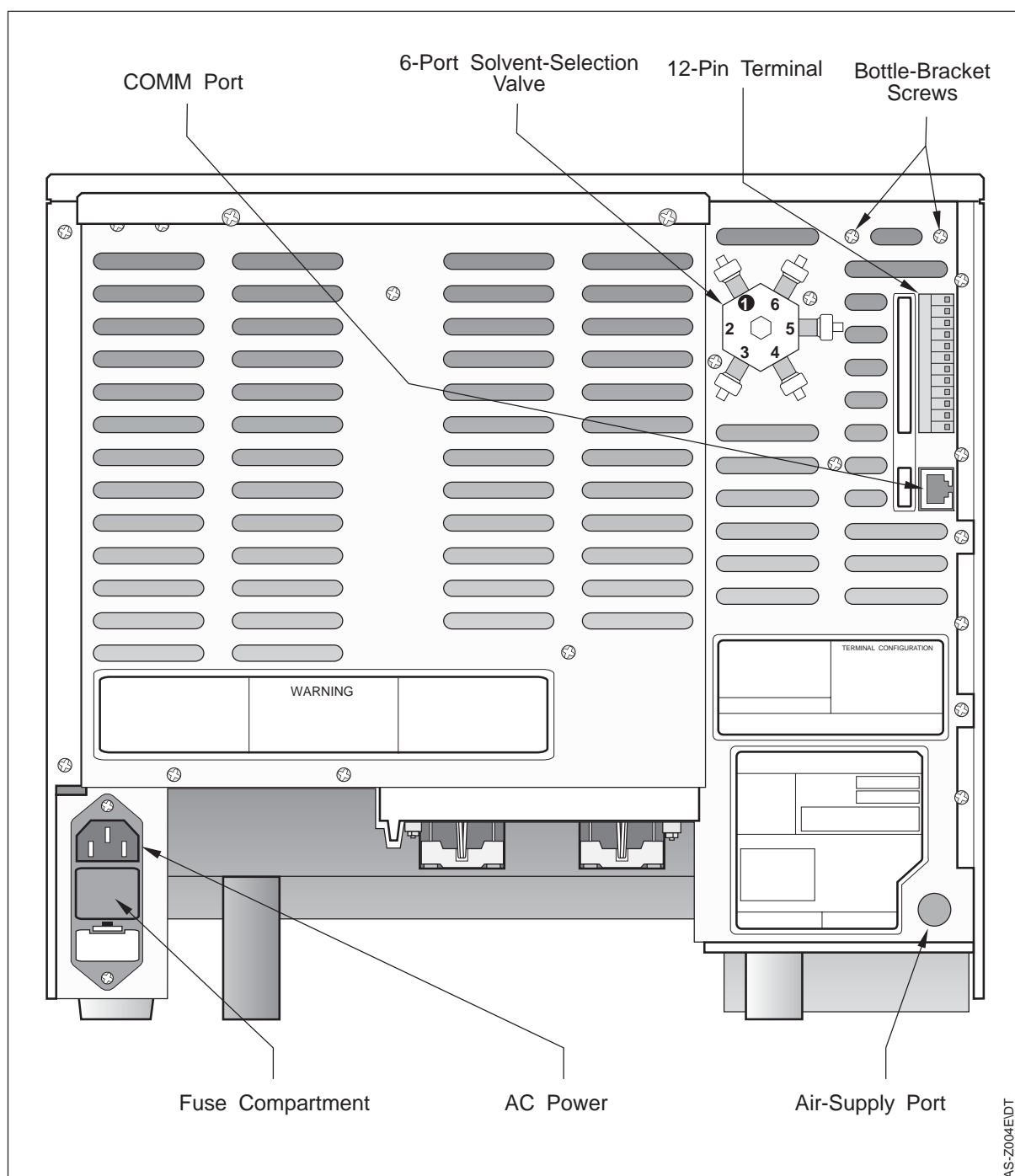
Figure 1.2 The autosampler's rear panel connections (with the Sample Preparation Option installed)

Flush Solvent Bottle

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

Use the following steps to install the flush bottle:

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

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

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

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

4. Place the flush bottle in the bracket.

> NOTE: If you did not purchase the Sample Preparation Option, proceed to "Solvent Tray/Waste Collection."

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

If you purchased the Sample Preparation Option, the internal solvent-line plumbing is already installed. You only need to set up your external solvent bottles and cap assemblies (not supplied), and connect the inlets and filters to the solvent-line extensions on the rear panel (Figure 1.2). Your accessory kit contains four solvent lines and four filter assemblies. Each solvent line has a flared (flanged) Cheminert nut on one end; the other end is flareless. To connect a solvent line, you simply connect the Cheminert nut to the Cheminert union on the appropriate solvent-line extension and attach the filter assembly to the other (flareless) end.

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

1. Designate four bottles as your solvent bottles (S1-S4), and plan the required space near your autosampler.

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

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

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

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

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

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

6. Remove a filter assembly from the accessory kit.

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

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

Heater/Mixer Air Supply

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

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

Connect the autosampler's air input line to your air supply. The air inlet line is located in the lower right-hand corner of the rear panel (Figure 1.2). It is flared and has a Cheminert nut on the end.

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

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

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

NOTE: Depending upon your regulator, you may need to cut off the Cheminert nut and flared end of the air supply line and use a standard 1/4" brass fitting in order to make the connection.

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

Waste Container

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

NOTE: The back of the compartment prevents the tray from sliding too far under the instrument.

Orient the tray so that the black-plastic, barbed elbow fitting is directed through the hole in the liquid collection trough (across the front of the instrument) (Figure 1.3).
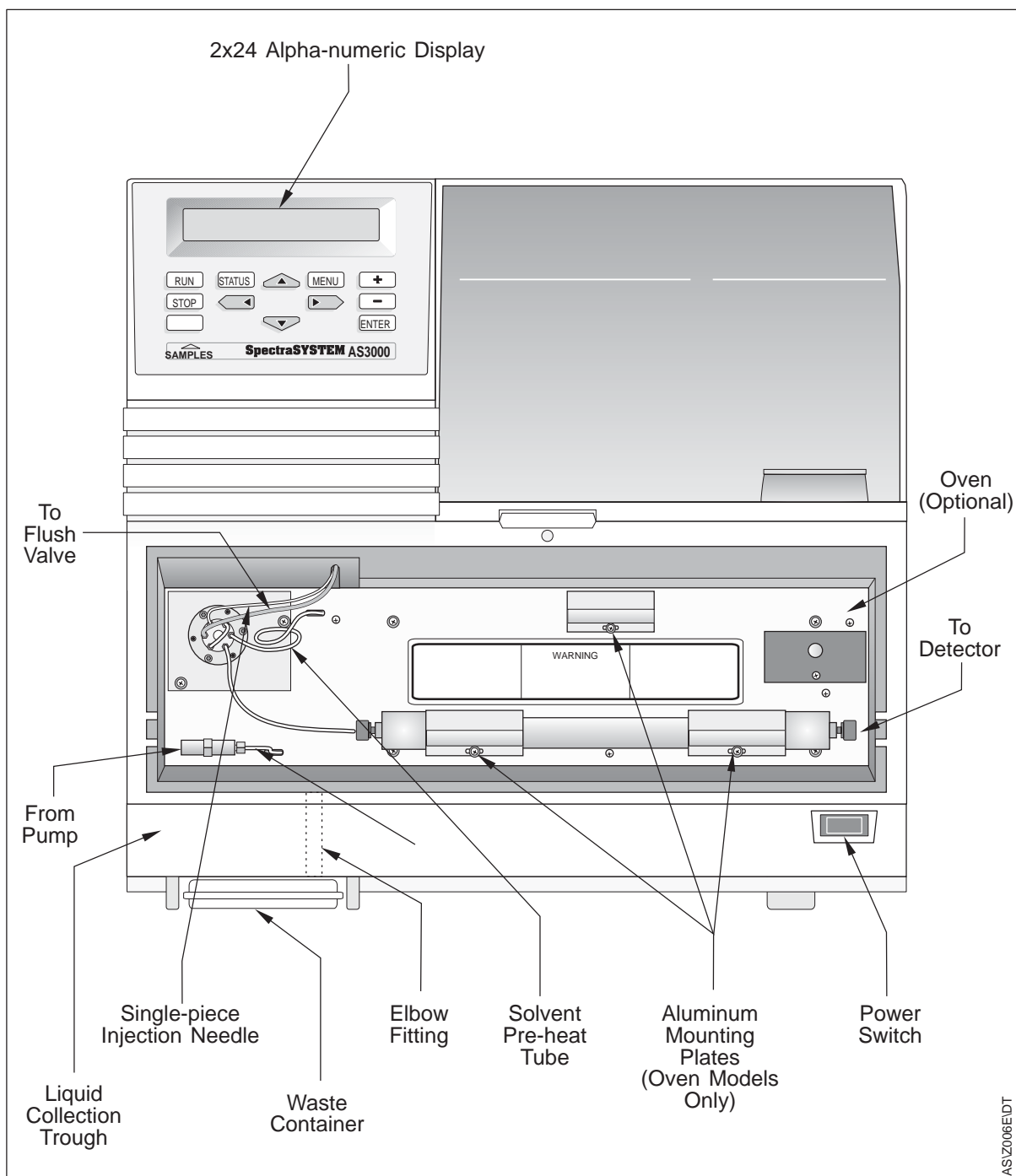
Figure 1.3 The autosampler's front panel connections (the optional column oven installed)

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

Other Waste-Collection Container
For larger-injection applications, or for those requiring frequent flushing, you may wish to install a piece of Tygon® tubing onto the waste port to allow the waste to flow to a carboy or other large container for waste-collection.

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

Pump-Autosampler Connection

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

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

Without the Column Oven Installed
The instrument is shipped with the needle and flush lines installed. To connect your pump to the autosampler's injection valve, use the following steps:

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

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

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

3. Connect the line to port 6 (Figure 1.4) on the injector valve. Finger-tighten, then use a 3/16" wrench to tighten the fitting an extra 1/8 turn.

NOTE: Do not under- or over-tighten the screws, or damage to the connections could occur. Undertightening causes leaks; overtightening may cause leaks or damage the connections.
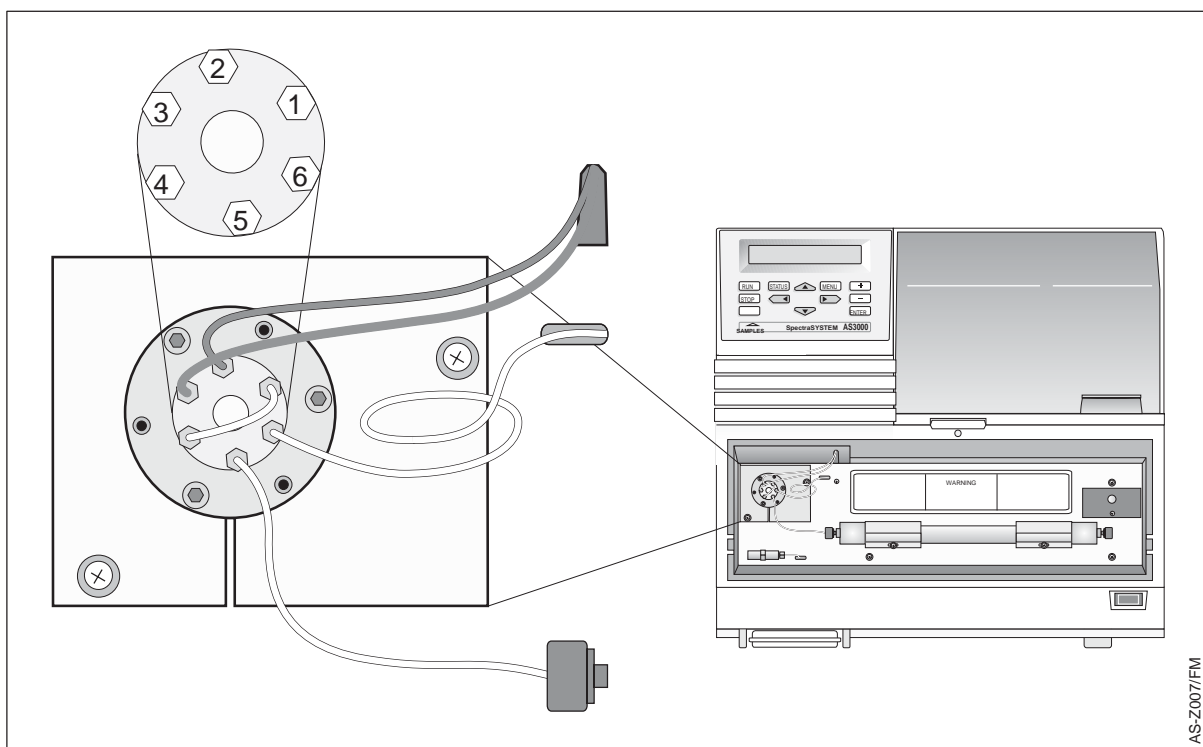
Figure 1.4 The autosampler's injector valve

With the Column Oven Installed

The instrument is shipped with the column oven assembly and the flush and needle lines already installed. The stainless-steel, low-dead-volume union that connects the pump outlet tubing to the column oven tubing is also factory-installed. You will have to connect the pump to the zero dead-volume union (ZDV), however. The preheat line runs behind the column oven, through the oven's mantle, up through the service loop, and into the injector valve. For standard analytical configurations, this provides approximately 180 µL of preheated solvent. Instruments with 0.007" narrow-bore preheat tubing provide approximately 22.7 µL preheated solvent.

Connect the pump to the preheat tube (Figure 1.5) as follows:

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

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

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

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

    a. Locate the ferrule included in your accessory kit.

    b. Thread the tubing through the fitting, and place the ferrule on the end of the preheat tube (Figure 1.5).

    c. Then use two 1/4" wrenches to tighten the connection. Use one wrench to hold the union body and one to tighten the nut 1/8-turn past the stop.

Refer to Chapter 3 for instructions on operating the oven.
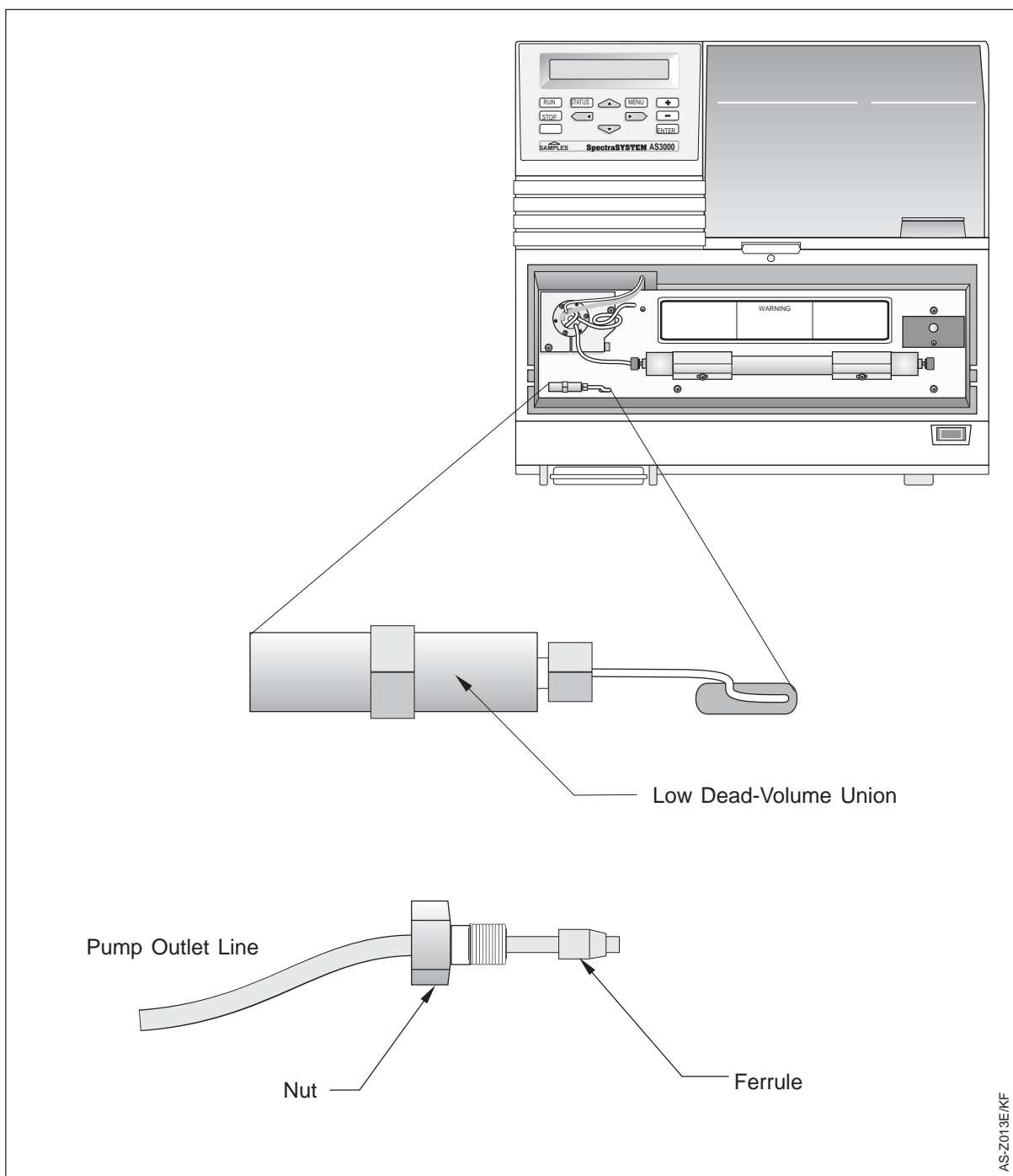
Figure 1.5 The pump-to-preheat tube connection

Sample Loop Installation

The AS3000, AS3500, and AS300 autosamplers come with a 100 μL sample loop installed; the AS1000 and AS100 autosamplers come

---

with a 20 μL loop. If you wish to change sample loops, refer to
Chapter 5, page 147.

Column Installation

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

Without the Column Oven Installed

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

*NOTE: Flush/purge the pump and autosampler lines before connecting the
column. This will ensure that no air gets into the column.*

Mount a column on your autosampler as follows:

1. Locate the 4-inch length of stainless steel tubing (0.010) and
   fittings included in your accessory kit.

2. Connect the 4-inch tubing to the inlet end of the column.

3. Connect the other end of the 4-inch tubing to port 5 on the
   injector valve (Figure 1.5).

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

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

*NOTE: Do not overtighten the fittings. You could damage, or cause
leaks to form around, the column connections.*

With the Column Oven Installed

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

*NOTE: Flush/purge the pump and autosampler lines before connecting the
column. This will ensure that no air gets into the column.*

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

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

---

2. Place the column between the column mounts, and replace the mounting screws to secure the column in place.

Autosampler-Detector Connection

The 12-inch piece of 0.010 ID tubing is provided in your accessory kit to connect your autosampler to your detector. Simply, connect the outlet end of the column to your detector (Figure 1.5).

NOTE: If you are using a Thermo Finnigan detector, you won't need the 12-inch tubing because the detector's inlet line is long enough to reach to the outlet end of your column.

You have completed the front-panel connections. Replace the column oven cover. It snaps firmly into place.

HINT: To replace the door, place the bottom edge into place and then snap the top closed.

Install Sample Trays

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

NOTE: Be sure to use a 40-60 mil (1.0-1.5 mm) rubber or silicone septum with a 5 mil (0.1 mm) Teflon liner on one side.

Each tray contains 40 (35, with Tray Temperature Control) vials arranged in eight (seven) rows of five. The first vial position is A01; the last is C40. The position labels are on the <u>left</u> of each position.

After your vials are in place, install sample trays A, B, and C as follows:

1. Hold the tray handle, tilting the back end down (Figure 1.6).
2. Insert the tray's "runners" into the slots in position A at the rear of the tub.
3. Lower the front of the tray into place and press down firmly to seat the tray.

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

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

NOTE: Be sure that each tray is properly seated. Each tray's upper front edge should fit under the upper lip of the tub. If a tray is not completely seated, the autosampler will not be able to correctly pick up or return vials to that sample tray.
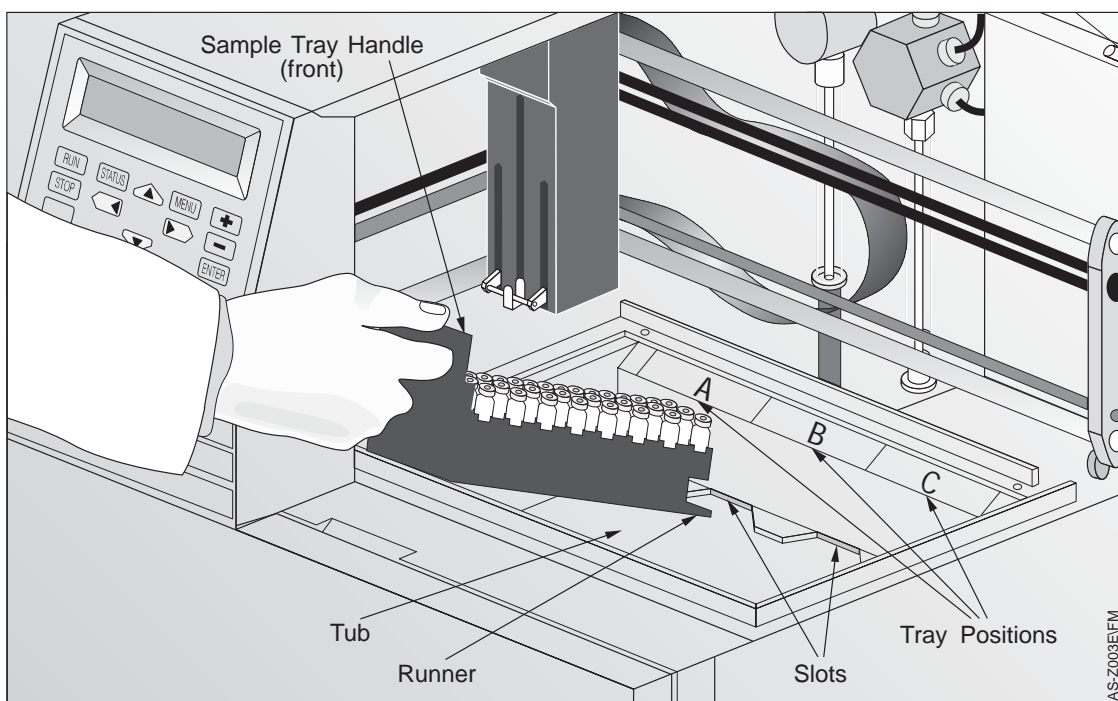
Figure 1.6 Installing a sample vial tray

POWERUP

Locate the power cord in the shipping container (not in your accessory kit). Insert one end into the AC power receptacle on the rear panel and the other end to a grounded power outlet. You don't have to worry about the fuses. Your autosampler is shipped with the correct fuses installed. Turn on your instrument by pressing the on/off button located in the lower right-hand corner just below the front (Figure 1.1).

During startup, the instrument's self-diagnostic sequence
- Completes a series of ROM checks
- Completes a series of RAM checks
- Activates the injection valve
- Verifies the proper connections on all installed options
- Verifies the proper operation of all sensors
- "Homes" (returns to starting position) all syringes and motors

Priming

After you have completed the plumbing and powerup procedures and started your instrument, you need to prime your autosampler to wet all of the lines. To do so, you'll need to flush all syringes and lines with an appropriate solvent.

NOTE: The autosampler is shipped with methanol in the lines. Be sure to use a miscible solvent.

HINT: If you purchased the Sample Preparation Option, proceed to the "2.5 mL" section to beginning priming the lines using the prep syringe. It will require less time. Then return to the "250 µL Syringe" section.

250 µL Syringe

To prime the autosampler:

1. Fill your flush solvent bottle, if you have not already done so.
2. Press [MENU] and select /COMMANDS/, (Figure 1.7).

```
Flush Sample Syringe
Initialize Hardware
Injector
Arm
```

Figure 1.7 The Commands Menu

```
Flush Sample Syringe
Flush Prep Syringe
Initialize Hardware
Injector
Arm
```

Figure 1.8 The AS3000 Commands Menu with the Sample Preparation Option installed

3. Select /Flush Sample Syringe/ to access the menu shown in Figure 1.9.
4. Use the [+] and [-] keys to select 5000 µL. Then press [ENTER] to initiate the flush operation.

```
Flush 5000 µL
```

Figure 1.9 The Flush Sample Syringe Menu

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

2.5 mL Syringe (AS3000 and AS3500 only)

If you purchased the Sample Preparation Option for your AS3000 or AS3500 autosampler, you must also flush the large (2.5 mL) sample preparation syringe and external solvent lines as follows:

1. Fill each solvent bottle with an appropriate solvent.

2. Press [MENU] and select /COMMANDS/.

3. Select /Flush Prep Syringe/ (Figure 1.10).

4. Use the [+] and [-] keys to select 5000 µL. Then move the cursor to the Solv field and select /Btl/. Press [ENTER] to initiate the flush sequence.

```
Flush 5000 µL Solv Btl
```

Figure 1.10 The Flush Prep Menu

NOTE: If there is residual air in the solvent-holding loop, flushing may expel solvent out the open port. To eliminate residual air that may be present during shipping, be sure to flush the lines with Btl selected before *flushing with S-1 through S-4 selected. Be sure that the S1-S4 solvent lines are connected.*

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

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

NOTE: If you are replacing syringe(s), be sure to install the 2.5 mL syringe on the left. Injecting small sample volumes with the large syringe can adversely affect reproducibility. Also, be sure to verify the correct syringe size in the Options/Configuration Menu.

Communication

The SpectraSYSTEM or SpectraSERIES autosamplers can be configured to send information to other Thermo Electron SpectraSYSTEM modules or to older SpectraSYSTEM and non-SpectraSYSTEM LC instruments, as follows:

- **SpectraNET Mode:** For AS1000 and AS3000, and AS3500 instruments only, SpectraNET mode as a part of a SpectraSYSTEM chromatograph connected to other SpectraSYSTEM modules through the SN4000, and controlled by your chromatography data system

- **Stand Alone Mode:** For any autosampler, stand-alone mode hardwired to other non-SpectraSYSTEM HPLC modules through the remote input/output connectors or connected to older SpectraSYSTEM and non-SpectraSYSTEM instruments by means of specialized cables and/or communication protocols. These options are described below.

NOTE: To connect to SpectraSYSTEM and non-SpectraSYSTEM integrators using your chromatography data system, refer to your chromatography data system Reference Manual.

SpectraNET MODE (AS1000, AS3000, AND AS3500 ONLY)

Configured as a SpectraSYSTEM module using SpectraNET communication and the SN4000, your autosampler is automatically controlled by your chromatography data system. The chromatgraphy data system assumes almost all keyboard control and coordinates the majority of the autosampler's operations. To configure your autosampler to use SpectraNET communication:

1. Turn off your autosampler.

2. Connect your autosampler to the SpectraNET Interface Module as described in your *chromatography data system Reference Manual*.

3. Turn on your autosampler, wait for the powerup sequence to complete, and then press /OPTIONS/Configurations/ to access the Configurations Menu (Figure 1.11).

```
   Sample Syringe                  250
   Prep Installed                  Yes
   (With sample prep)
-----------------------------------------------------------
   Oven Installed                  Yes
   TrayTemp Installed              Yes
   Key Repeat Rate              Medium
   Door Interlock                   On
   File Name                   Protect
   1: VITAMINS                      On
   2: BARBITUA                     Off
   3: EXPER 3                      Off
   4: STAT                         Off
   Mode                     SpectraNet
   Solv Viscosity               Normal
```

Figure 1.11 The Configurations Menu in SpectraNET mode

*NOTE: If you are running the RAM Hardware Test with "No System
Control" selected in the Mode Menu (38), the Controller (ISM100) will
cause the autosampler to reinitialize the hardware.*

4. Move the cursor to the Mode field, and use the [+] and [-] keys
    to select SpectraNET. Then press [ENTER] to accept the field
    value and exit the Configurations Menu.

Your autosampler is now configured as a SpectraSYSTEM module.

**HARDWIRED
STAND-ALONE
COMMUNICATION**

Stand alone mode is used to operate SpectraSYSTEM or
SpectraSERIES modules without data system control. Stand alone
mode is also used to connect older and non-SpectraSYSTEM
laboratory equipment.

The 12-pin, in-line connector on the instrument's rear panel allows
communications between your autosampler and other components in
an LC system (Figure 1.12). This simplistic communications system,
known as "hardwiring," provides injection synchronization between
your autosampler and the other components of your LC system.

A mating connector (provided in the accessory kit) allows you to
attach control wires from other LC devices to the 12-pin terminal by
loosening the appropriate screw, inserting the wire, and then
tightening the screw. Once all connections are made, you can quickly
make and break connections without disturbing individual wires.

Hardwire communications allow the autosampler to start and stop a
pump or detector, to begin a gradient program (gradient pumps only),
or to control other features of the externally connected instruments.
In addition, the terminal has four timed-function outputs that can be

used to control valve-switching devices or other devices on a timed basis. (Refer to Chapter 3, "Timed Events Program," page 72 to set up a timed-events program.)

This section describes the pin assignments, the recommended wiring configuration, and some optional wiring configurations.

TERMINAL CONFIGURATION

DESCRIPTION	SIGNAL
12. TIMED EVENT 1	OUT
11. TIMED EVENT 2	OUT
10. TIMED EVENT 3	OUT
9. TIMED EVENT 4	OUT
8. A/S READY	OUT
7. INJ HOLD	IN
6. INJ OUT	OUT
5. PUMP READY	IN
4. GRAD START	OUT
3. PUMP STOP	OUT
2. +5V 150MA	OUT
1. GROUND	—

DATE OF MANUFACTURE:

THIS PRODUCT IS COVERED BY ONE OR MORE OF THE FOLLOWING PATENTS:
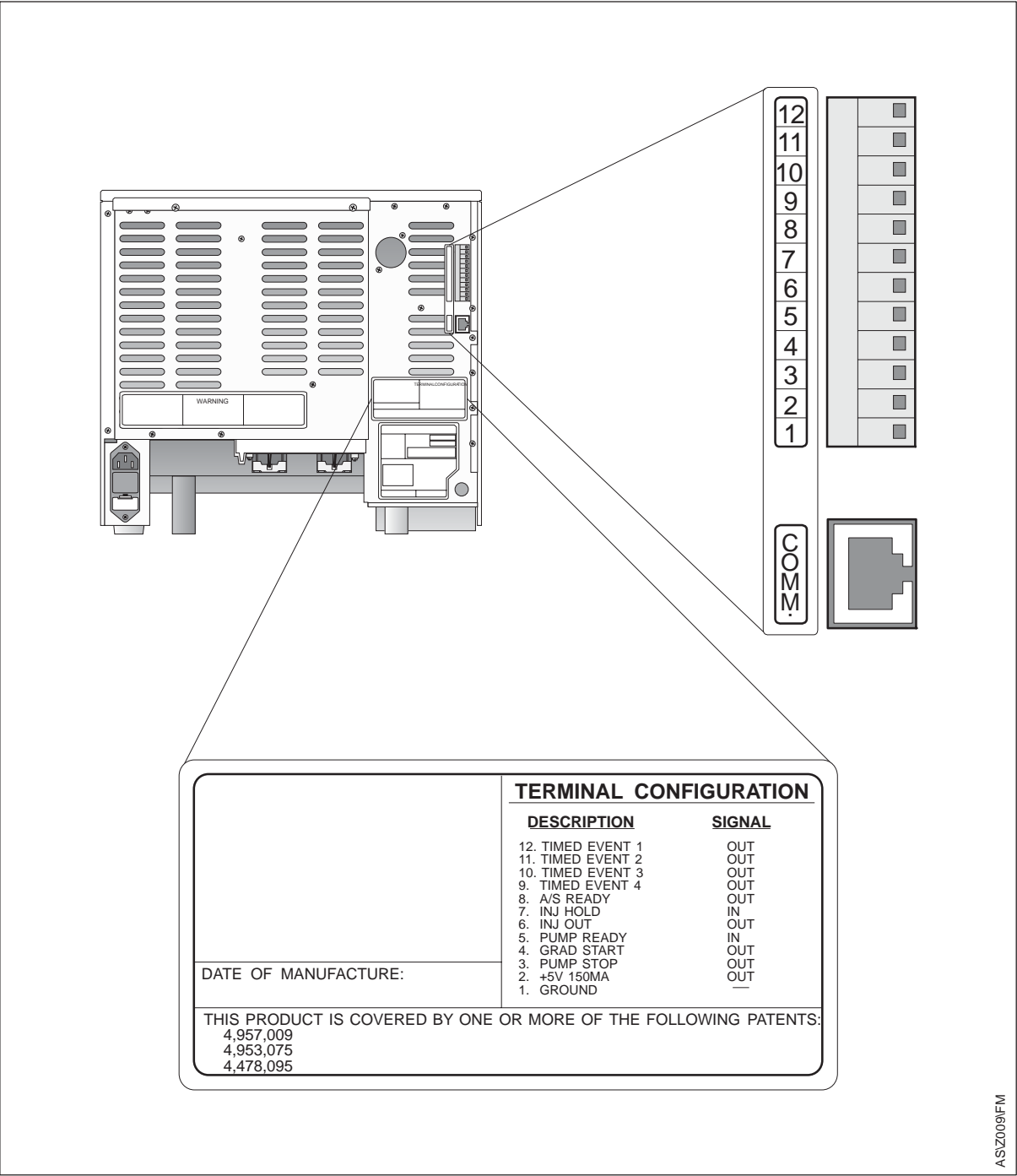4,957,009
4,953,075
4,478,095

Figure 1.12 The 12-pin terminal

Pin Assignments

Outputs

All output signals, including Time Functions 1 through 4 (TF1-4), are open-collector outputs capable of sinking (handling) currents up to 600 mA at voltages up to 24 VDC. The active state is user programmable in the Timed Events Menu (see Chapter 3).

Inputs

All inputs accept TTL-level inputs, with a transition activating each. The two inputs are tied to +5 VDC through pull-up resistors. A "Lo" level is from 0 to 0.8V; a "Hi" level is from 2 to 5.5V.

NOTE: All inputs and outputs are referenced to ground.

Pin 1 - GROUND: The ground reference used for all inputs and outputs.

Pin 2 - +5VDC: This regulated +5 VDC supply can be used to power pull-up resistors or external relays. The current is limited to a maximum of 300 mA to protect against external short circuits affecting the autosampler's operation.

Pin 3 - PUMP STOP: (output)This pulsed output is used to stop the pump after the last sample has been processed. It is activated when the last sample in the queue has completed its last injection, or when the shutdown file has been activated (using the special shutdown command). See Chapter 5 for more information on the Shutdown command.

Pin 4 - GRADIENT START OUTPUT: This signal activates the pump's gradient program. It occurs at a specified amount of time before the Inject Out line is activated. The time is specified as a Gradient Delay value in the More Menu (under Files). This momentarily active, pre-injection signal permits the gradient to start prior to sample injection to compensate for system delay volume. The result is an injection that occurs just as the gradient front reaches the injection valve. See the last section in this appendix for more information on the gradient delay function.

Pin 5 - PUMP READY (input): Through this input, the pump signals the autosampler that the mobile-phase flow rate is stable and that the pump is ready for an analysis to begin. (Other devices may also be connected here.) This input must be continuously active for the autosampler to proceed with injections. From the Input Polarity Menu you can select whether a high or low level on this line means "ready." The default is Hi.

Pin 6 - INJECT OUT (output): This line sends a momentary signal each time the autosampler makes an injection. This signal allows the synchronization of external devices (pumps, detectors, integrators, etc.) to the autosampler's injection. See "Outputs" below for the specifications of this output.

Pin 7 - INJECT HOLD (input): This signal puts the autosampler in the hold state with sample in the loop. Releasing the hold will cause an immediate injection. The signal must remain continuously active to hold injections. That is, when active, this signal will delay an imminent injection until this line goes to the inactive state. This function can be used with external equipment to precisely control the timing of the injection valve activation. The active level (Hi or Lo) of this input can be selected in the Input Polarity Menu (see Advanced Installation) at the end of this appendix.

Pin 8 - A/S READY: This line is used to signal to other devices that the autosampler is in a READY condition. See "Outputs" below for the specifications of this output.

Pins 9 thru 12 - TF1-TF4 (timed outputs): These user-programmed outputs are useful for triggering column switching valves or other external hardware. These outputs are controlled by the time functions defined in the currently active file (see Chapter 3 "Timed Events" page 72).

Recommended Hardwire Configuration

The recommended configuration requires a pump, autosampler, UV/VIS or fluorescence detector, and an integrator. We recommend that you connect the following signals:

- Pump Ready
- Inject Hold (detector ready)
- Detector Run/Start
- Integrator Run/Start

Refer to Table 1.1 to wire the appropriate pin connections. Pin connections for the SpectraSYSTEM modules are indicated in parentheses. Optional connections are indicated by asterisks (*).

Table 1.1 Pin Connections for the Recommended Configuration

Autosampler (Pin Connection)	Pump	Detector	Integrator
Ground (1)	Ground (3)	Ground (9)(11)	Ground (B1)
Pump Stop (3) (pulsed output)	Pump Stop (5)	N/A	N/A
Pump Ready (5) (input)	Pump Ready (1)	READY (4)	N/A
Inject Out (6)	N/A	RUN/START (10)	RUN/START "A" (B5) RUN/START "B" (B14)
*Inject hold (7)	Sync (8)	N/A	N/A
Gradient Start (4)	Run Grad (7)	N/A	N/A

Figure 1.13 illustrates the hardwire connections for the SpectraSYSTEM modules.
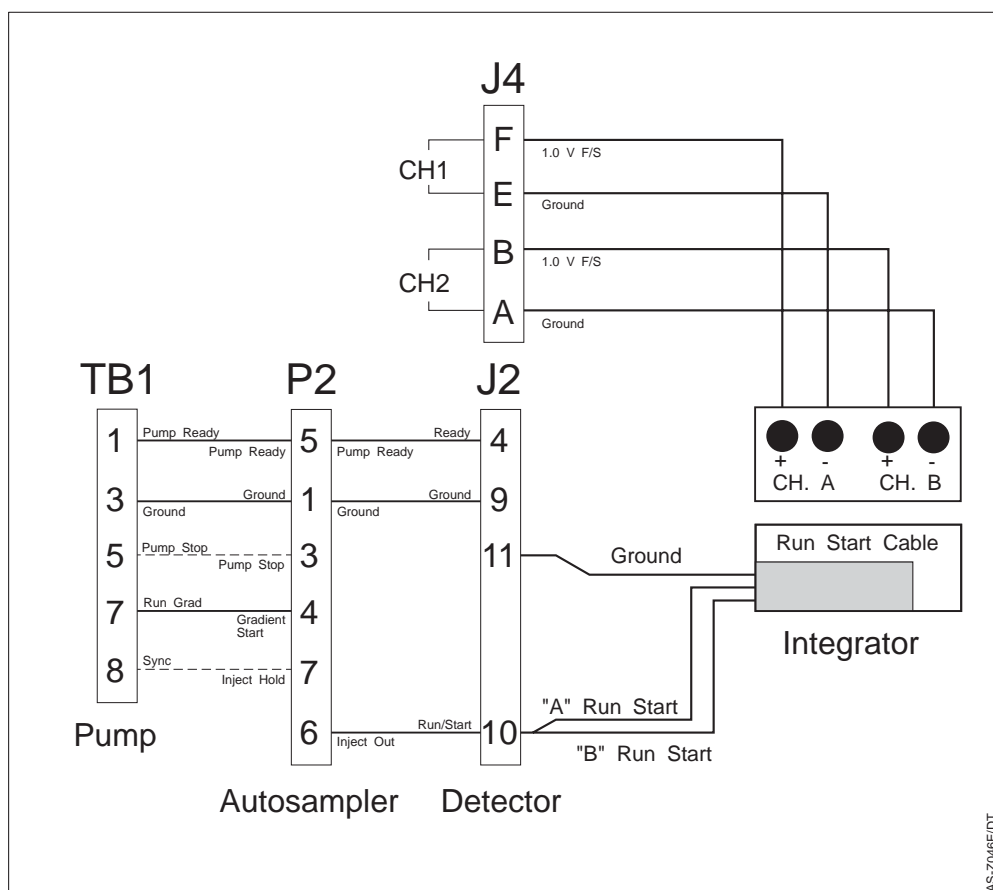

Figure 1.13 Hardwire connections for SpectraSYSTEM Modules

Communications Menu Parameters

For normal stand-alone operation, you do not need to need to change any of the values in the Communications Menu. Leave the communications parameters set to the default values (Figure 1.14). Details provided below for changing the polarity are provided for reference only.

```
Baud Rate      9600
Parity         NONE
Flow Control   NONE
Data Bits      8
Stop Bits      1
Echo           On
```

Figure 1.14 The Communications Menu default values used for normal stand-alone operation

The allowable entries and default values are listed below for each of the communications parameters.

Baud Rate: Allowable entries: 1200, 2400, 4800, or 9600 Bd; default: 9600Bd.

Parity: Allowable entries: None, Even, or Odd; default: NONE.

Flow Control: Allowable entries: NONE or XON/OFF; default: NONE.

Data Bits: Allowable entries: 8 or 7; default: 8.

Stop Bits: Allowable entries: 1 or 2; default: 1.

Echo: Allowable entries: On or Off; default: On.

NOTE: If the Communications Menu doesn't appear as one of the OPTIONS Menu choices, return to the OPTIONS Configurations Menu, and verify that Mode is set to Stand Alone. The Communications field will not appear in the OPTIONS Menu if Mode is set to SpectraNet.

Setting the Polarity

From the Options Menu you can change the sense of your autosampler's input and output signals as required by your other LC system modules. For example, when shipped, the Autosampler Ready Output is set to send a positive voltage (Hi) when the instrument is ready. If you change the Autosampler Ready Output Polarity to Lo, the instrument will send a 0-volt signal (Lo) when it's ready. The procedure for changing the polarity of the input and output signals is described below.

Input Polarity

To change the polarity of an input signal, select /Input Polarity/ from the Options Menu. Use the [+] and [-] keys to select either Hi or Lo for the desired input signal shown in Figure 1.15.

```
Pump Ready Active      Hi
Inj Hold Active        Lo
```

Figure 1.15 The Input Polarity Menu

This menu shows the level for the active state for each input signal.

NOTE: If you want to determine the current state of a particular signal, return to the Main Menu, and select /TESTS/Display Input States/.

Output Polarity

To change the polarity of an output signal, select /Output Polarity/ from the Options Menu. Use the [+] and [-] keys to select either Hi or Lo for the desired output signal shown in Figure 1.16.

```
Autosampler Ready      Lo
Inject Out Active      Lo
Grad Start Active      Lo
Pump Stop Active       Lo
```

Figure 1.16 The Output Polarity Menu

This menu shows the level for the active state for each output signal.

NOTE: If you want to determine the current state of a particular signal, return to the Main Menu, and select /TESTS/Display Output States/.

Table 1.2 and Table 1.3 show the output signal levels relative to the polarity settings and instrument status for the autosampler's input and output signals, respectively..

Table 1.2 Input Signal Levels

| | | ---------Polarity------- | |
		Hi	**Lo**
Pump Ready	Ready	Hi*	Lo
	Not Ready	Lo	Hi
Inj Hold	Active	Hi	Lo*
	Inactive	Lo	Hi
Note: The asterisked (*) values indicate default settings.			

Table 1.3 Output Signal Levels

| | | ---------Polarity------- | |
		Hi	Lo
Autosampler Ready	Ready	Hi	Lo*
	Not Ready	Lo	Hi
Inject Out	Active	Hi	Lo
	Inactive	Lo	Hi*
Gradient Start	Active	Hi	Lo
	Inactive	Lo	Hi*
Pump Stop	Active	Hi	Lo
	Inactive	Lo	Hi*
Note: The asterisked (*) values indicate default settings.			

STAND ALONE COMMUNICATION TO INTEGRATORS

Two specialized communication setups are available:

- Via an autosampler-to-integrator cable to ChromJet or DataJet integrators

- Via BCD interface to older Thermo Finnigan integrators or to non-Thermo Finnigan Integrators

Before using either of these communications setups, it is necessary to configure your autosampler as a stand-alone module as follows:

1. Turn on your autosampler, wait for the powerup sequence to complete, and then press /OPTIONS/Configurations/ to access the Configurations Menu (Figure 1.17).

```
  Sample Syringe                 250
  Prep Installed                 Yes
-----------------------------------------------------------
  Oven Installed                 Yes
  TrayTemp Installed             Yes
  Key Repeat Rate             Medium
  Door Interlock                  On
  File Name                  Protect
  1: VITAMINS                     On
  2: BARBITUA                    Off
  3: EXPER 3                     Off
  4: STAT                        Off
  Mode                    Stand Alone
  Solv Viscosity              Normal
```

Figure 1.17 Configurations Menu in stand-alone mode

2. Move the cursor to the Mode field, and use the [+] and [-] keys to select Stand Alone. Then press [ENTER] to accept the field value and exit the Configurations Menu.

Your autosampler is now configured as a stand-alone LC module.

ChromJet or DataJet Communication

Using an autosampler-to-integrator cable available from Thermo Electron, you can set up your autosampler to send vial information to a Thermo Finnigan ChromJet® (SP4400) or a DataJet (SP4600) integrator. For this connection you will need an RJ-45-to-RJ-45 cable and an integrator adapter (p/n A3981-010).

To make this connection:

1. Turn off your integrator.
2. Insert the RJ-45 connector (telephone connector) into the COMM. Port (telephone jack) on the autosampler's rear panel.
3. Connect the adapter to the other end of the cable. It orients in only one direction.
4. Plug the adapter-cable end into your DataJet or ChromJet integrator.
5. Turn on your integrator and type in the following:

   BAUD 9600

   POKE#C12D,#FF

6. Hold the [CTL] key and press [CALIB].

*NOTE: You must reenter this sequence after **any** integrator soft reset!*

This sequence provides you with the four-line report from your autosampler shown in Figure 1.18.

```
Sample Vial: A02 Inj Volume :        5.0    Oven Temp :   26
Inject Vial: A01 Inj/Vial   :        1/1    Tray Temp:     0
Vial Type  : SAMPLE    Cycle Time    0.8    Heater Temp:  24
File 1:  FILENAME      Set Number :     1
```

Figure 1.18 The vial location and injection report

BCD Communication

The BCD Communication Option provides BCD communication from the AS1000/AS3000/AS3500 SpectraSYSTEM autosamplers to older Thermo Electron and non-Thermo Electron integrators and computers. This option provides tray- and/or vial-location information to devices requiring either binary or binary-coded decimal data. The BCD assembly must be installed by a Thermo Finnigan service representative if ordered after initial instrument delivery. Installation instructions are provided in the BCD Option Kit. Contact your representative for further information.

Ready Participation

When the instrument is shipped, the following conditions must be satisfied in order for the Status Screen to indicate that the autosampler is "ready" for operation:

- The door must be closed.

- The oven must be at the set temperature (if installed).

- The heater must be at the set temperature (with the Sample Preparation Option only).

- The tray must be at the set temperature (with the Tray Temperature Control Option installed).

The Options Ready Participation Menu allows you to customize the "autosampler ready" requirements to fit your own needs. For example, you may wish to prepare your first sample set (without injection) while the column oven is still warming up. In this case, you would want to disable the column oven condition in order to complete the sample preparation run. Or, you may wish to run your autosampler with the door removed. In this case you would want to disable the door interlock.

To access the Ready Participation Menu (Figure 1.19), select /OPTIONS/Ready Participation/.

```
   Door Interlock         ENABLE
   Col Oven Ready         ENABLE
------------------------------------------------------------
   Heater Ready           ENABLE
```

Figure 1.19 The Ready Participation Menu

To change any of the above requirements, move the cursor to the appropriate line, and use the [+] and [-] keys to change ENABLE to DISABLE.

Instrument Configurations and Other Features

Your AS300, AS3000, or AS3500 autosampler is shipped with a 100 µL sample loop (the AS1000 or AS100 instruments come with a 20 µL loop), a 250 µL sample syringe, and a 2.5 mL sample preparation syringe (with the Sample Preparation Option installed). Should you wish to change your syringe sizes, you can do so from the Configurations Menu (Figure 1.20). This menu also reflects your other installed options (oven and tray temperature control). If you would like to adjust the rate at which fields cycle through the available choices, you can change the Key Repeat Rate entry. Chapter 3 discusses the File Protect function also available from this menu.

```
    Sample Syringe Size    250
    Prep Syringe Size      2500
----------------------------------------------------------
    Prep Installed         Yes
    Oven Installed         Yes
    TrayTemp Installed     Yes
    Key Repeat Rate        Medium
    File                   Protect
    1:                     Off
    2:                     Off
    3:                     Off
    4:                     Off
    Solv Viscosity         Normal
```

Figure 1.20 The Configurations Menu

**DISPLAY
CONTRAST**

You can vary the display's contrast to make it easier to read.

To change the display's contrast, first press [STATUS] to access the Status Screen. Then simultaneously press [>] and [+] to *increase* the contrast, or [>] and [-] to *reduce* the contrast.

Performance Verification

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

NOTE: Before beginning the performance verification runs, you may wish to familiarize yourself with the autosampler's menu structure and keypad. This information is found in Chapter 2 or on the quick reference and menu cards in the front pocket of the manual.

REPRODUCIBILITY

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

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

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

Test Setup

Set up the test run as follows:

1. Fill five vials with the test sample and place them in tray positions A01-A05.
2. Create a file using the Injection Menu parameters shown in Figure 1.21.

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

Figure 1.21 The Injection Menu performance verification parameters

3. Return to the Edit Menu.

4. Access the More Menu and verify that the Needle Height is set to 2.0. (Do not change any other parameters in this menu.)

5. Return to the Main Menu and Load your file.

6. Press the [SAMPLES] key and set up a sample queue that contains five sample sets of one vial each. For each sample set, enter the following Sample Menu values:

    Inj/Sample   10
    Cycle Time   1.5
    No. Vials   1

    Vary the injection volume (Inj Vol) for each set using the values listed in Table 1.4.

Table 1.4 Reproducibility Test Sample Queue Parameters

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

7. Return to the Main Menu.

8. Flush the syringe with 500 μL of flush solvent. (Select /COMMANDS/, /Flush Sample Syringe/).

9. Inspect all sample lines for bubbles. (Be sure to check the Teflon line that connects to port 3 of the injection valve.) If bubbles are present, repeat step 8 until all bubbles are removed.

10. Check for leaks.

11. Initialize your pump.

12. Prepare your integrator for a run.

13. Press [RUN].

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

%RSDs

Determine the percent relative standard deviations (%RSD) for the injection volumes and compare them with the desired results in Table 1.5.

Table 1.5 %RSD Results for the Reproducibility Test

Volume (in μL)	%RSD
1	$<1^*$
3	$<0.7^*$
7	$<0.5^*$
10	$<0.5^{**}$
* Typical value	
** Instrument specification	

If your results are other than expected, refer to Appendix A, *Troubleshooting*.

SAMPLE CARRYOVER

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

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

In this procedure you will inject your sample, a subsequent blank (BLANK1), additional blanks to return the system to baseline, and then a standard (STANDARD) that is 0.01% of your sample. You will determine the % carryover by multiplying the ratio of the carryover by the 0.01% specification:

Carryover = [Peak Area$_{BLANK1}$ / Peak Area$_{STANDARD}$] x 0.01%

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

Materials

To perform this procedure you'll need:

- Your sample
- 7 vials, septa, and caps for each run
- Autosampler with deflected point needle
- Any HPLC pump, detector, and data system

Sample Preparation

Prepare your SAMPLE and STANDARD as follows:

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

Example:

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

Blank Preparation

Prepare your blanks as follows:

1. Pipette fresh starting mobile phase into five vials.
2. Label vials as BLANK, BLANK1, BLANK2, BLANK3, and BLANK4 for each test set.

Test Procedure

System Setup

Set up your system as follows:

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

Autosampler Setup

Set up your autosampler as follows:

1. Set the flush volume to 2000 µL:

   a. Press [MENU], /Files/Flush Volume/
   b. Select 2000 µL

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

2. In the Injection Menu, set the injection volume (loop size) to 5, 10, or 20 µL as appropriate.

3. Set the number of injections to 1.

4. In the /Files/More/ Menu, set the injection type to Push.

5. Set the vial positions as follows:

> A01: BLANK
> A02: SAMPLE
> A03: BLANK1
> A04: BLANK2
> A05: BLANK3
> (Add additional Blanks as necessary to allow the system to return to baseline before running the STANDARD.)
> B01: STANDARD
> B02: BLANK4
> (Add additional Blanks as necessary to allow the system to return to baseline before replicating the test.)

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

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

Carryover Calculation

To calculate the % sample carryover:

1. Divide the peak area of BLANK1 by the peak area of the STANDARD. This gives you the ratio of the carryover to the 0.01% specification.

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

Example:

> If the peak area for BLANK1 is 110,958 counts,
> and the Peak area for the STANDARD is 656,283 counts,
> the ratio of the carryover to the specification is
> > 110,958/656,283 or 0.1691
> > % carryover = 0.1691 x 0.01%
> > % carryover = 0.001691

Specification

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

**DILUTION
REPRODUCIBILITY
(AS3000 AND AS3500
ONLY)**

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

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

Column:	Spheri-5 RP18, 5 micron, 100 x 4.6 mm
Flow Rate:	1.5 mL/min
Mobile Phase:	100% HPLC grade Methanol (MeOH)
Flush Solvent (Btl and S-1):	100% HPLC grade Methanol (MeOH)
Test Sample:	50% Toluene in Methanol
Detection:	254 nm

Test Setup

Setup the test run as follows:

1. Fill five vials with the test sample and place them in tray positions A01, A03, A05, A07, and A09.

2. Place empty vials in tray positions A02, A04, A06, A08, and A010.

3. Advance to the Sample Prep menu in Method 1. Enter 1 for the dilution template and setup the parameters as follows:

    - Template 1

    - Dilution

    - # of dilution cycles 1

    - 1 - Load 1000 µL Solv S-1

    - 2 - Pickup 10 µLSample

    - 3 - Pickup 0 µL Sample

    - 4 - PickLG 0 µL Sample

    - 5 - Add 1000 µL to Sam + 1

    - Mix for 0.5 minute

    - Overlap enable - Yes

4. Flush sample syringe with 500 μL. Next flush the prep syringe with 4800 μL from Btl and S-1. Check lines for air bubbles, repeat if necessary.

5. Press the [SAMPLES] key and setup a sample queue that contains five sample sets of one vial each. For each sample set, enter the following Sample Menu values:

   Inj/Sample   3
   Cycle Time   1.5
   No. Vials   1

Vary the sample vial position for each sample set:

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

6. Initialize your pump and check for leaks.

7. Prepare the integrator for a run.

8. Press [RUN].

The autosampler will prepare 5 dilution of the test sample that make a 0.5% Toluene in methanol sample. It will then inject 10 μL of the diluted sample three times.

% RSD

When the test run is complete, gather the area counts for each of the sample set's third injection. Calculate the average of these five area counts and their standard deviation. Divide the standard deviation by the average area count and multiply by 100 to obtain the % RSD.

The specification is < 1%.

If your results are not within the specification range, refer to *Troubleshooting*, Appendix A.

Specifications

Vial capacity	120 vials in three removable trays 105 vials with tray temperature control
Variable-Volume	
Injection Precision	<0.5% RSD @ 10 µL or greater
Injection Volume	0.1 - 100 µL injection standard (250 µL syringe) up to 1000 µL injections with larger loops and syringes
Fixed-Loop	
Injection Precision	<0.5% RSD @ 10 µL or greater
Injection Volume	20 µL standard (250 µL syringe) 1000 µL injections with larger loops and syringes
Sample Carryover	Typically <0.01% at 400 µL flush volume Typically 5 mL flush volumes can be set
Minimum Sample Volume	1 µL can be injected from 10 µL with a standard needle
Needle Height	Programmable in 0.1 mm increments
Column Oven (optional)	Settable in 1° increments from 20° - 80°C, starting at 5°C above ambient Temperature stability typically ± 0.2°C Accommodates up to 30 cm columns
Tray Temperature Control (optional)	Settable in 1°C increments from 0° - 60°C Temperature stability typically ± 1°C Typical vial temperature from 4° - 50°C
Sample Preparation (optional)	
Reagent Vials	Three vials can be specified per file
Dilution Solvents	Four external solvents can be selected
Heating/Mixing	Single vial, vortex heater/mixer Settable in 1°C increments from 30° - 100°C
Dilution Precision	Typically <1.0% RSD for a 1/100 dilution (combined dilution and injection)
Dispensing Precision	Typically <0.2% RSD for 1 mL
Method Files	Protected in non-volatile memory
Communications	Pump Ready, Inject Hold
Outputs	Pump Stop Autosampler Ready Inject Gradient Start Four timed-event outputs BCD (optional) SpectraNET/RS-232 port
Dimensions	14.5" (37 cm) x 16" (41 cm) x 20"(51 cm) (H x W x D)
Weight	35-53 lb. (16 -24 kg) depending on options installed
Power requirements	100-120/200-240VAC 50/60 Hz, 450VA
Environmental	10-40°C; 5-95%RH noncondensing
Safety/EMC Compliance:	CSA, TÜV, FCC, CE Mark

2

A Quick Example

Introduction

This chapter provides you with the three basic rules you'll need for using your AS3500, or AS3000, AS300, or AS100 SpectraSYSTEM or SpectraSERIES autosampler. It also introduces you to the instrument's command center and describes the conventions we'll use in this manual.

Before you start this chapter, be sure that you read the Safety Information section beginning on page vii and install your autosampler as described in Chapter 1.

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

Learning Your Way

AS EASY AS 1-2-3!

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

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

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

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

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

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

VISUAL CLUES

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

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

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

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

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

Instrument Control

Take a look at the keypad and two-line display located on the front panel (Figure 2.1). This is the command center from which you'll access menus and control the instrument's operations. A brief explanation of the keys and the main menus and screens follows.
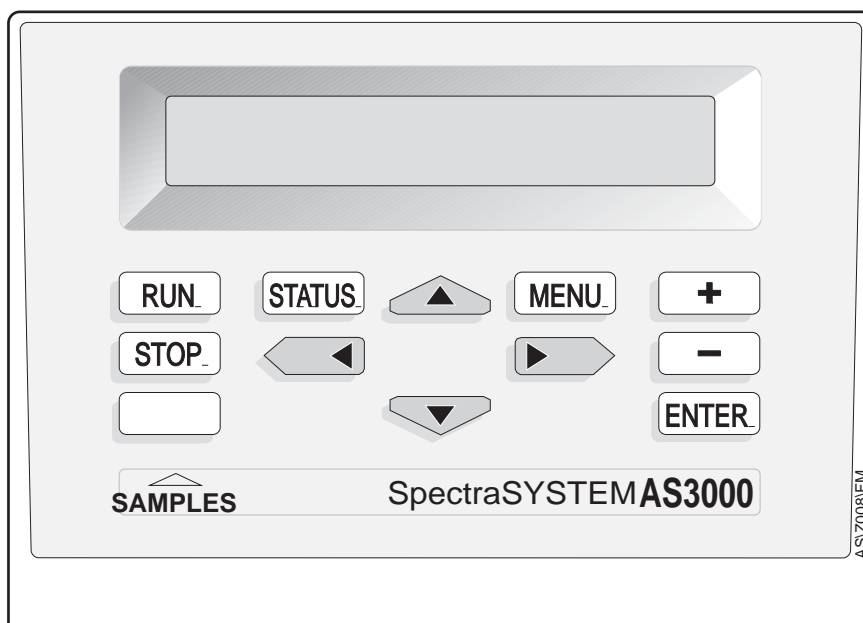
Figure 2.1 The autosampler's front panel
(With the exception of the model number, the front panel is identical for all SpectraSYSTEM and SpectraSERIES autosamplers.)

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

[RUN]

Pressing [RUN] starts the run.

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

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

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

[STOP]

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

[STATUS]

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

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

[SAMPLES]

The unlabeled key is the only variable key on the SpectraSYSTEM and SpectraSERIES instruments. (It has a different function on each of the SpectraSYSTEM modules.) On the autosampler, the blank key is the [SAMPLES] key. The key's name appears on the nameplate below the key.

The [SAMPLES] key displays the Samples Menu that lets you define the resources (samples, reagents, and standards) and assign a file to each sample set. From the Samples Menu you also add sample sets to the sample queue.

Because you manage resources and files from the Samples Menu, the [SAMPLES] key gives you the real-time editing capability provided by the [STATUS] key on the other SpectraSYSTEM instruments. That is, from the Samples Menu you can make changes to the file that is currently running. Pressing the [SAMPLES] key during a run lets you obtain information about, or edit the locations and injection volumes of, the sample and calibration vials. Chapter 3 contains more information on the [SAMPLES] key.

[MENU]

Pressing [MENU] displays the Main Menu (Figure 2.2). See below for more information.

[ENTER]

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

[∧], [∨], [<], and [>]

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

[+] and [-]

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

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

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

**MENUS AND
SCREENS**

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

Main Menu

The Main Menu (Figure 2.2) is the top level of the menu structure. It gives you access to five other menus: FILES, COMMANDS, QUEUE, OPTIONS, and TESTS. To see the Main Menu, press the [MENU] key at any time.

```
FILES                    QUEUE                TESTS
           COMMANDS              OPTIONS
```

Figure 2.2 The Main Menu

From the Files Menu you can edit, load, or delete files. From the Queue Menu you can edit or change the order of sample sets in the sample queue. The Tests Menu gives you access to the instrument's built-in diagnostics. The Commands Menu lets you run built-in instrument routines. In the Options Menu, you can set up or change your instrument's configuration. Refer to Chapters 3 and 4 for more information on any of the instrument's menus.

Status Screen

The Status Screen automatically appears whenever you turn on the instrument or press the [STATUS] key. The Status Screen (Figure 2.3) displays the status of the sample queue, the current sample set, the current sample vial, and the total number of samples in the set. Pressing the down-arrow once displays the Inject Status Screen (Figure 2.4). Pressing the down-arrow again displays the Oven/Tray Temperature Status Screen (Figure 2.5), if these options are present. Chapter 3 contains more information on the Status Screen.

```
QStatus    Set     #Samples
IDLE        1        1 / 1
```

Figure 2.3 The Queue Status Screen

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

Figure 2.4 The Injector Status Screen

```
Oven              TrayTemp
OFF  23           OFF  25
```

Figure 2.5 The Oven/Tray Temperature Status Screen

MESSAGES

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

User messages

User messages tell you about an existing instrument condition or ask for further actions. Some of these will only appear on the display for three seconds. An example of a message requiring further action is shown in Figure 2.6.

```
            * File Protected *
            No Editing Allowed
```

Figure 2.6 An example of a user message

Confirmation messages

Confirmation messages (Figure 2.7), indicated on the display by asterisks, appear for one second after an operation has been carried out successfully.

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

Figure 2.7 An example of a confirmation message

Error messages

Error messages (Figure 2.8), indicated on the display by exclamation points, are shown whenever an undesirable condition exists that prevents the instrument from carrying out an operation. Error messages remain on the display until you press a key.

```
  !!   Unexpected Vial in Hook   !!
```

Figure 2.8 An example of an error message

Practice Examples

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

The steps for setting up a run are as follows:

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

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

Before you start the practice runs, be sure that you review the Safety Information section and complete the Startup Checklist. This portion of the chapter assumes that you have installed your instrument as described in Chapter 1 and that your sample trays are installed.

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

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

HINT: If at any time you lose your place, you can do one of the following:

- *Press [∧] to return to a previous screen,*

  OR

- *Press [MENU] to return to the Main Menu (the top of the menu structure) and retrace your steps.*

PREPARE YOUR CHEMICALS

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

1. Fill a vial from your accessory kit with distilled water. Cap it securely, and place it in position A01, in the front left corner of tray A (Figure 2.9).

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

2. Fill the flush-solvent bottle with a 70:30 MeOH:H_2O solution. Refer to Chapter 1 for the flush-bottle installation procedure.

ENTER YOUR PARAMETERS

There are two ways to enter your run parameters into your autosampler: from the Samples Menu and from the Edit Menu. If you have an AS1000 or AS100, you will use the Sample Menu method most often. Most likely you will want to enter into the Edit Menu those parameters that are least likely to change and those that change with each run into the Samples Menu. The Samples Menu allows you to vary individual parameters with successive runs. Should you decide to enter your parameters into the Edit Menu, there is no need to re-enter them into the Samples Menu. They are automatically copied into the Samples Menu. Refer to Chapter 3 for more information on entering your run parameters. For now, you may wish to familiarize yourself with both approaches for entering your parameters.

Vial Position
A01

Hook

Samples
Key

On/Off Switch

Sample Tray Handle
(front)

ASZ001E\DT
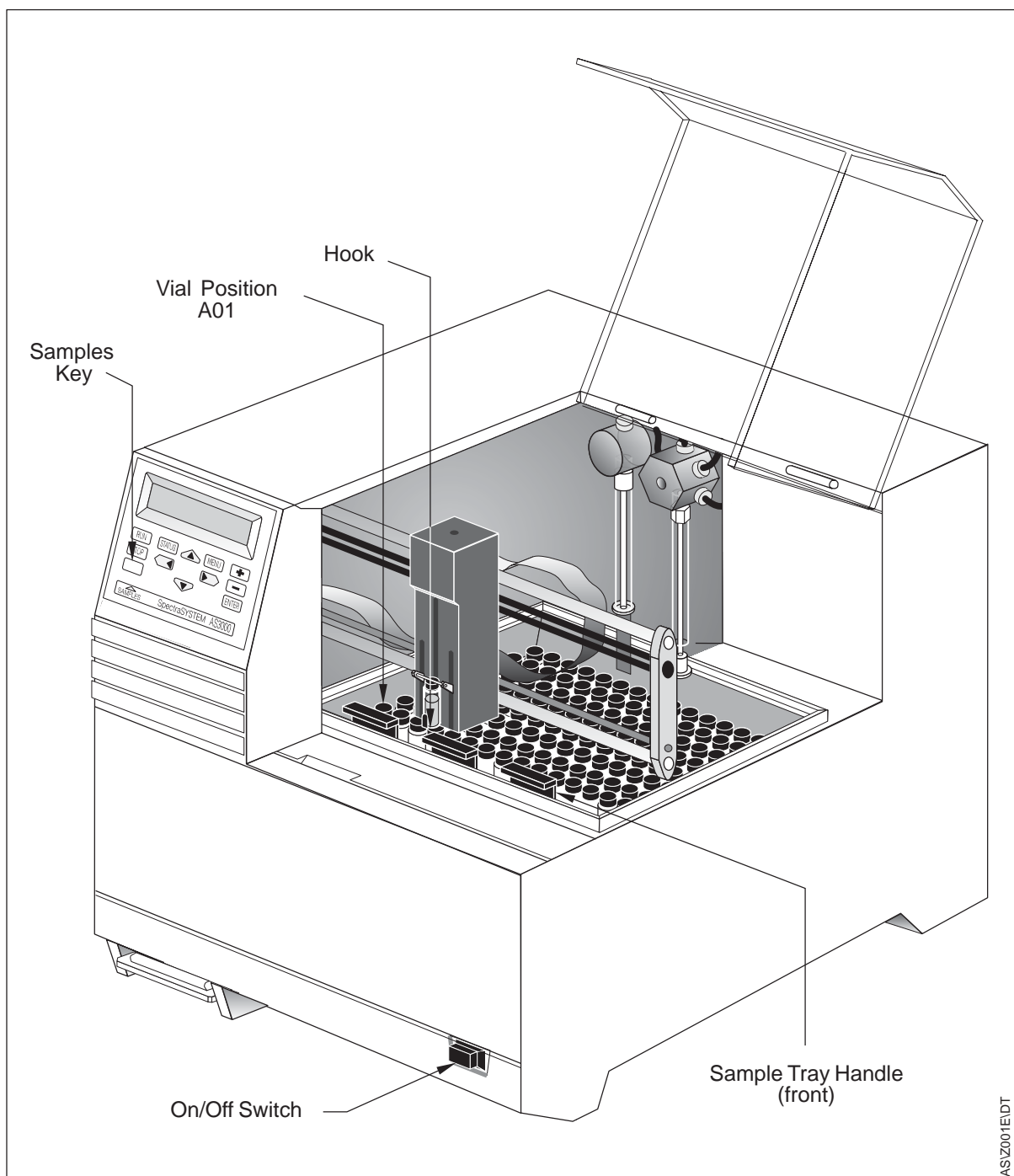
Figure 2.9 Front of the autosampler

**From the Samples
Menu**

To enter your parameters from the Samples Menu:

1. Turn on your autosampler by pressing the on/off switch located in the lower right-hand corner of the front of the autosampler (Figure 2.9).

When the startup sequence is complete, the Status Screen appears (Figure 2.10).

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

Figure 2.10 The Status Screen

2. Press the [SAMPLES] key to display the Samples Menu (Figure 2.11 and Figure 2.12). When the STATUS Menu first appears, the file name field is blank. Figure 2.11 and Figure 2.12 show how this field will appear when a file name is entered.

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

Figure 2.11 The Samples Menu (AS1000 and AS100)

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

Figure 2.12 The Samples Menu (AS3000 and AS300)

3. Use the [∨], [<], [>], [+] and [-] keys to change the Cycle Time to 1.0.
4. Press the [∨] key to move down the Samples Menu and press [ENTER] to select "Yes" to add your sample set (1) to the queue.

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

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

From the Edit Menu

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

Use the following steps to create your autosampler file.

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

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

1. Press [MENU] and select /FILES/, /Edit/ to display the Edit Menu (Figure 2.13). Notice that File 1 is already entered into the Edit File field. Press [ENTER] to edit File 1.

```
>   Edit File                               1_
      File Name: PRACTICE
    ----------------------------------------------------------
      Injection
      Calibration
      Timed Events
      Sample Prep   (Appears only with the Sample Preparation Option installed)
      More
```

Figure 2.13 The Edit Menu

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

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

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

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

LOAD YOUR FILE

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

1. Select /Load/ from the Edit Menu.
2. Verify that File 1 is entered, and press [ENTER] to load it into the autosampler's active memory. The message "**File Loaded**" appears briefly. Then the Status Screen is displayed (Figure 2.14).

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

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

Figure 2.14 The Queue Status Screen with the queue idle

BUILD YOUR QUEUE

Build your sample queue by adding Sample Set 1 (that contains your practice sample) to the sample queue as follows:

1. Press [SAMPLES] to display the Samples Menu (Figure 2.11 and Figure 2.12).
2. Press [ENTER] in each field to accept the Samples Menu's default values.

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

START YOUR RUN

To start your run, press [RUN]. If everything's running properly, the Status Screen's QStatus field will read "RUN" (Figure 2.15). The injection status field displays the time elapsed since the run was started (Figure 2.16).

```
 QStatus         Set     #Samples
  RUN             1        1 / 1
```

Figure 2.15 The Queue Status Screen with the queue running

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

Figure 2.16 The Injector Status Screen with a run in progress

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

3
Basic Operations

Introduction

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

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

There are four options available for SpectraSYSTEM autosamplers:

- Column oven (standard or narrow-bore configuration)
- Tray temperature control (AS3000 and AS3500 only)
- Sample preparation (AS3000 and AS3500 only)
- BCD communication

The menus and fields used to control these options appear only if they are installed. This chapter includes the column oven and tray temperature control procedures. Chapter 4, Advanced Operations, describes the sample preparation menus (templates) and procedures. If you purchased the Sample Preparation Option, you'll first want to learn the procedures described in this chapter and then proceed to Chapter 4 to learn how to use the sample preparation templates and the heater/mixer. Refer to Chapter 1 and contact your representative for information about the BCD option since this option requires specific installation procedures.

BEFORE YOU START

Before beginning this chapter, be sure that you've completed the Startup Checklist found in the beginning of this manual and installed your instrument according to the procedures described in Chapter 1.

Theory of Operation

**BASIC
AUTOSAMPLER**

The autosampler consists of a number of subsystems. It is important for you to be familiar with how these subsystems contribute to the instrument's operation in order for you to be able to trace a problem or symptom to its probable cause.

The basic autosampler consists of the following components: 1) flush valve, 2) small (250 µL) syringe, 3) injector valve, 4) sample loop, 5) needle and hook, 6) flush funnel, 7) pump output line, 8) column input line, and 9) sample vial. Figure 3.1 illustrates the autosampler's major components without the Sample Preparation Option. Figure 3.2 illustrates the major components with sample preparation installed.

Vials filled with samples to be analyzed are loaded into three trays that hold up to 40 vials each (35 with the Tray Temperature Control Option installed). During operation, a mechanical arm (the XYZ arm) locates a sample vial. The hook on the end of the arm moves under the selected vial and lifts it from the sample tray. The XYZ arm transports the vial to the sample tower and the sample needle for sample injection. The injection valve switches between the Inject and Fill positions (Figure 3.3) as necessary to draw sample or solvent into the autosampler lines and to inject sample onto the column. All of the SpectraSYSTEM autosamplers perform full-loop injection. The AS300, AS3000, and AS3500 offer both pull-loop injection and Thermo Electron's PushLoop™ injection modes. The injection routines are described here.

Full-loop injection

In full-loop injection, an air bubble is drawn into the needle to isolate the incoming sample slug from the flush solvent already present in the lines (Figure 3.4). With the injector valve in the FILL position, the syringe retracts to pull the sample from the vial into the sample loop. The injector valve rotates to the INJECT position, and the pump pushes the sample from the sample loop onto the column.

Pull-loop injection

The pull-loop injection routine is useful when you have a limited amount of sample. Only the desired sample amount is pulled from the vial into the sample loop. Specifically, an air bubble is drawn into the needle to isolate the incoming sample slug from the flush solvent already present in the lines (Figure 3.5). With the injector valve in the FILL position, sample is drawn into the needle. The sample vial

is lowered from the end of the needle and sufficient air drawn into the needle to pull the sample slug into the sample loop. The injector valve then rotates to the INJECT position and sample is expelled from the sample loop onto the column.
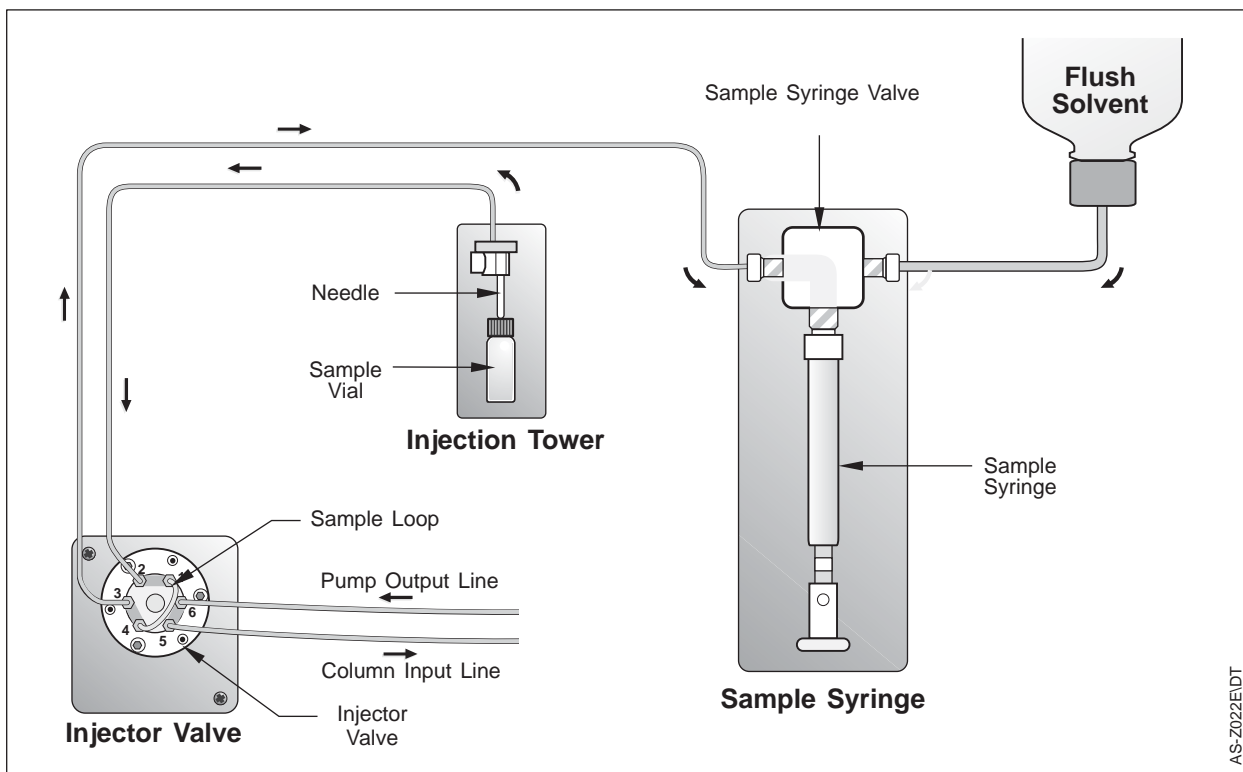
Figure 3.1 The autosampler's major components without the Sample Preparation Option
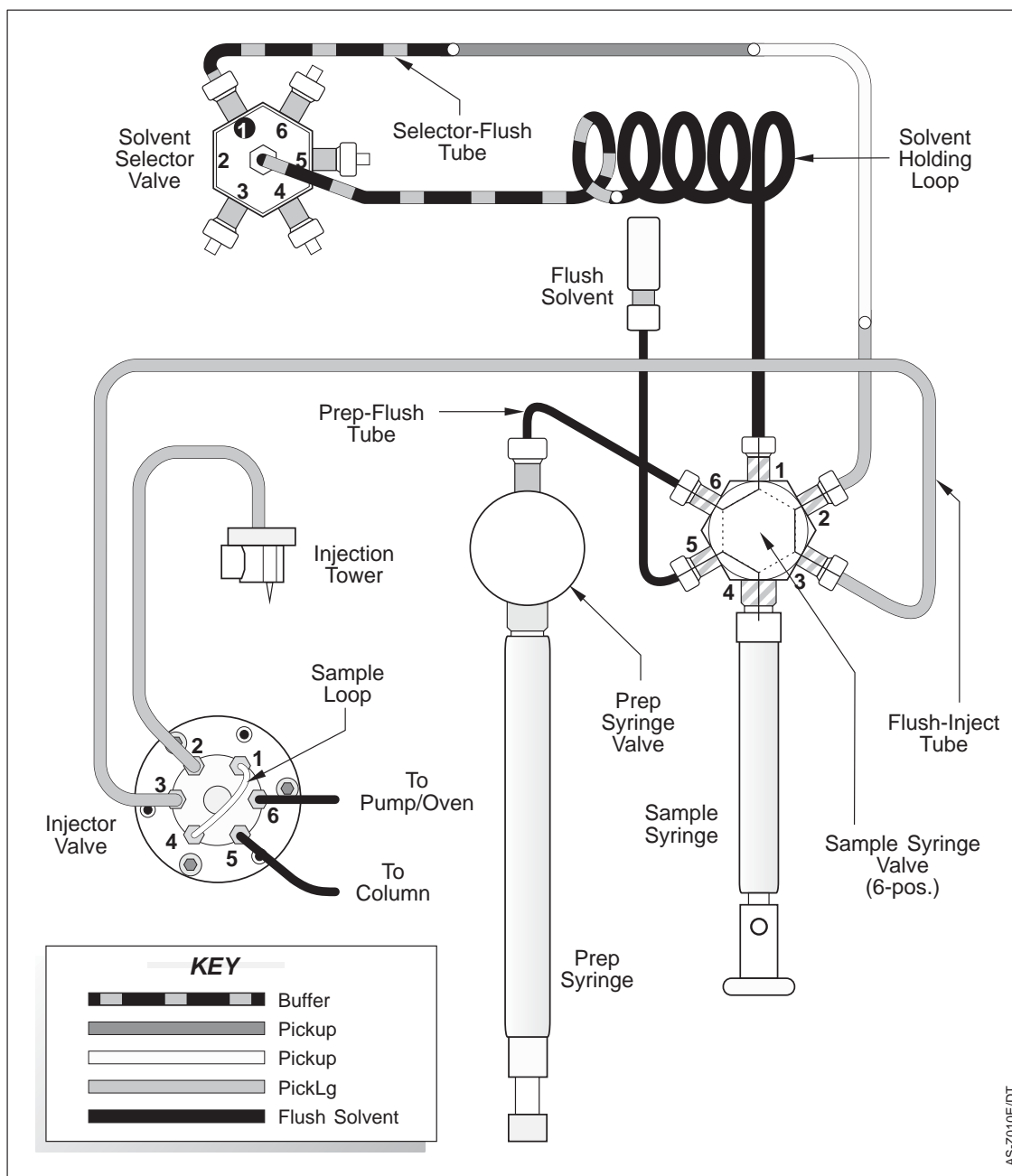
Figure 3.2 The autosampler's major components with the Sample Preparation Option installed

Figure 3.3 The injector valve's inject and fill positions

Figure 3.4 Full-loop injection just prior to sample injection
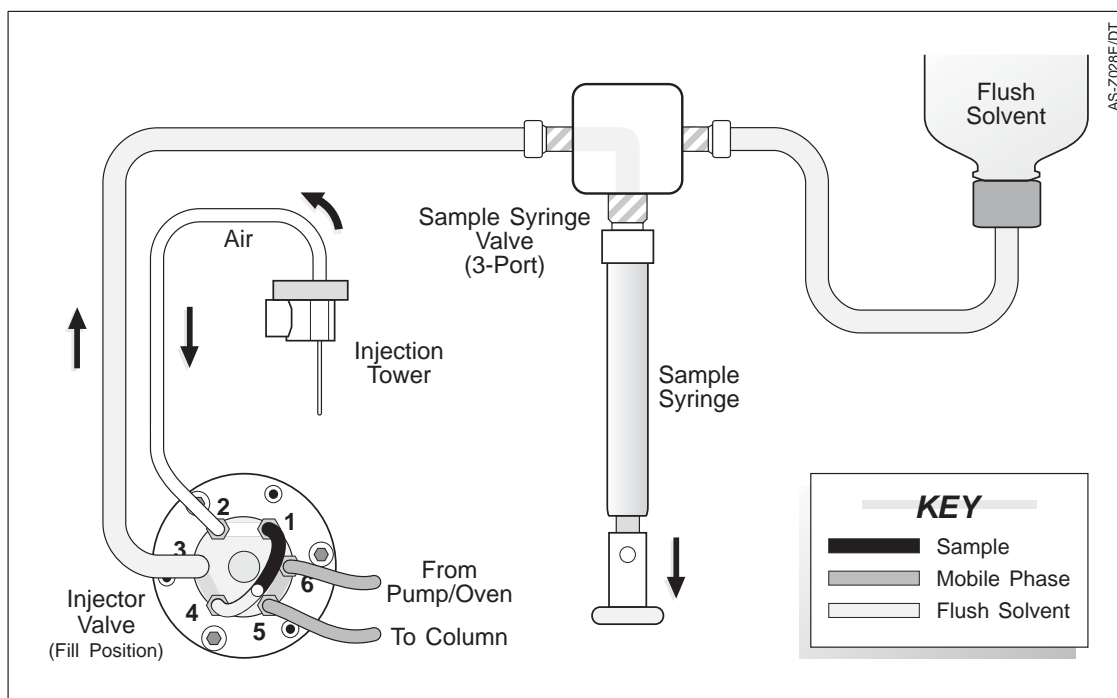
Figure 3.5 Pull-loop injection sequence

PushLoop Injection

The concept behind PushLoop injection is that it is more accurate to push a column of fluid than it is to pull it. Pulling liquids through a small tube can cause "cavitation" within the tubing. Cavitation is the outgassing of dissolved gases caused by reducing the pressure on a column of liquid. This forms bubbles within the column of liquid, resulting in inaccuracies and nonreproducible volumes.

With PushLoop injection, sample volume is separated from the previous flush solvent by an air bubble. With the valve in the Inject position, the sample slug is pulled up to and past the injection valve. After compensating for the syringe backlash, the valve is switched to the Fill position and the desired volume of sample is pushed into the sample loop. In PushLoop injection, sample volumes are "pushed" into the sample loop from solution directly adjacent to the loop. The sample solution is essentially at the "bottom" of the column of liquid. Any residue from the previous solvent has been minimized. The starting boundary of the delivered sample slug is well defined by the valve. There is no uncertainty about the air bubble or solvent droplets that may affect accuracy or reproducibility.   Finally, the injector valve is then switched back to the Inject position, and sample is expelled from the sample loop onto the column. Figure 3.6, Figure 3.7, and Figure 3.8 illustrate this routine.

Thermo Electron
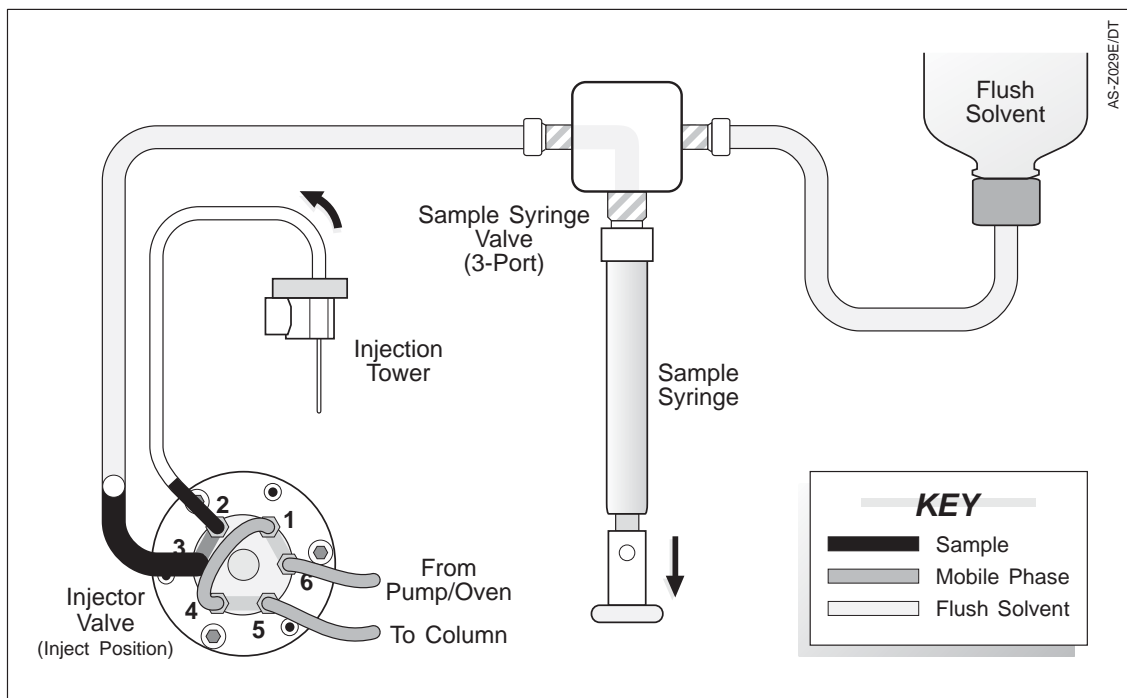
Figure 3.6 PushLoop injection
(step 1: The sample slug is pulled up to and past the injection valve.)
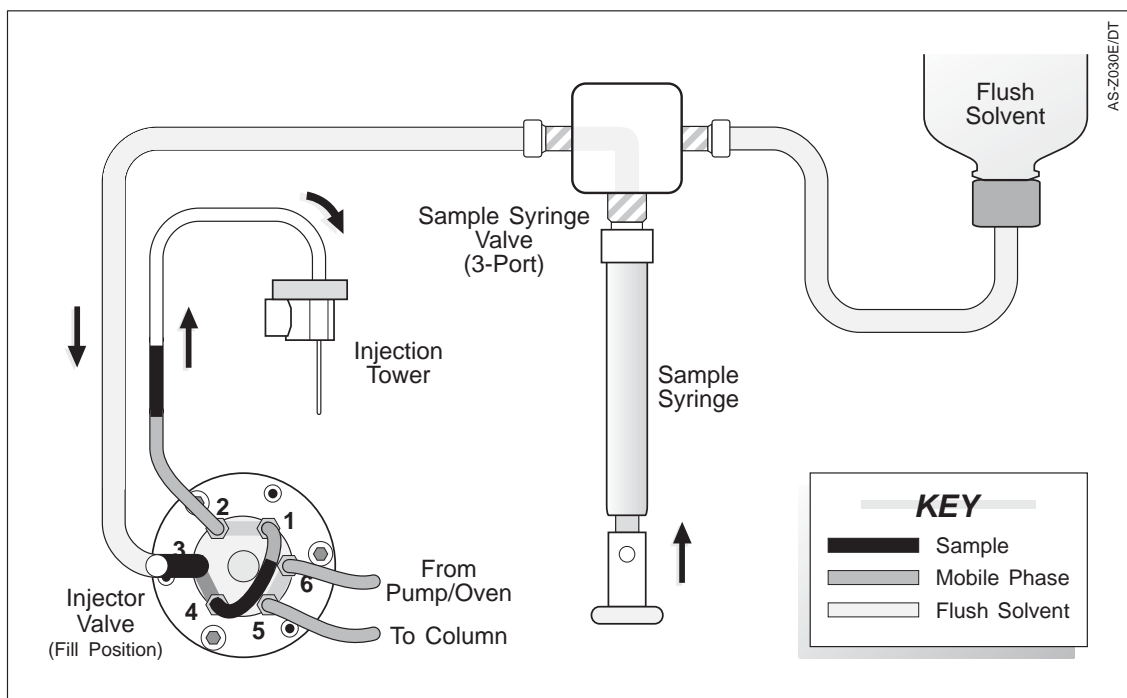
Figure 3.7 PushLoop injection
(step 2: The injection valve is switched to the Fill position, and the desired volume of
sample is pushed into the sample loop.)
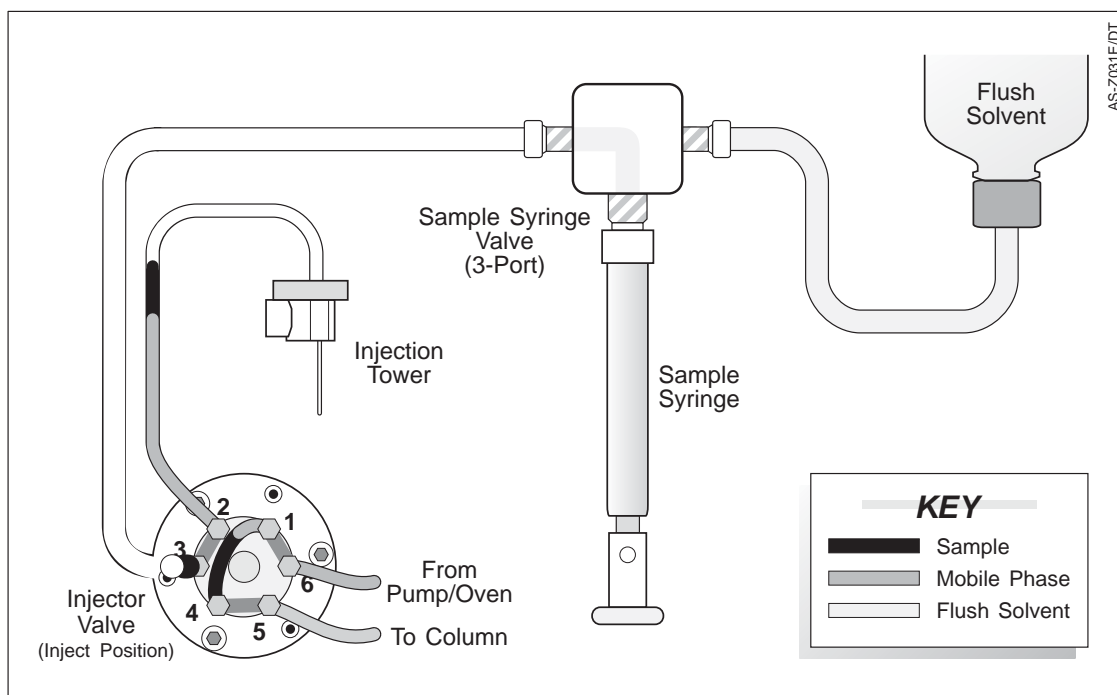
Figure 3.8 PushLoop injection
(step 3: The injection valve is switched back to the Inject position, and sample
is expelled from the sample loop onto the column.)

SpectraSYSTEM Autosamplers

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

AS1000

The AS1000 fixed-loop autosampler can function as a stand-alone HPLC module or through the COMM. port as part of Thermo Electron's SpectraSYSTEM. In this configuration, all HPLC modules are controlled by a system controller via SpectraNET™ communication. This instrument offers additional optimization capability: it holds four user-programmable built-in files and the sample queue can process up to 39 sample sets plus one priority sample set.

AS3000

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

AS3500

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

AS100

The AS100 is a fixed-loop autosampler that comes with a 20 μL sample loop and a built-in file that allows you to quickly and easily enter and modify your run parameters. The sample queue holds up to 39 sample sets plus one priority set that can be run automatically.

AS300

The AS300 is a variable-volume injection autosampler. This instrument offers three injection methods: 1) full-loop, 2) traditional pull-loop where sample conservation is of primary concern, and 3) Thermo Electron's patented PushLoop injection. The AS300 is shipped with a 100 μL sample loop and built-in customizable file. The AS300 sample queue can hold and process up to 39 sample sets plus an additional priority set.

OPTIONS

Column Oven

The column oven contains a resistive thermal device that heats up to stabilize the column temperature. A temperature sensor detects the temperature and sends the temperature reading back to the microprocessor, which turns the resistive device on or off to regulate the temperature. This is a closed assembly and requires no routine maintenance.

**Tray Temperature
Control**

The tray temperature control unit consists of four Peltier devices, two high-speed fans, and a heat sink. Heat is pumped from the sample compartment into the heat sink and then blown out the back of the instrument by the two fans. A temperature sensor sends the temperature reading back to the microprocessor, which controls the operation of the four Peltier devices to regulate the sample compartment temperature. Similar to the column oven, the tray temperature control unit is a closed assembly and requires no routine maintenance.

Sample Preparation

The sample preparation hardware consists of the solvent selection system and the heater/vortex mixer. (Refer to Chapter 4 for information on the sample preparation software.)

The solvent selection system is located at the back of the instrument and includes the sample preparation syringe(s) and the solvent selection valve.

The heater/mixer or vortex mixer, located behind the sample tower, uses compressed air to spin the sample vial creating the vortex inside the vial that mixes the vial's contents. The microprocessor clock tracks the run time and turns the mixer on and off at the desired times. A resistive thermal device (heater) and a temperature sensor, similar to the column oven, maintain the desired temperature during the vortex-mixing process.

Run Preparation

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

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

Once your run has started, you can:

- Monitor the progress of the run

- Modify the currently running file (the run file) to affect subsequent vials in the active sample set

- Change the run order of, add, or delete sample sets from the sample queue

- Add a priority sample set to the queue without disrupting the calibration routine of the active sample set

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

PREPARE YOUR CHEMICALS

Prepare your samples, solvents, and vials. Be sure that your sample(s) is (are) completely soluble in the mobile phase and that you have filtered your samples and solvents through a 0.45-micron filter. These techniques minimize sample precipitation in the lines and remove any particulate matter that could obstruct the flow through the autosampler's injector or column. Fill the solvent reservoir with flush solvent.

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

CREATE YOUR FILE

The group of autosampler parameters applied to a specific sample set is contained in a file. When your AS1000, AS3000, or AS3500 is shipped, each of its four built-in files contains the default values. The AS100 and AS300 have only a single method file. To create your own file, simply edit one of the existing files by entering your own parameters into the autosampler's Edit Menu. From the Edit Menu you can name your file and access additional menus to enter your injection, calibration, timed events, sample preparation, and other run parameters.

NOTE: When you have entered all of your parameters in the Edit Menu, you'll have finished creating your edit file. You can't run your edit file by pressing the [RUN] key. Instead, you first have to load your edit file into the run file. For more information, see "Load your file" on page 77.

To create your edit file:

1. Press the [MENU] key to display the Main Menu. From there, select /FILES/, /Edit/ to display the Edit Menu (Figure 3.9).

```
   Edit File      1
   File Name      VITAMINS
------------------------------------------------------------
   Injection
   Calibration
   Timed Events
   Sample Prep  (If installed)
   More
```

Figure 3.9 The Edit Menu

Edit File

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

File Name

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

Injection Menu

Selecting /Injection/ from the Edit Menu accesses the Injection Menu (Figure 3.10 and Figure 3.11) from which you can enter your injection parameters. The next section describes the Injection Menu's fields.

```
   Loop Size                         10.0
   Injections/Sample                    1
------------------------------------------------------------
   Cycle Time                        10.0
   Col Oven: OFF_  Temp 18      (If installed)
   TrayTemp:  OFF   Temp  0      (If installed)
```

Figure 3.10 The Injection Menu (AS1000 and AS100)

```
   Injection Volume                  10.0
   Injections/Sample                    1
------------------------------------------------------------
   Cycle Time                        10.0
   Oven:      OFF_  Temp 18      (If installed)
   TrayTemp:  OFF   Temp  0      (If installed)
```

Figure 3.11 The Injection Menu (AS3000 and AS300)

Injection Volume (Loop Size)

Use the first field of the Injection Menu to specify the sample Loop Size (if you are using an AS1000 or AS100) or the sample injection volume (if you are using an AS3000, AS3500, or AS300). The injection volume value depends upon the injection method and range that you entered in the More Menu's Injection Type and Injection Range fields, respectively, (see page 74). To optimize injection volume, see page 94.

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

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

Variable-Loop Injection: You can choose either Pull or PushLoop injection mode from the More Menu. These methods are described on page 75. If you use pull-loop injection, you can select a sample-injection volume from 1-1500 µL. If you use PushLoop injection, you can specify a sample-injection volume from either 0.1-10.0 µL or 1-200, depending upon which Push type (Hi or Lo) you selected in the More Menu's Injection Type field. You should limit the partial injection volume to approximately one-half the sample loop size.

Injections/Sample

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

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

Cycle Time

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

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

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

Column Oven

This field appears only if you purchased the Column Oven Option and set it to ON in your Configurations Menu. To use the column oven, move the cursor to the Col Oven field and press the [+] and [-] keys to toggle the oven's state between on and off. In the Temp field in the same line, you can specify the oven temperature in one-degree increments from 15 - 80°C.

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

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

HINT: The autosampler automatically delays the start of the run until the set temperatures have been reached. Once activated, the oven requires about 15 minutes to reach a temperature of 70°C. To save time, you may wish to load your file so that the oven can be warming up while you're completing your run preparation and entering the file parameters for your run. See page 77 for more information on loading your file.

NOTE: The vial temperature will typically reach 4°C with a set temperature at 0°C and 58°C with a set temperature at 60°C.

Tray Temperature Control

This option allows you to heat or cool the sample tray within a range of 0°C to 60°C (in 1° increments). This field appears only if you purchased the tray temperature control option and specified Yes in the Configurations Menu Tray Temp field. If you plan to use tray temperature control, use the [+] and [-] keys to turn on the tray heater/cooler in the Tray field. Then specify the temperature in its Temp field. If set to "On" in the Injection Menu, the heater/cooler will not be activated until you load the file manually (by pressing Load from the Files Menu), or automatically when you press [RUN] to start your queue.

HINT: The autosampler automatically delays the start of the run until the set temperatures have been reached. Once activated, if empty, the tray requires about 30 minutes to reach a temperature of 0 °C and about 10 minutes to reach a temperature of 60 °C. To save time, you may wish to load your file so that the heater/cooler can be coming to temperature while you are completing your run preparation. See page 77 for more information on loading your file. You can also save time by preheating or precooling your samples.

The autosampler's Tray Temperature Control Option is primarily designed to maintain the temperature of the sample compartment within ± 2 °C. The actual temperature inside the autosampler vial is dependent upon the specified tray temperature, type of vial, sample buffer, etc. The following graph (Figure 3.12) indicates the approximate vial temperature given a specified tray set temperature.

Vial vs. Tray Temperature
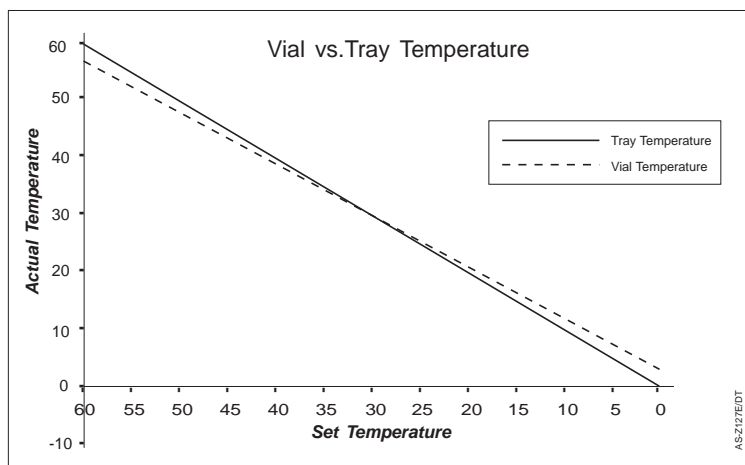
Figure 3.12 Plot showing the actual vial and tray temperature at a specified set temperature

The Tray Temperature Control Option can also be used to heat and cool samples. The time lag in heating and cooling is due to variations in ambient temperatures, indirect contact with temperature control units, and vial type. The accompanying graph (see Figure 3.13) indicates the time required to heat (to 60°C) or cool (to 0°C) the tray and vials from an ambient temperature of 23°C.

Heating and Cooling Times
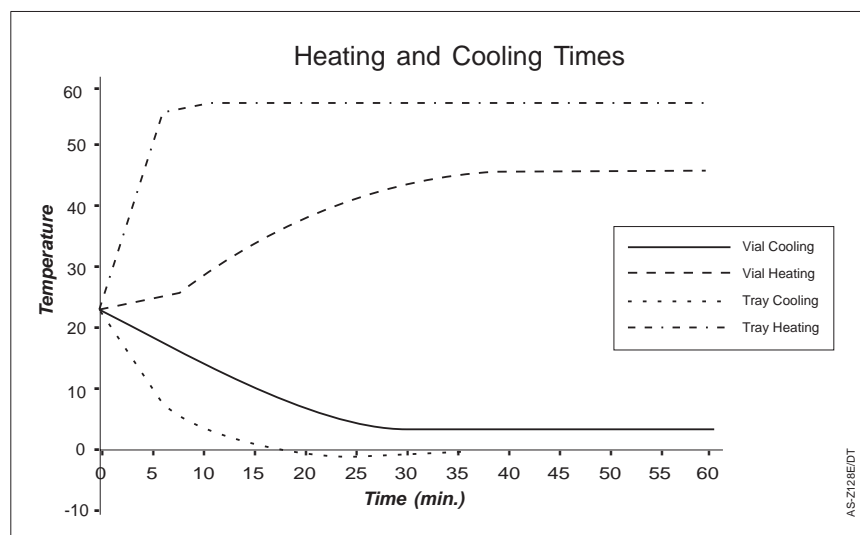
Figure 3.13 Plot of heating and cooling times of a standard glass vial and the autosampler tray. The starting ambient temperature was 23°C

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

Calibration Menu

By selecting Calibration from the Edit Menu, you can call up the Calibration Menu (Figure 3.14). This section tells you how to enter your calibration parameters and how each is used by other autosampler menus.

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

Figure 3.14 The Calibration Menu

Number of Levels
Specify the number of calibration levels (0-9). The autosampler automatically copies this value to the Calib 1Vial 1 (of X) field, in the Samples Menu, where X equals the number of levels. (Refer to page 78 for more information.) A value of zero in the Number of Levels field indicates that no calibration injections will be made for the specified sample set.

Samples/Calibration
From this field you can specify the calibration interval (the number of sample vials to be injected between each recalibration).

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

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

Similar to the Injection Volume field in the Injection Menu, this value depends upon the injection method you select in the More Menu's Injection Type field. As with the sample injection volume, you can choose between 0.1-10.0 µL and 1-200 µL if you are using PushLoop injection. (See page 74.)

NOTE: This field is not displayed if you have selected Full from the More Menu's Injection Type field or if you are using an AS1000 or AS100.

Using variable-volume injection, you can choose either Pull or PushLoop injection mode from the More Menu. These methods are described on page 74. If you use pull-loop injection, you can select a calibration-vial injection volume from 1-200 µL. If you use PushLoop

injection, you can specify a calibration-vial injection volume from 1-10.0 µL or from 1-1500 µL, depending upon which Push type you selected in the Injection Type field. You should limit the injection volume to approximately one-half the sample loop size.

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

NOTE: If you want to determine optimum syringe and sample loop size, see page 94.

Type

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

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

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

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

Intersperse vials programs the autosampler to use each calibration vial only once. You must manually intersperse your calibration vials between your sample vials within the sample set. The autosampler makes injections in tray-placement order. If you've specified a different injection volume for calibration vials versus sample vials, the autosampler uses the Samples/Calibration field value to determine whether the next vial is a calibration or a sample vial. It determines the location of the first calibration vial from the value entered in the Samples Menu's Calib 1(of X) field. Figure 3.15 illustrates the vial order used by the two different calibration routines.

Injection	Reuse	Intersperse Order
C	A01	A01
C	A02	A02
S	A03	A03
S	A04	A04
C	A01	A05
C	A02	A06
S	A05	A07
S	A06	A08
Total	6	8

Figure 3.15 The different injection orders and total number of vials used by the Reuse and Intersperse calibration routines

Bracket Samples

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

Timed Events Menu

Accessed from the Edit Menu, the Timed Events (external events) Menu (Figure 3.16) allows you to create a time program to turn on/off external devices (such as column-switching valves and fraction collectors) at preset times during your run. Pins 9-12 of the autosampler's 12-pin terminal are used for Timed Events. In this section, we'll show you how to create a time program. Refer to Chapter 1 for connecting external devices to your autosampler.

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

Figure 3.16 The Timed Events Menu

The Timed Events Menu holds a maximum of nine lines, each of which sets Hi/Lo one or more timed event outputs at the time specified. For example, in Figure 3.17 through Figure 3.19, Time Function 1 (TF1) is set Hi (activated) at 0.5 minutes into the run; at 2 minutes into the run, Time Function 2 (TF2) is set Hi while Time Function 1 is set Lo (deactivated). By changing the Time value in each line, you can add, delete, or insert menu lines.

Adding a Line

To add a line to the end of your program:

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

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

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

Deleting a Line

To remove a line:

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

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

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

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

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

Inserting a Line

To insert a line between two existing lines:

1. Move the cursor to the blank line at the end of the program.

2. Enter a time that falls between two existing times.

3. Move the cursor off the new line. The autosampler automatically inserts the new line.

4. Then move the cursor to the newly inserted line(s) and set the Timed Events Outputs' states as desired.

Figure 3.17 though Figure 3.19 illustrate this process.

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

Figure 3.17 The original timed events program

```
   Time     TF1     TF2     TF3     TF4
   0.00     Lo      Lo      Lo      Lo
---------------------------------------------------------
   1.00     Lo      Lo      Hi      Lo
   2.00     Lo      Hi      Lo      Lo
   0.50     Lo      Hi      Lo      Lo
```

Figure 3.18 The original program with an added line -- unsorted

```
    Time    TF1      TF2      TF3      TF4
    0.00    Lo       Lo       Lo       Lo
--------------------------------------------------------------
    0.50    Lo       Hi       Lo       Lo
    1.00    Lo       Lo       Hi       Lo
    2.00    Lo       Hi       Lo       Lo
```

Figure 3.19 The timed events program with an added line -- sorted

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

Sample Prep (AS3000/AS3500 only)

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

More Menu

The More Menu (Figure 3.20 and Figure 3.21) allows you to enter the remainder of your run parameters, each of which is discussed below.

```
   Equilibration Time    0.0
   Gradient Delay 0.00
--------------------------------------------------------------
   Viscosity      Normal
   Prep Viscosity Normal
   Flush Volume   400
   Needle Height  2.0
```

Figure 3.20 The More Menu (AS1000 and AS100)

```
   Equilibration Time              0.0
   Gradient Delay                  0.00
--------------------------------------------------------------
   Viscosity                       Normal
   Prep Viscosity                  Normal
   Flush Volume                    400
   Injection Type                  Push
   Injection Range                 .1-10.0
   Needle Height                   2.0
```

Figure 3.21 The More Menu (AS3000 and AS300)

Equilibration Time

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

Gradient Delay

The gradient delay field is used to specify the amount of time required to allow the mobile phase to travel from the pump's proportioning valve to the column. This field is used only if you are running a gradient. To determine the gradient delay value, you'll need to calculate the precolumn volume, which is best done during installation. Refer to Chapter 4 for a procedure for calculating your precolumn volume and gradient delay value.

Viscosity

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

HINT: Use the Normal setting for running most applications.

Prep Viscosity (AS3000/AS3500 only with sample prep)

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

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

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

Flush Volume

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

Injection Type (AS3000, AS3500 and AS300 only)

With the AS3000, you can choose from four different injection methods: 1) full-loop, 2) pull-loop 3) Thermo Finnigan's PushLoop injection using the high (Push Hi) injection volume range (1-200 µL) and 4) PushLoop injection using the low (Push Lo) injection volume range (0.1-10 µL). The advantages of each type are briefly described here. Refer to "Theory of Operation" page 56 for a description of how each method operates and for the excess sample volume required for injection.

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

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

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

Pull: Pull-loop injection (traditional variable-volume injection) is useful when sample conservation is important and when large injection volumes (>100 µL) are required.

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

Injection Range
The Injection Range field displays the sample- and calibration-injection volume range, depending upon the Injection Type that you selected. For full-loop injection, the range is 5-1000 µL. For pull-loop injection, the Injection Range field is automatically filled in with the 1-1500-microliter range. For PushLoop injection, the ranges are 1) 0.1-10.0 microliters (Push Lo) or 2) 1-200 microliters (Push Hi), depending upon your sample-loop size.

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

NOTES: With this value, you are actually controlling the vial's height relative to the needle tip. The vial moves; the needle is stationary. If you are using a standard, 65 mil septa in the vial cap, keep needle height below 50 mm to maximize injection precision.

SAVE YOUR FILE

Your file is saved automatically. Once you have filled in all of the Edit Menu's parameters, you have finished creating your edit file. Exiting the Edit Menu automatically saves your edit file but does not load it. For more about file management, see page 91.

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

LOAD YOUR FILE

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

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

```
   Load File 1:  FILENAME
```

Figure 3.22 Load File field

For AS3000, AS3500, and AS300 only: If you want to load the last file you edited, use the [+] and [-] keys to select its file number and press [ENTER]. If, instead, you want to load a different file, select the correct file number and then press [ENTER]. The message **File Loaded** will be displayed. Once a file is loaded, it becomes your run file.

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

**BUILD YOUR
SAMPLE QUEUE**

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

**Adding Items to the
Sample Queue**

To add an item to the queue, press [SAMPLES] to access the Samples Menu. Then enter each of the values shown in the Samples Menu (Figure 3.23 and Figure 3.24):

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

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

Figure 3.23 The Samples Menu (AS1000 and AS100)

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

Figure 3.24 The Samples Menu (AS3000 and AS300)

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

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

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

*NOTE: The injection volume field does not appear in the AS1000 or
AS100 Samples Menu.*

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

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

First Sample Vial
Specify the first sample-vial location.

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

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

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

Ordering Sets in the Sample Queue

You add items into the queue from the Samples Menu, which you access by pressing [Samples]. You can reorder them in the Queue Menu (Figure 3.25). You can reorder any sets not currently running.

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

Figure 3.25 The Queue Menu with no priority-vial sample sets in the queue

Changing the Order of Sets Within the Queue

To change the order of items in the queue, move the cursor to the line where the change in order should occur and increment/decrement to the new Order value. Refer to the example in Figure 3.26. To run sample set 5 before sample set 3, move the cursor to the order column of the sample-set 5 line, and decrement the Order value to 1 (Figure 3.26).

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

Figure 3.26 Changing the order of sets in the queue

The lines rearrange themselves after you exit the field (Figure 3.27).

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

Figure 3.27 The Queue Menu after the Order has been changed

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

**Deleting Sets
from the Queue**

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

**Managing a
Priority Set
Within the Queue**

A priority set is inserted into the active set and its samples run before other samples in the active set. It must be assigned to the active file. It appears in the Queue Menu with a P in the Set field (Figure 3.28). You can't assign or change its priority status from the Queue Menu. A priority set will automatically be deleted from the queue upon completion of the active set or when the queue (QStatus) returns to its IDLE state (if you press [STOP]). For more information on running priority vials, see page 88.

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

Figure 3.28 A sample queue that contains a priority set

**ENTER YOUR
OTHER LC
PARAMETERS**

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

- Initialize your pump and degas your solvents as required for your application.

- Be sure your detector is stabilized. Don't start your analysis until you've achieved a stable baseline.

- Set up your data-output device.

For more information on connecting your autosampler to other LC modules, see Chapter 1.

**LOAD
SAMPLE TRAYS**

Place your calibration and sample vials into the sample trays included in your Accessory Kit. Trays A, B, and C are arranged in the autosampler from left to right. Each tray contains 40 (35, with Tray Temperature Control) vials arranged in eight (seven) rows of five (Figure 3.29). The first vial position is A01; the last is C40. The position labels are on the <u>left</u> of each position.

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

NOTE: Be sure to use a 40-60 mil (1.0-1.5 mm) rubber or silicone septum with a 5 mil (0.1 mm) Teflon® liner on one side.
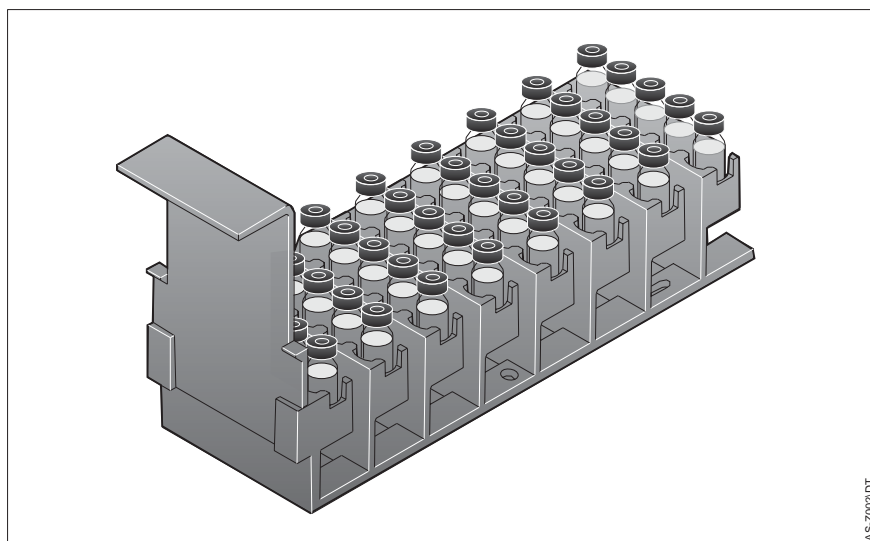
Figure 3.29 An autosampler tray

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

1. Hold the tray handle, tilting the back end down (Figure 3.30).

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

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

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

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

NOTE: Be sure that each tray is properly seated. Each tray's upper front edge should fit under the upper lip of the tub. If a tray is not completely seated, the autosampler will not be able to correctly pick up or return vials to that sample tray.
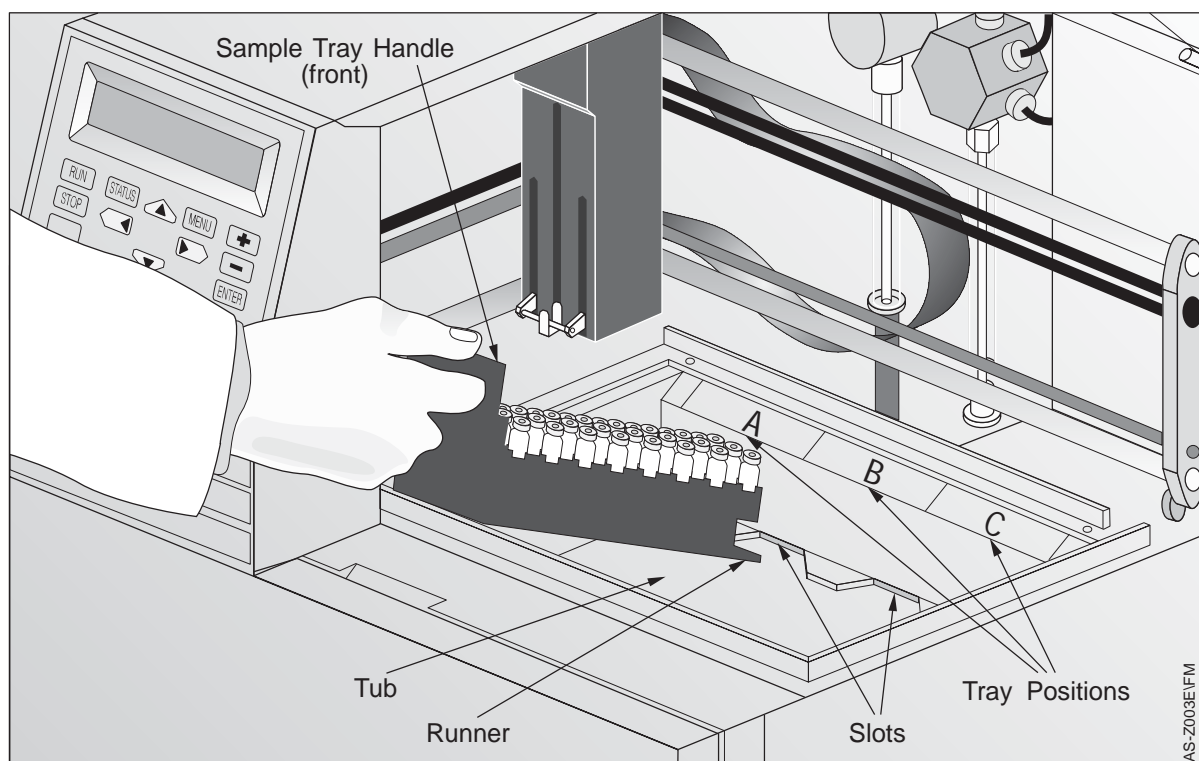
Figure 3.30 Installing a sample vial tray

Starting Your Run

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

Press [RUN] to start your run. If there are sample sets in the sample queue, the sample queue's "RUN" Status is displayed on the Status Screen (Figure 3.31).

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

MONITORING THE RUN STATUS

When [RUN] is pressed, the autosampler begins processing vials once the oven, heater/mixer and/or tray set temperatures have been reached, the equilibration delay time (entered in the More Menu) has elapsed, the door is closed, and the pump is ready. During the run, the Status Screen is displayed (Figure 3.31).

NOTE: You can change the "Ready Requirements" from the Options, Ready Participation Menu. See "Ready Participation" on page 31 in Chapter 1.

```
   QStatus        Set    #Samples
   RUN            1      1 /  1           ♦
---------------------------------------------------------
   Status  Vial   Vol    #Inj
   0.10    A01-S   1000   1 / 2
   File 1:
   Oven           TrayTemp
   ON:80          ON:4
```

Figure 3.31 The Status Screen

The status screen gives you four kinds of information (described below):

- Autosampler queue status
- Injection status
- Oven and tray temperatures
- Sample preparation status (if the Sample Preparation Option is installed)

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

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

Sample Queue information

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

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

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

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

PAUSE indicates that a sample set is loaded, but that processing has been suspended. If the queue is paused (the [STOP] key has been pressed once), you can either resume processing with the current or subsequent vial, or abort processing of the current sample set. For more information, see "Stopping a Run" on page 89.

RUN indicates that a sample set in the queue is being processed. queue (see also Sample queue)states ran

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

ABORT indicates that the processing of the current sample set has been stopped (the [STOP] key has been pressed and ABORT has been selected from the Abort/Continue Menu) or that a system error has occurred. Abort is displayed until the autosampler has returned the active vial to the sample tray and flushed all the lines. For more information about Abort, Continue, and Pause, see "Stopping a Run" on page 89.

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

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

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

Injection Status

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

NOTE: The Status Screen is updated every two seconds.

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

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

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

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

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

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

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

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

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

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

File
Pressing [∨] displays the number and name of the file assigned to the sample set currently being processed.

Temperature Status
Pressing [∨] accesses Oven/Tray Temperature Status screen which displays the actual—not the set temperatures of the oven and tray, if the Column Oven and Tray Temperature Control Options are installed. The temperatures are displayed even if these options are turned off (not set). "OFF" indicates that an option is installed but not turned on in the current file's Injection Menu (Figure 3.32).

```
   Oven    TrayTemp
   OFF:75  RDY:18
```

Figure 3.32 The Temperature Status screen with the oven turned off

The temperature field remains blank if one of these options is not present (Figure 3.33). This screen does not appear if neither option is installed.

```
   Oven    TrayTemp
   RDY:75
```

Figure 3.33 The Temperature status screen without the Tray Temperature Control Option installed

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

Changing the Run in Progress

Once a run has started, you may wish to change the injection volume, cycle time, calibration interval, or set assignments. You make changes to the run currently in progress (the run file) from the Samples Menu. Figure 3.24 and Figure 3.35 list the changeable parameters in bold-face type and indicates when those changes take effect. With the exception of the Cycle Time and Calib 1(of X) values, changes made from the Samples Menu take effect on the next sample injection. Changes to the Cycle Time take effect on the current sample injection; changes to the Calib 1(of X) field take effect on the next recalibration. Samples Menu changes remain in effect until the next sample set.

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

Figure 3.34 The Samples Menu (AS1000 and AS100)

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

Figure 3.35 The Samples Menu (AS3000 and AS300)

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

Priority sample sets

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

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

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

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

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

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

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

To insert a priority sample set into the sample queue, use the following steps (Figure 3.36):

1. Press [SAMPLES].

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

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

4. Add the priority set to the queue.

```
Sample Set 1:                               P
   File 1: VITAMINS
---------------------------------------------------------
   Injection Volume                        10
   Injections/Sample                        1
   Cycle Time                              10
   First Sample Vial                       C23
   Number of Samples                        1
   Add to Queue?                           Yes
```

Figure 3.36 The Samples Menu with a priority sample set

NOTE: The door is equipped with a safety interlock that automatically moves the autosampler's arm to the "home" position to allow you to add samples to the tray during a run. Once the door is closed, the arm returns to its position and resumes the operation in progress before the door was opened. However, you can disable the door interlock if you wish. Press [MENU], /OPTIONS/, Ready Participation, move to Door Interlock (page 31), and use the [+] and [-] keys to select DISABLE.

The autosampler assigns a P (priority) order to the priority set as shown in the Queue Menu (Figure 3.37):

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

Figure 3.37 The Queue Menu with one priority sample set

STOPPING A RUN

The [STOP] key has several functions depending upon the instrument's status when the [STOP] key is pressed. Refer to Figure 3.38 as you read about the STOP/ABORT sequence.
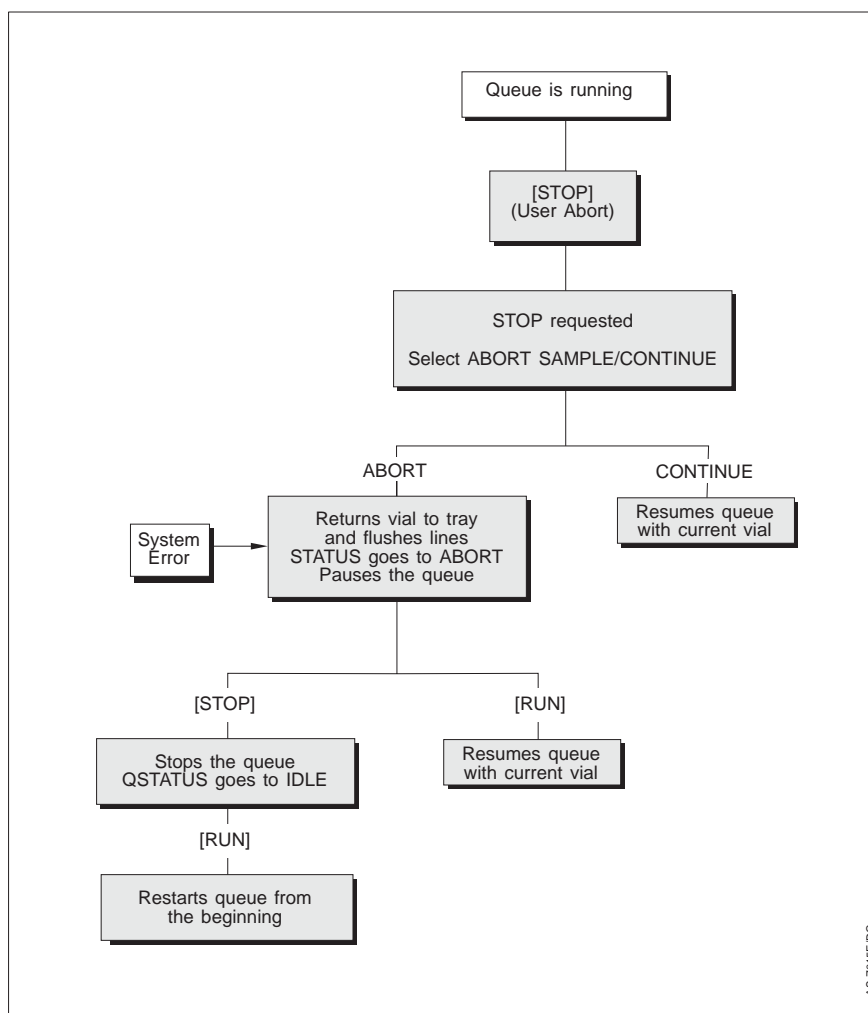
Figure 3.38 The Queue Stop/Abort Sequence

If the queue is in RUN when you press the [STOP] key, the autosampler suspends its current operation and waits for you to make a selection to ABORT (Figure 3.39) or CONTINUE (Figure 3.40):

```
Stop requested.
   Select               ABORT Sample
```

Figure 3.39 The Abort prompt

```
Stop requested.
   Select                   CONTINUE
```

Figure 3.40 The Continue prompt

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

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

- Aborts the processing of the current sample

- Returns the active vial to the vial tray

- Flushes the lines

- Puts the queue in the PAUSE state (Figure 3.41)

```
   QStatus        Set    #Samples
   PAUSE          1      1 /  1          ♦
```

Figure 3.41 The Queue Status line with the autosampler in the PAUSE state

When the queue is PAUSED, you can either:

1. Press [RUN] to resume processing of the current vial or

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

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

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

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

File Management

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

SAVING FILES

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

COPYING FILES (AS1000, AS3000, AND AS3500 ONLY)

To copy a file, press [MENU], and select /FILES/, /Copy/ to display the Copy Menu (Figure 3.42).

```
   Copy File      1:
   To File  2:
```

Figure 3.42 The Copy Menu

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

DELETING FILES

Deleting a file restores all parameters to their default values. To delete a file, press [MENU], select /FILES/, and /Delete/. The Delete Menu appears (Figure 3.43).

```
   Delete File    1:
```

Figure 3.43 The Delete Menu

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

**PROTECTING FILES
(AS1000, AS3000,
AND AS3500 ONLY)**

You can protect a file from being edited or deleted by using the file protection feature in the OPTIONS Menu. Because this feature enables you to control the changes to an original run file, you can easily incorporate your autosampler files into your standard good-laboratory-practice (GLP) protocols. To display the Configurations Menu (Figure 3.44), press [MENU], select /OPTIONS/, /Configuration/.

```
  Sample Syringe        250
  Prep Installed Yes
-------------------------------------------------------
  Oven Installed Yes
  TrayTemp Installed    Yes
  Key Repeat Rate        Medium
  File Name      Protect
  1: VITAMINS    On
  2: BARBITUA    Off
  3: EXPER 3     Off
  4: STAT Off
  Mode    Stand Alone
  Solv Viscosity Normal
```

Figure 3.44 The Configurations Menu

To protect a file, move the cursor down to the Protect field in the line that contains the file to be secured and press the [+]/[-] keys to turn ON the protect function. For example, File 1 VITAMINS in Figure 3.45 cannot be edited until the protect function is turned off. Attempting to edit a protected file displays the following message:

```
*File Protected*
No Editing Allowed
```

Figure 3.45 The confirmation message to prevent editing of a protected file

Changes to real-time editing

If a file is protected, you will not be able to change the injection volume, number of injections per vial, or cycle time for the run in progress. These lines will not appear on the Samples Menu (Figure 3.46).

```
Sample Set 1
File 1:   VITAMINS
First Sample Vial        A01
Number of Samples 10
Set Already in Queue
```

Figure 3.46 The Samples Menu for a protected file

Needle Height

For some applications, such as liquid-liquid extractions and low-injection volumes, you may need to change or optimize the "needle height." On SpectraSYSTEM autosamplers, the needle doesn't move. Instead, the bottom of the vial moves relative to the needle tip. The "needle height" is the approximate distance between the needle tip and the bottom of the vial (0 - 20 mm).

The More Menu's default setting of 2 mm (Figure 3.47) is sufficient for most applications and accommodates a wide variety of vials. However, in cases where you have very limited sample volumes, further optimization may be required.

NOTE: The thickness of the bottom of the vial varies considerably between vial types (1.8 mL vials, microvials, and vials with micro-inserts, etc.) and materials (glass, plastic, etc.).

```
  Equilibration Time              0.0
  Gradient Delay                  0.00
----------------------------------------------------------
  Viscosity                     Medium
  Flush Volume                   250.0
  Injection Type                  Push
  Injection Range               1-1500
  Needle Height                    2.0
```

Figure 3.47 The More Menu (Needle Height)

NOTE: Operating the autosampler with a needle height different from the default height may bend the needle. Do not adjust the needle height without a spare needle available.

Use the following procedure to optimize the needle height for your sample vials:

1. Place 200 - 300 µL water in a vial in position A01.

2. Create an autosampler file that will make two 200 µL injections (see Chapter 3 for more information).

   a. Set the Injection Menu's Injection Volume field equal to 200.

   b. Set the Injection Menu's Injections/Sample to 2.

3. Run the file and observe the amount of fluid remaining in the vial. The fluid height indicates the position of the needle tip from the vial's bottom.

4. Adjust the More Menu's Needle Height value until the needle tip is located approximately 1 - 2 mm from the bottom of the vial.

5. Repeat steps 1 - 4 to confirm the needle height.

Optimization

Injection Volume

This section describes how to select the sample loop and syringe size to obtain maximum reproducibility when performing large-volume injections.

The maximum injection volume depends upon:

- The volume of the syringe currently installed as designated under /OPTION/Configuration/Syringe Size/;

- The Injection Mode selected under /FILE/More/Injection Type; and

- The volume of the installed sample loop.

By changing the sample syringe and/or the sample loop, you can set up your autosampler to deliver injection volumes larger than can be delivered by the 250 µL syringe shipped with the instrument. Use the following steps to prepare your instrument for large-volume injections:

1. Determine the correct sample loop size for the injection volume desired. (See Table 3.1.)

2. Use the injection volume and algorithms outlined below to determine the required syringe size.

   The following algorithms define the amount of sample needed for your injection volume:

   PushLoop   (Injection Volume + 15 µL)
   Pull Loop   (Injection Volume + 1.1 µL)
   Full Loop   (Injection Volume x 1.33) + 70 µL

3. Change your sample loop and syringe. (Refer to Chapter 5 for instructions.)

4. Enter the syringe size in the /FILE/Configuration/Syringe Size field.

5. Enter the Injection Mode in the /OPTIONS/More/Injection Type/ field.

6. Enter your injection volume in the Injection Menu.

NOTE: If the Full-loop mode is chosen, the maximum value depends only on Loop Size (assuming that the syringe size is larger than the loop size). (Loops come only in the sizes included in the Parts and Accessories list located at the front of your Reference Manual).

NOTE: For Pull and PushLoop injection, to ensure maximum reproducibility, we recommend that you do not inject more than half of your loop volume.

Table 3.1 Recommended Injection Size For Selected Sample Loop Sizes[*]

Sample Loop Size (µL)	Injection Volume (in µL)		
	Injection Mode		
	PushLoop	Pull	Full
5	2.5	2.5	5
10	5.0	5	10
20	10	10	20
50	25	25	50
100	50	50	100
200	100	100	200
500	200	250	500
1000	200	500	1000
These recommended volumes will provide the best chromatographic performance. Maximum injection sizes will be determined by the particular system being used.			

The syringe size needed will be the total volume determined by these algorithms. For example, for a 500 µL full-loop injection (using a 1000 µL loop), the Total Volume (TV) needed is

TV = (500 µL x 1.33) + 70 µL
TV = 735 µL

Therefore you will need a 1000 µL syringe since there are no 735 µL syringes available. (See Table 3.2.)

Table 3.2 Maximum Injection Size for Syringe Size

Injection Type	Syringe Size (in µL)				
	250	500	1000	2500	5000
Push Hi	200	200	200	200	200
Pull	212	461	956	1500	1500
Full	100	200	500	1000	1000

SOLVENT VISCOSITY

You can adjust the syringe speed (draw rate) for your solvent viscosity from the Solv Viscosity field in the Configurations Menu (Figure 3.48).

```
   Sample Syringe Size    250
   Prep Syringe Size      2500
--------------------------------------------------------
   Prep Installed         Yes
   Oven Installed         Yes
   Tray Temp Installed    Yes
   Key Repeat Rate        Medium
   File                   Protect
   1:                     Off
   2:                     Off
   3:                     Off
   4:                     Off
   Solv Viscosity         Normal
```

Figure 3.48 Configurations Menu -- Solv Viscosity field

Using the [+]/[-] keys you can choose among three different syringe speeds: 1) Normal (fastest), 2) Medium, and 3) Viscous (slowest). "Normal" lets you optimize your cycle time and is optimal for most applications. With either very viscous solvents or normal-phase (low viscosity) solvents, you may wish to use the "Viscous" setting to slow down the syringe rate to prevent cavitation and nonreproducible injection volumes.

Special Commands: Commands Menu

The autosampler has several built-in commands that allow you to manually manipulate some of the autosampler's components (the arm, injector valve, etc.) outside of a run. These commands, useful at times during maintenance and troubleshooting procedures, are accessible from the Commands Menu (Figure 3.49).

```
   Flush Sample Syringe
   Flush Prep Syringe
   Initialize Hardware
   Injector
   Arm
```

Figure 3.49 The Commands Menu

**FLUSH SAMPLE
SYRINGE**

This command lets you manually flush the 250 µL sample syringe. You used this command when you primed your autosampler. It can also be useful for maintenance and troubleshooting. To access this test:

1. Fill your flush solvent bottle, if you have not already done so.
2. Press [MENU] and select /COMMANDS/, (Figure 3.49).
3. Select /Flush Sample Syringe/ to bring up the menu shown in Figure 3.50.
4. Use the [+] and [-] keys to select the desired flush volume in microliters. Then press [ENTER] to initiate the flush operation.

```
   Flush 5000 µL
```

Figure 3.50 Flush Syringe Menu

The autosampler automatically flushes the sample syringe and flush lines with the specified volume of flush solvent and returns the syringe to its starting position. During the flush sequence a confirmation message is displayed.

**Flush Prep Syringe
(AS3000 and
AS3500 only)**

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

1. Fill each solvent bottle with an appropriate solvent.
2. Press [MENU] and select /COMMANDS/.
3. Select Flush Prep Syringe (Figure 3.51).
4. Use the [+] and [-] keys to select the desired flush volume in microliters. Then move the cursor to the Solv field and select the desired solvent (S-1, S-4, Btl). Press [ENTER] to initiate the flush sequence.

```
   Flush 5000 µL Solv Btl
```

Figure 3.51 Flush Prep Menu

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

**INITIALIZE
HARDWARE**

The Initialize Hardware command allows you to manually control the autosampler's syringes and injector valve. This command brings the syringe(s) to the initial position(s) and removes any backlash.

INJECTOR

The Injector commands allows you to manually switch the injector valve between the inject and fill positions. Selecting /Injector/ displays the menu shown in Figure 3.52.

```
    Rotate Injection Valve INJECT
```

Figure 3.52 The Injector Menu

Pressing the [+] or [-] key and then pressing [ENTER] changes the injector valve's position.

ARM

The Arm Menu contains two routines for returning the XYZ arm to its home position and for aligning the arm. Figure 3.53 shows the Arm Menu. The two routines are described below.

```
    Home Arm
    Align Arm
    Clear Mixer
```

Figure 3.53 The Arm Menu

Home Arm

Pressing [ENTER] with the cursor in this field moves the arm from its present position to its home location to the left of the rear-left corner of the tub (Figure 3.54).
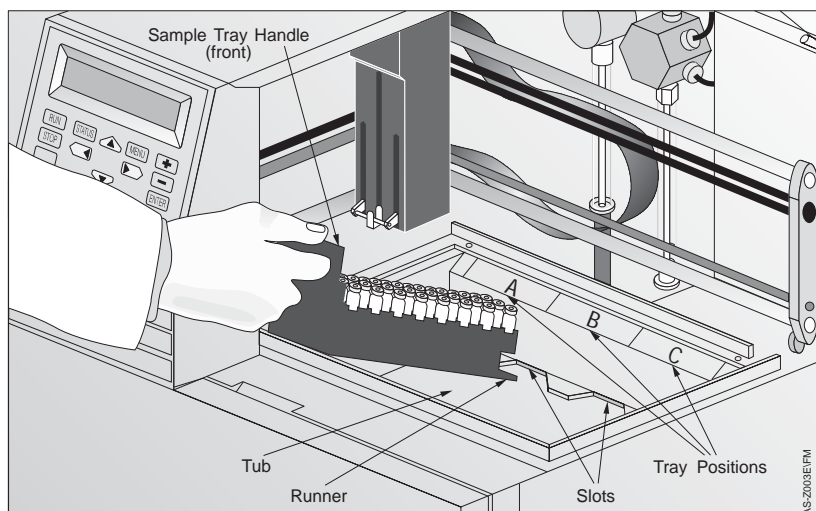
Figure 3.54 The arm in home position

Align Arm

This routine moves the arm to the vial position requested
(Figure 3.55). It is used primarily by Thermo Electron service
engineers to properly adjust the arm's x-y-z reference points. If you
suspect that the arm's alignment is incorrect, call your
Thermo Electron Service representative.

```
   Align Arm      A01
```

Figure 3.55 Align Arm Menu

*NOTE: The arm "senses" its position relative to its home position.
Therefore, if you request the arm to go to position A01, and it goes to
position A06, the system configuration may incorrectly indicate that the Tray
Temperature Control Option is installed. Check the system configuration in
the OPTIONS Configurations Menu against the installed options.*

Clear Mixer

This routine is used to remove a vial from the mixer if a power failure
occurs during a mix step in a sample preparation procedure. The arm
picks up the vial from the mixer and returns to the home position.
You must manually return to the vial to its proper location in the
sample tray.

*NOTE: This routine appears on the Arm Menu only with the Sample
Preparation Option installed.*

4

Advanced Operations

Introduction

This chapter describes the Sample Preparation Option and function in the AS3000 and AS3500 autosamplers as well as gradient delay programming, and built-in diagnostics available on all SPECTRASYSTEM autosamplers.

This chapter is organized into three parts:

- Sample Preparation beginning on page 101
- Gradient Delay beginning on page 127
- Diagnostics beginning on page 129

Sample Preparation

This section tells you how to use the optional sample preparation system to automate sample treatment, heating, and mixing. In this section you will learn:

- The terminology used in the sample preparation templates
- How the sample preparation system works
- How to use the built-in templates
- How to create your own customized file
- How to use the Edit and Samples Menus and Status Screen with the Sample Preparation Option installed
- How to run an autosampler file that uses automated sample preparation

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

BEFORE YOU START

In presenting the material in this chapter, we assume that you have installed your sample preparation bottles and lines as described in Chapter 1, that you have read and used the procedures described in Chapter 3, and that you are comfortable with the routine operations of your autosampler.

OVERVIEW

Preparing to run a sample preparation file is similar to preparing to run any other autosampler file. First, you prepare your chemicals and fill the solvent bottle(s). Then you enter your file information into the Injection, Calibration, Timed Events, and More Menus as always. Next you enter your sample preparation parameters into one of the four built-in sample preparation menus (templates) accessible from the Edit Menu's Sample Prep field (Figure 4.1). Your sample preparation parameters are stored in the same file as your other run parameters. You load your file, enter your sample set information (build your sample queue) and start your run as we discussed in Chapter 3.

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

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

```
Edit File  1
File Name
-------------------------------------------------------
Injection
Calibration
Timed Events
Sample Prep
More
```
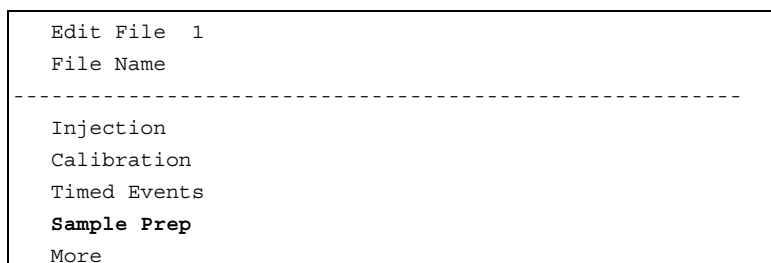
Figure 4.1 The Edit Menu

TERMINOLOGY

Templates

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

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

1) Simple dilution

2) DABS-CL derivatization

3) Rainbow dilution

4) Linear dilution

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

Dilution Cycles

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

Dilution cycles differ across templates in the number of steps and types of operations they perform. You can program the autosampler to repeat dilution cycles up to four times within a template to carry out more complex procedures. We'll see how this works in the section titled "Template Descriptions" on page 112. But first we'll discuss what happens in the simple dilution cycle used in Template 1 because its steps are used in all four templates. Once you understand how these steps control the autosampler's operation, you'll be able to apply the same principles to Templates 2-4.

```
Dilution Cycles   1
1- Load 500 µL Solv S-1
2- Pickup  100 µL Sample
3- Pickup  0 µL Sample
4- PickLG  0 µL Sample
5- Add 500 µL to Sam+1
6- Mix for 0.5 minute
Overlap Enable:   No
This Procedure requires
that 1 Extra Vial be
placed immediately after
each Sample Vial.
```

Figure 4.2 Template 1

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

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

delivers them to the target vial simultaneously. The vortex mixer then mixes the target vial for the specified amount of time.

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

HOW DILUTION CYCLES WORK

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

1) Prep syringe

2) Sample syringe with 6-port flush valve

3) Solvent holding loop

4) Six-port solvent selection valve with your four solvent bottles

5) Heater/vortex mixer

6) Needle

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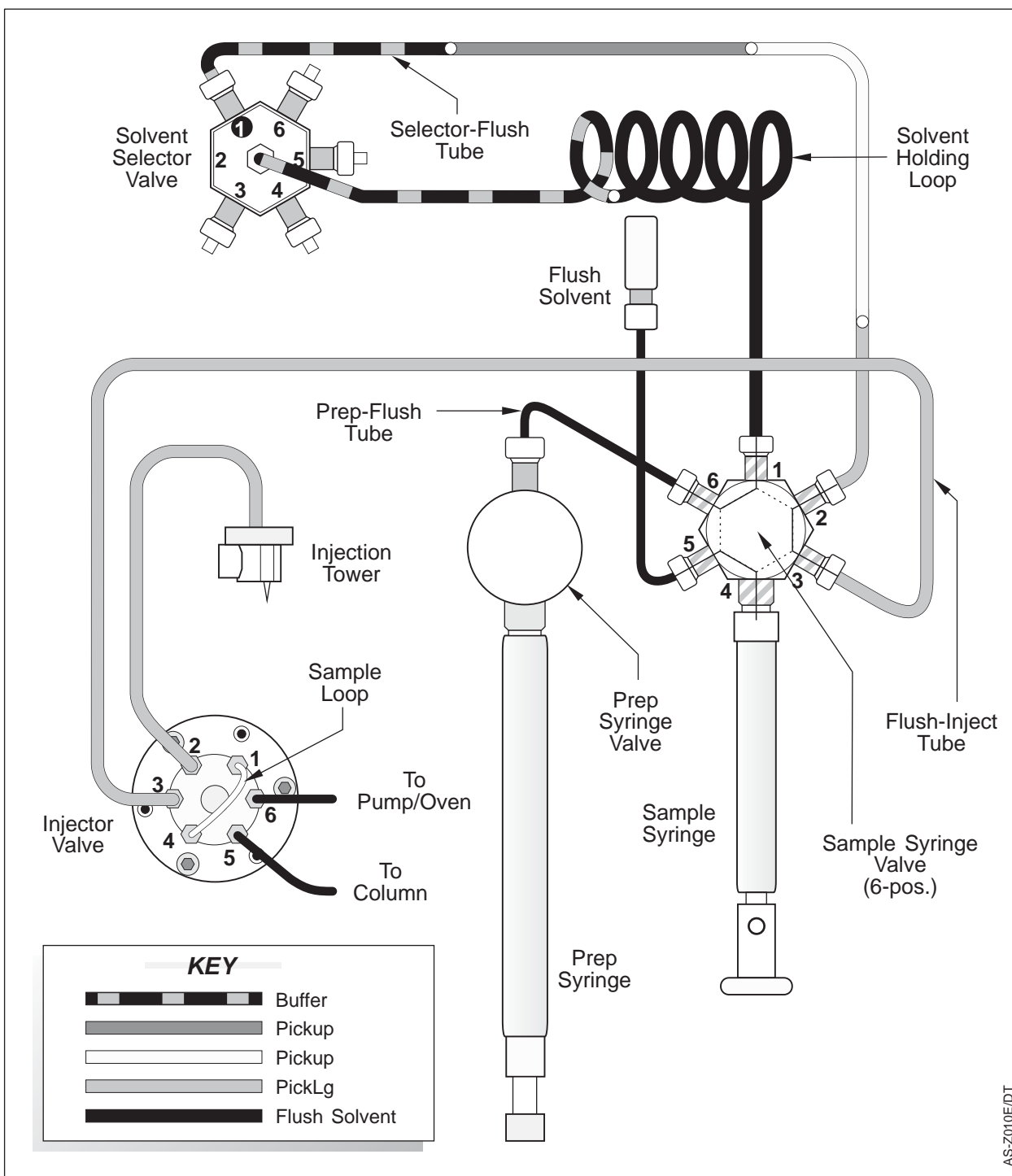
Figure 4.3 The sample preparation dilution process

**The Simple Dilution
Cycle**

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

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

Load

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

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

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

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

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

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

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

Pickup

The Pickup step causes the sample syringe to withdraw the specified volume of liquid from the source vial. The source can be the vial in the Sample (active) position, the vials in the Reagent A, B, or C locations (anywhere in the trays), or vials in the three positions adjacent to the Sample vial: Sam+1, Sam+2, or Sam+3. For example, if the Sample vial position is A01, Sam+1 would be A02, Sam+2 would be A03, etc. The following operations occur during Pickup:

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

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

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

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

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

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

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

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

PickLg

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

Add

During the Add step two events happen:

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

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

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

Mix

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

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

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

**TEMPLATE
PROGRAMMING
RULES**

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

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

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

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

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

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

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

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

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

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

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

SAMPLE PREPARATION MENU DIFFERENCES

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

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

Edit Menu

With the Sample Preparation Option installed, the Edit Menu has the Sample Prep field that you saw in Figure 4.1.

Sample Preparation Menu

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

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

Figure 4.4 The Sample Prep Menu

Samples Menu

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

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

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

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

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

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

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

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

Changes to real-time editing

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

Table 4.1 When Changes Affect the Run in Progress

File #	Can't edit
Injection Volume	Next injection*
Injections/Sample	Next injection
Cycle Time	Current injection
ReagentA(B,C)	Next sample
Reagent A-C	Next sample
Calib Vial 1 of (x)	Next recalibration
First Sample Vial	Can't edit
Number of Samples	Next injection
*Changes to this field affect sample vials only, not calibration vials.	

Protecting files from real-time editing

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

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

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

Status Screen

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

```
    QStatus        Set      #Sample
    RUN            1        5  / 120                    ♦
----------------------------------------------------------
    Status  Vial   Vol      #Inj
    NRDY    A01    1000     1 / 2
    File 1:VITAMINS
    Oven           TrayTemp
    RDY:80         RDY:4
    Prep    Vial   Htr/Mix  (only with sample preparation)
    PickLG  A01    RDY:35
```

Figure 4.7 The Status Screen for a file with sample preparation

Prep

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

Vial

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

Htr/Mix

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

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

TEMPLATE DESCRIPTIONS

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

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

Each template will be discussed in two ways: with a boxed figure showing how the display looks, and with a line-by-line description of each step. You can use the line-by-line description to assist you in

creating a customized method file for your own applications. Each example uses the template's default values.

Template 1: Dilution

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

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

Figure 4.8 Template 1: Dilution

Line-by-Line Description of Template 1

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

How the simple dilution steps work together

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

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

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

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

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

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

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

Template 2: DABS-CL

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

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

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

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

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

Figure 4.11 The first dilution cycle of Template 2

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

Line-by-line description of Template 2

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

**Template 3:
RAINBOW**

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

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

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

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

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

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

Figure 4.12 The last dilution cycle of Template 3

Line-by-line description of Template 3

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

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

Template 4: LINEAR

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

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

Figure 4.13 The last dilution cycle of Template 4: Linear

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

Line-by-line description of Template 4

`Use Template 4`	Template 4 is selected.
`Description  Linear`	Template 4 does linear dilution.
`Dilution Cycles  3`	One cycle per delivery to a target vial.
`Heater: On  Temp:  30 C`	Turn on heat; set temperature.
`1- Load 1000 µL Solv S-1`	Load 1000 µL buffer into solvent holding loop.
`2- Pickup 5 µL Sample`	Sample syringe draw sample (for the Level 1 standard) into the sample/needle line.
`3- Pickup 0 µL Sample`	Skip this step.
`4- PickLG 0 µL Sample`	Skip step.
`5- Add 1000 µL to Sam+1`	Deliver buffer and sample to the first Extra vial (Level 1 standard).
`6- Mix for 0.3 minute`	Mix the first Extra vial for 0.3 minutes.
`7- Heat for 0.0 minute`	Skip step.
`8- Mix for 0.0 minute`	Skip step.
`9- Wait for 0.0 minute`	Skip step.
`10- Remove 0 µL to` `waste at 2.0mm height`	Skip step.
`Repeat Dilution Steps`	Begin dilution cycle 2.
`11- Load 1000 µL Solv S-1`	Draw 1000 µL buffer into the solvent holding loop.
`12- Pickup 50 µL Sample`	Sample syringe draw sample (for the Level 2 standard) into sample/needle line.
`13- Pickup 0 µL Sample`	Skip step.
`14- PickLG 0 µL Sample`	Skip step.
`15- Add 1000 µL to Sam+2`	Add buffer and sample to second Extra vial (Level 2 calibration vial).
`16- Mix for 0.3 minute`	Mix the Level 2 calibration vial.
`17- Heat for 0.0 minute`	Skip step.
`18- Mix for 0.0 minute`	Skip step.
`19- Wait for 0.0 minute`	Skip step.
`20- Remove 0 µL to` `waste at 2.0mm height`	Skip step.
`Repeat Dilution Steps`	Begin dilution cycle 3.
`21- Load 1000 µL Solv S-1`	Load 1000 µL buffer into the solvent holding loop.
`22- Pickup 100 µL Sample`	Sample syringe draws 100 µL sample into the sample/needle line.
`23- Pickup 0 µL Sample`	Skip step.
`24- PickLG 0 µL Sample`	Skip step.
`25- Add 1000 µL to Sam+3`	Deliver sample and buffer for the Level 3 calibration vial into the third Extra vial.

`26- Mix for 0.3 minute`	Mix Level 3 calibration vial for 0.3 minute.
`27- Heat for 0.0 minute`	Skip step.
`28- Mix for 0.0 minute`	Skip step.
`29- Wait for 0.0 minute`	Skip step.
`30- Remove 0 μL to` `waste at 2.0mm height`	Skip step.
`FlushP  0 μL Solv S-1`	Flush lines with selected solvent.
`Overlap Enable  No`	Do not begin sample preparation on next set until current set has completed injection.

`This procedure requires`
`that 3 Extra Vial(s) be`
`placed immediately`
`after each Sample Vial.`
	You need an extra vial for each calibration vial.

STARTING A SAMPLE PREPARATION RUN

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

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

PREPARING AND RUNNING YOUR SAMPLES AUTOMATICALLY

A Simple Dilution Run

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

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

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

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

Preparing Calibration Vials and Injecting Them

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

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

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

Figure 4.15 The Samples Menu for the Prep Set

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

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

Figure 4.16 The Samples Menu for the Injection Set

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

STOPPING A RUN

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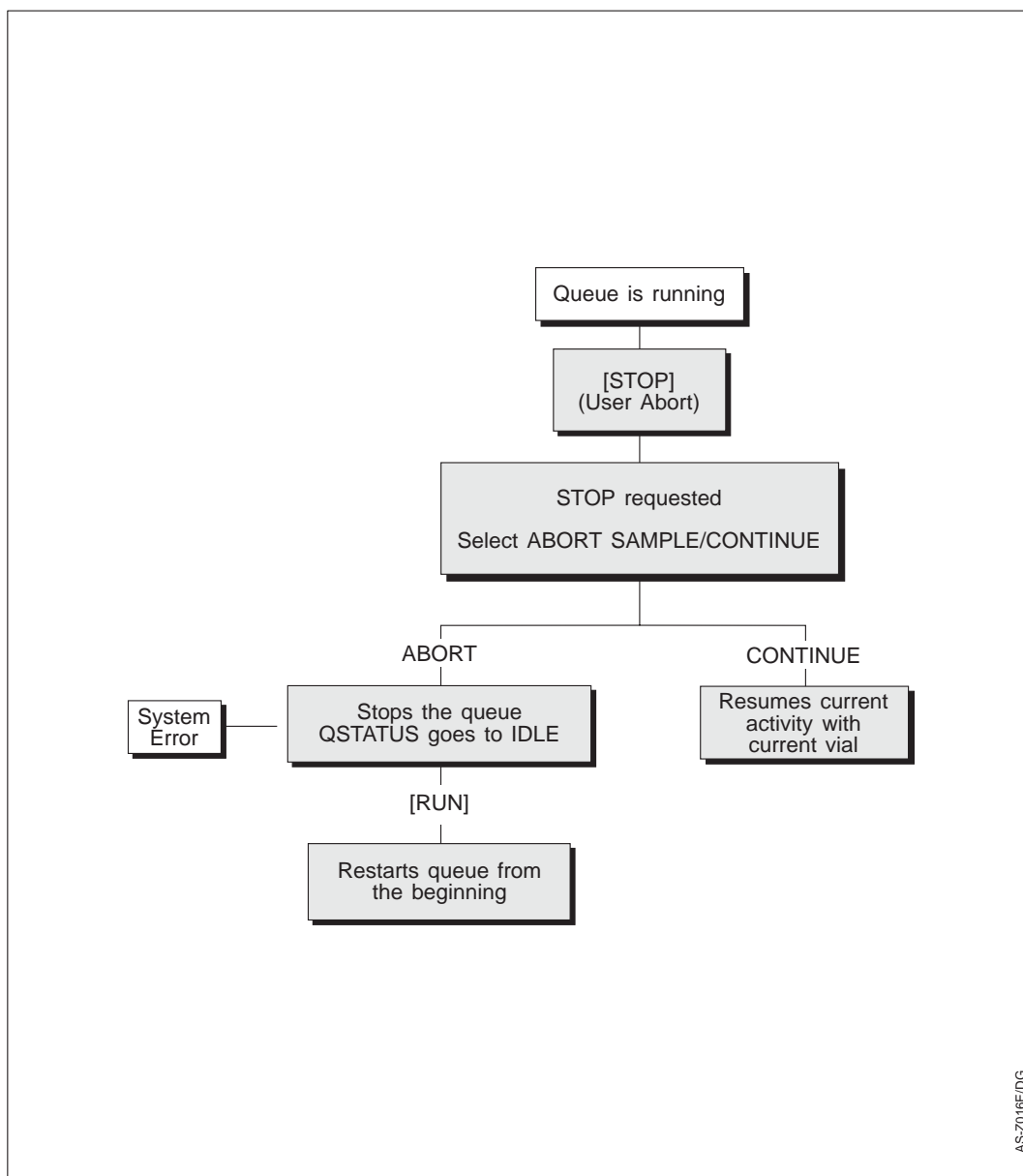
Figure 4.17 The Queue Stop/Abort Sequence

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

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

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

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

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

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

CUSTOMIZING THE TEMPLATES

Guidelines

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

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

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

3. You can use the vial positions in any order you wish within a dilution cycle. For example, even though the autosampler cannot skip Sample vial positions, you can program it to prepare interspersed calibration vials by Adding to the Sam + 2 position, without using the Sam + 1 position in any of the steps in a given dilution cycle.

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

Gradient Delay

Gradient delay is a user-defined value that delays the injection of a sample. This delay is the amount of time required for a change in the mobile phase to travel from the gradient proportioning valve to the injection valve. The volume of mobile phase contained between the two valves is also referred to as the precolumn volume (PV).

Although not required for normal autosampler operation, a gradient delay is recommended when using large volume pre-filters or low flow rates (<0.5 mL/min). The gradient delay time can be found under the FILE/Edit/More Menu. You then use the [+] or [-] keys to enter the desired time in minutes (Figure 4.18).

The following procedure is used to calculate the gradient delay time for your HPLC system. (See Figure 4.19.)

1. Inject an unretained sample into your LC system with a column in-line and using 100% A as the mobile phase (*e.g.,* MeOH).

2. Change to 100% B (*e.g.*, 0.1% Acetone in MeOH) as fast as possible (0.1 minutes for SpectraSYSTEM pumps).

3. Subtract the retention time (R_t) of the unretained peak from the retention time of the start of the baseline shift (T_2-T_1).

4. Subtract the time required to switch mobile phases from 100% A to 100% B.

5. The resulting time is the gradient delay time.

EXAMPLE

The following example illustrates how to apply this procedure.

An unretained peak has a retention time of 3.0 min. with a baseline shift at 4.5 min. and a complete mobile-phase switch time of 0.1 min. The gradient delay is calculated as follows:

Gradient Delay   = (T2-T1) - 0.1 min

                         = (4.5 - 3.0) - 0.1

                         = 1.4 min

```
  Equilibration Time                0.0
  Gradient Delay                    0.00
----------------------------------------------------------
  Viscosity                       Medium
  Flush Volume                     250.0
  Injection Type                    Push
  Injection Range                 1-1500
  Needle Height                      2.0
```

Figure 4.18 The More Menu (Gradient Delay)
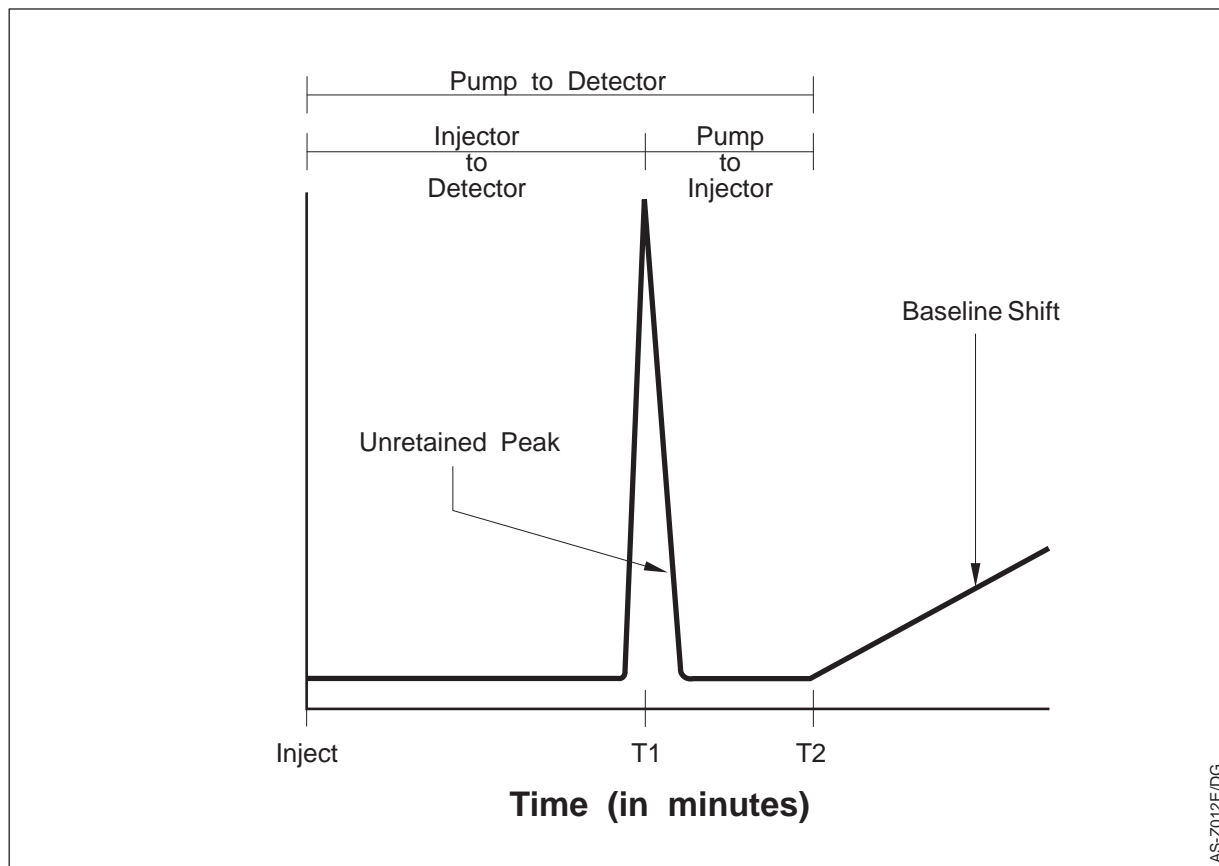
Figure 4.19 The delay time calculation using a hypothetical chromatogram

Diagnostics (Tests Menu)

The SpectraSYSTEM autosamplers have built-in diagnostic routines accessible from the Tests Menu. These tests enable you to access the operation of your autosampler. Before describing each of these tests, we'll describe how the diagnostic tests are run and how to interpret the error screen information, should a test fail.

All of the test screens are arranged similarly. A test is initiated by pressing [ENTER]. While a test is in progress, the name of the test is displayed along with three dots (Figure 4.20).

```
  Test External Contacts:  ...
```

Figure 4.20 The In-progress Screen

INTERPRETING THE RESULTS

When the test is complete, either a PASS or FAIL result is displayed. Should a test fail, an error screen similar to that shown in Figure 4.21 will appear that contains the following information:

- Test identification
- Fault code (diagnostic error)
- Module identification
- [RUN]/[STOP]

```
  Connector:      ERROR #3
  Syringe1       [RUN]/[STOP]
```

Figure 4.21 An example of an error screen

NOTE: The error number in this screen is just an example to show you the screen layout.

Test Identification

The test identification is the name of the test being run.

Fault Code

The fault code is the error number associated with the specific failed result. Document and report the fault code to your service representative to receive further assistance in diagnosing and solving the problem.

**Module
Identification**

> This field displays the specific autosampler board or assembly where the problem occurred.

[RUN]/[STOP]

> This field prompts you to press [RUN] to repeat the test or to press [STOP] to exit the current test and return to the Tests Menu.

**TEST
DESCRIPTIONS**

> All diagnostics and built-in assessment routines are accessible from the Tests Menu (Figure 4.22). Each of the routines is described here.

```
Software Version
Display Input States
Display Output States
ROM Test
RAM Test
Hardware Test
Test External Contacts
Test Comm Port
Initialize EEPROM
```
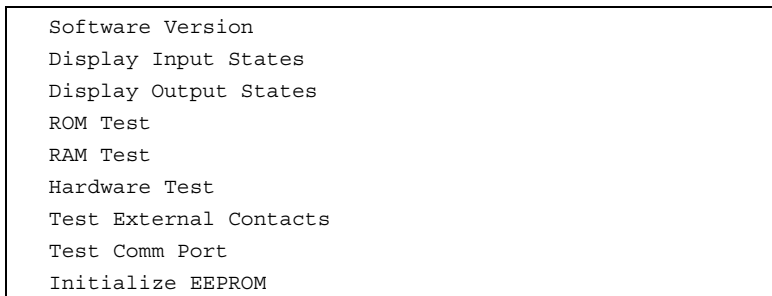
Figure 4.22 Tests Menu

Software Version

> Selecting this field displays the current software version number, x.yy.zz.

Display Input States

> This menu (Figure 4.23) shows the current states of the TTL-input device signals (Hi or Lo). This information can be used to check the level of the Pump Ready and Injection Hold input lines from other LC system modules. You can use this information to diagnose situations where the autosampler is waiting for ready or waiting to inject. Make sure the Input Polarity is set to the proper level for each of these signals. Pressing the up- or down-arrow keys returns you to the Test Menu's Display Input States field should you wish to repeat the test.

> *NOTE: You can change the active polarity of a specific input signal from the OPTIONS Input Polarity Menu.*

```
Pump Ready      Inj Hold
Hi              Hi
```

Figure 4.23 Display Inputs States Menu

Display Output States

This menu shows the current states of the TTL-output device signals (Hi or Lo). This information is used when hardwiring your autosampler to other LC system modules. Pressing the up- or down-arrow keys returns you to the Test Menu's Display Output States field should you wish to repeat the test.

NOTE: You can change the active polarity of a specific input signal from the OPTIONS Input Polarity Menu. This menu shows the states of the TTL output device signals (Hi or Lo) as shown in Figure 4.24.

```
Display Output States
AS Rdy  Inject  Grad    Stop
Lo      Lo      Lo      Lo
```

Figure 4.24 Display Output States Menu

Pressing the up- or down-arrow keys returns you to the Test Menu's Display Output States field should you wish to repeat the test.

Display Timed Events

This menu (Figure 4.25) displays the current states of the timed events outputs. This information is used when hardwiring your autosampler to other LC system modules. Refer to Appendix A "System Communication" for more information.

```
TF1     TF2     TF3     TF4
Lo      Lo      Lo      Lo
```

Figure 4.25 Display Timed Events Menu

RAM Test

This test (Figure 4.26) writes to and then reads the contents of memory. If the write information is the same as the read information, the test is successful. You may wish to run this test once a month to verify proper software operation.

```
Short Memory Test:
```

Figure 4.26 RAM Test Menu

If successful, the autosampler returns a PASS result (Figure 4.27). If unsuccessful, the autosampler returns a "FAIL" result and displays an error message. Document the test results and error message, and call your Thermo Electron service representative.

```
Short Memory Test: PASS
```

Figure 4.27 Successful RAM Test

Press [STOP] to return to the Tests Menu.

ROM Test

It evaluates check-sums on the contents of the instrument's ROM and compares the results to known values.

If successful, the autosampler returns a PASS result. If unsuccessful, the autosampler displays the number of the failed chip(s) along with the regular error screen information (Figure 4.28). If the test fails, document the test results (if failed) and call your Thermo Electron service representative.

```
ROM Test:                          Error #: U78, U79


CPU                                [RUN]/[STOP]
```

Figure 4.28 Failed ROM Test

Hardware Test

This is the most important autosampler diagnostic. It checks all of the cable connections, sensors, motors, circuitry, and attached components.

Before running this test, be sure that the XYZ arm is in the home position (at the left rear of the sample compartment), or false errors may be reported. You can use the Home Arm command in the Commands Menu (see "Special Commands" on page 96) if the arm is not at home.

If the Hardware Test fails, the screen displays the test identification, fault code, applicable module or connection, and the [RUN]/[STOP] fields. For example, the message "J10/J40/J45" in Figure 4.29 indicates that further troubleshooting is required. Document the screen information and report it to your Thermo Electron service representative when you call for assistance.

```
Connector:      ERROR #3
J10/J40/J45    [RUN]/[STOP]
```

Figure 4.29 An example of an error screen

Pressing [STOP] returns you to the Tests Menu.

Most of the failed Hardware Test results require that you contact your service representative for assistance. However, there are several messages that indicate problems that you can most likely resolve without a service call. These are described here.

Opt Config
This message indicates an incorrect configuration. Check the Configurations Menu (under OPTIONS) and verify that the options field entries match your installed options.

Arm,*
This message indicates that the XYZ arm was not in its "home" position when the Hardware Test was initiated. Select /COMMANDS/, /Arm/, /Home arm/.

Test External Contacts

This test activates all of the external contacts and tests for proper activation. It should not be run while the external contacts are wired to other equipment, or the test will report false errors. Before running this test, disconnect the 12-pin connector block from the rear of the instrument. After the test is complete, plug the connector back in to resume normal instrument operation.

Pressing [ENTER] with the cursor in the Test External Contacts field displays a warning prompt (Figure 4.30).

```
Disconnect all external contacts      CONTINUE
```

Figure 4.30 The External Devices Menu

From this menu, you can use the [+] and [-] keys to select CONTINUE or ABORT. Selecting Continue starts the test.

Selecting ABORT discontinues the test and returns you to the Test External Contacts field of the Tests Menu. If ABORT is displayed and you want to resume to test, press the left- or right-arrow keys. The display will again show CONTINUE.

Test COMM Port

A loop-back connector is required to run this test used to assesses the operation of the communications port located on the instrument's rear panel. This test sends a signal to the communications port and expects a signal in return. A successful result is reported as a PASS; an unsuccessful result is indicated by the regular error screen.

NOTE: If you wish to perform this test, contact your Thermo Electron Field Service Engineer for the procedure and necessary parts information.

Initialize EEPROM

The Electronically Erasable Programmable Read Only Memory (EEPROM) stores all files, user-set variable values, and system configuration information when the power is turned off. Reinitializing the EEPROM restores the default values to all files and erases the system configuration information. Pressing [ENTER] in this field displays the message shown in Figure 4.31:

```
 Reset all system values      YES
```

Figure 4.31 The Initialize EEPROM Menu

If you want to exit this routine before running it, you can:

- Press the [+] [-] keys to select No
- Press [MENU] to call up the Main Menu
- Press [Status] to display the Status Screen
- Press the up-arrow key to return to the Tests Menu.

NOTE: This routine erases any current file values and returns all field values to their default values. Initialize EEPROM also erases all configuration information. After running this routine, be sure to reset all installed options to "YES" in the Configurations Menu.

5

Required Maintenance

Introduction

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

SPECIFIC HAZARDS

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

Caution!
A caution alerts you to situations that could result in personal injury. It also tells you how to avoid them.

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

Hot Surface!
This icon alerts you to potential injury that could occur from coming in contact with a heated surface or area on or in an instrument. It also tells you how to avoid contact with the heated surfaces in your instrument.

Maintenance Schedule

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

Table 5.1 Maintenance Schedule

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

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

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

Procedures

**EMPTYING THE
FLUSH-SOLVENT
TRAY**

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

1. Be sure the autosampler is in idle status.

2. Carefully slide the tray out without spilling the collected solvent.

3. Empty the tray, rinse it off with water, and slide it back into place under the black elbowed, barbed piece of tubing.

⚠ **CAUTION! Wear skin and eye protection and dispose of hazardous waste according to local, state, and federal regulations.**

NOTE: Be sure that the level of the liquid in the waste container is below the level of the instrument or waste will collect in the lines and could backflush into the instrument.

**REPLACING THE
ROTOR SEAL**

Replacing the rotor seal includes the following procedures:

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

NOTE: If you are only replacing the rotor seal, you don't have to remove the injector valve from the instrument; however, it's easier. If you wish to replace the rotor seal with the valve installed, proceed to the section entitled, "Disassembling the Injector Valve" on page 140.

**Removing and
reinstalling the
Injector Valve**

To disassemble the injector valve (Figure 5.1):

1. Using a 1/4" open-end wrench, disconnect tubing from ports 2, 3, 5, and 6.

2. If you have the Column Oven Option and are using the 100 μL loop shipped with your instrument, you remove the sample loop. Otherwise, proceed to step 3.

3.   If you have purchased the column oven, loosen and remove the two Phillips-head screws that secure the injector valve collar to the front of the oven. Then remove the collar.

4.   Remove the two Phillips screws that secure the valve mounting plate.

5.   Carefully pull the valve out of the instrument.

6.   Reverse these steps to reinstall the valve.
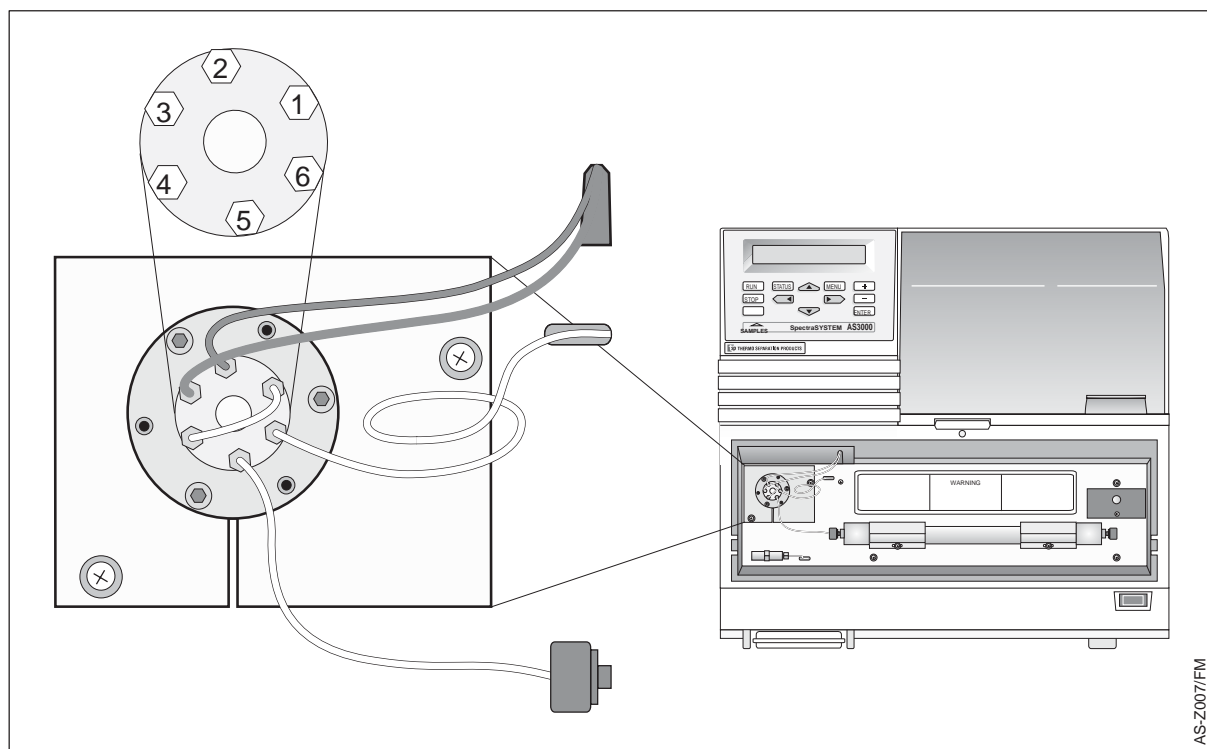
Figure 5.1 The injector valve

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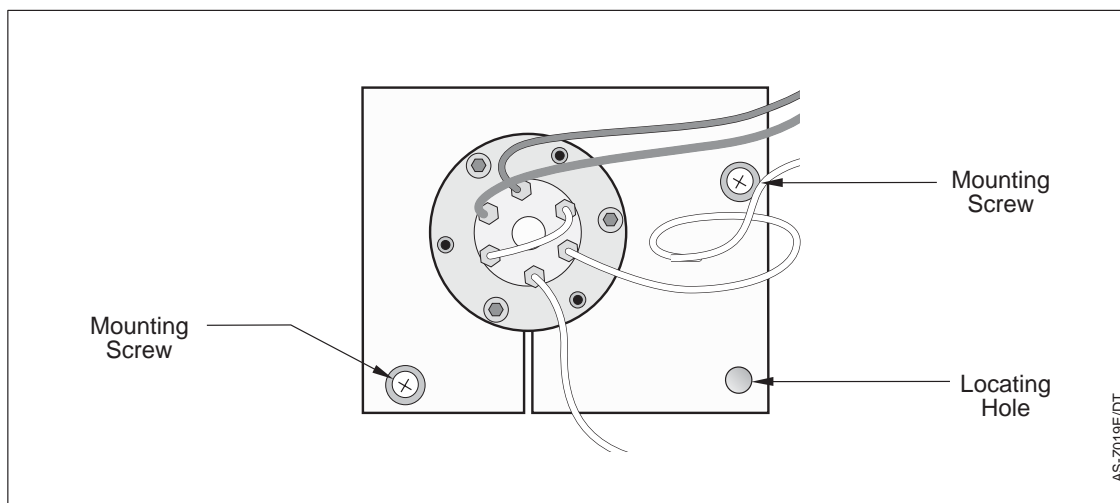
Figure 5.2 The correct injector valve bracket orientation

Disassembling the Injector Valve

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

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

To disassemble the injector valve:

1. Follow the procedures above for removing the injector valve.

2. Using an Allen wrench, remove the 9/64" hex cap screws from the top of the valve (Figure 5.3).

3. Carefully lift the stator from the valve body and set it onto a clean surface.
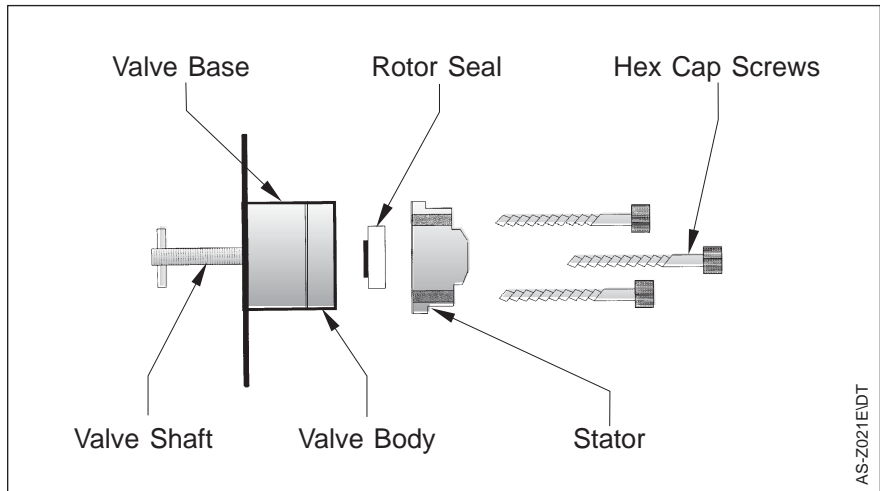
Figure 5.3 The injector valve's components

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

4. Remove the valve body.

5. Before removing the worn rotor seal, note the following on the rotor seal (Figure 5.4):

   - the three circular port grooves on one side of the rotor (the other side is smooth)

   - the small locating notch on the rotor's rim (*not* the V stamped on the seal).
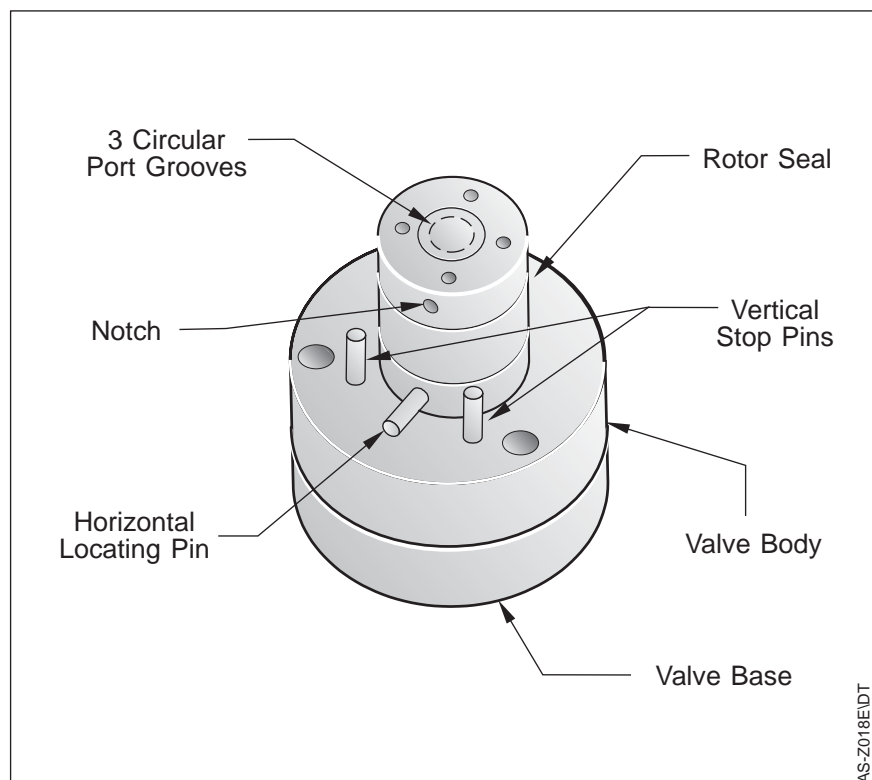
Figure 5.4 The injector valve with valve body removed and rotor seal exposed (NOTE: The mounting bracket is not shown.)

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

Cleaning the Stator

Clean the stator as follows:

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

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

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

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

To install the rotor seal:

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

2. Install the valve body.

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

NOTE: Be sure to hold the valve shaft so that the horizontal locating pin touches the left stop pin as you reassemble the valve. This puts the valve into the INJECT mode (the same mode as when removed from the instrument). If the pins are not in the correct orientation, the valve shaft may not be in the proper orientation for reinsertion into the instrument.
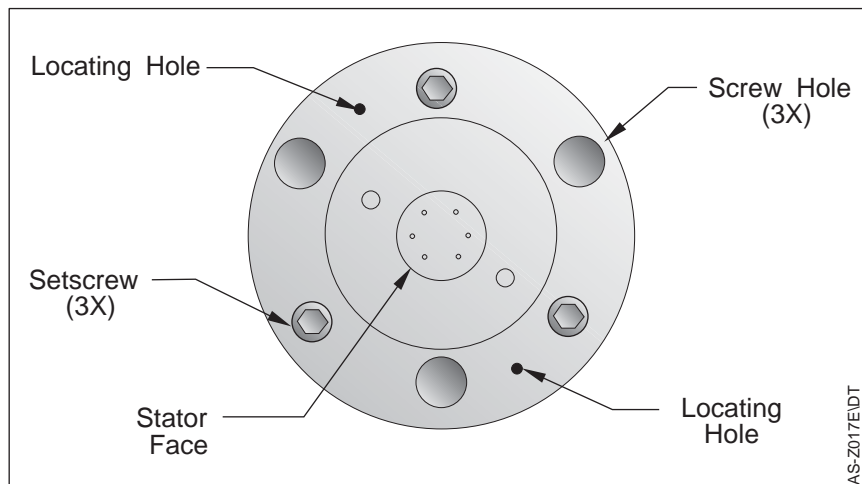
Figure 5.5 The bottom of the stator assembly

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

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

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

5. Tighten each cap screw an equal amount until all setscrews are in equal contact with the valve body.

REPLACING THE FLUSH SOLVENT INLET FILTER

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

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

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

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

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

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

SAMPLE NEEDLE

Module

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

Removal

Use the following steps to remove the sample needle module:

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

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

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

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

Cleaning

To clean the sample needle module:

1. Inspect the needle module.

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

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

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

Installation

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

1. Remove the new module from the replacement kit.

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

3. Snap the needle assembly into the mounting brackets on the backside of the sample tower (Figure 5.6).

4. Place the tube fitting and ferrule onto the needle and install it into port 2 of the injection valve (Figure 5.1).

Drop Catch

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

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

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

Labels in figure: Needle Module Assembly, Front Bulkhead, Drop-Catch Location, Needle, Injection Tower, Sample Tub, THIS SIDE OUT, AS-Z020EIDT
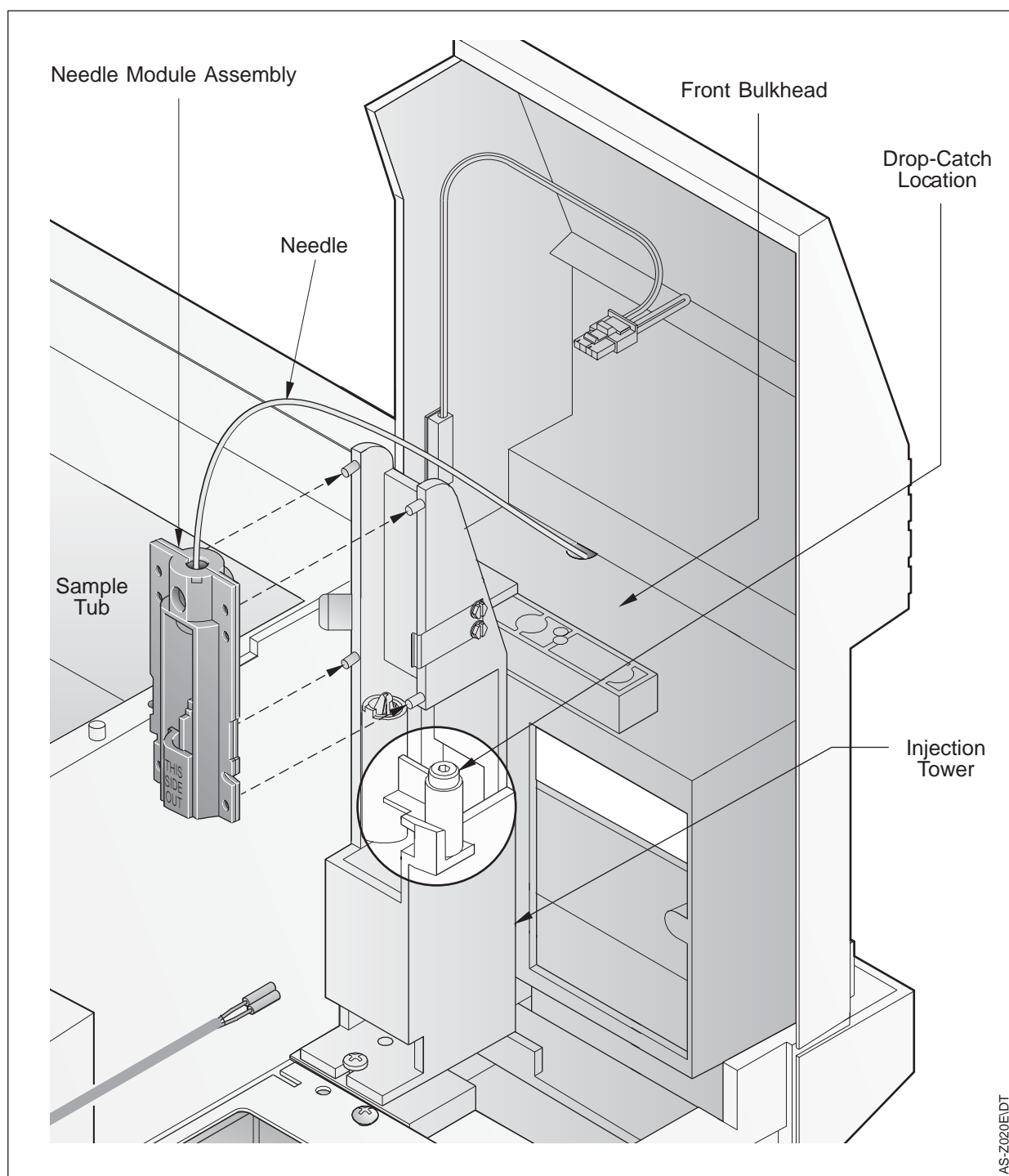
Figure 5.6 Replacing the sample needle module

**SYRINGE
REPLACEMENT**

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

**250 µL Sample
Syringe**

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

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

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

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

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

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

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

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

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

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

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

**2.5 mL Preparation
(prep) Syringe**

If you have the Sample Preparation Option, you can position the large (2.5 mL) syringe's plunger in the down position by interrupting a flush cycle, as follows:

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

2. Move the XYZ arm out of your way by pressing [MENU], Commands, /Arm/, /Align Arm/, and selecting position A01. Then press [ENTER].

3. To start the flush cycle, from the Main Menu, choose Commands, and Flush Prep Syringe.

4. When the plunger has lowered, turn off the power.

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

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

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

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

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

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

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

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

SAMPLE LOOP

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

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

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

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

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

FUSES

Instrument power is supplied by two T5A fuses housed in the fuse compartment on the lower left corner as you are facing the instrument's rear panel (Figure 5.7).

COMM Port

6-Port Solvent-Selection Valve

12-Pin Terminal

Bottle-Bracket Screws

1 6
2 5
3 4

WARNING

TERMINAL CONFIGURATION

AS-Z004ElDT

Fuse Compartment

AC Power

Air-Supply Port
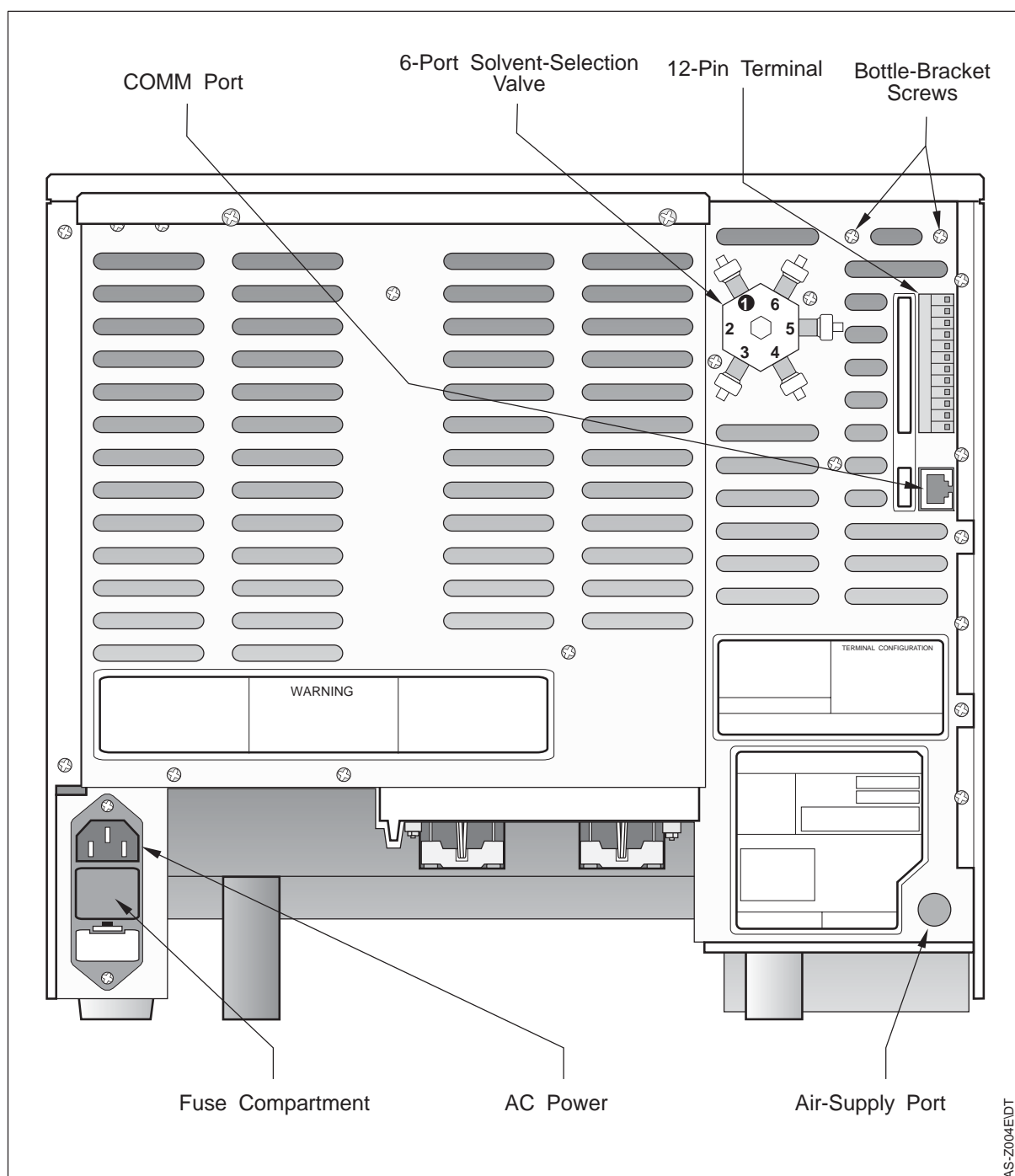
Figure 5.7 The autosampler's rear panel

Thermo Electron

To replace the fuses:

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

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

3. Snap the fuse holder back into place.

Maintenance Options

SHUTDOWN

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

```
Configurations
Ready Participation
Input Polarity
Output Polarity
Shutdown
Maintenance Log
Communications
```

Figure 5.8 The Options Menu

Figure 5.9 illustrates the autosampler's Shutdown Menu.

```
Delay Time           Off
Oven Shutoff         Yes  (Only if oven installed)
TrayTemp Shutoff     No   (Only if tray temperature control installed)
Heater Shutoff       No   (Only if sample prep installed)
Pump Shutoff         Yes
Flush Volume         400
Prep Flush Volume    1000  (Only if sample prep installed)
```

Figure 5.9 The Shutdown Menu

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

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

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

TrayTemp Shutoff

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

Heater Shutoff

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

Pump Shutoff

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

Flush Volume

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

Prep Flush Volume;

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

MAINTENANCE LOG

The Maintenance Log Menu (Figure 5.10), accessible from the OPTIONS Menu, monitors the instrument's maintenance status and counts and displays the number of activations for each of the autosampler's routine maintenance parts listed in the menu.

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

Figure 5.10 The Maintenance Log Menu

Instrument Maintenance Status

The Maintenance Log Menu provides a convenient way to keep track of autosampler maintenance. When you first access the Maintenance Log Menu from the OPTIONS Menu, the "Maintenance Required" confirmation message is displayed if instrument maintenance is required. This message is displayed if any of the #Cycles field values

in any of the Maintenance Log menus is greater than the number of cycles entered in the Due fields. See below for more information on these fields.

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

Total System Cycles Menu

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

```
#Cycles    Note
   35       Feb 93
```

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

Injector Valve Menu

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

```
#Cycles    Due     Note
  202     24000    021593  ♦
---------------------------------------------------------
Reset #Cycles      YES
```

Figure 5.12 The Injector Valve Menu

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

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

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

Note: Similar to the Total System Cycles Menu, this is a six-character, alphanumeric, user-programmable field. We recommend that you use the [+]/[-] keys to enter the last service date in this field. However, you can also use this field to document contact information or other comments regarding the maintenance of the specific autosampler part. *Reset #Cycles*: Pressing the down-arrow key displays the Reset #Cycles field used to reset the #Cycles counter for each autosampler part. To reset the counter to 0 after maintenance is complete, press the [+]/[-] keys to select YES, and press [ENTER]. The confirmation message ** Counter Reset ** is displayed. Most likely, your service representative will reset the #Cycles field value when the instrument is serviced.

Needle Wear Menu

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

Sample and Prep Syringe Menus

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

A
Troubleshooting

Introduction

This appendix provides information to help you troubleshoot possible autosampler problems. We have included the following:

- Table 1.1: Troubleshooting guide to general chromatographic problems
- Table 1.2: Troubleshooting guide to autosampler-specific chromatographic problems
- Table 1.3: Troubleshooting guide to autosampler hardware problems
- Display Messages
- Information on testing tubing restrictions

If, after reviewing the troubleshooting table and diagnostics included in this chapter, you would like additional assistance in troubleshooting a particular problem, don't hesitate to contact the Thermo Electron Technical Support Group or your service representative. The telephone numbers are found on the back cover of this manual.

General Chromatography Troubleshooting

This section focuses on general chromatographic problems and is designed to help you define the particular instrument or condition causing the problem. Once you have ascertained the cause, use the ordered outline to fix the problem or refer to the individual SpectraSYSTEM instrument's troubleshooting guide. Each of these problems can be detected by monitoring the chromatography running conditions or by observing the chromatogram.

Table A.1 General LC Troubleshooting Table

Symptom	Cause/Remedy
1. No flow.	a) Check mobile phase connections
	b) Check for leaks
	c) Check pump troubleshooting guide
2. High back pressure.	a) Check flow rate and system/column specifications
	b) Check for tubing or column blockage
	c) Check pump troubleshooting guide
3. Unstable baseline or drift.	a) System/column not equilibrated; allow more time
	b) Check detector troubleshooting guide
	c) Check pump troubleshooting guide
4. Baseline noise.	a) Check for air bubbles in system, degas solvents
	b) Check for system/solvent contamination
	c) Check pump troubleshooting guide
	d) Check your chromatography data system troubleshooting guides
5. No peaks.	a) Check detector and data system connections
	b) Check autosampler troubleshooting guide
	c) Check sample retention with chromatographic conditions
6. Contaminating/ghost peaks.	a) Clean system and column
	b) Check autosampler troubleshooting guide
	c) Check pump troubleshooting guide

Table A.1 General LC Troubleshooting Table, *continued*

7. Poor peak shape.	a) Check system for leaks
	b) Check fittings and tubing lengths
	c) Check column performance
	d) Check autosampler troubleshooting guide
	e) Check pump troubleshooting guide
	f) Check detector troubleshooting guide
8. Poor retention time reproducibility.	a) Check system for leaks and bubbles
	b) System/column not equilibrated, allow more time
	c) Check column performance
	d) Check pump troubleshooting guide
	e) Check autosampler troubleshooting guide
	f) Check data system troubleshooting guide
9. Poor peak area reproducibility.	a) Check column performance
	b) Check autosampler troubleshooting guide
	c) Check data system troubleshooting guide
10. Non-integrated or too many peaks.	a) Check integrator or data system troubleshooting guide
11. No instrument of device control.	a) Check cable connections
	b) Check system configuration
	c) Check individual instrument troubleshooting guide
	d) Check integrator, ISM, or data system troubleshooting guide

For more detailed chromatographic troubleshooting, refer to any HPLC troubleshooting reference book or call your local sales or service representative.

Table A.2 Autosampler-specific Chromatographic Problems

Symptom	Possible Problem	Remedy
1) Baseline drift when the injector valve changes from INJECT to FILL.	a) Lack of thermal stability in the column oven compartment, detector, or mobile phase.	a) Eliminate drafts around the column compartment. Wait until the column oven has reached thermal equilibrium. If the problem persists, contact your local Thermo Electron service representative.
2) Shifting retention times.	a) Temperature variations in the lab.	a) Stabilize temperature.
	b) Column-heater problems.	b) Call your Thermo Electron service representative.
3) Sensitivity changes.	a) Inconsistent sample injection volumes.	a) Check for a partially blocked injector valve and observe the pressure. If the problem persists, replace the injection valve (refer to Chapter 5).
	b) Leaks between the injector valve and the column causing inconsistent sample-volume injection.	b) Tighten/remake fitting.
	c) Air bubbles aspirated into the lines along with sample.	c) Replenish/prepare fresh sample and repeat the injection.
		c') Slow down syringe speed.
	d) Incomplete sample mixing during sample preparation.	d) Check mix time (Sample Preparation Option, only). Thaw frozen samples completely prior to sample preparation to prevent sample "layering."
	e) Sample adsorption to the walls of the injector valve or sample loop.	e) Change the mobile phase concentration.

Table A.2 Autosampler-specific Chromatographic Problems, *continued*

Symptom	Possible Problem	Remedy
3) Sensitivity changes, continued	f) Sample solvent incompatible with the mobile phase.	f) Change solvent, use mobile phase if possible.
	g) Sample insoluble in solvent.	g) Be sure sample solvent is lower elution strength than mobile phase.
	h) Air in syringe/sample lines.	h) Flush sample lines.
4) Irregular peak shapes.	a) The volume of sample injected exceeds the column's capacity.	a) Decrease the volume injected or dilute the sample.
	b) The strength of the solvent used to dilute the sample exceeds the initial concentration of the mobile phase.	b) If appropriate for your application, ensure that the flush solvent is of the same composition as the solvent used to dissolve the sample and that it never exceeds the initial composition of the mobile phase.
	c) Degradation of sample component(s).	c) Prepare fresh sample and repeat injection.
5) Band broadening.	a) Fitting problem at the injector-column connection.	a) Inspect for damage, mismatch, incorrect assembly.
	b) Tubing ID too large.	b) Use 0.010-inch ID tubing.
6) Smaller than expected peak heights.	a) Small injection due to wrong-size syringe.	a) Check syringe size.
	b) Wrong injection.	b) Check injection volume value in file.
	c) Air in lines.	c) Flush.
	d) Misadjusted syringe-drive mechanism.	d) Call your Thermo Electron service representative.

Table A.2 Autosampler-specific Chromatographic Problems, *continued*

Symptom	Possible Problem	Remedy
7) Peaks during a blank injection (from a previous injection).	a) Sample carryover due to residual sample in the sample loop.	a) Insufficient flush volume. Increase Injection Volume in Edit Menu; add a blank vial between sample vials; or use a stronger flush solvent.
	b) Sample carryover due to incorrectly assembled sample-loop fittings (adding dead volume that can contain residual sample).	b) Check and remake fittings.
	c) Sample too concentrated.	c) Dilute sample.
8) No peaks.	a) Plugged or bent needle.	a) Clean/replace needle. Tighten fittings and syringe.
	b) Leaking flush valve fitting.	b) Tighten fitting.
	c) Plugged or leaking tubing.	c) Replace faulty tubing.
9) Incorrect precision and/or accuracy problem on a sample of known content-random error.	a) Air leak.	a) Tighten all fittings and repeat the run.
	b) Worn out syringe.	b) Replace.
10) Irreproducibility of peak areas.	a) Plugged or bent needle.	a) Clean/replace needle. Tighten fittings and syringe.
	b) Leaking flush valve fitting.	b) Tighten fitting.
	c) Plugged or leaking tubing.	c) Replace faulty tubing.
	d) Sample viscosity too low or too high.	d) Change viscosity setting in the FILE/Edit/More Menu.
	e) Solvent viscosity too low or too high.	e) Change Solv Viscosity setting in the OPTIONS/Configurations Menu.

Table A.3 Autosampler Hardware Problems

Symptom	Possible Problem	Remedy
Startup		
1) Nothing works when instrument is turned on.	a) Power cord unplugged or faulty.	a) Plug in/replace cord.
	b) Blown fuse.	b) Replace fuse.
	c) Defective power switch, driver, transformer.	c) Call your local Thermo Electron service representative.
2) Fan is running but display doesn't work.	a) Display component(s) failure.	a) Call your local Thermo Electron service representative.
3) No response from keypad but display works.	a) Defective keypad.	a) Call your Thermo Electron service representative.
4) Column oven and/or tray temperature control won't turn on.	a) File isn't loaded.	a) Load file.
Communications		
5) No RS-232-C communications.	a) Further troubleshooting required.	a) Call your Thermo Electron service representative.
6) EEPROM fails to retain memory.	a) Reinitialize the EEPROM.	a) If unsuccessful, may be a defective PCB. Call your Thermo Electron service representative.
7) External devices (timed events) don't work.	a) Wrong settings.	a) Check Chapter 1 for proper pinouts.
	b) Interface equipment problem.	b) Refer to respective instrument manuals.

Table A.3 Autosampler Hardware Problems, *continued*

Symptom	Possible Problem	Remedy
Vials		
8) Vial stuck in tower.	a) Needle bent.	a) Replace needle module (see Chapter 5).
9) Vial stuck in mixer.	a) Run Clear Mixer routine in the Commands Menu.	a) See page 99.
10) Instrument can't locate vial.	a) Incorrect vial position entered into Samples Menu.	a) Check Samples Menu.
	b) TrayTemp Option incorrectly selected in Configurations Menu. (Instruments with tray temperature control have fewer tray rows.)	b) Check configuration.
	c) Sample incorrectly placed in sample tray.	c) Check tray arrangement.
	d) Arm misaligned.	d) Home arm. See "Special Commands" on page 96.
	e) Arm movement restricted.	e) Remove restriction and home arm.
11) Vials don't fit in tray.	a) Incorrect vials.	a) Vial diameter should be 0.44 - 0.47 inches.
12) Broken vials.	a) Tray not aligned or not seated properly.	a) Seat tray completely in tray chamber and **pull handle back toward you**.
13) Torn septa.	a) Bent needle.	a) Replace needle.

Table A.3 Autosampler Hardware Problems, *continued*

Symptom	Possible Problem	Remedy
Injector		
14) Injector valve won't switch.	a) Slipping injector drive.	a) Call your Thermo Electron service representative.
15) Injector valve continues to rotate until fault is generated.	a) Loose or faulty sensor cable.	a) Call your Thermo Electron service representative.
16) Injector valve does not rotate to the proper position.	a) Valve requires excessive torque.	a) Call your Thermo Electron service representative.
17) Motor fails to stop after syringe is fully returned.	a) Possible defective sensor.	a) Call your Thermo Electron service representative.
18) Sample loop won't fill.	a) Blocked injector valve.	a) Follow procedures on page 143 to isolate blockage and flush. Or take apart valve and sonicate. DO NOT clean valve with a cleaning wire or you could damage it.
	b) Blocked sample loop.	b) Backflush loop. If problem persists, replace the loop.
	c) Worn syringe.	c) Substitute a "known-good" syringe and determine if symptoms persist.
	d) Blocked needle.	d) Clean with a cleaning wire.
19) Rapid valve-seal wear.	a) Too much valve seal pressure against valve.	a) Decrease LC system pressure.
	b) Particulate matter in valve.	b) Use an in-line filter before the injector valve.
	c) Inadequately filtered sample.	c) Filter with a 0.5 μm filter before injection.

Table A.3 Autosampler Hardware Problems, *continued*

Symptom	Possible Problem	Remedy
Injector, continued		
20) Rapid valve-seal wear, continued	d) Buffer crystallization.	d) Don't allow buffers to stand in system. Run a flush sequence from the Commands Menu when the system is not in use.
21) Injection from incorrect vial.	a) Incorrect vial position entered in Samples Menu.	a) Check Samples Menu.
	b) Sample placed in incorrect position in vial tray.	b) Check tray arrangement.
22) Samples injected incorrectly in a pattern.	a) Error in file (calibration vials placement/sample preparation resource placement).	a) Check file (calibration/sample preparation template).
Needle		
23) Needle bent or broken.	a) Incorrect vials.	a) See list of recommended replacement parts in the front of the manual.
	b) Alignment problem.	b) Call Thermo Electron.
	c) Arm sticking.	c) Call your Thermo Electron service representative.
24) Needle plugging.	a) Multiple/incorrect septa.	a) Verify a single septum of type recommended.

Table A.3 Autosampler Hardware Problems, *continued*

Symptom	Possible Problem	Remedy
<u>**Syringe**</u>		
25) Loud buzzing from syringe drive.	a) Defective limit switch.	a) Call your Thermo Electron service representative.
26) Flush valve won't activate.	a) Defective valve/coupler/motor.	a) Call your Thermo Electron service representative.
27) Flush valve actuated, but syringe can't draw flush solvent.	a) Plugged inlet filter in solvent reservoir.	a) Replace inlet filter (Chapter 5).
<u>**Arm**</u>		
28) Hook doesn't lift vial.	a) XYZ arm motor stepping failure.	a) Call your Thermo Electron service representative.
<u>**Leaks**</u>		
29) Leakage during loop filling.	a) Blocked loop or waste line or valve not deflected far enough.	a) Flush sample loop or replace; check and flush waste line. Check the sensor.
30) Cross-port leakage.	a) Worn rotor seal.	a) Replace according to instructions in Chapter 5.
31) Liquid on vial caps.	a) Leaky flush valve.	a) Replace flush valve.
	b) Leaky injector valve.	b) Replace rotor seal (Chapter 5).

Table A.3 Autosampler Hardware Problems

Symptom	Possible Problem	Remedy
Other		
32) An automatic sequence is interrupted, or instrument will not start.	a) Column-oven, heater, door interlock(s) activated.	a) Check the Ready Participation Menu.
33) In Stand Alone mode, Status Screen indicates a run in progress, but the instrument is locked up.	a) Configuration is incorrectly set to SpectraNET mode.	a) In the Configurations Menu, set Mode to Stand Alone.
34) Contaminated sample.	a) Dirty vials.	a) Replace vials.
	b) Improper septa made of silicone or other rubber-like material.	b) Use recommended septa. *NOTE: Thermo Electron septa are made of Teflon-faced silicone.*
35) Elevated system pressures.	a) Block between column and autosampler.	a) Crack each fitting and observe pressure. See instructions in Appendix A "Testing for Tubing Restrictions" on page 169.

Display Messages

As we mentioned in Chapter 1, there are three different kinds of messages that can appear on your autosampler's display: user messages, confirmation messages, and error messages. This section describes the possible conditions that generate these messages.

USER MESSAGES

User messages are displayed as a result of an incorrect operation on the part of the user.

`Autosampler Busy`

You can't issue a command from a menu or press a key while the autosampler is in the middle of a previously requested operation.

`Autosampler Busy  No Loading Allowed`

You can't load a sample set while the queue is running.

`Can not add Priority Set  during final calibration`

You cannot add a priority set during bracketing (after all the sample vials have been injected).

`Can not add Priority Set at this time`

You cannot add a priority set to other than set 1. You cannot add a priority set unless the file's Calibration Menu calls for interspersed calibration vials (you must select reuse calibration vials in order to add priority sets to the queue).

`File in Queue Cannot be Copied to`

You cannot copy to a file assigned to a sample set in the queue.

`File in Queue Cannot be Deleted`

You cannot delete a file assigned to a sample set in the queue.

`File in Queue Cannot be Edited`

You cannot edit a file assigned to a sample set in the queue from the Edit Menu.

NOTE: You can edit the run-file copy of the active file from the Samples Menu.

`File Protected Cannot Be Copied To`

You can't copy to a file that is protected (Protect is set to On) in the Configurations Menu.

`Use SAMPLES key to Add Samples to Queue`

You can't start a run by pressing the [RUN] key when the queue is empty. You must first load or add sample set(s) to the queue.

CONFIRMATION MESSAGES

Confirmation messages inform you that the autosampler is completing a requested operation or command. These messages disappear when the operation is complete.

Aligning Arm   The Align Arm command is in progress.

Clearing Mixer   The Clear Mixer command is in progress.

File Copied   The requested file has been copied.

File Deleted   The requested file has been deleted.

File Loaded   The requested file has been loaded into the queue.

Flush Samp Syringe   The Flush Sample Syringe command is in progress.

Flush Prep Syringe   The Flush Prep Syringe command (page 97) is in progress.

Homing Arm   The Home Arm command is in progress.

Initializing   The Initialize Hardware command (page 98) is in progress.

Set Added to Queue   The requested sample set has been added to the sample queue.

ERROR MESSAGES

There are three kinds of errors: 1) hardware errors, 2) system errors, and 3) diagnostics errors. Hardware and system errors are discussed below. Diagnostic errors are discussed in the diagnostics section on page 129.

HARDWARE ERRORS

Hardware errors occur during the normal use and operation of the instrument. Most of the time you will be able to clear them. For some, you may need to call your Thermo Electron service representative. Pressing any key clears the error message. Then take the steps suggested for each message to resolve the problem.

Arm Failed to Home on X, Y, Z

The XYZ arm could not make the final X-, Y-, or Z-axis motion required return to the home position. Check for a dislodged vial, tray, or other obstruction. Run the Initialize Hardware routine. If the error persists, contact your Thermo Electron service representative.

Arm Jam on X, (Y, Z) Axis

The XYZ arm is obstructed on its left-to-right (X) axis; its front-to-back (Y) axis; or its vertical (Z) axis. Check for a dislodged vial, tray, or other obstruction. Run the Initialize Hardware routine. If the error persists, contact your Thermo Electron service representative.

Flush Valve Misaligned	The flush valve did not turn all the way to the required position. Perform the Initialize Hardware routine. If the error persists, run the Test Connectors routine. If the error persists, contact your Thermo Electron service representative.
Hardware Failure	A problem occurred with the valves or syringes. Run the Initialize Hardware diagnostic on page 98 and/or the Injector Test (page 98). If you are unable to clear the error, contact your Thermo Electron service representative.
Hardware Not Homed	The solvent valve was requested to move to a certain position, but the hardware was not initialized properly. Or, on powerup, the solvent valve could not home properly. Run the Initialize Hardware diagnostic on page 98. If you are unable to clear the error, contact your Thermo Electron service representative.
Hardware Timeout	A requested mechanical operation was not completed in the allotted time. The problem could be with the arm, valves, or syringe(s). Run the Initialize Hardware routine on page 98. If the problem persists, try manually flushing the syringe(s) using the Flush Sample (page 97) and Flush Prep Syringe (page 97) commands. If the problem persists, contact your Thermo Electron service representative.
Injector Valve Failure	The injector valve failed to move (between the INJECT and FILL positions, or vice versa) for the requested operation. Issue the Injector command from the Commands Menu. If the error persists, call your Thermo Electron service representative.
Inject Valve Misaligned	The injector valve is positioned between the INJECT and FILL positions. Run the Injector test (page 98), and call your Thermo Electron service representative if the error persists.
IValve Move Is Impossible	The injector valve cannot move to the requested position from its current position. Run the move the injector valve to another position using the Injector command, and call your service representative if the error persists.
IValve Move Not Detected	The system did not detect a requested movement to the required position. Check for a loose connection to the injector. Try to manually move the injector to another position. If the error persists, call your service representative .
Inject Valve Overshoot	The injector valve moved past the desired position. Run the Injector test (page 98), and call your Thermo Electron service representative if the error persists.
Injector Valve Undershoot	The injector valve failed to move far enough to the desired position. Run the Injector test (page 98), and call your Thermo Electron service representative if the error persists.
Parameter Out of Range	A value too large or too small was entered. For example, an injection volume too large for the installed syringe was entered. Check the installed syringe/sample loop and versus the edit file parameters.

`Solvent Valve` `Misaligned`	The 6-port solvent selection valve failed to turn far enough to reach the next required port or turned past the next required port. Issue the Initialize Hardware command on page 98. If the error persists, issue the Flush Prep Syringe (page 97) using various external solvent positions (S-1, S-2, S-3, S-4). This message appears only on instruments with the Sample Preparation Option installed.
`SValve Not Present`	A Load or Flush Prep Syringe command was issued without the Sample Preparation Option installed. The Configurations Menu incorrectly indicates that the Sample Preparation Option is installed (Sample Prep Yes). Check the Configurations Menu under OPTIONS. If the problem persists, call your Thermo Electron service representative.
`Syr 1(2) Not Enough to` `Expel`	One of the syringe(s) is not retracted far enough to expel the requested volume in the ADD step (sample preparation syringe) or flush operation (both syringes). Run the Initialize Hardware test (page 98), and restart the run (sample preparation) or the flush cycle.
`Syringe Failure`	A syringe failed to moved to the appropriate position required for an operation. Run the Initialize Hardware test (page 98). If the error repeats, call your Thermo Electron service representative.
`Syringe Overfill`	A volume has been requested for injection or ADD that is too large for the size of the installed syringe. Check the method and syringe volume(s).
`TrayTemp Failure`	The Tray Temperature Control Option was unable to reach the desired set point and has turned itself off returning the instrument to a READY state. Any method loaded will be executed without the Tray Temperature Control Option. Pressing any key clears the error. This error indicates a problem with the Tray Temperature Control Option. Call your Thermo Electron service representative for assistance.
`Unexpected Vial in` `Hook`	This message appears if a vial is found in the hook where no vial is expected for the requested operation. To clear the error, remove the vial and restart or resume the requested operation.
`Vial Not Found in Hook`	This message appears if a vial is not found in the hook when a vial is expected. Verify that a vial exists in the requested tray location, check the file and vial assignments in the Samples Menu, and check to see if a vial is lodged in the sample tower. (If so, the needle may be bent.)

SYSTEM ERRORS

System errors occur very rarely and abort normal operation. Since they are not usually problems you can correct, we will list some of them, but not describe them in detail. Should you see any of the following messages or other messages not described in this section, document them, and contact your Thermo Electron service representative for assistance.

> Invalid Current Location
> Invalid Command
> Not in GET/RET Sequence
> Z Must be Up/No Vial
> Move Z Not Allowed
> Get LocNum: Bad Row, Col
> Zup Only in Serv Block
> Invalid Target Location
> SValve Extra Interrupt
> Bad Vial Number
> Unexpected Syr Event

Testing for Tubing Restrictions

If there is a restriction in the solvent lines, you will probably hear the a loud grinding sound from the syringe-drive mechanism due to increased resistance to syringe travel.

NOTE: Before beginning this procedure, be sure to set up a beaker or other container to catch the solvent.

To isolate a block to a section of tubing:

1. Place the Autosampler injection valve into the FILL position (press [MENU], COMMANDS, Injector Test, and use the [+] and [-] keys to select /Fill/).

2. Disconnect the injection valve-to-flush valve tubing at the injection valve. Initiate a flush sequence (press [MENU], /COMMANDS/, /Flush Sample Syringe/, page 97 or /Flush Prep Syringe/, page 97. Solvent will flow freely on the return stroke of the plunger if there are no restrictions present. Flow restriction is unlikely at this point due to the large internal tubing diameter.

3. Reconnect the tubing.

4. Disconnect the needle and check the flow.

5. If no restriction is found, reattach all of the tubing, and flush the syringe to verify that the restriction still exists.

6. If a restriction is still present, remove and clean the sample needle as described in Chapter 5, page 143.

B

Glossary

Introduction

We have included a glossary to define certain technical terms used throughout the manual's text. These terms should be consistent with standard definitions used throughout the analytical industry, and are added here as a quick reference only.

A

active set/vial   The sample set or vial currently being processed. The active vial refers to the vial currently being injected or currently undergoing sample preparation.

Add   1) A step used in sample preparation used to deliver all Pickup volumes and the Load volume to the target vial. 2) The process of putting a sample set into the sample queue.

B

backlash   The slack in the syringe and its drive mechanism.

C

calibration interval   The number of sample vials to be injected between calibration-vial injection(s).

calibration level   The number of different concentration levels used in the calibration series.

calibration vial   A vial that contains a known concentration of the compound(s) of interest; a standard.

cycle time   The time between autosampler injections.

D

dead volume   The volume from the solvent-selection valve to the autosampler needle tip.

decrement   Press the [-] key to decrease the value of a parameter.

diluent   The solvent or buffer used to dilute a sample. On the autosampler, the volume of diluent is specified in the Load step of all of the sample preparation templates.

dilution cycle   A preset sequence used in the sample preparation templates that transfers solvents, sample, and reagents from the solvent bottle(s) or source vial(s) to the target vial(s).

E

extra vials   In sample preparation, an empty or extra reagent vial where samples and solvents are mixed; an empty reaction vial. It is always placed in relationship to the sample vial (Sam+1, Sam+2, Sam+3).

F

file/method   "File" and "method" are used interchangeably to describe the autosampler run parameters. However, the word "file" is often used in a more specific sense to mean the autosampler files in which the parameters are stored.

flush solvent   The solvent used to wash the internal tubing, syringes, and sample needle after an injection. This solvent should be of sufficient strength to easily remove the small amounts of sample that can cling to the tubing walls.

full-loop injection   A technique that injects a sample volume equal to the loop volume.

G

guard column   A small column placed just before the separation column. The guard column is used to remove contaminants that might otherwise damage the main column or interfere with the separation.

H

holding loop   The isolated section of connecting line used in sample preparation to hold measured amounts of sample, reagents, and solvents until they are delivered into a vial.

home   The position to the left of the rear-left corner of the sample tub where the XYZ arm resides when it is not transporting a vial.

hook	The attachment on the end of the pod that moves a vial up and down (along the Z axis). The hook lifts the vial from the sample tray and holds it during transport to the sample tower for injection.

I

increment	Press the [+] key to increase the value of a parameter.
initialization	The process that takes place between dilution cycles that brings the autosampler to a "known" state. During this process the lines and syringes are automatically flushed with flush solvent. Any residual resources present from a previous dilution cycle are flushed out of the lines to prepare the autosampler for the next injection.

J - L

linear dilution	A dilution sequence in which resource A (buffer) is added to resource B, C, and D.
loading a file	Loading a file copies the original edit file into the run-file location to be run. The original edit file remains.

M

method	A group of parameters applied to one or more vials.
mobile phase	In HPLC, the liquid used to move the sample through the column.

N - O

open-collector outputs	An output, usually a transistor, that has no connection to the collector.

P

PickLg	A step used in the sample preparation templates that retracts the prep syringe to draw a large volume of solvent or buffer into the holding loop.
Pickup	A step used in the sample preparation templates that retracts the sample syringe to draw a small volume of sample or reagent into the holding loop.
prep syringe	The large 2.5 mL sample preparation syringe used in to transfer large volumes of liquid (usually buffer or solvents).
pod	The autosampler component attached to the XYZ arm that contains the hook.

precision	The degree to which results are reproducible (analysis of the same sample yields similar results).
prime	The process that flushes lines with flush solvent, etc. Priming is done during installation and whenever a syringe is replaced.
PCB	A printed circuit board.
pull-loop injection	Variable-loop injection.
pull-up resistor	Resistors used to pull inputs or nonactive open-collector outputs to "Hi" states.

Q

queue	One or more vial sequences/sets subjected to one or more methods run in a series that use(s) the same column and solvents. The sample queue lists the order and file name for each sample set to be processed during a given run.

R

reagent transfer step	In a sample preparation program, a step used to deliver solvents, reagents, or samples from one location (vial or solvent bottle) to another (target or sample vial).
real-time editing	The ability to edit the run file for a run currently in progress. On the SpectraSYSTEM autosamplers, this function is provided by the [SAMPLES] key; on other SpectraSYSTEM instruments, this function is accessed by pressing the [STATUS] key.
recalibration	The reinjection of the calibration vial(s) after the injection of each series of sample vials within a sample set.
recalibration interval	The number of sample vials injected between each calibration series injection. The value in the Samples/Calibration field.
resource	One of the samples, calibration vials, reagents, or solvents used in an autosampler run.
routine	The type of order in which calibration vials are arranged within a sample set. Calibration vials may be reused throughout the set (Reuse same vials) or they may be interspersed (Intersperse) among the sample vials in the sample tray.
run file	The copy of the edit file that has been loaded and the file that is currently running. From the Samples Menu, changes can be made to the run file during a run, but those changes can't be saved until after the run has completed. Also, changes can't be copied to a file that is in use (assigned to a sample set in the queue).

S

sample needle
The needle that pierces the septum of the vial cap and through which vial contents are drawn into the holding loop.

sample syringe
The 250 μL syringe. In sample preparation, this syringe is used in sample preparation to transfer small volumes of liquid (usually samples or reagents).

sample tower
The autosampler location and component where injection takes place.

sample tub
The autosampler compartment that holds the sample trays.

sample tray
One of the three racks that holds the sample vials in position in the autosampler.

sampling valve
The valve through which sample is injected onto the column.

sample vial
The container that holds the sample in the sample tray. Sample vials must have a 12 mm diameter.

set
A group of samples vials and associated calibration vials run with a particular method.

solvent-selection valve
The 6-port valve that regulates the flow of solvents and air into the sample holding loop. This valve allows the flow of waste out of the loop.

solvent reservoir
One of the four bottles on the back of the autosampler that contains the external solvents used in sample preparation. The solvent in the first position usually contains the buffer.

standard
A vial that contains a known concentration of sample. Peaks generated from this vial are used to plot the calibration curve used to determine the concentration of the compound of interest. (See also calibration vial.)

syringe refill and loop-isolation valve
The valve in the sample preparation system that regulates the flow of liquid from the syringes.

T - V

template
In sample preparation, one of the four file formats that contain a unique, preset, unchangeable sequence of steps used to carry out a series of sample preparation procedures. The four types of templates include simple dilution, heating, extraction, and heating and extraction.

W - X

Wait

A step used in the sample preparation templates that pauses all autosampler activity. This step may be used to allow a mixture to settle or to react.

X

In the Calibration Menu's Calib 1 (of X) field, X indicates the number of calibration levels in the calibration sequence. X is not visible on the screen; it is used for discussion purposes only.

XYZ arm

Also referred to as the "arm," this component carries the sample vials to and from the sample tray and to the various workstations (sample tower, heater mixer) in the autosampler.

Y - Z

Z

In the sample preparation templates, the variable used to represent the number of clean (extra) vials subsequent to the sample vial. Z equals 1, 2, or 3.

C

Kits and Parts Lists

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

Clear Vials and Vial Kits

Shell Vials

A4946-010	Shell Vials, 100/package
A4948-010	Shell Vial Kit

Standard Vials

A4951-010	Standard Vials, 100/package
A4952-010	Standard Vial Septa, 100/package
A4953-010	Standard Vial Caps, 100/package
A4954-010	Standard Vial Kit, 100/package

Crimp Vials/Caps

A4955-010	Crimp Caps, 100/package
A4956-010	Crimp Vials, 100/package, 1.8 mL
A4957-010	Crimp Vial Kit, 100/package

Poly Kits

A4958-010	Polypropylene Kits, 600 mL, 100/package
A4959-010	Polypropylene Kits, 100 mL, 100/package

Inserts

A4981-010	Spring for Insert, 100/package
A4960-010	Insert, 50 mL, 100/package
A4961-010	Insert, 100 mL, 100/package

Amber Vials and Vial Kits

Crimp Vials

A4964-010	Amber Crimp Vials, 100/package
A4965-010	Amber Vial Crimp Kit, 100/package

Standard Vials

A4966-010	Standard Amber Vials, 100/package
A4967-010	Standard Amber Vial Kit, 100/package

PFTE Liners/Septa

A4968-010	PTFE Liners, 100/package
A4969-010	PTFE Vial Caps/Septa, 100/package
A4970-010	Standard Vial Station

Sample Trays

A3695-010	Sample Trays (3), 40-vial capacity
A3696-010	Sample Trays (3), 35-vial capacity
	(For autosamplers with Tray Temperature Control.)

Sample Trays

A3695-010	Sample Trays (3), 40-vial capacity

| A3696-010 | Sample Trays (3), 35-vial capacity |
| | (For autosamplers with the Peltier cooling option/upgrade.) |

Cables and Adapters

A3981-010	**Autosampler-to-integrator Cable/Adapter**
A3663-010	**BCD Communications Option**
	(This option requires field installation by Thermo Electron unless purchased simultaneously with a SpectraSYSTEM autosampler.)

Standard Autosamplers

| A3566-010 | **Injector Valve Assembly for the AS1000 autosamplers, 20 mL, SS** |
| | (Assembly includes the complete autosampler valve assembly.) |

| A3566-030 | **Injector Valve Assembly for the AS3000, 100 mL, SS** |
| | (Assembly includes the complete autosampler valve assembly.) |

| A4101-010 | **Standard Maintenance Kit** |
| | (Kit includes everything necessary to maintain the instrument for one year: inlet filter, needle replacement kit, a valve rotor seal, syringe, lubricant, cleaner and an LC test sample.) |

| A4051-010 | **Standard Fittings Kit** |
| | (Kit includes stainless steel fittings and tubing used in a SpectraSYSTEM LC system.) |

| A4719-010 | **Autosampler Needle Replacement Kit** |
| | (Kit includes the standard, stainless-steel, deflected-point replacement needle.) |

| A4719-020 | **Autosampler Needle Replacement Kit** |
| | (Kit includes the standard, inert, deflected-point replacement needle.) |

Standard Autosamplers, continued

Standard Sample Loops and Syringes

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

Test Mixes

A4991-010	Autosampler Test Mix
A5135-010	Autosampler Dilution Test Mix

Inert/Biocompatible Autosamplers

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

Inert/Biocompatible Sample Loops and Syringes

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

Test Mixes

A4991-010	Autosampler Test Mix
A5135-010	Autosampler Dilution Test Mix

Upgrade Kits

Upgrade kits are available for SpectraSYSTEM and SpectraSERIES stainless steel and inert/biocompatible autosamplers. Contact your local Thermo Electron Representative for details.

INDEX

D

S