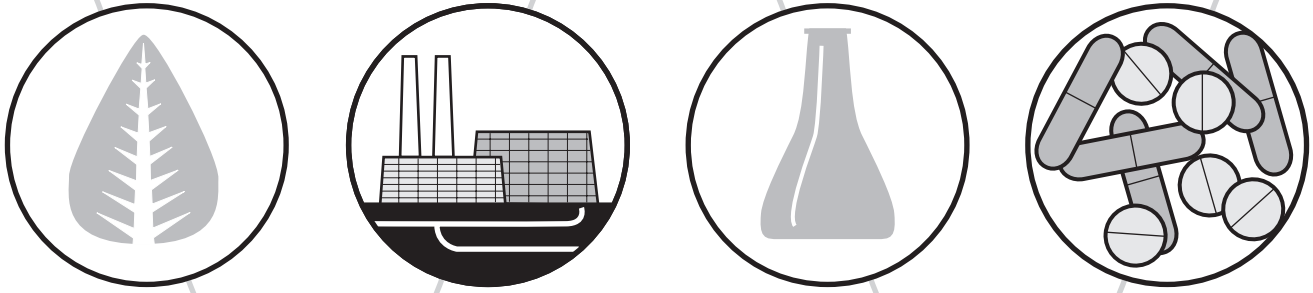
Waters 600E Multisolvent Delivery System

User's Guide



Waters

34 Maple Street
Milford, MA 01757

WAT174-02TP, Revision 3

NOTICE

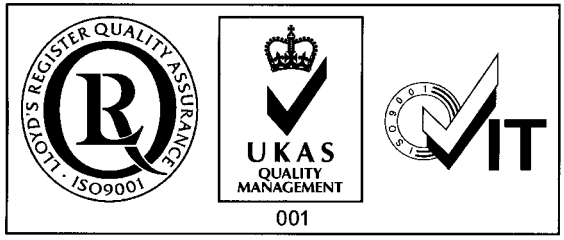
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Note: *When you use the instrument, follow generally accepted procedures for quality control and methods development.*

If you observe a change in the retention of a particular compound, in the resolution between two compounds, or in peak shape, immediately determine the reason for the changes. Until you determine the cause of a change, do not rely on the separation results.

Note: *The Installation Category (Overvoltage Category) for this instrument is Level II. The Level II Category pertains to equipment that receives its electrical power from a local level, such as an electrical wall outlet.*



Attention: *Changes or modifications to this unit not expressly approved by the party responsible for compliance could void the user's authority to operate the equipment.*

Important : *Toute modification sur cette unité n'ayant pas été expressément approuvée par l'autorité responsable de la conformité à la réglementation peut annuler le droit de l'utilisateur à exploiter l'équipement.*

Achtung: *Jedwede Änderungen oder Modifikationen an dem Gerät ohne die ausdrückliche Genehmigung der für die ordnungsgemäße Funktionstüchtigkeit verantwortlichen Personen kann zum Entzug der Bedienungsbefugnis des Systems führen.*

Avvertenza: *eventuali modifiche o alterazioni apportate a questa unità e non espressamente approvate da un ente responsabile per la conformità annulleranno l'autorità dell'utente ad operare l'apparecchiatura.*

Atención: *cualquier cambio o modificación efectuado en esta unidad que no haya sido expresamente aprobado por la parte responsable del cumplimiento puede anular la autorización del usuario para utilizar el equipo.*

注意：未經有關法規認證部門允許對本設備進行的改變或修改，可能會使使用者喪失操作該設備的權利。

注意：**未经有关法规认证部门明确允许对本设备进行的改变或改装，可能会使使用者丧失操作该设备的合法性**。

주의 : 기기 검교정 담당자의 승인 없이 무단으로 기기를 변경 또는 수정하는 경우에는, 그 기기 운영에 대한 허가가 취소될 수 있습니다.

注意：規制機関から明確な承認を受けずに本装置の変更や改造を行うと、本装置のユーザとしての承認が無効になる可能性があります。



Caution: *Use caution when working with any polymer tubing under pressure:*

- *Always wear eye protection when near pressurized polymer tubing.*
- *Extinguish all nearby flames.*
- *Do not use tubing that has been severely stressed or kinked.*
- *Do not use nonmetallic tubing with tetrahydrofuran (THF) or concentrated nitric or sulfuric acids.*
- *Be aware that methylene chloride and dimethyl sulfoxide cause nonmetallic tubing to swell, which greatly reduces the rupture pressure of the tubing.*

Attention : *Manipulez les tubes en polymère sous pression avec precaution:*

- *Portez systématiquement des lunettes de protection lorsque vous vous trouvez à proximité de tubes en polymère pressurisés.*
- *Eteignez toute flamme se trouvant à proximité de l'instrument.*
- *Evitez d'utiliser des tubes sévèrement déformés ou endommagés.*
- *Evitez d'utiliser des tubes non métalliques avec du tétrahydrofurane (THF) ou de l'acide sulfurique ou nitrique concentré.*
- *Sachez que le chlorure de méthylène et le diméthylesulfoxyde entraînent le gonflement des tuyaux non métalliques, ce qui réduit considérablement leur pression de rupture.*

Vorsicht: *Bei der Arbeit mit Polymerschläuchen unter Druck ist besondere Vorsicht angebracht:*

- *In der Nähe von unter Druck stehenden Polymerschläuchen stets Schutzbrille tragen.*
- *Alle offenen Flammen in der Nähe löschen.*
- *Keine Schläuche verwenden, die stark geknickt oder überbeansprucht sind.*
- *Nichtmetallische Schläuche nicht für Tetrahydrofuran (THF) oder konzentrierte Salpeter- oder Schwefelsäure verwenden.*
- *Durch Methylenchlorid und Dimethylsulfoxid können nichtmetallische Schläuche quellen; dadurch wird der Berstdruck des Schlauches erheblich reduziert.*

Attenzione: *prestare attenzione durante l'utilizzo dei tubi di polimero pressurizzati:*

- *Indossare sempre occhiali da lavoro protettivi nei pressi di tubi di polimero pressurizzati.*
- *Estinguere ogni fonte di ignizione circostante.*
- *Non utilizzare tubi soggetti che hanno subito sollecitazioni eccessive o son stati incurvati.*
- *Non utilizzare tubi non metallici con tetraidrofurano (THF) o acido solforico o nitrico concentrato.*
- *Tenere presente che il cloruro di metilene e il dimetilsolfossido provocano rigonfiamento nei tubi non metallici, riducendo notevolmente la resistenza alla rottura dei tubi stessi.*

Advertencia: *se recomienda precaución cuando se trabaje con tubos de polímero sometidos a presión:*

- *El usuario deberá protegerse siempre los ojos cuando trabaje cerca de tubos de polímero sometidos a presión.*
- *Si hubiera alguna llama las proximidades.*
- *No se debe trabajar con tubos que se hayan doblado o sometido a altas presiones.*
- *Es necesario utilizar tubos de metal cuando se trabaje con tetrahidrofurano (THF) o ácidos nítrico o sulfúrico concentrados.*
- *Hay que tener en cuenta que el cloruro de metileno y el sulfóxido de dimetilo dilatan los tubos no metálicos, lo que reduce la presión de ruptura de los tubos.*

警告：當在有壓力的情況下使用聚合物管線時，小心注意以下幾點：

- 當接近有壓力的聚合物管線時一定要戴防護眼鏡。
- 熄滅附近所有的火焰。
- 不要使用已經被壓癟或嚴重彎曲管線。
- 不要在非金屬管線中使用四氫呋喃或濃硝酸或濃硫酸。
- 要了解使用二氯甲烷及二甲基亞楓會導致非金屬管線膨脹，大大降低管線的耐壓能力。

警告：当在有压力的情况下使用管线时，小心注意以下几点：

- 当接近有压力的聚合物管线时一定要戴防护眼镜。
- 熄灭附近所有的火焰。
- 不要使用已经被压瘪或严重弯曲的管线。
- 不要在非金属管线中使用四氢呋喃或浓硝酸或浓硫酸。
- 要了解使用二氯甲烷及二甲基亚枫会导致非金属管线膨胀，大大降低管线的耐压能力。

경고: 폴리머재질의 튜빙을 압력하에서 사용할 때는 다음 사항에 유의하십시오.

- 압력을 받은 폴리머 튜빙 부근에서는 반드시 보호안경을 착용할 것
- 모든 화기의 접근을 금함
- 늘리거나 뒤틀린 튜빙은 사용하지 말 것
- 비금속 튜빙을 테트라히드로퓨란(THF)이나 염산 및 황산과 함께 사용하지 말 것
- 디글로로메탄(methylene chloride)와 디메틸설폭시드(dimethyl sulfoxide)는 비금속 튜빙을 팽창시켜 쉽게 파열되므로 주의할 것

警告：ポリマーチューブに圧力をかけて取り扱う場合は、次のように注意してください。

- 加圧したポリマーチューブの付近では、常に保護めがねを着用してください。
- 付近の火はすべて消してください。
- 激しい応力やねじれを受けたチューブは使用しないでください。
- テトラヒドロフラン（THF）、濃硝酸、あるいは濃硫酸には、非金属製のチューブを使用しないでください。
- ジクロロメタンやジメチルスルホキシドは非金属製のチューブを膨張させ、チューブの破断圧力を大幅に低下させますので、注意してください。



Caution: *The user shall be made aware that if the equipment is used in a manner not specified by the manufacturer, the protection provided by the equipment may be impaired.*

Attention : *L'utilisateur doit être informé que si le matériel est utilisé d'une façon non spécifiée par le fabricant, la protection assurée par le matériel risque d'être défectueuses.*

Vorsicht: *Der Benutzer wird darauf aufmerksam gemacht, dass bei unsachgemäßer Verwenddung des Gerätes unter Umständen nicht ordnungsgemäß funktionieren.*

Attenzione: *l'utente deve essere al corrente del fatto che, se l'apparecchiatura viene usta in un modo specificato dal produttore, la protezione fornita dall'apparecchiatura potrà essere invalidata.*

Advertencia: *el usuario deberá saber que si el equipo se utiliza de forma distinta a la especificada por el fabricante, las medidas de protección del equipo podrían ser insuficientes.*

警告：使用者必須非常清楚如果設備不是按照制造廠商指定的方式使用，那么該設備所提供的保護將被消弱。

警告: **使用者必须非常清楚如果设备不是按照制造厂商指定的方式使用, 那么该设备所提供的保护将被消弱**

경고 : 제조사가 지정한 것 이외의 방법으로 기기를 사용하는 경우에는, 사용자가 위험으로부터 보호될 수 없는 경우가 발생할 수 있음에 유념하십시오.

警告: ユーザは製造業者が指定していない方法で装置を使用した場合は装置が提供する保護が損なわれることがあるということを承知しているものとします。



Caution: *To protect against fire hazard, replace fuses with those of the same type and rating.*

Attention : *Remplacez toujours les fusibles par d'autres du même type et de la même puissance afin d'éviter tout risque d'incendie.*

Vorsicht: *Zum Schutz gegen Feuergefahr die Sicherungen nur mit Sicherungen des gleichen Typs und Nennwertes ersetzen.*

Attenzione: *per una buona protezione contro i rischi di incendio, sostituire i fusibili con altri dello stesso tipo e amperaggio.*

Advertencia: *sustituya los fusibles por otros del mismo tipo y características para evitar el riesgo de incendio.*

警告：為了避免火災的危險，應更換同种類型及規格的保險絲。

警告: 为了避免火灾的危险, 应更换同种类型及规格的保险丝。

경고 : 화재를 방지하기 위해서는 퓨즈 교체 시 같은 종류, 같은 등급의 것을 사용하십시오.

警告: 火災の危険防止のために、ヒューズの交換は同一タイプおよび定格のもので行なってください。



Caution: *To avoid possible electrical shock, disconnect the power cord before servicing the instrument.*

Attention : *Afin d'éviter toute possibilité de commotion électrique, débranchez le cordon d'alimentation de la prise avant d'effectuer la maintenance de l'instrument.*

Vorsicht: *Zur Vermeidung von Stromschlägen sollte das Gerät vor der Wartung vom Netz getrennt werden.*

Attenzione: *per evitare il rischio di scossa elettrica, scollegare il cavo di alimentazione prima di svolgere la manutenzione dello strumento.*

Precaución: *para evitar descargas eléctricas, desenchufe el cable de alimentación del instrumento antes de realizar cualquier reparación.*

警告：要避免觸電，請在修理或保養器材前把電源線拔出。

警告：为避免可能引起得触电危险，在修理前请切断电源连接。

경고: 전기 충격의 가능성을 피하기 위해서는, 기기를 수리하기 이전에 전원 코드를 차단하십시오.

警告：感電の危険性を避けるために、装置の保守を行う前には装置の電源コードを引き抜いてください。

Commonly Used Symbols

⎓	Direct current Courant continu Gleichstrom Corrente continua Corriente continua 直流電 直流电 직류 直流
~	Alternating current Courant alternatif Wechselstrom Corrente alternata Corriente alterna 交流電 交流电 교류 交流
⏚	Protective conductor terminal Borne du conducteur de protection Schutzleiteranschluss Terminale di conduttore con protezione Borne del conductor de tierra 保護的導線端子 保护性的接地端 보호 도체 단자 接地

Commonly Used Symbols (Continued)

	Frame or chassis terminal Borne du cadre ou du châssis Rahmen- oder Chassisanschluss Terminale di struttura o telaio Borne de la estructura o del chasis 結構或底盤端子 机架或底盘接地端 프레임 또는 틀 단자 フレームまたはシャーシアース
	Caution or refer to manual Attention ou reportez-vous au guide Vorsicht, oder lesen Sie das Handbuch Prestare attenzione o fare riferimento alla guida Actúe con precaución o consulte la guía 小心或查閱手冊 小心或查阅手册 경고 또는 사용설명서 참조 警告またはマニュアルを参照
	Caution, hot surface or high temperature Attention, surface chaude ou température élevée Vorsicht, heiße Oberfläche oder hohe Temperatur Attenzione, superficie calda o elevata temperatura Precaución, superficie caliente o temperatura elevada 警告，熱表面或高溫 警告,热表面或高温 경고, 뜨거운 표면 또는 고온 警告、熱くなっている面、あるいは高温

Commonly Used Symbols (Continued)

	Caution, risk of electric shock (high voltage) Attention, risque de commotion électrique (haute tension) Vorsicht, Elektroschockgefahr (Hochspannung) Attenzione, rischio di scossa elettrica (alta tensione) Precaución, peligro de descarga eléctrica (alta tensión) 警告，小心触電（高壓電） 警告,小心触电（高压电） 경고, 전기충격의 위험 (고압) 警告、電気ショックの危険性（高電圧）
	Caution, risk of needle-stick puncture Attention, risques de perforation de la taille d'une aiguille Vorsicht, Gefahr einer Spritzenpunktierung Attenzione, rischio di puntura con ago Precaución, riesgo de punción con aguja 警告，小心尖狀物刺傷 警告,小心尖状物刺伤 경고, 뾰족한 것으로부터의 상해 위험 警告、ニードルで穴をあける危険性
	Caution, ultraviolet light Attention, rayonnement ultrviolet Vorsicht, Ultraviolettes Licht Attenzione, luce ultravioletta Precaución, emisiones de luz ultravioleta 警告，紫外光 警告,紫外光 경고, 자외선 警告、紫外線

Commonly Used Symbols (Continued)

	Fuse Fusible Sicherung Fusibile Fusible 保險絲 保险丝 퓨즈 ヒューズ
1	Electrical power on Sous tension Netzschalter ein Alimentazione elettrica attivata Alimentación eléctrica conectada 開啓電源 接通电源 전원 켜기 電源オン
0	Electrical power off Hors tension Netzschalter aus Alimentazione elettrica disattivata Alimentación eléctrica desconectada 關閉電源 切断电源 전원 끄기 電源オフ

United States – FCC Emissions Notes

This device complies with Part 15 of the FCC Rules. Operation is subject to the following two conditions: (1) this device may not cause harmful interference, and (2) this device must accept any interference received, including interference that may cause undesired operation.

Changes or modifications to this unit not expressly approved by the party responsible for compliance could void the user's authority to operate the equipment.

Note: *This equipment has been tested and found to comply with the limits for a Class B digital device, pursuant to Part 15 of the FCC Rules. These limits are designed to provide reasonable protection against harmful interference in a residential installation. This equipment generates, uses, and can radiate radio frequency energy and, if not installed and used in accordance with the instructions, may cause harmful interference to radio communications. However, there is no guarantee that interference will not occur in a particular installation. If this equipment does cause harmful interference to radio or television reception, which can be determined by turning the equipment off and on, the user is encouraged to try to correct the interference by one or more of the following measures:*

- *Reorient or relocate the receiving antenna.*
- *Increase the separation between the equipment and receiver.*
- *Connect the equipment into an outlet on a circuit different from that to which the receiver is connected.*
- *Consult the dealer or an experienced radio TV technician for help.*

Shielded cables must be used with this unit to ensure compliance with the Class B FCC limits.

Canada – Spectrum Management Emissions Notes

Cet appareil numérique de la classe B est conforme à la norme NMB-003.

This Class B digital apparatus complies with Canadian ICES-003.

600E Pump Information

Intended Use

When you develop methods, follow the “Protocol for the Adoption of Analytical Methods in the Clinical Chemistry Laboratory,” *American Journal of Medical Technology,* 44, 1, pages 30–37 (1978). This protocol covers good operating procedures and techniques necessary to validate system and method performance.

Biological Hazard

When you analyze physiological fluids, take all necessary precautions and treat all specimens as potentially infectious. Precautions are outlined in “CDC Guidelines on Specimen Handling,” *CDC – NIH Manual,* 1984.

Calibration

Follow acceptable methods of calibration with pure standards to calibrate methods. Use a minimum of five standards to generate a standard curve. The concentration range should cover the entire range of quality-control samples, typical specimens, and atypical specimens.

Quality Control

Routinely run three quality-control samples. Quality-control samples should represent subnormal, normal, and above-normal levels of a compound. Ensure that quality-control sample results are within an acceptable range, and evaluate precision from day to day and run to run. Data collected when quality-control samples are out of range may not be valid. Do not report this data until you ensure that chromatographic system performance is acceptable.

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Preface

The *Waters 600E Multisolvent Delivery System User's Guide* describes the features and use of the Waters® 600E Multisolvent Delivery System. This guide also includes information on the use of these systems with other Waters components, including autosamplers, detectors, and computer control/data management systems.

This guide is intended for use by a wide variety of HPLC system users whose familiarity with chromatography systems ranges from novice to expert.

Organization

This guide contains the following:

Chapter 1 describes the system modules and features.

Chapter 2 describes the powerup and power-down procedures, running diagnostics, using the controller front panel, and controller modes of operation.

Chapter 3 provides procedures for preparing eluents, priming the pump, flushing the system, equilibrating the system, and changing eluents.

Chapter 4 provides instructions for configuring the controller, verifying PowerLine (IEEE-488) devices, and setting up the pump, autoinjector, and detector parameters.

Chapter 5 describes how to make a Direct Control (isocratic) run using the operating conditions you set up in Chapter 4.

Chapter 6 describes the creation of time-based tables for unattended multi-method gradient operation.

Chapter 7 describes how to run method tables.

Chapter 8 provides instructions for configuring the gradient controller and setting up the pump parameters for system control by a data system (Empower or Millennium Chromatography Manager) or for stand-alone operation.

Chapter 9 describes how to make an isocratic run using the operating conditions described in Chapter 8.

Chapter 10 provides instructions for creating time-based tables for unattended gradient operation.

Chapter 11 describes how to run gradient tables.

Appendix A contains 600E system specifications.

Appendix B describes Eluent Cosiderations.

Appendix C contains the Analysis Plan and File Sheet.

Related Documentation

***Waters Licenses, Warranties, and Support*:** Provides software license and warranty information, describes training and extended support, and tells how Waters handles shipments, damages, claims, and returns.

Online Help

A convenient way to look up information while using the 600E System. You access Help by pressing the Help screen key whenever it appears on the controller screens.

Printed Documentation for the Base Product

***Waters 600E System Quick Start Guide*:** Provides concise setup and operational information that is designed to get you up and running right away.

***Waters 600E System Installation and Maintenance Guide*:** Provides installation and maintenance procedures required to set up, troubleshoot, and repair your Waters 600E System. Includes warranty and service information.

Documentation on the Web

Related product information and documentation can be found on the World Wide Web. Our address is http://www.waters.com.

Documentation Conventions

The following conventions can be used in this guide:

Convention	Usage
Italic	Italic indicates information that you supply such as variables. It also indicates emphasis and document titles. For example, “Replace *file_name* with the actual name of your file.”
`Courier`	Courier indicates examples of source code and system output. For example, “The `SVRMGR>` prompt appears.”
`Courier Bold`	Courier bold indicates characters that you type or keys you press in examples of source code. For example, “At the `LSNRCTL>` prompt, enter **`set password oracle`** to access Oracle.”

Convention	Usage
Keys	The word *key* refers to a computer key on the keypad or keyboard. *Screen keys* see the keys on the instrument located immediately below the screen. For example, “The A/B screen key on the 2414 Detector displays the selected channel.”
…	Three periods indicate that more of the same type of item can optionally follow. For example, “You can store *filename1*, *filename2*, … in each folder.”
>	A right arrow between menu options indicates you should choose each option in sequence. For example, “Select File > Exit” means you should select File from the menu bar, then select Exit from the File menu.

Notes

Notes call out information that is helpful to the operator. For example:

Note: *Record your result before you proceed to the next step.*

Attentions

Attentions provide information about preventing damage to the system or equipment. For example:



Attention: *To avoid damaging the detector flow cell, do not touch the flow cell window.*

Cautions

Cautions provide information essential to the safety of the operator. For example:



Caution: *To avoid burns, turn off the lamp at least 30 minutes before removing it for replacement or adjustment.*



Caution: *To avoid electrical shock and injury, unplug the power cord before performing maintenance procedures.*



Caution: *To avoid chemical or electrical hazards, observe safe laboratory practices when operating the system.*

Chapter 1 Introduction

This chapter introduces you to the Waters® 600E Multisolvent Delivery System. It describes the system and each individual system module.

1.1 Waters 600E System Overview

The Waters 600E system is a high-performance liquid chromatography (HPLC) multisolvent delivery system that includes the following features:

- 225 µL pump head volume at 45 mL/min flow rate or 100 µL pump head volume at 20 mL/min flow rate
- Maximum system pressure

Maximum Pressure	Flow Rate
6000 psi (420 kg/cm^2)	10 mL/min
1000 psi (70 kg/cm^2)	45 mL/min

- 0.01 to 45.0 mL/min operating flow rate range adjustable in 0.01 mL/min increments
- Four-eluent gradient capability (Auto•Blend™ method)
- Automatic eluent degassing by helium sparge
- Pump vent valve to facilitate priming and purging of eluent lines and changing eluents
- Rheodyne® 7725i variable-volume, syringe-loading sample injector
- Single keypad control with PowerLine™ system controller
- Methods storage allowing access to previously created gradient, event, autosampler, and detector programs
- Eleven preprogrammed gradient profiles including:
  - Step change at start of segment
  - Step change at end of segment

  - Linear change
  - Four convex gradient curves
  - Four concave gradient curves
- System control of PowerLine detectors and autoinjectors through IEEE-488 interface
- RS-232 serial communications interface for connection to optional integrator
- Optional shelf unit to house system modules
- Polymeric and stainless steel flow path with low-permeability CO_2/O_2 tubing
- Optional column heater

Figure 1-1 illustrates the 600E system. Table 1-1 describes its major modules.

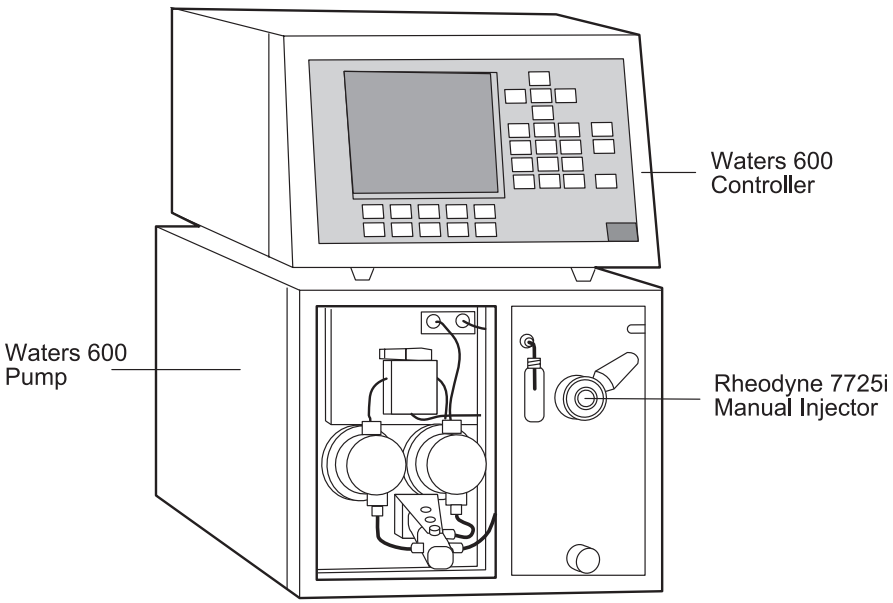


Figure 1-1 Waters 600E System

Table 1-1 Waters 600E System

Module	Description
Waters 600 Controller	Controls eluent gradient, flow rate, external events, and sparging for the Waters 600E system. Provides connection terminals and communication ports for operation with and control of external devices, such as detectors, autosamplers, and data systems.
Waters 600E Pump	Contains components required to blend and to deliver eluents from the eluent reservoir bottles to the injector and column. Consists of the pump with 225 µL or 100 µL volume pump heads, eluent sparge valve assembly, eluent proportioning valve assembly, manual injector, and vent valve.
Rheodyne 7725i Manual Injector Load/Inject Handle	Connects and disconnects the sample loading loop from the system. Allows variable-volume injections from a fraction of a µL to 20 µL without changing loops.

1.2 Waters 600E System Modules

This section discusses the major modules of the Waters 600E system:

- Waters 600 Controller
- Waters 600E Pump

1.2.1 Waters 600 Controller

The Waters 600 controller (Figure 1-2) automatically controls the eluent gradient, the flow rate, column heating, external events, and sparging. You can configure the 600 Controller as a PowerLine system controller or as a Gradient controller (see Section 1.3, PowerLine Controller Features, and Section 1.4, Gradient Controller Features, for details on these controller configurations).

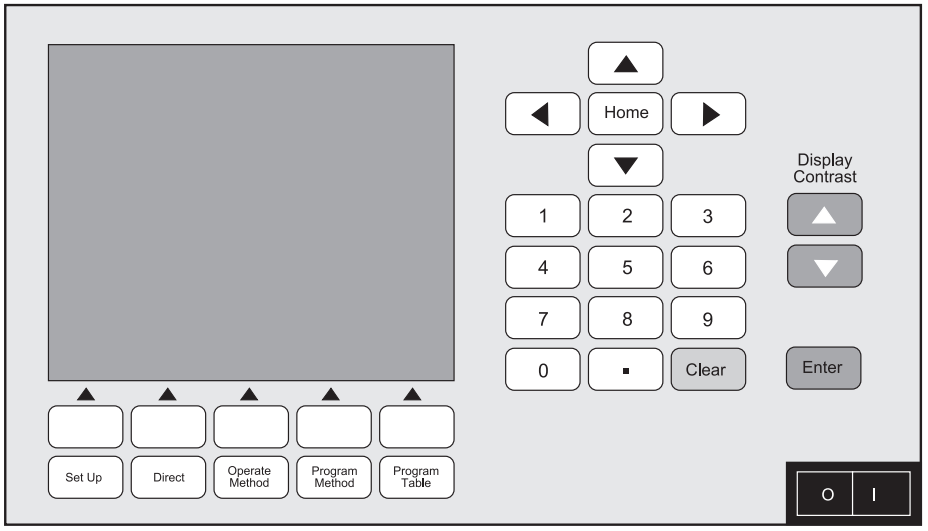


Figure 1-2 Waters 600 Controller Front Panel

The 600 Controller rear panel (Figure 1-3) provides connection terminals and communication ports for operation with external devices.

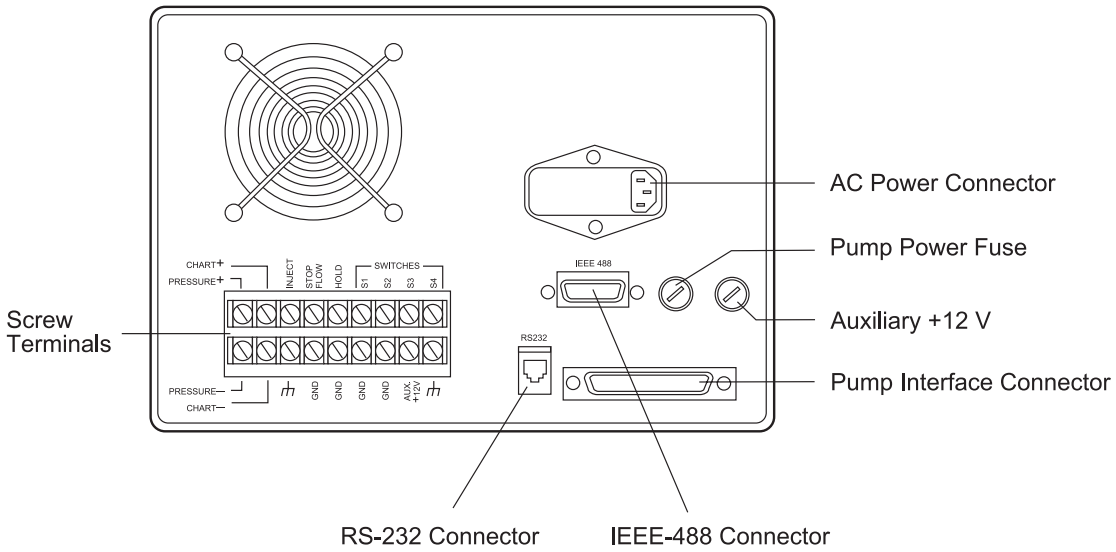


Figure 1-3 Waters 600 Controller Rear Panel

Screw Terminals

The 600 Controller rear panel contains the following screw terminals:

- Pressure
- Chart
- Inject
- Stop Flow
- Hold
- S1 through S4 (event switches)
- Aux +12 V

Pressure/Chart

Function as output terminals (10 mV full scale analog signals). Located on the controller rear panel. Produce plots of system pressure and percentage of eluent composition, respectively, on any standard recorder or integrator.

Inject

Accepts the signal from the free-standing manual injector or autosampler to initiate a run. Located on the controller rear panel.

When you configure the 600 controller as a PowerLine system controller, you initiate operation with an autoinjector through:

- IEEE-488 communications interface (for use with a PowerLine autoinjector)
- Inject terminal (for use with a non-PowerLine autoinjector)

When no injector is being used for the separation, and the method start is initiated from the 600 controller front panel using screen keys, the Inject terminal functions as an output. You can connect fraction collectors, integrators, or other devices that require a start signal to the Inject terminal to synchronize operation with the 600 controller.

Stop Flow/Hold

Both the Stop Flow and the Hold terminals on the controller rear panel are TTL-compatible.

- **Stop Flow input switch** - Allows you to stop the pump unit with a signal from an external device

- **Hold output switch** - Transmits a signal from the 600 controller to an external device when the controller receives a:
  - Stop Flow signal
  - Hold screen key signal
  - Pressure limit error
  - Pump flow rate error

Event Switches/Aux +12 V

Four TTL-compatible connectors (S1 through S4) on the controller rear panel:

- Control column-switching valves, fraction collectors, or similar external devices
- Operate manually or automatically through the 600 controller screens
- Function in conjunction with a built-in auxiliary power supply terminal (Aux +12 V) for devices that require an external power source

1.2.2 Waters 600E Pump

The Waters 600E pump (Figure 1-4 and Figure 1-5) contains the components required to blend and deliver eluents from the reservoir bottles to the injector.

The Waters 600E pump features:

- Four-eluent blending (Auto•Blend)
- Manual, variable-volume injector (optional)
- Vent valve (optional)

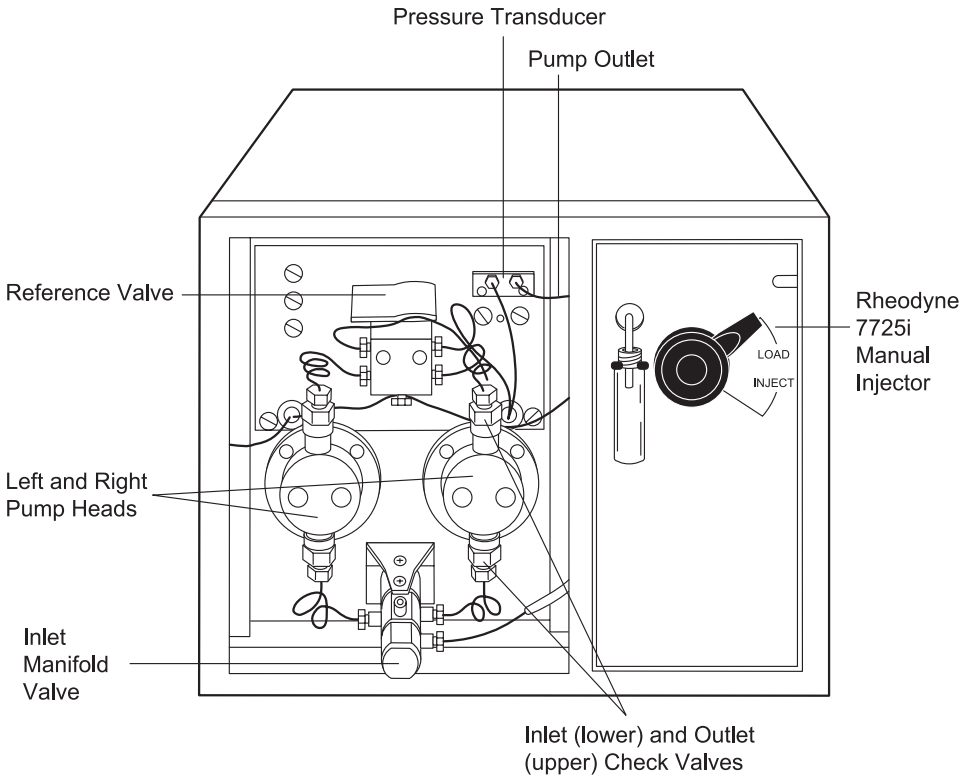


Figure 1-4 Waters 600E Pump (Front View)

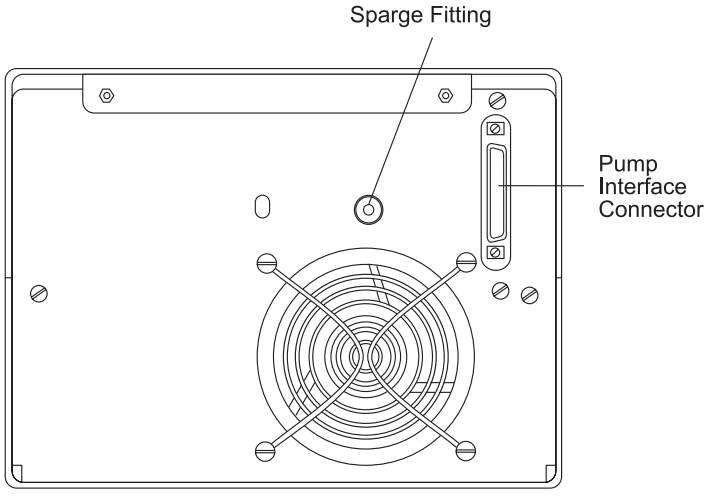


Figure 1-5 600E Pump Rear Panel

Eluent Blending

The gradient proportioning valve blends up to four eluents or buffers in any combination. It produces predictable gradient segments regardless of eluent compressibility and system backpressure.

Eluent selection and proportioning take place on the low-pressure (intake) side of the 600E system.

Flow Path

The pump flow path begins with the eluent reservoirs and ends with the column as follows:

1. In the eluent reservoirs, sparging with helium degasses the eluents.
2. The eluents flow through Teflon tubing to the gradient proportioning valve, which blends the eluents.
3. The inlet manifold directs flow to the two inlet check valves.
4. The pump heads move a volume of 100 or 225 µL with each stroke at a flow rate of 0.01 to 20 or 45 mL/min, depending on the size of the installed pump head.
5. From the pump heads, the eluent converges at a reference valve before flowing into the high pressure noise filter and then to the pressure transducer.
6. Flow continues from the pressure transducer outlet to the injector.
7. Flow continues out of the system to the column and the detector.

Injector

The optional Rheodyne 7725i Manual Injector is designed for variable-volume, full- or partial-loop filling operation.

The volume of the sample injected is determined by the volume of the sample loop plus the valve passages.

Vent Valve

The vent valve on each injector allows you to direct eluent flow from the pump to the column or to divert the eluent flow waste.

1.3 PowerLine Controller Features

PowerLine Controller Description

Use the PowerLine Controller configuration when you want to control other devices from the 600E system over the IEEE-488 bus.

The PowerLine Controller is the default configuration for the Waters 600 controller. You can also configure the controller as a Gradient Controller (see Section 1.4, Gradient Controller Features). PowerLine configuration includes programming methods, external device control, and an RS-232 interface.

The PowerLine Controller:

- Provides automatic setup and control of all gradient, injection (manual and automatic), event, and detection parameters directly from the 600 controller keypad.
- Creates multi-method programs that link time-based control tables. Each time-based table comprises a gradient table, an event table, and a detector table.
- Interfaces with and control Waters PowerLine injectors and detectors over the IEEE-488 bus.

Using Dissolution Control

The Waters 600E system can control dissolution testing with the following optional components:

- Waters 712D WISP™ Multiport Sample Processor
- Dissolution bath
- Hanson Transfer Controller (including volumetric manifold and transfer/air vacuum source)

For information on using the Waters 600E dissolution control, see the *Waters Dissolution System Operator's Manual*.

1.4 Gradient Controller Features

The 600 Gradient Controller allows you to:

- Program isocratic and gradient methods. For gradient methods, you can run concave, convex, and linear composition profiles with up to four eluents.

- Program tables that define the varying percentage composition of the eluent, the flow rate, and the time range for each phase of the gradient operation. You develop tables for gradient programs and programmed events and save them in memory.
- Create multi-method programs that link time-based control tables. Each time-based table comprises a gradient table and an event table.

1.5 Options and Accessories

This section describes several options that allow you to expand the capability of your LC system.

Waters U6K Injector

Optional septum-less manual injector that allows variable-volume loop filling and injecting. Stainless steel and TFE components permit use with a wide range of eluents.

Rheodyne 7725i Injector

Optional manual injector that allows variable-volume loop filling and injecting. Stainless steel components permit use with any eluent.

Column Heater

Heats column up to 99 °C for separations that require controlled temperatures.

RCM Module

Accommodates Radial-Pak™ cartridges for methods development. Radial-Pak cartridges are available in a wide variety of packings.

Fraction Collector

You can configure the Waters Fraction Collector with the Waters 600E system. You can program event outputs to control fraction collector operation, or you can program the fraction collector directly.

SE-120 Chart Recorder

With the Waters SE-120™ Chart Recorder, you can simultaneously plot two analog signals ranging from 0 to 10 mV full scale to 0 to 10 V full scale.

Automatic Switching Valves

Allow automatic column selection, large-volume sample injection, or flow-path diversion.

746 Data Module

The Waters 746 Data Module (integrator) provides documentation of PowerLine system programs, a permanent annotated chromatographic record, and integrated data.

Reference Valve

Routes eluent to the reference cell of a detector. Replaces the mixing tee on the front of the pump.

Chapter 2
Basic Operation

This chapter introduces you to the operation of the Waters 600E Multisolvent Delivery System. This chapter assumes that you are using the system with other Waters system components.

2.1 Powering Up the System

Before You Begin

This section provides instructions on powering up your Waters 600E system, including:

- IEEE-488 component powerup sequence
- Powering up the stand-alone 600E system



Attention: *Operate the system with covers installed to ensure proper ventilation. Failure to operate the system properly will shorten the life expectancy of some parts and invalidate the warranty.*

If this is the first time you are powering up the system, make sure all components in the system are properly installed (see the *Waters 600E Multisolvent Delivery System Installation and Maintenance Guide*).

2.1.1 Powering Up IEEE-488 Components

This section describes:

- Powering up a 600E system with a data system
- Powering up a 600E system in PowerLine mode

Note: *You must perform the steps in the following procedures in the exact order outlined to ensure proper operation of your system (data system-based or PowerLine-based). If you do not perform the exact steps, the data system or PowerLine Controller may not recognize the other devices on the bus.*

Powering Up a 600E System When Used with a Data System

This section contains the powerup sequence to follow when using the 600E system with a data system (Empower or Millennium® Chromatography Manager).

Note: *The 600 Controller must be in the Gradient Controller configuration to be controlled from an IEEE-488 based data system. See Section 2.4.2, Gradient Controller Configuration, for details.*

Note: *Power up all devices connected to your data system* ***before*** *you power up the computer. As you power up each component, wait a brief period to allow its internal diagnostic tests to run. These tests ensure that each module is functional and serve to quickly isolate a failure.*

To power up the 600E system when used with a data system:

1. Power up all equipment *not* controlled by the data system (for example, a printer).
2. Power up all equipment controlled by the data system but *not* under direct IEEE-488 control (for example, a non-IEEE-488 detector):
3. Power up all equipment controlled through the IEEE-488 bus.

   To power up the 600E system, press the On/Off switch on the front panel of the 600 controller to the 1 (On) position. The Powerup screen appears (Figure 2-1).

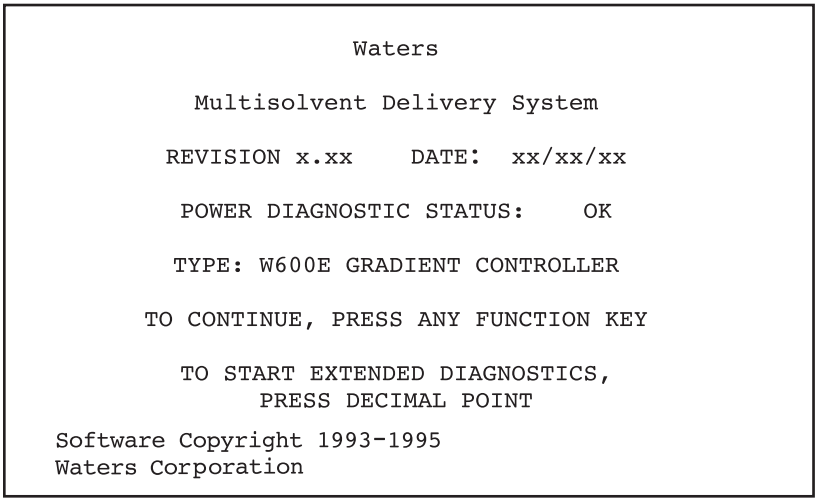


Figure 2-1 Gradient Controller Powerup Screen

The Powerup screen lists the diagnostic status, controller configuration, and software version number.

Note: *You cannot run methods or method sets, or use the QuickSet Control window unless all devices on the IEEE-488 bus that are assigned to a system on the Empower or Millennium Chromatography Manager are powered up and calibrated.*

Note: *You do not need to power up instruments that are not assigned to a system, or that are assigned to a system you do not intend to use.*

4. Power up the printer and monitor.
5. Power up the computer.

Powering Up a 600E System in PowerLine Mode

This section contains the powerup sequence to follow when using the 600 controller as a PowerLine Controller. To power up your system:

1. Power up all equipment *not* controlled through the IEEE-488 bus (non-PowerLine).
2. Power up all equipment controlled through the IEEE-488 bus (PowerLine).
3. Power up the 600E system by pressing the On/Off switch on the front panel of the 600 controller to the 1 (On) position. The Powerup screen appears (Figure 2-2).

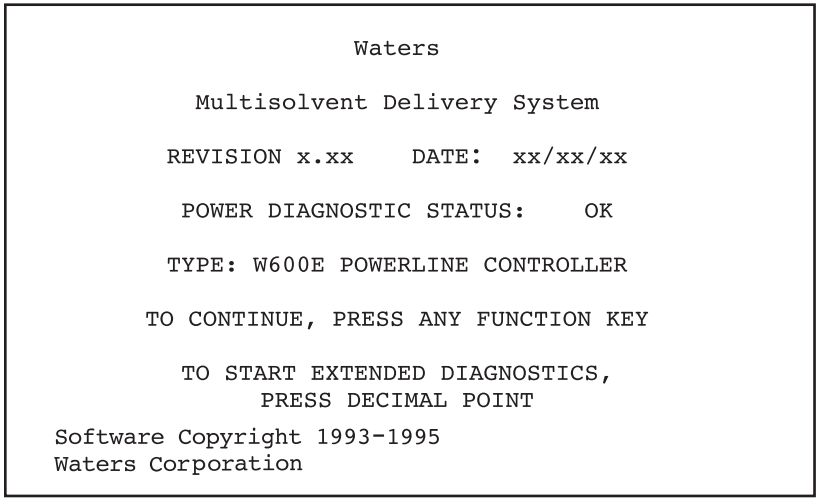


Figure 2-2 PowerLine Controller Powerup Screen

2.1.2 Powering Up the Stand-Alone 600E System

This section contains the powerup sequence to follow when using the 600E system with the stand-alone Gradient Controller configuration. To power up your stand-alone system:

1. Power up all external equipment (chart recorder, injector).
2. Power up the 600E system by pressing the On/Off switch on the front panel of the 600 controller to the 1 (On) position.

2.2 Running Diagnostics

This section provides an overview of the diagnostics available in your 600E system, including:

- Powerup self-diagnostics
- Running extended diagnostics

2.2.1 Powerup Self-Diagnostics

During powerup, the controller automatically runs self-diagnostic tests and then displays the Powerup screen (see Figure 2-1). The powerup self-diagnostics test each PowerLine module in the Waters 600E system. These modules include:

- Waters 600 controller and 600E pump
- The following Waters detectors:
  - Waters 410 Refractive Index Detector
  - Waters 431/432 Conductivity Detector
  - Waters 486 Tunable Absorbance Detector
  - Waters 490E Programmable Multiwavelength Detector
- Waters 717plus Autosampler

If all tests run successfully, the Powerup screen indicates that the powerup diagnostic status is “OK.”

Diagnostic failure on powerup

If any of the tests fail, a failure message appears as follows:

- If the controller or pump fails the diagnostic test, the Powerup screen indicates the failed test.
- If a detector or an autoinjector fails the diagnostic test, the failed device reports the problem on its own front panel.

If any failure occurs, turn the controller Off, then On again. If the diagnostics fail a second time, contact Waters Technical Service. To reach Waters Technical Service, contact your local Waters sales/service representative.

2.2.2 Running Extended Diagnostics

In addition to the powerup self-diagnostics, your 600E system contains extended diagnostic routines.

To start the extended diagnostics, press the decimal point (.) key from the Powerup screen. For further information on running extended diagnostics, see your *Waters 600E Multisolvent Delivery System Installation and Maintenance Guide*, Section 5.3, Performing 600 Controller Extended Test Routines.

2.3 Using the Controller Front Panel

Overview

The controller front panel (Figure 2-3) consists of:

- On/Off switch
- Display screen
- Controller keys

The controller keys comprise:

- **Keypad** - Cursor movement keys, display contrast keys, Home key, Enter key, Clear key, and number keys.
- **Function Keys** - The row of labeled keys located just below the screen keys that access modes of system operation.
- **Screen Keys** - The row of unlabeled keys located directly below the screen. The screen keys vary with different screens used during operation. The Help screen key

displays screen-specific help. The specific function for a given screen key appears at the bottom of the screen above the key.

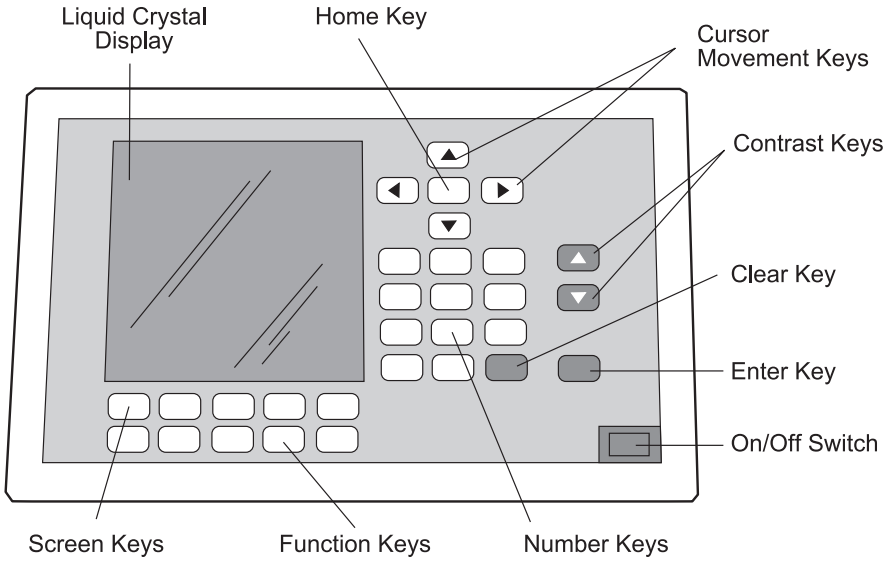


Figure 2-3 Waters 600 Controller Front Panel

2.3.1 Keypad

The Waters 600 controller main keypad (see Figure 2-3) includes:

- **Cursor movement keys** - Move the cursor up, down, left, and right. The cursor location appears on the screen as a bright rectangle.
- **Display contrast keys** - Adjust the contrast (lightness and darkness) of the screen to improve readability in different lighting situations.
- **Number keys** - Enter numeric data on the screens. When pressed, these keys display the numbers 0 to 9 and a decimal point.
- **Home key** - Moves the cursor directly to the home, or first entry, position on that screen.
- **Enter key** - Enters, or registers, the value just typed for a parameter.
- **Clear key** - Erases the value just typed if you have not pressed the Enter key.

To enter values:

1. Use the arrow keys to move the cursor to the desired field.

2. Type a value for the selected parameter. Press the Help screen key to display the allowable range for the field.
3. Press Enter. The system checks the new value to see if it is the correct type of entry and within the proper range.
    - If the entry is acceptable, it becomes the current parameter value and the cursor moves to the next logical entry position.
    - If the entry is not acceptable, an error message appears at the bottom of the screen and the previous value remains as the current parameter value. Press Clear to clear the error message. Repeat steps 2 and 3 above.

2.3.2 Function Keys

Function keys are the labeled keys located directly below the screen keys. Function keys:

- Control modes of system operation
- Access related screens

Using Function Keys

To use the function keys, press the appropriate key.

Note: *In PowerLine mode, function key names correspond to the screens they access. In Gradient mode, function key names do not always correspond to the screens they access. See Section 2.4, Comparing Controller Types, for information on the two controller configurations.*

PowerLine Controller Keys

When you configure the controller as a PowerLine Controller, function keys include:

Key	Accesses	Function
Setup	Pump Setup screen	Sets up pump hardware
Direct	Direct Control screen	Programs conditions for attended operation
Operate Method	Operate Method screen	Starts a stored method run
Program Method	Program Methods screen	Sets up/stores the method table
Program Table	Program Gradient, Program Event, and Detector Tables screens	Sets up/stores gradient, event, and detector tables

Gradient Controller Keys

In Gradient Controller mode, function keys access different screens:

Key	Accesses	Function
Setup	Pump Setup screen	Sets up pump hardware
Direct	Isocratic screen	Programs conditions for attended operation
Operate Method	Operate Gradient screen	Starts a gradient run
Program Method	Program Gradient screen	Sets up/stores gradient tables
Program Table	Program Event screen	Sets up/stores event tables

2.3.3 Screen Keys

Screen Key Description

Screen keys are the five unlabeled keys directly below the display screen. Their functions are indicated by labels that appear on the bottom of the displayed screen immediately above each key.

Note: *The functions of the screen keys vary from screen to screen.*

Using Screen Keys

To use the screen keys, press the key directly below the label on the display screen.

Certain screens display the More screen key (such as the Operate Methods and Operate Gradient screens). Pressing the More screen key displays a second set of screen keys associated with that screen.

Screen keys:

- Act as toggles. When you press a toggle screen key, the screen label changes to display the alternate function. For example, when you press the Lock Keyboard screen key, the screen label changes to Unlock Keyboard.
- Indicate system status. For example, the leftmost key on the Operate Method or Operate Gradient screen indicates whether or not a run is in progress. The label changes from `Idle` to `Inject Wait` to `Running`, depending on the operating mode of the system.

Figure 2-4 shows the location of the Clear Line screen label relative to the screen key.

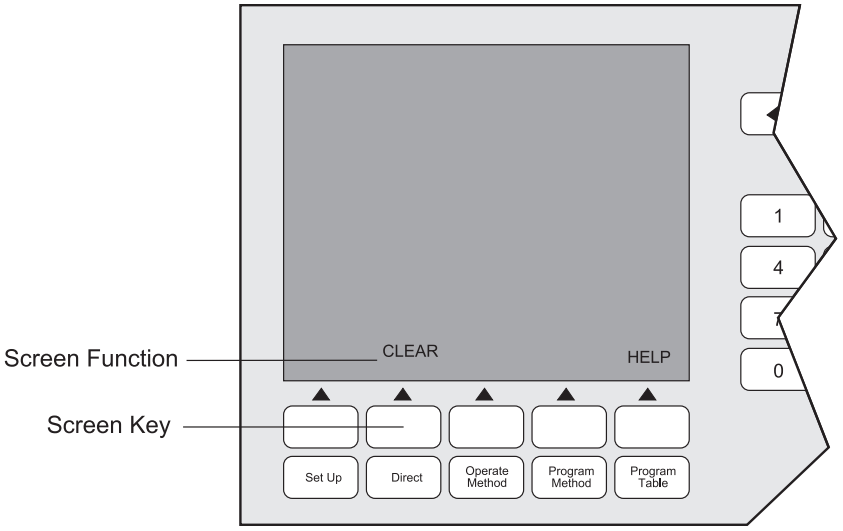


Figure 2-4 Clear Line Screen Key

2.4 Comparing Controller Types

Use this section to help you determine which controller configuration best fits your needs. This section describes:

- PowerLine controller configuration
- Gradient controller configuration

Note: *After you have decided upon a controller type, continue with Chapter 3, Preparing Your 600E System for Operation, before proceeding to the controller-specific chapters outlined in the table below.*

Using PowerLine or Gradient Controller Mode

To use the controller as a:	**Refer to:**
PowerLine Controller	Chapters 4 through 7
Gradient Controller	Chapters 8 through 11

2.4.1 PowerLine Controller Configuration

This section includes:

- PowerLine Controller description
- PowerLine Controller operation

When to Use PowerLine Mode

Use PowerLine mode to:

- Control external PowerLine devices (such as, autosamplers, detectors and integrators) from the Waters 600E system through the IEEE-488 bus.
- Develop complex multi-method applications.

  Note: *Your Waters 600 controller was preconfigured at the factory as a PowerLine Controller.*

PowerLine Description

The PowerLine Controller:

- Automatically sets up and controls all flow rate, gradient, injection, external event, and detector parameters directly from the 600 controller keyboard.
- Uses a Method table that links time-based control tables. These control tables consist of a gradient table, events table, and a detector table.
- Interfaces with:
  - PowerLine detectors (Waters 410 Refractive Index, Waters 431/432 Conductivity, Waters 486 Tunable Absorbance, Waters 490E Programmable Multiwavelength).
  - PowerLine autoinjectors (Waters 717plus Autosampler).
  - Integrator (Waters 745B/746 Data Module).
- Communicates with:
  - PowerLine modules (autoinjector and detectors) through the IEEE-488 interface.
  - Waters 745B/746 Data Module through the RS-232 serial interface.

See Chapter 4, Setting PowerLine Controller Operating Parameters, for information on setting up PowerLine devices.

PowerLine Operation

The PowerLine controller provides product-specific setup and operating parameters for PowerLine detectors and autoinjectors.

Detector parameters inlude:

- Detector filter constant
- Autozero (except for 431/432 detector)
- Polarity
- Absorbance Units Full Scale (AUFS)
- UV wavelength (λ)
- Sensitivity
- Lamp On/Off
- Filter
- Cell temperature

Injector parameters include:

- Syringe draw rate
- Inject delay
- Standards (how many and how often)
- Methods specifying vial numbers
- Number of injections from each vial
- Injection volume

Storage for Tables

The controller provides storage for 15 sets of tables. Each set of tables can include a:

- **Gradient table** - Contains a maximum of 15 operational steps that define the varying eluent composition, flow rate, time range, and rate of change for each phase of the gradient.
- **Event table** - Contains a maximum of 15 operational steps to control output signals to external devices, internal alarms, sparge rate, column heater temperature, detector lamp On/Off, and linking operation to other tables.
- **Detector table** - Contains a maximum of 15 operational steps to change wavelength and attenuation during a run.

- **Method table** - A time-based program that contains up to 48 individual steps. Each step accesses a set of gradient/event/detector tables. You can program the Method table to automatically:
  - Apply multiple separation methods to a sample set
  - Equilibrate the system for a specified period of time
  - Change over eluents
  - Purge the autoinjector
  - Purge the 410 detector flow cell
  - Activate automatic system purge and shutdown
  - Lower the system flow rate after an analysis

  Once you have programmed the Method table, you may instruct the system to start at a specific step number and vial number.

The PowerLine configuration also provides control for optional dissolution testing. For details on dissolution control, see the *Waters Dissolution System Operator's Manual*.

2.4.2 Gradient Controller Configuration

When to Use Gradient Controller Mode

Use the Gradient Controller configuration to:

- Control the 600E system from an IEEE-based data system, such as the Waters Empower or Millennium Chromatography Manager Data System.
- Operate the 600E system in stand-alone mode.

Description

The Waters 600E system in a Gradient Controller configuration:

- Controls isocratic separation conditions.
- Allows you to program and run gradient tables that define the varying eluent composition, flow rate, time range, and rate of change for each phase of the gradient.
- Allows you to program and run external event tables that control output signals to external devices, internal alarms, sparge rate, column heater temperature, and linking operation with other tables while running a gradient.

Note: *The gradient controller does not control any external devices through the IEEE-488 or RS-232 connections.*

Operation

The Gradient Controller provides storage for 15 sets of tables. Each set of tables consists of a gradient table and an event table that run together during gradient operation. The gradient and event tables can each contain a maximum of 15 operational steps.

You can program separate tables to:

- Equilibrate the system
- Change over eluent
- Automatically purge and shutdown the system
- Lower the system flow rate after an analysis

At its completion, you can program a table to initiate the start of another table. You can also link a table back to itself to cause a method to repeat.

2.5 Powering Down the System

If you do not plan to use the Waters 600E system for a long period of time (overnight or longer), power down the system.

Before You Power Down

Observe these precautions before powering down your system:

- To prevent microbial growth, do not leave water or buffers in the fluidic lines while the system is not in use.
- Flush the lines with high-purity filtered water (such as that provided by reverse-osmosis/ion-exchange water systems), followed by an aqueous 10% methanol solution.
- Remove the column if it is incompatible with this storage protocol.

Turn off the Waters 600E by pressing the On/Off switch on the front panel of the controller to the 0 (Off) position.

Chapter 3
Preparing Your 600E System for Operation

This chapter contains procedures for preparing your Waters 600E Multisolvent Delivery System for operation.

You perform some of these procedures only once (the first time you operate the system). You must perform other procedures every time you use the system.

Preparing the System in PowerLine and Gradient Mode

This chapter assumes the 600E system is in PowerLine Controller mode when you prepare the system. Each time you press the Direct key, the Direct Control screen appears (see Figure 3-2).

You can perform the procedures listed in this chapter when the 600E system is in Gradient Controller mode. However, when you press the Direct key, the Isocratic screen appears. Set the parameters on the Isocratic screen as instructed in the procedures for the Direct Control screen.

Good Operating Practices

To ensure proper operation of your system:



Caution: *Always follow safe laboratory practices when handling eluents. Know the physical and chemical properties of the eluents. See the Material Safety Data Sheets for the eluents in use.*

- Use only clean, HPLC-grade eluents. Filter all eluents. This is especially critical with aqueous buffers.
- Never pump eluents that react with the material of the wetted surfaces in the fluid system. See Section Appendix B, Eluent Considerations, for more information.
- Avoid placing eluents on top of the system unless the bottles are in a container that can hold the total volume of all of the eluents in case bottle leakage occurs.

Eluent Considerations

When purging the Waters 600E system:

- Always wear safety glasses when working with eluents.
- Before operation, always check for miscibility with the previous eluent used, or change eluents.
- If organic and aqueous buffers are used together, ensure that they do not precipitate salt in the pump heads and valves. Allow proper changeover steps when priming and recycling programs from final to initial conditions.
- After using halide ions, flush the system thoroughly.
- Maintain sufficient eluent in the reservoirs to ensure that the reservoirs do not run dry.

3.1 Sparging Eluents

Use the Waters 600E system helium sparge feature to sparge all enabled reservoirs at a flow rate between 0 and 100 mL/min. At 100 mL/min, the sparge valves are On at all times. At flow rates less than 100 mL/min, the sparge valves turn on and off periodically to achieve an average flow rate over time.

Use an initial sparge rate of 100 mL/min for a minimum of 15 minutes to degas the eluents followed by a reduced rate (for example, 30 to 50 mL/min) to maintain the degassed condition. See Appendix B, Eluent Considerations, for additional information on sparging.



Caution: *To avoid respiratory problems do not sparge into open air. This action may be harmful when performed with certain eluents. Use eluent reservoir caps as described in the Waters 600E Multisolvent Delivery System Installation and Maintenance Guide, Section 2.2.1, Setting Up the Eluent Reservoirs. Use an exhaust hood to remove fumes emitted from the reservoirs.*

When to Sparge

Sparge at 100 mL/min for a minimum of 15 minutes when:

- You have not sparged reservoirs for eight hours or more (air can diffuse into the system)
- You add fresh eluent to the system

During pump operation, sparge all active reservoirs at a reduced rate (for example, 30 mL/min) with helium to:

- Maintain pump performance with blended eluents
- Prevent accumulation of dissolved air in the eluents

Note: *A sparge rate of 50 mL/min is recommended for gradients involving methanol and aqueous solutions.*

If helium is not available or is undesirable for sparging, see Appendix B, Eluent Considerations, for other degassing methods.

Preparing to Sparge

Adjust the helium tank pressure to a setting between 345 KPa and 1035 KPa (50 to 150 psi).

1. Confirm that sparge and eluent tubing are properly assembled and connected (see the *Waters 600E Multisolvent Delivery System Installation and Maintenance Guide*, Chapter 2, making Fluidic Connections to the 600E Pump).
2. Press the Setup function key. The Pump Setup screen appears (Figure 3-1).

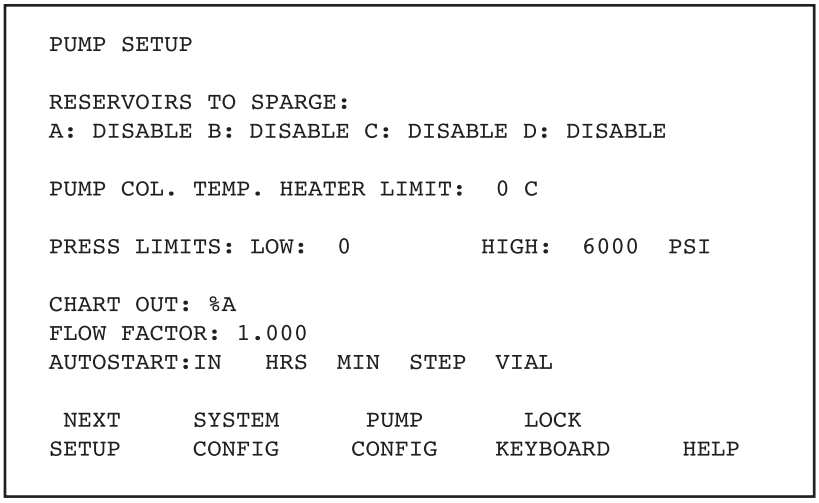


Figure 3-1 Pump Setup Screen

Note: *For eluents that contain minor volatile components, you can first sparge the eluents without the component. Then, reduce the sparge rate and add the volatile component to the sparged eluent.*

Enabling Sparging

To enable sparging for the reservoirs containing eluent:

1. Move the cursor to the Reservoir to Sparge field for the first reservoir that contains eluent (A, B, C, or D).
2. Type 1 and press Enter. This enables the reservoir for sparging. Gas does not flow when you enable the reservoir to be sparged.
3. Repeat steps 1 and 2 above for each reservoir that contains eluent.

Sparging

To initiate sparging:

1. Press the Direct function key. The Direct Control screen appears (Figure 3-2).

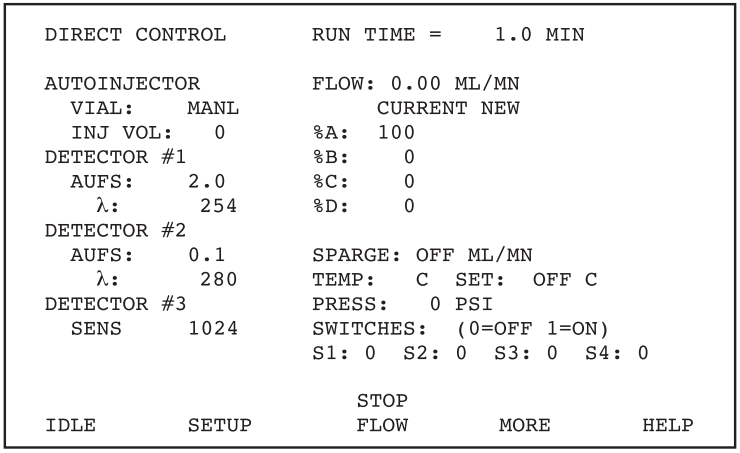


Figure 3-2 Direct Control Screen

2. Set the sparge flow rate.
   - Move the cursor to the Sparge field.
   - Type 100 and press Enter to set an initial flow rate of 100 mL/min for the sparge gas. Gas flow begins in all enabled reservoirs.
3. Flow helium at 100 mL/min for 20 minutes, then repeat step 2 and reduce flow to:
   - 10 to 15 mL/min for aqueous mobile phases
   - 20 to 30 mL/min for aqueous-acetonitrile mobile phases
   - 50 mL/min for aqueous-methanol mobile phases

Maintain these helium flow conditions continuously while the system is running.

3.2 Priming the Pump

Priming is necessary to ensure proper pump operation. Priming involves:

- Initiating flow
- Drawing off eluent
- Purging air from the pump heads

When to Prime

Prime the pump when:

- Starting up the system for the first time
- Adding fresh eluent to the system
- Changing eluent

Considerations

Consider the following when priming the 600E pump.



Caution: *Always follow safe laboratory practices when handling eluents. Know the physical and chemical properties of the eluents. See the Material Safety Data Sheets for the eluents in use.*

- The optional 225 µL pump heads usually do not require priming once eluent has been introduced to the draw-off valve. With some eluents, however, the standard 100 µL pump heads may require additional priming after you introduce eluent into the inlet manifold.
- You must prime with a suitable syringe (such as the one available from Waters). During priming, do not apply excessive force to the syringe by injecting eluent more quickly than the pumps can accept.

Venting the Eluent

Before you can prime the pump, you must vent the eluent at some point in the flow path before the column. Use one of the following procedures to vent the eluent.

Note: *For the first two procedures, place a small glass beaker under the open tubing or injection port to catch the vented eluent.*

- Open the reference valve (mounted on the pump, above the pump heads).

- Open the slide-out drawer. Disconnect the fluid tubing at the column inlet. See the *Waters 600E Multisolvent Delivery System Installation and Maintenance Guide*, Section 2.3.1, Installing a Column. Disconnecting the fluid line avoids introducing air into the column and reduces backpressure resistance to priming.
- If you are using a Waters 717plus Autosampler, put the autosampler in purge mode.

Initiating Flow

1. Rotate the inlet manifold valve handle located on the front of the pump to the Run position (Figure 3-3).
2. Place a waste beaker under the vent tube of the reference valve to collect the diverted eluent.
3. Open the reference valve (see Figure 1-4) by rotating the handle from left to right to isolate the column from the flow path.

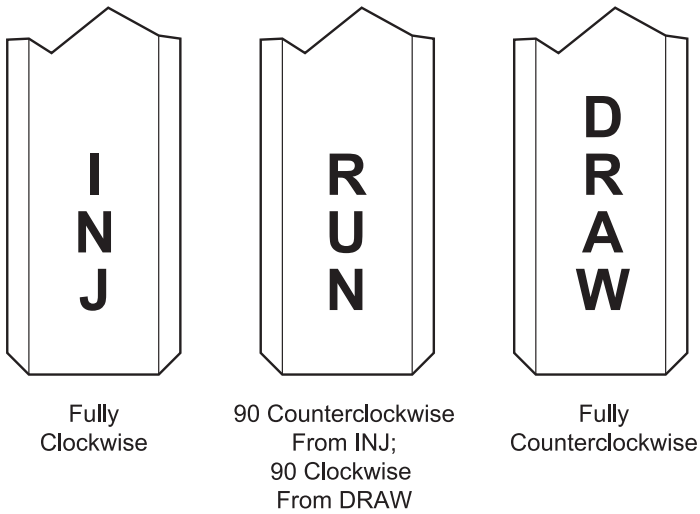


Figure 3-3 Pump Inlet Manifold Valve Positions

4. Press the Direct function key and the Direct Control screen appears. At the Flow field, type a flow rate of 1.0 mL/min (the gradient proportioning valve is not actuated at 0 mL/min).

Drawing Off Eluent

Once you have initiated flow, draw off eluent as follows:

1. Move the cursor to the %A field for the first eluent and type 100 in the New column. Press Enter.
2. Type 0 and press Enter for the remaining eluent composition fields.
3. Attach the priming syringe to the Luer™ fitting on the inlet manifold valve (Figure 3-4).
4. Rotate the inlet manifold valve handle fully counterclockwise to the Draw position (see Figure 3-3). Draw off 10 mL of eluent, rotate the handle to the Run position, remove the syringe and discard the eluent properly.

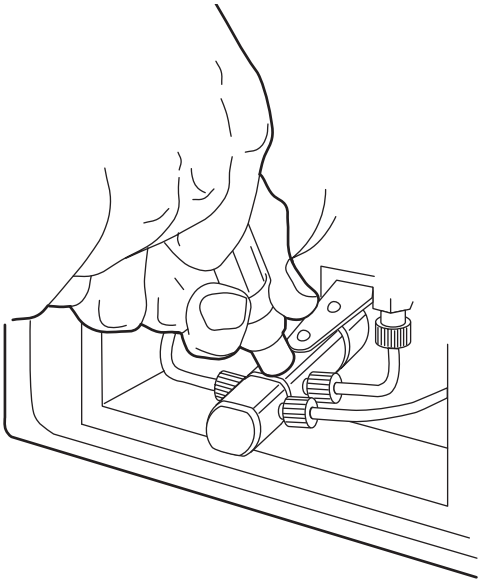


Figure 3-4 Drawing Off Eluent

5. Repeat steps 1 through 4 for each of the remaining reservoirs that you intend to use.

Priming

1. Attach the priming syringe to the Luer fitting on the inlet manifold valve (see Figure 3-4).
2. Move the cursor to the %A field for the first eluent and type 100. Press Enter. Type 0 and press Enter for the remaining % composition fields.

3. Move the cursor to the Flow field and press 10. Press Enter. This sets the flow rate to 10 mL/min for eluent reservoir A.



Attention: *Confirm that the reference valve is open (i.e., injector and column bypassed) when selecting high flow rates or when making rapid changes in flow rate. Sudden flow and backpressure variations or high flow rates may damage installed columns.*

4. Rotate the inlet manifold valve handle fully counterclockwise to the Draw position (see Figure 3-3). Draw off some eluent, rotate the valve handle to the Inj position and apply slight pressure to force eluent through the heads. Rotate the valve handle to the Run position and observe the flow. If flow is not constant repeat this step. When flow is constant repeat this procedure for other reservoirs that you intend to use.
5. When the pump has been primed using all applicable reservoirs, press the Stop flow screen key to stop the pump.

3.3 Daily Routine Operation

This section provides the information you need to prepare the system on a daily basis.

Initial Startup

1. Fill the eluent reservoirs with sufficient eluent for the day's operation.
2. Power up the system.

   Note: *Oxygen can dissolve into the eluent overnight. If you are not using fresh eluent, sparge each filtered eluent reservoir for a minimum of 15 minutes at 100 mL/min before flushing the system (see Section 3.4, Flushing the System) to replace the older eluent.*

Flushing the Pump

To ensure a thorough flushing:

1. Disconnect the pump outlet tube from the column inlet and place the outlet tube in a waste container.

   If thorough flushing is not required, leave the pump outlet tube connected and flush fresh eluent through the system to waste by opening the reference valve.
2. Press the Direct function key. The Direct Control screen appears.
3. Move the cursor to the Flow Rate field and type a flow rate of 2.00 mL/min.

4. Flush for 2 to 5 minutes.
5. If your system backpressure fluctuates more than 100 psi (7 kg/cm^2) once the system has reached the operating flow rate, prime the system again as described in Section 3.2, Priming the Pump.
6. If the column in the system has been used before, use sufficient eluent to equilibrate the system. If the column is new, allow eluent to go to waste before connecting the eluent outlet to the detector. See the associated *Column Care and Use Manual* for equilibration instructions.

3.4 Flushing the System

Flushing the system ensures that all eluent in the fluid system is replaced with new eluent before running samples.



Caution: *Be careful when venting eluents from a pressurized system. Follow good laboratory practices including the wearing of eye protection.*

Flushing involves:

- Flushing system fluid lines
- Flushing the manual injector
- Purging the autoinjector
- Connecting the column after flushing

When to Flush

Flush the system when:

- Adding fresh eluent
- Changing eluent

Before You Begin

Before flushing the system:

1. Filter and sparge the fresh eluent. See Appendix B, Eluent Considerations, and Section 3.1, Sparging Eluents, for more information.
2. Prime the pump. See Section 3.2, Priming the Pump, if this is the first time you are starting up your system or if you are using miscible eluents. See Section 3.6, Changing Eluents, to change from one eluent to another.

3. Install a union in place of the column during system flush.
4. Place the rest of the system back in the flow path by closing the reference valve.
5. Flush the complete system, including detectors (see Section 3.4.1, Flushing the System Fluid Lines).

3.4.1 Flushing the System Fluid Lines

1. Press the Direct function key to display the Direct Control screen (see Figure 3-2).
2. Move the cursor to the Flow field and type 2.0. Press Enter. Eluent now flows through the system at 2.0 mL/min.
3. Flush the system for 2 to 5 minutes.

3.4.2 Flushing the Rheodyne Manual Injector

Caution: *Always observe safe laboratory practices when handling eluents. Know the chemical and physical properties of eluents. See the Materials Safety Data Sheets for the eluents in use.*

1. Set the injector handle to the Inject position. Setting the handle in the Inject position inserts the sample loop in the flow path.
2. Start flow of the flushing eluent at 1.0 mL/min. Flow for 2 to 3 minutes.
3. Fill a syringe (fitted with a 22-gauge, 90° point needle) with the new or intermediate eluent.
4. Set the injector handle to the Load position (Figure 3-5).

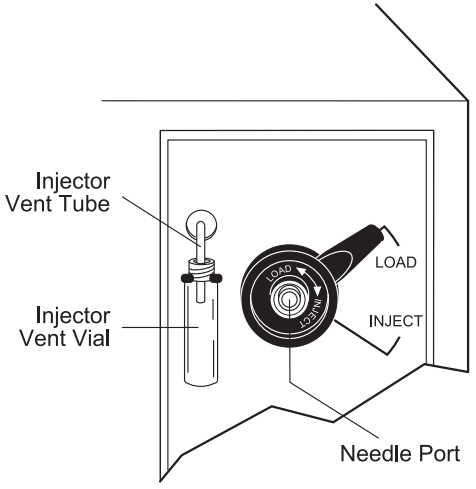


Figure 3-5 Injector Handle in the Load Position

5. Fully insert the syringe needle into the injector port.
6. *Gently* discharge the contents of the syringe to completely fill the sample loop and expel any old or intermediate eluent from the injector vent tube. This step replaces the eluent in the sample loop with fresh eluent.
7. Leave the syringe in position and turn the injector handle to the Inject position.
8. Remove the syringe.
9. Allow the eluent to flow for several minutes at 1.0 mL/min.

3.4.3 Purging the Autoinjector

Perform the following steps to purge a Waters autoinjector. If you are using a non-Waters autoinjector, see the documentation provided with the autoinjector for the appropriate steps to flush the autoinjector flow path.

1. Move the cursor to the Vial field on the Direct Control screen (see Figure 3-2).
2. Type 103 and press Enter. (103 is the special vial code that places the autoinjector in its purge mode.)

   The default run time is 4.5 minutes. Enter a longer time if 4.5 minutes is too short for your application.
3. If necessary, continue to one of the following sections:
   - Section 3.4.4, Reconnecting the Column After Flushing the System, if the column is not in the flow path
   - Section 3.5, Equilibrating the System, if the column is in the flow path

3.4.4 Reconnecting the Column After Flushing the System

Perform the following procedure if the column has been replaced with a union, and if the eluent inlet tubing, gradient proportioning valve, pump, injector, and detector flow cells are filled with new eluent.

1. Press the Stop Flow screen key to stop the pump.
2. Remove the union and reinstall the column. See the column *Care and Use Manual* for column operating guidelines.
3. Continue with Section 3.5, Equilibrating the System, to prepare the system for samples.

3.5 Equilibrating the System

To equilibrate the system, set the following initial parameters on the Direct Control screen (see Figure 3-2):

- Flow rate
- Eluent composition
- Sparge rate
- AUFS
- Sensitivity

Note: *When using the Millennium Chromatography Manager or the ExpertEase 845/860 Data System, see Section 8.1.2, Communicating with a Data System, for information on configuring the data system. The data system will help determine when the baseline is stable and the system is ready for injections.*

When to Equilibrate

Equilibrate the system:

- When starting up your system for the first time (after eluents are sparged)
- After changing eluents
- After changing eluent composition
- After changing columns
- When preparing to make a gradient separation
- After an idle period

Good Operating Practices

When you equilibrate your system:

- Maintain sufficient eluent in the mobile phase reservoirs to ensure that the reservoirs do not run dry while operating. Running the pump without eluent adversely affects seal life.
- Equilibrate both the eluent and the system at ambient temperature before pump operation. Cold eluents that flow through a system at room temperature outgas as they warm up.
- If you are operating in a cold room, equilibrate the system at the reduced temperature.

Procedure

To equilibrate the system:

1. Press the Direct function key to access the Direct Control screen (see Figure 3-2).

Sparging

2. If desired, set the sparging conditions as follows:
   a. Move the cursor to the Sparge field.
   b. Type in the maintenance rate for your eluents. Press Enter. All reservoirs that are enabled for sparging on the Pump Setup screen sparge at that rate.
   c. The actual sparge rate is dependent on your analytical requirements. An initial high sparge flow rate that reduces to a low maintenance rate, is recommended. For guidelines and information about sparging, see Section 3.1, Sparging Eluents, and Appendix B, Eluent Considerations.

Setting Eluent Composition

3. To set the initial eluent composition:
   a. Move the cursor to the New %A field.
   b. Type the % composition for the mobile phase (0 to 100, no decimals, in 1% increments) from reservoir A. Press Enter.
   c. Repeat the previous two actions for the remaining three reservoirs. The sum of the four % composition entries must equal 100.
   d. The system implements the changes when you press Enter after typing the value for eluent D. The Current column displays the new composition.

Setting Flow Rate

4. Set the initial flow rate as follows.



Attention: *Be sure that the values for the maximum and minimum operating pressures programmed on the Pump Setup screen are appropriate for your column. See the column Care and Use Manual for your column. For information on setting the minimum and maximum pump pressure values, see Section 4.3.3, Exiting the Pump Setup Screen (for PowerLine Controller operation), or Section 8.2.3, Exiting the Pump Setup Screen (for Gradient Controller operation).*

Note: *Some columns require a flow ramp up. See the column Care and Use Manual for guidelines.*

   a. Move the cursor to the Flow field.

b. Type in the desired flow rate value (0 to 20 or 45 mL/min). Press Enter.

   The pump immediately begins operating at the new flow and eluent composition conditions.

Setting PowerLine Detector Parameters

5. Set the PowerLine detector parameters as necessary by moving the cursor to the appropriate detector column.

   For a PowerLine UV/Vis absorbance detector:

   - At the AUFS field, type the required absorbance units full scale (AUFS) value. Press Enter. Valid values are 0.001 to 2.00 AU.
   - At the l field, type the required detector operating wavelength. Press Enter. Valid entries are 190 to 600 nm.

   For a PowerLine refractive index detector (Waters 410), at the Sens field, type the required sensitivity. Press Enter. Valid values are 1, 2, 4, 8, 16, 32, 64, 128, 256, 512, and 1024.

   For a PowerLine conductivity detector (Waters 431/432), at the Sens field, type the required sensitivity in microSiemens (µS). Press Enter. Valid values are 0.1, 0.05, 0.02, 0.01, 0.005, 0.002, 0.001, and 0.0005.

6. Allow a minimum of five to six column volumes to pass through the system before checking the stability of the baseline.
7. Observe the Press (pressure) value on the Direct Control screen. The pressure fluctuations should be within 5 to 10 percent of the average pressure value.
8. If the pressure fluctuates greatly, or if every other pump stroke produces a significant pressure drop, repeat the procedures in Section 3.2, Priming the Pump and Section 3.4, Flushing the System, to purge the pump of air. If this does not correct the problem, see the *Waters 600E Multisolvent Delivery System Installation and Maintenance Guide*, Chapter 5, Troubleshooting, for additional help.

3.6 Changing Eluents

This section outlines the recommended procedures for changing eluents in the Waters 600E System. The procedures include:

- Adding fresh eluent
- Changing between incompatible eluents



Attention: *Never change directly between immiscible eluents or between buffered solutions and organic eluents. Immiscible eluents form emulsions in the flow path. Buffered solutions and organic eluents in combination may result in salt precipitation in the gradient proportioning valves, pump heads, check valves, or other parts of the system.*

Eluent Compatibility

All eluents are compatible with the 600E system. However, long-term *static* exposure to halide salts (for example, fluoride, bromide, chloride, and iodide) will cause pitting and corrosion of stainless steel parts. Flush the system thoroughly with water if the pump will be idle for more than two days. See Section 3.4, Flushing the System.

3.6.1 Adding Fresh Eluent

Before You Begin

This section assumes that you have already performed the procedures in the preceding sections:

- Section 3.1, Sparging Eluents
- Section 3.2, Priming the Pump
- Section 3.3, Daily Routine Operation
- Section 3.4, Flushing the System
- Section 3.5, Equilibrating the System

Considerations

Before adding eluent to your system:

- Always follow safe laboratory practices when working with eluents. See the Material Safety Data Sheets for the eluents in use.

- Always wear safety glasses when working with eluents.
- If your column is not compatible with an eluent, disconnect the column and replace the column in line with a union.
- If organic eluents and aqueous buffers are used together, make certain that salt does not precipitate at any composition. Salts damage pump heads and valves.

Adding Fresh Eluent

Procedure

1. Filter and sparge the fresh eluent. Confirm that the vent lines are properly routed to an exhaust hood. See Appendix B, Eluent Considerations, and Section 3.1, Sparging Eluents, for more information.

   Attention: *Before priming and flushing, place the reference valve in the open position, or disconnect the pump outlet tube from the column inlet tube to take the column out of the flow path. If you disconnect the pump outlet tube, be sure to place it in a waste beaker.*

2. Prime and flush the system with new eluent using the procedures described in Section 3.2, Priming the Pump, and Section 3.4, Flushing the System.

3.6.2 Changing Between Incompatible Eluents

This section describes the procedures for changing between incompatible eluents, including:

- Flushing the system with an intermediate eluent (described below)
- Adding the final eluent to the system (see Section 3.6.1, Adding Fresh Eluent)

You must perform the above-mentioned procedures whenever you change between the following types of incompatible eluents:

- Organic eluents and buffered solutions
- Immiscible eluents

Considerations

Before you begin, take into account the following considerations:

- If organic eluents and aqueous buffers are used together, be careful not to precipitate salt in the pump heads and check valves during eluent changes.

- Changes involving two miscible eluents can be made immediately, without an intermediate eluent.
- To prevent eluent contamination, use the same container to prepare, supply, and store an eluent. Once a container is used for one eluent, it should not be used for any other eluent without a thorough cleansing.



Caution: *Do not use vacuum to degas eluents contained in the 1-gallon brown bottles. Transfer eluents from these bottles to clean vacuum-safe reservoirs for degassing.*

- Consider the column chemistry. If the column will be damaged by the intermediate or new eluent, remove the column from the flow path by performing one of the following actions:
  - Opening the reference valve
  - Connecting the pump outlet tube to the detector inlet tube with a union.

  Note: *If you are changing to a miscible eluent, follow the procedure in Section 3.6.1, Adding Fresh Eluent.*

Flushing with an Intermediate Eluent

This section describes the procedure for flushing with an intermediate eluent.

Choosing an intermediate Eluent

To choose an intermediate eluent, use the following guidelines:

- When changing between organic eluents and buffered solutions, use water or a predominantly aqueous eluent to prevent buffer salts from precipitating.
- When changing between immiscible eluents, use an appropriate intermediate eluent that is freely miscible in both eluents (see Appendix B, Eluent Considerations, for more information).

Flushing

Procedure

1. Filter and sparge the fresh intermediate eluent. See Section 3.1, Sparging Eluents.
2. Confirm that the vent lines are properly routed to an exhaust hood. See Appendix B, Eluent Considerations, and Section 3.1, Sparging Eluents, for more information.

3. Prime and flush the system with intermediate eluent using the procedures described in Section 3.2, Priming the Pump, and Section 3.4, Flushing the System.



Attention: *Before priming and flushing the system with intermediate eluent, remove the column from the flow path by opening the reference valve or by connecting the pump outlet to the detector inlet with a union.*

4. Flush the system with 20 to 40 mL of eluent at a flow rate of 2.0 mL/min for 10 to 20 minutes or until the detector signal is constant.

Adding the Final Eluent to the System

To add the final eluent to the system, follow the instructions in Section 3.6.1, Adding Fresh Eluent.

Chapter 4
Setting PowerLine Controller Operating Parameters

This chapter contains the information you need to set the operating parameters for the PowerLine modules using the 600 controller.

The operating parameters apply to all controller operations, whether you are running a direct-control or a timed multimethod application. Once you set these parameter values, they remain in memory until you change them.

Note: *For a detailed description of using the 600 controller as a Gradient Controller, see Section 2.4.2, Gradient Controller Configuration. For instructions on configuring and using your Waters 600 Controller as a Gradient Controller, see Chapters 8 through 11.*

When to Use the PowerLine Controller

Use PowerLine Controller mode when you want to control other devices (such as, detectors, autosamplers, and integrators) from the 600E system.

For a detailed description of PowerLine Controller mode, see Section 2.4.1, PowerLine Controller Configuration.

Background Information

This chapter assumes that you have installed your system according to the instructions in the *Waters 600E Multisolvent Delivery System Installation and Maintenance Guide*. Ensure that you have read Chapters 1 through 3, in this manual and that you have:

- Acquainted yourself with the system (Chapter 1, Introduction)
- Familiarized yourself with basic operation and decided upon a controller configuration (Chapter 2, Basic Operation)
- Prepared your system for operation (Chapter 3, Preparing Your 600E System for Operation)

If you have not completed the tasks listed above, do so now.

Screen Layout

Figure 4-1 illustrates the relationship of the screens associated with the Waters 600 controller in its PowerLine configuration. See Figure 4-1 when you set PowerLine parameters in preparation for a run.

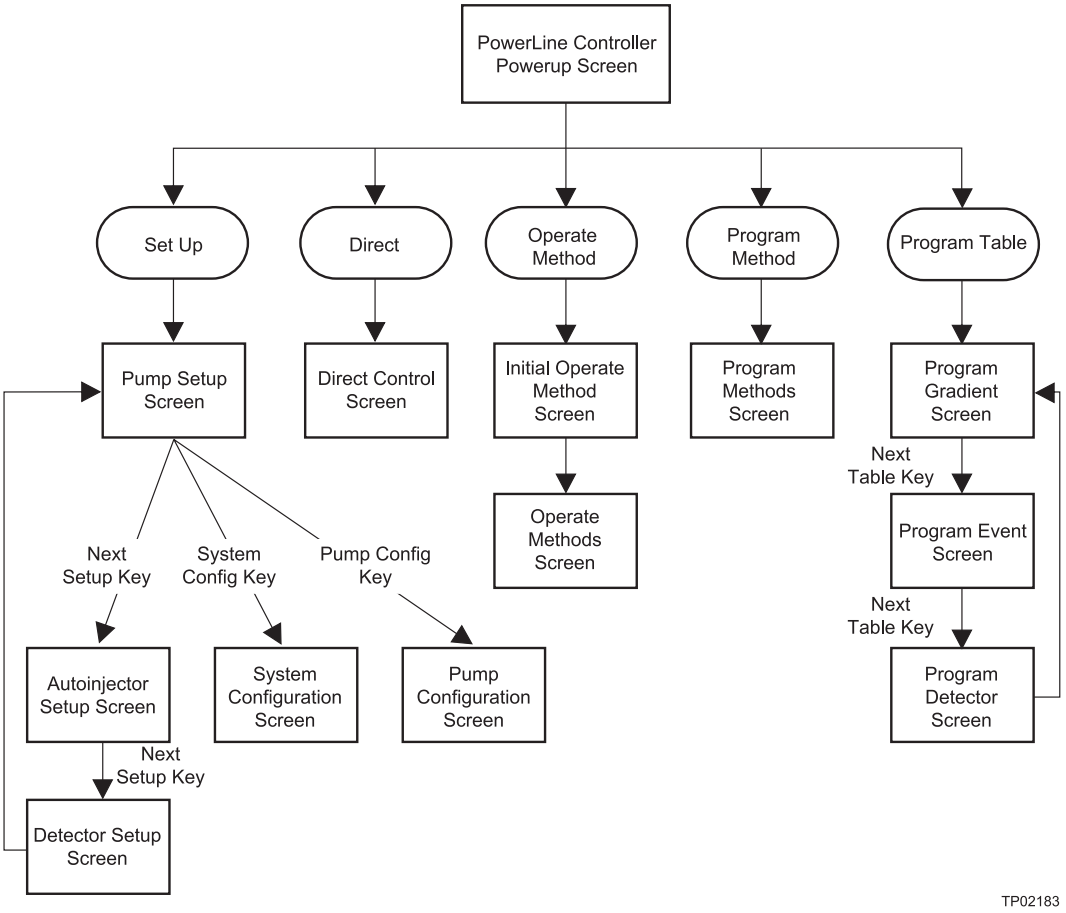


Figure 4-1 PowerLine Controller Screens

4.1 Setting Up the 600 as a PowerLine Controller

This section describes how to use the Pump Configuration screen to configure the Waters 600 Controller as a PowerLine Controller. It includes procedures for:

- Configuring the controller type
- Communicating with a data module (integrator)
- Selecting the report type

Figure 4-2 illustrates the steps involved in using the Pump Configuration screen. Use this flowchart as a quick reference for setting the parameters on the Pump Configuration screen.

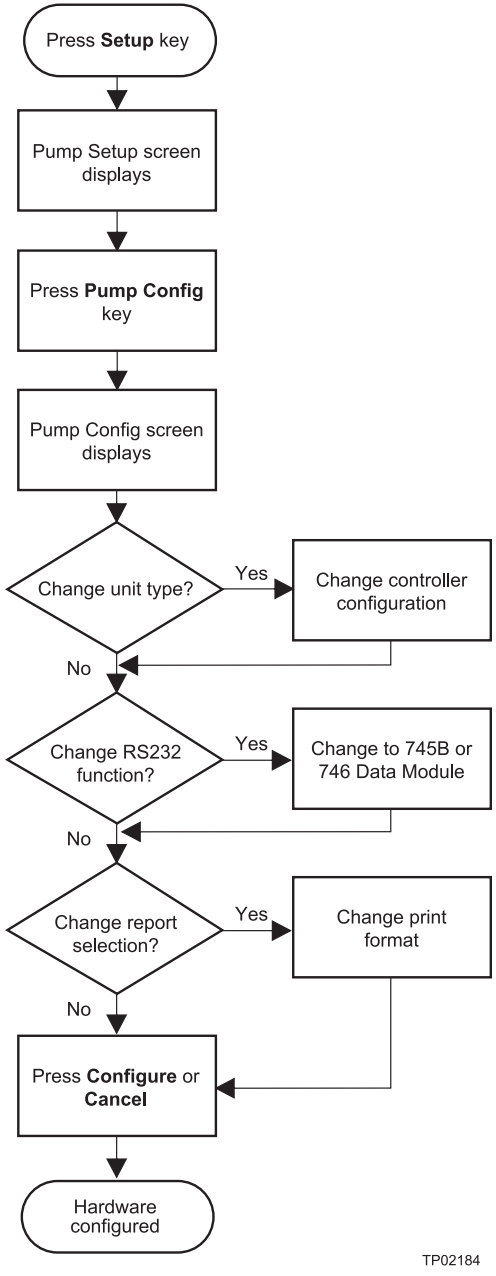


Figure 4-2 Pump Configuration Flowchart

Exiting the Pump Configuration Screen

Do one of the following:

- Press the Configure screen key to save the parameter values displayed on the screen. The system reboots and displays the Powerup screen.

  Pressing the Configure screen key also aborts a run in process and sets the flow rate to 0.

- Press the Cancel screen key to return to the Pump Setup screen without saving any changes made to the Pump Configuration screen.

4.1.1 Configuring the Controller Type

This section describes how to configure the Waters 600 Controller as a PowerLine Controller.

Waters preconfigures the 600 controller as a PowerLine Controller at the manufacturing site. Use this section to verify that the controller type is correct or to change the controller type if you previously configured the controller as a Gradient Controller (see Chapter 8, Setting Gradient Controller Operating Parameters).

To verify or configure the controller type:

1. Turn on the 600 controller by pressing the On/Off switch to the 1 (On) position. The Powerup screen appears (Figure 4-3).

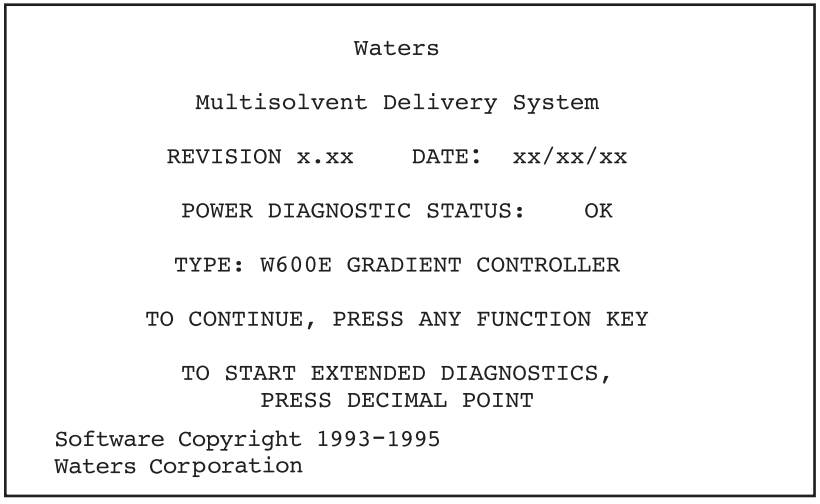


Figure 4-3 Gradient Controller Powerup Screen

2. Check the controller Type field. If the Type field contains:
   - **W600E PowerLine Controller** - Skip the rest of this section and proceed to Section 4.1.2, Selecting an Integrator.
   - **W600E Gradient Controller** - Proceed to step 3.
3. Press the Setup function key to display the Gradient Controller Pump Setup screen (Figure 4-4).

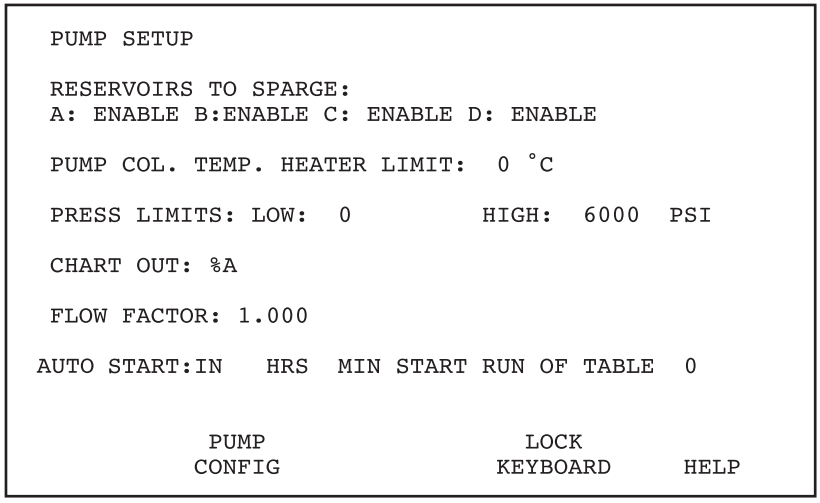


Figure 4-4 Gradient Controller Pump Setup Screen

It is not necessary to set any parameters on this screen right now. A description of this screen and parameters appears in Section 4.3, Setting Up the 600E Pump.

4. Press the Pump Config screen key to display the Gradient Controller Pump Configuration screen (Figure 4-5).

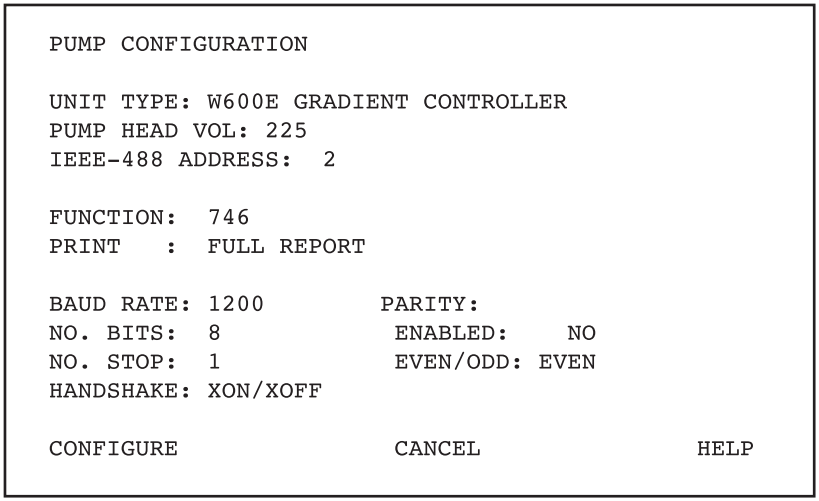


Figure 4-5 Gradient Controller Pump Configuration Screen

5. Move the cursor to the Unit Type field.

   Note: *Press the Help screen key to display the available controller configuration options.*

6. Type 1 to select the PowerLine controller configuration. Press Enter.
7. Press the Configure screen key to effect the change. The controller reboots and returns to the Powerup screen. The Type field contains:

   `W600E PowerLine Controller`

8. Continue with the following sections:
   - Section 4.1.2, Selecting an Integrator
   - Section 4.1.3, Selecting the Report Type
   - Section 4.2, Verifying that PowerLine Devices Are Active
   - Section 4.3, Setting Up the 600E Pump

4.1.2 Selecting an Integrator

This section describes how to set the parameters for an optional Waters 745B/746 Data Module (integrator). An integrator allows you to print out a report at the conclusion of a run.

Accessing the Pump Configuration Screen

1. Press the Set Up function key from the PowerLine Controller Powerup screen. The PowerLine Controller Pump Setup screen appears (Figure 4-6).

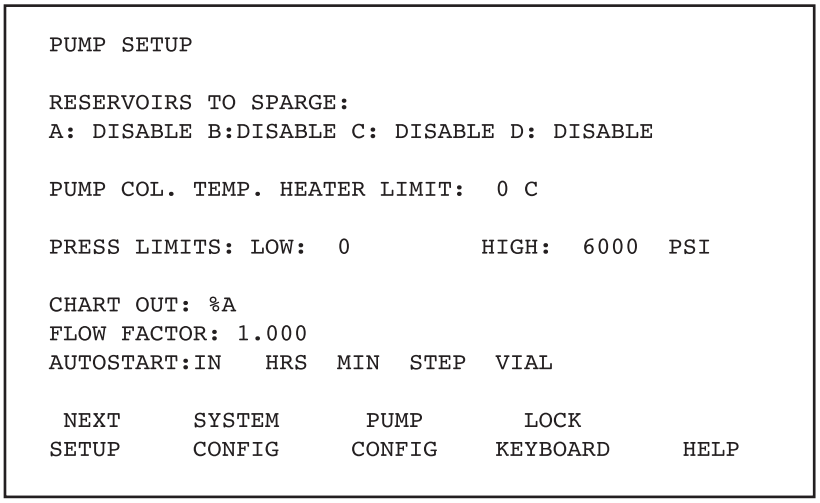


Figure 4-6 PowerLine Controller Pump Setup Screen

2. Press the Pump Config screen key. The PowerLine Controller Pump Configuration screen appears (Figure 4-7).

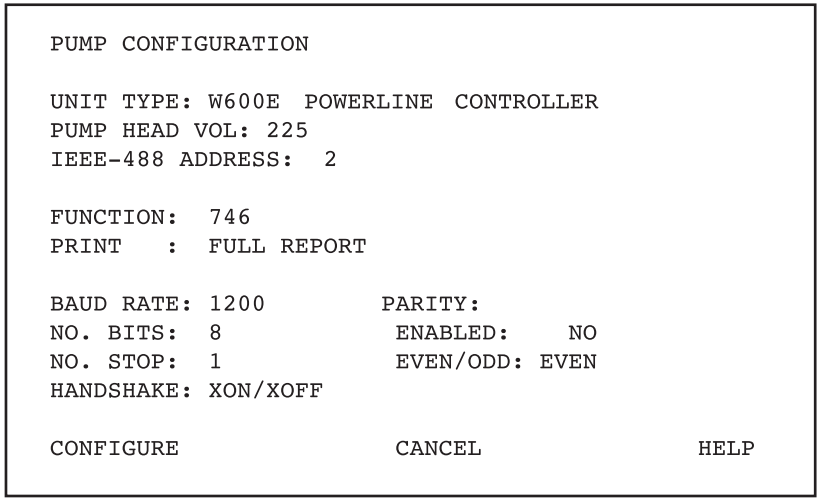


Figure 4-7 PowerLine Controller Pump Configuration Screen

The Function parameter on the Pump Configuration screen (Figure 4-7) identifies the module connected to the RS-232 interface. This module can be either the:

- 745B Data Module
- 746 Data Module

Table 4-1 lists the default RS-232 communication parameter values defined by the controller.

Table 4-1 Default RS-232 Communication Parameter Values

Parameter	Function=0 (Host Link)	Function=1 (745B)	Function=2 (746)
Baud Rate	9600	1200	1200
Bit Number	8	8	8
Stop bits number	1	1	1
Handshake	CTS/RTS	CTS	XON/XOFF
Parity: Enabled Even/Odd	No Even	No Even	No Even



Attention: *Do not change the default RS-232 communication parameters at the bottom of the screen. Otherwise, communication problems will result.*

Selecting an Integrator on the RS-232 Connector

1. Move the cursor to the Function field on the Pump Configuration screen (see Figure 4-7).
2. Type the number for the module you want to use:
   - 0 for no device connected
   - 1 for a Waters 745B Data Module
   - 2 for a Waters 746 Data Module
3. Press Enter. The system automatically displays the default RS-232 communication parameters in the fields at the bottom of the screen.
4. If you want to select or change the report type, continue with Section 4.1.3, Selecting the Report Type.

5. If you do not want to select or change the report type, press the Configure screen key to save your selections. The controller reboots and returns to the Powerup screen.
6. Proceed with the following sections:
   - Section 4.2, Verifying that PowerLine Devices Are Active
   - Section 4.3, Setting Up the 600E Pump

4.1.3 Selecting the Report Type

If you connected a Waters 745B/746 Data Module to the RS-232 port, specify the type of report to print as follows:

1. Move the cursor to the Print field.
2. Type the number for the desired report option:
   - 0 for no report
   - 1 for a short report (see Figure 4-8)
   - 2 for an abridged report (see Figure 4-9)
   - 3 for a full report (see Figure 4-10)
3. Press the Configure screen key to save your selections. The controller reboots and returns to the Powerup screen.
4. Continue with Section 4.3, Setting Up the 600E Pump, to set up the pump for the first time or to change your pump setup parameter values.

```
PUMP SETUP

RESERVOIRS TO SPARGE:
   A: ENABLED B: OFF    C: OFF     D: OFF
PUMP COL. TEMP HEATER LIMIT:   0 C
PRESS LIMITS: LOW:   0  HIGH: 4000 PSI

CHART OUT:   %A

AUTOINJECTOR SETUP

       SYRINGE DRAW RATE: NORMAL
       INJECT DELAY: 0.0 ml

     AUTO STDS: NO

DISSOLUTION ENABLED: NO

DETECTOR SETUP:

DET #1          490
 DATA CHAN:       0
 TIME CONST:    1.0
 AUTOZERO:       ON



 POLARITY:      POS

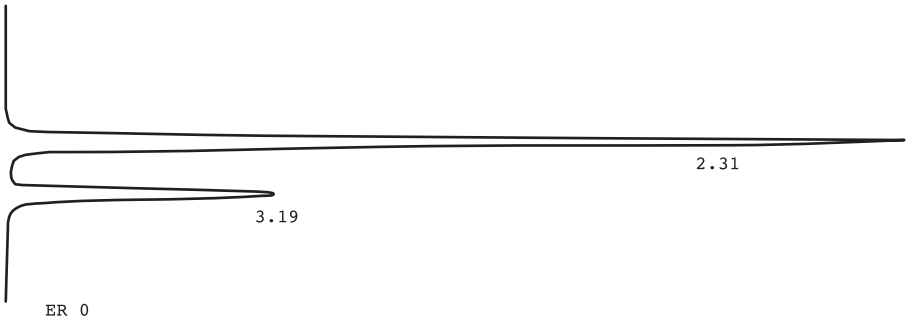
DET #2          490
 DATA CHAN:       0
 TIME CONST:    1.0
 AUTOZERO:       ON
 POLARITY:      POS

USING TABLE # 1
_______________


Working on STEP 1, VIAl  1, VOLUME   10, INJECTION #1
```

Figure 4-8 Example of a Short Report (First Injection)

```
CHANNEL A  INJECT      17-12-89 14:59:48  STORED TO BIN #  36
```




```
DATA SAVED TO BIN # 36

LYNN                          17-12-89  14:59:48    CH= "A"  PS= 1.

FILE 1.     METHOD  0.     RUN 36       INDEX 36            BIN 36

PEAK#     AREA%        RT       AREA  BC

    1      76.786     2.31  10019814 01
    2      23.214     3.19   3029213 01

TOTAL     100.              13049027


  Run Completed Successfully
```

Figure 4-8 Example of a Short Report (First Injection) (Continued)

```
PUMP SETUP

RESERVOIRS TO SPARGE:
   A: ENABLED B: OFF    C: OFF     D: OFF
PUMP COL. TEMP HEATER LIMIT:   0 C
PRESS LIMITS: LOW:   0  HIGH: 4000 PSI

CHART OUT:   %A

AUTOINJECTOR SETUP

       SYRINGE DRAW RATE: NORMAL
       INJECT DELAY: 0.0 ml

     AUTO STDS: NO

DISSOLUTION ENABLED: NO

DETECTOR SETUP:

DET #1          490
 DATA CHAN:       0
 TIME CONST:    1.0
 AUTOZERO:       ON
 POLARITY:      POS

DET #2          490
 DATA CHAN:       0
 TIME CONST:    1.0
 AUTOZERO:       ON
 POLARITY:      POS

       GRADIENT TABLE #1

   TIME     FLOW      %A      %B      %C      %D     CURVE
INITIAL     0.50     100       0       0       0       *
  20.00     0.50     100       0       0       0       6




  25.00     0.00     100       0       0       0       6


       EVENT TABLE #1

TIME    EVENT ACTION
 0.00    ALRM    OFF
```

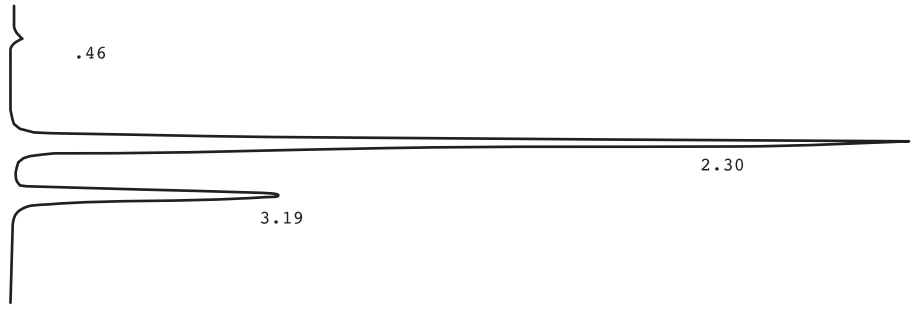
Figure 4-9 Example of an Abridged Report (First Injection)

```
       DETECTOR TABLE #1

   TIME        CHANNEL  nm      AUFS
INITIAL           1    254     1.000
INITIAL           2    210     1.000

Working on STEP 1,  VIAL 1,  VOLUME    10,  INJECTION #  1

CHANNEL A  INJECT      17-12-89 14:36:18  STORED TO BIN #  34
```




```
 ER 0
DATA SAVED TO BIN # 34

LYNN                          17-12-89  14:36:18    CH= "A"  PS= 1.

FILE 1.     METHOD  0.     RUN 34       INDEX 34            BIN 34

PEAK#     AREA%        RT       AREA  BC

    1       0.563     0.46      74994 01
    2      77.881     2.3    10375990 01
    3      23.214     3.19    2871959 01

TOTAL     100.              13322943

WARNING - MEMORY AT 0. K - UNPROTECTED CHROMATOGRAMS WILL BE REPLACED

  Run Completed Successfully
```

Figure 4-9 Example of an Abridged Report (First Injection) (Continued)

```
PUMP SETUP

RESERVOIRS TO SPARGE:
   A: ENABLED B: OFF    C: OFF     D: OFF
PUMP COL. TEMP HEATER LIMIT:   0 C
PRESS LIMITS: LOW:   0  HIGH: 4000 PSI




CHART OUT:   %A

AUTOINJECTOR SETUP

       SYRINGE DRAW RATE: NORMAL
       INJECT DELAY: 0.0 ml

     AUTO STDS: NO

DISSOLUTION ENABLED: NO

DETECTOR SETUP:

DET #1          490
 DATA CHAN:       0
 TIME CONST:    1.0
 AUTOZERO:       ON
 POLARITY:      POS

DET #2          490
 DATA CHAN:       0
 TIME CONST:    1.0
 AUTOZERO:       ON
 POLARITY:      POS

       GRADIENT TABLE #1

   TIME     FLOW      %A      %B      %C      %D      CURVE
INITIAL      0.50     100       0       0       0        *
  20.00      0.50     100       0       0       0        6
  25.00      0.00     100       0       0       0        6


       EVENT TABLE #1

TIME     EVENT ACTION
 0.00     ALRM    OFF
```

Figure 4-10 Example of a Full Report (All Injections)

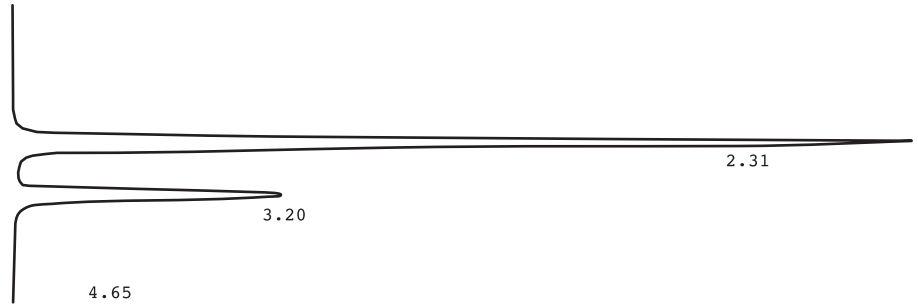
```
       EVENT TABLE #1

 TIME   EVENT   ACTION
 0.00    ALRM      0FF


       DETECTOR TABLE #1

   TIME        CHANNEL  nm     AUFS
INITIAL           1    254    1.000
INITIAL           2    210    1.000


Working on STEP 1,  VIAL 1,  VOLUME    10,  INJECTION #
 1
CHANNEL A  INJECT      17-12-89 13:37:28  STORED TO BIN #  32
```




```
 ER 0
DATA SAVED TO BIN # 32


LYNN                          17-12-89  13:37:28    CH= "A"  PS= 1.

FILE 1.      METHOD 0.     RUN 32        INDEX 32           BIN 32

PEAK#     AREA%      RT       AREA  BC

    1      78.335   2.31  10537023 01
    2      21.357   3.2    2872703 01
    3       0.308   4.65     41424 01

TOTAL     100.            13451150


   Run Completed Successfully
```

Figure 4-10 Example of a Full Report (All Injections) (Continued)

4.2 Verifying that PowerLine Devices Are Active

Verify that PowerLine devices are active only if you will be using a PowerLine autoinjector (717plus autosampler) or a PowerLine detector (410 Refractive Index, 431/432 Conductivity, 486 Tunable Absorbance, or 490E Programmable Multiwavelength) in your system. Continue with Section 4.3, Setting Up the 600E Pump, if you are not using these devices.

Powerup Verification of PowerLine Devices

When you power up the 600 controller, it automatically scans the IEEE-488 bus to locate active PowerLine devices.

The 600 can control one PowerLine autoinjector (Waters 717plus) and up to three PowerLine detectors (Waters 410, 431/432, 486, or 490E) at one time.

For information on connecting PowerLine devices to the IEEE-488 bus, see the *Waters 600E Multisolvent Delivery System Installation and Maintenance Guide*, Section 3.3, Making IEEE-488 Interface Connections.

Verifying PowerLine Devices

To verify that the PowerLine devices in your 600E system are active on the IEEE-488 bus:

1. Press the Setup function key to display the Pump Setup screen (Figure 4-6).
2. Press the System Config screen key from the Pump Setup screen to display the System Configure screen (Figure 4-11).

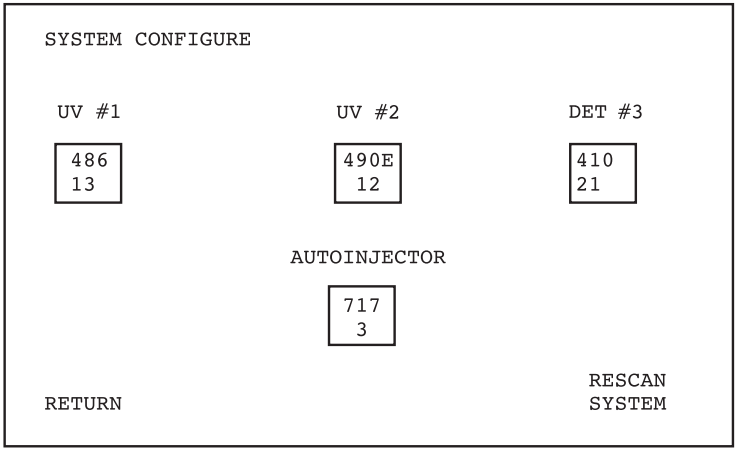


Figure 4-11 System Configure Screen

Active PowerLine Devices

The System Configure screen indicates which PowerLine devices are active on the IEEE-488 bus. This screen displays *device boxes* that include the device names and IEEE addresses of all PowerLine devices that the system recognizes.

3. Verify that the PowerLine devices installed in your system appear on the System Configure screen.
4. If a connected PowerLine device is not listed on the System Configure screen, press the Rescan System screen key. The controller rescans the IEEE-488 bus to locate active devices.

   The controller must rescan the system to recognize PowerLine devices and to make them active if:

   - You powered up a PowerLine device after you powered up the controller
   - You powered up the controller before a PowerLine device finished its powerup calibration sequence
5. If rescanning the system does not list all PowerLine installed devices, check your IEEE-488 connections. See the *Waters 600E Multisolvent Delivery System Installation and Maintenance Guide*, Section 3.3, Making IEEE-488 Interface Connections, for the PowerLine device connection procedure.

   Note: *If you do not set a PowerLine device IEEE-488 address correctly, the controller will not recognize the device. See the Waters 600E Multisolvent Delivery System Installation and Maintenance Guide, Section 3.3, Making IEEE-488 Interface Connections, for information on setting addresses and powerup sequences.*
6. Press the Return screen key to exit the System Configure screen and return to the Pump Setup screen.
7. If you are setting up your system for the first time, or if you wish to change one or more pump parameters, proceed to Section 4.3, Setting Up the 600E Pump.

4.3 Setting Up the 600E Pump

This section describes how to access and use the Pump Setup screen in PowerLine Control mode. It includes the following information:

- Accessing the Pump Setup screen
- Setting Pump Setup screen parameters
- Exiting the Pump Setup screen

Pump Setup Overview

The PowerLine Controller Pump Setup screen includes the following configuration selections:

- Enable/disable reservoir sparging
- High-temperature limit for an optional column heater
- High and low operating-pressure limits for the system
- External chart output function
- Enable/disable a flow factor to account for flow path elasticity or eluent compressibility
- Enable/disable and program autostart
- Lock/unlock keyboard

Figure 4-12 illustrates the steps for using the Pump Setup screen. Use Figure 4-12 as a quick reference for setting the parameters on the Pump Setup screen.

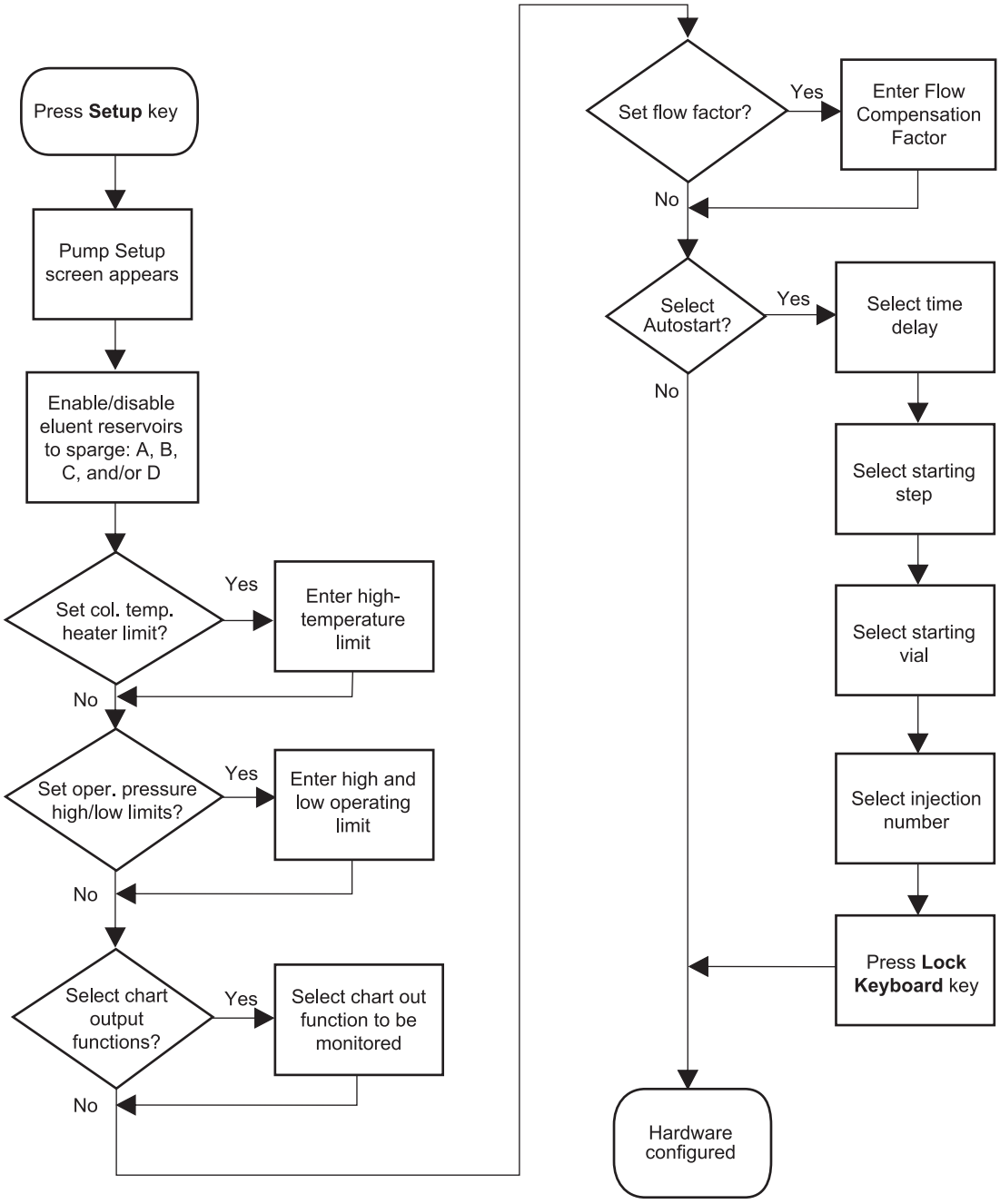


Figure 4-12 PowerLine Controller Pump Setup Flowchart

4.3.1 Accessing the Pump Setup Screen

1. Press the Set Up function key from any screen except the PowerLine Controller Pump Configuration screen. The PowerLine Controller Pump Setup screen appears (Figure 4-13).
2. If you are at the PowerLine Controller Pump Configuration screen and do not need to save changes, press the Cancel screen key to return to the PowerLine Controller Pump Setup screen.
3. If you are at the PowerLine Controller Pump Configuration screen and do need to save changes, press the Configure screen key to save your changes. The controller reboots and returns to the Powerup screen.
4. Press the Set Up function key. The PowerLine Controller Pump Setup screen appears.

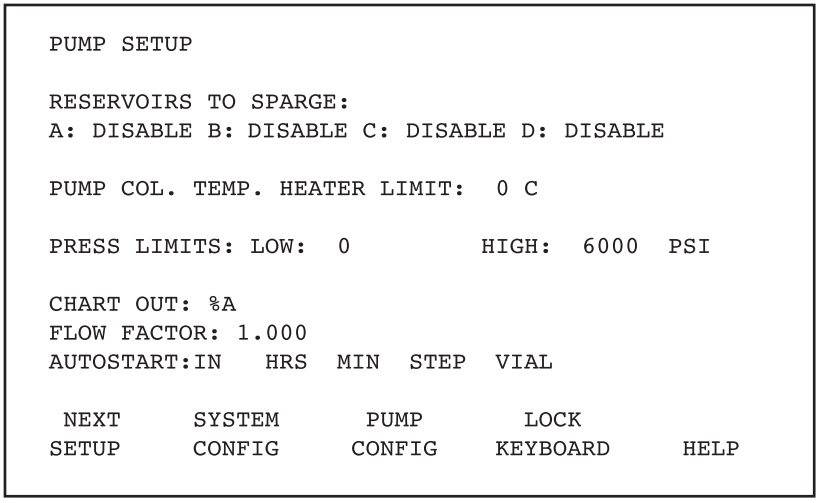


Figure 4-13 PowerLine Controller Pump Setup Screen

In addition to the parameters shown on the screen, the PowerLine Controller Pump Setup screen also displays the screen keys described in Table 4-2.

Table 4-2 Pump Setup Screen Keys

Key	Function
Next Setup	Displays the configuration screens for the autoinjector and detectors.
System Config	Identifies the PowerLine modules active in the system.

Table 4-2 Pump Setup Screen Keys (Continued)

Key	Function
Pump Config	Displays additional configuration parameters related to the pump and controller.
Lock Keyboard	Locks or unlocks the keyboard.
Help	Displays the Help screen for the Pump Setup screen.

4.3.2 Setting Pump Setup Parameters

This section describes the pump parameters on the Pump Setup screen (see Figure 4-13) and provides procedures for setting each parameter.

The Pump Setup parameters are:

- Reservoirs to sparge
- Pump col. temp. heater limit
- Press limits
- Chart out
- Flow factor
- Autostart

Reservoirs to Sparge

Indicates which reservoirs you want to enable for helium sparging. The Reservoirs to Sparge parameter only enables a particular reservoir for sparging; it does not initiate the sparging process. The sparge flow rate can be set later on the Direct Control, Operate Methods, or Program Event screens, depending on the mode of operation.

Note: *If helium is unavailable or undesirable, see Appendix B, Eluent Considerations, for alternative sparging methods.*

To set the Reservoirs to Sparge parameter:

1. Move the cursor to the field for the reservoir that you want to enable for sparging.
2. Press 0 to disable sparging, or 1 to enable sparging for that reservoir.
3. Press Enter.
4. Repeat the previous three steps for each active reservoir that you want to sparge.

Pump Col. Temp. Heater Limit

Defines the high-temperature limit for the optional column heater. The upper limit of the heater module is on the module label.

Note: *To prevent damage to the column, the heater module shuts down when the temperature exceeds the programmed limit for more than 1.2 seconds.*

To set the pump column temperature heater limit:

1. Move the cursor to the Pump Col. Temp. Heater Limit field.
2. Type the appropriate temperature value (0 to 99) in °C.
3. Press Enter.

Press Limits

Defines the high and low operating-pressure limits of the Waters 600E pump.

Your system column and packing chemistry determine the low and high values. See the *Care and Use Manual* supplied with the column to determine the maximum pressure to which you can expose the column.

You typically set the high-pressure limit to 50 to 100 psi (3.5 to 7.0 kg/cm^2) below the normal operating-pressure limit for the column. For more information, see the *Care and Use Manual* for the column.

Note: *To prevent damage to the pump or column, the system shuts down when the pressure exceeds the high-pressure limit for more than 1.2 seconds or falls below the low-pressure limit for 60 seconds.*

Maximum Pressure

Maximum Pressure	Flow Rate
6000 psi (420 kg/cm^2)	10 mL/min
1000 psi (70 kg/cm^2)	45 mL/min

Low Pressure and High Pressure

To set the pressure limits:

1. Move the cursor to the Low field.
2. Enter a low-pressure limit value within the range 0 to 5950 psi (0 to 416.5 kg/cm^2).
3. Press Enter.

4. Move the cursor to the High field.
5. Enter a high-pressure limit value within the range 51 to 6000 psi (3.6 to 420 kg/cm^2).
6. Press Enter.

Chart Out

Determines which signal is available at the Chart Out terminal on the rear panel of the controller. This 0 to 10 mV signal can indicate the composition percentage of any one of the four eluents (%A, %B, %C, or %D), the eluent flow rate (Flow), or the column temperature (Temp).

To use this feature, connect the Chart Out terminals on the rear panel of the controller to the 0 to 10 mV terminal on the recorder or integrator.

You can also connect this output signal to the second pen of a chart recorder or to a data system. It is frequently used during troubleshooting to reflect a selected condition, such as pump flow or eluent composition.

A time delay between the eluent composition shown by the Chart Out signal and the eluent composition at the column inlet always occurs. This time delay is the system, or delay, volume. The 600E system has been optimized to reduce the delay volume.

The detector signal is further delayed by the volume of the column. At a low flow rate, with a column attached, the delay between an eluent changeover instruction and its subsequent detection may be several minutes.

To set the Chart Out parameter:

1. Move the cursor to the Chart Out field.
2. Type the number for the desired output:
   - 1, 2, 3, or 4 for the % composition of one of the four eluents (A, B, C, or D)
   - 5 for flow rate
   - 6 for column heater temperature
3. Press Enter.

Flow Factor

Speeds up or slows down the pump to compensate for differences in pressure or eluent compressibility. You enter a value here only if you need to match flow rate conditions between systems or between different eluents.

To determine the flow factor for your application:

1. Set the flow rate at 1.0 mL/min.
2. Measure the actual flow rate.
3. If the actual flow rate is less than or greater than 1.0 mL/min, type a flow factor value to compensate for the difference. The valid range is 0.950 to 1.050. For example, if your actual flow rate is 0.98 mL/min, use a flow factor of 1.02.
4. Press Enter.

Autostart

Specifies a time delay before a run starts and tells the system where to begin execution in a Method Table (step number and vial number). You can use Autostart at any time.

While the autostart countdown is in progress, you can continue to run a method started before you initiated Autostart. When the Autostart countdown completes, the system immediately aborts any run in progress, and the Autostart parameters take precedence.

To Autostart a method:

1. Press the Set Up function key to display the Pump Setup screen.
2. Move the cursor to the Autostart field.
3. Type the total delay time in the Hrs and Min fields and press Enter.
   - Enter a value between 0 and 99 for Hrs (hours).
   - Enter a value between 0 and 59 for Min (minutes).
4. At the Step field, type the step number of the Method Table where program execution will start. Press Enter.
5. At the Vial field, type the vial number in the specified step number where sample analysis will start. Press Enter.

Sample Method Table

For example, your Method table may appear as shown in Figure 4-14.

STEP	FIRST VIAL	LAST VIAL	# INJ	INJ VOL	RUN TIME	TABLE #
1	PRG 1				4.5	3
2	EQUIL				3	
3	5	17	2	35	8.5	3
4	PRG 1				4.5	5
5	EQUIL				12	5
6	20	31	1	50	11	5

Figure 4-14 Example of a Method Table

If you want to equilibrate your system 12 hours from now and start on vial 15, type the following in the Autostart field:

```
In 12 Hrs 0 Min Step 3 Vial 15
```

6. Activate autostart by pressing the Lock Keyboard screen key. The countdown time appears on the Pump Setup screen.

   Note: *When the keyboard is locked, pressing the function keys will display screens, but you cannot use the screens to make changes. Make sure you have programmed all gradient, event, and detector tables before locking the keyboard.*

Canceling Autostart

To cancel a countdown in progress, unlock the keyboard by pressing the Unlock Keyboard screen key.

Keyboard Lock/Unlock

Locking the keyboard:

- Prevents accidental alteration of gradient flow conditions
- Starts the autostart condition

When the keyboard is locked, the screen key legend changes to Unlock Keyboard.

Unlock Keyboard, which is the only operational key on the Pump Setup screen, cancels the Autostart condition.

4.3.3 Exiting the Pump Setup Screen

1. Press any one of the following screen keys to select another screen:
   - Next Setup - Displays the Autoinjector Setup screen
   - System Config - Displays the System Configuration screen
   - Pump Config - Displays the Pump Configuration screen
2. Press one of the function keys to enter a different mode.

4.4 Setting Up the Autoinjector

Refer to this section when using a PowerLine autosampler (Waters 717plus) in your system.

This section describes:

- Accessing the Autoinjector Setup screen
- Setting Autoinjector Setup parameters
- Exiting the Autoinjector Setup screen

Note: *Make sure the Waters 600 controller has recognized the autoinjector on the IEEE-488 bus before performing this procedure. See Section 4.2, Verifying that PowerLine Devices Are Active, for more information.*

Figure 4-15 illustrates the steps involved in using the Autoinjector Setup screen. Use this flowchart as a quick reference for setting the parameters on the Autoinjector Setup screen.

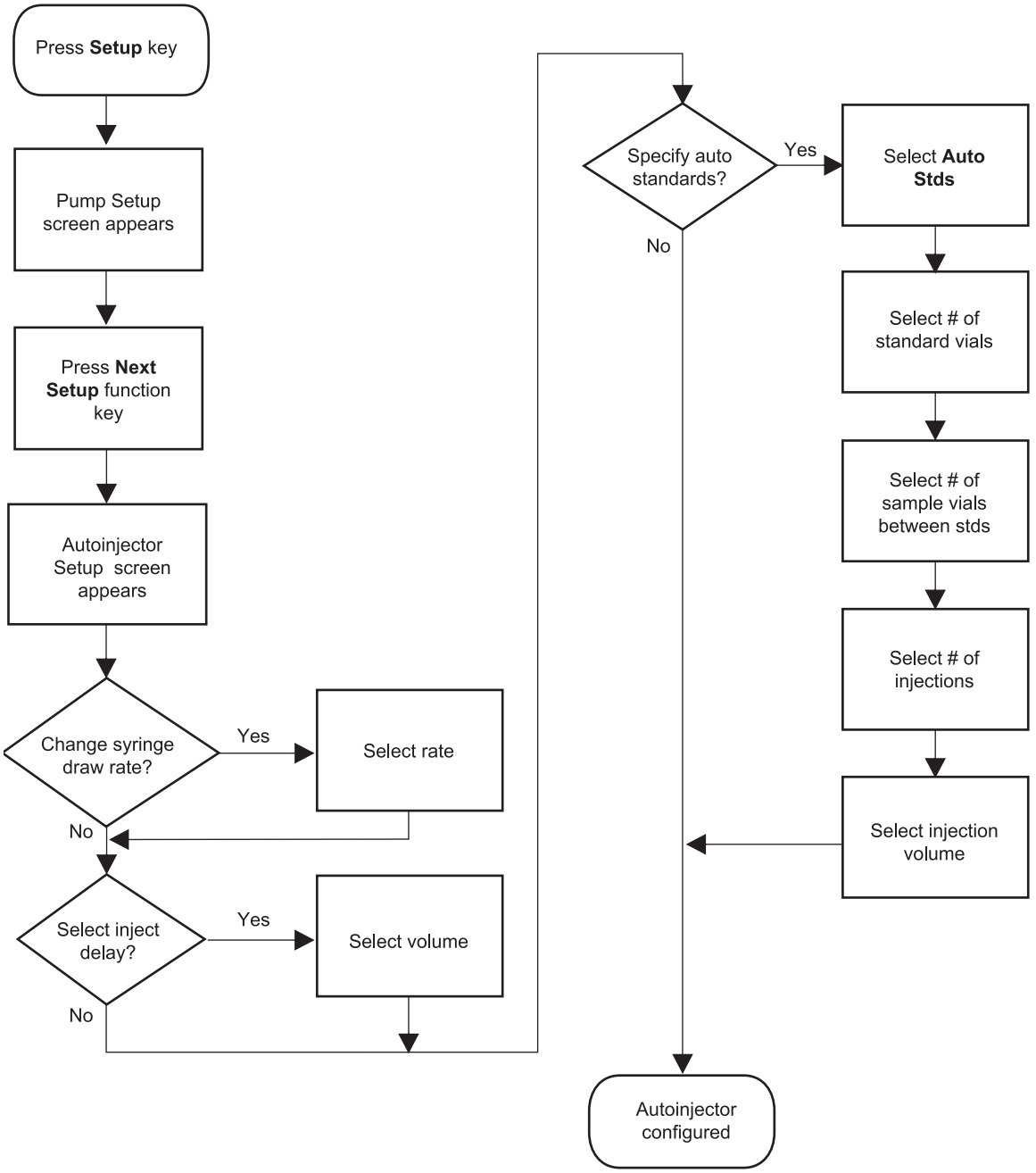


Figure 4-15 Autoinjector Setup Flowchart

4.4.1 Accessing the Autoinjector Setup Screen

You set up the autoinjector through the Autoinjector Setup screen. To access the Autoinjector Setup screen (Figure 4-16):

1. Press the Set Up key to display the Pump Setup screen (Figure 4-13).
2. Press the Next Setup screen key to display the Autoinjector Setup screen (Figure 4-16).

The Autoinjector Setup screen includes the following parameter selections:

- Syringe draw rate
- Inject delay
- Auto standards

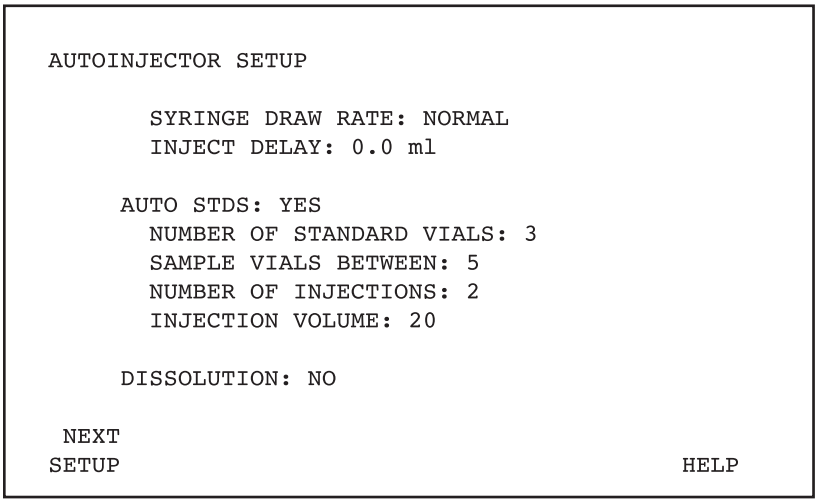


Figure 4-16 Autoinjector Setup Screen

In addition to the parameters shown on the screen, the Autoinjector Setup screen displays screen keys described in Table 4-3.

Table 4-3 Autoinjector Setup Screen Keys

Key	Function
Next Setup	Displays the Detector Setup screen.
Help	Displays the Help screen for the Autoinjector Setup screen.

4.4.2 Setting Autoinjector Setup Parameters

This section describes the autoinjector setup parameters and the procedures for setting them.

Syringe Draw Rate

Controls the rate at which the autoinjector draws the sample from the vial. The slow rate is useful for viscous or gassy samples.

To set the Syringe Draw Rate parameter:

1. Move the cursor to the Syringe Draw Rate field.
2. Type 1 for normal (3.7 µL/sec - default) or 0 for slow (1.8 mL/sec) operation.
3. Press Enter.

Inject Delay

Instructs the system to pump a selected volume of eluent before making an injection.

This feature allows a gradient to start or external events to complete before a sample is injected. (See Chapter 6, Creating PowerLine Methods, for more information on gradients and external events.)

To set the Inject Delay parameter:

1. Move the cursor to the Inject Delay field.
2. Type a volume value between 0.0 and 9.9 (mL).
3. Press Enter.

Auto Standards

Specifies the sequence of standards or samples for the Waters autoinjector.

Note: *Special function vial codes (for example Purge Inj., Equilibrate) are executed in priority over the auto standards routine at the beginning of a method run (see Table 5-1).*

Standards can occupy up to the first five consecutive vial positions in the Waters autoinjector carousel and must start with Vial 1. If you are using fewer than five standards, samples can start in the next vial position after the last standard.

Standards are run at the beginning of the method and are repeated after the specified number of sample vials has been run.

To set the Auto Standards parameter:

1. Move the cursor to the Auto Stds field.
2. Type 0 (No, default) to turn off auto standard sequencing, or type 1 (Yes) to select auto standard sequencing. Press Enter.
3. If you typed 1 in step 2, enter values for the following parameters:
   - **Number of Standard Vials** - Type the number of standard vials to run (maximum 5) and press Enter.
   - **Sample Vials Between** - Type the number of sample vials to be run between the set of standards (1 to 48/96) and press Enter.
   - **Number of Injections** - Type the number of injections to be made from each standard vial (maximum 9) and press Enter.
   - **Injection Volume** - Type the volume in µL (0 to 2000) for a standard injection and press Enter.

Dissolution

Specifies whether you will be using a dissolution bath with your Waters WISP 712D Autoinjector.

To set the Dissolution parameter:

1. Move the cursor to the Dissolution field.
2. Type 0 (No) or 1 (Yes).
3. Press Enter.

4.4.3 Exiting the Autoinjector Setup Screen

To exit the Autoinjector Setup screen:

- Press the Next Setup screen key to display:
  - The Detector Setup screen (if the value of the Dissolution parameter on the Autoinjector Setup screen is No)
  - The Dissolution Setup screen (if the value of the Dissolution parameter on the Autoinjector Setup screen is Yes). See Section 4.5, Setting Up Dissolution Control.
- Alternatively, press any function key to select another mode.

4.5 Setting Up Dissolution Control

Use the Dissolution Setup screen to coordinate the collection and transfer of dissolution samples from a Hanson Transfer Controller into the WISP 712D autoinjector sample vials.

Accessing the Dissolution Setup Screen

1. Press the Set Up function key. The Pump Setup screen appears (see Figure 4-13).
2. Press the Next Setup screen key. The Autoinjector Setup screen appears (see Figure 4-16).
3. Enable dissolution by moving the cursor to the Dissolution field, typing 1, and pressing Enter.
4. Press the Next Setup screen key. The Dissolution Setup screen appears.

When you use the 600E system for dissolution control, the collection of dissolution samples becomes the highest-priority function of the system. When the 712D is not in the process of collecting dissolution samples, you can run chromatography on previously collected samples or calibration standards.

For details on Dissolution Control, see the *Waters Dissolution System Operator's Manual*.

4.6 Setting Up the Detectors

Refer to this section when using PowerLine detectors (Waters 410, 431/432, 486, or 490E) in your system.

This section describes:

- Accessing the Detector Setup screen
- Setting Detector Setup parameters
- Exiting the Detector Setup screen

Note: *Make sure that the Waters 600 controller has recognized all detectors on the IEEE-488 bus before performing this procedure. See Section 4.2, Verifying that PowerLine Devices Are Active, for more information.*

Using Multiple Detectors

You may use up to three PowerLine detectors in the system at one time:

- Two UV/Vis absorbance detectors (two Waters 486 detectors, two channels of a Waters 490E detector, or one 486 and one 490E channel) and
- One refractive index (RI) detector (Waters 410) or one conductivity detector (Waters 431/432)

Detector Considerations

Be aware of the following considerations when using a PowerLine detector:

- Det #1 and Det #2 are dedicated to the UV/Vis detectors, and Det #3 is always dedicated to the RI or the conductivity detector.
- If the system contains both a 486 and a 490E, the IEEE-488 address of the 486 must be less than the address of the 490E. If the 486 address is higher, the controller assigns both detector channels to the 490E and does not recognize the 486.
- When controlling a Waters 431 or Waters 432 conductivity detector, both detectors are recognized as a 431 by the 600 controller.
- The 486 detector (firmware revision 4.10) operating with PowerLine version 2.1 or greater and/or ExpertEase version 3.0 or greater should have its DIP switch #7 set to the ON position (the factory default position). This setting is required for the 486 to be recognized by the 600 controller. Previous 486 detector firmware versions require DIP switch #7 to be in the OFF position in order for the 600 controller to recognize the 486 as a 484 detector.

Figure 4-17 summarizes the steps involved in using the Detector Setup screen. Use this flowchart as a quick reference for setting the parameters on the Detector Setup screen.

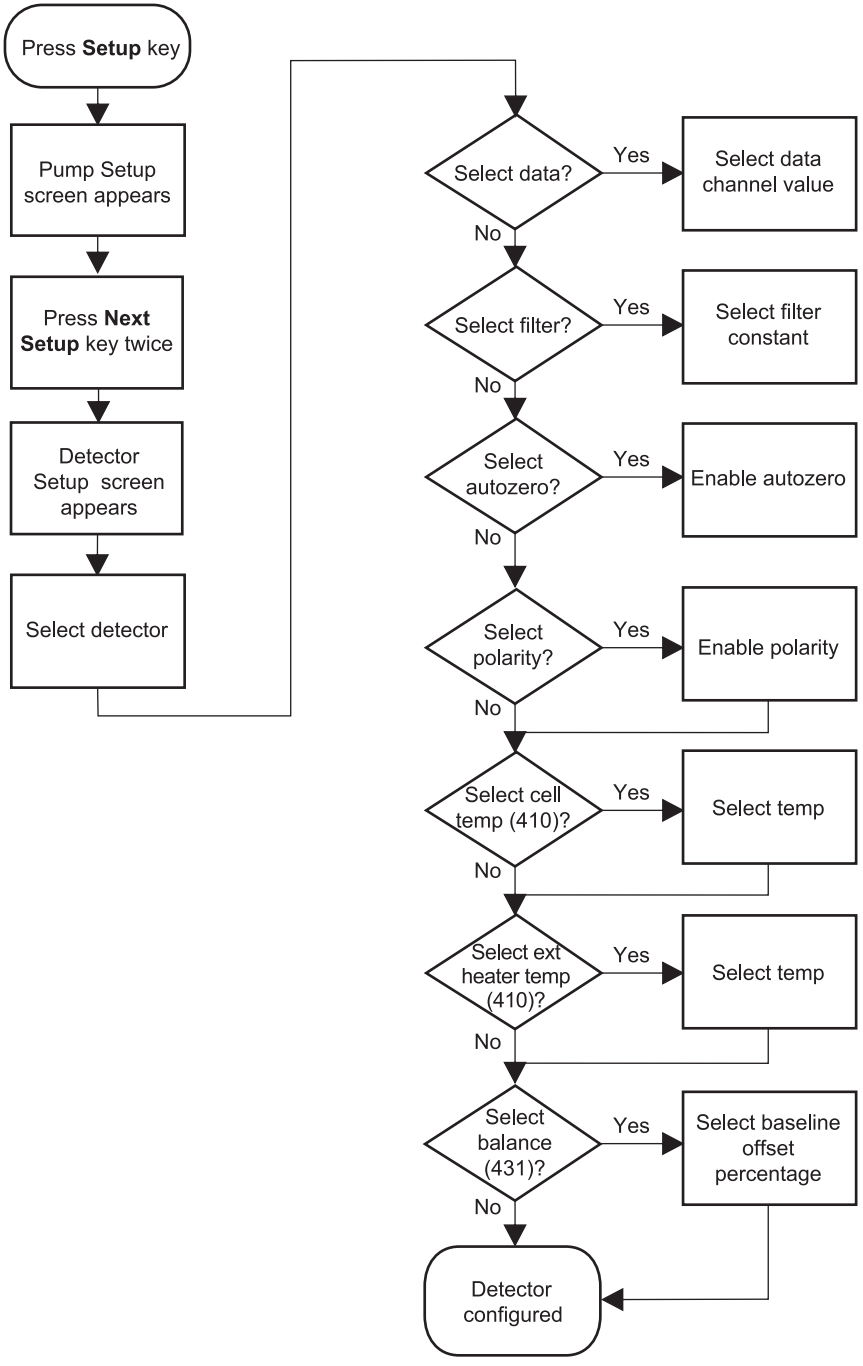


Figure 4-17 Detector Setup Flowchart

4.6.1 Accessing the Detector Setup Screen

To access the Detector Setup screen (Figure 4-18):

1. Press the Set Up function key to display the Pump Setup screen (see Figure 4-13).
2. Press the Next Setup screen key to display the Autoinjector Setup screen (see Figure 4-16).
3. Press the Next Setup screen key to display:
   - Detector Setup screen (if Dissolution Control is not enabled on the Autoinjector Setup screen)
   - Dissolution Control screen (if Dissolution Control is enabled on the Autoinjector Setup screen)
4. If the Dissolution Control screen appeared in step 3, press the Next Setup screen key again to display the Detector Setup screen.

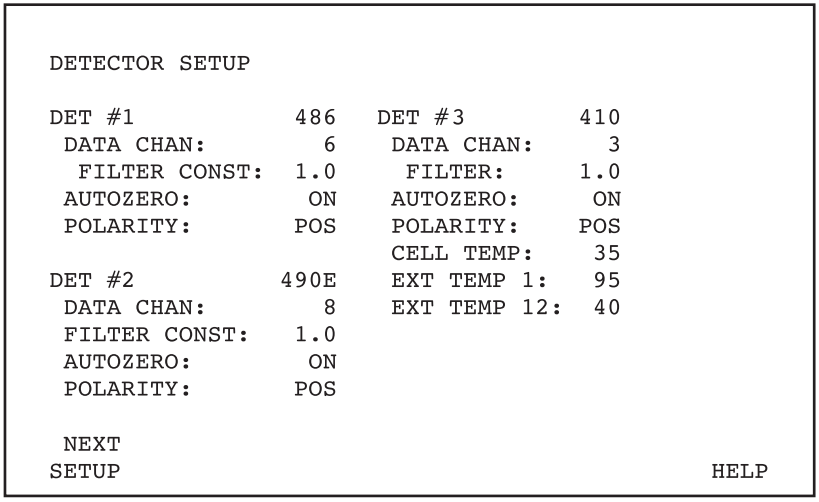


Figure 4-18 Detector Setup Screen (with 410 Detector Parameters)

The Detector Setup screen also supports setup of a Waters 431/432 Conductivity detector as an alternate Det #3 (Figure 4-19). The type of detector connected (410 or 431/432) determines which screen appears.

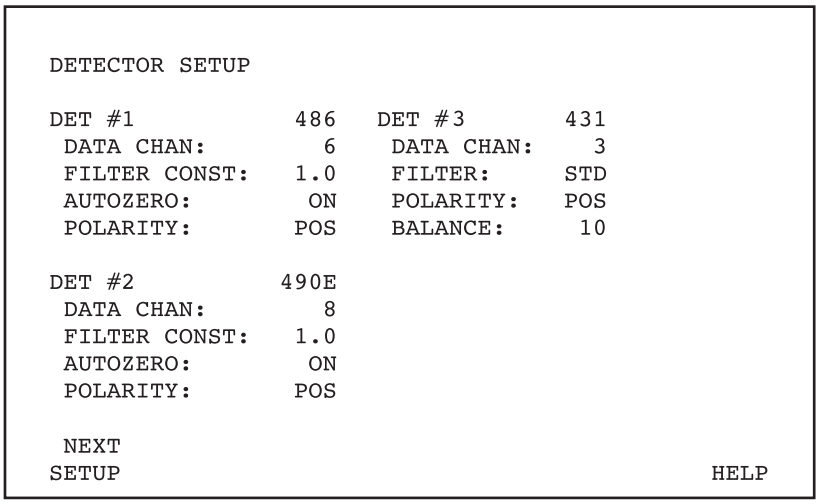


Figure 4-19 Detector Setup Screen (with 431/432 Detector Parameters)

In addition to the parameters shown on the screen, the Detector Setup screen displays the screen keys described in Table 4-4.

Table 4-4 Detector Setup Screen Keys

Key	Function
Next Setup	Displays the Pump Setup screen.
Help	Displays the Help screen for the Detector Setup screen.

4.6.2 Setting Detector Setup Parameters

This section describes the detector setup parameters and the procedures for setting them.

The Detector Setup screen includes the following parameter selections:

For UV/Vis detectors:

- Data channel
- Filter
- Autozero
- Polarity

For RI detectors (Waters 410):

- Data channel
- Filter
- Autozero
- Polarity
- Internal cell temperature
- Temperature of external heaters 1 and 2

For conductivity detectors (Waters 431/432):

- Data channel
- Filter
- Polarity
- Balance

Data Chan

Assigns an identifying number to a channel of data when using an integrator or data system (for example, the Waters Millennium Chromatography Manager or the Waters ExpertEase 845/860 Data System) with your Waters 600E system.

Note: *The channel number is arbitrary and is for documentation purposes only.*

To set the Data Chan parameter:

1. Move the cursor to the Data Chan field for the particular detector.
2. Type an appropriate data channel number between 0 and 99.
3. Press Enter.

Filter

Specifies the time constant of the digital noise filter applied to the output of the detector.

To set the Filter parameter:

1. Move the cursor to the Filter field for the particular detector.

2. Type a value:
    - For a UV detector, between 0.1 and 99.99 (seconds). (The default is 1 second). Press Enter.
    - For an RI detector, 0.2 (default), 1.0, 3.0, or 10.0. Press Enter.
    - For a 431/432 conductivity detector, Slow (1.0 sec.), Std (0.5 sec.), or Fast (0.25 sec.).

Autozero

Specifies whether the detector should zero its output at every injection.

To set the Autozero parameter:

1. Move the cursor to the Autozero field for the particular detector.
2. Type 1 (default) to enable, or 0 to disable, the autozero feature.
3. Press Enter.

Note: *See the appropriate conductivity detector operator's manual for details about the autozero operation that is controlled by 600 Controller events.*

Polarity

Specifies whether the data output of the detector is positive or negative.

Note: *This parameter affects only the detector 10 mV full scale output, except for the Waters 431/432 in which case both the 1 V output and the 10 mV output are affected.*

To set polarity:

1. Move the cursor to the Polarity field for the particular detector.
2. Type 1 for positive (default) or 0 for negative polarity.
3. Press Enter.

Balance

Besides the parameters described above, the 431/432 detector (Det #3) setup parameters contain a field for manually setting the percentage of baseline offset of the external chart recorder attached to the detector.

To set the baseline offset:

1. Move the cursor to the Balance field.
2. Type the appropriate offset percentage value.
3. Press Enter.

Cell temp

Sets the internal oven temperature of the 410 detector and defines an alarm to be set at 5 °C above that temperature.

To set the Cell Temp parameter:

1. Move the cursor to the Cell Temp field for Det #3.
2. Type a value between 30 and 50 (°C) to set a temperature, or 0 (default) to turn off this feature.
3. Press Enter.

Ext temp 1, Ext temp 2

Specifies the set temperature of up to two external column heaters connected to the Waters 410 detector.

To set the Ext Temp parameters:

1. Move the cursor to the appropriate Ext Temp field for Det #3.
2. Type 0 (default) to turn off this feature, or 1 to turn on this feature.
3. Press Enter.
4. If you typed 1, enter a temperature value between 0 and 99 (°C). Press Enter.

Note: *Although the Det #3 Help message states that the Radial Heater Module (RHM) has a range between 0 to 100 °C and that the Column Heater Module (CHM) has a range between 0 and 150 °C, the 600 controller temperature field only allows entries between 0 and 99 °C.*

4.6.3 Exiting the Detector Setup Screen

To exit the Detector Setup screen do the following:

- Press the Next Setup screen key or the Set Up function key to return to the Pump Setup screen.
- Press one of the other function keys to enter another mode.

Chapter 5
Running PowerLine in Direct Control Mode

Once you have familiarized yourself with your system and prepared your system according to the procedures in Chapters 1 through 4, you can run your Waters 600E Multisolvent Delivery System in the direct control mode.

Direct Control

All direct-control operations are *isocratic*. Isocratic operations keep flow rate, eluent composition, or other time-dependent conditions, constant. To change flow rate or eluent composition, or to switch output states, you must manually program new values to override the original parameter settings. The system implements these changes immediately.

Direct control mode is useful for preparing the system to make a run. You program direct-control operation through the Direct Control screen (see Section 5.1, Setting Run Conditions).

By setting parameters on the Direct Control screen, you can do the following without making an injection:

- Sparge eluents
- Prime the system with new eluent
- Equilibrate the system
- Monitor the baseline
- Set external switch parameters

Before You Begin

This chapter assumes that you have:

- Installed your system and all external devices according to the procedures in the *Waters 600E Multisolvent Delivery System Installation and Maintenance Guide*

- Familiarized yourself with basic operation and have decided upon a controller configuration (Chapter 2, Basic Operation)
- Prepared your system as outlined in Chapter 3, Preparing Your 600E System for Operation
- Set operating parameters as described in Chapter 4, Setting PowerLine Controller Operating Parameters

5.1 Setting Run Conditions

Before you make a run with the PowerLine controller in the Direct Control mode, you must first set the run conditions. This section describes the procedures for setting the following run conditions:

- Pump parameters
- External event switches
- Autoinjector parameters
- Detector parameters

Figure 5-1 illustrates the steps involved in using the Direct Control screen. Use this figure for quick reference once you become familiar with the 600E system.

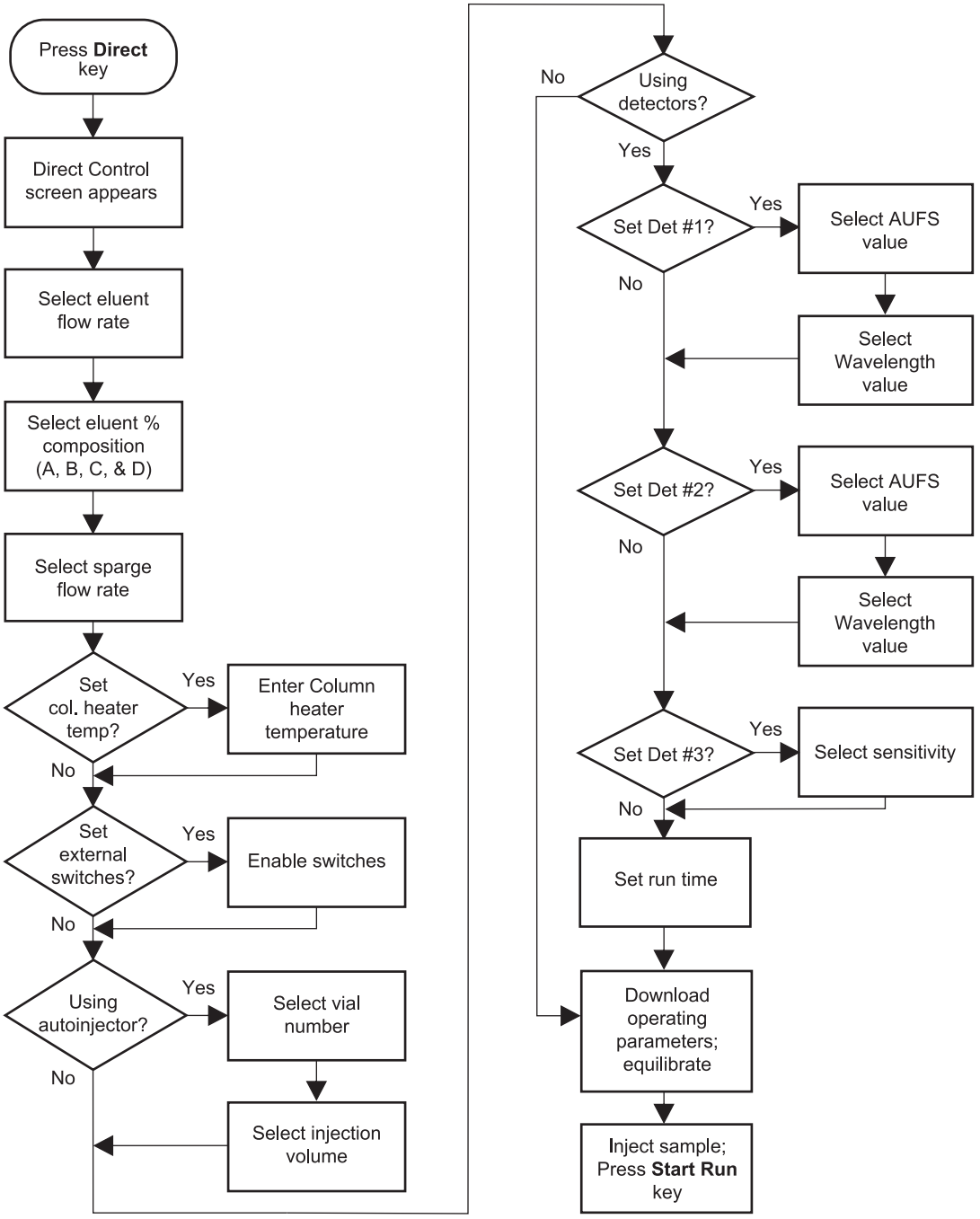


Figure 5-1 Direct Control Flowchart

Accessing the Direct Control Screen

You set the run conditions from the Direct Control screen. To access the Direct Control screen, press the Direct function key. The PowerLine Controller Direct Control screen appears (Figure 5-2).

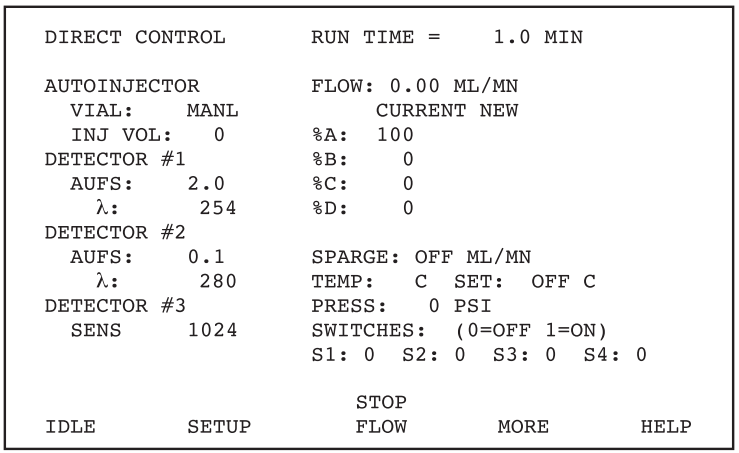


Figure 5-2 PowerLine Controller Direct Control Screen

Exiting the Direct Control Screen

To exit the Direct Control screen, press any function key to select another mode.

5.1.1 Setting Pump Parameters

Use the Direct Control screen (Figure 5-2) to make manual changes to pump parameters. The pump parameters are:

- Flow
- %A, %B, %C, %D
- Sparge
- Temp
- Press



Attention: *Be sure that the values for the maximum and minimum operating pressures programmed on the Pump Setup screen are appropriate for your column. See Section 4.3.2, Setting Pump Setup Parameters (for PowerLine Controller operation), or Section 8.2.3, Exiting the Pump Setup Screen (for Gradient Controller operation). See the Column Care and Use Manual for guidelines.*

Flow

Specifies the eluent flow rate.

To set the Flow Rate:

1. Move the cursor to the Flow field if necessary.
2. Type the appropriate eluent flow rate (0.00 to 20.0 or 45.00 mL/min) for the application and press Enter.

   The system immediately sets the pump to the flow rate you entered.

Note: *Some columns require a flow ramp-up. See the column Care and Use Manual for guidelines.*

%A,%B,%C,%D

Indicates the percentage (0 to 100%) of each eluent that will be used in the mobile phase.

To set the eluent composition:

1. Move the cursor to the New %A field.

   Note: *The Current values indicate how the pump is presently set to operate. The system copies the values from the Current column when you move the cursor to the New column.*

2. Type the % composition (0 to 100, no decimals, in 1% increments) for eluent reservoir A. Press Enter.
3. Repeat steps 1 and 2 for the three remaining eluent reservoirs. The sum of all four entries must equal 100 percent.

   After you enter the percentage for eluent D and press Enter, the system implements the composition changes for all eluents. The Current column reflects the new eluent composition.

Sparge

Determines the rate at which the system sparges the eluent reservoirs.

To set the sparge rate:

1. Move the cursor to the Sparge field.
2. Type the sparge rate value (0 to 100 mL/min). Press Enter.

Note: *The system uses the sparge rate for all eluent reservoirs that you enable on the Pump Setup screen. Use an initial high sparge flow rate and then reduce it to a low maintenance rate. The sparge method you use depends on your analytical requirements. See Appendix B, Eluent Considerations, and Chapter 3, Preparing Your 600E System for Operation, for more information.*

Temp

Controls and maintains a stable column-operating temperature. The range of temperature within which the column heater can operate is listed on the heater label. The Temp value must be less than or equal to the high-temperature limit programmed on the Pump Setup screen (see Section 4.3.2, Setting Pump Setup Parameters).

Note: *If there is no column heater installed in the 600E system, the Temp field displays a value of None.*

To set the optional column heater temperature:

1. At the Temp field, type the appropriate temperature for the chromatography that you wish to run. Valid values are 0 to 99 °C.
2. Press Enter.

Note: *Verify separation methods that require elevated temperatures at those temperatures.*

5.1.2 Setting External Event Switches

S1, S2, S3, S4

The Direct Control screen allows you to manually operate external devices by setting the S1 through S4 external switch parameters.

Use S1 through S4 on the controller rear panel to control devices such as fraction collectors, switching valves, or other non-PowerLine components.

Connecting External Devices

Before setting any of the event switches, ensure that all external devices are connected to the appropriate switch terminals on the rear panel of the controller. See the *Waters 600E Multisolvent Delivery System Installation and Maintenance Guide* for more information on making connections.

Note: *S4 defaults to the Off position when a stop-flow condition or a critical fault occurs. Use this feature by connecting to S4 a device, such as an autosampler, that must be shut off when an error, such as a high-pressure shutdown or a critical fault, occurs.*

Turning On Switches

To turn on a switch:

1. Move the cursor to the appropriate switch field (S1, S2, S3, or S4).
2. Type 1 (On). Press Enter.

Turning Off Switches

To turn off a switch:

1. Move the cursor to the appropriate switch field (S1, S2, S3, or S4).
2. Type 0 (Off). Press Enter.

Pulsing Switches

To pulse a switch:

1. Move the cursor to the appropriate switch field (S1, S2, S3, or S4).
2. Type 2 (Pulse). Press Enter.

Note: *The On time for a pulsed event is 600 milliseconds.*

5.1.3 Setting Autoinjector Parameters

When you are using a PowerLine autosampler (Waters 717plus), use the Direct Control screen (see Figure 5-2) to set up the autoinjector.

The autoinjector parameters are:

- Vial
- Inj Vol

Vial

Indicates the vial you wish to use for the injection.

To select a vial:

1. Move the cursor to the Vial field.
2. Type the vial number from which to make an injection and press Enter.

   Valid vial numbers are 1 to 48 or 1 to 96, depending on the capacity of the carousel in the autoinjector. You can also enter special vial codes (described below) to perform special functions.

Special Vial Codes

Vial numbers from 100 to 107 in the Vial field instruct the system to perform the special functions described in Table 5-1.

Table 5-1 Special Vial Codes

Vial Code	Function	Description
100	Manual Inj	Tells the controller that you will make the injection with a manual injector or a non-PowerLine autoinjector.
101	Equilibrate	Equilibrates the system at initial conditions for the specified run time.
102	Purge 410	Purges the Waters 410 detector by placing its flow path in the purge mode and running initial conditions for the specified run time.
103	Purge Inj	Purges the PowerLine autoinjector (Waters 717plus) for 4.5 minutes using the autoinjector purge routine and initial conditions.
104	Prep	Runs blank gradients or reequilibrates a column.
105	Auto Base	Instructs the 431/432 detector (Det #3) to execute its auto-baseline routine.
106	Pos Polarity	Instructs the 410 or 431/432 detector (Det #3) to operate with positive polarity.
107	Neg Polarity	Instructs the 410 or 431/432 detector (Det #3) to operate with negative polarity.

Inj Vol

Specifies the size of the sample you wish to inject.

To select the injection volume:

1. Move the cursor to the Inj Vol field.
2. Type the appropriate sample value to be injected.
3. Press Enter.

Valid entries are 0 to 200 mL with the standard sample loop installed, or 0 to 2000 mL with the optional sample loop installed.

5.1.4 Setting Detector Parameters

Use the Direct Control screen (see Figure 5-2) to:

- Set attenuation and wavelength values for Waters PowerLine UV/Vis detectors (Waters 486 and 490E)
- Turn the 486 detector lamp On/Off
- Set sensitivity for a PowerLine refractive index (RI) detector (Waters 410) or a conductivity detector (Waters 431/432)

Setting Attenuation and Wavelength for UV/Vis Detectors

To set the attenuation and wavelength for an absorbance detector:

1. Move the cursor to the AUFS field for a UV/Vis detector (Detector #1).
2. Type an AUFS value between 0.001 and 2.00 (AU) for the detector. Press Enter.
3. At the λ field, type an operating UV/Vis wavelength between 190 and 600 (nm). Press Enter.
4. Repeat steps 1 through 3 if using a second UV/Vis detector.

Note: *Ensure that the 600 controller recognizes the Waters 486 detector on the IEEE-488 bus. See Section 4.2, Verifying that PowerLine Devices Are Active.*

Controlling the 486 Detector Lamp

Note: *Waters recommends that in order to conserve lamp life, do not turn the lamp off for less than 4 hours.*

To turn On/Off the 486 detector lamp:

1. Press the More screen key.

2. Press the Lamp On/Lamp Off screen key for the desired function. The key toggles between the two functions.

Waters 486 lamp status is checked at the beginning of each run. If the lamp is off at the start of a run, the system returns to IDLE and the error message `Unable to Set Up Detector` appears.

Setting Sensitivity for the 410 Detector

1. If you are using the 410 RI detector, move the cursor to the Sens field.
2. Type the required RI sensitivity. (Valid values are 1, 2, 4, 8, 16, 32, 64, 128, 256, 512, 1024.)
3. Press Enter.

Setting Sensitivity for the 431/432 Detector

1. If you are using the 431/432 conductivity detector, move the cursor to the Sens field.
2. Type the required conductivity sensitivity in microSiemens (µS). (Valid values are 0.1, 0.05, 0.02, 0.01, 0.005, 0.002, 0.001, 0.0005.)
3. Press Enter.

5.2 Performing a Direct Control Run

Once you have set the run conditions (see Section 5.1, Setting Run Conditions), use the Direct Control screen (see Figure 5-2) to make a run. Making a run involves performing the following procedures:

- Setting the run time
- Downloading the operating parameters
- Equilibrating the system
- Starting the run
- Stopping the pump
- Aborting a run
- Changing parameters during a run

In addition to the parameters shown on the screen, the Direct Control screen displays the screen keys described in Table 5-2.

Table 5-2 Direct Control Screen Keys

Key	Function
Setup	Sends the operating parameters (displayed on the setup screens) to the PowerLine modules. Toggles with Start Run and Abort.
Start Run	Starts the isocratic run and the run time clock. Toggles with Setup and Abort.
Stop Flow	Stops the pump. To start the pump again, type in a value for Flow and press Enter.
More	Displays a second set of screen keys.
Lamp On	Available after you press More. Turns on the 486 detector lamp. Toggles with Lamp Off.
Lamp Off	Available after you press More. Turns off the 486 detector lamp. Toggles with Lamp On.
Abort	Stops the run in progress. Toggles with Setup and Start Run.
Help	Displays the Hhelp for the Direct Control screen.

The lower-left corner of the Direct Control screen displays a system status flag. This flag is similar to the screen keys except that it appears in reverse video. The flag indicates the statuses described in Table 5-3.

Table 5-3 Direct Control System Statuses

Status	Function
Idle	No run in progress.
Setup	System is downloading the module operating parameters.
Initial	Module parameters have been downloaded; system is ready to start a run.
Inject Wait	System is waiting for an inject signal from a contact closure at the Inject terminal or a signal over the IEEE-488 bus to start analysis.
Running	Analysis in progress.

5.2.1 Setting the Run Time

Use the Direct Control screen (see Figure 5-2) to set the Run Time parameter.

Run Time

Specifies the duration of the analysis after the injection is made. The system does not recognize another Inject signal while a run is in progress.

To set the run time:

1. Move the cursor to the Run Time field.
2. Type a run-time value between 0.0 and 655.34 minutes. Press Enter.

When the controller receives the Inject signal (see Section 5.2.4, Starting a Run), the controller resets the run-time value to 0.00 and the clock starts counting up to the value you entered.

5.2.2 Downloading Operating Parameters

When you enter parameter values on the Direct Control screen (see Figure 5-2), the system immediately activates the parameters.

Before making an injection, however, the system must download other operating parameters to the PowerLine modules (see Chapter 4, Setting PowerLine Controller Operating Parameters). These are the parameters found on the Pump Setup, Autoinjector Setup, and Detector Setup screens (see Figure 4-13, Figure 4-16, Figure 4-18, and Figure 4-19).

Downloading PowerLine Parameters

To download the PowerLine operating parameters, press the Setup screen key.

The system status field in the lower left corner of the screen changes from `Idle` to `Setup` while the system sends the parameters to the PowerLine modules.

Stopping Downloading

To stop downloading the parameters, press the Abort screen key.

When downloading completes, the system status changes to `Initial` and the `Setup` screen key name changes to `Start Run`.

5.2.3 Equilibrating the System

When the system status displays `Initial`, the system is running with the conditions displayed on the Direct Control screen and with the downloaded PowerLine module operating parameters. The system runs at these conditions until you equilibrate the system. For more details, see Section 3.5, Equilibrating the System.

To equilibrate your system, allow at least 5 to 6 column volumes of eluent (as a minimum, 10 is recommended) to pass through your system flow path. When you have equilibrated your system, continue with Section 5.2.4, Starting a Run.

5.2.4 Starting a Run

Note: *The 600 controller examines the status of the 490E detector before initiating a run. If the detector is in the standby state, the controller automatically turns on the lamp.*

Using the Rheodyne 7725i Manual Injector

To begin a run with the Rheodyne 7725i manual injector:

1. Load the injector loop with sample. see Section 7.1.1, Using the Rheodyne 7725i Manual Injector.
2. Turn the injector handle to the Inject position to make the injection. The injector sends an Inject signal to the controller.

Using a PowerLine Autosampler

To begin a run with a PowerLine autosampler (717plus):

1. Load the sample vials into the autosampler.
2. Press the Start Run screen key. The 600 controller automatically starts the autosampler. The system status changes from Initial to Inject Wait.
3. The autosampler draws the sample and makes the injection. The controller receives the Inject signal through the IEEE-488 bus.

Using a Non-PowerLine Autoinjector

Note: *If you are using an autoinjector, see the documentation provided with that product for information on sample loading.*

To begin a run with an external non-PowerLine autoinjector:

1. Load the sample vials into the autoinjector.
2. Program and start the autoinjector. See the autoinjector documentation.

3. The autoinjector draws the sample and makes the injection. The autoinjector sends a contact closure signal to the Inject input terminal on the rear panel of the controller.

Using the Start Run Screen Key with a Non-PowerLine Autoinjector

For methods development and for runs that are not time-sensitive, you may also use the Start Run screen key to start a run with a non-PowerLine autoinjector.

To begin a run with an autoinjector using the Start Run key:

1. Load the sample vials into the autoinjector.
2. Program and start the autoinjector. See the autoinjector documentation.
3. Press the Start Run key as soon as possible after the injection occurs. Note that the Start Run key does not initiate an inject switch contact closure.

Starting the Run Time Clock

When the controller receives an Inject signal from an injector, the system status changes to `Running` and the run time clock begins counting at 0.00. The system does not accept another Inject signal until the run concludes or you abort the run.

Completing a Run

When a run completes, the system status changes from `Running` to `Idle`.

5.2.5 Stopping the Pump

Stopping the Pump without Aborting the Run

To stop the pump without aborting the run do one of the following:

- Press the Stop Flow screen key.
- Type a value of 0.0 at the Flow field and press Enter.

Resuming Flow

To resume flow, type in a flow rate value at the Flow field and press Enter.

Note: *These actions do not affect the eluent composition percentages.*

5.2.6 Aborting a Run

1. Press the Abort screen key. The Caution screen appears (Figure 5-3).

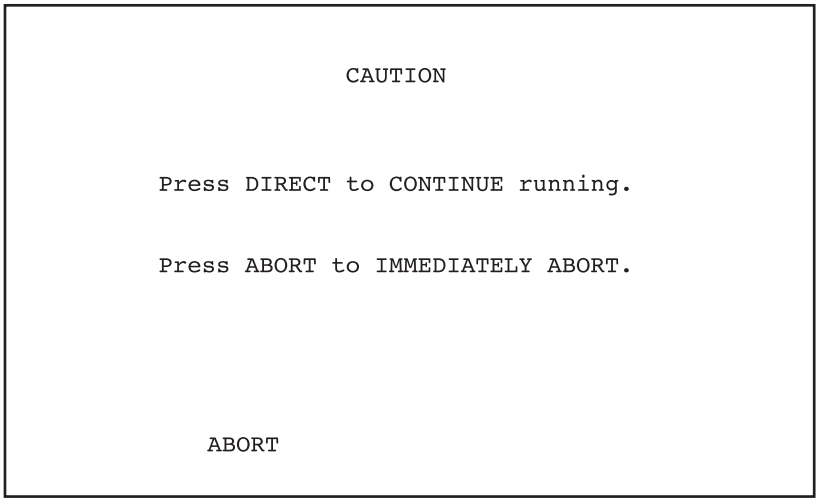


Figure 5-3 Caution Screen

2. Press either the:
   - Abort screen key to immediately abort the run
   - Direct function key to continue running

5.2.7 Changing Parameters During a Run

The pump, external switch, injector, and detector conditions displayed on the Direct Control screen can be changed while a run is in progress.

To change a parameter:

1. Move the cursor to the appropriate field.
2. Type in the new value and press Enter. The change takes effect immediately.

5.3 Running a Method from the Direct Control Screen

After equilibrating or flushing the system using the Direct Control screen, you can run a previously created method (see Chapter 6, Creating PowerLine Methods).

Accessing the Operate Method Screen

To run a method from the Direct Control screen:

1. Press the Operate Method function key. The initial Operate Method screen appears with the current direct control flow conditions shown in the Current field (Figure 5-4).

   Note: *If there is a direct-control run in progress when you press the Operate Method function key, the system warns that, if you proceed to the Operate Method screen, the system will abort the direct control run. Pressing the Operate Method function key a second time aborts the run and the initial Operate Method screen appears.*

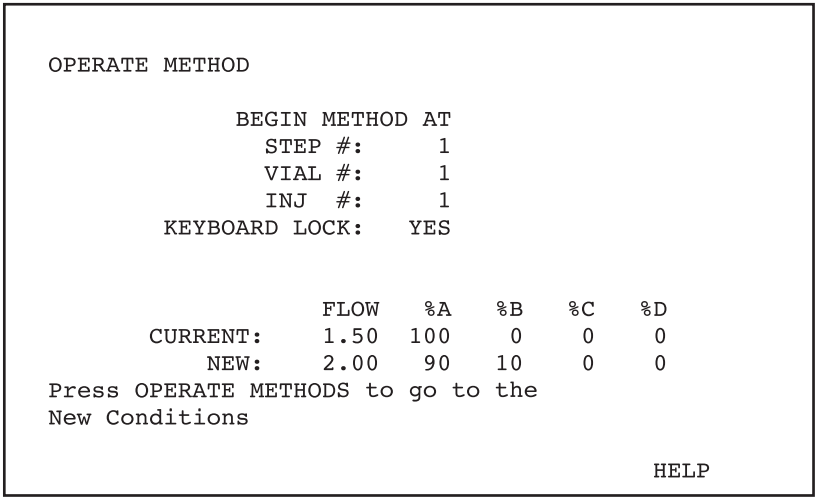


Figure 5-4 Initial Operate Method Screen

2. Type the Step #, Vial #, and Inj # values that you want the system to use while running the method. The initial flow conditions of the step you specified appear in the New line. This line shows any impending changes in eluent or flow rate.

   If you prefer not to begin the method, press any function key except Operate Method.

Starting the Method

To start the method, press the Operate Method function key again.

For complete information on running the method, see Chapter 7, Running PowerLine Methods.

Chapter 6
Creating PowerLine Methods

This chapter describes how to create the time-based tables required for unattended operation of the Waters 600E Multisolvent Delivery System. You can combine these tables to compose a method.

A method is a set of instructions that controls how and when a run is made. In the Waters 600E system, a method comprises a set of timed-based tables consisting of:

- Gradient tables to control the 600E pump
- Event tables to control external devices
- Detector tables to control the PowerLine detectors in your system

The time-based tables of the same set are identified by the same number, which is listed in a Method table (Figure 6-1). When the system executes that table number, the set of gradient, event, and detector tables having that number run simultaneously.

The 600E system can store up to 15 sets of tables that allow you to:

- Run a series of samples under the same or different conditions
- Implement flow and eluent compositional gradients
- Actuate external devices
- Change wavelengths and attenuations
- Program special method functions (special vial codes)

Figure 6-1 illustrates the relationship between time-based tables and a method table. A Gradient, an Event, and a Detector table with the same number combine to form one line of a Method table.

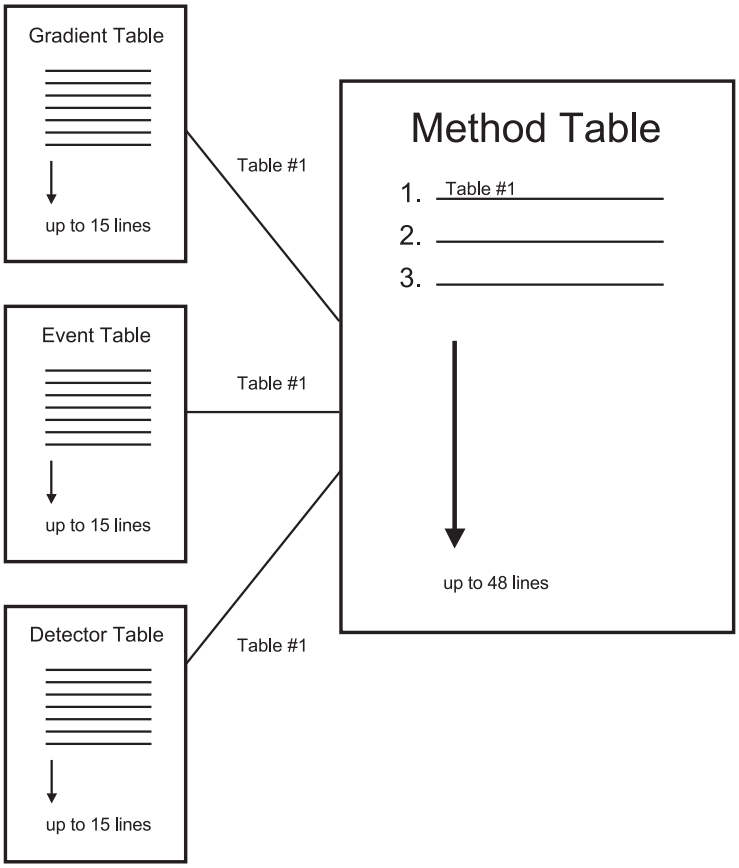


Figure 6-1 Methods and Tables

Once you create these tables, you can run them as described in Chapter 7, Running PowerLine Methods.

6.1 Creating a Gradient Table

This section provides the information you need to create the first element of a method table, the gradient table. Use the gradient table to control the 600E pump.

This section describes:

- Documenting the gradient table
- Programming the gradient table

Note: *When creating gradient, event, and detector tables, be sure that you design the time-related parameters specified in each table to work effectively with the time-related parameters in the other two tables of the same set.*

6.1.1 Documenting the Gradient Table

Define the information you want in a gradient table before you actually program a gradient table. This will minimize the need for you to reprogram the table later.

Using the Analysis Plan and File Sheet

Appendix C, Analysis Plan, contains an Analysis Plan and File Sheet to assist you in defining and documenting gradient-table information.

1. Make a photocopy of the Analysis Plan and File Sheet.
2. On each line of the Analysis Plan and File Sheet, write:
   - Gradient segment time (time is cumulative from injection)
   - Flow rate
   - Eluent composition
   - Gradient profile curve number

   The number of changes needed to execute the gradient determines the total number of lines. The gradient table can contain up to 15 lines (14 plus initial conditions).

   Note: *You must create a gradient table to include isocratic pump parameters in a method. A gradient table with isocratic pump parameters consists of a single line.*

   See Section 6.1.2, Programming the Gradient Table.
3. When the table entries are complete, keep the Analysis Plan and File Sheet as a permanent record of your table.

Returning to Initial Conditions

When making multiple injections that require flow or compositional gradients, include a line in the gradient table to return the system to initial conditions or to reequilibrate the system before the next injection.

This line in the gradient table should:

- Be executed by the controller after the separation is complete (run time has elapsed) and allow enough time for the column to equilibrate
- Change the flow rate to that used in the Initial line of the gradient table
- Have the same eluent composition as the Initial line of the gradient table

Reducing Flow

To conserve eluent, include a final line in the gradient table to reduce the flow rate after the last injection.

This line in the gradient table should:

- Appear as the last line in the table.
- Be set to a time at least 5 to 10 minutes longer than the time required for another injection to occur.
- Specify a flow rate of either 0.0 mL/min, or a reduced flow rate, usually 0.1 mL/min.
- Use the eluent composition from the previous line.
- Specify curve #11 (see Analysis Plan and File Sheet) so that the flow rate does not decrease until the last line is reached. If an injection occurs before this line executes, the flow rate does not decrease.

Sample Gradient Table

Figure 6-2 is an example of an Auto•Blend gradient table for an analysis with a run time of 30 minutes. It includes a 5-minute segment that returns the eluent composition to initial conditions at the end of the run. It also includes a segment that reduces the flow rate to 0.0 mL/min if an injection is not made within 50 minutes of the previous injection.

TIME	FLOW	%A	%B	%C	%D	CURVE
Initial	1.00	10	10	0	80	*
30.00	1.00	10	10	80	0	6
35.00	1.00	10	10	0	80	6
50.00	0.00	10	10	0	80	11

Figure 6-2 Example of an Auto•Blend Gradient Table

Figure 6-3 illustrates the flow, %C, and %D profiles for this gradient table.

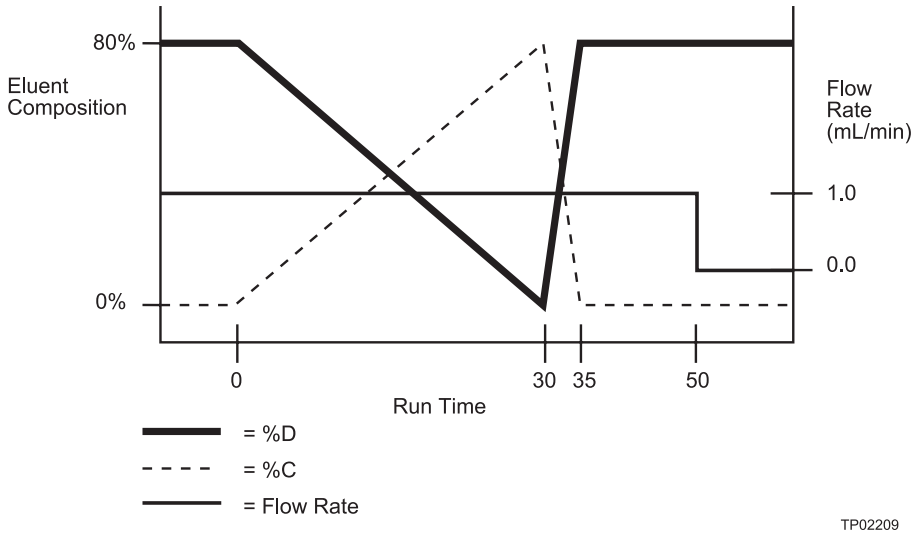


Figure 6-3 Auto•Blend Gradient Profile

6.1.2 Programming the Gradient Table

This section provides instructions for:

- Entering parameter values in the gradient table
- Editing the gradient table

Figure 6-4 illustrates the steps involved in using the Program Gradient screen to create a gradient table. Use this flowchart for quick reference once you become familiar with your Waters 600E system.

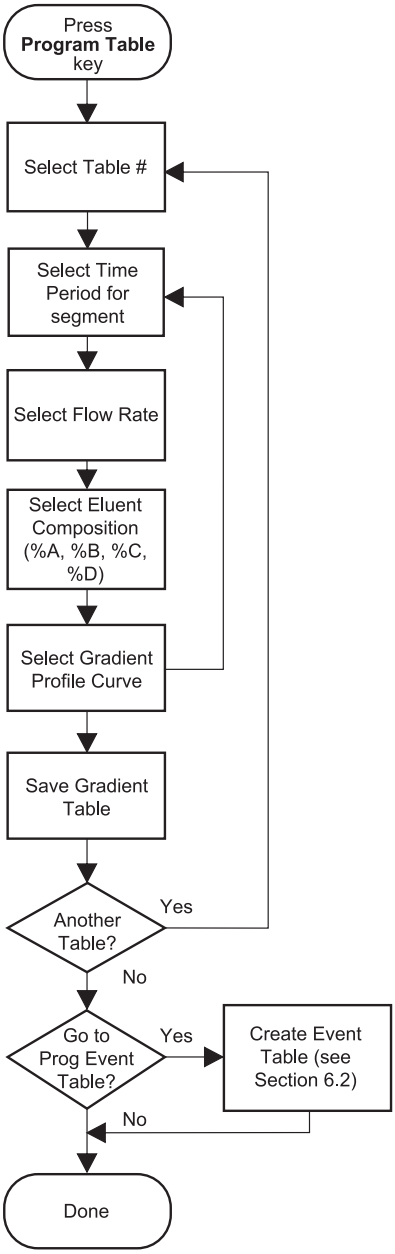


Figure 6-4 Program Gradient Flowchart

Accessing the Program Gradient Screen

Press the Program Table function key to display the PowerLine Controller Program Gradient screen (Figure 6-5).

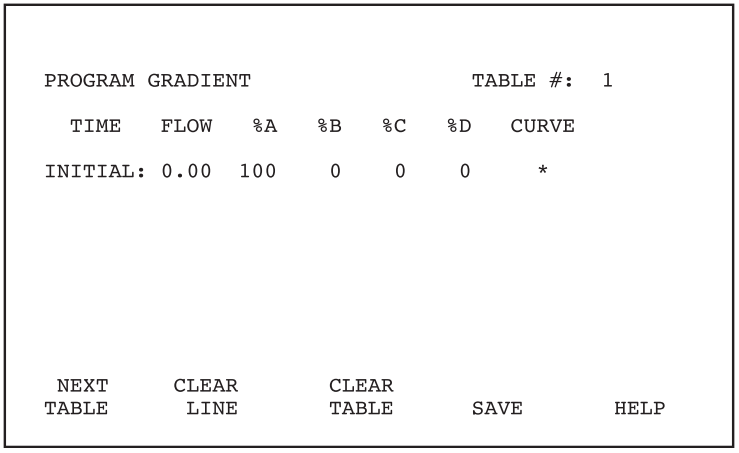


Figure 6-5 PowerLine Controller Program Gradient Screen

In addition to the gradient table parameters, there are five active screen keys. These screen keys have the functions described in Table 6-1.

Table 6-1 Program Gradient Screen Keys

Key	Function
Next Table	Cycles through the Program Event screen, Detector Table screen, and Program Gradient screen.
Clear Line	Clears the line on which the cursor currently resides. The line is not erased from permanent memory.
Clear Table	Clears the entire table currently displayed on the screen and returns the Initial conditions line to default status. The table is not erased from permanent memory until a new table is saved.
Save	Saves the table currently displayed and erases the previous values. You must save the table before advancing to the next screen.
Help	Displays the Help screen associated with the Program Gradient screen.

Exiting the Program Gradient Screen

To exit the Program Gradient screen, press either:

- The Next Table screen key to access the Program Event screen
- Any function key, except the Program Table key, to enter another mode

6.1.3 Entering Parameter Values in the Gradient Table

To enter the parameter values into the Program Gradient table (see Figure 6-5):

1. Move the cursor to the Table # field.
2. Type a table number between 1 and 15. Press Enter.

   If a gradient table is already stored under this number, the table appears on the screen. You can overwrite or edit this table, or enter another table number.

   Note: *The gradient table always runs simultaneously with the event and detector table of the same number within a method.*
3. If you are entering the initial conditions, continue with step 4.

   Otherwise, type the time elapsed since injection (0.00 to 655.34 minutes) at which the new gradient segment is to start or the previous segment is to end. Press Enter.

   Each time value entered into the table must be unique.
4. At the Flow field, type a flow rate value between 0.00 and 20.0 or 45.00 mL/min. Press Enter.
5. At the %A field, type the appropriate percentage of the mobile phase (0 to 100, in 1% increments, no decimals) for eluent reservoir A. Press Enter.
6. Repeat step 5 for each of the remaining three eluent reservoirs. The sum of the four eluent percentages must equal 100 percent.

   When entering information for subsequent lines in the table, press Enter (without typing a number) to copy the values from the previous line to the new line.

7. At the Curve field, type the gradient curve profile number for the gradient segment. Figure 6-6 shows the gradient curve profiles.

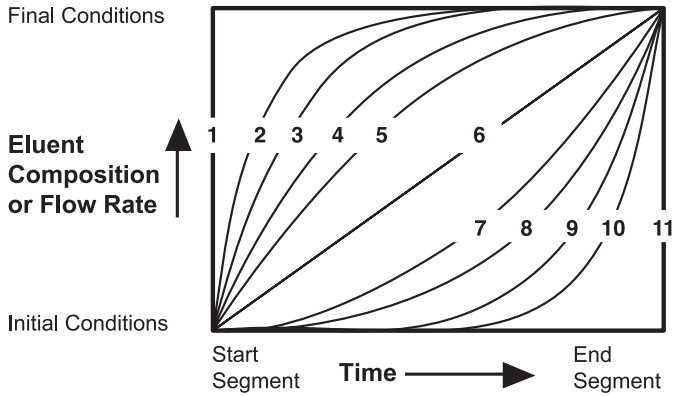


Figure 6-6 Gradient Curve Profiles

8. Repeat steps 3 through 7 for each line in the gradient table.
9. If you wish, enter the line to return to initial conditions.
10. If you wish, enter a line to reduce the flow rate.
11. Press the Save screen key to save the table in permanent memory. When prompted, enter a table number between 1 and 15. Press Enter.

6.1.4 Editing the Gradient Table

This section describes the following editing functions:

- Deleting and inserting lines
- Saving tables
- Replacing and deleting tables

These functions apply to:

- Gradient tables
- Event tables
- Detector tables

Deleting Lines

To delete individual lines from a table:

1. Move the cursor to any field on the line you want to delete.
2. Press the Clear Line screen key. This immediately clears the line the cursor is on and moves succeeding lines in the table up one line. The cursor remains in the original field.

Inserting Lines

To insert a line into a table:

1. Move the cursor to the end of the table in the Time column.
2. Type the time for the new line. The table automatically sorts the lines according to time and inserts the new line in the proper location.
3. Enter values for the other parameters on the new line.

   If the new line appears at the end of the table, the system copies the values from the previous line into the new line. Edit the copied conditions as necessary.

Saving Tables

Note: *Whenever you change a table, you must save the changes before leaving the screen or powering down the system.*

The system does not retain parameter values that you have not saved. When you turn on the system at a later time, the last table saved is the default.

To save a table after all changes have been entered:

1. Press the Save screen key. The system displays:

   ```
   Enter Table Number (1-15)
   ```

2. Type the number under which to save the table. Press Enter. The screen displays:

   ```
   Replace With New Table? 1 = Yes 0 = No
   ```

   If you enter a table number for a table that already exists, that preexisting table appears.

3. To overwrite the preexisting table, type 1. This erases the contents of the preexisting table.

   If you do not want to overwrite the preexisting table, type 0. The screen displays the newly created table again.

To save the new table, repeat steps 1 and 2, and assign a different table number to the table. Continue this process until you locate a table that can be replaced with the new table.

Replacing Existing Tables

A maximum of 15 tables of one type can be stored in permanent memory in the controller. Once 15 tables are saved, you must replace one of these tables to save a new table.

1. Move the cursor to the Table # field.
2. Type the number of the table to replace. Press Enter.
3. If desired, clear the table by pressing the Clear Table screen key.
4. Enter the new information.
5. After you have entered all the information, save the table by pressing the Save screen key. This replaces the existing table in permanent memory with the new table.

Deleting Tables

To delete a table without replacing it with a new table:

1. Move the cursor to the Table # field.
2. Type the number of the table to delete and press Enter.
3. Press the Clear Table screen key.
4. Press the Save screen key.
5. At the `Enter Table NUMBER (1-15)` prompt, type the number of the table to delete. Press Enter. The table you want to delete is displayed again.
6. At the `Replace With New Table?` prompt, type 1 (Yes). This deletes the table from memory.

6.2 Creating an Event Table

This section provides the information you need to create the second element of a method table, the event table.

This section describes:

- Documenting the event table
- Programming the event table

Note: *When creating gradient, event, and detector tables, be sure that you design the time-related parameters specified in each table to work effectively with the time-related parameters in the other two tables of the same set.*

6.2.1 Documenting the Event Table

Define the information you want in an event table before you actually program the event table. This will minimize the need for you to reprogram the table later.

Using the Analysis Plan and File Sheet

Appendix C, Analysis Plan, contains an Analysis Plan and File Sheet to assist you in defining and documenting event table information.

1. Make a photocopy of the Analysis Plan and File Sheet.
2. On each line of the Analysis Plan and File Sheet, write:
   - Time of event
   - Event type
   - Event action or setting

   The number of events needed to execute the method determines the total number of lines. The event table can contain up to 15 lines (14 plus initial conditions). Multiple events can start at the same time.

   Note: *If you do not specify Event 8 or 9, the last condition established remains in effect when the run time expires.*
3. When the table entries are complete, keep the Analysis Plan and File Sheet as a permanent record of the table.

Event Types

There are 10 types of events that you can select, as described in Table 6-2.

Table 6-2 Event Types

Event type	Function
1 through 4	Contact closure switches on the rear panel for timed output to external devices.
5	Internal alarm (reminder to user).
6	Sparge rate for any reservoir enabled on the Pump Setup screen.
7	Column temperature.
8	Go to initial conditions of specified Table #.

Table 6-2 Event Types (Continued)

Event type	Function
9	Start running Table #. The method begins immediately at the time this event executes. No inject signal is necessary.
10	Lamp on/off.

Once set, events remain in that state until you set them to new conditions.

Hint for Event 5

To program a beeping alarm, specify Pulse instead of On.

If you turn on the alarm, include another step to turn off the alarm. Otherwise, manually turn off the alarm using the Cancel Alarm screen key on the Operate Method screen.

Hints for Events 8 and 9

Note: *Do not program events 8 and 9 in the same table.*

Program event 8 or 9 at the end of the table to initiate the use of new tables.

Event 8 maintains the initial conditions of the new table until the next injection occurs.

Event 9 executes the new table without waiting for an inject signal. Event 9 is useful for executing a table that changes conditions and equilibrates the system before the next injection occurs. Event 9 is useful for automated separations development.

Note: *The Detector table is not activated when you specify event 9.*

Hint for Event 10

Note the following considerations when using Event 10:

- Event 10 is designed to turn the lamp off at the end of an unattended (e.g., overnight) run. You should physically turn the lamp on (not use event 10) prior to the next analysis to assure sufficient lamp warm-up and baseline stability.
- Waters 486 lamp status is checked at the beginning of each run. If the lamp is turned off via Event 10 at the start of a run, the system returns to IDLE and the error message `Unable to Set Up Detector` appears.
- Do not program event 10 to use a Pulse action.

Event Actions

The action selections for events 1 through 5 are:

- **0** = Off
- **1** = On
- **2** = Pulse (generates 600 millisecond pulse)

Event 6 (sparge) requires specification of a sparge rate between 0 and 100 mL/min.

Event 7 requires that you specify a column heater temperature value in °C that is greater than ambient temperature but less than the maximum temperature (99 °C for the standard heater) permitted by the column heater.

Events 8 and 9 require specification of a table number between 1 and 15.

Event 10 requires 1 to turn On the lamp or 0 to turn Off the lamp.

Note the following considerations when using Event 10:

- Event 10 is designed to turn the lamp off at the end of an unattended (e.g., overnight) run. You should physically turn the lamp on (not use event 10) prior to the next analysis to assure sufficient lamp warm-up and baseline stability.
- Waters 486 lamp status is checked at the beginning of each run. If the lamp is turned off via Event 10 at the start of a run, the system returns to IDLE and the error message `Unable to Set Up Detector` appears.
- Do not program event 10 to use a Pulse action.

Sample Event Table

Figure 6-7 illustrates a simple example of an event table.

Time	Event	Action
Initial	1	Pulse
3.00	2	On
3.55	2	Off

Figure 6-7 Event Table Example

6.2.2 Programming the Event Table

Figure 6-8 illustrates the steps involved in using the Program Event screen to create an event table. Use this flowchart for quick reference once you become familiar with the Waters 600E system.

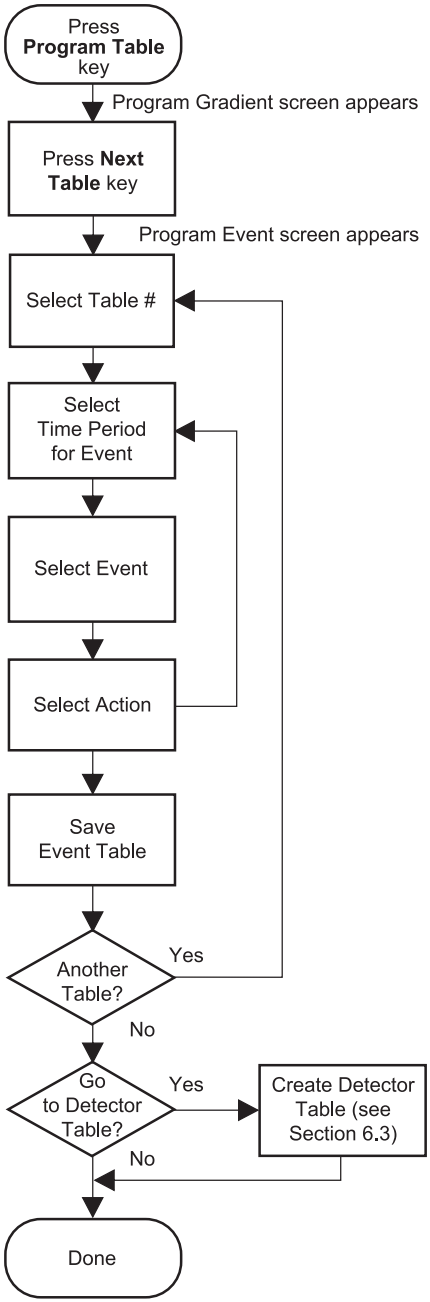


Figure 6-8 Program Event Flowchart

The remainder of this section describes how to access the Program Event screen and enter an event table.

Accessing the Program Event Screen

1. Press the Program Table function key to display the Program Gradient screen (see Figure 6-5).
2. Press the Next Table screen key. The PowerLine Controller Program Event screen appears (Figure 6-9).

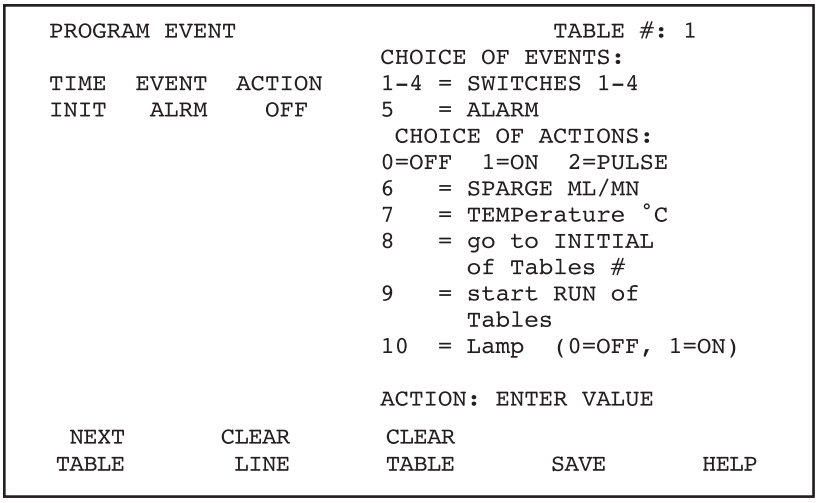


Figure 6-9 PowerLine Controller Program Event Screen

In addition to the event table parameters, the Program Event screen displays five active screen keys that function as described in Table 6-3.

Table 6-3 Program Event Screen Keys

Key	Function
Next Table	Cycles through the Detector Table screen, Program Gradient screen, and back to the Program Event screen.
Clear Line	Clears the line on which the cursor currently resides. The line is not erased from permanent memory.
Clear Table	Clears the entire table currently displayed on the screen and returns the initial conditions line to default status. The table remains in permanent memory until you save a new table.

Table 6-3 Program Event Screen Keys (Continued)

Key	Function
Save	Saves the table currently displayed and erases the previous values. You must save the table before leaving this screen. Otherwise, you lose any changes that you made.
Help	Displays the Help screen associated with the Program Event screen.

6.2.3 Entering Parameter Values in the Event Table

To enter the parameter values into the Program Event table (see Figure 6-9):

1. Move the cursor to the Table # field.
2. Type a table number between 1 and 15. Press Enter.

   If an event table is already stored under this number, it now appears on the screen. You can overwrite or edit this table or enter another table number.

   Note: *The event table always runs simultaneously with the gradient and detector tables of the same number within a method.*

3. If you enter the initial conditions, continue with step 4 below.

   Otherwise, type the time since injection (0.00 to 655.34 minutes) at which you want the event to occur. Press Enter.

   Note: *If you are on the Initial line, the Time field represents the default starting point for program event operation. You cannot enter a value on this line. When you add a new line to the table, the values from the previous line appear in the new line after you enter the time for the new line.*

4. At the Event field, type the event type (1 to 10). Press Enter. (Event types and functions are listed on the screen.)

   Note: *Events 8 and 9 specify the next table to be run in sequence. Since these two events begin new method tables, they must be the last event in the table. Event 8 holds your analysis at the initial conditions of the new table until an inject signal is received. Event 9 begins executing the table without an inject signal. Do not program events 8 and 9 in the same table.*

5. At the Action field, type the appropriate value for the action required for the event. Press Enter.
6. Repeat steps 3 through 5 for each line in the event table.
7. Press the Save screen key to save the table in permanent memory.

6.2.4 Editing the Event Table

For details on editing the event table, see the procedure for editing the gradient table in Section 6.1.4, Editing the Gradient Table.

6.3 Creating a Detector Table

Skip this section if you are not using PowerLine detectors in your Waters 600E system.

This section provides the information you need to create the third and last component of a method table, the detector table.

This section describes:

- Documenting the detector table
- Programming the detector table

Note: *When creating gradient, event, and detector tables, be sure that you design the time-related changes specified in each table to work effectively with the changes in the other two tables of the same set.*

6.3.1 Documenting the Detector Table

Define the information you want in a detector table before you actually program the detector table. This will minimize the need for you to reprogram the table later.

Using the Analysis Plan and File Sheet

Appendix C, Analysis Plan, contains an Analysis Plan and File Sheet to assist you in defining and documenting table information.

1. Make a photocopy of the Analysis Plan and File Sheet.
2. On each line of the Analysis Plan and File Sheet, write:
   - Time at which you want a detector change to occur
   - Detector number
   - Detector parameters to change (λ and AUFS)

   The total number of lines depends on the number of detector changes needed to execute the method. The detector table can contain up to 15 lines (14 plus initial conditions).

   Note: *More than one detector change can occur at the same time as long as you specify the changes for different detectors.*

3. When the table entries are complete, keep the Analysis Plan and File Sheet as a permanent record of your table.

Detector table entries affect the two UV/Vis detectors (Waters 486 and 490E). There is a separate sensitivity parameter for the Waters 410 RI detector and Waters 431/432 Conductivity detector. Sensitivity is not under time control for these detectors.

Sample Detector Table

Figure 6-10 illustrates a detector table.

410 Sens 1024		**431/432 Sens 0.0005**	
Time	**Det/Chan**	**Wavelength**	**AUFS**
Initial	1	254	0.1
3.10	1	240	0.005
4.62	1	245	0.01

Figure 6-10 Detector Table Example

6.3.2 Programming the Detector Table

Figure 6-11 illustrates the steps involved in using the Detector Table screen to create a detector table. Use this flowchart for quick reference once you become familiar with your Waters 600E system.

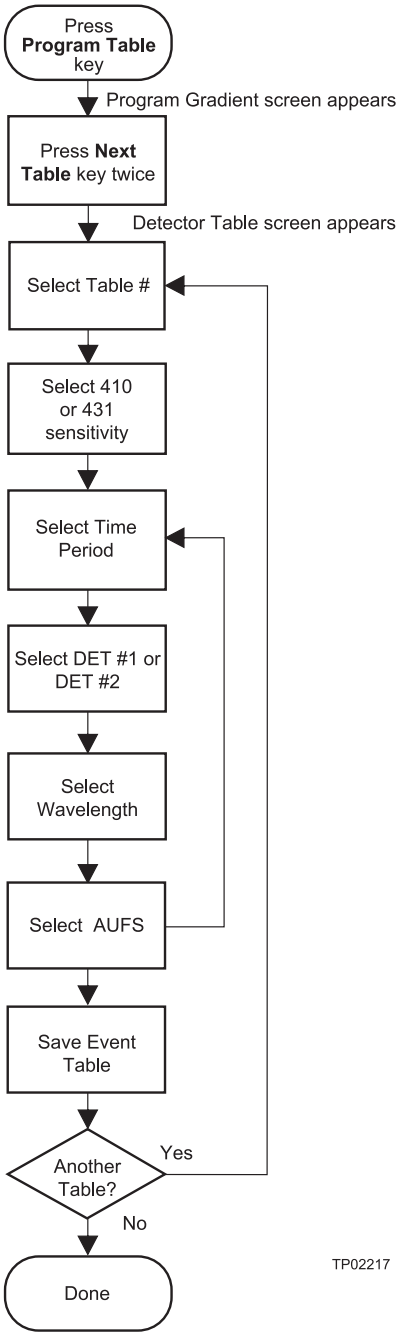


Figure 6-11 Detector Table Flowchart

Accessing the Detector Tables Screen

1. Press the Program Table function key to display the Program Gradient screen (see Figure 6-5).
2. Press the Next Table screen key to display the Program Event screen (see Figure 6-9).
3. Press the Next Table screen key. The Detector Tables screen appears (Figure 6-12).

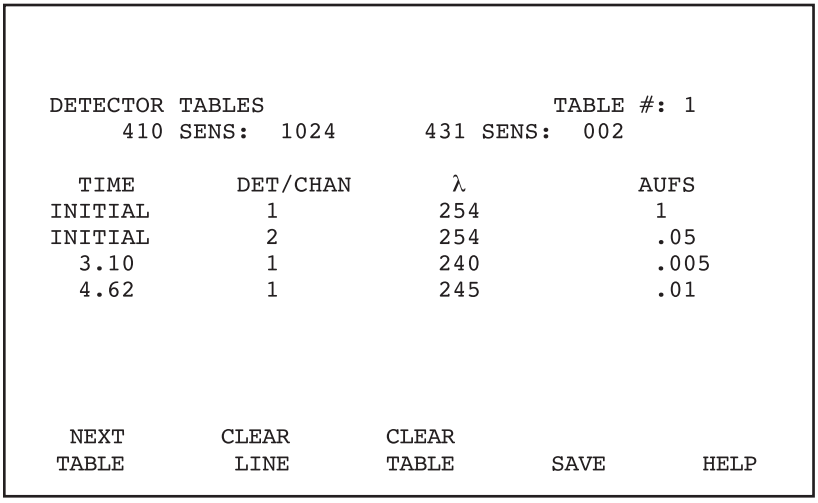


Figure 6-12 Detector Tables Screen

In addition to the detector tables parameters, there are five active screen keys on the Detector Tables screen. These screen keys have the functions described in Table 6-4.

Table 6-4 Detector Table Screen Keys

Key	Function
Next Table	Cycles through the Program Gradient screen, Program Event screen, and back to the Detector Tables screen.
Clear Line	Clears the line on which the cursor currently resides. The line remains in permanent memory.
Clear Table	Clears the entire table currently displayed on the screen and returns the initial conditions line to default status. The table remains in permanent memory until you save a new table.

Table 6-4 Detector Table Screen Keys (Continued)

Key	Function
Save	Saves the table currently displayed and erases the previous values. You must save the table before leaving this screen.
Help	Displays the Help screen associated with the Detector Tables screen.

6.3.3 Entering Parameter Values in the Detector Table

To enter the parameter values into the Detector Table (see Figure 6-12):

1. Move the cursor to the Table # field.
2. Type a table number between 1 and 15. Press Enter.

   If a detector table is already stored under this number, it now appears on the screen. You can overwrite or edit this table, or enter another table number.

   The detector table always runs with the gradient and event tables of the same number within a method.
3. If you are using an RI detector:

   a. Move the cursor to the 410 Sens field.

   b. Type the required operating sensitivity. Valid values are 1, 2, 4, 8, 16, 32, 64, 128, 256, 512, and 1024.

   c. Press Enter.

   You cannot change this value during a run nor can you program it by time.
4. If you are using a conductivity detector:

   a. Move the cursor to the 431/432 Sens field.

   b. Type the required conductivity sensitivity in µS. Valid values are 0.1, 0.05, 0.02, 0.01, 0.005, 0.002, 0.001, 0.0005.

   c. Press Enter.
5. If you are using a UV/Vis detector (Det #1 and/or Det #2):

   a. Move the cursor to the Time field.

   b. Type an appropriate value for the time (0.00 to 655.34 minutes) at which a change is to occur. Press Enter. (You can use the same time value for Det #1 and Det #2.)

c. At the Det/Chan field, type a value to specify which UV/Vis detector (1 = Det #1 or 2 = Det #2) or channel to change. Press Enter.

d. At the l field, type a wavelength value between 190 and 600 nm. Press Enter.

Note: *By default, wavelength changes are accompanied by an Autozero, unless you disable this feature using the detector diagnostics.*

e. At the AUFS field, type a value between 0.001 and 2.0 for the detector. Press Enter.

6. Repeat step 5 for all remaining lines in the detector table.
7. Press the Save screen key to save the table.

6.3.4 Editing the Detector Table

For details on editing the detector table, see the instructions for editing the gradient table in Section 6.1.4, Editing the Gradient Table.

6.4 Creating a Method Table

This section provides the information you need to link together the gradient, event, and detector tables in a method table.

This section describes:

- Entering parameter values into the Method table
- Editing the Method table

6.4.1 Method Table Overview

You can program a Method table to:

- Initiate automated separation development
- Specify separation methods for individual samples or sets of samples
- Change the eluent
- Equilibrate the system
- Purge the PowerLine autoinjector fluidics
- Purge the 410 detector flow cell
- Flush out the system
- Shut down the flow in the system after a series of runs are complete

A Method table can contain up to 48 lines. These lines specify a combination of tables (gradient, event, and detector), and include such information as vial numbers, number of injections, injection volumes, and run times. Each line in the method table executes in order and runs for the specified time.

Note: *You must save a Method table in memory before you can run the table.*



Attention: *If you make changes to the Method table and do not save the table before leaving the Program Methods screen, the controller uses the conditions in the last-saved method table to operate the system.*

6.4.2 Programming the Method Table

Figure 6-13 illustrates the steps involved in using the Program Methods screen to create a Method Table. Use this flowchart for quick reference once you become familiar with your Waters 600E system.

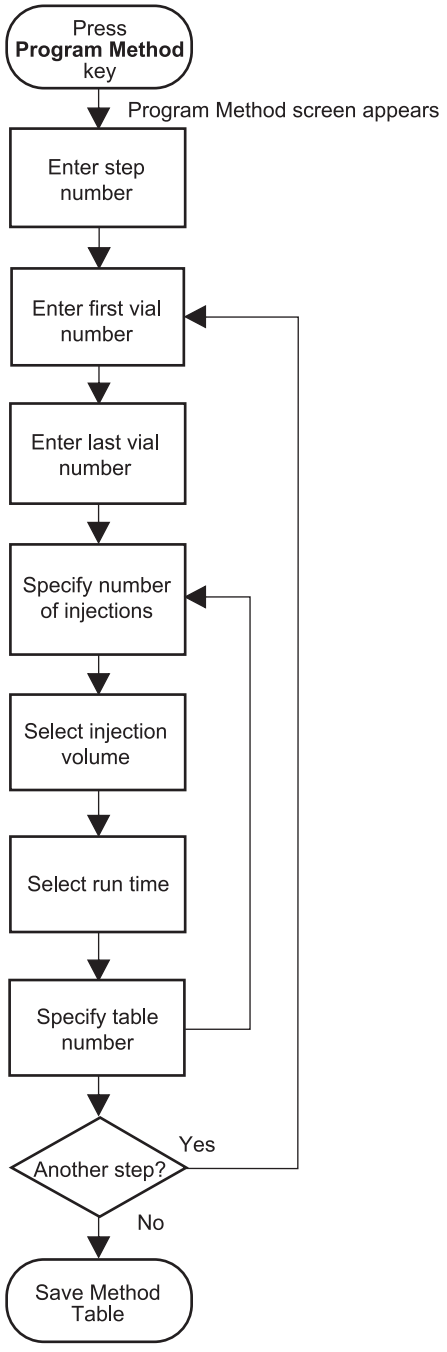


Figure 6-13 Program Methods Flowchart

Accessing the Program Methods Screen

To access the Program Methods screen, press the Program Method function key. The Program Methods screen appears with the cursor at the Step field (Figure 6-14).

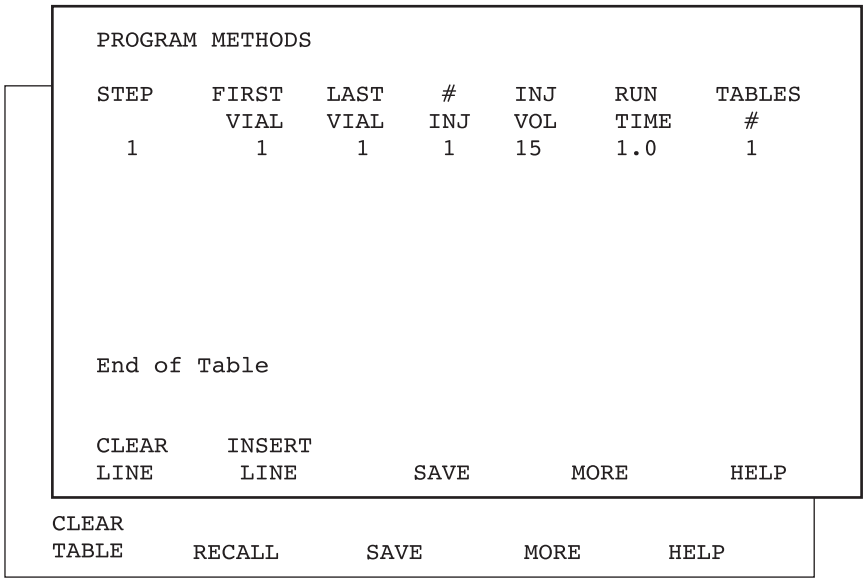


Figure 6-14 Program Methods Screen

In addition to the methods table parameters, the Program Methods screen displays seven active screen keys that function as described in Table 6-5.

Table 6-5 Program Methods Screen Keys

Key	Function
Clear Line	Clears the line on which the cursor currently resides. The line is not erased from permanent memory.
Clear Table	Clears the table currently displayed and returns the initial conditions line to default status. The table remains in permanent memory until you save a new table. Available after you press More.
Insert Line	Copies the line on which the cursor is positioned to the line immediately below.
Recall	Displays the last table you saved. Toggles with Insert Line. Available after you press More.

Table 6-5 Program Methods Screen Keys (Continued)

Key	Function
Save	Saves the table currently displayed and erases the previous values. You must save the table before leaving this screen.
More	Displays additional screen keys (Clear Table and Recall) associated with the method table.
Help	Displays the Help screen associated with the Program Methods screen.

6.4.3 Entering Parameter Values in the Method Table

Note: *When the Program Methods table (see Figure 6-14) first appears, the Step 1 line contains default values. You can modify these values if you wish. For subsequent lines, the system automatically provides step numbers when you press the Insert Line screen key or when you type values on a new line and press Enter.*

To enter parameter values into the Program Methods table (see Figure 6-14):

1. Move the cursor to the First Vial field.
2. Type the number of the vial from which you want to make the first injection. Press Enter. The system automatically assigns a step number.
   - When using a Waters PowerLine autoinjector, the vial number can be any value between 1 and 48 or 1 and 96, depending on the autoinjector carousel.

Special Vial Codes

- When using the manual injector or a non-PowerLine autoinjector, enter one of the special vial codes described in Table 6-6.

Table 6-6 Special Vial Codes

Vial Code	Function	Description
100	Manual Inj	Tells the controller that you will make the injection with the manual Rheodyne injector or a non-PowerLine autoinjector.
101	Equilibrate	Equilibrates the system at initial conditions for the specified run time.

Table 6-6 Special Vial Codes (Continued)

Vial Code	Function	Description
102	Purge 410	Purges the Waters 410 detector by placing its flow path in the purge mode and running initial conditions for the specified run time.
103	Purge Inj	Purges the PowerLine autoinjector (Waters 717plus) for 4.5 minutes using the autoinjector purge routine and initial conditions.
104	Prep	Tells the controller to run in the preparative chromatography mode when the sample is introduced into the flow path from one of the eluent lines or through the inlet manifold on the pump. Allows the system to inject the sample without an inject signal. Also runs blank gradients or reequilibrates a column.
105	Auto Base	Instructs the 431/432 detector (Detector 3) to execute its auto-baseline routine.
106	Pos Polarity	Instructs the 410 or 431/432 detector (Detector 3) to operate with positive polarity.
107	Neg Polarity	Instructs the 410 or 431/432 detector (Detector 3) to operate with negative polarity.

Entering a special vial code affects which parameters appear on that line. Some parameters are not available when you use certain special vial codes.

3. At the Last Vial field, type the number of the vial from which you wish to make the last injection. Press Enter.

   Note: *If you entered a special vial code (100 to 107) in step 2, do not enter a value in the Last Vial field.*

4. At the #Inj field, type a value between 1 and 9 to indicate the number of injections to make from each sample vial. Press Enter.

   Note: *If you specified special vial codes 100 or 104 for the First Vial field, enter the number of injections (1 to 9).*

5. At the Inj Vol field, type the sample volume for all injections. Press Enter.
    - When using a Waters autosampler (717plus), the maximum volume is determined by the loop size (200 µL or 2000 µL) installed in the autosampler.
    - When using the manual injector, a non-PowerLine autoinjector, or making prep injections, this value is for documentation purposes only.
6. At the Run Time field, type the analysis run time (0.0 to 655.34 minutes). Press Enter.
7. At the Tables # field, type the table number for the set of tables (gradient, event, and detector) for this step. Press Enter.
8. Repeat steps 1 through 7 for each line in the Method table.
9. Save the table by pressing the Save screen key.

6.4.4 Editing the Method Table

This section describes the following editing functions:

- Deleting and inserting lines
- Saving and clearing tables

Deleting Lines

To delete individual lines from a table:

1. Move the cursor to the line you want to delete by doing the following:
    a. Move the cursor to the Step column of the current line
    b. Type the step number of the line you want to delete and press Enter. The cursor moves immediately to the line you specified.
2. Press the Clear Line screen key. This immediately clears the line the cursor is on and moves succeeding lines in the table up one line. The cursor remains in the original field.

Inserting Lines

To copy and insert a line into a table:

1. Move the cursor to the line preceding the location of the new line by doing the following:
    a. Move the cursor to the Step column of the current line.
    b. Type the step number of the line to which you want to move and pressing Enter. The cursor moves immediately to the line you specified.

2. Press the Insert Line screen key. This copies the line the cursor is presently on to the new line and reorders the step numbers.
3. Edit the information on the new line as necessary.

Saving Tables

Whenever you change a table, you must save the changes before powering down the system.

To save a table after you have entered all of the changes, press the Save screen key.

The system does not retain settings that you have not saved. When you turn on the system later, the last-saved table is the default.

If you exit the Program Methods screen without pressing the Save screen key, the system erases the information.

Clearing Tables

1. Press the More screen key.
2. Press the Clear Table screen key. The system erases the table displayed on the screen.
3. To recall the last-saved table, press the Recall screen key.

Chapter 7
Running PowerLine Methods

This chapter describes how to run a PowerLine method using the Operate Method mode. The Operate Method mode executes the time-based tables that you created in Chapter 6, Creating PowerLine Methods.

Before You Begin

This chapter assumes that you have:

- Prepared your system (see Chapter 3, Preparing Your 600E System for Operation).
- Set the operating parameters for all Waters PowerLine modules (see Chapter 4, Setting PowerLine Controller Operating Parameters).
- Created a method table (see Chapter 6, Creating PowerLine Methods).

If you have not performed these tasks, please take the time to do them now.

7.1 Using an Injector

The Waters 600E system supports three methods for injecting samples. The following sections present programming instructions for each method and a description of how to make injections using:

- The Rheodyne 7725i manual injector
- A PowerLine autoinjector (Waters 717plus Autosampler)
- A non-PowerLine autoinjector

7.1.1 Using the Rheodyne 7725i Manual Injector

This section is an overview on using the optional Rheodyne 7725i manual injector. See the *Rheodyne 7725i Manual Injector Installation and Maintenance Guide* for specific operating instructions.

Caution: *Always follow safe laboratory practices when handling eluents. Know the physical and chemical properties of the eluents. See the Material Safety Data Sheets for the eluents in use.*

Caution: *When using sample loops larger than 100-µL, protect yourself from the rapid ejection of mobile phase from the needle port when you turn the valve from Inject to Load. A 1-mL loop expands by 20-µL upon decompression from 2900 psi (200 bar).*

Attention: *Be sure to use the proper size of syringe needle to avoid damage to the valve. Proper needle dimensions are 0.028 inches x 2 inches (0.071 cm x 5.08 cm) OD, without electro taper. The point must be 90° (square).*

To load the sample into the Rheodyne 7725i:

1. Turn the handle on the manual injector to the Load position (Figure 7-1).

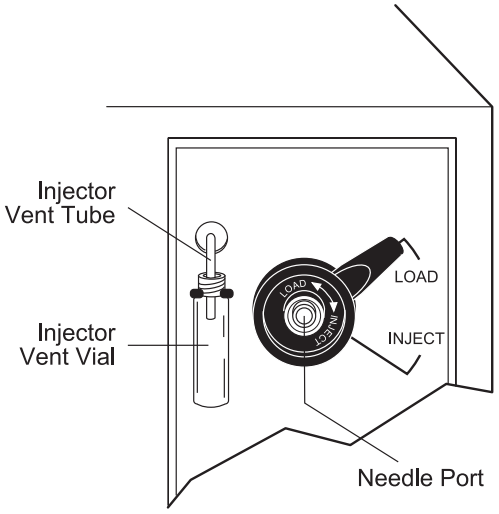


Figure 7-1 Rheodyne 7725i Injector in the Load Position

2. Load a syringe with sample. The loop may be partially filled or overfilled. The injector is equipped with a 20-µl loop.
3. Fully insert the 22-gauge, 90° point syringe needle into the filler port.
4. Gently discharge the contents of the syringe.
5. Turn the Load/Inject handle to the Inject position.

6. Allow sufficient time for the sample to clear the injector before repositioning the Load/Inject handle. Reposition the Load/Inject handle as follows:
   - If you are operating in the gradient mode, or if the next sample is not ready to be loaded, leave the handle in the Inject position.
   - If you are operating in the direct or isocratic mode, or if the next sample is ready to be loaded, turn the Load/Inject handle clockwise to the Load position.

7.1.2 Using a PowerLine Autoinjector

Note: *For complete information on the Waters 717plus Autosampler, see the Waters 717plus Autosampler Operator's Manual.*

You configure a PowerLine autoinjector such as the Waters 717plus Autosampler on the IEEE-488 bus. The autoinjector is under the control of the Waters 600 Controller.

Programming Considerations

When you make injections with a PowerLine autoinjector through either the Direct Control mode or Operate Method mode:

- Set the autoinjector parameters to the desired values on the Autoinjector Setup screen (see Section 4.4, Setting Up the Autoinjector).
- Specify the vial from which you want to make the injection:
  - In the Vial # field, when in the Direct Control mode (see Section 5.1.3, Setting Autoinjector Parameters)
  - In the vial range, when in the Operate Method mode (see Section 6.4.3, Entering Parameter Values in the Method Table)
- Specify the injection volume in the Inj Vol field.
- When programming a method table, specify the number of injections.

Injecting Samples

When you start the run or the method, the PowerLine autoinjector receives the sample injection information from the controller, draws the sample from the appropriate vial, and signals the controller when it makes the injection.

7.1.3 Using a Non-PowerLine Autoinjector

Note: *For complete information on your non-PowerLine autoinjector, see the operator's manual included with the injector.*

Overview

The Waters 600E system treats a non-PowerLine autoinjector as an external device.

Programming Considerations

When you make injections using a non-PowerLine autoinjector through either the Direct Control mode or Operate Method mode:

- Specify the special vial code 100 in the Vial # field. This code tells the controller that you are using an external injector. The inject start signal will be presented at the Inject terminal on the rear panel.
- If desired for documentation purposes, enter the injection volume in the Inj Vol field.
- When programming a method table, specify the number of injections.

Injecting Samples

To make sample injections using a non-PowerLine autoinjector:

1. Ensure that you have connected the injector according to the instructions in the *Waters 600E Multisolvent Delivery System Installation and Maintenance Guide*.
2. Load the sample vials into the autoinjector.
3. Program the autoinjector as necessary.
4. Start the autoinjector when you are ready to make the run.

7.2 Setting Initial Operating Parameters

This section describes how to use the Operate Methods screens to set the initial operating parameters for running a method table that you programmed (see Chapter 6, Creating PowerLine Methods).

Figure 7-2 summarizes the steps involved in using the Operate Methods screens. Use this flowchart for quick reference once you become familiar with your Waters 600E system.

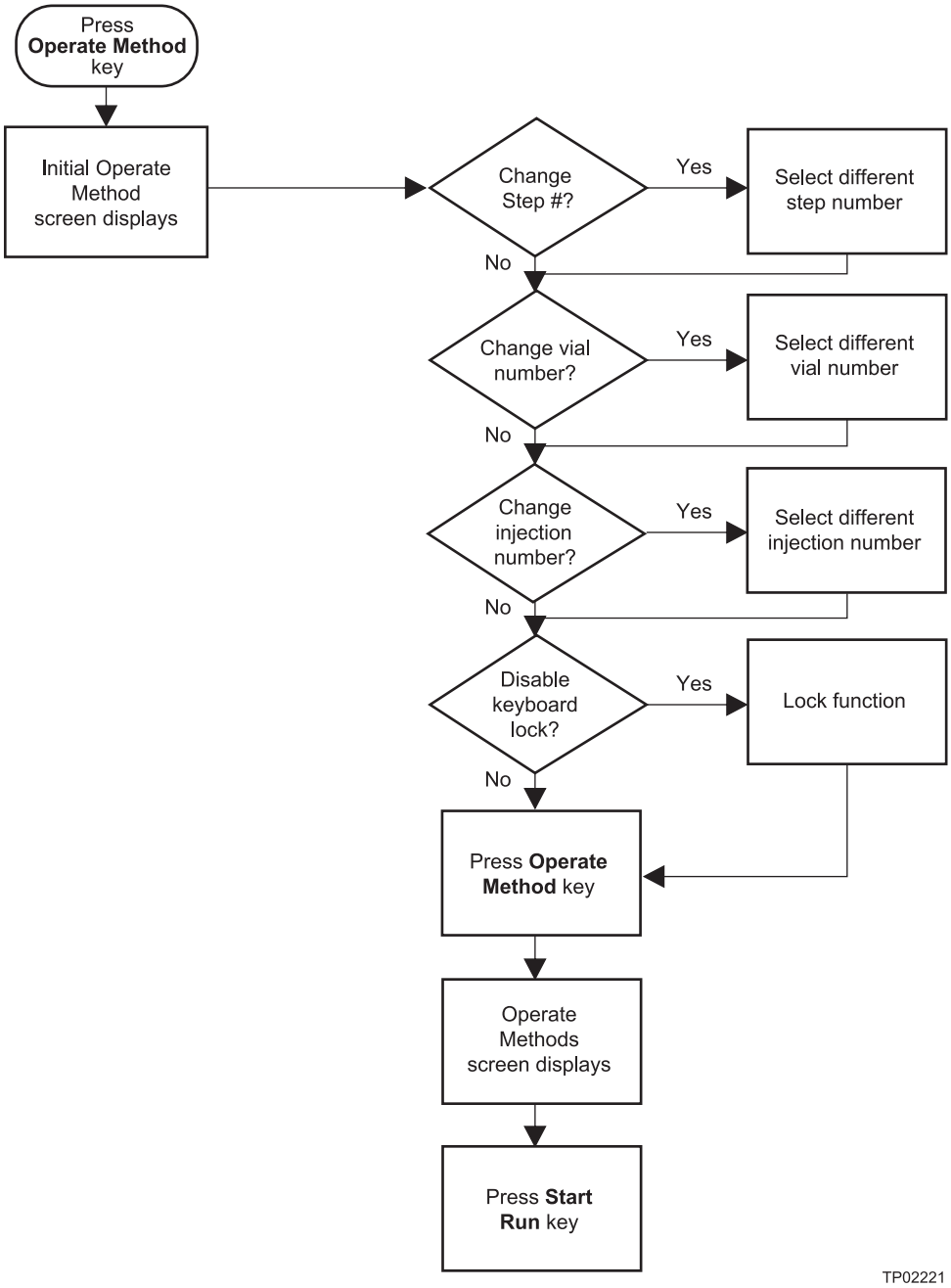


Figure 7-2 Operate Method Flowchart

Accessing Initial Operate Method Screen

Press the Operate Method function key. The initial Operate Method screen appears with the cursor at the Step # field (Figure 7-3).

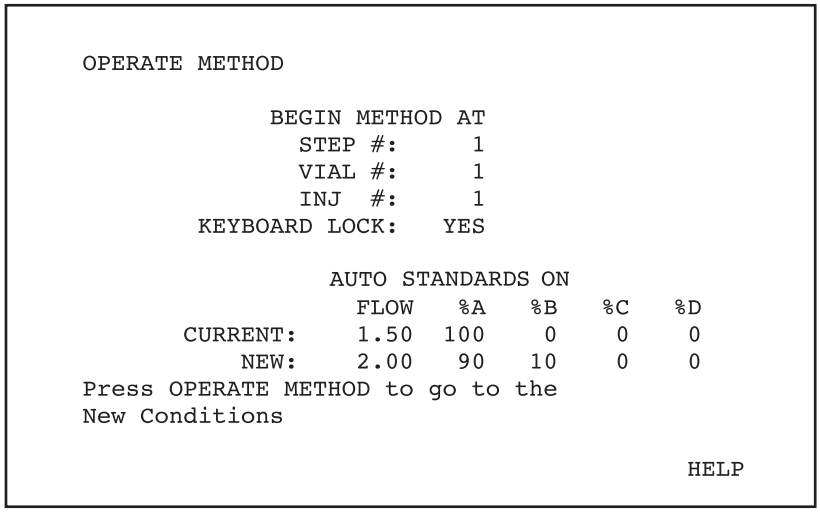


Figure 7-3 Initial Operate Method Screen

This screen displays the following:

- Step number, vial number, and injection number at which the run will start, and the keyboard status (locked or unlocked) during the run. These fields are modifiable.
- Current pump conditions in the Current line.
- Initial conditions of the first gradient table listed in the method table in the New line.
- `Auto Standards On` message in the middle of the screen if you enabled Auto Standards from the Autoinjector Setup screen (see Section 4.4.2, Setting Autoinjector Setup Parameters).
- `Dissolution On` message at the top of the screen if you enabled Dissolution from the Autoinjector Setup screen (see Section 4.4.2, Setting Autoinjector Setup Parameters).

Setting Parameters

The following changes are necessary only if you do not want to begin the run using the current parameters.

If Autostart is active at this time, the keyboard will be locked. To abort the Autostart, unlock the keyboard on the Pump Setup screen (see Section 4.3.2, Setting Pump Setup Parameters). After the keyboard is unlocked, make your changes to the Initial Operate Method screen as follows:

1. To run the method at any step in the method table, move the cursor to the Step # field and type the number of the desired step (1 to 48). Press Enter.
2. To select a new starting vial:
   a. Move the cursor to the Vial # field.
   b. Type the number of the vial (any vial number in the range of vials indicated in the Program Methods step) at which to begin the method (see Section 6.4.3, Entering Parameter Values in the Method Table).
   c. Press Enter.
3. To change the number of the injection at which to start, move the cursor to the Inj # field and type the new value (any injection number in the range of injections indicated in the Program Methods step). Press Enter.
4. To keep the keyboard unlocked during the run (the default condition is locked), move the cursor to the Keyboard Lock field and type 0 (No). Press Enter.

Canceling the Run

To cancel the run at this time, press any function key, except the Operate Method key, to select another mode.

7.3 Starting a Run

This section assumes that you have already set your initial operating conditions. If you have not already done so, see Section 7.2, Setting Initial Operating Parameters, for a list of requirements. Otherwise, proceed with the following topics:

- Accessing the Operate Methods screen
- Downloading the operating parameters
- Running a method

7.3.1 Accessing the Operate Methods Screen

1. If the controller screen is not displaying the initial Operate Method screen (see Figure 7-3), press the Operate Method function key. Otherwise, go to step 2.

2. Press the Operate Method function key again. The Operate Methods screen appears (Figure 7-4).

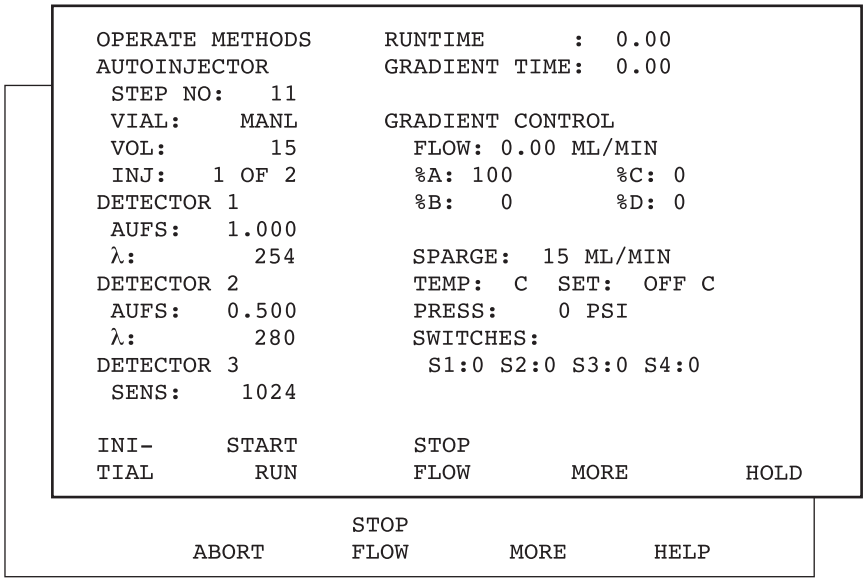


Figure 7-4 Operate Methods Screen

Operate Methods Screen Keys

In addition to the current system conditions, the Operate Methods screen displays the screen keys described in Table 7-1.

Table 7-1 Operate Methods Screen Keys

Key	Function
Start Run	Starts the gradient and the run-time clocks at 0.00. Toggles with Abort.
Stop Flow	Sets the pump flow rate to zero. Toggles with Resume.
More	Displays an additional set of screen keys associated with the Operate Methods screen.
Hold	Holds conditions at the current state and suspends the run-time clock. Toggles with Resume.
Abort	Displays a Caution screen that presents choices on how to prematurely end the method. Toggles with Start Run.

Table 7-1 Operate Methods Screen Keys (Continued)

Key	Function
Resume	Allows the run-time clock to continue after the Hold or Stop Flow keys have been pressed.
Alarm Cancel	Appears when you press More. Cancels the alarm.
Help	Displays Help for the Operate Methods screen.

System Status Flag Values

The lower-left corner of the Operate Methods screen displays a system status flag. This flag is similar to the screen keys except that it appears in reverse video. The flag assumes one of the statuses listed in Table 7-2.

Table 7-2 System Statuses

Status	Function
Idle	No run in progress.
Setup	Controller is sending the setup parameters to the Power-Line modules.
Initial	Module parameters have been downloaded; system is ready to start the run.
Inject Wait	System is waiting for an inject signal (contact closure at the Inject terminal or signal over the IEEE-488 bus) to start analysis.
Running	Analysis in progress.
Reporting	Report is printing on the Waters 746 integrator.

7.3.2 Downloading Operating Parameters

When you press the Operate Method function key to display the Operate Methods screen, the controller automatically downloads to the PowerLine modules the parameters that you programmed on the Setup screens (see Chapter 4, Setting PowerLine Controller Operating Parameters).

As the controller sends the parameter values to the modules, the system status is Setup. When parameter loading completes, the status changes to `Initial`, and the Start Run screen key appears.

7.3.3 Running a Method

Note: *The 600 controller examines the status of the 490E detector before initiating a run. If the detector is in the standby state, the controller automatically turns on the lamp.*

Using the Rheodyne 7725i Manual Injector

To begin a run with the Rheodyne 7725i manual injector:

1. Load the injector loop with sample. See Section 7.1.1, Using the Rheodyne 7725i Manual Injector.
2. Turn the injector handle to the Inject position to make the injection. The injector sends an Inject signal to the controller.

Using a PowerLine Autosampler

To begin a run with a PowerLine autosampler (717plus):

1. Load the sample vials into the autosampler.
2. Press the Start Run screen key. The 600 controller automatically starts the autosampler. The system status changes from Initial to Inject Wait.
3. The autosampler draws the sample and makes the injection. The controller receives the Inject signal through the IEEE-488 bus.

Using a Non-PowerLine Autoinjector

Note: *If you are using an autoinjector, see the documentation provided with that product for information on sample loading.*

To begin a run with an external, non-PowerLine autoinjector:

1. Load the sample vials into the autoinjector.
2. Program and start the autoinjector. See the autoinjector operating instructions.

   When the autoinjector makes the injection, it sends a contact closure signal to the Inject input terminal on the rear panel of the controller.

Using the Start Run Screen Key with a Non-PowerLine Autoinjector

For methods development and for runs that are not time-sensitive, you may also use the Start Run screen key to start a run with a non-PowerLine autoinjector.

To start a run with an autoinjector using the Start Run key:

1. Load the sample vials into the autoinjector.

2. Program and start the autoinjector.
3. Press the Start Run key as soon as possible after the injection occurs.

Note: *An Autostart method can override a method that you started with the Start Run screen key.*

Starting the Run-Time Clock

When the controller receives an Inject signal from an injector, the system status changes to Running and the run-time clock begins counting at 0.00. The system does not accept another inject signal until the run concludes or you abort the run.

Completing the Method

The system status flag changes from `Running` to `Idle` when the method table completes.

The external events remain as set when each analysis completes. They do not automatically reset to Off. Events that need to be turned on at a specific time in an analysis must be programmed to turn off before the start of the next injection.

The internal clock continues to run after each analysis completes and only resets to 0.00 with a new injection. Events or gradient segments programmed to occur after the run time for each injection still occur.

7.4 Modifying Conditions While Running

This section describes how to modify conditions while the method is running, including:

- Changing parameters
- Holding conditions constant
- Stopping flow
- Modifying gradient, event, and detector tables
- Modifying the method table
- Aborting the method

Note: *If the keyboard is locked, unlock it by pressing the Unlock Keyboard screen key on the Pump Setup screen (see Section 4.3.2, Setting Pump Setup Parameters).*

7.4.1 Changing Parameters

You can change the following parameters during a run:

- Sparge rate
- Column heater temperature
- Event switch status (On/Off)
- Operating parameters for any of the detectors

Note: *You can change parameters only if the keyboard is unlocked.*

To change a parameter on the Operate Methods screen:

1. Move the cursor to the appropriate parameter field on the Operate Methods screen (see Figure 7-4).
2. Type the new value. Press Enter.

When you manually enter a change, the new value remains in effect until the next timed program change.

7.4.2 Holding Conditions Constant

The Hold function keeps the eluent flow rate and composition constant during a run, which is a useful feature in methods development. The system suspends the run time and external events program while this function is in effect.

To suspend system operation at the current conditions:

1. Press the Hold screen key on the Operate Methods screen. The Hold key acts as a toggle, and changes to Resume when you press it.

   When you press Hold both the gradient clock and the Hold clocks stop.
2. To continue system operation from the point at which you suspended it, press the Resume screen key. The key label changes back to Hold, and the gradient and run-time clocks continue from where they stopped.

7.4.3 Stopping Flow

You generally use the Stop Flow screen key at the end of an analysis. However, you can also use it to temporarily shut down your pump (for example, to adjust a fraction collector) or to function as an emergency shutdown.

The Stop Flow function also suspends the method table, but does not stop the run-time clock. The current event and detector tables continue to execute. However, pressing Stop

Flow causes switch S4 to default to the Off position. This action provides an additional level of control for non-IEEE devices.

Note: *The receipt of the Stop Flow signal (at the 600 controller's Stop Flow rear panel terminal) suspends the controller's run, gradient, and event clocks. The three clocks resume operation when the Stop Flow signal terminates.*

To stop flow:

1. Press the Stop Flow screen key. This key acts as a toggle and changes to Resume when you press it.
2. When you press Stop Flow:
   - Flow rate is set to zero
   - Gradient clock stops
   - Run-time clock continues
3. To continue running the method, press the Resume screen key. This key changes back to Stop Flow again.
4. When you press Resume, the pump ramps up to the flow rate at which it was running when you pressed Stop Flow, and the method continues.

7.4.4 Modifying Gradient, Event, and Detector Tables

While a run is in progress, you can modify any existing table except the one currently running.

To modify a table during a run:

1. Press the Program Table function key.
2. Press the Next Table screen key until the table you want to modify appears.
3. Move the cursor to the Table # field and type the number of the table you want to modify. Press Enter.

   Note: *You cannot modify the table that is currently running.*
4. Modify the table.
5. Save the new table by pressing the Save screen key.

   Note: *You cannot assign the number of the table that is currently running to the modified table.*

To modify a table that is currently running:

1. Stop the run by pressing the Abort screen key from the Operate Methods screen. The Caution I screen appears.
2. Press the Abort screen key from the Caution I screen (Figure 7-5).
3. Modify the table.
4. Save the table by pressing the Save screen key.

7.4.5 Modifying the Method Table

To modify any line in the method table that follows the currently running line:

1. Press the Program Method function key. The Program Methods screen appears.
2. Revise the table as necessary, then press the Save screen key.
3. Press the Operate Method function key to resume monitoring the run.

7.4.6 Aborting the Method

There are two ways to abort the method:

- Pressing the Abort screen key
- Pressing the Direct function key

Using the Abort Screen Key

To abort the method using the Abort screen key:

1. Press the Abort screen key. The Caution I screen appears (Figure 7-5).

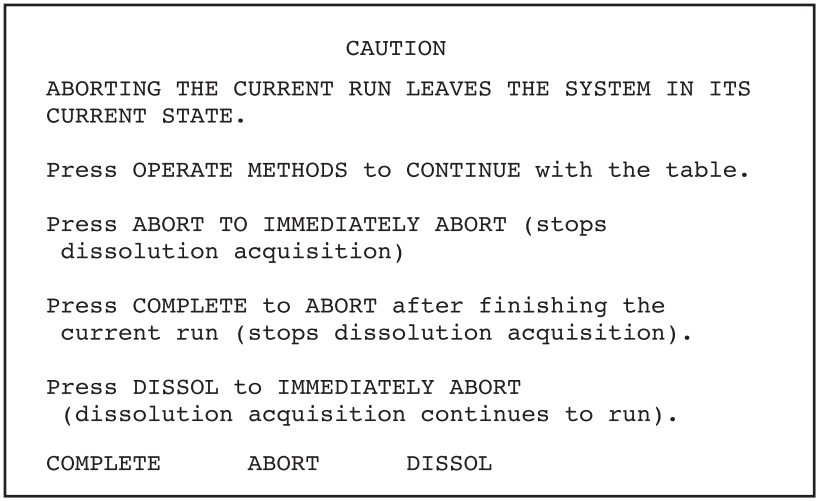


Figure 7-5 Caution I Screen

2. Take one of the following actions:
   - To cancel the abort and resume monitoring the method, press the Operate Method function key. The Operate Methods screen appears.
   - To immediately abort the run, press the Abort function key. This action also stops dissolution acquisition.
   - To abort the method after the current run completes, press the Complete screen key.
   - To abort the run but continue with dissolution acquisition, press the Dissolution screen key.

   Note: *When you abort a method, execution of gradient, event, and detector tables stops. All external switches remain in the current or last-set state. You can change the switch states on the Direct Control screen, if necessary. See Chapter 5, Running PowerLine in Direct Control Mode.*

Using the Direct Function Key

To abort the method using the Direct function key:

1. Press the Direct function key. The Caution II screen appears (Figure 7-6).

   This Caution screen prompts you to abort the method immediately and enter Direct Control mode or to finish the current run and then abort the method.

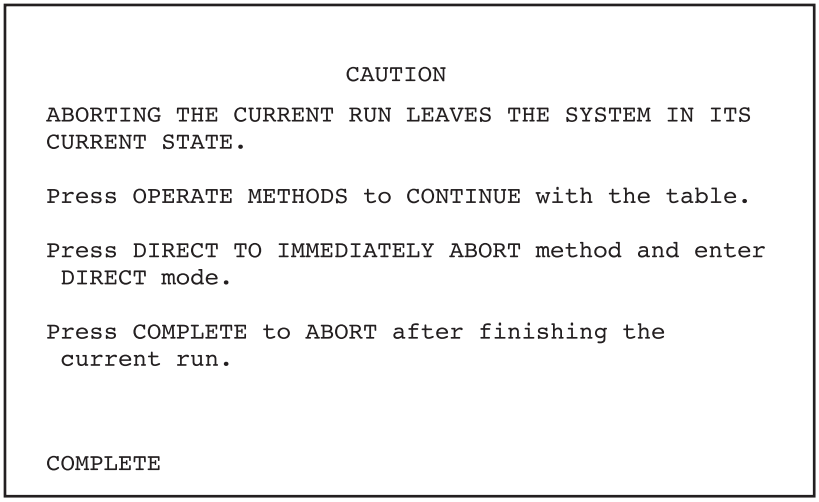


Figure 7-6 Caution II Screen

2. Take one of the following actions:
   - To cancel the abort and continue monitoring the method, press the Operate Method function key. The Operate Methods screen appears.
   - To abort the method immediately and enter the direct control mode, press the Direct function key. The Direct Control screen appears.
   - To abort the method after the current run completes, press the Complete screen key.

   Note: *When you abort a method, execution of gradient, event, and detector tables stops. All external switches remain in the current or last-set state. You can change the switch states on the Direct Control screen, if necessary. See Chapter 5, Running PowerLine in Direct Control Mode.*

Chapter 8
Setting Gradient Controller Operating Parameters

This chapter contains the information you need to set the operating parameters when you want to use the Waters 600 Controller as a Gradient Controller.

The operating parameters apply to all controller operations, whether you are running a direct control (isocratic) or timed (gradient) application. Once you set these parameter values, they remain in memory until you change them.

Note: *For instructions on configuring and using your Waters 600 Controller as a PowerLine Controller, see Part 2, Using the PowerLine Controller.*

When to Use Gradient Controller Mode

Use Gradient Controller mode when you want to control the Waters 600E Multisolvent Delivery System from a data system (Millennium Chromatography Manager or ExpertEase 845/860 Data System), or when you want to run the 600E system in stand-alone operation.

For a detailed description of Gradient Controller mode, see Section 2.4.2, Gradient Controller Configuration.

Background Information

This chapter assumes that you have installed your system according to the instructions in the *Waters 600E Multisolvent Delivery System Installation and Maintenance Guide*. Ensure that you have read Chapter 1 to Chapter 3 and that you have:

- Acquainted yourself with the system (Chapter 1, Introduction)
- Familiarized yourself with basic operation and have decided upon a controller configuration (Chapter 2, Basic Operation)
- Prepared your system for operation (Chapter 3, Preparing Your 600E System for Operation)

If you have not completed these tasks, do so now.

Gradient Controller Screens

Figure 8-1 illustrates the relationships of the screens associated with the Waters 600 Controller in its Gradient Controller configuration. Use Figure 8-1 to navigate through Gradient Controller operation.

Note: *In Gradient Controller mode, function key labels do not correspond exactly to the screens they access. For more information, see Section 2.3.2, Function Keys.*

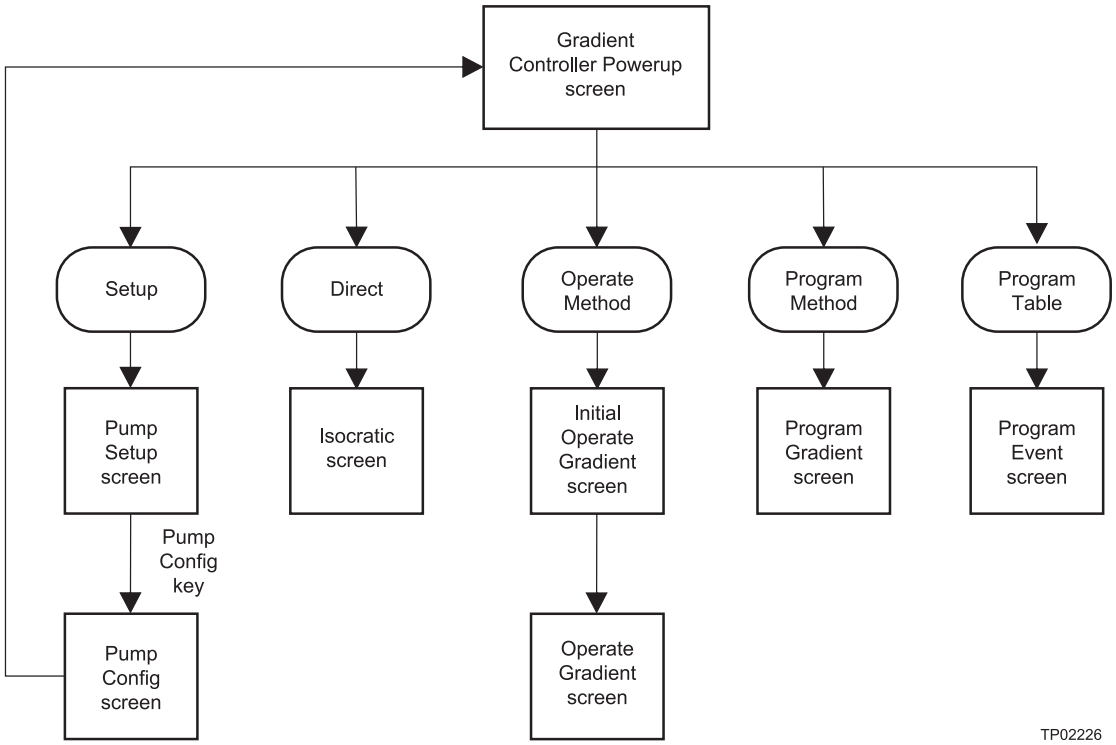


Figure 8-1 Gradient Controller Screens

8.1 Setting Up the 600 as a Gradient Controller

This section describes how to use the Pump Configuration screen to configure the Waters 600 Controller as a Gradient controller. It includes procedures for:

- Configuring the controller type
- Communicating with a data system

Figure 8-2 illustrates the steps involved in using the Pump Configuration screen. Use this flowchart as a quick reference for setting the parameters on the Pump Configuration screen.

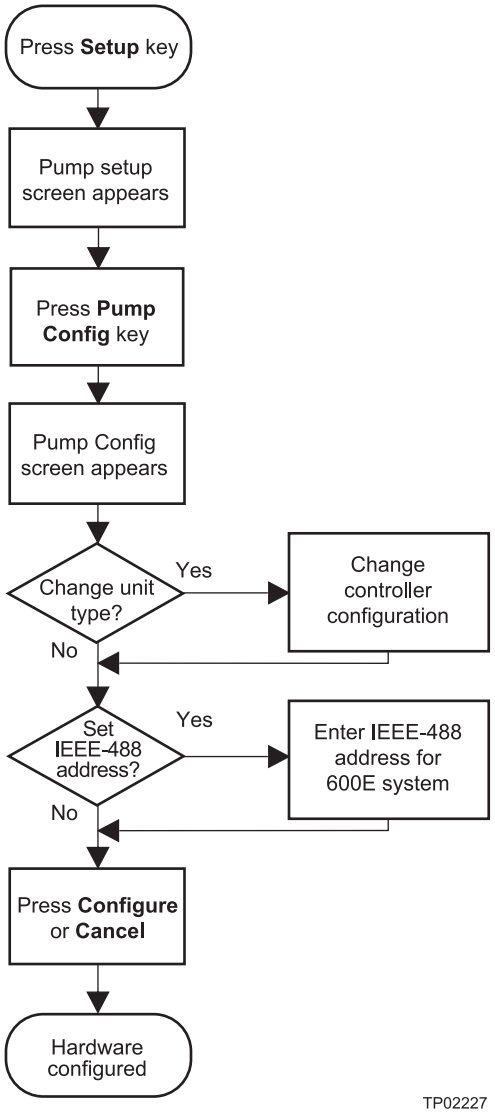


Figure 8-2 Pump Configuration Flowchart

Exiting the Pump Configuration Screen

- If you want to save the parameter values displayed on the screen, press the Configure screen key. The system reboots and displays the Powerup screen.

  Pressing the Configure screen key also aborts a run in process and sets the flow rate to 0.

- If you want to return to the Pump Setup screen without saving any changes made to the Pump Configuration screen, press the Cancel screen key.

8.1.1 Configuring the Controller Type

This section describes how to configure the Waters 600 Controller as a Gradient Controller.

Waters preconfigures the 600 controller as a PowerLine Controller at the manufacturing site. Use this section to:

- Verify that the controller type is correct
- Change the controller type to Gradient, if you did not previously configure the 600 as a Gradient controller

To verify or configure the controller type:

1. Turn on the 600 controller by pressing the On/Off switch to the 1 (On) position. The Powerup screen appears (Figure 8-3).

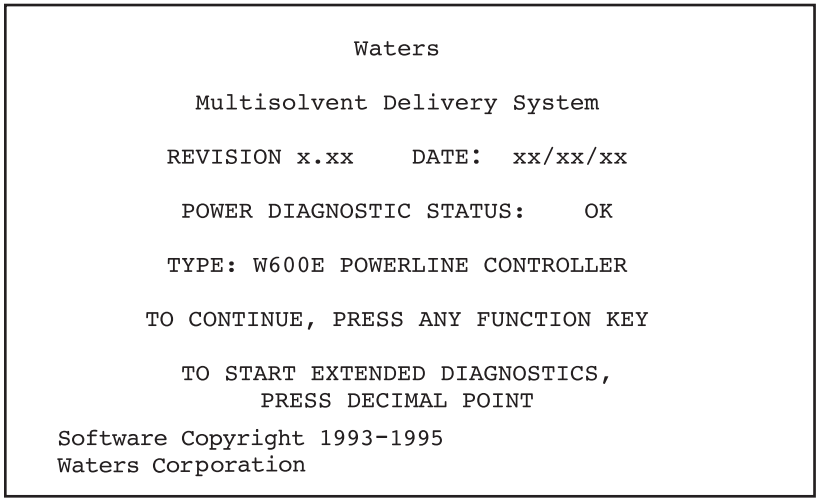


Figure 8-3 PowerLine Controller Powerup Screen

2. Check the controller Type field. If the Type field contains:
   - **W600E Gradient Controller** - Skip the rest of this section and proceed to Section 8.1.2, Communicating with a Data System.
   - **W600E PowerLine Controller** - Continue with step 3.
3. Press the Set Up function key to display the PowerLine Controller Pump Setup screen (Figure 8-4).

```
PUMP SETUP

RESERVOIRS TO SPARGE:
A: DISABLE B: DISABLE C: DISABLE D: DISABLE

PUMP COL. TEMP. HEATER LIMIT:  0 C

PRESS LIMITS: LOW:  0         HIGH:  6000  PSI

CHART OUT: %A
FLOW FACTOR: 1.000
AUTOSTART:IN   HRS  MIN  STEP  VIAL

 NEXT     SYSTEM      PUMP       LOCK
SETUP     CONFIG     CONFIG    KEYBOARD     HELP
```

Figure 8-4 PowerLine Controller Pump Setup Screen

It is not necessary to set any parameters on the Pump Setup screen now. For a description of the gradient controller version of the Pump Setup screen and pump parameters, see Section 8.2, Setting Up the 600E Pump.

4. Press the Pump Config screen key to display the PowerLine Controller Pump Configuration screen (Figure 8-5).

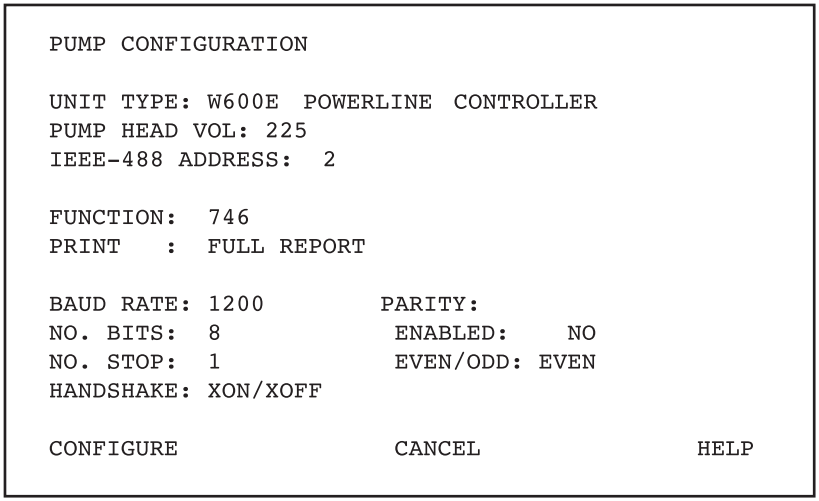


Figure 8-5 PowerLine Controller Pump Configuration Screen

5. Move the cursor to the Unit Type field.
6. Type 0 to select the Gradient Controller. Press Enter.
7. Press the Configure screen key. The controller reboots and returns to the Gradient Controller Powerup screen. The Type field contains:

   `W600E Gradient Controller`

8. Continue with the following sections:
   - Section 8.1.2, Communicating with a Data System
   - Section 8.2, Setting Up the 600E Pump

8.1.2 Communicating with a Data System

Use this section only if you intend to use the Waters Millennium Chromatography Manager (PC-based) or the Waters ExpertEase 845/860 Data System (VAX/VMS-based) to control the 600E system. Otherwise, proceed to Section 8.2, Setting Up the 600E Pump, to set up your pump for the first time or to change one or more pump parameters.

This section does not explain how to use the data systems or how to program the data systems for other applications. For information on using and programming the data systems, see the appropriate user's manuals.

This section assumes that your system was installed as described in the *Waters 600E Multisolvent Delivery System Installation and Maintenance Guide* and you have made the appropriate IEEE-488 cable connections to the data system.

Setting the IEEE-488 Address

To configure the 600E system to communicate with a data system, you must set the IEEE-488 address of the 600E system on the Gradient Controller Pump Configuration screen as follows:

1. From the Gradient Controller Powerup screen, press the Set Up function key. The Gradient Controller Pump Setup screen appears (Figure 8-6).

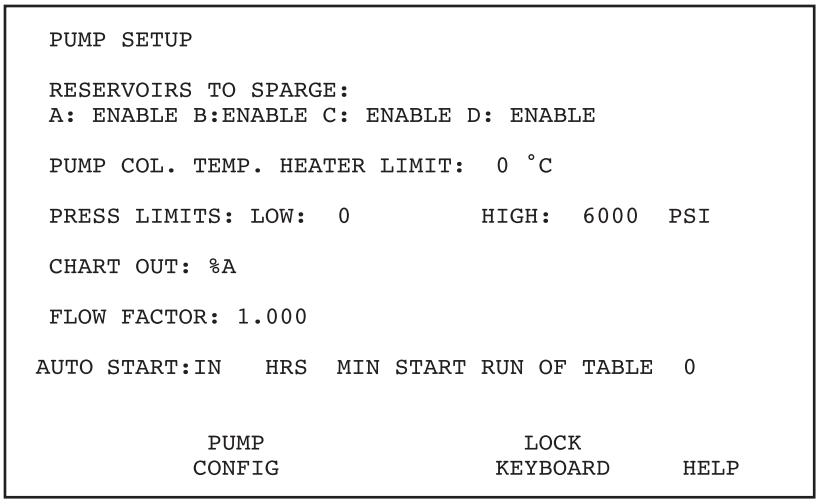


Figure 8-6 Gradient Controller Pump Setup Screen

2. Press the Pump Config screen key. The Gradient Controller Pump Configuration screen appears (Figure 8-7).

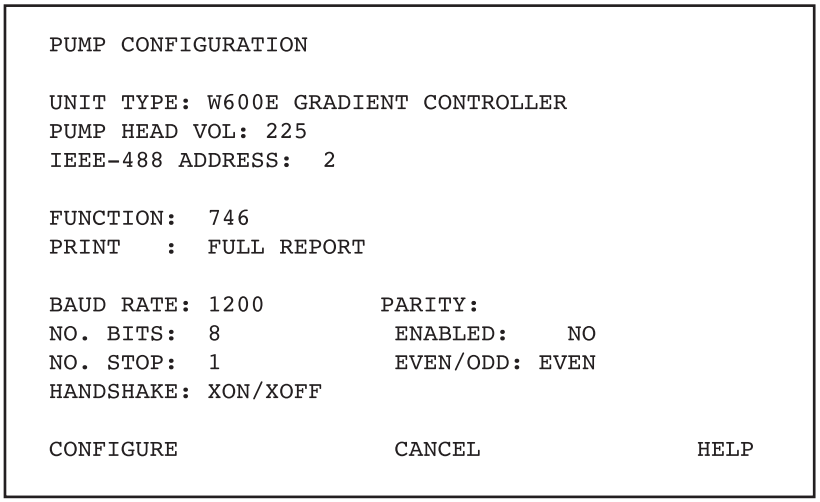


Figure 8-7 Gradient Controller Pump Configuration Screen

3. Move the cursor to the IEEE-488 field.
4. Type a number from 2 to 29 that is a unique address on the IEEE-488 network. Press Enter.

   Note: *Ensure that the address is unique within your IEEE-488 network. Otherwise, communications problems will result.*

5. Press the Configure screen key to reset the system software and to enable your IEEE-488 address selection. The controller reboots and returns to the Gradient Controller Powerup screen.

If you are setting up your system for the first time or you want to change one or more pump parameter values, proceed to Section 8.2, Setting Up the 600E Pump. Otherwise, proceed to:

- Chapter 9, Running Gradient Controller in Isocratic Mode
- Chapter 10, Creating Gradient Controller Time-Based Tables
- Chapter 11, Running Gradients

8.2 Setting Up the 600E Pump

This section describes how to access and use the Pump Setup screen in Gradient Control mode. It includes the following information:

- Accessing the Gradient Controller Pump Setup screen
- Setting Gradient Controller Pump Setup parameters
- Exiting the Gradient Controller Pump Setup screen

Pump Setup Overview

The Gradient Controller Pump Setup screen includes the following parameters:

- Enable/disable reservoir sparging
- High-temperature limit for an optional column heater
- High and low operating-pressure limits for the system
- External chart output function
- Enable/disable a flow factor to account for flow-path elasticity or eluent compressibility
- Enable/disable Autostart
- Lock/unlock keyboard

Figure 8-8 illustrates the steps for using the Pump Setup screen. Use Figure 8-8 as a quick reference for setting the parameters on the Pump Setup screen.

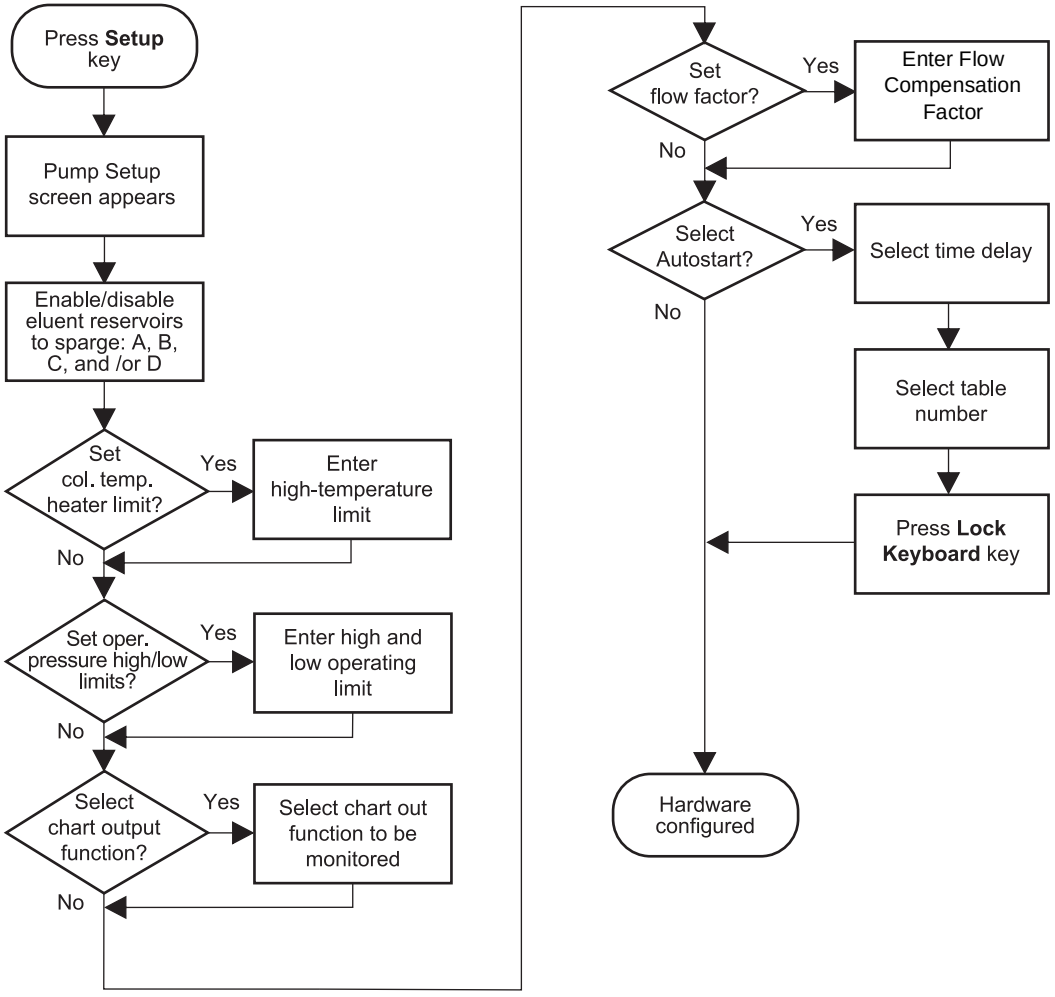


Figure 8-8 Pump Setup Flowchart

8.2.1 Accessing the Pump Setup Screen

To access the Gradient Controller Pump Setup screen (Figure 8-9):

1. Press the Set Up function key from any screen except the Gradient Controller Pump Configuration screen. The Gradient Controller Pump Setup screen appears (Figure 8-9).

2. If you are at the Gradient Controller Pump Configuration screen (see Figure 8-7) and do not need to save changes, press the Cancel screen key to return to the Gradient Controller Pump Setup screen (Figure 8-9).
3. If you are at the Gradient Controller Pump Configuration screen (see Figure 8-7) and do need to save changes:
   - Press the Configure screen key to save your changes. The controller reboots and returns to the Gradient Controller Powerup screen.
   - Press the Set Up function key. The Gradient Controller Pump Setup screen appears (Figure 8-9).

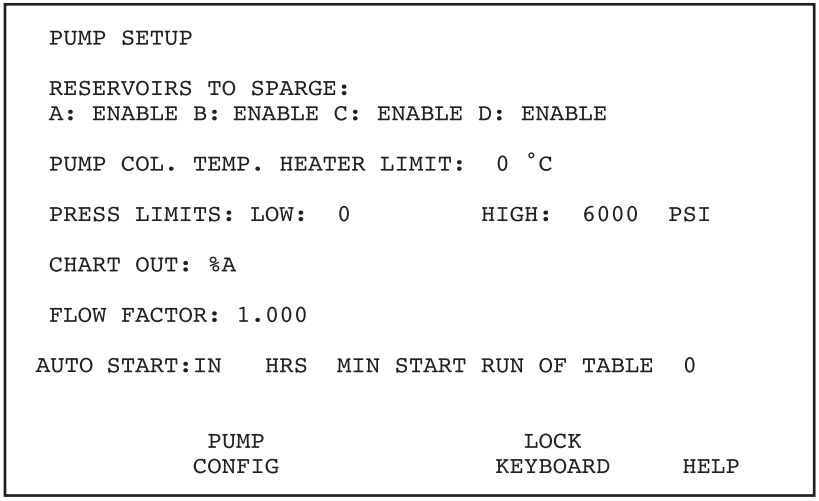


Figure 8-9 Gradient Controller Pump Setup Screen

Pump Setup Screen Keys

In addition to the parameters shown on the screen, the Gradient Controller Pump Setup screen also displays the screen keys described in Table 8-1.

Table 8-1 Pump Setup Screen Keys

Key	Function
Pump Config	Displays additional configuration parameters related to the pump and controller.
Lock Keyboard	Locks or unlocks the keyboard.
Help	Displays the Help screen for the Pump Setup screen.

8.2.2 Setting Pump Setup Parameters

This section describes the pump parameters and provides procedures for setting each parameter on the Pump Setup screen.

The pump parameters are:

- Reservoirs to sparge
- Pump col. temp. heater limit
- Press limits
- Chart out
- Flow factor
- Autostart
- Keyboard lock/unlock

Reservoirs to Sparge

Indicates which reservoirs you want to enable for helium sparging. The Reservoirs to Sparge parameter only enables a reservoir for sparging; it does not initiate the sparging process. The sparge flow rate can be set on the Isocratic, Event, or Operate Gradient screen, depending on the mode of operation.

If helium is unavailable or undesirable for sparging, see Appendix B, Eluent Considerations, for alternative degassing methods.

To configure the reservoirs to sparge:

1. Move the cursor to the field for the reservoir that you want to sparge.
2. Type 0 to disable sparging, or 1 to enable sparging for that reservoir.
3. Press Enter.
4. Repeat steps 1 through 3 for each active reservoir that you want to sparge.

Pump Col. Temp. Heater Limit

Defines the high-temperature limit for the optional column heater. The upper limit of the heater module is indicated on the module label.

Note: *To prevent damage to the column, the heater module shuts down when the temperature exceeds the programmed limit for more than 1.2 seconds.*

To set the pump column-temperature heater limit:

1. Move the cursor to the Pump Col. Temp. Heater Limit field.
2. Type the appropriate temperature value (0 to 99) in °C.
3. Press Enter.

Press Limits

Defines the high and low operating-pressure limits of the Waters 600E pumps.

Your system, column, and packing chemistry determine the low- and high-pressure limits. See the *Care and Use Manual* supplied with the column to determine the maximum pressure to which you can expose the column.

You typically set the high-pressure limit to 50 to 100 psi below the normal maximum operating pressure for the column. For more information, see the *Care and Use Manual* for the column.

To prevent damage to the pump or column, the system shuts down when the system pressure exceeds the high-pressure limit for more than 1.2 seconds or falls below the low pressure limit for 60 seconds.

Low-Pressure Limit and High Pressure Limit

To set the pressure limits:

1. Move the cursor to the Low field.
2. Enter a low-pressure limit value within the range shown below:

Pump	Range
600E	0 to 5950 psi (0 to 416.5 kg/cm^2)

3. Press Enter.
4. Move the cursor to the High field.
5. Enter a high-pressure limit value within the range shown below:

Pump	Range
600E	51 to 6000 psi (3.6 to 420 kg/cm^2)

6. Press Enter.

Chart Out

Determines which signal is available at the Chart terminal on the rear panel of the controller. This 0- to 10-mV signal can be the eluent flow rate (Flow) or the composition percentage of any one of the four eluents (%A, %B, %C, or %D). To use this feature, connect the Chart Out terminals on the rear panel of the controller to the 0- to 10-mV terminal on the recorder or integrator.

This output signal can be connected to a data system or the second pen of a chart recorder. It is frequently used during troubleshooting to reflect a selected condition (such as pump flow or eluent composition).

A time delay between the eluent composition shown by the Chart Out signal and the eluent composition at the column inlet always occurs. This time delay is the system volume or delay volume. The 600E system has been optimized to reduce the delay volume.

The detector signal is further delayed by the volume at the column. At a low flow rate, with a column attached, the delay between an eluent changeover instruction and its subsequent detection may be several minutes.

To set the Chart Out parameter:

1. Move the cursor to the Chart Out field.
2. Type the number for the desired output:
   - 1, 2, 3, or 4 for the % composition of one of the four eluents (A, B, C, or D)
   - 5 for flow rate
   - 6 for column heater temperature
3. Press Enter.

Flow Factor

Speeds up or slows down the pump to compensate for differences in pressure or eluent compressibility. Enter a value here only if you need to match flow rate conditions between systems or between different eluents.

To determine the flow factor for your application:

1. Set the flow rate to 1.0 mL/min.
2. Measure the actual flow rate.

3. If the actual flow rate is less than or greater than 1.0 mL/min, type a flow factor value to compensate for the difference. The valid range is 0.950 to 1.050. For example, if your actual flow rate is 0.98 mL/min, use a flow factor of 1.02.
4. Press Enter.

Autostart

Specifies a time delay before a run starts and tells the system where to begin execution (Gradient Table number). You can use Autostart at any time. While the Autostart countdown is in progress, you can continue to run a method started before you initiated Autostart. When the countdown completes, the system immediately aborts any run in progress and the Autostart parameters take precedence.

To Autostart a gradient:

1. Press the Set Up function key to display the Pump Setup screen.
2. Move the cursor to the Autostart field.
3. Type the total delay time in the Hrs and Min fields.
   - Enter a value between 0 and 99 for Hrs (hours).
   - Enter a value between 0 and 59 for Min (minutes).
4. Press Enter.
5. At the Table field, type the number of the Gradient table at which you want program execution to start. Press Enter.

Sample Gradient Table

For example, your Gradient Table may appear as shown in Figure 8-10.

Time	Flow	%A	%B	%C	%D	Curve
Initial	1.00	100	0	0	0	*
30.00	1.00	0	100	0	0	6
35.00	1.00	100	0	0	0	6
50.00	1.00	100	0	0	0	11

Figure 8-10 Example of a Gradient Table

If you want to run your system 12 hours from now and start on Table 1, type the following in the Autostart field:

```
In 12 Hrs 0 Min Start Run of Table 1
```

6. Activate Autostart by pressing the Lock Keyboard screen key. The countdown time displays on the Pump Setup screen.

   When the keyboard is locked, pressing the function keys will display the correct screens, but no changes to the parameter values are allowed. Make sure you have programmed all gradient and event tables before you lock the keyboard.

Canceling Autostart

To cancel a countdown in progress, unlock the keyboard by pressing the Unlock Keyboard screen key.

Keyboard Lock/Unlock

Locking the keyboard:

- Prevents accidental alteration of gradient flow conditions
- Starts the Autostart condition

When the keyboard is locked, the screen key legend changes to Unlock Keyboard.

Unlock Keyboard:

- Is the only operational key on the Pump Setup screen
- Cancels the Autostart condition

8.2.3 Exiting the Pump Setup Screen

To exit the Pump Setup screen, perform one of the following actions:

- Press any function key to select another mode
- Press the Pump Config screen key to display the Pump Configuration screen.

Chapter 9
Running Gradient Controller in Isocratic Mode

Once you have familiarized yourself with the Waters 600E Multisolvent Delivery System and prepared it according to the procedures in Chapters 1 through 3 and 8, you may make a run in the isocratic mode.

Isocratic Control

Isocratic operations do not allow time-actuated changes in flow rate or eluent composition or other time-dependent conditions to occur. To change flow rate or eluent composition, or to switch output states, you must manually program new values to override the original parameter settings. The system implements these changes immediately.

Isocratic mode is also useful for preparing the system to make a run. You program isocratic operation through the Isocratic screen (see Section 9.1, Setting Run Conditions).

By setting parameters on the Isocratic screen, you can do the following without making an injection:

- Sparge eluents
- Prime the pump (Section 3.2, Priming the Pump)
- Equilibrate the system
- Monitor the baseline (see your detector manual)
- Set external switch parameters

Before You Begin

This chapter assumes that you have:

- Installed your system and all external devices according to the procedures in the *Waters 600E Multisolvent Delivery System Installation and Maintenance Guide*
- Familiarized yourself with basic operation and decided upon a controller configuration (Chapter 2, Basic Operation)

- Prepared your system as outlined in Chapter 3, Preparing Your 600E System for Operation
- Set operating parameters as described in Chapter 8, Setting Gradient Controller Operating Parameters

9.1 Setting Run Conditions

Before you make a run with the PowerLine controller in the Isocratic mode, you must first set the run conditions. This section describes the procedures for:

- Setting pump parameters
- Setting external event switches

Run Requirements

To perform an isocratic run in the isocratic mode, you must:

- Set the run conditions (flow rate, eluent composition, sparge rate, heater module temperature, and external switch output settings)
- Set the run time
- Equilibrate the system
- Start the run

Figure 9-1 illustrates the steps involved in using the Isocratic screen. Use Figure 9-1 for quick reference once you become familiar with your 600E system.

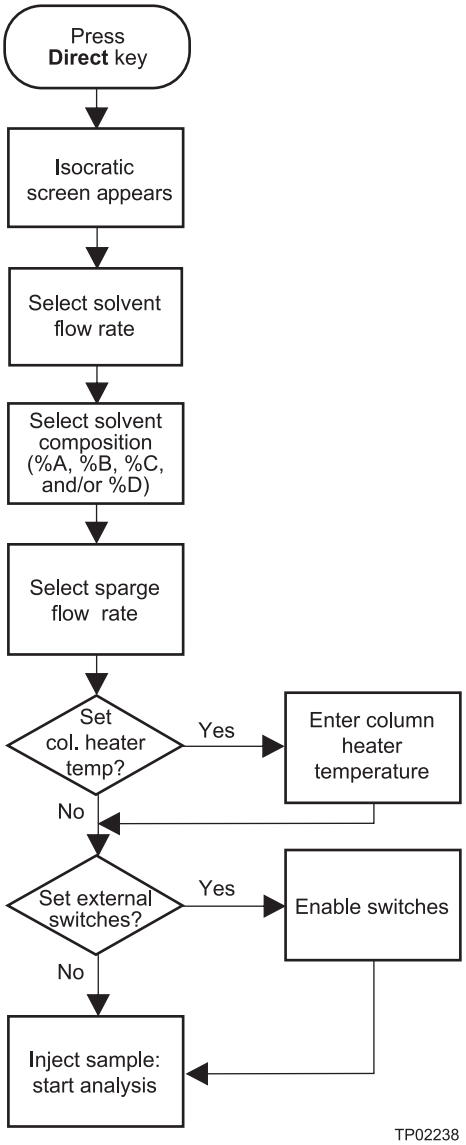


Figure 9-1 Isocratic Control Flowchart

Accessing the Isocratic Screen

To access the Isocratic screen (Figure 9-2), press the Direct function key on the controller front panel.

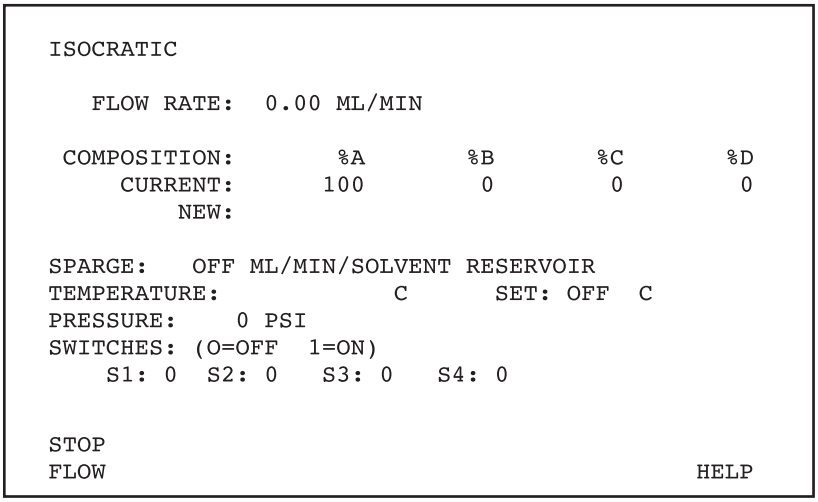


Figure 9-2 Isocratic Screen

In addition to the parameters shown on the screen, the Isocratic screen displays the screen keys described in Table 9-1.

Table 9-1 Isocratic Screen Keys

Key	Function
Stop Flow	Stops the pump.
Help	Displays the Help screen for the Isocratic screen.

Exiting the Isocratic Screen

Press any function key to select another mode.

9.1.1 Setting Pump Parameters

Use the Isocratic screen (see Figure 9-2) to make manual changes to the pump parameters. The pump parameters are:

- Flow rate
- Composition

- Sparge
- Temperature

Flow Rate

Specifies the eluent flow rate.

Note: *Some columns require a flow ramp up. See the column Care and Use Manual for guidelines.*

Be sure that the values for the maximum and minimum operating pressures programmed on the Pump Setup screen are appropriate for your column. See Section 4.3.2, Setting Pump Setup Parameters (for PowerLine Controller operation), or Section 8.2.3, Exiting the Pump Setup Screen (for Gradient Controller operation). See the column Care and Use Manual for pressure guidelines.

To set the Flow Rate:

1. Move the cursor to the Flow Rate field.
2. Type the appropriate eluent flow rate (0.00 to 20.0 or 45.00 mL/min) for the application. Press Enter.

   The system immediately sets the pump to the flow rate you entered.

Composition

Indicates the percentage (0 to 100%) of each eluent that will be used in the mobile phase.

To set the eluent composition:

1. Move the cursor to the New %A field.

   Note: *The Current values indicate the present eluent composition. The system copies the values from the Current line when you move the cursor to the New line.*

2. Type the % composition for the mobile phase (0 to 100, no decimals, in 1% increments) for eluent reservoir A. Press Enter.
3. Repeat steps 1 and 2 for the remaining three eluent reservoirs. The sum of all four entries *must* equal 100 percent.
4. After you enter the percentage for eluent D and press Enter, the system implements all the eluent composition changes. The Current line reflects the new eluent composition.

Sparge

Determines the rate at which the system sparges the eluent reservoirs.

To set the sparge rate value:

1. Move the cursor to the Sparge field.
2. Type the appropriate sparge rate value (0 to 100 mL/min). Press Enter.

Note: *The system uses the same sparge rate for all eluent reservoirs that you enable on the Pump Setup screen. Use an initial high sparge flow rate and then reduce it to a low maintenance rate. The sparge method you use depends on your analytical requirements. See Appendix B, Eluent Considerations, and Chapter 3, Preparing Your 600E System for Operation, for more information.*

Temperature

Controls and maintains a stable column-operating temperature. This value must be less than or equal to the high-temperature limit programmed on the Pump Setup screen (see Section 8.2.3, Exiting the Pump Setup Screen). The range of temperature within which the column heater can operate is listed on the module label.

To set the column heater temperature:

1. At the Temperature field, type the appropriate temperature in °C for the analysis you want to run.
2. Press Enter.

Note: *Verify separation methods requiring elevated temperatures at the elevated temperatures. If no column heater is installed, the Temperature field contains a value of None.*

9.1.2 Setting External Event Switches

S1, S2, S3, S4

The Isocratic screen (see Figure 9-2) allows you to manually operate external devices by setting the S1 through S4 external event switch parameters.

Use the S1 through S4 event switch terminals on the controller rear panel to control devices such as fraction collectors, switching valves, or other non-PowerLine components.

Connecting External Devices

Before setting any of the event switches, connect the external device to the switch terminals on the rear panel of the controller. See the *Waters 600E Multisolvent Delivery System Installation and Maintenance Guide* for more information on these connections.

Note: *S4 defaults to the Off position when a stop-flow condition or a critical fault occurs. Use this feature by connecting to S4 a device, such as an autosampler, that must be shut off when an error, such as a high-pressure shutdown or a critical fault, occurs.*

Turning On Switches

1. Move the cursor to the appropriate switch field (S1, S2, S3, or S4).
2. Type 1 (On). Press Enter.

Turning Off Switches

1. Move the cursor to the appropriate switch field (S1, S2, S3, or S4).
2. Type 0 (Off). Press Enter.

9.2 Performing an Isocratic Run

Once you have set the run conditions (see Section 9.1, Setting Run Conditions), use the Isocratic screen (see Figure 9-2) to make a run by performing the procedures in the following sections:

- Equilibrating the system
- Starting a run
- Changing parameters while running
- Stopping the pump

9.2.1 Equilibrating the System

The 600E system operates with the conditions displayed on the Isocratic screen. The system runs at these conditions until the system is equilibrated. For more details, see Chapter 3, Preparing Your 600E System for Operation.

To equilibrate your system, allow at least 5 to 6 column volumes of eluent to pass through the system. When your system is equilibrated, continue with Section 9.2.2, Starting a Run.

9.2.2 Starting a Run

Using the Rheodyne 7725i Manual Injector

1. Load the injector loop with sample. See Section 11.1.1, Using the Rheodyne 7725i Manual Injector.
2. Turn the injector handle to the Inject position to make the injection.

Using a Non-PowerLine Autoinjector

Note: *If you are using an autoinjector, see the documentation provided with that product for information on sample loading and operation.*

To begin a run with an external non-PowerLine autoinjector:

1. Load the sample vials into the autoinjector.
2. Program and start the autoinjector. The autoinjector makes the injection.

Completing the Run

In the isocratic mode, there is no run-time clock, no system status, and no change in eluent composition. The run continues until you stop the pump. See Section 9.2.4, Stopping the Pump.

9.2.3 Changing Parameters While Running

The pump and external event switch parameters displayed on the Isocratic screen can be changed while a run is in progress.

To modify a parameter while a run is in progress:

1. Move the cursor to the appropriate field.
2. Type the new value and press Enter. The change takes effect immediately.

9.2.4 Stopping the Pump

To stop the pump, perform one of the following actions:

- Press the Stop Flow screen key.
- Type a value of 0.0 at the Flow Rate field and press Enter.

To resume flow after you have stopped the pump, type in a flow-rate value at the Flow Rate field and press Enter.

Note: *These actions do not affect the eluent composition.*

Chapter 10
Creating Gradient Controller Time-Based Tables

This chapter describes how to create time-based tables (gradient tables and event tables) for unattended operation of the Waters 600E Multisolvent Delivery System.

Gradient tables control the 600E pump, whereas event tables control external devices connected to the 600 controller. Each time-based table has an identification number. When the system executes a table number, the gradient and event tables with that number run simultaneously.

The 600E system can store up to 15 sets of tables that allow you to:

- Run a series of samples
- Implement flow and eluent compositional gradients
- Actuate external devices

Once you create these tables, you can run them as described in Section Chapter 11, Running Gradients.

10.1 Creating a Gradient Table

This section describes how to define and document the information you will program into the gradient table to control the 600E pump, including:

- Documenting the gradient table
- Programming the gradient table

Note: *When you create gradient and event tables, be sure that you design the time-related changes specified in each table to work effectively with the time-related changes in the other table of the set.*

10.1.1 Documenting the Gradient Table

Define the information you want in a gradient table before you actually program a gradient table. This minimizes the need for you to reprogram the table later.

Using the Analysis Plan and File Sheet

Appendix C, Analysis Plan, contains an Analysis Plan and File Sheet to assist you in defining and documenting gradient-table information.

1. Make a photocopy of the Analysis Plan and File Sheet.
2. On each line of the Analysis Plan and File Sheet, write:
   - Gradient segment time (time is cumulative from injection)
   - Flow rate
   - Eluent composition
   - Gradient profile curve number

   The number of changes needed to execute the gradient determines the total number of lines in the table. The gradient table can contain up to 15 lines (14 plus initial conditions).

   Note: *A gradient table with isocratic pump parameters consists of a single line.*

   See Section 10.1.2, Programming the Gradient Table.
3. When the table entries are complete, keep the Analysis Plan and File Sheet as a permanent record of your gradient table.

Returning to Initial Conditions

When making multiple injections that require flow or eluent compositional gradients, include a line in the gradient table to return the system to initial conditions or to reequilibrate the system before the next injection.

This line in the gradient table should:

- Be executed by the controller after the separation is complete (run time elapsed) and allow enough time for the column to equilibrate
- Specify the same flow rate as the Initial line of the table
- Specify the same eluent composition as the Initial line of the gradient table

Reducing Flow

Include a final line in the gradient table to reduce the flow rate after the last injection to conserve eluent when the sample set completes.

This final line in the gradient table should:

- Appear as the last line in the table.
- Be set to a time at least 5 to 10 minutes longer than the time required for another injection to occur.
- Specify a flow rate of either 0.0 mL/min, or a reduced flow rate, usually 0.1 mL/min.
- Use the eluent composition from the previous line.
- Specify curve #11 (see Analysis Plan and File Sheet) so that the flow rate does not decrease until the last line in the table executes. If an injection occurs before this line executes, the flow rate does not decrease.

Sample Gradient Table

Figure 10-1 is an example of an Auto•Blend gradient table for an analysis with a run time of 30 minutes. It includes a 5-minute segment that returns the eluent composition to initial conditions at the end of the run and a segment that reduces the flow rate to 0.0 mL/min if an injection is not made within 50 minutes of the previous injection.

Time	Flow	%A	%B	%C	%D	Curve
Initial	1.00	10	10	0	80	*
30.00	1.00	10	10	80	0	6
35.00	1.00	10	10	0	80	6
50.00	0.00	10	10	0	80	11

Figure 10-1 Example of an Auto•Blend Gradient Table

Figure 10-2 illustrates the flow rate and the %C, and %D profiles for the gradient table in Figure 10-1.

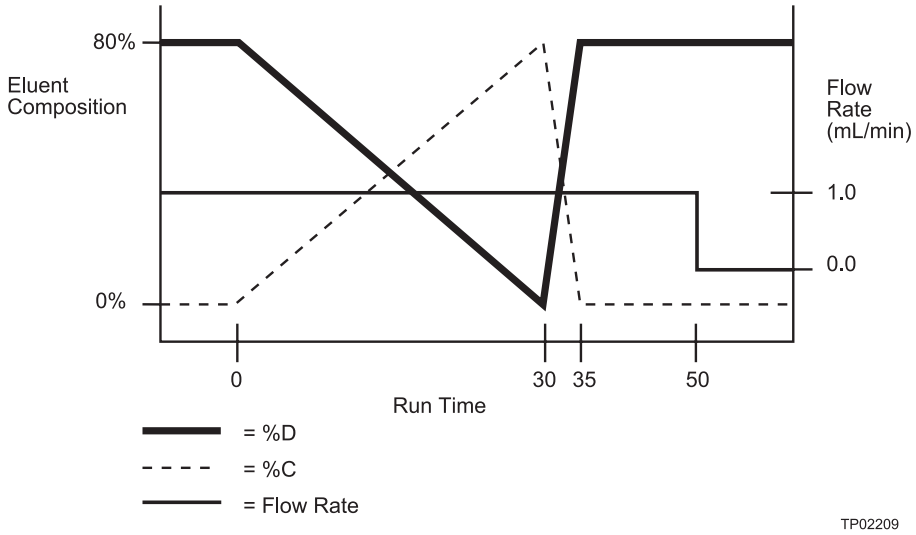


Figure 10-2 Auto•Blend Gradient Profile

10.1.2 Programming the Gradient Table

Figure 10-3 illustrates the steps involved in using the Program Gradient screen to create a gradient table. Use this flowchart for quick reference once you become familiar with the Waters 600E system.

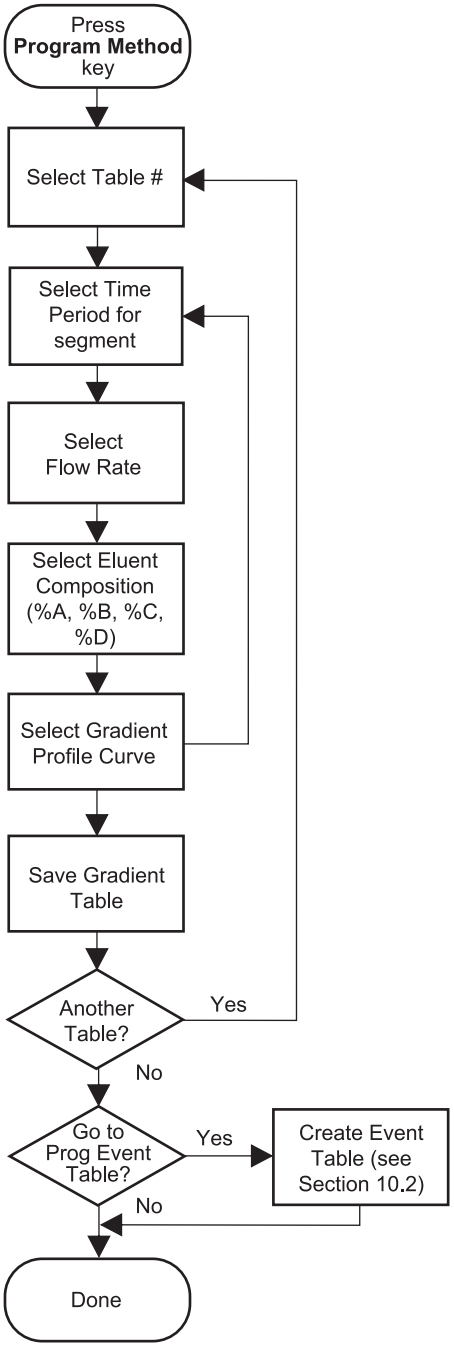


Figure 10-3 Program Gradient Flowchart

Accessing the Program Gradient Screen

To display the Gradient Controller Program Gradient screen (Figure 10-4), press the Program Method function key.

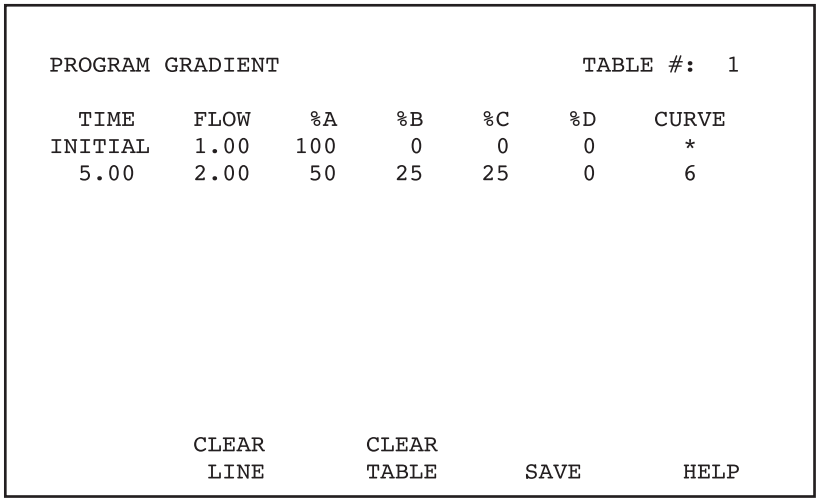


Figure 10-4 Gradient Controller Program Gradient Screen

In addition to the gradient table parameters, the Program Gradient screen displays four active screen keys, as described in Table 10-1.

Table 10-1 Program Gradient Screen Keys

Key	Function
Clear Line	Clears the line on which the cursor resides. The line remains in permanent memory.
Clear Table	Clears the entire table currently displayed on the screen and returns the initial conditions line to default status. The table remains in permanent memory until you save a new table.
Save	Saves the table currently displayed and erases the previous values. You must save the table before advancing to the next screen.
Help	Displays the Help screen associated with the Program Gradient screen.

Exiting the Program Gradient Screen

To exit the Program Gradient screen and enter another mode, press any function key except the Program Method key.

10.1.3 Entering Parameter Values into the Gradient Table

To enter the parameter values into the table displayed on the Program Gradient screen:

1. Move the cursor to the Table # field.
2. Type a table number between 1 and 15. Press Enter.

   If a gradient table is already stored under this number, that gradient table appears on the screen. You can overwrite or edit this table, or enter another table number.

   Note: *The gradient table always runs with the event table of the same number.*
3. If you are entering the initial conditions, continue with step 4 below.

   Otherwise, type the elapsed time after injection (0.00 to 655.34 minutes) at which the gradient segment is to start (or the previous segment is to end). Press Enter.

   Each time entered into the table must be unique.
4. Move the cursor to the Flow field, type a flow-rate value between 0.00 and 20.0 or 45.00 mL/min. Press Enter.
5. Move the cursor to the %A field, type the appropriate percentage of the mobile phase (0 to 100, in 1% increments, no decimals) for eluent reservoir A. Press Enter.
6. Repeat step 5 for the remaining three eluent reservoirs. The sum of the four eluent percentages must equal 100 percent.
7. At the Curve field, type the gradient curve profile number for the gradient segment. Figure 10-5 shows the gradient curve profiles.

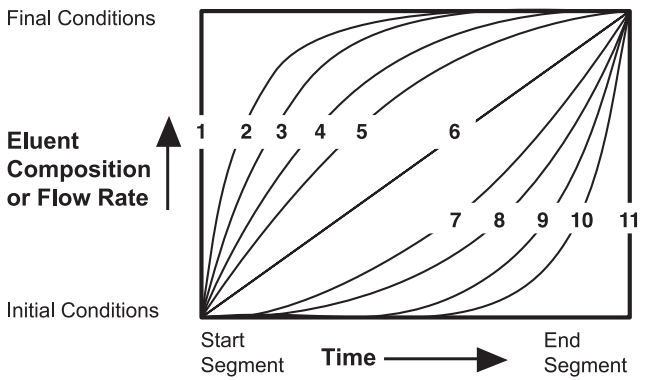


Figure 10-5 Gradient Curve Profiles

8. Repeat steps 3 through 7 for each line in the gradient.

   When entering information for subsequent lines in the table, enter the time value for each line and press Enter. This action copies the values from the previous line to the new line.

9. If desired, enter a line to return to initial conditions.
10. If desired, enter a line to reduce the flow rate.

Press the Save screen key to save the table in permanent memory. When prompted, enter a table number between 1 and 15 and press Enter.

10.1.4 Editing the Gradient Table

This sections describes the following editing functions:

- Deleting lines
- Inserting lines
- Saving tables
- Replacing and deleting tables

These procedures apply to:

- Gradient tables
- Event tables

Deleting Lines

To delete individual lines from a table:

1. Move the cursor to any field on the line you wish to delete.
2. Press the Clear Line screen key. This immediately deletes the line the cursor is on and moves succeeding lines in the table up one line. The cursor remains on the original field.

Inserting Lines

To insert a line into a table:

1. Move the cursor to the Time field in a new line just below the last line in the table.
2. Type the time for the new line. The table automatically sorts the lines according to time and inserts the new line in the proper location.
3. Enter the parameter values for the new line.

If the new line appears at the end of the table, the values from the previous line are copied into the new line when you enter the time for that line. Edit the copied conditions as necessary.

Saving Tables

Whenever you change a table, you must save the changes before powering down the system.

The system does not retain parameters that you did not save. When you turn on the system at a later time, the last table you saved is the default.

To save a table after you have entered all changes:

1. Press the Save screen key. The system displays:

   ```
   Enter Table Number (1-15)
   ```

2. Type the number under which you want to save the table. Press Enter. The screen displays:

   ```
   Replace with New Table?  1 = YES  0 = NO
   ```

3. If you entered a table number under which no table is currently saved, type 1 and the new table is saved under that table number.
4. If you entered the number of a table that has been previously saved, that preexisting table appears on the screen.
   - To overwrite the preexisting table, type 1. This erases the contents of the preexisting table and replaces it with the new table.
   - If you do not want to overwrite the preexisting table, type 0. The screen displays the newly created table again.
5. To save the new table, repeat steps 1 through 3 and assign a different table number to the table. Continue this process until you locate a table that can be replaced with the new table.

Replacing Existing Tables

The controller can store a maximum of 15 tables (15 gradient and 15 event) in permanent memory. Once you save 15 tables, you must replace one of these tables when you want to save a new table.

To replace an existing table:

1. Move the cursor to the Table # field.
2. Type the number of the table you wish to replace. Press Enter.

3. If desired, clear the table by pressing the Clear Table screen key.
4. Enter the new information.
5. When you have entered all information, save the table by pressing the Save screen key. This replaces the preexisting table with the new table in permanent memory.

Deleting Tables

To delete a table without replacing it with a new table:

1. Move the cursor to the Table # field.
2. Type the number of the table to delete and press Enter.
3. Press the Clear Table screen key.
4. Press the Save screen key.
5. At the Enter Table Number (1-15) prompt, type the number of the table to delete. Press Enter. The table you want to delete appears again.
6. At the Replace with New Table? prompt, type 1 to answer "yes." This deletes the table from memory.

10.2 Creating an Event Table

This section describes how to define and document the information you will program into the event table to control external devices. It includes information on:

- Documenting the event table
- Programming the event table

Note: *When creating gradient and event tables, be sure that you design the time-related changes specified in both tables of a set to work together effectively.*

10.2.1 Documenting the Event Table

Define the information you want in an event table before actually programming the event table. This minimizes the amount of reprogramming you will have to do later.

Using the Analysis Plan and File Sheet

To assist you in defining and documenting table information, Appendix C, Analysis Plan, contains an Analysis Plan and File Sheet.

1. Make a photocopy of the Analysis Plan and File Sheet.

2. Before you program the event table (see Section 10.2.2, Programming the Event Table), develop the table by writing each change as an individual line on the Analysis Plan and File Sheet.

   On each line of the Analysis Plan and File Sheet, write:

   - Time of event
   - Event type
   - Event action or setting

   The number of events needed to execute the gradient determines the total number of lines in the table. The event table can contain up to 15 lines (14 plus initial conditions). Multiple events can start at the same time.

   Note: *If Event 8 or 9 is not specified, the last condition established remains in effect when the run time expires.*

3. When the table entries are complete, keep the Analysis Plan and File Sheet as a permanent record of your event table.

Event Types

There are 10 types of events that you can select, as described in Table 10-2.

Table 10-2 Event Types

Event Type	Function
1 through 4	Contact closure switches on the rear panel for timed output to external devices.
5	Internal alarm (reminder to user).
6	Sparge rate for any reservoir enabled on the Pump Setup screen.
7	Column temperature.
8	Go to initial conditions of specified Table #.
9	Start running Table #. The method begins immediately at the time this event executes. No inject signal is necessary.
10	Detector lamp on/off.

Once set, each event remains in its set state until you change it.

Hint for Event 5

To program a beeping alarm, specify Pulse instead of On.

If you turn on the alarm, include another step to turn off the alarm. Otherwise, manually turn off the alarm by pressing the Cancel Alarm screen key on the Operate Gradient screen (see Figure 11-4).

Hints for Events 8 and 9

Note: *Do not program events 8 and 9 in the same table.*

Program events 8 or 9 at the end of the table to initiate the use of new tables.

Event 8 maintains the initial conditions of the new table until the next injection occurs.

Event 9 executes the new table without waiting for an inject signal. Use Event 9 to execute a table that changes conditions and equilibrates the system before the next injection occurs. Event 9 is useful in automated separations development.

Hints for Event 10

Note the following considerations when using Event 10:

- Event 10 is designed to turn the lamp off at the end of an unattended (e.g., overnight) run. You should physically turn the lamp on (not use event 10) prior to the next analysis to assure sufficient lamp warm-up and baseline stability.
- Waters 486 lamp status is checked at the beginning of each run. If the lamp is turned off via Event 10 at the start of a run, the system returns to IDLE and the error message `Unable to Set Up Detector` appears.
- Do not program event 10 to use a Pulse action.

Event Actions

The action selections for events 1 through 5 are:

- **0** = Off
- **1** = On
- **2** = Pulse (generates 600 millisecond pulse)

Event 6 (sparge) requires specification of a sparge rate between 0 and 100 mL/min.

Event 7 requires that you specify a column heater temperature value in °C that is greater than ambient temperature but less than the maximum temperature (99 °C for the standard heater) permitted by the column heater.

Events 8 and 9 require specification of a table number between 1 and 15.

Event 10 requires 1 to turn On the lamp or 0 to turn Off the lamp. See “Hints for Event 10” on page 184 for background information on using event 10

Sample Event Table

Figure 10-6 is a simple example of an event table.

Time	Event	Action
Initial	1	Pulse
3.00	2	On
3.55	2	Off

Figure 10-6 Example of an Event Table

10.2.2 Programming the Event Table

Figure 10-7 illustrates the steps involved in using the Program Event screen to create an Event table. Use this flowchart for quick reference once you become familiar with your Waters 600E system.

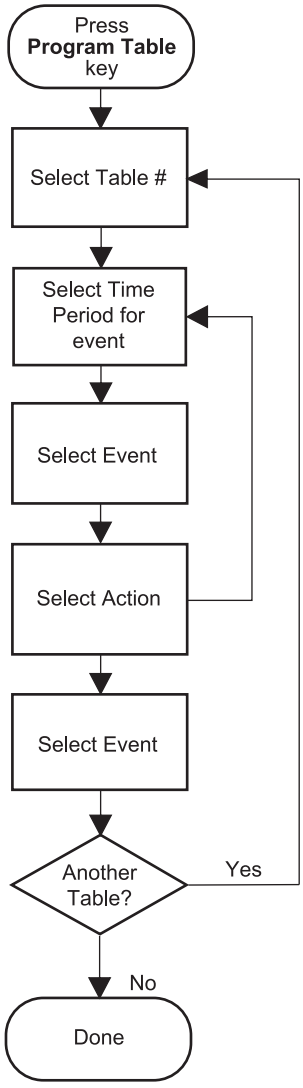


Figure 10-7 Program Event Flowchart

Accessing the Program Event Screen

Press the Program Table function key to display the Program Event screen (Figure 10-8).

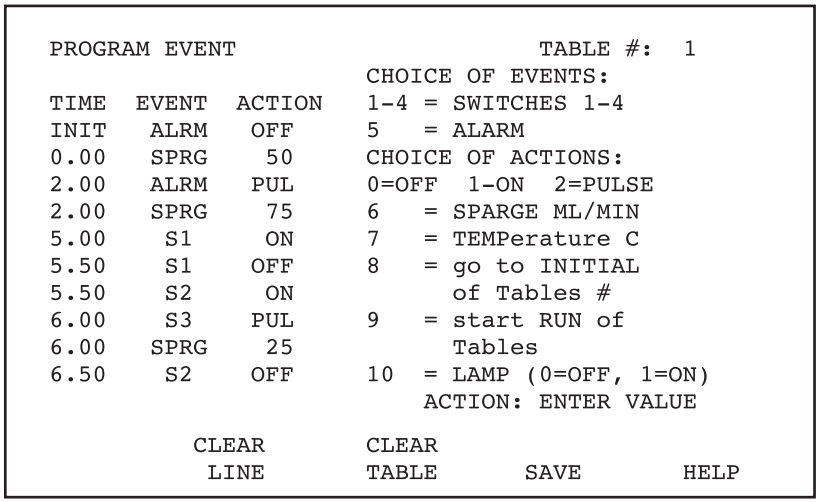


Figure 10-8 Gradient Controller Program Event Screen

In addition to the event table parameters, the Program Event screen displays four active screen keys as described in Table 10-3.

Table 10-3 Program Event Screen Keys

Key	Function
Clear Line	Clears the line on which the cursor resides. The line remains in permanent memory.
Clear Table	Clears the entire table currently displayed on the screen and returns the initial conditions line to default status. The table remains in permanent memory until a new table is saved.
Save	Saves the table currently displayed and erases the previous values. You must save the table before leaving the Program Event screen. Otherwise, any changes are lost.
Help	Displays the Help screen associated with the Program Event screen.

Exiting the Program Event Screen

To exit the Program Event screen, press any function key except the Program Table key to enter another mode.

10.2.3 Entering Parameter Values into the Event Table

To enter the parameter values into the Program Event table (see Figure 10-8):

1. Move the cursor to the Table # field.
2. Type a table number between 1 and 15. Press Enter.

   If an event table is already stored under this number, that table appears on the screen. You can overwrite or edit this table or enter another table number.

   Note: *The event table always runs with the gradient table of the same number.*

3. If you are entering the initial conditions, continue with step 4.

   Otherwise, type the elapsed time after injection (0.00 to 655.34 minutes) at which the event is to occur. Press Enter.

   Note: *If you are on the Initial line, the Time field represents the default starting point for program event operation. You cannot enter a value on this line. When you add a new line to the table, the values from the previous line are inserted into the new line.*

4. At the Event field, type the event type (1 to 10). Press Enter. Event types and functions are listed on the screen.

   Note: *Events 8 and 9 specify the next table to be run in sequence. Since these two events begin new tables, they must be the last line in the table. Event 8 holds your analysis at the initial conditions of the new table until an inject signal is received. Event 9 begins executing the table without an inject signal. Do not program events 8 and 9 in the same table.*

5. At the Action field, type the appropriate value for the action required for the event. Press Enter.
6. Repeat steps 3 through 5 for each line in the event table.
7. Press the Save screen key to save the table in permanent memory.

10.2.4 Editing the Event Table

For details on editing the event table, see the procedures for editing the gradient table in Section 10.1.4, Editing the Gradient Table.

Chapter 11
Running Gradients

This chapter describes how to run gradient and event tables using the Operate Gradient mode of the Gradient Controller. Execution of these time-based tables allows you to:

- Implement flow and compositional gradients
- Activate external events

This chapter assumes that you have:

- Prepared your system (see Chapter 3, Preparing Your 600E System for Operation).
- Set the operating parameters (see Chapter 8, Setting Gradient Controller Operating Parameters).
- Created gradient and event tables (see Chapter 10, Creating Gradient Controller Time-Based Tables).

If you have not performed these tasks, please take the time to do them now.

11.1 Using an Injector

The Waters 600E systems supports two methods for injecting samples in the Operate Gradient mode: manual injection and autoinjection. This section provides instructions for using:

- The Rheodyne 7725i manual injector
- A non-PowerLine autoinjector

11.1.1 Using the Rheodyne 7725i Manual Injector

This section is an overview on using the optional Rheodyne 7725i manual injector. See the *Rheodyne 7725i Manual Injector Installation and Maintenance Guide* for specific operating instructions.

Caution: *Always follow safe laboratory practices when handling eluents. Know the physical and chemical properties of the eluents. See the Material Safety Data Sheets for the eluents in use.*

Caution: *When using sample loops larger than 100-µL, protect yourself from the rapid ejection of mobile phase from the needle port when you turn the valve from Inject to Load. A-1 mL loop expands by 20-µL upon decompression from 2900 psi (200 bar).*

Attention: *Be sure to use the proper size of syringe needle to avoid damage to the valve. Proper needle dimensions are 0.028 inches x 2 inches (0.071 cm x 5.08 cm) OD, without electro taper. The point must be 90° (square).*

To load the sample into the Rheodyne 7725i:

1. Turn the handle on the manual injector to the Load position (Figure 11-1).

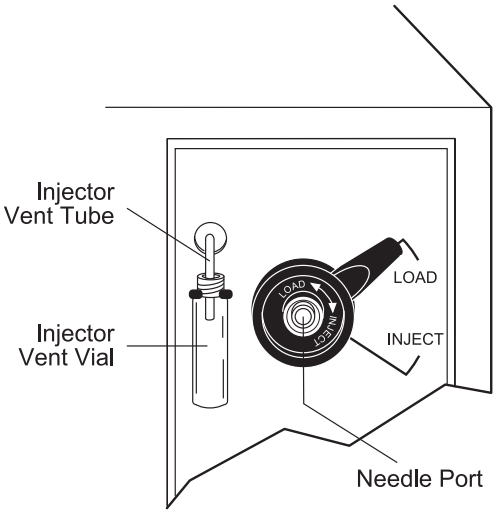


Figure 11-1 Rheodyne 7725i Injector in the Load Position

2. Load a syringe with sample. The loop may be partially filled or overfilled. The injector is equipped with a 20-µl loop.
3. Fully insert the 22-gauge, 90° point syringe needle into the filler port.
4. Gently discharge the contents of the syringe.
5. Turn the Load/Inject handle to the Inject position.

6. Allow sufficient time for the sample to clear the injector before repositioning the Load/Inject handle. Reposition the Load/Inject handle as follows:
   - If you are operating in the gradient mode, or if the next sample is not ready to be loaded, leave the handle in the Inject position.
   - If you are operating in the isocratic mode, or if the next sample is ready to be loaded, turn the Load/Inject handle clockwise to the Load position.

11.1.2 Using a Non-PowerLine Autoinjector

For complete information on your non-PowerLine autoinjector, see the operator's manual included with the injector.

Injecting Sample

1. Ensure that you have connected the injector according to the instructions in the *Waters 600E Multisolvent Delivery System Installation and Maintenance Guide* (see Section 3.5.2, Connecting a Non-IEEE-488 Autosampler).
2. Load the sample vials into the autoinjector.
3. Program the autoinjector as necessary.
4. Start the autoinjector.

11.2 Setting Initial Operating Parameters

This section describes how to set the initial operating parameters for running the gradient and event tables that you program (see Chapter 10, Creating Gradient Controller Time-Based Tables).

Figure 11-2 summarizes the steps involved in running gradients. Use this flowchart for quick reference once you become familiar with your Waters 600E system.

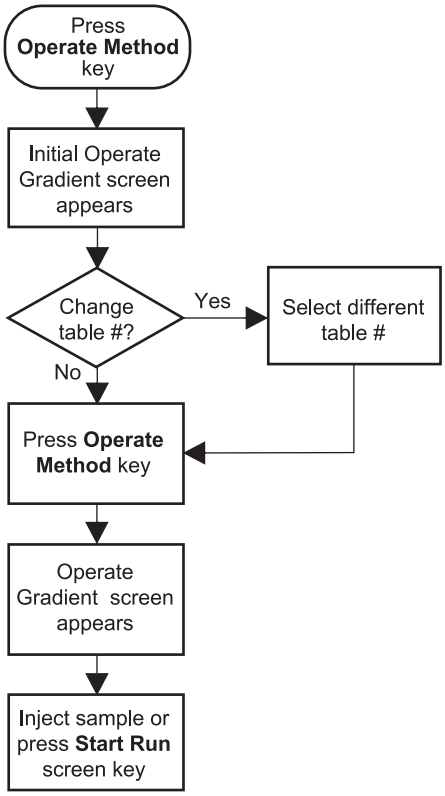


Figure 11-2 Operate Gradient Flowchart

Accessing the Initial Operate Gradient Screen

To access the initial Operate Gradient screen, press the Operate Method function key. The initial Operate Gradient screen appears (Figure 11-3).

```
OPERATE GRADIENT

GRADIENT AND EVENTS TABLE #: 1
           TABLES   FLOW   %A   %B   %C   %D
CURRENT:     8      0.00  100    0    0    0
    NEW:     1      2.00  100    0    0    0

Enter TABLES number to view
 the INITIAL Conditions

Enter OPERATE GRADIENT to enter Gradient Mode
 and go to the new conditions

Press ISOCRATIC to remain in Isocratic Mode

                                              HELP
```

Figure 11-3 Initial Operate Gradient Screen

This screen displays:

- Gradient and events table numbers (sets) to be run from the Operate Gradient screen
- Current pump conditions on the Current line
- Initial conditions of the gradient table listed on the New line

Setting Parameters

To change the gradient and event tables that you set to run:

1. If Autostart is active, the keyboard will be locked. To abort the Autostart, unlock the keyboard on the Pump Setup screen by pressing the Unlock Keyboard screen key.
2. After you unlock the keyboard, make your changes to the initial Operate Gradient screen as follows:
   a. Move the cursor to the Gradient and Events Tables # field.
   b. Type the table number of the desired set of gradient and event tables.
   c. Press Enter.

Canceling the Run

To cancel the run, press any function key, except the Operate Method key, to select another mode.

11.3 Starting a Run

This section assumes that you have already set your initial operating parameters. If you have not, review Section 11.2, Setting Initial Operating Parameters, for requirements. Otherwise, proceed with the following topics:

- Accessing the Operate Gradient screen
- Running a gradient

11.3.1 Accessing the Operate Gradient Screen

1. If the controller screen is not displaying the initial Operate Gradient screen (see Figure 11-3), press the Operate Method function key and the initial Operate Method screen appears. Otherwise, go to step 2.
2. Move the cursor to the Table field on the initial Operate Gradient screen. Type a table number (1 to 15) and press Enter.
3. Press the Operate Method function key. The second Operate Gradient screen appears (Figure 11-4).

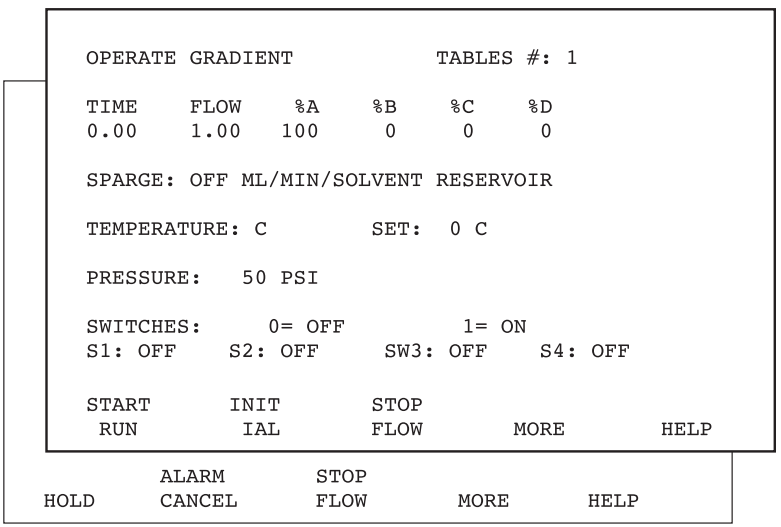


Figure 11-4 Second Operate Gradient Screen

In addition to the current system conditions, the Operate Gradient screen displays the screen keys described in Table 11-1.

Table 11-1 Second Operate Gradient Screen Keys

Key	Function
Start Run	Starts the gradient and the run-time clock at 0.00 minutes.
Initial	Starts the pump at the initial conditions of the table. Press the key to ensure that the system is at the initial conditions of the gradient table you want to run before starting the run or making an injection.
Stop Flow	Sets the pump flow to zero and suspends the run time.
More	Displays an additional set of screen keys associated with the Operate Gradient screen.
Hold	Holds conditions at the current state and suspends the run time.
Alarm Cancel	Shuts off the reminder alarm.
Resume Run	Allows the run-time clock to continue after you press the Hold or the Stop Flow key.
Help	Displays the Help screen for the Operate Gradient screen.

11.3.2 Running a Gradient

Setting Initial Conditions

To start the pump at the initial conditions of the gradient table you want to run, press the Initial screen key from the second Operate Gradient screen (see Figure 11-4).

Using the Rheodyne 7725i Manual Injector

To begin a run with the Rheodyne 7725i manual injector:

1. Load the injector loop with sample. See Section 11.1.1, Using the Rheodyne 7725i Manual Injector.
2. Turn the injector handle to the Inject position to make the injection. The injector sends an Inject signal to the controller.

Using a Non-PowerLine Autoinjector

Note: *If you are using an autoinjector, see the documentation provided with that product for information on sample loading.*

To begin a run with an external non-PowerLine autoinjector:

1. Load the sample vials into the autoinjector.
2. Program and start the autoinjector. See the autoinjector manual.
3. When the autoinjector makes the injection, it sends a contact closure signal to the Inject input terminal on the rear panel of the controller.

Using the Start Run Screen Key with a Non-PowerLine Autoinjector

For methods development and for runs that are not time-sensitive, you may also use the Start Run screen key to start a run with a non-PowerLine autoinjector.

To start a run with an autoinjector using the Start Run key:

1. Load the sample vials into the autoinjector.
2. Program and start the autoinjector. See the autoinjector manual.
3. Press the Start Run key as soon as possible after the injection occurs.

Note: *An Autostart gradient can override a gradient that you started with the Start Run screen key.*

Starting the Run-Time Clock

When the controller receives an Inject signal from an injector, the run-time clock begins counting at 0.00. The system does not accept another Inject signal until the run completes or you abort the run.

Completing the Gradient

The Operate Gradient screen displays the current conditions during the run. The gradient and event tables continue to run until you manually stop them by pressing the Initial screen key, making another injection, or aborting the run.

The external events remain in their set conditions when each analysis completes. They do not automatically reset to Off. Events that need to be turned on at a specific time in an analysis must be programmed to turn off before the start of the next injection.

The run-time clock continues to run after each analysis completes and resets to 0.00 only with a new injection. Events or gradient segments programmed to execute after the run time for each injection still occur.

11.4 Modifying Conditions While Running

This section describes how to modify conditions during a run, including:

- Changing parameters
- Holding conditions constant
- Stopping flow
- Modifying gradient and event tables
- Switching to another table
- Aborting the run

Note: *If the keyboard is locked, unlock it by pressing the Unlock Keyboard screen key on the Pump Setup screen (see Section 8.2.3, Exiting the Pump Setup Screen).*

11.4.1 Changing Parameters

You can change the following parameters during a run:

- Sparge rate
- Event switch status (On/Off)

To change a parameter on the Operate Gradient screen:

1. Move the cursor to the appropriate parameter field on the screen.
2. Type the new value. Press Enter.

When you manually enter a change, the new value remains in effect until the next timed program change. You can change the parameter only if the keyboard is unlocked.

11.4.2 Holding Conditions Constant

The Hold function keeps the eluent flow rate and composition constant during a run, which is a useful feature in methods development. The system suspends the run time and external events while this function is in effect.

To suspend system operation at the current conditions:

1. Press the Hold screen key. The system displays the Hold key in reverse video and changes the Start Run screen key to Resume Run.

When you press Hold:

- Gradient clock stops
- Run-time clock stops

2. To continue system operation from the point at which you suspended it, press the Resume Run screen key. The gradient and run-time clocks continue from where they stopped.

11.4.3 Stopping Flow

You generally use the Stop Flow screen key at the end of an analysis. However, you can also use it to temporarily shut down your pump (for example, to adjust a fraction collector) or to function as an emergency shutdown.

The Stop Flow function also suspends the method table, but does not stop the run-time clock. The current event and detector tables continue to execute. However, pressing Stop Flow causes switch S4 to default to the Off position. This action provides an additional level of control for non-IEEE devices.

Note: *The receipt of the Stop Flow signal (at the 600 controller's Stop Flow rear panel terminal) suspends the controller's run, gradient, and event clocks. The three clocks resume operation when the Stop Flow signal terminates.*

To stop flow:

1. Press the Stop Flow screen key. The system displays the Stop Flow key in reverse video and changes the Start Run screen key to Resume Run.

   When you press Stop Flow:

   - Flow rate is set to zero
   - Gradient clock stops
   - Run-time clock continues

2. To continue running the gradient, press the Resume Run screen key. The pump ramps up to the rate at which it was running just before you pressed Stop Flow, and the gradient continues. Note that this ramping affects any current analysis.

11.4.4 Modifying Gradient and Event Tables

While an analysis is in progress, you can modify any existing table *except* the one currently in process.

To modify a table during a run:

1. Press the Program Method function key to display the gradient table or the Program Table function key to display the event table.
2. Move the cursor to the Table # field and type the number of the table to modify. Press Enter.

   Note: *You cannot modify the table that is currently running.*
3. Modify the table.
4. Press the Save screen key to save the new table.

Note: *You cannot assign the number of the table that is currently running to the modified table. To modify a table that is currently running, stop the run. See Section 11.4.6, Aborting the Run.*

11.4.5 Switching to Another Table

To switch to another gradient-event table set while a run is in progress:

1. Move the cursor to the Tables # field on the Operate Gradient screen.
2. Type the new table number and press Enter. The initial Operate Gradient screen (see Figure 11-3) appears with the current gradient-/event-table flow conditions shown on the Current line.

   The initial conditions of the newly selected table are displayed on the New line, but are not in effect.
3. Review the flow and eluent conditions on the New line to determine if there is any eluent or flow rate incompatibility with the current conditions.
4. If you selected the incorrect table, move the cursor to the Table # field in the New line and enter another table number. Repeat step 3.

   Otherwise, press the Operate Method function key to continue. The second Operate Gradient screen appears (see Figure 11-4).
5. Press the Initial screen key to go to the initial conditions of the new table.

11.4.6 Aborting the Run

Using the Direct Function Key

1. Press the Direct function key. The Caution screen appears (Figure 11-5). The Caution screen prompts you to immediately abort the gradient or cancel the abort.

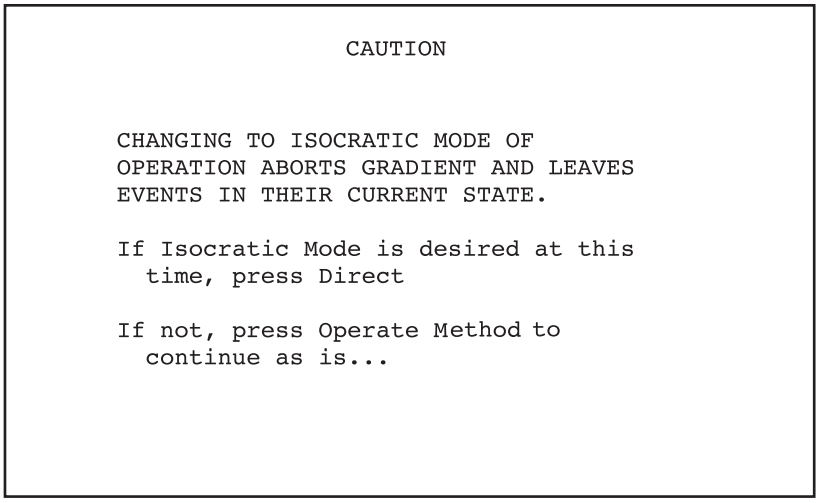


Figure 11-5 Caution Screen

2. Take one of the following actions:
   - To abort the run immediately, press the Direct function key. The system goes into the direct control mode and displays the Isocratic screen. If you want, you can change the switch states on the Isocratic screen.
   - To cancel the abort, press the Operate Method function key to return to the Operate Gradient screen and to resume monitoring the gradient.

Note: *When you abort the run, gradient- and event-table execution stops. Any enabled external switches remain in the current (or last-set) state.*

Appendix A
Specifications

This appendix contains 600E system specifications for the following areas:

- Operational
- Electrical
- Environmental
- Physical
- Optional Rheodyne 7725i manual injector

Table A-1 Operational Specifications

Item	Specification
Number of eluents	One to four.
Modes of operation	Gradient, isocratic, and flow programming
Operating flow range	0.00 to 45 mL/min (225 µL heads), in 0.01-mL increments. 0.00 to 20 mL/min (100 µL heads), in 0.01-mL increments.
Composition range	0 to 100% programmable in 1% increments for each of four reservoirs: A, B, C and D. Total composition must sum to 100%.
Compositional accuracy	Better than 1.0%, independent of pressure.
Automatic eluent sparging	Standard, helium gas, input gas pressure range 50 to 150 psi (3.5 to 10.5 kg/cm^2). Helium flow rate range 0 to 100 mL/min, programmable in 1 mL/min increments.
Pressure maxima	6000 psi (420 kg/cm^2) at 10 mL/min. 1000 psi (70 kg/cm^2) at 45 mL/min.
Programmable pressure limits	Lower: 0 to 5950 psi (0 to 416.5 kg/cm^2) Upper: 51 to 6000 psi (3.6 to 420 kg/cm^2)

Table A-1 Operational Specifications (Continued)

Item	Specification
Program storage	Storage for 15 sets of tables (gradient, program event, and detector), with a maximum of 15 steps per table. Storage for a time-based multi-method program with up to 48 individual steps. EEPROM stores setup parameters and tables, and preserves memory indefinitely following power loss or extended shutdown.
Gradient profiles	Eleven gradient curves (including linear, Step [2], concave [4], and convex [4]). Up to 80 curve segments can be linked.
Liquid crystal display (LCD)	5-inch (12.7-cm) diagonal screen. Sixteen lines with 40 characters per line. 320 x 200 pixels. Displays current operating conditions. Allows operator to monitor complete gradient and timed-event tables on single screens. Reverse video cursor prompts operator through program setup.
Keypad, function keys, and screen keys	Dedicated keys provide direct access to all display screens. Cursor movement keys simplify programming, editing, and parameter entering. All keys are eluent-resistant.
Programmable timed events	You can program and store 15 timed events for each table. Switches S1 through S4 are located on the controller's rear panel for timed output to external devices. The following processes may also be controlled by timed-events: • Audible alarm • Sparge rate • Column temperature • Proceed to initial conditions of specifed table • Start run of specified table • Turn detector lamp off

Table A-2 Electrical Specifications

Item	Specification
Power Requirements	Maximum power: 250 VA
Frequency	47 to 63 Hz
Fuse	**Nominal Voltage** / **Voltage Range** / **Fuse (North America)** / **Fuse (Europe)** 100 / 10% / 2 A, 250 V UL/CSA, Time Delay / 1.6, 250 V IEC, Time Delay 120 / 10% / 2 A, 250 V UL/CSA, Time Delay / 1.6, 250 V IEC, Time Delay 230 / 10% / 1 A, 250 V UL/CSA, Time Delay / 0.8, 250 V IEC, Time Delay 240 / 10% / 1 A, 250 V UL/CSA, Time Delay Time Delay / 0.8, 250 V IEC, Time Delay Delay
Time- or Operator-Controllable Switch Closures	Four controllable switch closures to ground as follows: • TTL-compatible terminals pulled up with 10-K ohm resistors to 5 V. May be pulled up to 12 V. • Maximum allowable current = 1.0 A • Maximum allowable voltage = 12 V. Outputs may be controlled directly from keyboard or from events program. • Two modes available from keyboard and Program Events screen: ON = switch closed to ground OFF = switch open, +5 V to ground • Additional mode available from Program Events screen: Pulse = single contact closure to ground for 600 msec
Auxiliary Power Supply	12 V to ground fused supply. 1 A max current.

Table A-2 Electrical Specifications (Continued)

Item	Specification
Hold Output	One TTL-compatible switch closure to ground. Maximum current sink: 100 mA. Low output indicates pumps stopped due to: • Exceeding high-pressure limit • Below low-pressure limit • Abort input • "Powerup" condition Used to communicate with Hold input on Waters 717plus autosamplers or other compatible input to prevent further injections.
Chart Output	Two terminals (+,–) for recording pump flow-rate traces or monitoring eluent composition changes. 10 mV analog output.
Pressure Output	Two terminals (+,–) for recording pump pressure traces. 10 mV analog output; bypasses microprocessor.
Inject	Receives a signal from an external injector (autosampler or manual injector) to initiate the chromatographic run. The Inject terminal also provides a signal to start a Waters 745B/746 Data Module (integrator). The Inject terminal accepts signals from several outputs, including TTL signals, open collector outputs, and contact closures.
Ground Terminals	Connected to signal ground and used as reference for one of the following: • Outputs (Hold, Inject and switches S1 to S4) • Inputs (Inject and Abort) or • 12-V power supply (not for use with 10-mV outputs)

Table A-3 Environmental Specifications

Item	Specification
Temperature	Operating Range: 4 to 38 °C (39.3 to 100.4 °F) Storage Range: 0 to 50 °C (32 to 122 °F)
Humidity	Operating Range: 20 to 90% Relative Humidity Storage Range: 0 to 90% Relative Humidity (non-condensing)
Eluent Compatibility	Compatible with eluents consistent with materials of construction

Table A-4 Physical Specifications

Item	Specification
Wetted Surface Material	316 stainless steel, ruby, sapphire, fluorocarbon polymer
Controller	Height: 7.19 inches (18.26 cm) Depth: 21.75 inches (55.25 cm) Width: 11.32 inches (28.75 cm) Weight: 33 lb (15 kg)
Pump	Height: 9.625 inches (24.4 cm) Depth: 20.5 inches (52.1 cm) Width: 11.125 inches (28.3 cm) Weight: 60.2 lb (27.3 kg)

Table A-5 Optional Rheodyne 7725i Manual Injector Specifications

Item	Specification
Syringe Needle Size	Length: 2.0 inches (5.08 cm) O.D: 0.028 inches (0.071 cm) (22 gauge)
Pressure Limit	6000 psi (420 kg/cm^2)
Wetted Surface Material	316 stainless steeel, TFE
Sample Load Size	Sample injections from 1 µL to 20 µL with no loop change. Injector mounts directly to pump.

Appendix B
Eluent Considerations

Caution: *Always follow good laboratory practices when handling eluents. Know the chemical and physical properties of the eluents. See the Material Safety Data Sheets for the eluents in use.*

This appendix describes eluents for use with your Waters 600E Multisolvent Delivery System. It also provides information on eluent properties, including:

- Eluent compatibility
- Clean eluent
- Eluent miscibility
- Head height
- Eluent viscosity
- Eluent degassing

B.1 Eluent Compatibility with the 600E System

The Waters 600E System is constructed of high-quality, 316 stainless steel components that, with some minor restrictions, can be used with all eluents.

This section lists the eluents that have, and have not, been approved for use with the 600E System.

Eluents to Avoid

You can use any eluent with the 600E system. However, long-term *static* exposure to halide salts (for example, fluoride, bromide, chloride, and iodide) will cause pitting and corrosion of stainless steel parts. When using these salts, flush your system thoroughly with water if the pump will be idle for more than two days. See Section 3.4, Flushing the System.

Eluents to Use

Materials of construction used in the 600E system are nonreactive with most acids, bases, salts, and organic eluents.

The following list of eluents has been approved for use with the 600E system. These include salts, acids and bases in concentrations up to 1 M (unless otherwise noted), and organic eluents in concentrations of up to 100% (unless otherwise noted). Higher concentrations could be used in many instances.

Information on the use of a specific mobile phase or concentration that is not listed in this manual may be obtained by contacting your local Waters sales/service representative.

Aqueous Buffers

The following is a list of aqueous-buffer components that may be used with the 600E system:

- Acetate
- Al_2SO_4
- $Ca(OCl)_2$
- $CaCl_2$
- Citrate
- H_2O_2 up to 10%
- HIBA
- K_2CO_3
- $K_2Cr_2O_3$
- K_2S
- K_2SO_4
- $K_3Fe(CN)_6$
- $K_4Fe(CN)_6$
- KBr
- KCl
- $KHCO_3$
- $KMnO_4$
- KNO_3

- $LiCl0_4$
- $Na_2B_4O_7$
- Na_2CO_3
- Na_2S
- Na_2SO_4
- NaCl
- Sodium acetate
- NaH_2BO_3
- $NaHCO_3$
- $NaHSO_4$
- $NaNO_3$
- NaOCl
- NH_4Cl
- Perfluorobutyric acid
- Phosphate
- Tartrate
- Trilithium citrate
- Tris
- 4-(2-pyridylazo)resorcinol monosodium salt monohydrate

Acids

The following is a list of acids that may be used with the 600E system:

- Acetic acid, glacial
- Benzoic acid
- Chromic acid
- Citric acid
- Formic acid
- Glyceric acid
- Phosphoric acid
- Sulfuric acid, up to 0.20 M
- Hydrochloric acid

- Perchloric acid
- Nitric acid, up to 30% (6 N)
- Lactic acid
- Methanesulphonic acid
- Octanesulphonic acid
- Oxalic acid
- Pyridine-2,6-dicarboxylic acid
- Trifluoroacetic acid (TFA), up to 10%

Bases

The following is a list of bases that may be used with the 600E system:

- $Ba(OH)_2$
- KOH
- LiOH
- NaOH, up to 10 M
- NH_4OH, up to 3 M
- Tetramethylammonium hydroxide pentahydrate

Organic Eluents

The following is a list of organic eluents that may be used with the 600E system:

- 4-cyanophenol
- Acetone
- Acetonitrile
- Amyl acetate
- Benzaldehyde
- Benzene
- Benzyl alcohol
- Butanol
- Carbon tetrachloride
- Chloroform
- Cyclohexane

- Cyclohexanone
- Dibutyl phthalate
- Dimethyl formamide
- Dimethyl sulfoxide
- Ethanol
- Ethyl acetate
- Ethylene dichloride
- Ethylene glycol
- Formaldehyde
- Heptane
- Hexane
- iso-Octane
- iso-Propanol
- Lysine hydrochloride
- Methanol
- Methyl ethyl ketone
- Methylene chloride
- n-Propanol
- Phenol
- Tetrahydrofuran (THF)
- Toluene
- Waters PIC™ Reagents
- Xylene

B.2 Clean Eluent

Using Clean Eluents

Use clean eluents to obtain reproducible results and for operation with minimal maintenance. An unclean eluent can cause baseline noise and drift. Purchase only the highest quality HPLC-grade eluents. Table B-1 lists the physical properties of some commonly used eluents.

When using a buffer, obtain a high-quality reagent and filter it through a 0.45 micron filter such as the type in the Eluent Clarification Kit available from Waters.

Using Clean Water

When operating a system with aqueous eluents, use high-purity, filtered water such as that provided by reverse-osmosis, ion-exchange water systems.

Using Clean Filters

The 600E system contains an eluent filter. To avoid drawing foreign particles into the pump where they can accumulate and affect pump operation, the eluent reservoir filter should be kept clean.

Cleaning Filters

If a stainless steel filter becomes plugged, clean it by sonicating in 6 N nitric acid (HNO_3) for 5 to 10 minutes.

B.3 Eluent Miscibility

Changing Between Eluents

Before changing from one eluent to another, see Table B-1 to determine the miscibility of the eluents to be used. The following considerations apply when changing eluents:

- Make a change directly from one eluent to another if the two eluents are miscible.
- When changing between two eluents that are not totally miscible (for example, from hexane to water), flush the system with an intermediate eluent (such as methanol). See Section 3.6.2, Changing Between Incompatible Eluents.

Considering Temperature

You must consider the effect of temperature on eluent solubility if operating at an elevated or reduced temperature. Temperature does affect eluent miscibility.

Using Buffers

Aqueous buffers may precipitate when mixed with organic eluents. Therefore, take the following precautions:

- When switching from a high-ionic-strength buffer to an organic eluent, flush the buffer out of the system with distilled water before adding the organic eluent.

- When using a high-ionic-strength buffer, flush all fluid pathways with distilled water before shutting down the system. Leave distilled water in the system (flush with 10% methanol in water for shutdowns scheduled to be more than one day). See Section 2.5, Powering Down the System.

B.3.1 Eluent Properties Table

Table B-1 lists the physical properties of a series of eluents. Use this table in conjunction with Section B.3.2, Using Miscibility Numbers (M-Numbers).

Table B-1 Physical Properties of Eluents

Polarity Index	Eluent	Viscosity [η] CP, 20 °C	Boiling Point °C (1 atm)	Miscibility Number (M)
0.0	Hexane	0.313	68.7	29
0.0	Cyclohexane	0.98	80.7	28
0.3	n-Decane	0.92	174.1	29
0.4	Octane	0.50	99.2	29
1.7	Butyl ether	0.70	142.2	26
1.8	Triethylamine	0.38	89.5	26
2.2	i-Propyl ether	0.33	68.3	26
2.3	Toluene	0.59	101.6	23
2.4	p-Xylene	0.70	138.0	24
3.0	Benzene	0.65	80.1	21
3.3	Benzyl ether	5.33	288.3	26
3.4	Methylene chloride	0.44	39.8	20
3.4	Chloroform	0.57	61.2	19
3.7	Ethylene chloride	0.79	83.5	20
3.9	i-Butyl alcohol	3.00	117.7	15
4.2	Tetrahydrofuran	0.55	66.0	17
4.3	Ethyl acetate	0.47	77.1	19
4.3	1-Propanol	2.30	97.2	15
4.3	2-Propanol	2.35	117.7	15
4.4	Methyl acetate	0.45	56.3	15, 17
4.5	Methyl ethyl ketone	0.43	80.0	17

Table B-1 Physical Properties of Eluents (Continued)

Polarity Index	Eluent	Viscosity [η] CP, 20 °C	Boiling Point °C (1 atm)	Miscibility Number (M)
4.5	Cyclohexanone	2.24	155.7	17
4.5	Nitrobenzene	2.03	210.8	14, 20
4.6	Benzonitrile	1.22	191.1	15, 19
4.8	p-Dioxane	1.54	101.3	17
5.2	Ethanol	1.20	78.3	14
5.3	Pyridine	0.94	115.3	16
5.3	Nitroethane	0.68	114.0	13, 20
5.4	Acetone	0.32	56.3	15, 17
5.5	Benzyl alcohol	5.80	205.5	13
5.7	Methoxyethanol	1.72	124.6	13
6.2	Acetonitrile	0.37	81.6	11, 17
6.2	Acetic acid	1.26	117.9	14
6.4	Dimethylformamide	0.90	153.0	12
6.5	Dimethyl sulfoxide	2.24	189.0	9
6.6	Methanol	0.60	64.7	12
7.3	Formamide	3.76	210.5	3
9.0	Water	1.00	100.0	--

Adapted from Godfrey, Norman B., *Solvent Selection via Miscibility Number*, CHEMTECH, 359-363 (1972).

B.3.2 Using Miscibility Numbers (M-Numbers)

Miscibility numbers (M-numbers) are used to predict the miscibility of a liquid with one of the standard eluents (see Table B-1).

A liquid is classified in the M-number system by testing for miscibility with a sequence of standard eluents. A correction term of 15 units is then either added or subtracted from the cutoff point for miscibility.

To predict the miscibility of two liquids, subtract the smaller M-number value from the larger.

- If the difference between the two M-numbers equals 15 or less, the two liquids are miscible in all proportions at 15 °C.

- A difference of 16 units indicates that the two liquids possess a critical solution temperature between 25 and 75 °C, with 50 °C as the optimal temperature.
- If the difference equals 17 or more, the two liquids are immiscible, or their critical solution temperature is above 75 °C.

Interaction between the molecules of the two liquids can sometimes change the expected degree of miscibility. For example, ethers or tertiary amines show unpredicted miscibility with hydroxylic eluents due to hydrogen bonding. Unusually strong hydrogen bonding is also responsible for the miscibility of long-chain alcohols or carboxylic acids with standard eluents of low M-number. Conversely, long-chain alcohols and carboxylic acids show anomalous immiscibility with aprotic eluents of low M-number.

Some eluents prove immiscible with eluents at both ends of the lipophilicity scale. These eluents receive a dual M-number as follows:

- The first number, always lower than 16, indicates the degree of miscibility with highly lipophilic eluents.
- The second number indicates the degree of miscibility with highly lipophobic eluents.

A large difference between the two M-numbers of an eluent indicates a limited range of miscibility. For example, some fluorocarbons are immiscible with all the standard eluents and have M-numbers 0 and 32. Two liquids with dual M-numbers are usually miscible with each other.

B.4 Head Height

The 600E system allows you flexibility in the placement of the eluent reservoirs. Ideally, the eluent elevation should be close to that of the pump heads or higher. Avoid placing eluents on top of the system unless the bottles are in a container that can hold the total volume of all of the eluents in case bottle leakage occurs.

B.5 Eluent Viscosity

Generally, when you operate with a single eluent or under low pressure, viscosity is not important. However, when you run a gradient, the viscosity changes that occur as the eluents are mixed in different proportions can result in pressure changes during the run.

For example, a 1:1 mixture of water and methanol produces twice the pressure of either water or methanol alone. If the extent to which the pressure changes affect the analysis is not known, monitor the pressure during the run using the controller Chart output terminal provided for this purpose (select %A or %B). See Section 4.3.2, Setting Pump Setup Parameters.

B.6 Eluent Degassing

Mobile phase difficulties account for most liquid chromatographic problems. Degassing eluents that are used in the mobile phase is one of the most effective measures to eliminate these problems. The benefits are:

- Stability in the baseline and enhanced sensitivity in some types of chromatographic detectors
- Reproducible retention times for eluting peaks
- Reproducible inject volumes for quantitation

B.6.1 Conditions Affecting Gas Dissolution

Overview

Only a finite amount of gas dissolves in a given volume of liquid under specific conditions. This amount depends on:

- Chemical affinity
- Temperature
- Pressure

Chemical Affinity

Generally, a gas is most soluble in an eluent where the attractive forces between molecules of the eluent are similar to those between molecules of the gas (“like dissolves like”). Also, when the main intermolecular attractive forces in an eluent are Van der Waals forces, a gas is more soluble than when dipole forces or hydrogen bonding predominate. Thus, larger amounts of helium, nitrogen, oxygen, and hydrogen dissolve in alkanes and benzene than in water.

Temperature

Temperature affects the solubility of gases in two ways:

- The higher the boiling point of a gas, the more soluble the gas is in a given eluent.
- Increasing the temperature of the gas-liquid solution affects the percentage of gas in solution.

If the heat of solution results in:

- An exothermic reaction, the percentage of gas in solution decreases.
- An endothermic reaction, the percentage of gas in solution increases.

For example, the solubility of helium in water decreases with an increase in temperature, but its solubility in benzene increases in direct proportion to the temperature.

Pressure

The mass of gas dissolved in a given volume of eluent is proportional to the partial pressure of the gas in the vapor phase of the eluent. If the pressure of the gas decreases, the amount of that gas in solution also decreases.

Dissolved Oxygen

Dissolved oxygen affects UV/Vis detector performance in several ways.[1] Oxygen dissolved in eluents may form a UV-absorbing complex, the concentration of which is considerably different in different eluents. The absorbance effect is particularly strong with wavelengths below 260 nm. Therefore, any change in dissolved oxygen content may affect a UV baseline considerably. This consequence is particularly evident in the eluent tetrahydrofuran (THF).

Dissolved oxygen does not seem to affect the absolute sensitivity of a UV system, but primarily causes baseline drift. This effect is especially noticeable during gradient operation in which the dissolved oxygen content varies among the different eluents. As the eluent composition changes, the dissolved oxygen content causes erratic baselines or even peak-shaped artifacts in the baseline.

Dissolved oxygen in a fluorescence detector has quite a different effect. It results in a great loss of sensitivity. Bowen and Williams have discussed the quenching of aromatic hydrocarbons by dissolved oxygen in fluorescence detectors.[2] Parker and Barnes have

1. S.R. Bakalyar, M.B.T. Bradley, R. Hoganen, *Journal of Chromatography*, 158 (1978) 277.
2. E.J. Bowen and A.H. Williams, *Trans. Faraday Soc.*, 35 (1939) 65.

reported a 95-percent reduction in sensitivity of the fluorescence of borate-benzoin complex in air-equilibrated ethanol.[1] The amount of quenching by oxygen varies with different types of compounds. Aromatic hydrocarbons, aliphatic aldehydes, and ketones are especially susceptible.

B.6.2 Eluent Degassing Methods

You can degas eluents by sparging, heating, sonication, and vacuum. Sparging, however, is the method of choice for online degassing. Sparging combines the convenience of short initial degassing time, ease of maintaining the eluent condition during operation, and complete control within the framework of the 600E system.

Sparging

Sparging, or bubbling a gas through eluent, removes unwanted gas from solution while saturating the eluent with the sparging gas.

Sparging brings the eluent to a state of equilibrium that can be maintained by keeping either a blanket of gas over the eluent or a constant, low-rate dispersion of the sparge gas through the eluent.

Sparging with either nitrogen or helium is effective in reducing background absorbance in a UV detector and the quenching phenomenon caused by dissolved oxygen in a fluorescence detector.

Sparging with Helium

Helium sparging tends to give a more stable baseline on a refractive index detector than air-equilibrated eluents. Also, the absolute mass of dissolved gas is considerably lower than with air-equilibrated eluents. Somewhat better pumping reproducibility is obtained from helium-sparged eluents in a piston pump.

Helium sparging gives stable baselines and improved sensitivity in a fluorescence detector, and prevents the reabsorption of atmospheric gases. Also, eluents sparged with helium will generally be well-degassed by the time they reach the pump because the helium diffuses out of the eluent into the atmosphere through the Teflon tubing that connects the reservoirs to the pump. The eluent arrives at the pump with less than 1 atmosphere partial pressure of helium.

1. C.A. Parker and W.J. Barnes, *Trans. Faraday Soc*., 82 (1957) 606.

Heat

Heating to remove dissolved gas is usually not effective unless you boil the eluent, and this is not practical for mixed eluents. However, low levels of heat will raise the partial pressure of the eluent and thereby reduce the rate of resolubilization of a gas. This may affect the separation on the column.

Sonication

Sonication plus vacuum will degas eluent very quickly. This technique will not change the composition of mixed eluents appreciably.



Caution: *It is dangerous to apply vacuum to the brown gallon bottles in which eluent is shipped. There is a high risk of implosion under these conditions.*

Conclusions

With any of the above techniques, except sparging, the eluent will reequilibrate to air saturation in 12 to 24 hours (depending on the eluent).

Degassing by vacuum or sonication is often performed for improved pump performance in high-pressure applications. However, degassing may not yield the required baseline stability for high-sensitivity absorbance detection. The most practical degassing method for most applications is sparging. Due to the low solubility of the sparging gasses, sparging will not impair pump performance in most eluents.

Appendix C
Analysis Plan

Use the Analysis Plan and File Sheet that follows to help you define and document:

- The gradient, event, and detector tables that compose a method table in PowerLine Control mode (see Chapter 6, Creating PowerLine Methods)
- The gradient and event tables in Gradient Control mode (see Chapter 10, Creating Gradient Controller Time-Based Tables)

Analysis Plan and File Sheet

Analysis Name:_____ Memory Location (Table Number):____

Gradient Table

Time	Flow	%A	%B	%C	%D	Curve

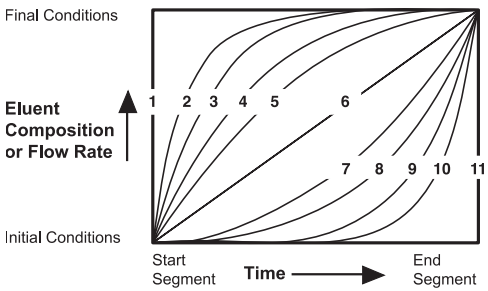
Event Table

Time	Event	Action

Detector Table

410 Sensitivity	
431/432 Sensitivity	

Time	Det Chan	Sens	AUFS



Choice of Gradient Curves:

1	Go to end condition immediately
2-5	Convex
6	Linear
7-10	Concave
11	Maintain start conditions to end of segment (step grad.)

Choice of Events:

1-4 = Switches 1-4
5 = Alarm
6 = Sparge mL/min
7 = Temperature
8 = Go to Initial of Tables #
9 = Start Run of Tables #
10 = Lamp (0=Off, 1=On)

Events 1-5 Actions:
0 = Off 1 = On 2 = Pulse

Events 6-10 Actions: Select a value

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