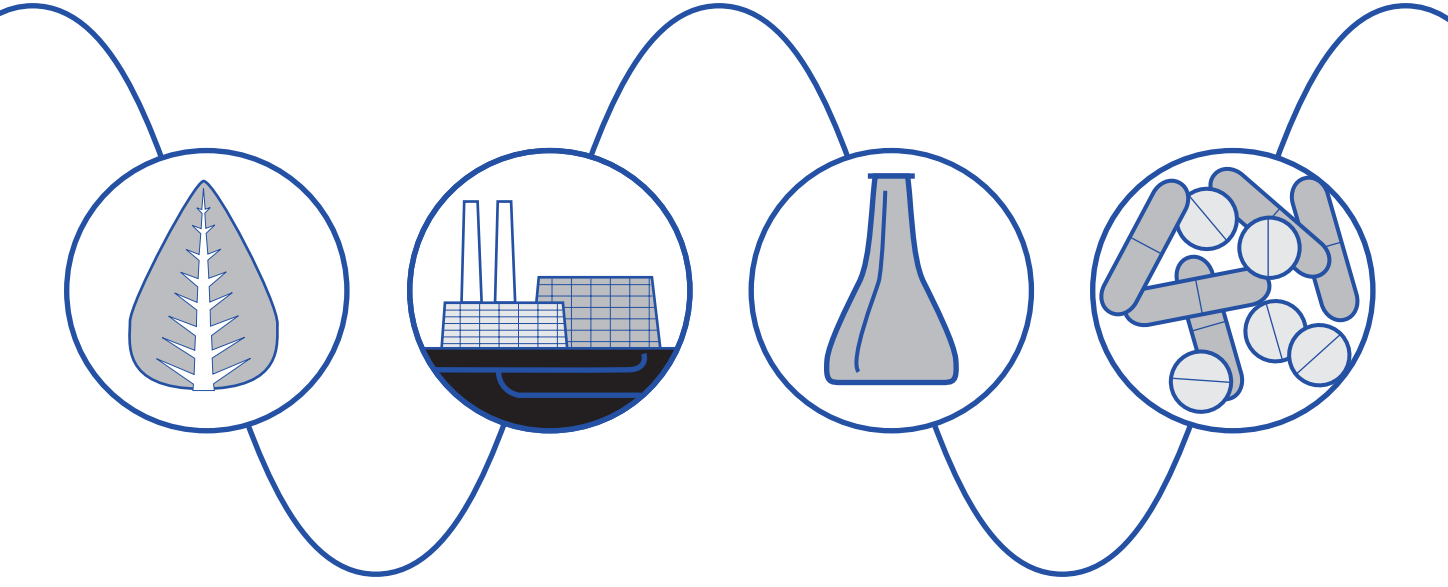
Guide to Successful Operation of Your LC System



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How to Use This Guide

Purpose

This guide is an aid in identifying problems within your liquid chromatographic system and helping to return your system to a proper level of performance. Your system may be a combination of Waters and non-Waters instrumentation.

The primary intent of this guide is to:

- Provide a logical approach to troubleshooting an LC system
- Outline good chromatography/operating practices
- Maximize system operation time

This guide includes a separate quick reference chart that synopsizes the system troubleshooting information.

Audience

This guide is intended for use by a wide variety of LC system operators, whose experience may range from novice to expert.

Structure of This Document

This guide is divided into our chapters and a series of Appendixes. Each page includes a footer, providing easy access to information within the chapter.

The table below describes the material covered in each chapter and Appendix.

Chapter 1, Introduction to System Troubleshooting	Includes a general discussion on the system troubleshooting process.

Chapter 2, Troubleshooting an LC System	Divides the system troubleshooting process into primary LC system problems:
	• System pressure (high, no or low, erratic)
	• Baseline noise
	• Changes in chromatographic resolution or results, including:
	  • Incorrect retention time
	  • Abnormal peak shape
	  • Incorrect qualitative/ quantitative results
	  • Loss of resolution
	Possible symptoms, causes, and corrective actions included for each topic.

Chapter 3, Troubleshooting System Components	Troubleshooting tables for individual hardware components in an LC system: • Pump • Manual injector • Autoinjector • Column • Detector • Data-handling device Possible symptoms, causes, and corrective actions included for each topic.

Chapter 4, Good Chromatography/ Operating Practices	Proper care and use of a system, intended as a set of operator preventative actions: • Solvent preparation and use • System plumbing • Chromatographic performance tests (resolution, k, α, and N) • Measuring System bandspreading
Appendix A, Reference Information	Reference tables

Related Documents

Refer to the appropriate instrumentation and column operator's manuals and service manuals for specific troubleshooting information.

Related Adobe™ Acrobat Reader Documentation

For detailed information about using the Adobe Acrobat Reader, refer to the *Adobe Acrobat Reader Online Guide*. This Online Guide covers procedures such as viewing, navigating and printing electronic documentation from Adobe Acrobat Reader.

Printing From This Electronic Document

Adobe Acrobat Reader lets you easily print pages, pages ranges, or the entire electronic document by selecting **Print** from the File menu. For optimum print quantity, Waters recommends that you specify a Postscript printer driver for your printer. Ideally, use a printer that supports 600 dpi print resolution.

Conventions Used in This Guide

This guide uses the following conventions to make text easier to understand.

- **Purple Text** indicates user action. For example:

  Press **0**, then press **Enter** for the remaining fields.

- *Italic* text denotes new or important words, and is also used for emphasis. For example:

  An *instrument method* tells the software how to acquire data.

- Underlined, Blue Color text indicates hypertext cross-references to a specific chapter, section, subsection, or sidehead. Clicking this topic using the hand symbol automatically brings you to this topic within the electronic document. Right-clicking and selecting **Go Back** from the popup context menu brings you back to the originating topic. For example:

  To begin troubleshooting, refer to Chapter 1.2, Check Simple Things First.

Notes, Attentions, and Cautions

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

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

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

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

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

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

Caution: *To avoid the possibility of electrical shock and possible injury, always turn off the detector and unplug the power cord before performing maintenance procedures.*

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

1 Introduction to System Troubleshooting

Troubleshooting a LC system can be a frustrating and sometimes mysterious process. Has one of these happened your lab?

- You left your autoinjector running samples over the weekend. When you return on Monday, you realize that your results gradually progressed from highly reproducible results to no peaks in the chromatogram.
- The retention times of sample peaks have suddenly shifted within the past hour.

What do you do now? Where do you start?

This chapter outlines a common-sense process for troubleshooting an LC system. The troubleshooting information in this chapter is presented with the following assumptions:

- Your analysis method has already been developed
- Proper system benchmarks have been recorded in the normal operation log
- System or instrument performance has degraded as compared with previously established system benchmarks (see Section 1.3, Compare System Performance to Established Benchmarks)

Troubleshooting Stragety

A troubleshooting strategy includes five primary processes:

- Identifying the symptom(s).
- Understanding possible causes of the symptom. Is it hardware, software, operation, or chemistry related?
- Isolating the exact possible causes of the problem.
- Determining if you have the expertise and items to correct the problem?
- Resolving the problem and resuming operation or contacting your local Customer Support Representative.

Troubleshooting process

To implement this strategy, you need a systematic troubleshooting process that works with all types of problems. This process is to:

1. Get the facts.
2. Check simple things first.
3. Compare system performance to established benchmarks.
4. Indentify possible causes.
5. Use a systematic troubleshooting approach.
6. Getting help.

The flowchart in Figure 1-1 illustrates the troubleshooting process. The remainder of this chapter discusses each phase of the strategy.

1.1 Get the Facts

Before troubleshooting, be sure to get all the facts. The fact gathering stage of system troubleshooting is an important first step in determining how the system is malfunctioning.

Gather general facts

When you initially believe there is a problem with the performance of your LC system, step back and consider:

- What makes you think something is malfunctioning? What is the evidence (shifted peaks, no peaks, baseline drift)?
- Is anything different now with the system as compared with established performance (as documented in a log)?

Gather important facts

After this initial gathering process, write down answers to the following questions in your log:

- Could someone have changed something in the system (such as detector sensitivity)?
- Are you asking the system to do something that may be beyond its capabilities?
- Has the problem ever occurred before?
- Is the problem reproducible?
- Does this problem occur at any particular time of the day or when another instrument is turned on and off?

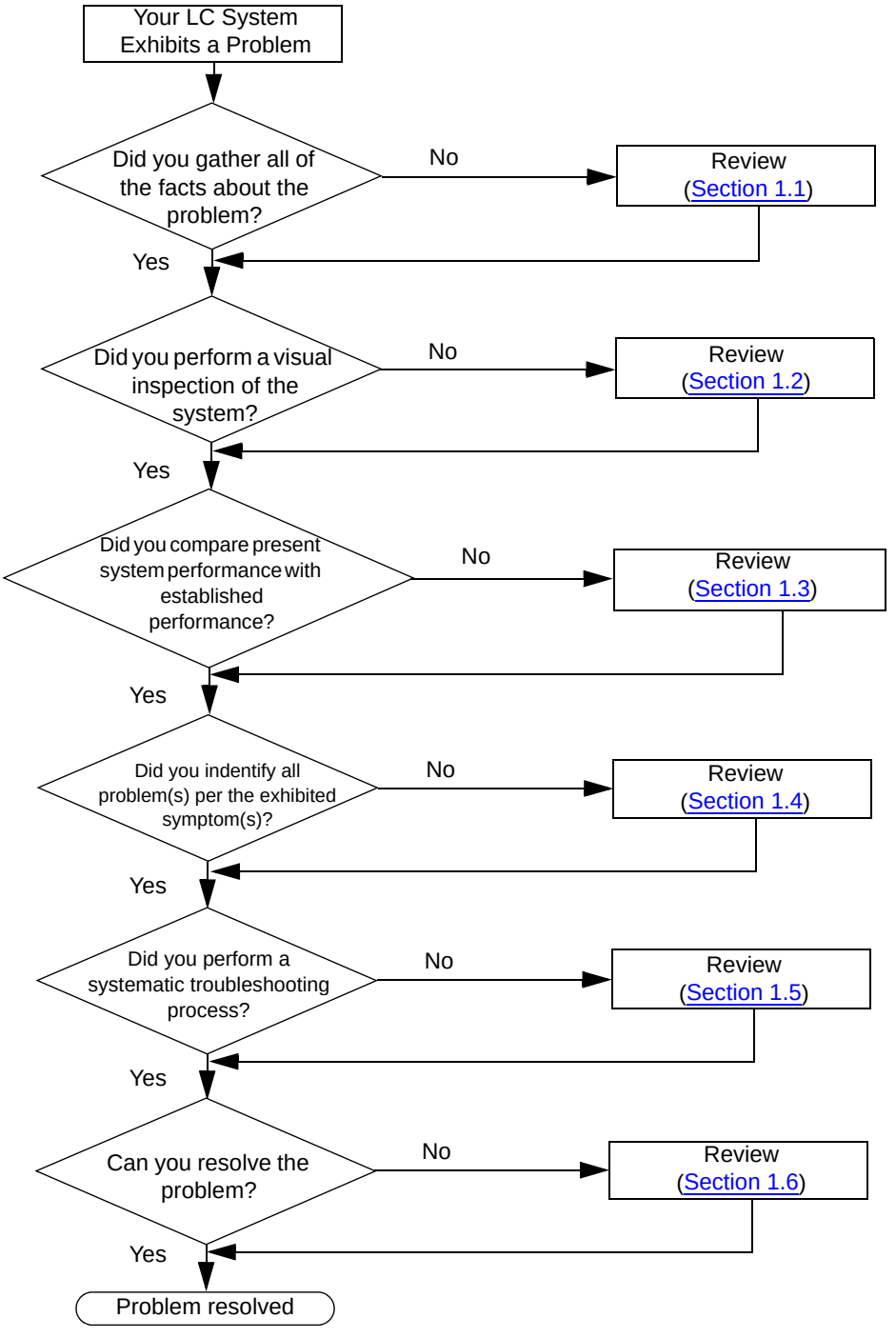


Figure 1-1 The System Troubleshooting Process

1.2 Check Simple Things First

When you are certain that something is affecting system performance, always check the simple things. Finding the easy solution saves time and frustration.

Attention: *Always observe safe laboratory practices when troubleshooting. Know the chemical and physical properties of the solvents. Refer to the Material Safety Data Sheet for the solvents in use.*

Attention: *To avoid electric shock, power down the instrument and disconnect the power cord before removing the cover and examining the instrument.*

Look for obvious "clues"

Make a visual inspection of the system, investigating:

- Alarms triggered on any components?
- Fluid leaks?
- Pump pressure reading normal?
- Power cord inserted in both the outlet and the instrument rear panel?
- Power switch on?
- Fuses blown?
- Sufficient solvent in the reservoir?
- Electrical cables connected between devices? Are connections correct?
- Instrument settings changed or incorrectly set?
- Solvent flowing out of detector waste tubing?
- Correct column?

If the visual inspection does not uncover anything obvious, compare current system performance to established system operation (see Section 1.3, Compare System Performance to Established Benchmarks).

1.3 Compare System Performance to Established Benchmarks

To help you identify normal operation conditions:

- Record a map of your LC system
- Keep a log
- Run test chromatograms regularly

These three practices allow you to compare present system operation with established system performance.

Note: *A log is especially important in a lab where the system is shared by multiple operators.*

Recording a System Map

When your LC system is initially installed, sketch a general map of the fluidic and electrical configuration. Label the individual connections. Use this map to review these fluidic and electrical connections and reconfigure when necessary. Figure 1-2 is an example of a system map.

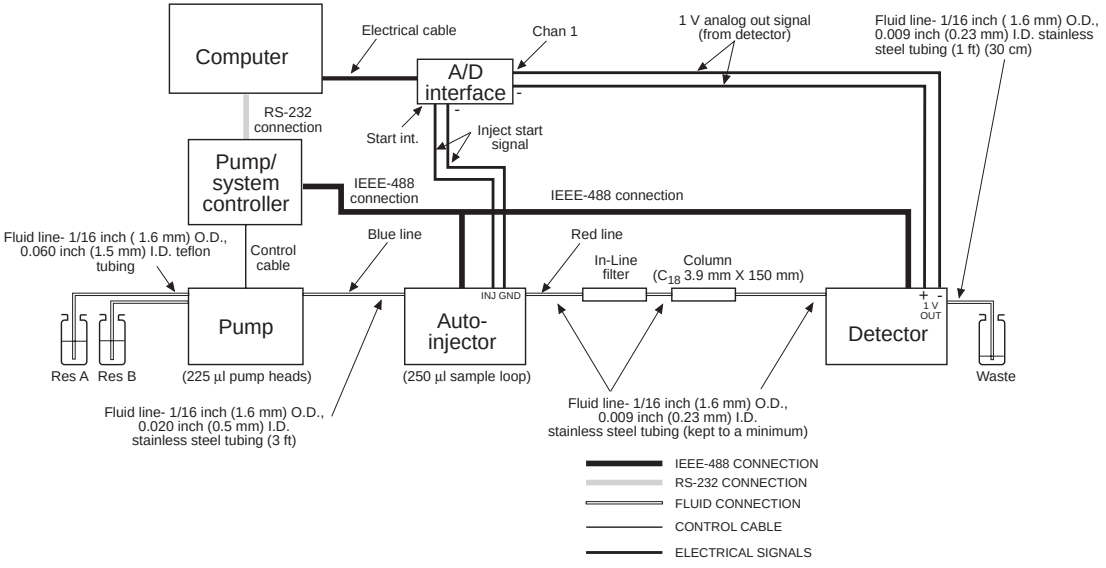


Figure 1-2 System Map Example

Keeping a log

Record operating conditions (pressure, flow rate, and so on) in a logbook. When you have problems with your system, use the logbook to compare system information, such as:

- Instrument settings
- Parts and/or components recently replaced (with manufacturer serial number and date that the change was made)
- Maintenance procedures (what and when)
- Number of samples run (system throughput) in your method
- Sample, standard, and mobile phase information for each method used
- Test chromatogram, including specific operating conditions (column, flow rate, mobile phase, backpressure, and so on) for each column used
- Method table(s) used for your application(s) (such as gradient conditions, integration parameters)

Running a Test Chromatogram

Always run a test chromatogram when:

- A new system is installed
- An instrument is replaced or added
- A column is replaced or added
- New mobile phase is prepared

When running the standard, establish a set of standard conditions. Collect the data and record:

- Pressure
- Resolution (R_s) of critical peak pairs
- Capacity factor (k') for each peak
- Selectivity (α)
- Column efficiency (N)

Refer to Chapter 4, Good Chromatography/ Operating Practices for information on calculating and troubleshooting resolution, capacity factor, selectivity, and column efficiency.

Keep the test chromatogram and associated data in your logbook. When you believe there is a problem, rerun the standard. Compare the results to establish:

- If the test results are not significantly different from those previously recorded, the problem is method-specific.

- If the test results are different, either the column or an instrument have changed. Repeat the test with a new (or known good) column. If the results of the second column are satisfactory, the problem is with the first column. If both columns fail, the problem is most likely with an instrument.

1.4 Identify Possible Causes

Use the exhibited symptoms to narrow down the possible causes within the system. To identify a problem(s):

- Identify *all* of the symptoms
- Match the symptoms with the potential possible causes

Identifying the Symptoms

Perform a survey of your system to determine where the exhibited symptom(s) may originate.

Some symptoms are:

- Variable or unusual system pressure (high, no or low, erratic)
- Noisy or drifting baseline
- Incorrect or changing retention time(s)
- Abnormal peak shapes (such as broad, tailing, or fronting peaks)
- Incorrect qualitative/quantitative results (too many peaks, too few peaks, wrong answer)

Identifying the Possible Causes

From the isolated symptom(s), write down a list of suspected possible causes in your logbook.

For example:

Symptom	Possible Causes
Sudden high system backpressure	• Blocked column frit • Sample precipitation on column • Changed or incorrect flow rate • Blocked tubing • Defective pump pressure transducer • Drop in operating temperature

The troubleshooting tables in Chapter 2, Troubleshooting an LC System, and Chapter 3, Troubleshooting System Components are examples of possible causes for a particular symptom.

Isolating the Possible Cause

From your list of possible causes, isolate the problem areas within your LC system. This narrows down whether the problem is hardware, software, operation, or chemistry related. You can then follow a systematic troubleshooting approach to resolve the problem (see Section 1.5).

1.5 Use a Systematic Troubleshooting Approach

Once all of the possible causes are identified, resolve the problem using a systematic approach:

- Follow a logical sequence to correct the problem
- Make only one change at a time to the system, starting with the easiest changes
- Document the results of each change

Following a Logical Sequence

After listing the possible causes of the problem, follow a logical troubleshooting sequence to narrow down the exact source of the problem.

For example, if the symptom is unusually high backpressure, loosen each fitting, starting at the detector waste outlet, and observe if pressure drops. Continue to work toward the pump until the pressure drops, identifying the possible cause of the problem.

Note: *For detailed information on system troubleshooting, refer to* *Chapter 2, Troubleshooting an LC System**.*

Making Only One Change at a Time

During troubleshooting, make only one change to the system at a time to the system until the problem is resolved. Parts of the system you may make changes to include:

- Instrument settings
- Column
- Sample
- Operating conditions
- Mobile phase
- Replacement of suspected malfunctioning *parts*
- Replacement of suspected malfunctioning *instruments*

Note: *If the replacement part does not correct the problem, be sure to put back the original part. Conversely, rebuild or discard a malfunctioning part/assembly.*

Making only one change at a time allows you to understand the affects on system performance.

Documenting Changes

Always take notes as you make changes to the system, such as changing settings, swapping component parts, or swapping an instrument. Record-keeping is a valuable part of the systematic approach because it provides you with:

- A trail back to configuring the system in its original form if your troubleshooting path is incorrect
- A trail back if new problems or symptoms arise

If you proceed through the system troubleshooting process and the identical symptom(s) persist, reevaluate your diagnosis and investigate another area in the system.

1.6 Getting Help

It is important that you feel confident with your troubleshooting and maintenance abilities. If you feel that the problem is beyond your area of expertise, consult someone within your lab with more experience. If that does not help resolve the problem, call the Customer Support Department (or service specialist) for help.

Follow the troubleshooting process outlined in this guide to narrow down the symptom to the most likely problem source. With that knowledge, you are able to concretely discuss your diagnosis with the Customer Support representative, saving valuable time and confusion.

1.7 Troubleshooting References

For additional information on liquid chromatography system troubleshooting or liquid chromatography in general, refer to the following reference material:

- The operator's manuals and service manuals for your LC instrumentation and columns
- Dolan, John, W., and Snyder, Lloyd, R. (1989), *Troubleshooting LC Systems*, The Humana Press Inc., P.O. Box 2148, Clifton, NJ, 07015
- Snyder, L.R. and Kirkland, J.J., *Introduction to Modern Liquid Chromatography*, Second Edition, Wiley-Interscience, New York, 1979
- The "LC Troubleshooting" column in LC/GC Magazine

2 Troubleshooting an LC System

This chapter outlines LC system troubleshooting. It divides system troubleshooting into:

- Initial survey of system problems
- Isolating possible causes of system problems, including:
- System backpressure (high, no or low, erratic)
- Baseline noise
- Changes in chromatographic resolution or results, including:
    - Incorrect/changing retention times
    - No peaks or abnormal peak shapes
    - Incorrect qualitative/quantitative results
    - Loss of resolution

Troubleshooting information in this chapter is presented with the assumption:

- Your analysis method has already been developed
- Proper system benchmarks have been recorded in the normal operation log
- System or instrument performance has degraded as compared with previously established system benchmarks (see Section 1.3)

For background information on the system troubleshooting process, refer to Chapter 1, Introduction to System Troubleshooting.

For specific instrument and column troubleshooting, refer to Chapter 3, Troubleshooting System Components. When troubleshooting, keep the following safety considerations in mind:

Attention: *Always observe safe laboratory practices when troubleshooting. Know the chemical and physical properties of the solvents. Refer to the Material Safety Data Sheet for the solvents in use.*

Attention: *To avoid electric shock, power down the instrument and disconnect the power cord before removing the cover and examining the instrument.*

Attention: *Ultraviolet light is emitted during UV and fluorescence detector operation. To prevent eye damage, eye protection must be worn while troubleshooting the detector with its covers removed.*

- If handling integrated circuit boards, use an anti-static mat and wear an anti-static wrist strap to remove excess static charge and prevent damage to the board.
- Do not touch any of the integrated circuit chips or other components which do not specifically require manual adjustment.

When You Call

Many problems with your LC system Customer Support can be easily corrected by the process outlined in this chapter. If you cannot correct a condition for a Waters product, contact one of the following:

- Your Waters service specialist
- Waters Customer Support Department
- Waters Service offices (listed on the rear cover of this guide)

To expedite service, have the following information available when you call:

1. Completed Normal Operation Log and test sample chromatogram for method
2. Nature of symptom(s)
3. Type and model number of pump (single or multiple solvent)
4. Flow rate
5. Operating pressure
6. Mobile phase(s)
7. Type and model number of injector (manual or autoinjector)
8. Type and model number of detector (UV, RI, fluorescence, conductivity, electrochemical)
9. Detector settings (wavelength, sensitivity, and so on)
10. Type and serial number of column
11. Sample matrix and components
12. Data system
13. Software version (if applicable)

2.1 Initial Survey of System Problems

When a problem occurs, first perform a visual check of the system. Look for leaks, disconnected tubing, disconnected cables, changed instrument settings, and so on. Review your system map System map (as described in Section 1.3, Compare System Performance to Established Benchmarks) to verify all fluidic and electrical connections.

Table 2-1 is a summary of the most common problems affecting system operation.

If you determine one (or more) of these are the problem, refer to Chapter 3, Troubleshooting System Components, or the instrument/column operator's manual for the corrective action.

Table 2-1 System Problems

Component	Areas to Check
General System	• Instrument not plugged in/not turned on • No power at wall outlet • Blown power fuse • Incorrect instrument settings • Cooling fans in instrument not running • Leaks • Incorrect air or gas supplies • Disconnected or improper electrical cabling • Too many instruments on same circuit • Incorrect grounding between instruments

Table 2-1 System Problems *(Continued)*

Component	Areas to Check
Pump/Solvent Flow	• Incorrect flow rate setting • Solvent reservoir(s) empty • Solvent not degassed or sparged • Obstruction or crimp in tubing • Improper tubing ID • Improperly cut tubing • Incorrect or worn ferrules/compression screws • Plugged in-line filter • Plugged solvent inlet filter • Air in pump inlet lines or pump heads • Leaking plunger seal • Leaking fittings • Malfunctioning inlet/outlet check valve • Pressure transducer out of calibration • Solvent reservoir positioned lower than pump inlet
Manual Injector	• Obstruction in sample loop • Insufficient injection volume • Inconsistent injection volume • Incorrect or damaged syringe • Leaking vent tube or valve • Wrong sample loop • Inconsistent injection sequence • Leaking or worn seals • Contaminated syringe • Injector out of adjustment

Table 2-1 System Problems *(Continued)*

Component	Areas to Check
Autoinjector	• Air in syringe • Insufficient sample in vial • Improper sample vial position • Incorrect or improper settings • Leaking or worn seals • Valve failure • No or low air pressure • Wrong syringe size
Detector	• Defective source lamp • Incorrect or improper settings (sensitivity, attenuation, wavelength, time constant) • Insufficient time for lamp to stabilize • Auto zero left enabled • Incorrect optical filter and/or lamp • Dirty flow cell • Air bubble in flow cell • Dirty reference electrode • Dirty working electrode • Incorrect reference and sample solvent balance (RI detector) • Leaking flow cell
Computer	• Wrong A/D sampling time • Improper output voltage signal from detector • Improper attenuation, peak width, area reject, noise rejection parameters • Poor peak integration • Incorrect calibration or sample table

Table 2-1 System Problems *(Continued)*

Component	Areas to Check
Integrator	• Improper attenuation • Improper input voltage range • Auto zero left enabled by detector • Improper output voltage signal from detector • Improperly inserted paper
Chart recorder	• Improper chart speed • Improper input voltage range • Improper output signal from detector • Gain improperly adjusted • Reversed polarity • Auto zero left enabled on detector • Auto zero left enabled on chart recorder
Chromatography	• Operating environment • Column not equilibrated • Mobile phase problem (improperly degassed, prepared, or filtered; incorrect for application; contaminated) • Sample problem (improperly prepared or filtered; insoluble with mobile phase; degraded) • Contaminated column • Obstruction in guard column or column inlet frit • Improper column or precolumn

2.2 Isolating System Problems

This section covers the most common symptoms exhibited during operation, including:

Exhibited symptom	Refer to
System pressure (high, no/low, or erratic	Section 2.2.1
Baseline noise	Section 2.2.2
Changes to chromatographic resolution and results • Incorrect retention time • No peaks or abnormal peaks • Incorrect qualitative/quantitative results • Loss of resolution	Section 2.2.3 Section 2.2.3.1 Section 2.2.3.2 Section 2.2.3.3 Section 4.3

Note: *If a specific instrument or the column in your system exhibits a symptom not addressed in this section, refer to Chapter 3, Troubleshooting System Components, for instrumentation and column troubleshooting.*

2.2.1 System Pressure

Troubleshooting overview

This section covers troubleshooting:

- High system pressure
- Low system pressure
- Erratic (fluctuating) system pressure

System pressure troubleshooting is presented in a flow diagram format. Use Figure 2-2 through Figure 2-4 to investigate pressure problem sources.

System pressure reference point

To identify a pressure change from normal operation, it is critical that you have a pressure reference point. System pressure is affected by the column, flow rate, mobile phase, and temperature, and can vary greatly with different methods.When running a gradient, fluctuations in system pressure may be due to viscosity changes between solvents.

Each time you install a new column or start a new method, equilibrate the system and record the system pressure (both with and without the column in line) to use as a comparison. Use the normal operation log to record system pressure.

Gradual versus sudden pressure increase

When high system pressure occurs, it is important to note whether the pressure increase was gradual or sudden.This can help you isolate the problem source.

If the pressure has risen *gradually* (over a series of injections), it may be due to:

- Particulates in the sample or the mobile phase which have accumulated in the in-line filter or column frits
- Debris from failed fluid seals

If the pressure has risen *suddenly*, it may be due to:

- Particulates in one sample
- A system hardware problem (such as blocked tubing)
- Collapse of the column packed bed

High pressure locations within an LC system

To isolate the origin of high system pressure, loosen inlet or outlet fittings as instructed in Figure 2-2 and observe if pressure stays the same or reduces.

Attention: *Always observe safe laboratory practices when troubleshooting. Always wear safety glasses and gloves. Know the chemical and physical properties of the solvents. Refer to the Material Safety Data Sheet for the solvents in use.*

Figure 2-1 presents pressure release points in a typical LC system. Use Figure 2-1 as a reference when reviewing the high pressure troubleshooting flow diagram in Figure 2-2.

To relieve pressure, slowly loosen the fitting. Use a tissue to prevent solvent spray and also to collect any spilled solvent.

Attention: *f the source of high pressure is within the detector flow cell, use extreme caution when relieving high pressure buildup. Many detectors (especially RI and fluorescence) have fragile flow cells. Before backflushing the detector to remove the blockage, review the flow rate specifications and backpressure limits for that flow cell in the detector operator's manual.*

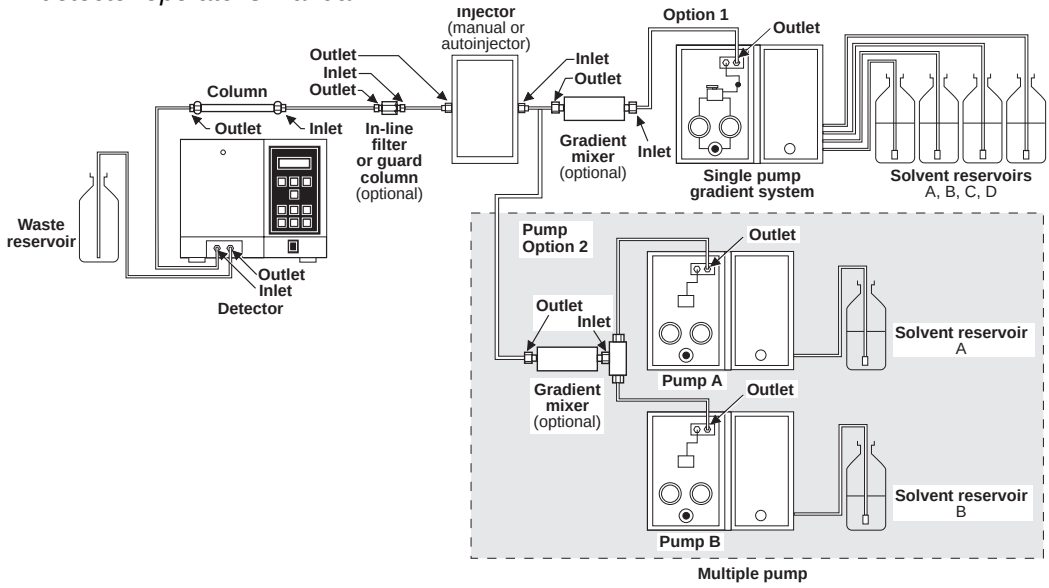


Figure 2-1 Pressure Release Points within a Typical LC System

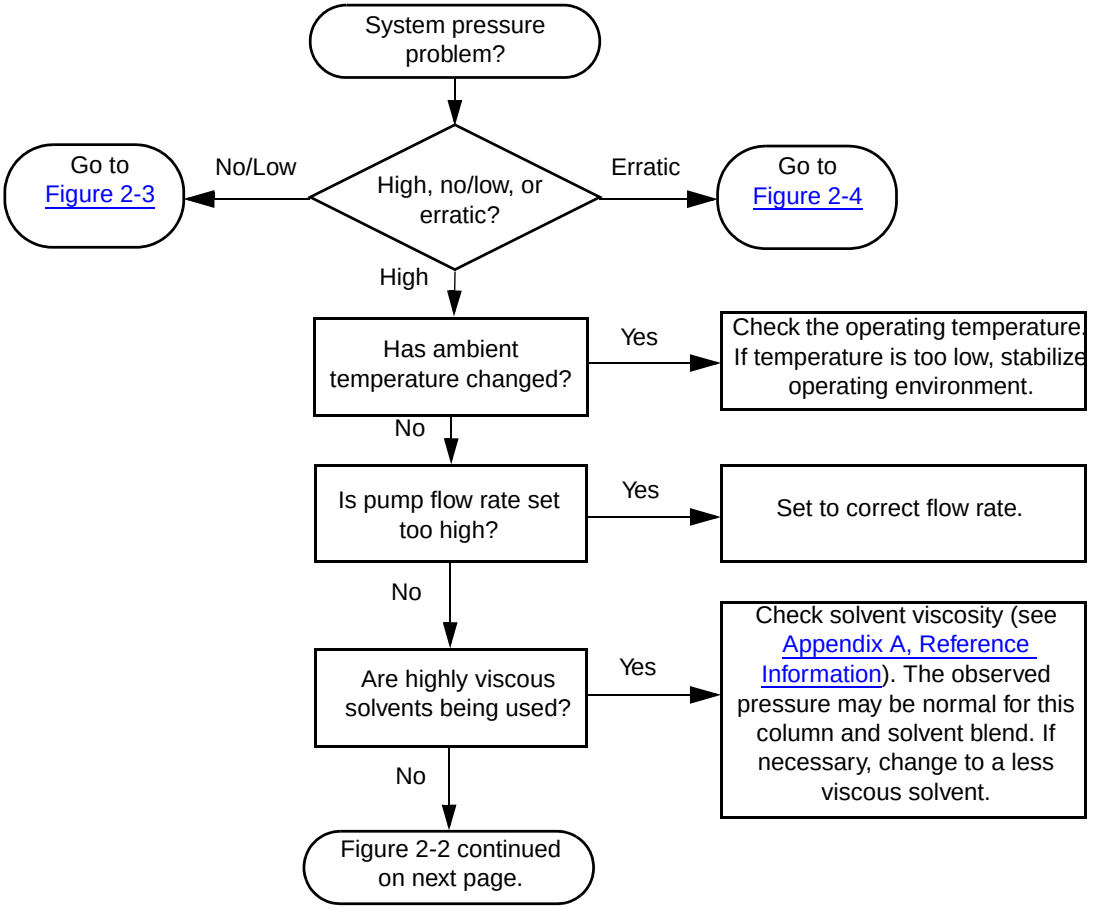


Figure 2-2 High System Pressure Troubleshooting

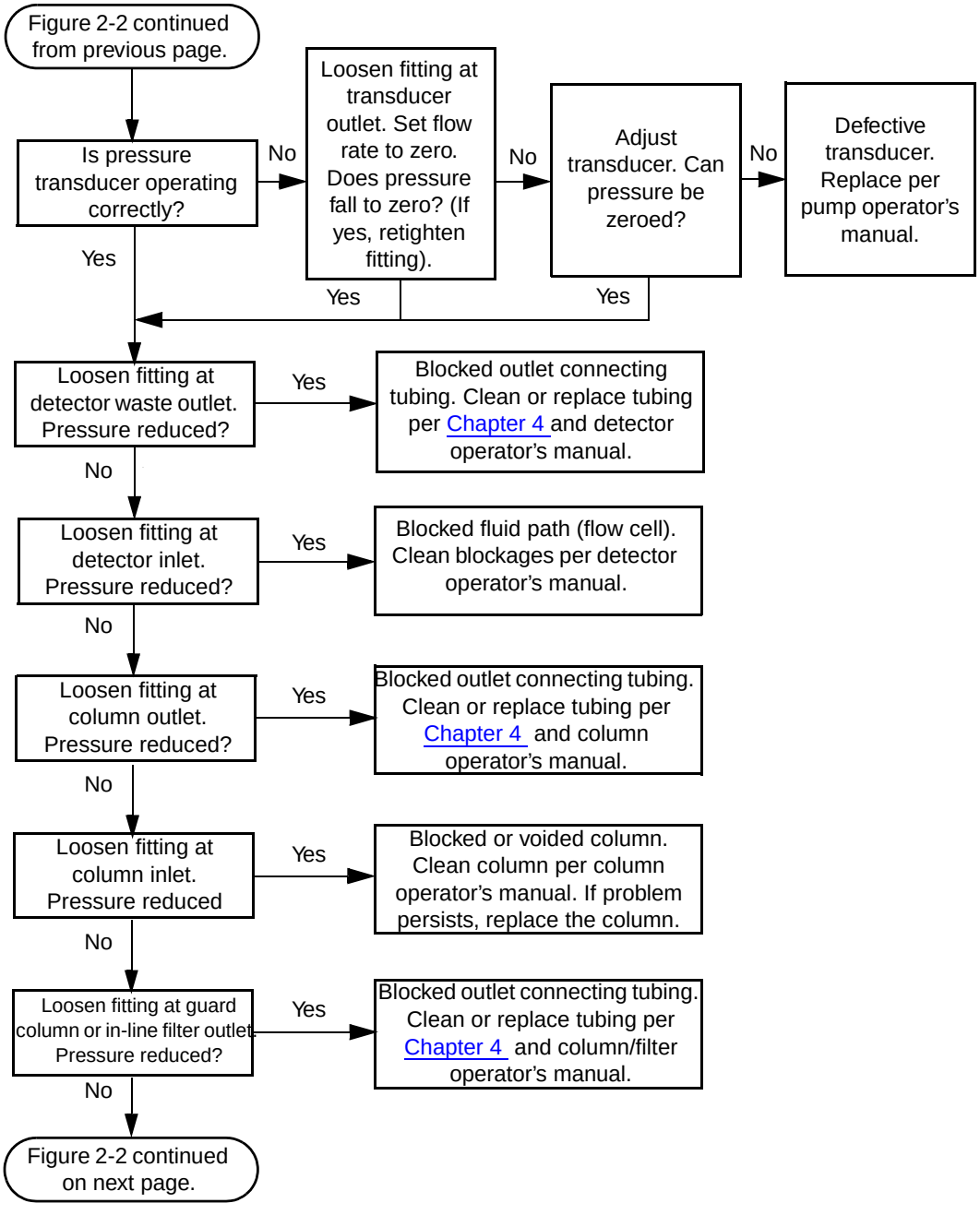


Figure 2-2 High System Pressure Troubleshooting *(Continued)*

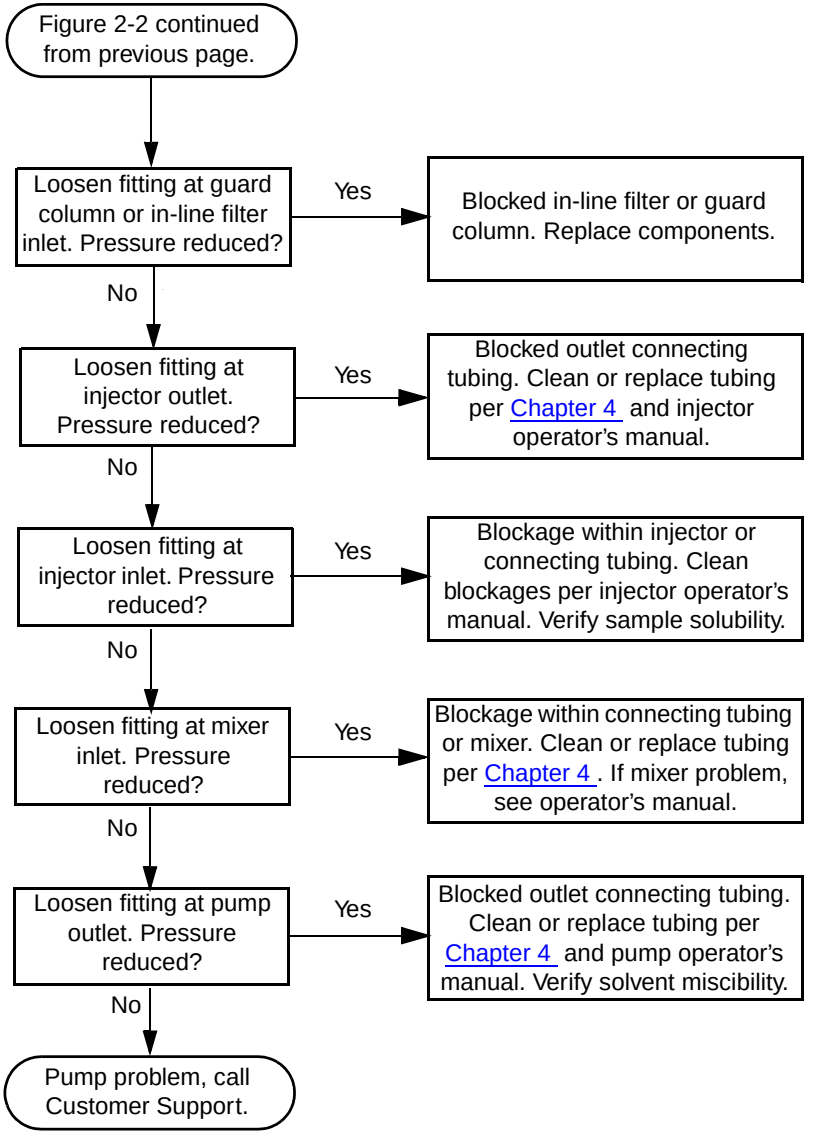


Figure 2-2 High System Pressure Troubleshooting *(Continued)*

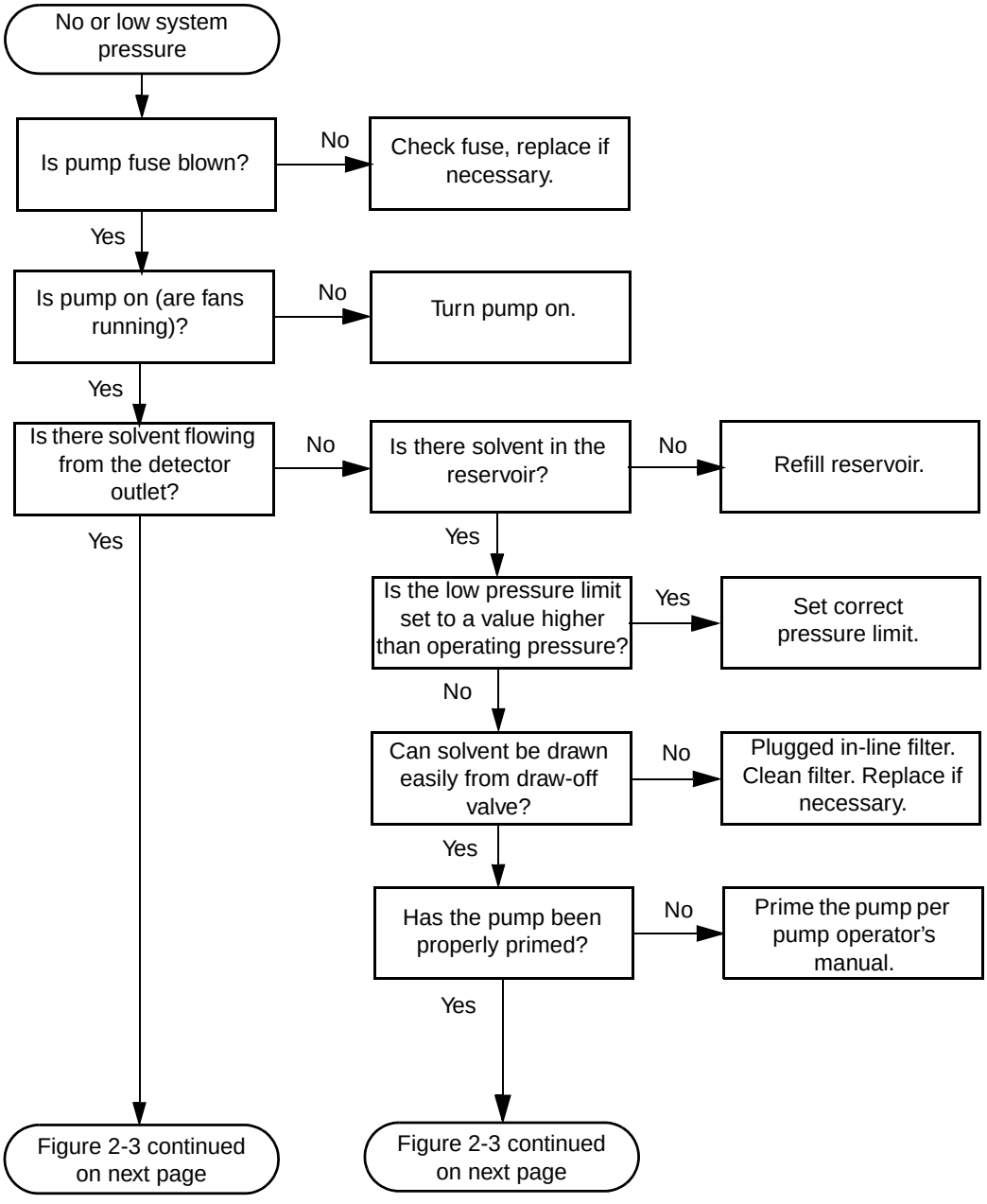


Figure 2-3 Low System Pressure Troubleshooting

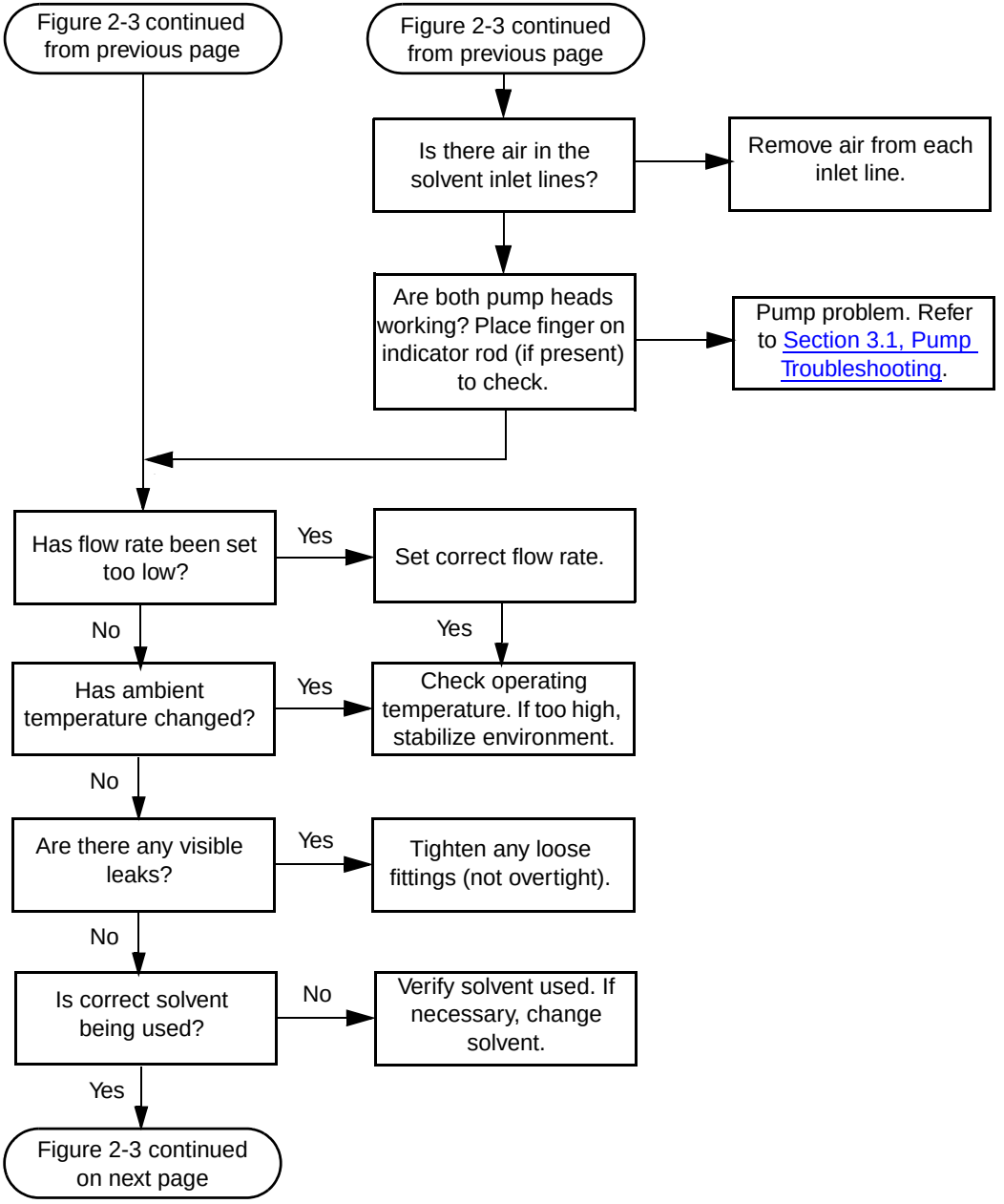


Figure 2-3 Low System Pressure Troubleshooting *(Continued)*

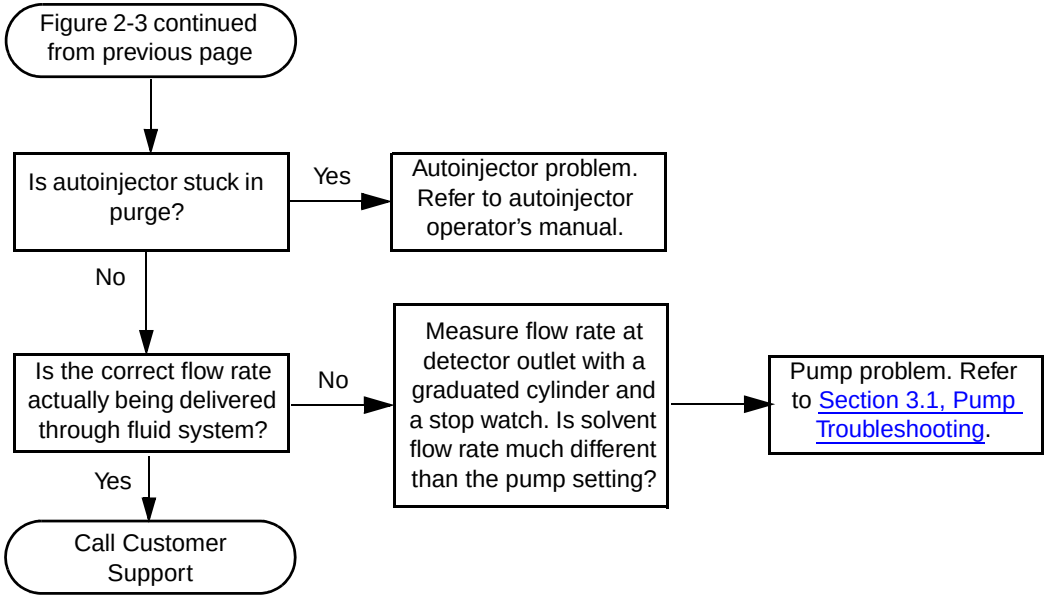


Figure 2-3 Low System Pressure Troubleshooting *(Continued)*

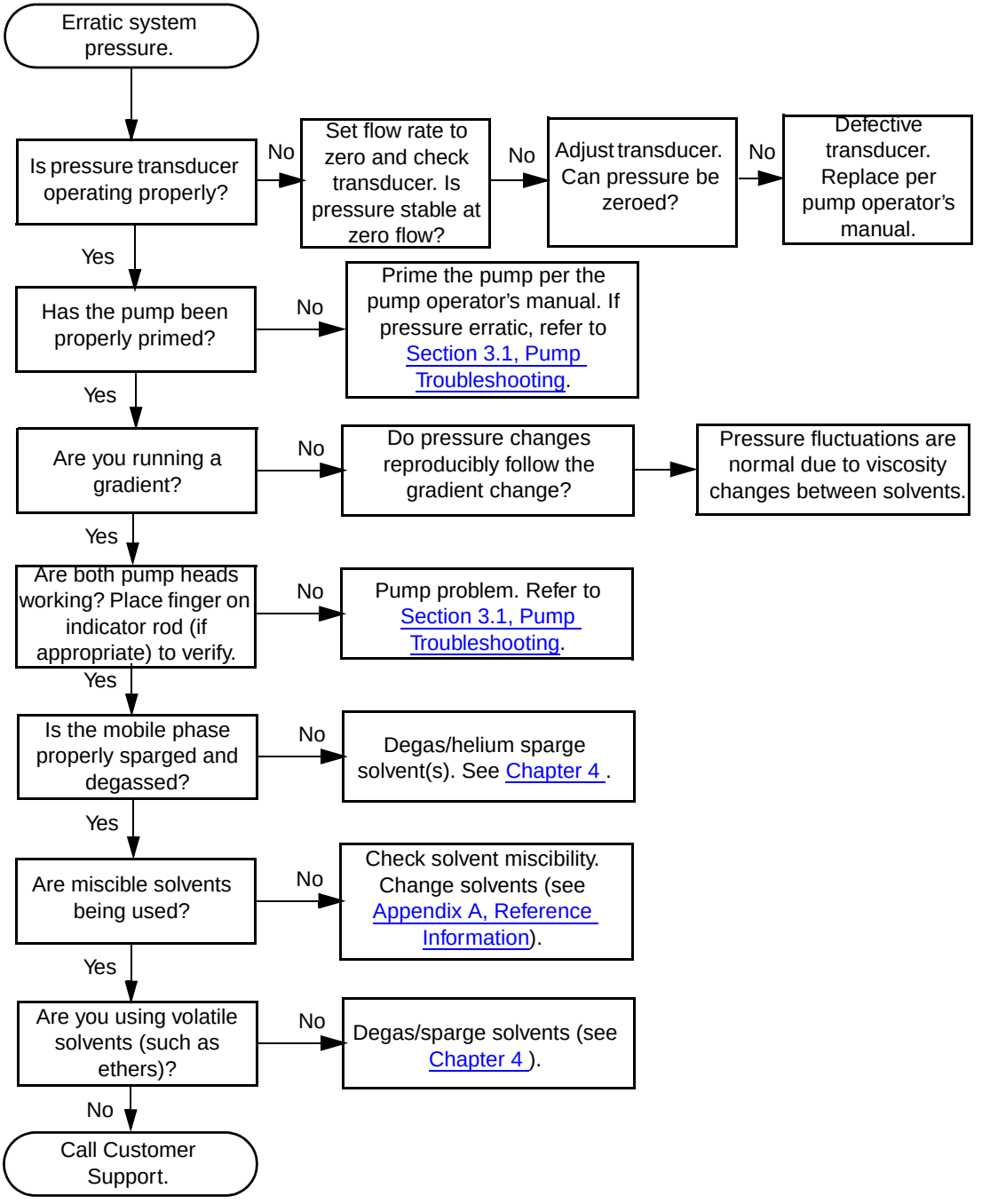


Figure 2-4 Erratic System Pressure Troubleshooting

2.2.2 Baseline Noise

Troubleshooting overview

This section covers troubleshooting baseline noise. It assumes baseline noise is either fluid path-related (mobile phase, pump, column) or detector electronics- related.

Baseline noise is characterized as:

- Non-cyclic (erratic) baseline noise
- Cycling (short or long-term) baseline noise
- Baseline drift
- Noise spikes on the baseline

Table 2-3 covers fluid path-related noise and Table 2-4 covers detector electronics-related noise.

Note: *Data-handling device electronics noise is covered in Section 3.6.*

Table 2-2 summarizes the baseline noise symptoms used in Table 2-3 and Table 2-4. From Table 2-2:

1. Review the baseline noise symptoms and select the one that best typifies your system problem.
2. Proceed to the page number of the baseline noise symptom (in Table 2-3 or Table 2-4).
3. Review the list of possible causes and follow the corrective actions.

Isolating the source of baseline noise

To isolate the source of the baseline noise:

1. Turn off your pump to stop solvent flow to the system.
2. Monitor the baseline for a few minutes. Note the following:
   - If there is significant improvement in the baseline, the problem is within the fluid path (pump/mobile phase/flow path/column). Refer to Table 2-3.

     Note: *Some flow sensitive detectors, such as RI and electrochemical, may require a significant time to stabilize once flow stops.*

   - If the noise continues, the problem is within the detector or its electrical connections. Proceed to step 3.
3. Disconnect the detector electrical cables from the data-handling device (A/D interface to the computer, computer, integrator, or chart recorder).

4. Attach a jumper source to the input terminals on the data-handling device (such as a wire or paperclip).
    - If the noise continues, the problem is within the data-handling device. Refer to Section 3.6, Data-Handling Device Troubleshooting.
    - If the noise stops, the problem is within the detector or its electrical connections. Refer to Table 2-4.

Baseline noise summary

Table 2-2 is a summary of the baseline noise symptoms listed in Table 2-3 and Table 2-4. These baseline noise examples were generated using the following operating conditions:

Parameter	Setting
Pressure	1000 psi
Detector absorbance units	1 AU
Recorder sensitivity	10 mV full-scale
Chart speed	1 cm/min
Output signal from detector	10 mV

Note: *The amplitude of the noise shown in the examples in this section have been generated in a lab environment. The signal amplitude can vary depending upon the severity of the problem.*

Table 2-2 Baseline Noise Summary

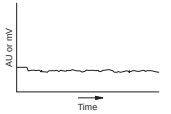
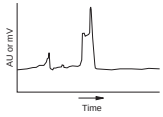
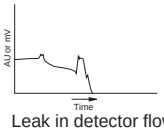
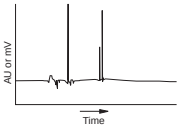
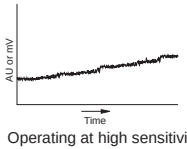
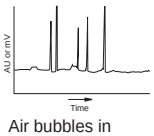
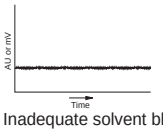
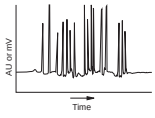
Baseline Noise Symptom	Refer to
Non-cyclic (erratic) noise Fluid path problem:  Unstabilized system  Air bubble in flow cell  Leak in detector flow cell	Table 2-3
Detector electronics problem:  Defective lamp  Operating at high sensitivity	Table 2-4
Short-term cycling noise Fluid path problem  Air bubbles in detector flow cell  Inadequate solvent blending  Inadequate solvent blending	Table 2-3

Table 2-2 Baseline Noise Summary *(Continued)*

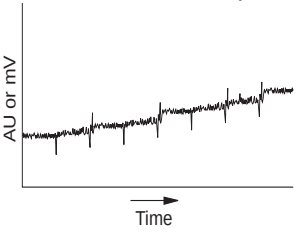
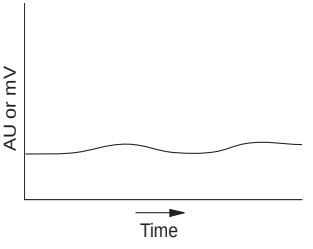
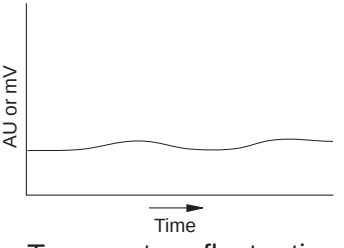
Baseline Noise Symptom	Refer to
Short-term cycling noise *(Continued)* Detector electronics problem:  Cycling equipment	Table 2-4
Long-term cycling noise Fluid path problem:  Temperature fluctuations	Table 2-3
Detector electronics problem:  Temperature fluctuations	Table 2-4

Table 2-2 Baseline Noise Summary *(Continued)*

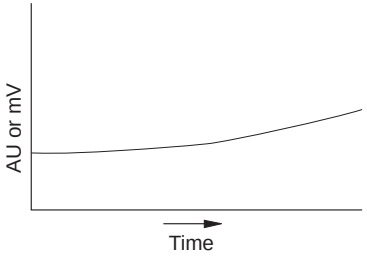
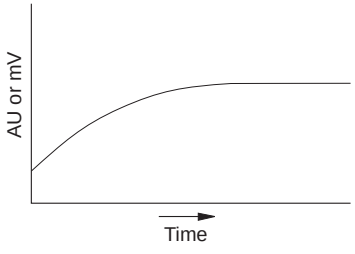
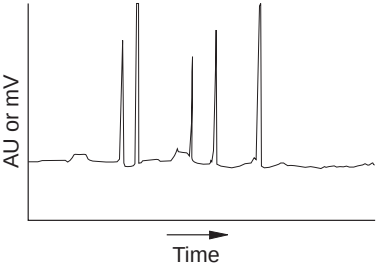
Baseline Noise Symptom	Refer to
Baseline drift Fluid path problem:  Contaminated column	Table 2-3
Detector electronics problem  Insufficient stabilization	Table 2-4
Noise spikes Fluid path problem:  Air bubbles in fluid path	Table 2-3

Table 2-2 Baseline Noise Summary *(Continued)*

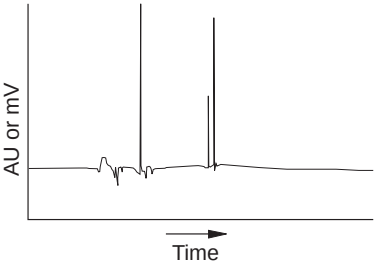
Baseline Noise Symptom	Refer to
Detector electronics problem:  Defective lamp	Table 2-4

Table 2-3 Fluid Path-Related Baseline Noise Troubleshooting

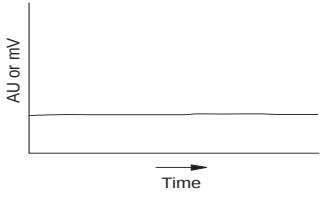
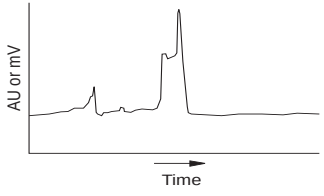
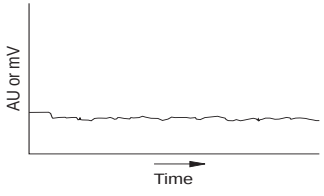
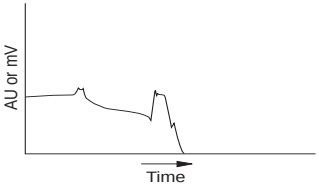
Symptom	Possible Cause	Corrective Action
Non-cyclic (erratic) noise (originating from fluid path)  Normal baseline  Air bubble in flow cell  Unstabilized system  Leak in detector flow cell	Large air bubble trapped in detector flow cell	To remove the air bubble, purge the detector flow cell or apply slight pressure on the detector waster outlet (see detector operator's manual). ***Note:*** *To prevent air bubbles from forming in the flow cell, add a 1 to 3 foot (30 to 90 cm) length of 0.009 -inch (0.23 mm) ID, 1/16 -inch (1.58 mm) ID, 1/16 -inch (1.58 mm) OD tubing to the detector waste outlet. This tubing functions as a flow restrictor to increase backpressure. A 3 foot (90 cm) piece of tubing provides 30 to 50 psi (2 to 3 atm) of backpressure at 1 mL/min in water.* *Keep in mind the backpressure limits of the flow cell (with RI, fluorescence, conductivity, and electrochemical detectors) before attaching this tubing.*
	Air bubble trapped in reference electrode (electrochemical detector only)	Remove the reference electrode and gently shake it to remove the air bubble. Replace the reference electrode.

Table 2-3 Fluid Path-Related Baseline Noise Troubleshooting *(Continued)*

Symptom	Possible Cause	Corrective Action
Non-cyclic (erratic) noise (originating from fluid path) *(Continued)*	Small air bubbles traveling through the flow path	Prime the pump to remove air (see pump operator's manual). To prevent additional air bubbles, ensure the mobile phase is properly degassed or helium sparged (see Chapter 4 for information).
	System not stabilized or chemically equilibrated	Allow all system components (such as the column and detector) sufficient time to stabilize and chemically equilibrate. Note the operating conditions of your application (such as mobile phase, detector settings, detector type). Refer to the instrument or column operator's manual for recommended equilibration times. If running an automated gradient method, ensure sufficient and reproducible equilibration times are used between injections. ***Note:*** *If using ion-pairing reagents, ensure that the first time you use the column you provide a sufficient time and volume of solvent to adequately equilibrate the column (for example, running a total volume of 100 mL of a 5 mM solution at 1 ml/min).*

Table 2-3 Fluid Path-Related Baseline Noise Troubleshooting *(Continued)*

Symptom	Possible Cause	Corrective Action
Non-cyclic (erratic) noise (originating from fluid path) *(Continued)*	Mobile phase contaminated	Discard the contaminated mobile phase and: • Clean the solvent reservoir. Clean or replace the solvent inlet filter. To clean the filter, remove and sonicate using 6N nitric acid, water (repeat 3 times), followed by methanol. • Prepare and filter fresh solvent using only high quality reagents and HPLC-grade solvents (see Section 4.1 for solvent preparation and use considerations) • Flush and re-equilibrate the system.

Table 2-3 Fluid Path-Related Baseline Noise Troubleshooting *(Continued)*

Symptom	Possible Cause	Corrective Action
Non-cyclic (erratic) noise (originating from fluid path) *(Continued)*	Detector flow cell leaking	Remove the detector cover and check for leaks. If leaks are not visible, perform the following: 1. Flush the detector with a non-buffered miscible solvent, followed by methanol. 2. Attach a nitrogen or helium source to the detector inlet. Slowly blow the flow cell dry. 3. Monitor the baseline for noise. If noise disappears, there is a leak within the detector flow cell. Repair/replace the detector flow cell (see detector operator's manual)

Table 2-3 Fluid Path-Related Baseline Noise Troubleshooting *(Continued)*

Symptom	Possible Cause	Corrective Action
Non-cyclic (erratic) noise (originating from fluid path) *(Continued)*	Column contaminated	To verify this problem as a possible cause, replace all columns in the system with a union (or with a known good column of the same type). Return the mobile phase, and monitor the baseline. ***Note:*** *If you require additional system backpressure during this verification, use a piece of 0.009 -inch (0.23 mm) ID tubing (or suitable restrictor) instead of a union.* If the problem stops, clean or replace the contaminated column as outlined in the column operator's manual. If the problem continues, this could be due to: • Solvent properties such as miscibility. Refer to Section 4.1 and Appendix A, Reference Information for information. • Contaminated mobile phase (refer to the "Mobile phase contaminated" Possible Cause listed above for the corrective action • Contaminated guard column in-line filter (clean or replace as outlined in the operator's manual).

Table 2-3 Fluid Path-Related Baseline Noise Troubleshooting *(Continued)*

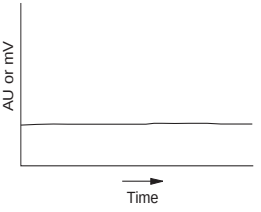
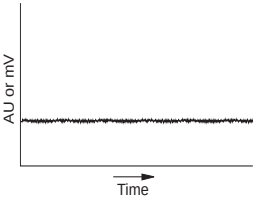
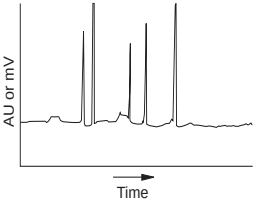
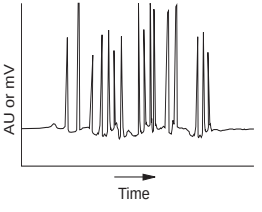
Symptom	Possible Cause	Corrective Action
Short-term cycling noise (seconds to minutes) (originating from fluid path)  Normal baseline  Inadequate solvent blending  Air bubbles in detector flow cell  Inadequate solvent blending	Erratic pump pressure/ pump pulsations	Refer to Figure 2-4 (in Section 2.2.1) to verify and correct the source of erratic pressure. If erratic pressure continues, refer to Section 3.1, Pump Troubleshooting.

Table 2-3 Fluid Path-Related Baseline Noise Troubleshooting *(Continued)*

Symptom	Possible Cause	Corrective Action
Short-term cycling noise (seconds to minutes) (originating from fluid path)	Inadequate solvent bending	To confirm a mixing problem: 1. With the column in-line, pump 5-10 column volumes of 100% A and monitor the baseline. This provides both a constant composition and sufficient volume to equilibrate the column and reach the detector flow cell. 2. Pump several pre-mixed solvents (for example, 50/50, 95/5, 70/30, or the mixture you are running) and monitor the baseline. If the baseline is acceptable with 100% A, but becomes noisier when running mixtures, there is a mixing problem. Verify the mixing problem and correct as outlined below: 1. Use of immiscible solvents. Verify miscibility of solvents (see Appendix A) and change to more miscible solvents. 2. Malfunction in the pump, the pump solvent proportioning valve, or the high-pressure mixer. Troubleshoot per Section 3.1, Pump Troubleshooting

Table 2-3 Fluid Path-Related Baseline Noise Troubleshooting *(Continued)*

Symptom	Possible Cause	Corrective Action
Short-term cycling noise (seconds to minutes) (originating from fluid path) *(Continued)*	Inadequate solvent bending *(continued)*	3. Inadequate blending after the pump. The solution is to add additional mixing. However, the mixing required depends on the severity of the problem. Verify and correct as follows: • Increase laminar flow mixing and residence time (the time to pass through the mixer). Add a 6 to 12 inch (150 to 300 mm) length of 0.040-inch (1.0 mm) ID tubing between the pump outlet and the injector. This tubing length adds only a small system delay. volume and does not distort gradient shape. • If the tubing does not resolve the problem, add a larger mixer volume for a more vigorous mixing. Add one or more Waters Gradient Flow Mixers between the pump outlet and injector. The mixing chamber provides consistent solvent blending with minimal volume and gradient distortion.

Table 2-3 Fluid Path-Related Baseline Noise Troubleshooting *(Continued)*

Symptom	Possible Cause	Corrective Action
Short-term cycling noise (seconds to minutes) (originating from fluid path) *(Continued)*	Inadequate solvent bending *(continued)*	***Note:*** *Ensure that you use a mixing chamber with the smallest possible volume. This prevents introducing additional delay volume or distorting gradient shape.* 4. Use premixed solvent(s).
	Pump Inlet tubing loose, bent, or blocked	Check the tubing. If loose, tighten. If bent, straighten. If blocked, replace (see Chapter 4, Good Chromatography/ Operating Practices).
	Large air bubble in detector flow cell	Refer to the Corrective Action under the “Non-cyclic (erratic) noise” symptom listed above.
	Dirty or malfunctioning pump inlet check valve	Refer to Section 3.1, Pump Troubleshooting
	Worn pump plunger seal	Refer to Section 3.1, Pump Troubleshooting
	Solvent from detector outlet dripping into waste container (primarily affecting flow sensitive detectors such as RI and electrochemical)	Position detector outlet against the side next to the waste container.

Table 2-3 Fluid Path-Related Baseline Noise Troubleshooting *(Continued)*

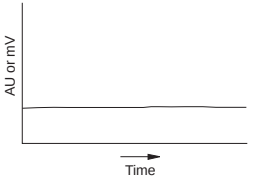
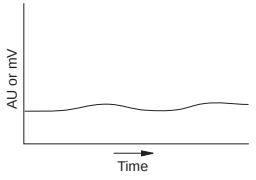
Symptom	Possible Cause	Corrective Action
Long-term cycling noise (minutes to hours) (originating from fluid path)  Normal baseline  Temperature fluctuations	Ambient temperature fluctuations	Stabilize operating environment temperature. If the problem continues: • Use a column heater (run 5° Celsius above ambient). • Relocate the system or column to a thermally stable environment. • Avoid placing the system in direct sunlight.
	Solvent is being recycled from detector waste outlet back through LC system	Unless absolutely necessary, do not recycle solvent through your LC system. Only use fresh and filtered solvent for your application.

Table 2-3 Fluid Path-Related Baseline Noise Troubleshooting *(Continued)*

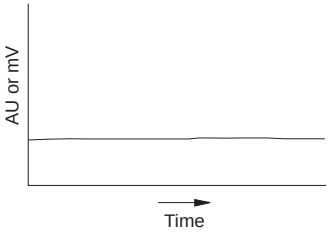
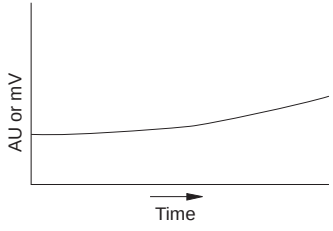
Symptom	Possible Cause	Corrective Action
Baseline drift (originating from fluid path)  Normal baseline  Contaminated column	System not stabilized or chemically equilibrated	Refer to the Corrective Action under the “Non-cyclic (erratic) noise” symptom listed above.
	Ambient temperature fluctuations	Refer to the Corrective Action under the “Long-term cycling noise” symptom listed above.
	Mobile phase contaminated (or decomposing)	Refer to the Corrective Action under the “Non-cyclic (erratic) noise” symptom listed above.
	Mobile phase improperly degassed or sparged	Degas or helium sparge the solvent(s) and re-equilibrate the system (see Section 4.1). ***Note:*** *Ensure that you limit the amount of helium sparging to avoid depleting mobile phase component(s).*

Table 2-3 Fluid Path-Related Baseline Noise Troubleshooting *(Continued)*

Symptom	Possible Cause	Corrective Action
Baseline drift (originating from fluid path) *(Continued)*	Detector flow cell leaking	Refer to Corrective Action under the “Non-cyclic (erratic) noise” symptom listed above.
	Contaminated column	Refer to the Corrective Action under the “Non-cyclic (erratic) noise” symptom listed above.
	Solvent is being recycled from detector waste outlet back through LC system	Unless absolutely necessary, do not recycle solvent through your LC system. Only use fresh and filtered solvent for your application.
	Leak(s) in system	Check all fitting for leaks. If there is a leaky fitting, tighten it (do *not* overtighten). If the leak continues, replace the fitting and ferrule (see Section 4.2 for considerations).

Table 2-3 Fluid Path-Related Baseline Noise Troubleshooting *(Continued)*

Symptom	Possible Cause	Corrective Action
Baseline drift (originating from fluid path) *(Continued)*	Stationary phase bleed	To verify this problem, replace all columns in the system with a union. Rerun the mobile phase and monitor the baseline. ***Note:*** *If you require additional system backpressure during this verification, use a piece of 0.009 -inch (0.23 mm) ID tubing instead of a union.* Ensure your operating conditions are suitable for the column (for example, solvent compatibility, pH range, and so on). If the operating conditions affect the column: • Select a different mobile phase • Select a different column Refer to the column operator's manual for information.
	Incorrect wavelength for solvent	Verify the background absorbance of the mobile phase using a spectrometer. If the background is high (you are unable to zero the detector baseline), the mobile phase contains a UV-absorbing compound, which causes baseline drift. Operate at a wavelength that is above the UV cutoff for the mobile phase (see Table A-3) or change the solvent(s).

Table 2-3 Fluid Path-Related Baseline Noise Troubleshooting *(Continued)*

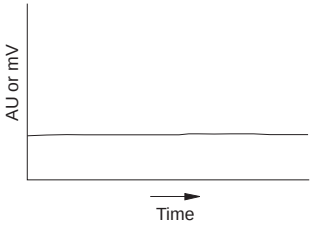
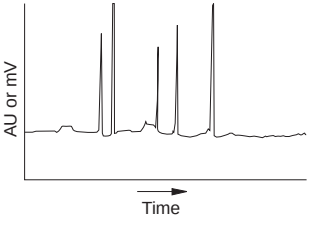
Symptom	Possible Cause	Corrective Action
Baseline drift (originating from fluid path) *(Continued)*	Mobile phase contains a stabilizer or there is a change in the stabilizer	Use a preservative-free solvent(s). ***Note:*** *Separations may require adjustment if changing to a preservative-free solvent.*
	Late-eluting sample component	Wash the column with an appropriate strong solvent.
	Unbalanced solvents (gradient operation)	Attempt to balance the UV absorbance with the mobile phase. If constrained by chemistry, run a gradient blank and subtract the baseline.
Noise spikes (originating from fluid path)  Normal baseline  Air bubbles in fluid path	Small air bubbles traveling through the fluid path	Refer to the Corrective Action under the “Non-cyclic (erratic) noise” symptom listed above.

Table 2-3 Fluid Path-Related Baseline Noise Troubleshooting *(Continued)*

Symptom	Possible Cause	Corrective Action
Noise spikes (originating from fluid path) *(Continued)*	Pump head cavitation	Refer to Section 3.1, Pump Troubleshooting.
	Particles in detector flow cell	Clean or backflush the detector flow cell (as described in the detector operator's manual).
	Improper grounding of pump or autoinjector electrical connections	Use a shielded signal cable and attach the shield to one device *only*. If shielding is not the problem, plug the pump or autoinjector into another outlet on a different electrical circuit, If a separate outlet is unavailable, use a line power conditioner.

Table 2-4 Detector Electronics-Related Baseline Noise Troubleshooting

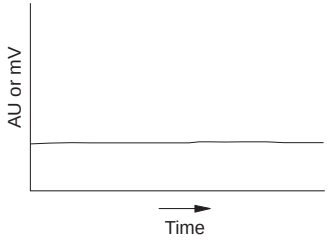
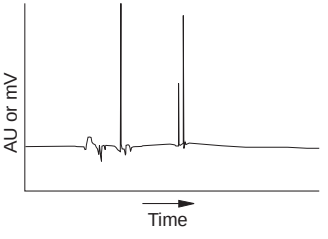
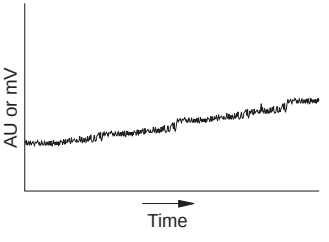
Symptom	Possible Cause	Corrective Action
Non-cyclic (erratic) noise (originating from detector)  Normal baseline  Defective lamp  Operating at high sensitivity	Detector not stabilized	Allow detector lamp sufficient time to stabilize (until baseline is stable). Detector equilibration time varies based on the type of detector used and operating parameters (such as wavelength, sensitivity, background, potential, and/or current). Refer to the detector operator's manual for recommended equilibration times.

Table 2-4 Detector Electronics-Related Baseline Noise Troubleshooting *(Continued)*

Symptom	Possible Cause	Corrective Action
Non-cyclic (erratic) noise (originating from detector) *(Continued)*	Defective detector lamp	Verify lamp energy using detector diagnostics. If energy is below specification (as compared when the lamp was new), replace the lamp. ***Note:*** *Some detectors allow you to adjust lamp energy to compensate for decreased energy. Refer to your detector operator's manual for information on adjusting lamp energy.*
	Contaminated detector flow cell	Refer to Section 3.5, Detector Troubleshooting.
	Detector electronics problem	Detector malfunction. Contact Customer Support.
	Cable loose or improperly connected between detector and data-handling system (computer, integrator, or chart recorder)	Verify that the correct detector output signal is properly connected to the data-handling device. Ensure that any related output signal switch settings are in the proper position. Refer to the detector's operator's manual and data-handling device operator's manual.

Table 2-4 Detector Electronics-Related Baseline Noise Troubleshooting *(Continued)*

Symptom	Possible Cause	Corrective Action
Non-cyclic (erratic) noise (originating from detector) *(Continued)*	Detector improperly grounded	Use a shielded signal cable and attach the shield to one device *only.* If shielding is not the problem, plug the detector into another outlet on a different electrical circuit. If a separate outlet is unavailable, use a line power conditioner.
	Cycling equipment or radio frequency (RF) interference	Isolate the detector from other equipment in the lab, especially devices with large electric motors. Then: • Check circuit grounding and line voltage quality (refer to the “Detector improperly grounded” Possible cause above for the corrective action). • Ensure that the detector and the data-handling device are on the same common ground. Relocate if necessary. • If necessary, relocate the detector to an area where RF is not a problem or use a Faraday cage around the detector.

Table 2-4 Detector Electronics-Related Baseline Noise Troubleshooting *(Continued)*

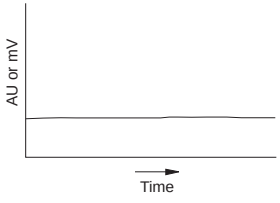
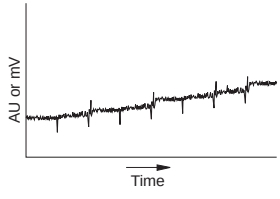
Symptom	Possible Cause	Corrective Action
Non-cyclic (erratic) noise (originating from detector) *(Continued)*	Data-handling device gain/sensitivity setting too high	Change to a lower gain/sensitivity setting (see data-handling device operator's manual).
	Leak from reference electrode (electrochemical detector only)	Repair or replace the reference electrode (see detector operator's manual).
	Foulded reference electrode (electrochemical detector only)	Renew reference electrode filling solution and/or replace the frit (see detector operator's manual).
	Contaminated or scratched working electrode (electrochemical detector only)	Clean or polish working electrode (see detector operator's manual). If problem persists, replace the working electrode.
Short-term cycling noise (originating from detector) (seconds to minutes)  Normal baseline  Cycling equipment	Cycling equipment or radio frequency (RF) interference	Refer to the Corrective Action under the "Non-cyclic (erratic) noise" symptom listed above.

Table 2-4 Detector Electronics-Related Baseline Noise Troubleshooting *(Continued)*

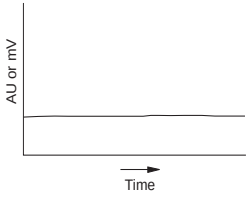
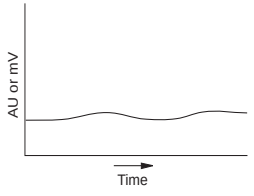
Symptom	Possible Cause	Corrective Action
Short-term cycling noise (originating from detector) (seconds to minutes) *(Continued)*	Internal detector temperature improperly set (heater cycles on and off too frequently)	Correctly set the detector internal heater (see detector operator's manual).
Long-term cycling noise (originating from detector) (minutes to hours)  Normal baseline  Temperature fluctuations	Ambient temperature fluctuations	Stabilize operating environment temperature to allow full equilibration. If problem continues: • Relocate the detector to a thermally stable environment or close any open air vents. • Avoid placing the system in direct sunlight.
	Cycling equipment or radio frequency (RF) interference	Refer to the Corrective Action under the "Non-cyclic (erratic) noise" symptom listed above.

Table 2-4 Detector Electronics-Related Baseline Noise Troubleshooting *(Continued)*

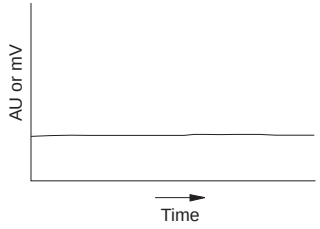
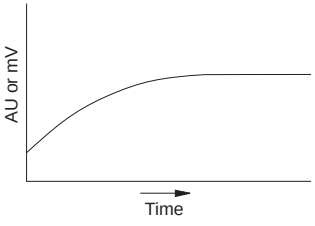
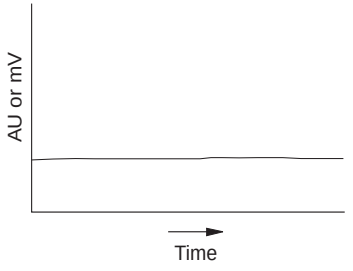
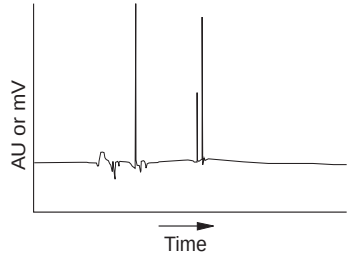
Symptom	Possible Cause	Corrective Action
Baseline drift (originating from detector)  Normal baseline  Insufficient stabilization	Detector not stabilized	Refer to the Corrective Action under the “Non-cyclic (erratic) noise” symptom listed above.
	Change in ambient temperature	Refer to the Corrective Action under the “Long-term cycling noise” symptom listed above.
	Contaminated detector flow cell	Refer to Section 3.5, Detector Troubleshooting.
	Fouled reference electrode (electrochemical detector only)	Renew reference electrode filling solution (see detector operator’s manual). If problem persists, replace the frit.

Table 2-4 Detector Electronics-Related Baseline Noise Troubleshooting *(Continued)*

Symptom	Possible Cause	Corrective Action
	Contaminated or scratched working electrode (electrochemical detector only)	Clean or polish working electrode (see detector operator's manual). If problem persists, replace the working electrode.
Noise spikes (originating from detector)  Normal baseline  Defective lamp	Defective detector lamp	Refer to the Corrective Action under the "Non-cyclic (erratic) noise" symptom listed above.
	Cycling equipment or radio frequency (RF) interference	Refer to the Corrective Action under the "Non-cyclic (erratic) noise" symptom listed above.
	Detector electronics problem	Detector malfunction. Contact your Customer Support Department.

2.2.3 Changes in Chromatographic Resolution or Results

Chromatographic problems typically exhibit themselves as:

- Incorrect or changing retention time(s)
- No peaks or abnormal peak shape(s)
- Loss of resolution
- Incorrect qualitative or quantitative results

When your chromatographic results are unacceptable (as compared with established performance), reinject your application standard(s) and translate your observations into specific problems with your system.

For example, problems that appear to be due to abnormal peak shape can be due to other issues with chromatographic performance. Before investigating solutions to abnormal peak shape, verify that incorrect retention time is not affecting your chromatography.

Troubleshooting unacceptable chromatographic results involves two stages:

- Sequentially evaluate your chromatographic results (retention time, peak shape, resolution, and qualitative/quantitative results)
- Isolate the source of the problem

The flow diagram in Figure 2-5 presents questions to help you narrow down your problem. Once you isolate the problem, refer to the appropriate troubleshooting section.

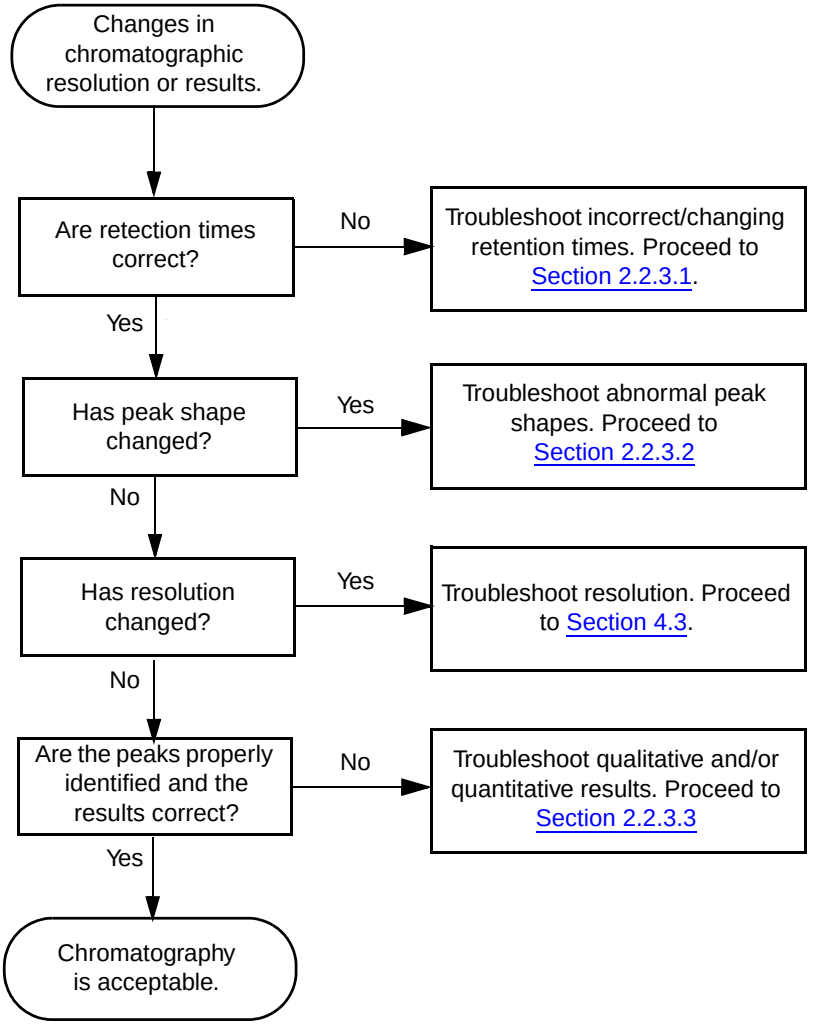


Figure 2-5 Isolating the Source of Changing Chromatographic Resolution/Results

2.2.3.1 Incorrect/Changing Retention Times

Troubleshooting overview

Incorrect retention time is characterized as:

- Erratic (changing back and forth from run to run)
- Steadily increasing
- Steadily decreasing
- Changed to a constant new value (incorrect but reproducible)

Table 2-5 covers incorrect/changing retention time. From the table:

1. Review the retention time symptoms and select the symptom that best typifies your problem.
2. Review the possible causes and follow the corrective actions.

Retention time stability factors

Retention time stability is affected by:

- System and column equilibration
- Mobile phase (composition, preparation, improper degassing, stability)
- Column age
- Operating temperature
- Pump performance (flow rate, pressure, loss of prime)

Isolating retention time changes

When evaluating retention times changes, it is critical that you have previously established system performance benchmarks. From those benchmarks, you determine whether retention times:

- Erratically change in a different direction (either increasing or decreasing) from run to run.
- Change in the same direction (increasing or decreasing) from run to run.
- Suddenly change to a new value (increased or decreased), which is outside the allowable range for the assay, and then hold at that value for a number of runs.

Table 2-5 Incorrect/Changing Retention Time Troubleshooting

Symptom	Possible Cause	Corrective Action
Erratic retention times (changing from run to run)	System not stabilized or chemically equilibrated	Allow all system components (such as the column and detector) sufficient time to stabilize and chemically equilibrate. Note the operating conditions of your application (such as mobile phase, detector settings, and detector type). Refer to the instrument or column operator's manual for recommended equilibration times. If running an automated gradient method, ensure sufficient and reproducible equilibration times are used between injections. ***Note:*** *If using Ion-pairing reagents, ensure that the first time you use the column you provide a sufficient time and volume of solvent to adequately equilibrate the column (for example, running a total volume of 100 mL of a 5 mM solution at 1 mL/min).*

Table 2-5 Incorrect/Changing Retention Time Troubleshooting *(Continued)*

Symptom	Possible Cause	Corrective Action
Erratic retention times (changing from run to run) *(Continued)*	Erratic pump pressure/pump pulsations (due to problems such as air bubbles, worn dirty/malfunctioning check valves, worn plunger seals, worn plunger)	Refer to Figure 2-4 (in Section 2.2.1) to verify the source of erratic pressure. If erratic pressure continues, refer to Section 3.1, Pump Troubleshooting.
	Injection volume/sample concentration too high (sample overload), disrupting equilibrium	Reduce the injection volume or dilute the sample with mobile phase. If using a weaker solvent, you can inject up to 10% of column void volume. If using a stronger solvent, you can inject up to 1% of column void volume.
	Ambient temperature fluctuations	Stabilize operating environment temperature to allow full equilibration. If problem continues: • Use a column heater (run 5° Celsius above ambient). • Ensure the solvent is stirred. • Relocate the system or column to a thermally stable environment or close any open air vents.

Table 2-5 Incorrect/Changing Retention Time Troubleshooting *(Continued)*

Symptom	Possible Cause	Corrective Action
Erratic retention times (changing from run to run) *(Continued)*	Inadequate solvent blending	To confirm a mixing problem: 1. Premix, filter, and degas the mobile phase. 2. With the column in-line, pump a minimum of 5 to 10 column volumes through a single pump (or solvent line) to equilibrate the column. 3. Inject a standard a minimum of 3 times and compare the reproducibility of the retention times to the previous injections (with erratic retention times). If retention times are reproducible with the premixed solvent, this indicates a solvent blending problem. Verify the mixing problem and correct as outlined below: 1. Use of immiscible solvents. Verify miscibility of solvents (see Appendix A, Reference Information) and change to more miscible solvents.

Table 2-5 Incorrect/Changing Retention Time Troubleshooting *(Continued)*

Symptom	Possible Cause	Corrective Action
Erratic retention times (changing from run to run) *(Continued)*	Inadequate solvent blending	2. Malfunction in the pump, the pump solvent proportioning valve, or the high-pressure mixer. Troubleshoot per Section 3.1, Pump Troubleshooting. 3. Inadequate blending after the pump. The solution is to add additional mixing. However, the mixing required depends on the severity of the problem. For the Corrective Action, refer to Table 2-3, the "Short-term cycling noise" symptom. 4. Use premixed solvent(s).

Table 2-5 Incorrect/Changing Retention Time Troubleshooting *(Continued)*

Symptom	Possible Cause	Corrective Action
Erratic retention times (changing from run to run) *(Continued)*	Column contamination	To verify this problem as a possible cause, replace the column with a known good column of the same type. Rerun the analysis and observe if retention times stabilize. If the problem stops, clean or replace the column as outlined in the column operator's manual. If the retention time continues to be erratic, this could be due to: • Solvent properties such as miscibility. Refer to Section 4.1 and Appendix A, Reference Information for information. • Contaminated mobile phase (refer to the "Mobile phase contaminated" Possible Cause later in this table for the corrective action). • Contaminated guard column or in-line filter (clean or replace as outlined in the operator's manual).
Increased or decreased retention times (continuously changing in the same direction)	Pump flow rate changed	Verify the solvent flow rate setting. Set to appropriate flow rate for the application.

Table 2-5 Incorrect/Changing Retention Time Troubleshooting *(Continued)*

Symptom	Possible Cause	Corrective Action
Increased or decreased retention times (continuously changing in the same direction) *(Continued)*	Ambient temperature change	Refer to the Corrective Action under the "Erratic retention times" symptom listed above.
	System not equilibrated or chemically stabilized	Refer to the Corrective Action under the "Erratic retention times" symptom listed above.
	Column contaminated	Refer to the Corrective Action under the "Erratic retention times" symptom listed above.
	Column degraded (loss of column chemistry)	Verify column performance by measuring capacity factor (k') and selectivity (see Chapter 4). If either measurement has changed, adjust as outlined in Chapter 4 .
	Mobile phase improperly degassed or sparged	Degas or helium sparge the solvent(s) and re-equilibrate the system (see Section 4.1 for information). ***Note:*** *Ensure that you limit the amount of helium sparging to avoid depleting mobile phase component(s).*

Table 2-5 Incorrect/Changing Retention Time Troubleshooting *(Continued)*

Symptom	Possible Cause	Corrective Action
Increased or decreased retention times (continuously changing in the same direction) *(Continued)*	Mobile phase contaminated	Discard the contaminated mobile phase and: • Clean the solvent reservoir. Clean or replace the solvent inlet filter. To clean, remove the filter and sonicate using 6N nitric acid, water (repeat 3 times), followed by methanol. • Prepare and filter fresh solvent using only high-quality reagents and HPLC-grade solvents (see Section 4.1 for solvent preparation and use considerations). • Flush and re-equilibrate the system.
	Solvent inlet filter or inlet lines blocked	Check the lines for blockages. Replace if necessary. Clean the solvent inlet filter frit (as outlined above). Replace the frit if necessary.

Table 2-5 Incorrect/Changing Retention Time Troubleshooting *(Continued)*

Symptom	Possible Cause	Corrective Action
Increased or decreased retention times (continuously changing in the same direction) *(Continued)*	Leak(s) in system	Check all fittings for leaks. If there is a leaky fitting, tighten it (do *not* overtighten). If the leak continues, replace the fitting and ferrule (see Section 4.2 for considerations).
Retention time changed to a new constant value (reproducible but incorrect)	Incorrect mobile phase or incorrect composition for the sample	Prepare fresh mobile phase.
	Pump flow rate changed	Verify the solvent flow rate setting. Set to appropriate flow rate for the application.

Table 2-5 Incorrect/Changing Retention Time Troubleshooting *(Continued)*

Symptom	Possible Cause	Corrective Action
Retention time changed to a new constant value (reproducible but incorrect) *(Continued)*	Incorrect flow rate being delivered (due to pump malfunction)	To verify this problem: 1. Place the detector waste line in a 5.0 mL graduated cylinder. 2. Set flow rate to 1.0 mL/min. (or a setting that is more appropriate for your application). 3. Using a stopwatch, measure the flow rate. Collect the mobile phase in the cylinder for at least 4 minutes to determine the actual flow rate of the system (in mL/min). 4. Divide the volume collected by the collection time to determine the actual flow rate of the system (in mL/min). If the measured flow rate is different, there is a pump malfunction. Refer to Section 3.1, Pump Troubleshooting.
	Ambient temperature change	Refer to the Corrective Action under the "Erratic retention times" symptom listed above.
	Incorrect temperature setting on column heater	Change to the correct operating temperature.

Table 2-5 Incorrect/Changing Retention Time Troubleshooting *(Continued)*

Symptom	Possible Cause	Corrective Action
Retention time changed to a new constant value (reproducible but incorrect) *(Continued)*	Incorrect column size or type	Verify the source or type of the column. Use a column identical to the column used during methods development.
	Mobile phase contains a stabilizer, or there is a change in the stabilizer	Use a preservative-free solvent. ***Note:*** *Separations may require adjustment if changing to a preservative-free solvent.*
	Column contaminated	Refer to the Corrective Action under the "Increased or decreased retention times" symptom listed above.
	Incorrect gradient delay volume for the fluidic system	Determine whether a change has been made to fluidic system (for example, addition of a gradient mixer). If a change has been made, recalculate the new gradient delay volume (see pump operator's manual).

2.2.3.2 Abnormal Peak Shape

Troubleshooting overview

Abnormal peak shape is characterized by:

- No peaks
- Smaller than expected peaks
- Broad peaks (only early-eluting peaks and all peaks
- Double/shoulder peaks
- Fronting peaks
- Tailing peaks
- Flat-topped peaks
- Negative peaks (one or more)

Table 2-7 covers no peaks and abnormal peak shapes. Prior to Table 2-7 is Table 2-6, a summary of abnormal peak shapes. From these examples:

1. Review peak shape symptoms and select the symptom that best typifies your system problem.
2. Proceed to the page number of the abnormal peak shape symptom.
3. Review the possible causes and follow the corrective actions.

Verify abnormal peak shape occurrence

Determine whether the abnormal peak shape occurs:

- For all peaks throughout the chromatogram (standards and samples)
- For selected peaks within the chromatogram

All peaks

If all peaks are abnormal, this indicates a system-related problem, such as loss of column efficiency or incorrect detector settings.

If early eluting peaks are abnormal, check for problems within the system such as the injector, incorrect tubing/fittings, leaking flow cell, and detector time constant.

Selected peaks

If one or more peaks in a chromatogram are abnormal, this may imply a specific chemistry problem. Examine the steps you used to develop the method in use.

In a gradient method, if the early eluting peaks are abnormal while the later peaks are acceptable, this is may be due to pre-column band-broadening, high injection volume, or sample diluent is too strong. If all the peaks have deteriorated, this implies post-column band-broadening, column deterioration, or other changes in the system.

In an isocratic method, if the early eluting peaks are abnormal while the later peaks are acceptable, this implies post-column band-broadening, injector failure, incorrect detector time constant, or wrong A/D sampling time for the data-handling device. If all the peaks have deteriorated, this implies post-column band-broadening, column deterioration, or other changes in the system.

Abnormal peak shape summary

Table 2-6 is a summary of the abnormal peak shapes listed in Table 2-7. Review individual peak shape symptoms and select the symptom that best typifies your system problem

Table 2-6 Abnormal Peak Shape Summary

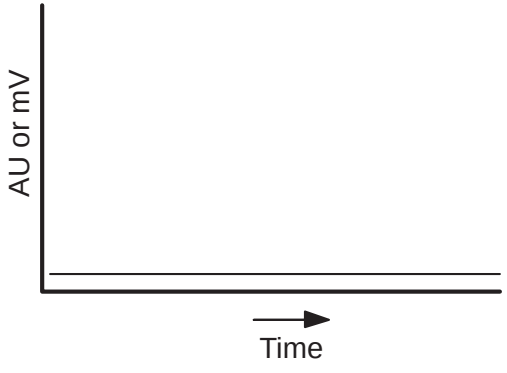
Abnormal Peak Shape Symptom
No peaks 

Table 2-6 Abnormal Peak Shape Summary *(Continued)*

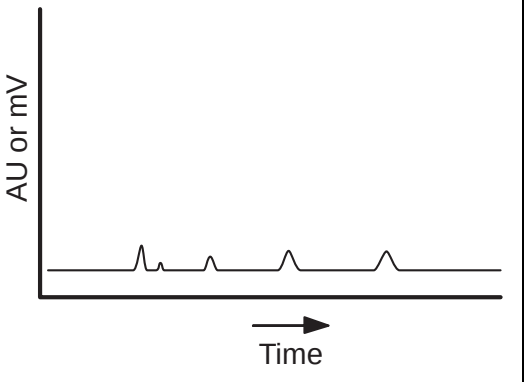
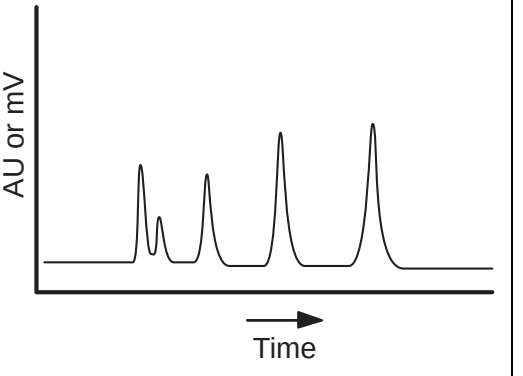
Abnormal Peak Shape Symptom	
Smaller than expected peaks	
Broad peaks (only early -eluting peaks)	

Table 2-6 Abnormal Peak Shape Summary *(Continued)*

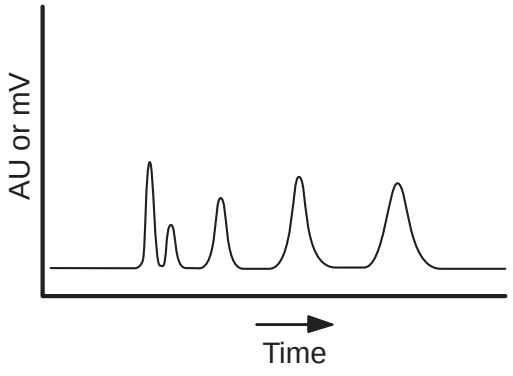
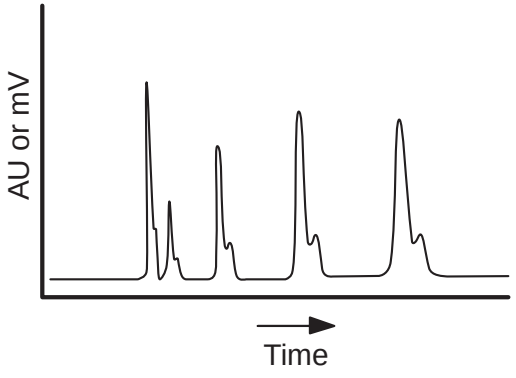
Abnormal Peak Shape Symptom	
Broad peaks (all peaks)	
Double/shoulder peaks	

Table 2-6 Abnormal Peak Shape Summary *(Continued)*

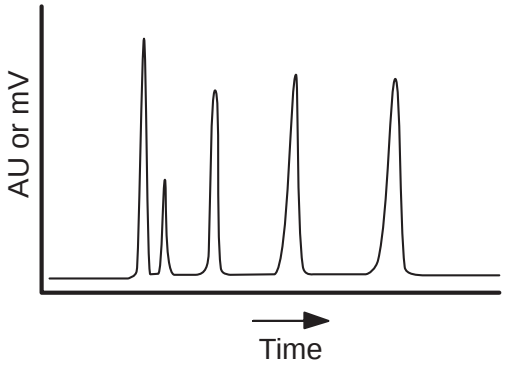
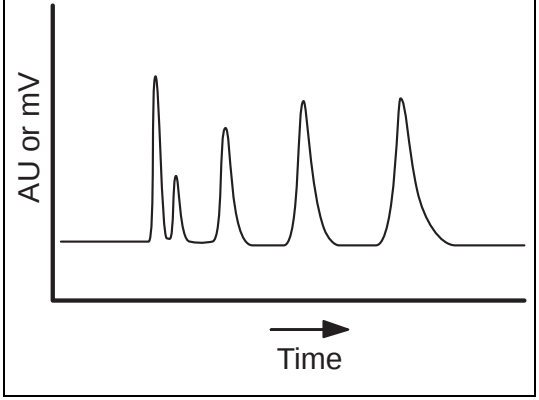
Abnormal Peak Shape Symptom	
Fronting peaks	
Tailing peaks	

Table 2-6 Abnormal Peak Shape Summary *(Continued)*

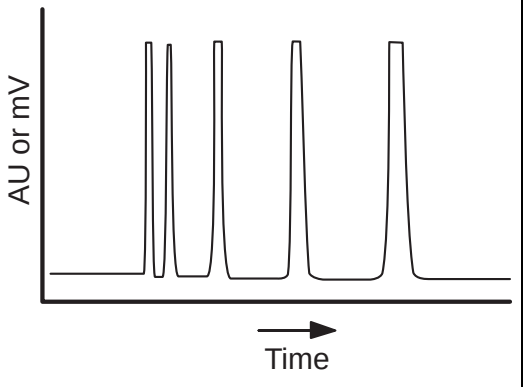
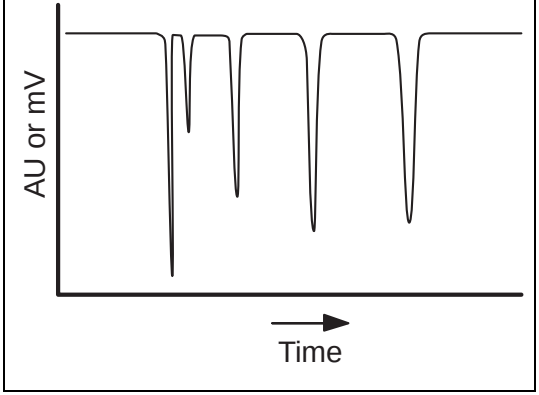
Abnormal Peak Shape Symptom	
Flat-topped peaks	
Negative peaks (all peaks)	

Table 2-6 Abnormal Peak Shape Summary *(Continued)*

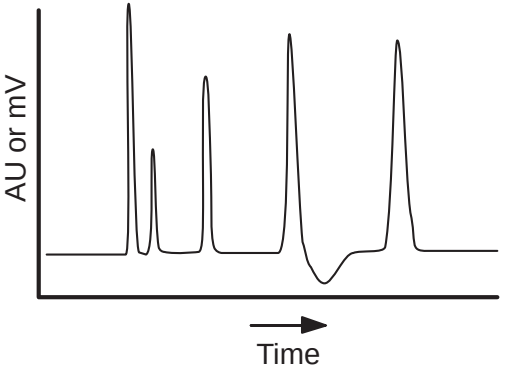
Abnormal Peak Shape Symptom	
Negative peaks (one or more peaks)	

Table 2-7 Abnormal Peak Shape Troubleshooting

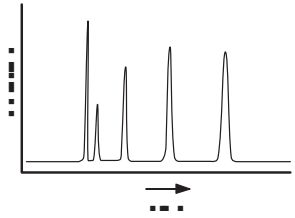
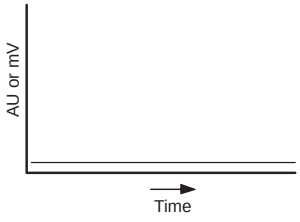
Symptom	Possible Cause	Corrective Action
No Peaks  Normal Peaks	Injector problem (due to wrong vial, no vial, insufficient sample volume, incorrect injection, blocked needle)	Refer to Section 3.2, Manual Injector Troubleshooting, or Section 3.3, Autoinjector Troubleshooting.
 No Peaks	No (or low) flow being delivered	Verify if this is due to: • **Pump power not on or not delivering solvent -** Observe if solvent is flowing from detector waste outlet. Ensure pump is on and is delivering solvent. • **Reservoir low or out of solvent** - Check level of solvent. If necessary, refill reservoir (degas/sparge solvent). • **Blocked solvent reservoir inlet filter or inlet lines** - Check lines for blockages. Replace if necessary. Clean or replace the solvent inlet filter frit. Remove and clean by sonicating using 6N nitric acid, water (repeat 3 times), followed by methanol. If you suspect a pump problem, refer to Section 3.1, Pump Troubleshooting.

Table 2-7 Abnormal Peak Shape Troubleshooting *(Continued)*

Symptom	Possible Cause	Corrective Action
No Peaks (continued)	Incorrect detector settings (such as wavelength, sensitivity, auto zero)	Verify and adjust any incorrect detector settings (see detector operator's manual).
	Detector output not zeroed	Zero detector baseline (see detector operator's manual).
	Detector problem (due to flow cell, power supply, electronics)	Refer to Section 3.5, Detector Troubleshooting.
	Cable improperly connected between detector and data-handling system (computer, integrator, or chart recorder)	Check that the correct detector output signal is properly connected to the data-handling device. Ensure that related output signal switch settings are in the proper position. Refer to the detector operator's manual and data-handling device operator's manual.
	Incorrect mobile phase	Prepare new mobile phase. ***Note:*** *If this problem occurs in addition to a high system backpressure, this could indicate precipitation of sample.*
	Degraded sample	Verify the integrity of sample and review sample preparation process. Replace with a fresh sample.

Table 2-7 Abnormal Peak Shape Troubleshooting *(Continued)*

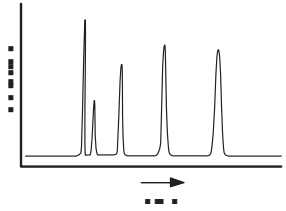
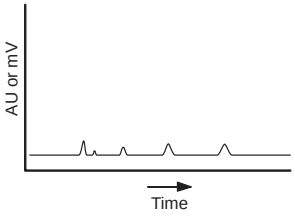
Symptom	Possible Cause	Corrective Action
Smaller than expected peaks (loss of sensitivity)  Normal Peaks  Smaller than expected peaks	Wrong injection volume	Change to appropriate injection volume.
	Incorrect size sample loop in injector	Verify sample loop in use. If necessary, replace with an appropriate size sample loop (see injector operator's manual).
	Incorrect detector settings (such as wavelength, sensitivity, or time constant)	Change to correct detector settings (see detector operator's manual).
	Detector output not zeroed	Zero detector baseline (see detector operator's manual).
	Incorrect output signal used between detector and data-handling system (computer, integrator, or chart recorder)	Refer to the Corrective Action under the "No peaks" symptom listed above.

Table 2-7 Abnormal Peak Shape Troubleshooting *(Continued)*

Symptom	Possible Cause	Corrective Action
Smaller than expected peaks (loss of sensitivity) *(Continued)*	Contaminated detector flow cell	Refer to Section 3.5, Detector Troubleshooting.
	Defective detector lamp	Check the lamp energy using detector diagnostics. If the energy is below specification (as compared when the lamp was new), replace the lamp. ***Note:*** *Some detectors allow you to adjust the lamp energy to compensate for decreased energy. Refer to your detector operator's manual for information on adjusting lamp energy.*
	Injector problem (due to wrong vial, no vial, insufficient sample volume, incorrect injection, blocked needle)	Refer to Section 3.2, Manual Injector Troubleshooting, or Section 3.3, Autoinjector Troubleshooting.
	Sample too viscous	Dilute sample or decrease syringe draw speed rate.

Table 2-7 Abnormal Peak Shape Troubleshooting *(Continued)*

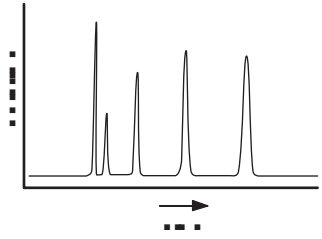
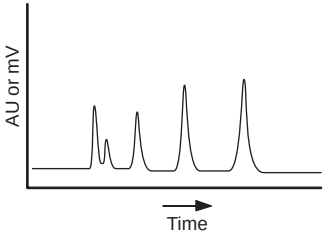
Symptom	Possible Cause	Corrective Action
Broad Peaks (only early-eluting peaks)  Normal Peaks  Broad Peaks (early eluters only)	Injection volume/sample concentration too high (sample overload), disrupting equilibrium	Reduce the injection volume or dilute the sample with mobile phase. If using a weaker solvent, you can inject up to 10% of column void volume. If using a stronger solvent, you can inject up to 1% of column void volume.
	Incorrect ID tubing, improperly cut tubing, improper fittings and ferrules	Perform a system band spread test (see Chapter 4). If the result is greater than established benchmarks, verify tubing ID, cut of tubing, and compatibility of fittings/ferrules. Refer to your system map to ensure that appropriate tubing, fittings, and ferrules are used. Replace if necessary. Refer to Chapter 4 for information.
	In-line filter, guard column inlet, column inlet, or connecting tubing partially blocked	Inspect these components for particle build-up. Replace any blocked tubing (see Chapter 4). If the problem is in the in-line filter, guard column, or column inlet, clean or replace (see operator's manual).
	Injector problem (such as a sticking or leaking valve, blocked or damaged needle, plugged injection port)	Refer to Section 3.2, Manual Injector Troubleshooting, or Section 3.3, Autoinjector Troubleshooting.

Table 2-7 Abnormal Peak Shape Troubleshooting *(Continued)*

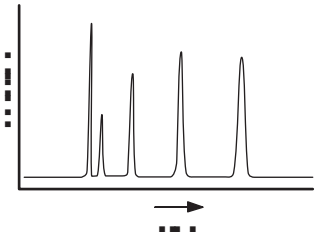
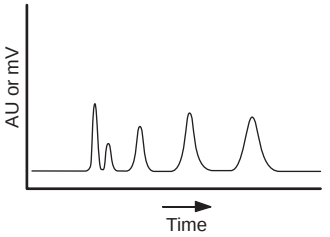
Symptom	Possible Cause	Corrective Action
Broad Peaks (only early-eluting peaks) *(Continued)*	Incorrect size sample loop in injector	Verify sample loop in use. If necessary, replace with an appropriate size sample loop (see injector operator's manual).
	Incorrect detector time constant setting	Verify the time constant setting and adjust if necessary (see detector operator's manual).
Broad Peaks (all peaks)  Normal Peaks  Broad Peaks (all peaks)	Column or guard column contaminated	Clean the column or guard column (see column operator's manual). If problem persists, replace the column.
	Column degraded (due to loss of efficiency or voided column)	Verify column performance by measuring column efficiency (N) (see Chapter 4). If the column efficiency measurement is low (as compared when the column was new), replace the column.
	Guard column degraded	Remove the guard column and resume operation. If results are normal, replace the guard column.

Table 2-7 Abnormal Peak Shape Troubleshooting *(Continued)*

Symptom	Possible Cause	Corrective Action
Broad Peaks (all peaks) *(Continued)*	Sample solvent too strong for the mobile phase	Perform one of the following: • Dissolve sample in mobile phase • Use a weaker diluent • Make smaller injections
	Incorrect column size or type	Verify the source or type of the column. Use a column identical to that used during methods development.
	Ambient temperature change	Use a column heater to control temperature. A change in temperature may also result in a change in retention time (reproducible but incorrect).

Table 2-7 Abnormal Peak Shape Troubleshooting *(Continued)*

Symptom	Possible Cause	Corrective Action
Broad Peaks (all peaks) *(Continued)*	System not stabilized or chemically equilibrated	Allow all system components (such as the column and detector) sufficient time to stabilize and chemically equilibrate. Note the operating conditions of your application (such as mobile phase, detector settings, detector type). Refer to the instrument or column operator's manual for recommended equilibration times. If running an automated gradient method, ensure sufficient and reproducible equilibration times are used between injections. If using ion-pairing reagents, ensure that the first time you use the column you provide a sufficient time and volume of solvent to adequately equilibrate the column (for example, running a total volume of 100 mL of a 5 mM solution at 1 mL/min).
	Incorrect chart recorder speed	Adjust chart recorder speed (see chart recorder operator's manual).

Table 2-7 Abnormal Peak Shape Troubleshooting *(Continued)*

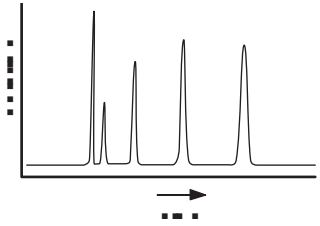
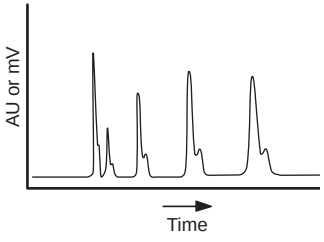
Symptom	Possible Cause	Corrective Action
Double Peaks/Shoulder Peaks  Normal Peaks  Double/Shoulder Peaks	Guard column or column inlet partially blocked	Refer to the Corrective Action under the "Broad peaks" symptom listed above.
	Column or guard column contaminated	Clean the column or guard column (see column operator's manual). If problem persists, replace the column or guard column.
	Column degraded (due to voiding)	Refer to the Corrective Action under the "Broad peaks" symptom listed above.
	Guard column degraded	Remove the guard column and resume operation. If results are normal, replace the guard column.
	Injection volume/sample concentration too high (sample overload), disrupting equilibrium	Refer to the Corrective Action under the "Broad peaks" symptom listed above.

Table 2-7 Abnormal Peak Shape Troubleshooting *(Continued)*

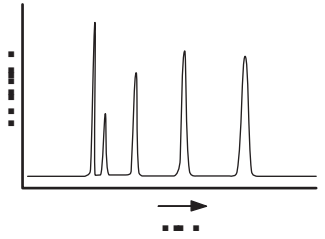
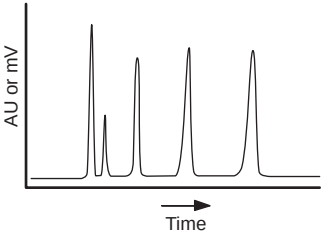
Symptom	Possible Cause	Corrective Action
Fronting Peaks  Normal Peaks  Fronting Peaks	Injection volume/sample concentration too high (sample overload), disrupting equilibrium	Refer to the Corrective Action under the “Broad peaks” symptom listed above
	Sample solvent too strong for the mobile phase	Refer to the Corrective Action under the “Broad peaks” symptom listed above
	Column or guard column contaminated	Clean the column or guard column (see column operator's manual). If problem persists, replace the column or guard column.
	Column degraded (due to voiding)	Refer to the Corrective Action under the "Broad peaks" symptom listed above.
	Guard column degraded	Remove the guard column and resume operation. If results are normal, replace the guard column.

Table 2-7 Abnormal Peak Shape Troubleshooting *(Continued)*

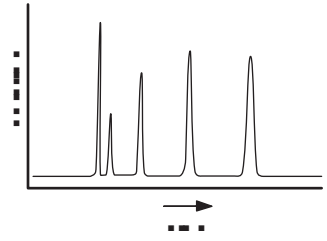
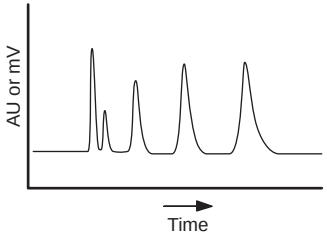
Symptom	Possible Cause	Corrective Action
Tailing Peaks  Normal Peaks  Tailing Peaks	Column or guard column contaminated	Clean the column or guard column (see column operator's manual). If problem persists, replace the column or guard column.
	Column degraded (due to voiding)	Refer to the Corrective Action under the "Broad peaks" symptom listed above.
	Guard column degraded	Remove the guard column and resume operation. If results are normal, replace the guard column.
	Injector problem (such as a sticking or leaking valve, blocked or damaged needle, plugged injection port)	Refer to Section 3.2, Manual Injector Troubleshooting, or Section 3.3, Autoinjector Troubleshooting.
	Incorrect detector time constant setting	Change to correct setting.

Table 2-7 Abnormal Peak Shape Troubleshooting *(Continued)*

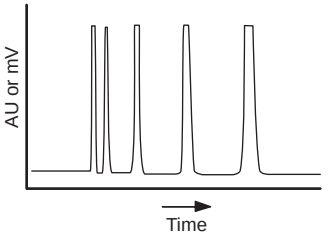
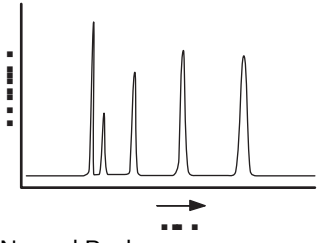
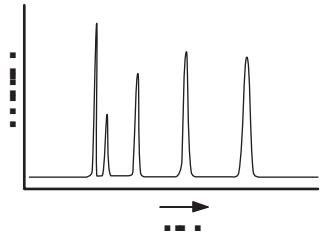
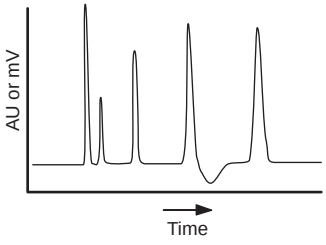
Symptom	Possible Cause	Corrective Action
Flat-topped Peaks  Flat Topped Peaks	Incorrect detector settings (such as wavelength, sensitivity, or auto zero)	Adjust any incorrect detector settings (see detector operator's manual).
	Incorrect recorder input voltage	Adjust recorder input voltage.
	Injection volume/sample concentration too high	Refer to the Corrective Action under the "Broad peaks" symptom listed above.
Negative Peaks (all peaks)  Normal Peaks	Signal cables to data-handling device reversed	Correctly attach cables (see detector operator's manual).
	Signal polarity setting improperly set on chart recorder or detector	Change the polarity setting (see detector or chart recorder operator's manual).
	Unbalanced optics (RI detector only)	Refer to detector operator's manual.

Table 2-7 Abnormal Peak Shape Troubleshooting *(Continued)*

Symptom	Possible Cause	Corrective Action
Negative Peaks (one or more peaks)  Normal Peaks	System peak in ion pair separation	Dissolve the sample in mobile phase. Modify the separation to move the system peak from the analytes.
 Negative Peaks (one or more)	Sample has a component with a RI lower than the mobile phase (RI detector only).	Determine whether the negative peak is due to the sample or solvent impurities. If the peak interferes with your analysis, modify your method. If the peak is due to solvent impurities, use fresh solvent.
	Highly absorbing mobile phase	Use the mobile phase as the sample diluent. If the problem continues, adjust the mobile phase so that the negative peak does not interfere with the peak of interest. Operate at a wavelength that is above the UV cutoff for the mobile phase (see Table A-3) or change the solvent(s).
	Injection of air by autoinjector	Refer to Section 3.3, Autoinjector Troubleshooting.

2.2.3.3 Incorrect Qualitative/Quantitative Results

Troubleshooting overview

Incorrect qualitative results are characterized as:

- Misidentification of peaks
- Chromatogram has more peaks than expected (sometimes known as ghost peaks)
- Chromatogram has fewer peaks than expected

Incorrect quantitative results are characterized as:

- Loss of accuracy
- Loss of precision

Table 2-8 covers inaccurate qualitative/quantitative analysis results. From Table 2-8:

1. Select the symptom that best typifies your system problem.
2. Review the possible causes and follow the corrective actions.

Troubleshooting accuracy

Accuracy is defined as the closeness of a result is to the true value. Precision is defined as the reproducibility of the result. The definition of required and expected accuracy/precision is an essential part of the method development process. This definition is part of your established benchmarks.

When there is a problem with either the accuracy or precision of a result, base your judgement on a minimum of three consecutive injections. If the results are incorrect but consistent for at least three injections, there is a problem with accuracy. If the results vary over time, there is a problem with precision.

Table 2-8 Incorrect Qualitative/Quantitative Results Troubleshooting

Symptom	Possible Cause	Corrective Action
Qualitative Results		
Misidentification of peaks	Incorrect values entered in data-handling system	Verify values entered in such areas as: • Sample tables • Calibration tables • Reference peaks • Peak windows (overlapping, too wide) • Peak threshold • Peak integration • Retention time Make the appropriate change(s), rerun standards, and verify that accuracy improves.
	Changing retention time	Refer to Section 2.2.3.1, Incorrect/Changing Retention Times.
No peaks found	Incorrect values entered in data-handling system	Refer to the Corrective Action under the "Misidentification of peaks" symptom listed above.
	Changing retention time	Refer to Section 2.2.3.1, Incorrect/Changing Retention Times.

Table 2-8 Incorrect Qualitative/Quantitative Results Troubleshooting *(Continued)*

Symptom	Possible Cause	Corrective Action
Chromatogram has more peaks than expected (ghost peaks)	Mobile phase contaminated	Discard the contaminated mobile phase and: • Clean the solvent reservoir. Clean or replace the solvent inlet filter. To clean, remove the filter and sonicate using 6N nitric acid, water (repeat 3 times), followed by methanol. • Prepare and filter fresh solvent using only high quality reagents and HPLC-grade solvents (see Section 4.1 for solvent preparation and use considerations) • Flush and re-equilibrate the system
	Degraded sample or impurities introduced during sample preparation	Verify chromatography running a standard. If results are normal, there is a problem with the sample. Verify the integrity of sample. Review the sample preparation process. Replace with a fresh sample.
	Late-eluting compounds from previous injection	Increase the overall run time between injections to allow for late-eluting components. If the problem continues, flush the column with a strong solvent between runs.

Table 2-8 Incorrect Qualitative/Quantitative Results Troubleshooting *(Continued)*

Symptom	Possible Cause	Corrective Action
Chromatogram has more peaks than expected (ghost peaks) (continued)	Needlewash system malfunction	Extra peaks as a result of the needlewash system could be due to: • Defective fluid valve • Defective needlewash pump • Autoinjector siphoning solvent from needlewash reservoir For the corrective action, refer to Section 3.3, Autoinjector Troubleshooting.
	Inadequate sample loop flushing	Flush sample loop with mobile phase between injections (see manual injector operator's manual).
	Dirty syringe	Use a clean syringe. Flush the injection port (see manual injector operator's manual).
	Contaminated injector	Flush the injector and sample loop (see manual injector operator's manual). If necessary. Replace seals and filters (see injector operator's manual).
	Column contaminated	Clean the column (see column operator's manual). If problem persists, replace the column.

Table 2-8 Incorrect Qualitative/Quantitative Results Troubleshooting *(Continued)*

Symptom	Possible Cause	Corrective Action
Chromatogram has more peaks than expected (ghost peaks) *(Continued)*	Mobile phase contains a stabilizer, or there is a change in the stabilizer	Use a preservative-free solvent. ***Note:*** *Separations may require adjustment if changing to a preservative-free solvent.*
Chromatogram has fewer peaks than expected	Degraded sample	Refer to the Corrective Action under the "Chromatogram has more peaks than expected" symptom listed above.
	Loss of resolution	Refer to Section 4.3, Chromatographic Performance Tests.
	Incorrect mobile phase used	Verify the mobile phase used with your sample. If the mobile phase is incorrect for the sample, prepare a fresh batch of mobile phase. ***Note:*** *If this problem is occurring in addition to a high system backpressure, this could indicate precipitation of sample.*

Table 2-8 Incorrect Qualitative/Quantitative Results Troubleshooting *(Continued)*

Symptom	Possible Cause	Corrective Action
Quantitative Results		
Loss of accuracy	Incorrect peak height or area integration	Verify values entered in such areas as: • Sample amount • Scale factor • Internal standard amount • Retention time Make the appropriate change(s), rerun standards, and verify that accuracy improves. ***Note:*** *If the data-handling system reports an error in component amount, compare the responses of the peak to standard values. If the response (area or height) is accurate, problem is in the quantitation. If responses are incorrect, the problem is integration (for example, software values) or chromatography.*
	Degraded sample or impurities introduced during sample preparation	Refer to the Corrective Action under the "Chromatogram has more peaks than expected" symptom listed above.

Table 2-8 Incorrect Qualitative/Quantitative Results Troubleshooting *(Continued)*

Symptom	Possible Cause	Corrective Action
Loss of accuracy *(Continued)*	Sample evaporation	Refrigerate sample, maintain appropriate temperature, seal and store the sample.
	Incorrect sample preparation	Check the sample preparation procedure (concentration, solvent filtration, and so on).
	Incorrect internal standard preparation	Verify standard preparation/composition procedure (weighed and diluted properly). Prepare a new internal standard.

Table 2-8 Incorrect Qualitative/Quantitative Results Troubleshooting *(Continued)*

Symptom	Possible Cause	Corrective Action
Loss of accuracy *(Continued)*	Injection problem (with external standard only)	Injection problems vary with different types of manual injectors. Depending upon the type of injector being used: • If using a fixed-loop manual injector, ensure 3 times the loop volume is loaded into the loop before making the injection. • If using a partial-loop manual injector, ensure that less than 50% of the sample loop volume is injected. • If using a syringe-type manual injector, ensure the syringe injection technique is constant, correct loop size is installed, the correct syringe is used, the syringe is clean, and the loading port is not leaking.

Table 2-8 Incorrect Qualitative/Quantitative Results Troubleshooting *(Continued)*

Symptom	Possible Cause	Corrective Action
Loss of accuracy *(Continued)*	Injection problem (with external standard only) *(Continued)*	• If using an autoinjector, ensure the correct loop and syringe are installed, the syringe does not contain air, vials have sufficient sample, and there are no leaks. • If using either a manual or autoinjector, ensure the flow path is equilibrated. This is performed by leaving the manual injector in the inject position or by purging the autoinjector.
Loss of precision	Incorrect peak integration	Refer to the Corrective Action under the "Loss of accuracy" symptom listed above.
	Injection problem	Refer to the Corrective Action under the "Loss of accuracy" symptom listed above.
	Injector problem (such as a sticking or leaking valve, blocked or damaged needle, plugged injection port)	Refer to Section 3.2, Manual Injector Troubleshooting, or Section 3.3, Autoinjector Troubleshooting.
	Degraded sample or impurities introduced during sample preparation	Refer to the Corrective Action under the "Chromatogram has more peaks than expected" symptom listed above.

Table 2-8 Incorrect Qualitative/Quantitative Results Troubleshooting *(Continued)*

Symptom	Possible Cause	Corrective Action
Loss of precision (continued)	Chromatographic problem (changing retention time, abnormal peaks shape)	Refer to Section 2.2.3.1, Incorrect/Changing Retention Times or Section 2.2.3.2, Abnormal Peak Shape
	Degraded detector response	Refer to Section 3.5, Detector Troubleshooting.

3 Troubleshooting System Components

This section contains troubleshooting tables for individual hardware components in an LC system. These components include:

- Pump
- Injector (manual or autoinjector)
- Column
- Detector (UV, RI, fluorescence, conductivity, or electrochemical)
- Data-handling device (computer, integrator, or chart recorder)

If the origin of a specific system symptom is unclear (such as high system pressure or baseline noise), refer to Chapter 2, Troubleshooting an LC System.

Attention: *To avoid electric shock, power down the instrument and disconnect the power cord before removing the cover and examining the instrument.*

Safety and Handling

When troubleshooting instrumentation, keep the following safety considerations in mind:

Attention: *Always observe safe laboratory practices when troubleshooting. Know the chemical and physical properties of the solvents. Refer to the Material Safety Data Sheet for the solvents in use.*

Attention: *To avoid electric shock, power down the instrument and disconnect the power cord before removing the cover and examining the instrument.*

Attention: *Ultraviolet light is emitted during UV and fluorescence detector operation. To prevent eye damage, eye protection must be worn while troubleshooting the detector with its covers removed.*

- If handling integrated circuit boards, use an anti-static mat and wear an anti-static wrist strap to remove excess static charge and prevent damage to the board.
- Do not touch any of the integrated circuit chips or other components which do not specifically require manual adjustment.

When You Call

Many problems with your LC system can be easily corrected by the process outlined in this chapter. If you cannot correct a condition for a Waters product, contact one of the following:

- Your Waters service specialist
- Waters Customer Support Department
- Waters Service offices (listed on the rear cover of this guide)

To expedite service, have the following information available when you call:

1. Completed normal operation log and test sample chromatogram for method
2. Nature of symptom(s)
3. Type and model number of pump (single or multiple solvent)
4. Flow rate
5. Operating pressure
6. Mobile phase(s)
7. Type and model number of injector (manual or autoinjector)
8. Type and model number of detector (UV, RI, fluorescence, conductivity, electrochemical)
9. Detector settings (wavelength, sensitivity, and so on)
10. Type and serial number of column
11. Sample matrix and components
12. Data system
13. Software version (if applicable)

3.1 Pump Troubleshooting

This section covers pump troubleshooting. It includes a:

- Pump troubleshooting table
- Modified ramp test for isolating a malfunctioning pump head and malfunctioning check valves

3.1.1 Pump Troubleshooting Table

Table 3-1 is a guide to troubleshooting pump problems. It lists pump-related symptoms, along with the possible cause and corrective action.

If you experience baseline noise-related pump problems, refer to Section 2.2.2, Baseline Noise.

For specific troubleshooting information on your pump, refer to the operator's manual.

Table 3-1 Pump Troubleshooting

Symptom	Possible Cause	Corrective Action
Pump does not run (fan and front panel lights off)	Pump not connected to power source	Ensure power cable is properly connected to power source and pump.
	No power at outlet	Check the outlet by connecting another electrical unit known to be working and verify if the unit operates. If that unit does not work, relocate the pump to a functioning electrical outlet.
	Blown fuse	Replace the fuse (see pump operator's manual).
Pump not delivering solvent	Blown fuse	Replace the fuse (see pump operator's manual).

Table 3-1 Pump Troubleshooting *(Continued)*

Symptom	Possible Cause	Corrective Action
Pump not delivering solvent *(Continued)*	Pump not connected to pump controller.	Ensure cable is properly connected to pump controller. Verify that IEEE-488 addresses (if IEEE-488 communication is in use) are properly set. • If properly connected and IEEE-488 addresses are correct, turn off the pump and controller and disconnect the cable. Turn on the pump and verify that it operates under its own control. ***Attention:*** *Never disconnect the pump from the controller while the two devices are powered on.* • If pump operates properly, refer to the controller operator's manual for troubleshooting information.
	Pump low pressure limit set to a value that is higher than operating pressure	Set to the correct pressure limit (see pump operator's manual).
	Flow rate set to zero	Increase pump flow rate.

Table 3-1 Pump Troubleshooting *(Continued)*

Symptom	Possible Cause	Corrective Action
Pump not delivering solvent *(Continued)*	Pressure transducer out of adjustment or defective	With flow set to zero, adjust the pressure transducer to zero (see pump operator's manual). If problem continues, repair or replace the pressure transducer.
	Pump not primed	Prime the pump (see pump operator's manual).
	Draw-off valve open or leaking	Close the draw-off valve. If solvent still leaks, replace defective valve seals (see pump operator's manual).
	Immiscible solvents in pump head	Purge pump with appropriate solvents. Verify miscibility of solvents being used (see Appendix A) and change to more miscible solvents.
	Dirty or malfunctioning inlet or outlet check valve	Refer to Section 3.1.2 to determine if this symptom is due to a problem with the inlet or outlet check valve.
	Damaged plunger seal	Check if solvent is leaking from behind the pump head or for salt crystal build-up around the back of the pump head. This is an indicator of a damaged plunger seal. Verify if both pump heads can maintain pressure as outlined in Section 3.1.2. If a problem is detected, replace the plunger seal (see pump operator's manual).

Table 3-1 Pump Troubleshooting *(Continued)*

Symptom	Possible Cause	Corrective Action
Pump not delivering solvent *(Continued)*	Pump head cavitation (high pressure pumps only)	Verify if the problem is due to: • **Solvent reservoirs positioned equal to or below the pump** - Raise solvent reservoirs above the pump (see Section 4.1.2) • **Loose, bent, or blocked pump inlet tubing** - Check tubing. Tighten, straighten, or replace tubing • **Improperly degassed solvent** - Degas or helium sparge solvents to prevent cavitation • **Dirty solvent reservoir inlet filter** - Remove and clean with 6N nitric acid, water (rinse 3 times), followed by methanol • **Volatile solvents in pump head** - Prime pump (see operator's manual) • **Tubing ID too small for solvent inlet** - Use correct tubing
	Ruptured pulse dampener/high pressure noise filter	Check for leaks, replace the pulse dampener or high pressure noise filter (see pump operator's manual).

Table 3-1 Pump Troubleshooting *(Continued)*

Symptom	Possible Cause	Corrective Action
Pump not delivering solvent *(Continued)*	Solvent proportioning valve or in-line mixer failure	Flush the solvent proportioning valve or in-line mixer (see pump operator's manual). If problem continues, replace the component.
	Defective pump motor	Contact Customer Support.
	Defective circuit board	Contact Customer Support.
Leak from pump head	Worn pump plunger seals	Replace defective plunger seals (see pump operator's manual).
	Worn plunger	Repair or replace the plunger (see pump operator's manual).
	Loose pump head	Tighten the two pump head screw(s). Ensure that both screws are tightened equally, otherwise seal wear may result. Do *not* overtighten.
	Loose inlet or outlet check valve	Tighten the loose check valve(s). Do *not* overtighten. Verify fittings and ferrules for under/overtightening and wear. Replace if necessary. Refer to Chapter 4 for fitting/ferrule information.
Leak from draw-off valve	Draw-off valve open or broken	Close the draw-off valve. If solvent still leaks, replace defective valve seals (see pump operator's manual).
	Solvent line damaged	Replace inlet line (see Chapter 4).

Table 3-1 Pump Troubleshooting *(Continued)*

Symptom	Possible Cause	Corrective Action
Erratic flow rate/pump pulsations	Mobile phase improperly degassed or sparged	Degas or sparge the solvent(s) and re-equilibrate the system (see Section 4.1). ***Note:*** *Ensure you limit the amount of helium sparging to avoid depleting mobile phase components.*
	Pump not primed	Prime the pump (see pump operator's manual). ***Note:*** *If using a volatile solvent (such as hexane or ether), prime the pump with a miscible, less volatile solvent such as THF and methanol. Ensure that the column is disconnected to avoid disrupting equilibrium.*
	Reservoir low or out of solvent	Check solvent level in reservoirs. If out of solvent, refill reservoir (degas and helium sparge solvent).
	Air bubble in pump head	Prime pump to remove bubble (see pump operator's manual). Ensure there are no air bubbles in the solvent inlet lines. Degas or helium sparge solvents.

Table 3-1 Pump Troubleshooting *(Continued)*

Symptom	Possible Cause	Corrective Action
Erratic flow rate/pump pulsations *(Continued)*	Dirty or malfunctioning check valve(s)	Refer to Section 3.1.2 to determine if this symptom is due to a problem with the inlet or outlet check valve. Clean the check valve as described in the pump operator's manual. Ensure solvent is properly filtered to prevent additional precipitation. ***Note:*** *Buffer precipitation on a check valve can occur when running a mixture of water and an organic solvent in a concentration of 50% or higher. The actual degree of precipitation can vary depending on the organic solvent in use and the buffer.*
	Solvent inlet filter or inlet lines blocked.	Check lines for blockages. Replace if necessary. Clean the solvent inlet filter frit by sonicating using 6N nitric acid, water (repeat 3 times), followed by methanol.
	Solvent proportioning valve or in-line mixer failure	Flush the solvent proportioning valve or in-line mixer (see pump operator's manual). If problem continues, replace the component.

Table 3-1 Pump Troubleshooting *(Continued)*

Symptom	Possible Cause	Corrective Action
Erratic flow rate/pump pulsations. *(Continued)*	Pump plunger seal leaking (under pump head)	Replace pump plunger seal (see pump operator's manual).
	Worn pump plunger	Replace the plunger (see pump operator's manual)
	Immiscible solvents in pump head	Refer to the Corrective Action under the "Pump not delivering solvent" symptom listed above.
	Pump head cavitation	Refer to the Corrective Action under the "Pump not delivering solvent" symptom listed above.
	Pump electronics failure	Contact Customer Support.
Inadequate solvent blending (gradient system)	Immiscible solvents in pump head	Refer to the Corrective Action under the "Pump not delivering solvent" symptom listed above.
	Solvent proportioning valve or in-line mixer failure	Flush the solvent proportioning valve or in-line mixer (see pump operator's manual). If problem continues, replace the component.
	Pump malfunction	Refer to pump operator's manual.
	Solvent blending problem after the pump	Additional mixing required. Refer to Table 2-3, the "Short-term cycling noise" symptom for a discussion on correcting this problem.

Table 3-1 Pump Troubleshooting *(Continued)*

Symptom	Possible Cause	Corrective Action
High system pressure due to pump (also refer to Section 2.2.1)	Pump flow rate set too high	Set to the correct operating flow rate.
	Pressure transducer out of adjustment or defective	Set flow rate to zero and adjust the pressure transducer to zero (see pump operator's manual). If problem continues, repair or replace the pressure transducer.
Squeaking noise	Pump seals dry	Verify and perform one of the following: • Refill plunger wash reservoir (if applicable) • Prime plunger wash system (if applicable) • Wet plunger with appropriate solvent through pump head access holes
	Binding pump plunger seal	Replace pump seal assembly (see pump operator's manual).
	Binding indicator rod (if applicable)	Replace indicator rod (see pump operator's manual).
	Improper plunger seal	Install correct pump plunger seal (see pump operator's manual).

3.1.2 Isolating a Defective Pump Head (Modified Ramp Test)

The following modified ramp test assists you in isolating a malfunction to either the right or left pump head. This test also assists in isolating a failed check valve in the pump head. To perform the pump ramp test:

Attention: *Always observe safe laboratory practices when troubleshooting. Wear safety glasses and gloves. Know the chemical and physical properties of the solvents. Refer to the Material Safety Data Sheet for the solvents in use.*

1. Disconnect the pump outlet.
2. If your pump supports a pressure output signal, attach the signal to a chart recorder. Set the chart recorder speed between.0.50 and 1.0 in/min (10 to 25 mm/min).

   Note: *If pump does not support a pressure output signal, you can watch the pump pressure gauge for the same change in pressure patterns outlined in this procedure.*

3. Flush the pump with a solvent that is miscible with methanol (used in step 7). To remove any buffers, flush with water, followed by methanol.
4. Insert a fitting plug into the pressure transducer outlet.
5. To check the right pump head, disconnect the left pump head outlet from the tee or reference valve. Insert a fitting plug into the tee or reference valve.
6. Place a tissue under the disconnected left pump head outlet tubing.
7. Set the high pressure limit to 1000 psi (70 atm) above its normal operating pressure. Run 100% methanol at a flow rate of 0.3 mL/min.
8. Allow the pump to reach the high pressure limit. At the high pressure limit, the pump should shut off and maintain high pressure for approximately 30 seconds. Slowly relieve system pressure by either turning the reference valve or loosening the fitting plug on the pressure transducer.

Attention: *If the reference valve outlet is plumbed to a refractometer reference cell, disconnect this fitting before relieving pressure to avoid overpressurizing the detector flow cell.*

If the pressure does not build up or holds under these conditions (due to a check valve problem), gradually increase the flow rate until pressure builds up.

Repeat this process another two times. If there is a sticking or improperly seated check valve problem, repeating the process may clear the problem.

9. Set the pump to 1000 psi (70 atm) over normal operating pressure and monitor the pump pressure on the chart recorder. When the pump head operates properly, the pressure should rise with each plunger stroke, then hold as the plunger recedes. This produces a staircase pattern on the chart recorder (see Table 3-2).

   If a pump head is malfunctioning:

   - If the inlet check valve is bad, pump pressure may stop rising at a certain point, or may not rise at all.
   - If the outlet check valve is bad, pump pressure may increase, then immediately decrease when the plunger starts to recede.

Table 3-2 presents pressure-recording examples, illustrating normal operation and check valve malfunctions.

Table 3-2 Pressure Recording Examples for Modified Ramp Test

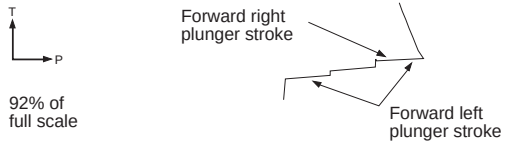
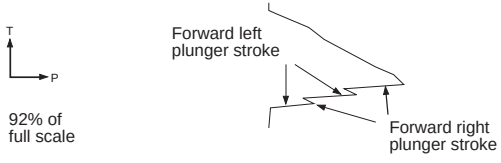
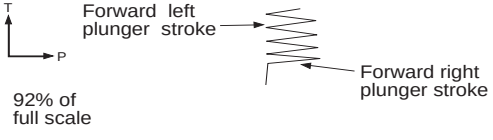
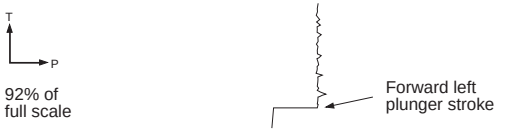
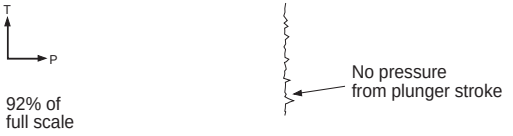
Pressure Recording Example	Indicating
	Normal pump operation
	Failing outlet check valve
	Failed outlet check valve

Table 3-2 Pressure Recording Examples for Modified Ramp Test *(Continued)*

Pressure Recording Example	Indicating
	Failing inlet check valve
	Failed inlet check valve

10. If operation is normal, repeat steps 5-9 for the left pump head.
11. If you determine that there is a problem with a check valve, remove it and clean (see pump operator's manual). If the pump problem persists (such as pump not delivering solvent or erratic flow rate), rebuild or replace the check valve (see pump operator's manual).

3.2 Manual Injector Troubleshooting

Table 3-3 is a guide to troubleshooting manual injector problems. It lists manual injector-related symptoms, along with the possible cause and corrective action.

Note: *The contents of Table 3-3 are specific primarily to the Waters U6K and the Rheodyne series of manual injectors.*

For specific troubleshooting information on your manual injector, refer to the operator's manual.

Table 3-3 Manual Injector Troubleshooting

Symptom	Possible Cause	Corrective Action
Leak from injection port	Defective injection port	Adjust or replace seal (see injector operator's manual).
	Syringe needle gauge larger or smaller than injection port seal	Use correct size syringe. Using too large a needle gauge damages the injection port seal. Replace the injection port seal (see injector operator's manual).
	Defective syringe (such as bent or burred tip)	Check syringe. If damaged, replace with a new syringe. In addition, replace the injection port seal (see injector operator's manual).
	Leak from disk valves	Tighten the valves. If leak reappears, replace the disk valves.
Leak from stator	Rotor stator screws need retightening	Tighten stator screws, using a feeler gauge to ensure equal tightening. Do *not* overtighten. If leaking continues, replace rotor stator (see injector operator's manual).
	Worn rotor stator face	Replace stator (see injector operator's manual).
Leak from compression screw(s)	Loose/overtightened fitting	Verify fittings and ferrules for under/overtightening and wear. Replace if necessary. Refer to Chapter 4 for fitting/ferrule information.

Table 3-3 Manual Injector Troubleshooting *(Continued)*

Symptom	Possible Cause	Corrective Action
Sample injection problem (such as difficult to inject sample, abnormal peak shape)	Blockage in injection port or vent tube pathway.	Check for blockages. Backflush injector (see manual injector operator's manual). If problem continues, repair the injector (see injector operator's manual).
	Defective syringe (such as bent or burred tip)	Refer to the Corrective Action under the "Leak from injection port" symptom listed above.
	Leak from disk valves	Tighten the valves. If leak reappears, replace the disk valves.
	Defective rotor seal	Replace rotor seal (see injector operator's manual).
	Blocked syringe.	Clean syringe. Ensure the solvent and sample are properly filtered. Ensure that the sample and solvent are miscible (see Appendix A, Reference Information).
Sample carryover	Inadequate sample loop flushing.	Flush sample loop with mobile phase between injections (see injector operator's manual).
	Dirty syringe or dirty injection port.	Use a clean syringe. Flush the injection port (see injector operator's manual).
	Injector cross-porting due to misalignment	Realign/rebuild the injector (see injector operator's manual).

3.3 Autoinjector Troubleshooting

Table 3-4 is a guide to troubleshooting autoinjector problems. It lists autoinjector-related symptoms, along with the possible cause and corrective action for the problem.

For specific troubleshooting information on your autoinjector, refer to the operator's manual.

Table 3-4 Autoinjector Troubleshooting

Symptom	Possible Cause	Corrective Action
Autoinjector does not operate (fan and front panel lights off)	Autoinjector not connected to power source	Ensure power cable is properly connected to power source and autoinjector.
	No power at outlet	Check the outlet by connecting another electrical unit known to be working and verify if the unit operates. If that unit does not work, relocate the autoinjector to a functioning electrical outlet.
	Blown fuse	Replace the fuse (see autoinjector operator's manual).

Table 3-4 Autoinjector Troubleshooting *(Continued)*

Symptom	Possible Cause	Corrective Action
Autoinjector does not operate (fan and front panel lights on)	Autoinjector not connected to controller.	Ensure cable is properly connected to controller. Verify that IEEE-488 addresses (if IEEE-488 communication is in use) are properly set. • If properly connected and IEEE-488 addresses are correct, turn off the autoinjector and controller and disconnect the cable. Turn on the autoinjector and verify that it operates under its own control. • If autoinjector operates properly, refer to the controller operator's manual for troubleshooting information.
	Air pressure problem (air actuated autoinjector)	Verify that the air pressure line is attached to the autoinjector (see autoinjector operator's manual). Check the air pressure regulator setting for proper operating range. Adjust if necessary.
	Power failure	Reset the autoinjector and resume operation.
	Sample compartment door opened	Open and reclose sample compartment door. If problem continues, contact Customer Support.
	Sample carriage error	Contact Customer Support
	Defective circuit board(s)	Contact Customer Support

Table 3-4 Autoinjector Troubleshooting *(Continued)*

Symptom	Possible Cause	Corrective Action
Leak from fluid system (needle, injector, seal pack, fluid pack)	Loose or overtightened compression fitting	Verify fittings and ferrules for under/overtightening and wear. Replace if necessary. Refer to Chapter 4 for fitting/ferrule information.
	Defective valve seals	Replace seals (see autoinjector service manual).
	Defective seal pack	Replace seal pack (see autoinjector service manual).
	Defective fluid pack	Replace fluid pack (see autoinjector service manual).
	Damaged needle	Purge the autoinjector to unplug the needle (see autoinjector operator's manual). If problem continues, replace the needle assembly and seal pack (see autoinjector service manual).
	Blocked or damaged syringe	Replace syringe (see autoinjector operator's manual).

Table 3-4 Autoinjector Troubleshooting *(Continued)*

Symptom	Possible Cause	Corrective Action
Leak from needlewash system	Loose or overtightened compression fitting	Verify fittings and ferrules for under/overtightening and wear. Replace if necessary. Refer to Chapter 4 for fitting/ferrule information.
	Defective fluid valve	Replace fluid valve (see autoinjector service manual).
	Defective needlewash pump	Replace needlewash pump (see autoinjector service manual).
	Autoinjector is siphoning solvent from needlewash reservoir	Lower the needlewash solvent reservoir.
Sample injection problem (such as sample not being injected, abnormal peak shape)	Defective injection valve	Purge the autoinjector (see autoinjector operator's manual). If problem continues, repair/replace the valve.
	Blocked needle due to particles in sample	Purge the autoinjector to unplug the needle (see autoinjector operator's manual). Ensure sample and solvent are properly filtered (see Chapter 4) to prevent further blockages. If problem continues, replace the needle assembly.

Table 3-4 Autoinjector Troubleshooting *(Continued)*

Symptom	Possible Cause	Corrective Action
Sample injection problem (such as sample not being injected, abnormal peak shape) *(Continued)*	Air bubble in syringe or sample loop assembly.	Purge the autoinjector (see autoinjector operator's manual). If air bubbles are repeatedly being drawn in, verify that a vacuum is not being created when needle pierces the septum (indicating seal is too tight around needle). Inject from the sample vial without a cap in place.
	Injection from empty sample vial	Compare sample vial entry and vial position. Enter correct sample vial number.
	Insufficient sample in vial	Ensure minimum sample volume requirement is in the vial (see autoinjector operator's manual).
	Cap is on too tight (creating a vacuum).	Loosen the cap.
	Sample too viscous	Dilute sample or decrease syringe draw speed rate.
	Vial septum not being pierced	Reinject from the sample vial with a cap in place. Inspect if a hole is being made by the injector. If no hole is made, there is a problem with the injector/needle (see autoinjector operator's manual).
	Air pressure problem (air actuated autoinjector)	Refer to the Corrective Action under the "Autoinjector does not run" symptom listed above.

Table 3-4 Autoinjector Troubleshooting *(Continued)*

Symptom	Possible Cause	Corrective Action
Sample injection problem (such as sample not being injected, abnormal peak shape) *(Continued)*	Misaligned sample transport system	Refer to autoinjector operator's manual.
	Defective injector seals	Replace injector seals (see autoinjector operator's manual).
No solvent flow through injector	Autoinjector not connected to pump	Attach fluid lines to pump (see pump operator's manual).
	Injection valve in wrong position	Restart operation to reposition valve. If problem continues, repair/replace valve (see autoinjector operator's manual).
	Blocked injection valve	Backflush the autoinjector (see autoinjector operator's manual). If problem continues, repair/replace the injection valve.
	Leak within autoinjector or prior to autoinjector	Check for leaks. Tighten loose fittings. If leaks continue, verify fittings and ferrules within autoinjector for wear. Replace if necessary.
	Autoinjector stuck in purge	Refer to autoinjector operator's manual.
High system pressure due to autoinjector (also refer to Section 2.2.1)	Blocked needle due to particles in sample.	Refer to the Corrective Action under the "Sample not being injected" symptom listed above.

Table 3-4 Autoinjector Troubleshooting *(Continued)*

Symptom	Possible Cause	Corrective Action
High system pressure due to autoinjector (also refer to Section 2.2.1) *(Continued)*	Blocked injection valve	Purge the autoinjector (see autoinjector operator's manual). If problem continues, repair/replace the valve.
	Blocked tubing between autoinjector and column	Verify tubing connections. Replace any blocked tubing (see Chapter 4).
	Blocked restrictor loop	If blocked, the backpressure problem should be obvious during the autoinjector injection cycle. To correct this problem: 1. Disconnect and reverse the autoinjector inlet and outlet connections. The solvent should now flow from the pump, into the injector outlet tubing, and out the injector inlet tubing to waste beaker. 2. Ensure that the primary flow path is closed (refer to autoinjector service manual). 3. Run the pump to backflush the restrictor loop and remove the blockage. If problem continues, replace the restrictor loop.

Table 3-4 Autoinjector Troubleshooting *(Continued)*

Symptom	Possible Cause	Corrective Action
High system pressure due to autoinjector (also refer to Section 2.2.1) *(Continued)*	Sample not miscible with mobile phase.	To verify solubility, place sample and mobile phase in a test tube and observe if the sample dissolves. If necessary, further dilute the sample or change the mobile phase.
	Blocked autoinjector filters	Clean or replace the filters (see autoinjector operator's manual).

Table 3-4 Autoinjector Troubleshooting *(Continued)*

Symptom	Possible Cause	Corrective Action
Sample carryover	Injection volume too large.	Reduce the injection volume or install a larger sample loop (see autoinjector operator's manual).
	Sample injection problem.	To verify the problem, test with blank injections of solvent after a sample injection. If the carryover problem occurs after a sample injection, this may be due to a problem with the needlewash system (see below).
	Needlewash system problem (due to loss of prime, empty solvent reservoir, contaminated frits, or defective needlewash pump).	Verify these potential problem areas and perform one of the following: • **Empty solvent reservoir** - Refill the needlewash reservoir • **Loss of prime** - Reprime the needlewash system • **Contaminated frits** - Replace frits (see autoinjector operator's manual) • **Defective needlewash pump** - See autoinjector service manual

Table 3-4 Autoinjector Troubleshooting *(Continued)*

Symptom	Possible Cause	Corrective Action
Injection from wrong sample vial	Incorrect sample vial number specified	Compare sample vial entry and vial position. Enter correct sample vial number in autoinjector.
	Sample vial mislabeled	Verify information on sample vial. Relabel if necessary.
	Misaligned sample transport system	Refer to autoinjector operator's manual.

Table 3-4 Autoinjector Troubleshooting *(Continued)*

Symptom	Possible Cause	Corrective Action
Incorrect sample volume injected.	Incorrect injection volume specified.	Compare injection volume value and vial position. Enter correct injection volume value in autoinjector.
	Incorrect sample loop or syringe installed in fluidic system.	Replace with appropriate size sample loop or syringe (see autoinjector operator's manual).
	Incorrect syringe size value specified	Compare syringe size value and installed syringe. Enter correct syringe size value in autoinjector.
	Insufficient sample in vial	Ensure minimum sample volume requirement is in the vial (see autoinjector operator's manual).
	Leaky syringe	Repair/replace syringe (see autoinjector operator's manual).
	Sample too viscous.	Dilute sample or decrease syringe draw speed rate.
	Vacuum created in vial	Make injections without a cap in place, remove excess sample from the vial, or loosen cap.

Table 3-4 Autoinjector Troubleshooting *(Continued)*

Symptom	Possible Cause	Corrective Action
Vials repeatedly break	Incorrect vials being used	Use manufacturer recommended vials.
	Incorrect caps being used	Use manufacturer recommended vials.
	Caps not put on properly	Ensure that caps are on properly.
	Needle or fluidic system malfunction	Refer to autoinjector operator's manual.
	Misaligned sample transport system	Refer to autoinjector operator's manual.
Needle bent	Solid cap not removed from sample vial	Remove sample vial cap and replace with a septum cap. Replace the autoinjector needle (see autoinjector service manual).
	Incorrect vials being used	Use manufacturer recommended vials. Replace the autoinjector needle (see autoinjector service manual).
	Septum too resistant	Inject from the sample vial without a cap in place. If the septum is too resistant, contact the vial manufacturer. Ensure that only one septum is being used at a time. Replace the autoinjector needle (see autoinjector service manual).
	Misaligned sample transport system	Refer to autoinjector operator's manual.

3.4 Column Troubleshooting

Table 3-5 is a guide to troubleshooting column problems. It lists column symptoms, along with the possible cause(s) and corrective action(s).

If you experience a separation problem, run your initial test mixture (when the column was new) to verify the problem.

- If the test results are not significantly different from those previously recorded, the problem is method-specific.
- If the test results are different, either the column or instrument have changed. Repeat the test with a new (or known good) column. If the results of the second column are satisfactory, the problem is with first column.

Note: *If a guard column is being used, it can often be a source of band-broadening and the component most likely to fail first. If you suspect a problem with column performance, also check the guard column.*

Depending on the nature of the problem, refer to the following sections:

- For retention time problems, refer to Section 2.2.3.1.
- For no peaks or abnormal peak shape problems, refer to Section 2.2.3.2.
- For system resolution problems, refer to Section 4.3.1.

For specific troubleshooting information on your column, refer to the operator's manual.

Table 3-5 Column Troubleshooting

Symptom	Possible Cause	Corrective Action
High system backpressure due to column (see also Section 2.2.3.1)	Blocked column inlet or outlet frit, or column	Clean the inlet or outlet frit or column (see column operator's manual). If problem continues, replace the frits or the column.
	Column degraded (due to loss of efficiency or voided column).	Verify column performance by measuring column efficiency (N) (see Chapter 4). If the column efficiency measurement is low (as compared to when the column was new), replace the column.
	Blocked interconnecting tubing	Replace the tubing (see Chapter 4).
Peak shape problems (see also Section 2.2.3.1)	Column not equilibrated.	Allow the column sufficient time to equilibrate. ***Note:*** *Typically 100 column volumes or longer is required for ion-pairing reagents.*
	Column contaminated	Clean the column (see column operator's manual). If problem persists, replace the column.

Table 3-5 Column Troubleshooting *(Continued)*

Symptom	Possible Cause	Corrective Action
Peak shape problems (see also Section 2.2.3.1) *(Continued)*	Column degraded	Verify column performance by measuring column efficiency (N) and capacity factor (k') (see Chapter 4). If the k' value is out of range or the N measurement is low (as compared to when the column was new), replace the column.
	Incorrect ID tubing, improperly cut tubing, improper fittings and ferrules	Perform a system bandspread test (see Chapter 4). If the result is greater than established benchmarks, verify tubing ID, cut of the tubing, and compatibility of fittings/ferrules. Refer to your system map to ensure that the appropriate tubing, fittings, and ferrules are used. Replace if necessary. Refer to Chapter 4 for information.
	Ambient temperature change.	Use a column heater to control temperature. ***Note:*** *A change in temperature may also result in a change in retention time (reproducible but incorrect).*

3.5 Detector Troubleshooting

Table 3-6 is a guide to troubleshooting detector problems. It lists detector-related symptoms, along with the possible cause and corrective action. The majority of the problems in Table 3-6 relate to most detectors, such as UV, RI, fluorescence, conductivity, and electrochemical.

Attention: *Ultraviolet light is emitted during UV and fluorescence detector operation. To prevent eye damage, eye protection must be worn while troubleshooting the detector with its covers removed.*

If you experience noise-related detector problems, refer to Section 2.2.2.

For specific troubleshooting information on your detector, refer to the operator's manual.

Table 3-6 Detector Troubleshooting

Symptom	Possible Cause	Corrective Action
Detector does not run (fan and front panel lights off)	Detector not connected to power source	Ensure power cable is properly connected to power source and detector.
	No power at outlet	Check the outlet by connecting another electrical unit known to be working and verify if the unit operates. If that unit does not work, relocate the detector to a functioning electrical outlet.
	Blown fuse	Replace the fuse (see detector operator's manual).

Table 3-6 Detector Troubleshooting *(Continued)*

Symptom	Possible Cause	Corrective Action
Detector does not run (fan and front panel lights on)	Detector under control of external controller with locked keyboard	Verify if controller keyboard is locked. Unlock keyboard and verify that the detector operates under its own control.
	Power failure	Reset the detector and resume operation.
	Defective circuit board(s)	Contact Customer Support.
Detector does not respond to external controller	Detector not connected to controller.	Ensure cable is properly connected to controller. If using an IEEE-488 interface, verify that IEEE-488 addresses are properly set.
	Defective circuit board(s)	Contact Customer Support.

Table 3-6 Detector Troubleshooting *(Continued)*

Symptom	Possible Cause	Corrective Action
Source lamp does not light (or reference energy failure)	Blown fuse(s)	Replace blown fuse.
	Defective lamp	Replace the lamp (see detector operator's manual).
	Lamp leads not connected	Connect lamp leads.
	Lamp in standby	Take out of standby mode (see detector operator's manual).
	Lamp switch (in a detector containing multiple lamps) set for wrong lamp	Verify switch setting, change to correct lamp.
	Lamp voltage set to zero	Change lamp voltage setting (see detector operator's manual).
	Defective lamp power supply/board	Contact Customer Support.
	Contaminated reference cell	To clean the reference cell: 1. Flush the reference cell with a miscible solvent, followed by methanol. 2. Attach a nitrogen or helium source to the detector inlet and blow the reference cell dry.

Table 3-6 Detector Troubleshooting *(Continued)*

Symptom	Possible Cause	Corrective Action
Source lamp continuously blows	Defective lamp power supply/board	Contact Customer Support.
	Detector improperly grounded (line voltage problem)	Plug the detector into another outlet on a different electrical circuit. If a separate outlet is unavailable, use a line power conditioner.
	Smudges or fingerprints put on deuterium lamp during installation	Carefully follow the lamp replacement procedure outlined in the detector operator's manual.
No detector response (straight baseline, no peaks)	Lamp burned out	Replace lamp (see detector operator's manual).
	Incorrect detector settings (such as wavelength, or sensitivity)	Verify and adjust any incorrect detector settings (see detector operator's manual).
	Cable improperly connected between detector and data-handling system (computer, integrator, or chart recorder).	Verify that the correct detector output signal is properly connected to the data-handling device. Ensure that any related output switch settings are in the proper position. Use a shielded signal cable and attach the shield to one device only. Refer to the detector operator's manual and data-handling device operator's manual.
	Detector output not zeroed	Zero detector baseline (see detector operator's manual).

Table 3-6 Detector Troubleshooting *(Continued)*

Symptom	Possible Cause	Corrective Action
No detector response (straight baseline, no peaks) *(Continued)*	Auto zero left enabled on detector	Turn auto zero off.
	Lamp switch (in a detector containing multiple lamps) off or set for wrong lamp	Verify switch setting. Turn on or change to correct lamp.
	Defective or incorrect photodiode	Replace photodiode (see detector operator's manual).
	Dirty or contaminated flow cell or reference cell.	Clean flow cell. To clean: 1. Remove the column and install a union. 2. For buffers, flush the detector with 100% water, 100% methanol (if miscible with the last solvent in the flow cell), again followed by 100% water. 3. For non-polar solvents, flush the detector with a 50/50 mixture of THF and water (if miscible with the last solvent in the flow cell), followed by 100% THF. If the problem continues, flush with a stronger solution (as outlined in the detector operator's manual).
	Dirty detector flow cell window	Clean window. If problem persists, replace the window.

Table 3-6 Detector Troubleshooting *(Continued)*

Symptom	Possible Cause	Corrective Action
No detector response (straight baseline, no peaks) *(Continued)*	Solvent leaking into reference cell	Repair or replace the flow cell. Refer to detector operator's manual.
	Reference cell not properly purged (RI detector only).	Purge reference cell (see detector operator's manual). Ensure equilibration of mobile phase in both the reference and sample sides of the flow cell.
	Unbalanced optics (RI detector only)	Refer to detector operator's manual.
	Incorrect excitation and/or emission filters or wavelength set (fluorescence detector only)	Use correct filters for your application (see detector operator's manual). Ensure that the wavelength differential is sufficient for detecting components.
	Excitation and/or emission filters aged from normal use (fluorescence detector only)	Replace filters (see detector operator's manual).
	Incorrect excitation lamp (fluorescence detector only)	Use the correct lamp (see detector operator's manual).
	Dissolved oxygen quenches sample response (fluorescence detector only)	Dissolved oxygen (levels as low as 10^{-3} M) can reduce fluorescence by as much as 20% for certain compounds. Remove dissolved oxygen by degassing or helium sparging the mobile phase.

Table 3-6 Detector Troubleshooting *(Continued)*

Symptom	Possible Cause	Corrective Action
No detector response (straight baseline, no peaks) *(Continued)*	Insufficient electrolyte in mobile phase (electrochemical detectors only)	Verify conductivity of solvent. If conductivity is low, prepare fresh solvent with a higher ionic strength.
	Fouled reference electrode (electrochemical detector only)	Renew reference electrode filling solution. If necessary, replace the frit.
	Contaminated or scratched working electrode (electrochemical detector only)	Clean or polish working electrode.
	Wrong reference or working electrode (electrochemical detector only)	Verify detector electrode configuration (see detector operator's manual).
	Wrong solution in reference electrode (electrochemical detector only)	Remove reference electrode and verify solution. Renew reference electrode solution. If necessary, replace the frit (see detector operator's manual).
	Air in reference electrode (electrochemical detector only)	Remove reference electrode, tap air bubbles from frit. Replace electrode.
	Current range incorrect (electrochemical detector only)	Adjust to lower current range.
	Applied potential incorrect (electrochemical detector only)	Use the correct value.

Table 3-6 Detector Troubleshooting *(Continued)*

Symptom	Possible Cause	Corrective Action
No detector response (straight baseline, no peaks) *(Continued)*	Defective circuit board(s)	Contact Customer Support.
Change in sample or reference energy (UV detector only).	Dirty or contaminated flow cell or reference cell	Clean flow cell as described in the Corrective Action under the "No detector response (straight baseline, no peaks)" symptom listed above.
	Dirty detector flow cell window	Clean window. If problem persists, replace the window.
	Leak in flow cell	Refer to the Possible Causes outlined in the "Leak from flow cell" symptom later in this table.
	Defective photodiode	Replace the photodiode (see detector operator's manual).

Table 3-6 Detector Troubleshooting *(Continued)*

Symptom	Possible Cause	Corrective Action
Change in sample or reference energy (UV detector only).	Mobile phase contaminated	Discard the contaminated mobile phase and perform the following: • Clean the solvent reservoir and solvent inlet filter. To clean, remove the filter and sonicate using 6N nitric acid, followed by water (repeat 3 times). Rinse with methanol. • Prepare and filter fresh solvent daily using only high quality reagents and HPLC-grade solvents (see Section 4.1 for solvent preparation and use considerations). • Flush and re-equilibrate the system.

Table 3-6 Detector Troubleshooting *(Continued)*

Symptom	Possible Cause	Corrective Action
Change in sample or reference energy (UV detector only). *(Continued)*	Large air bubbles trapped in flow cell	To remove the air bubble, purge the detector flow cell or apply slight pressure on the detector waste outlet (see detector operator's manual). ***Note:*** *To prevent additional air bubbles, ensure the mobile phase is properly degassed or helium sparged (see Chapter 4).* To prevent air bubbles from forming in the flow cell, add a 1 to 3 foot (30 to 90 cm) length of 0.009 - inch (0.23 mm) ID, 1/16 -inch (1.58 mm) OD tubing to the detector waste outlet. This tubing functions as a flowrestrictor to increase backpressure. A 3 foot (90 cm) piece of tubing provides 30 to 50 psi (2 to 3 atm) of backpressure at 1 mL/min in water. Keep in mind the backpressure limits of the flow cell (such as with RI, Fluorescence, conductivity, and electrochemical detectors) before attaching this tubing.
	Highly-absorbing mobile phase	Refer to the Corrective Action under the "No detector response (straight baseline, no peaks)" symptom listed above.

Table 3-6 Detector Troubleshooting *(Continued)*

Symptom	Possible Cause	Corrective Action
Change in *both* sample and reference energy (UV detector only)	Defective photodiode	Replace the photodiode (see detector operator's manual).
	Defective lamp	Verify the lamp energy using detector diagnostics. If the energy is below specification (as compared to when the lamp was new), replace the lamp. ***Note:*** *Some detectors allow you to adjust the lamp energy to compensate for decreased energy. Refer to your detector operator's manual for information on adjusting lamp energy.*
	Leak in flow cell	Refer to the Possible Causes outlined in the "Leak from flow cell" symptom listed above.
	Defective filter	Contact Customer Support.
Detector electronics can not be calibrated (from front panel control)	Decreased lamp energy	Refer to the Corrective Action under the "Change in both sample and reference energy" symptom listed above.
	Dirty or contaminated flow cell or reference cell	Clean flow cell as described in the Corrective Action under the "No detector response (straight baseline, no peaks)" symptom listed above.
	Dirty detector flow cell window	Clean window. If problem persists, replace the window.

Table 3-6 Detector Troubleshooting *(Continued)*

Symptom	Possible Cause	Corrective Action
Detector electronics can not be calibrated (from front panel control) *(Continued)*	Air bubble in detector flow cell	Refer to the Corrective Action under the "Change in both sample and reference energy" symptom listed above.
	Bad photodiode	Replace the photodiode (see detector operator's manual).
Detector overheating	Dirty ventilation filters	Clean air filters (see detector operator's manual).
	Malfunctioning fan	Replace the fan assembly (see detector operator's manual).
	Insufficient clearance and ventilation	Provide correct clearance and ventilation around detector (see detector operator's manual).
Leak from fittings	Loose/overtightened compression fitting	Verify fittings and ferrules for under/overtightening and wear. Replace if necessary. Refer to Chapter 4 for fitting/ferrule information.
Leak from flow cell.	Defective flow cell gasket	Replace gasket (see detector operator's manual). If leak continues, replace the flow cell.
	Blocked or damaged flow cell	Carefully backflush the detector flow cell (see detector operator's manual). If the flow cell is damaged or cracked, repair/replace (see detector operator's manual).

Table 3-6 Detector Troubleshooting *(Continued)*

Symptom	Possible Cause	Corrective Action
High system backpressure due to detector (also refer to Section 2.2.3.1)	Blocked detector inlet or outlet tube	Refer to the Corrective Action under the "Leak from flow cell" symptom listed above.
	Blocked or damaged flow cell	Carefully backflush the detector flow cell to remove any blockages (as described in the detector operator's manual). If the flow cell is damaged or cracked, repair/replace (see detector operator's manual).

3.6 Data-Handling Device Troubleshooting

This section is a guide to troubleshooting data-handling device hardware problems. It covers:

- Computer (Table 3-7)
- Integrator (Table 3-8)
- Chart recorder (Table 3-9)

Each table lists a series of symptoms, along with the possible cause and corrective action.

Note: *If you are receiving incorrect qualitative/quantitative results, refer to Section 2.2.3.3.*

For specific troubleshooting information on your data-handling device refer to the operator's manual.

Table 3-7 Computer Hardware Troubleshooting

Symptom	Possible Cause	Corrective Action
CRT		
Screen does not illuminate when power switch is turned on	Power cord is not connected to power source	Ensure power cable is properly connected to power source.
	No power at outlet	Check the outlet by connecting another electrical unit known to be working and verify if the unit operates. If not, relocate the computer to a functioning electrical outlet.
	Blown fuse	Replace the fuse (see computer operator's manual).
	Defective CRT tube	Contact Customer Support.
No CRT response	Cable improperly connected	Verify electrical connections (see computer operator's manual).
	HOLD or PAUSE screen key pressed	Verify if indicator light is on. If on, press the HOLD or PAUSE key to deactivate the condition.

Table 3-7 Computer Hardware Troubleshooting *(Continued)*

Symptom	Possible Cause	Corrective Action
Flickering CRT screen	Cycling equipment or radio frequency (RF) interference	Turn the computer off, then on and see if the flickering stops. If not, check the following: • Check circuit grounding and line voltage quality (refer to the "Computer improperly grounded" Possible Cause below). • Ensure that the computer and the detector are on the same common ground. Relocate if necessary. • If necessary, relocate the computer to an area where RF is not a problem.
	Computer improperly grounded	If shielding is not the problem, plug the computer into another outlet on a different electrical circuit. If a separate outlet is unavailable, use a line power conditioner.

Table 3-7 Computer Hardware Troubleshooting *(Continued)*

Symptom	Possible Cause	Corrective Action
Baseline noise	Cable loose or improperly connected between detector and computer or A/D interface	Refer to the Corrective Action under the "Flickering CRT screen" symptom listed above.
	Cycling equipment or radio frequency (RF) interference	Refer to the Corrective Action under the "Flickering CRT screen" symptom listed above.
	Computer or A/D interface improperly grounded	Refer to the Corrective Action under the "Flickering CRT screen" symptom listed above.
	A/D interface gain/sensitivity setting too high	Change to a lower gain/sensitivity setting (see A/D interface operator's manual).
	Defective A/D interface circuit board	Contact Customer Support.
Broken lines on the screen	Incorrect setup	Verify setup (see computer operator's manual).
	Graphics card failure	Contact Customer Support.

Table 3-7 Computer Hardware Troubleshooting *(Continued)*

Symptom	Possible Cause	Corrective Action
Keyboard		
No response when computer is turned on	Keyboard cable improperly connected	Verify electrical connections (see computer operator's manual).
	HOLD or PAUSE screen key pressed	Verify if indicator light is on. If on, press the HOLD or PAUSE key to deactivate the condition.
	Keyboard lock is enabled	Disable keyboard lock
	Liquid or particles in keyboard	Clean keys and keyboard.
	Defective keyboard	Contact Customer Support.
Certain keys do not work	Wrong keyboard attached to monitor	Verify keyboard being used, change with correct keyboard.
	Liquid or particles in keyboard	Clean keys and keyboard.
	Defective keyboard	Contact Customer Support.

Table 3-7 Computer Hardware Troubleshooting *(Continued)*

Symptom	Possible Cause	Corrective Action
Printer		
No response when printer is turned on	Power cord is not connected to power source	Ensure power cable is properly connected to power source.
	No power at outlet	Check the outlet by connecting another electrical unit known to be working and verify if the unit operates. If that unit does not work, relocate the printer to a functioning electrical outlet.
	Blown fuse	Replace the fuse (see printer operator's manual).
Printer does not print	Cable improperly connected	Reconnect the cable and ensure the printer is ON.
	SELECT or READY switch incorrectly set	Change to correct setting.(see printer operator's manual).
	Report sent to wrong printer location	Refer to computer operator's manual.
	Printer incorrectly setup	Verify printer hardware setup (see printer operator's manual).
	Power failure	Reset the printer and resume operation.
	Printer out of paper	Refill paper.
	Incorrect baud rate	Verify computer and printer baud rate setup (see computer *and* printer operator's manuals).

Table 3-7 Computer Hardware Troubleshooting *(Continued)*

Symptom	Possible Cause	Corrective Action
Unintelligible characters printed	Power failure	Check connection. Restart both the computer and printer. If problem persists, contact Customer Support.
	Printer incorrectly setup	Verify printer hardware setup (see printer operator's manual).
	Incorrect baud rate	Verify computer and printer baud rate setup (see computer *and* printer operator's manuals).
Poor print quality	Thermal paper improperly inserted	Remove and reinsert thermal paper.
	Ribbon worn out/toner cartridge out of ink	Replace ribbon or toner cartridge.
	Print head worn out or damaged	Contact Customer Support.

Table 3-8 Integrator Hardware Troubleshooting

Symptom	Possible Cause	Corrective Action
No response from integrator when power switch is turned on	Power cord is not connected to power source	Ensure power cable is properly connected to power source.
	No power at outlet	Check the outlet by connecting another electrical unit known to be working and verify if the unit operates. If that unit does not work, relocate the integrator to a functioning electrical outlet.
	Blown fuse	Replace the fuse (see integrator operator's manual).
	Cable loose or improperly connected between integrator and detector	Verify that the correct detector output signal is properly connected to the integrator. Ensure that any related output signal selections are set properly. Refer to the detector and integrator operator's manual.
	Cable loose or improperly connected between integrator and injector (no run start signal)	Verify connections. Correct if necessary.
	Autozero engaged on detector	Turn off auto zero on detector (see detector operator's manual).

Table 3-8 Integrator Hardware Troubleshooting *(Continued)*

Symptom	Possible Cause	Corrective Action
Baseline noise	Sensitivity incorrectly set at the integrator and/or detector	Adjust sensitivity setting (see integrator or detector operator's manual).
	Cable loose or improperly connected between integrator and detector	Refer to the Corrective Action under the "No response from integrator when power switch is turned on" symptom listed above.
	Cycling equipment or radio frequency (RF) interference	Check the following: • Check circuit grounding and line voltage quality (refer to the "Integrator Improperly Grounded" Possible Cause below for the corrective action). • Ensure that the integrator and the detector are on the same common ground. If necessary, relocate. • If necessary, relocate the integrator to an area where RF is not a problem.
	Integrator improperly grounded	Use a shielded signal cable and attach the shield to one device *only*. If shielding is not the problem, plug the integrator into another outlet on a different electrical circuit. If a separate outlet is unavailable, use a line power conditioner.
	Defective A/D interface circuit board	Contact Customer Support.

Table 3-8 Integrator Hardware Troubleshooting *(Continued)*

Symptom	Possible Cause	Corrective Action
Incorrect peak height/area	Incorrect detector output signal attached to integrator	Refer to the Corrective Action under the "No response from integrator when power switch is turned on" symptom listed above.
	Incorrect integrator attenuation setting	Verify setting, adjust if necessary (see integrator operator's manual).
Poor print quality	Worn, empty, or defective print cartridge	Verify cartridge and replace (see integrator operator's manual).
	Thermal pens out of adjustment	Refer to the integrator operator's manual.
	Incorrect paper installed	Verify paper and replace (see integrator operator's manual).
	Print head wrong for paper thickness	Readjust print head (see integrator operator's manual).
Unintelligible characters printed	Power failure	Check connection. Restart both the integrator and printer. If problem persists, contact Customer Support.
Paper jamming	Paper installed improperly	Re-install paper (see integrator operator's manual).
	Incorrect paper installed	Verify paper and replace (see integrator operator's manual).
	Paper feed binding	Check for binding, remove any objects from paper path. Clean particulates from paper path.

Table 3-9 Chart Recorder Troubleshooting

Symptom	Possible Cause	Corrective Action
No response from chart recorder when power switch is turned on	Power cord is not connected to power source	Ensure power cable is properly connected to power source.
	No power at outlet	Check the outlet by connecting another electrical unit known to be working and verify if the unit operates. If that unit does not work, relocate the chart recorder to a functioning electrical outlet.
	Blown fuse	Replace the fuse (see chart recorder operator's manual).
	Cable loose or improperly connected between chart recorder and detector	Verify that the correct detector output signal is properly connected to the chart recorder. Ensure that any related output signal selections are set properly. Refer to the detector operator's manual and chart recorder operator's manual.
	Auto zero engaged on detector or chart recorder	Turn off the auto zero function (see detector or chart recorder operator's manual).

Table 3-9 Chart Recorder Troubleshooting *(Continued)*

Symptom	Possible Cause	Corrective Action
Baseline noise	Gain incorrectly set at chart recorder and/or detector	Adjust gain setting (see chart recorder operator's manual).
	Cable loose or improperly connected between chart recorder and detector	Refer to the Corrective Action under the "No response from chart recorder when power switch is turned on" symptom listed above.
	Cycling equipment or radio frequency (RF) interference	Check the following: • Check circuit grounding and line voltage quality (refer to the "Chart recorder improperly grounded" Possible Cause below for the corrective action). • Ensure that the chart recorder and the detector are on the same common ground. Relocate if necessary. • If necessary, relocate the chart recorder to an area where RF is not a problem.
	Chart recorder improperly grounded	Use a shielded signal cable and attach the shield to one device *only*. If shielding is not the problem, plug the chart recorder into another outlet on a different electrical circuit. If a separate outlet is unavailable, use a line power conditioner.

Table 3-9 Chart Recorder Troubleshooting *(Continued)*

Symptom	Possible Cause	Corrective Action
Incorrect peak height/area	Incorrect detector output signal attached to chart recorder	Refer to the Corrective Action under the "No response from chart recorder when power switch is turned on" symptom listed above.
	Incorrect chart recorder attenuation setting	Verify setting, adjust if necessary (see chart recorder operator's manual).
Inverted peaks	Signal polarity setting switched on chart recorder	Verify polarity setting, change if necessary (see chart recorder operator's manual).
Poor print quality.	Pen(s) out of ink	Replace dry pens (see chart recorder operator's manual).
	Pen(s) jammed	See chart recorder operator's manual.
	Incorrect paper installed	Verify paper and replace (see chart recorder operator's manual).
	Pen not fully lowered	Ensure level is fully depressed.
Paper jamming	Paper installed improperly	Re-install paper (see chart recorder operator's manual).
	Incorrect or damaged paper installed	Verify paper and replace (see chart recorder operator's manual).
	Roller guides binding	Adjust roller guides. Replace if roller guides are damaged.
	Dirty bed	Clean with alcohol.
	Damaged roller teeth	Inspect roller. If teeth are damaged, replace roller.

4 Good Chromatography/ Operating Practices

Understanding and maintaining proper operation of your system is essential to minimizing system downtime. This chapter outlines a set of good chromatography and operating practices to keep your LC system in proper operating condition, including:

- Mobile phase preparation and use
- System plumbing
- Chromatographic performance tests
- Measuring system band spreading

4.1 Mobile Phase Preparation and Use

This section presents recommended operating practices for:

- Mobile phase preparation
- Solvent degassing
- Solvent use
- Solvent changeover

Effects of Improper Solvent Practices

In many instances, problems with an LC system stem from improper solvent preparation and use. Improper practices may result in:

- Outgassing in pump heads
- Air bubbles or particles trapped in the detector flow cell
- Mobile phase contamination
- Damage to pump check valves and seals
- Plugged in-line filters, frits, check valves, or connecting tubing
- Poor injection precision

which affect system operation in a number of ways, such as:

- High system backpressure
- Flow-related baseline noise
- Shifting retention times
- Abnormal peak shapes
- Incorrect qualitative/quantitative results

Attention: *Always observe safe laboratory practices when handling solvents. Know the chemical and physical properties of the solvents. Refer to the Material Safety Data Sheet for the solvents in use.*

4.1.1 Mobile Phase Preparation

Recommended mobile phase preparation practices include:

Use Clean Solvents

Always use clean, high-purity HPLC-grade solvents and reagents when preparing your mobile phase.

Filter Solvents

All solvents should be filtered through a 0.45 micron (or smaller) filter. This is especially critical with salts and buffers.

Note: *Ensure that the filter material is compatible with the solvent.*

The benefits of solvent filtering include:

- Improved pump performance (including seals, check valves, plunger, and so on)
- Increase in injector life

After filtering, store the solvent in a covered reservoir to prevent dust and debris from entering the solvent.

Precision During Mobile Phase Preparation

During mobile phase preparation, weigh out the individual components rather than use a volumetric cyclinder. Volumetric preparation using a graduated cylinders may not provide sufficient precision, and may lead to irreproducible results.

Use and Storage of Water

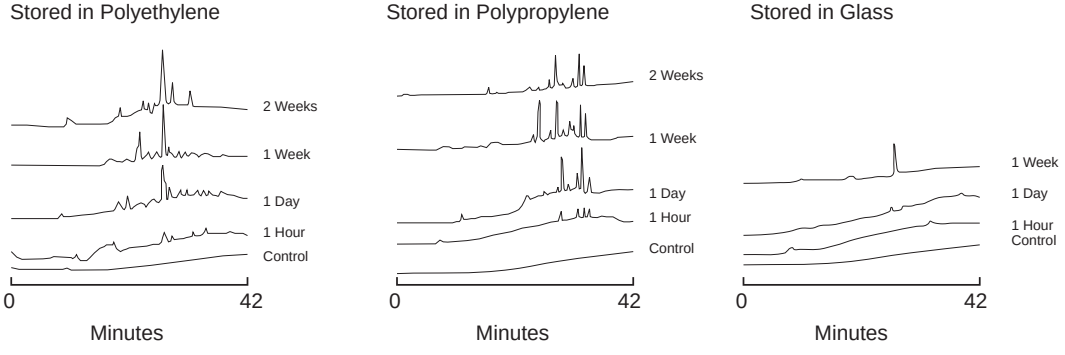
Only use ultrapure water (18 megaohm resistivity), such as that supplied by the Milli-Q™ system (or equivalent).

The quality of water is critical in gradient chromatography. Impurities in the water tend to collect on the column during equilibration with the weak solvent.

Note: *Deionized water is not acceptable because it contains organic compounds that can alter column selectivity.*

For most applications, use a clean glass container to store the prepared water instead of a plastic container. Some plastic containers contain plasticizers, monomers, and molding agents (used in the manufacture of the containers) which may leach out and contaminate the water.

Figure 4-1 illustrates chromatograms of high purity water (40 mL injections) stored in three different containers. Measurements are taken at various intervals for 2 weeks.



Operating Conditions:

Flow rate: 2.0 mL/min

Column: C_{18}

Solvent: Linear gradient of 100% Milli-Q water to 100% acetonitrile

Detector Settings: 214 nm, 0.02 AUFS

Figure 4-1 Comparison of Water Stored in Glass and Plastic Containers

As shown Figure 4-1, contaminants in plastic containers become more pronounced over time. When using a glass container, solvent degradation occurs at a much slower rate.

When storing water and aqueous buffers in glass, be aware that they:

- Absorb chemicals from the air at variable rates
- Support microbial growth
- Leach silicates from glass, affecting certain applications

If you are uncomfortable about storing water or aqueous buffers, discard them.

Dedicate Glassware

Dedicate specific glassware for HPLC-grade solvents and reagents. To prevent solvent contamination, use the same container to prepare and store a solvent.

Wash Solvent Reservoir

Always wash the solvent reservoir with mobile phase prior to use. Avoid using detergents to wash this glassware. Residues may cause severe baseline problems, especially in gradient chromatography.

If detergents are used, perform the following after washing with detergent:

1. Thoroughly rinse the glassware with Milli-Q water
2. Wash the glassware with methanol and air dry

Use of Stabilizers

Stabilizers used in certain solvents alter the optical and chromatographic characteristics of the solvent. To prevent this problem, purchase preservative-free solvents (when appropriate).

If you develop a separation with a solvent containing a stabilizer, continue to use that solvent throughout your analysis to ensure consistency.

Note: *Different lots of solvents, or solvents from different vendors, may use different stabilizers or may use varying concentrations of stabilizers.*

If the stabilizer interferes with the detector's background, switch to an unmodified solvent. Be aware that the separation may also change.

4.1.2 Solvent Degassing

Overview

Improperly degassed mobile phases cause 70% or more of all problems in liquid chromatography. Degassing is one of the most effective measures to eliminate many of these problems. The benefits are:

- Reproducible retention times
- Stable pump operation
- Stability in the baseline and enhanced sensitivity in many types of chromatographic detectors

Recommended degassing methods are described below.

Degassing Methods

There are three commonly used degassing methods:

- Vacuum filtration
- Sonication
- Sparging with helium

These methods may be used individually or in combination. The recommended order is:

- Vacuum filtration in conjunction with sonication
- Vacuum filtration without sonication
- Sonication
- Helium sparging

Vacuum Filtration and Sonication

Vacuum filtration reduces the pressure on the surface of the solvent. However, vacuum alone is too slow to be an acceptable means of degassing solvent. A 0.45 µm membrane filter with 300 mm of vacuum can filter and degas 4 liters of solvent in approximately 8 minutes.

The sonication method uses high energy sound waves to drive energy into the solvent and causes submicron-sized "bubbles" of gas to aggregate. As the gas bubbles aggregate, they become large enough to float out of the solvent and dissipate.

The use of vacuum filtering in conjunction with sonication quickly degasses a liter of solvent. This combination is less likely to change the composition of mixed solvents because the mixed solvents are not held under vacuum as long (less than a minute is usually sufficient). Conversely, sonication alone degasses 4 liters of solvent in approximately 22 minutes.

Attention: *Do not apply vacuum to the brown bottles in which solvent is shipped. There is a high risk of implosion under these conditions. Use a thick-walled container designed for vacuum applications.*

Sparging

Sparging removes gases from solution by saturating the solvent with a less soluble gas, usually helium. Helium sparging brings the solvent to a state of equilibrium, which may be maintained by slow sparging or by keeping a blanket of helium over the solvent. Blanketing inhibits reabsorption of atmospheric gases and also slows microbial growth.

Sparging is most effective in low pressure gradient mixing systems.

Well-sparged solvent:

- Improves pump performance
- Provides a stable baseline and better sensitivity in a detector
- Prevents reabsorption of atmospheric gases

4.1.3 Solvent Use

Recommended solvent use practices include:

Use a Solvent Inlet Filter

Ensure that your solvent reservoir includes a solvent inlet filter (sinker) to prevent foreign particles from being drawn into the pump. Use the solvent inlet filter in addition to the practice of filtering solvents.

Routinely check and clean the solvent inlet filter. Replace the filter if it is severely plugged. A plugged filter is evidenced by a lack of solvent flow through the tubing. Lack of solvent flow results in pump starvation.

Vent the Solvent Reservoirs

Provide venting for the solvent reservoir(s) to allow pressure equalization and to avoid pump starvation. Do *not* seal the reservoir.

Solvent Reservoir Position

Position the solvent reservoir higher (as possible) than the pump inlet manifold.

To determine if the solvent reservoir is high enough, open the pump draw-off valve two to three turns while running the pump at a desired flow rate.

- If solvent continuously drips out of the draw-off valve, the pump head height is sufficient.
- If solvent does not drip, raise the solvent reservoir.

Generally, use a head height of six inches (15 cm) as a minimum.

Note: *Elevating the solvent reservoir in a low-pressure mixing system may lead to solvent siphoning and to eventual failure of the automated sparging function.*

Ensure that the solvent inlet filter is clean and that the connecting tubing is not bent.

Maintain Sufficient Solvent

Always maintain sufficient solvent in the reservoirs to ensure that the reservoirs do not run dry. Running the pump for extended periods without solvent can severely damage the pump seals.

Observe pH Range of Mobile Phase

To ensure maximum column life, use mobile phases in the pH range supported by your column. The use of solvents outside of the specified pH range significantly reduces column life.

Solvents with UV-absorbing Impurities

Solvents used for gradient elution separations must be free of impurities. These impurities may result in high background absorbances, and spurious peaks on the chromatogram. Always run a blank gradient to ensure the solvent peaks on the chromatogram do not interfere with the analyte peaks. The use of high-quality solvents can minimize impurity problems.

Use of Volatile Solvents

The use of highly volatile solvents (such as hexane or ether) may make pump priming difficult. If using a highly volatile solvent, prime your pump with a miscible solvent (such as THF or methanol), or cool the pump.

Use of Aqueous Buffers or Water

Do not use aqueous buffers or water for longer than 48 hours. If using buffers, rotate the reservoirs with clean bottles to protect from contamination on the inside of the reservoir walls. Rinse the reservoir with water and methanol. If necessary, clean the reservoir with acid (avoid detergents).

Attention: *Always observe safe laboratory practices when handling solvents. Know the chemical and physical properties of the solvents. Refer to the Material Safety Data Sheet for the solvents in use.*

Aqueous buffers and water are susceptible to microbial growth, causing peaks to appear during gradient operation and increase background absorbance during isocratic operation.

Microbial growth also blocks filters, frits, columns, and pump check valves, which cause high column or pump backpressure. This backpressure could result in a system shutdown.

Steps to Avoid Microbial Growth

To avoid microbial growth in your mobile phase, perform one of the following:

- Prepare, filter, and degas mobile phase daily
- Store in a brown bottle
- Sparge and blanket with helium
- In certain instances, it is acceptable to add 0.02 percent sodium azide to the mobile phase

Attention: *Sodium azide is highly UV-absorbing and hazardous.*

To prevent microbial growth in your system during shutdown or over a period of time (over the weekend), flush the system completely with water, followed by 10 percent (minimum) of an appropriate organic solvent (such as acetonitrile or methanol).

Note: *Do not store the system in water or buffers alone. This results in bacterial growth in the system and precipitation of the buffers.*

Discard Old Mobile Phase

Discard aged or degraded mobile phase to avoid microbial growth or solvent composition changes.

Flush Pump Heads

When running with buffers, routinely flush behind the pump heads, or rinse the plunger seals with water to prevent particulate matter from building up on the pump plunger and seals. Most pumps have access holes or a provision for automatic washing behind the seals.

4.1.4 Solvent Changeover Practices

Recommended solvent changeover practices include:

Switching from Aqueous to Organic Solvents

When changing between an organic solvent and water containing salts, use Milli-Q water as an intermediate solvent. Use care when adding organic solvents to aqueous buffer solutions, as salt precipitation can occur. Precipitated salts can seriously degrade the pump check valves and other parts of the LC system.

Solvent Physical Properties

Know the physical properties of the solvents you use. Refer to Table A-1, Physical Properties of Solvents, to review solvent physical properties, such as viscosity, boiling point, and so on.

Check Solvent Miscibility

Ensure solvents are miscible before changing from one to another. Changes involving two miscible solvents may be made immediately, without an intermediate solvent. Before changing from one solvent to another, refer to Section A.1, Solvent Properties, to determine the miscibility of the solvents being used. If unsure, mix the two solvents in a beaker and look for the phase separation.

Changes involving two solvents which are not totally miscible (for example, from hexane to water) require an intermediate solvent (such as isopropanol or THF).

Column/Solvent Compatibility

Ensure that your column is compatible with all solvents used. If it is not compatible, disconnect the column when changing solvents.

4.2 System Plumbing

This section presents recommended operating practices for:

- Tubing and connector installation
- Cutting tubing

System Plumbing Troubleshooting Overview

A typical LC system includes a variety of tubing and connectors to facilitate connections between the:

- Solvent reservoirs and the pump
- Pump outlet and injector
- Injector and column
- Column and detector
- Detector and waste

The objective of properly plumbing an LC system is to provide leak-tight connections between components and to minimize dead volume within the system. Within those constraints, the tubing should not unnecessarily restrict solvent flow or increase system dead volume.

Effects of Improper Plumbing

The typical plumbing mistakes that can occur when connecting components include:

- Improper tubing/fitting assembly
- Interchanging different manufacturer compression fittings and/or ferrules
- Use of wrong internal diameter tubing

resulting in:

- Band broadening
- Leaks
- High system backpressure
- Baseline noise
- Damage to column end fittings

Once one of these problems is inadvertently introduced in a system, it may be difficult to detect. Other than a visual examination of all fitting, ferrules, and tubing for proper fit and wear, a system bandspreading test (see Section 4.4, Measuring System Bandspreading) is one of the best means to determine if tubing is causing a system performance problem.

Reference Material

For detailed information on connecting tubing, refer to the following reference material:

- Paul Upchurch, *Interchangeability of HPLC Fittings*, Upchurch Scientific, Inc., Oak Harbor, Washington, 1983
- J.W. Dolan, P. Upchurch, *Troubleshooting LC Fittings, Part I,* LC•GC Magazine, **6**(9), 788 (1988)
- J.W. Dolan, P. Upchurch, *Troubleshooting LC Fittings, Part II*, LC•GC Magazine, **6**(10), 886 (1988)

4.2.1 Tubing Connection Practices

This section outlines recommended practices for installing tubing and connectors. It covers:

- Interchanging fittings and ferrules
- Using unions and adapters
- Installing tubing
- Removing tubing blockages
- Repairing leaking fittings

Interchanging Fittings and Ferrules

Never interchange compression fittings and ferrules from different manufacturers. Fittings and ferrules may vary in:

- Fitting length and thread types
- Nut thread types
- Ferrule shape
- Distance the tubing extends past the ferrule once the ferrule is swagged onto the tubing

Mixing fittings and ferrules may result in leaks (due to inadequate ferrule seating) or stripped fitting threads (due to misthreading), or damage to column end fittings.

In addition, improper seating of tubing can create dead volume (small "mixing" chambers due to improperly fitted tubing), which increase system bandspreading. System bandspreading can affect system performance in the form of broad peaks, poor column efficiency, and reduced resolution. The use of correct fittings and ferrules ensures the proper tubing distance past the ferrule, minimizing dead volume.

Note: *When using hardware parts from a number of manufacturers, use a small parts box to store miscellaneous connecting hardware. Use separate compartments to store different vendor tubing components to avoid mixing different compression fittings and ferrules in the same box.*

Figure 4-3 illustrates a proper tubing assembly within a compression screw union.

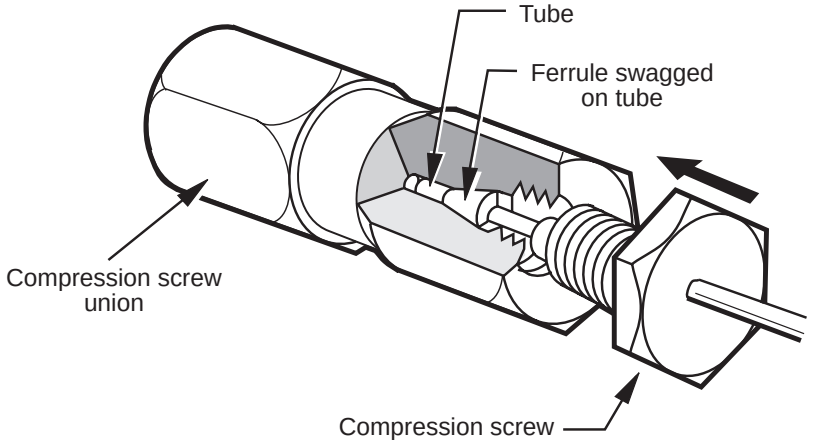


Figure 4-2 Typical Tubing/Compression Fitting Connection

Using an Adapter Assembly

To attach fittings/ferrules from different manufacturers, use an adapter assembly and a union to make the connection. The adapter assembly is a short piece of narrow diameter tubing with different fittings on each end. The adapter assembly allows compression fittings and nuts from different components to be interconnected.

Note: *Ensure that you use a union or adapter compatible with your existing system hardware.*

Figure 4-3 illustrates the use of an adapter assembly and union to attach a detector to a different manufacturer column.

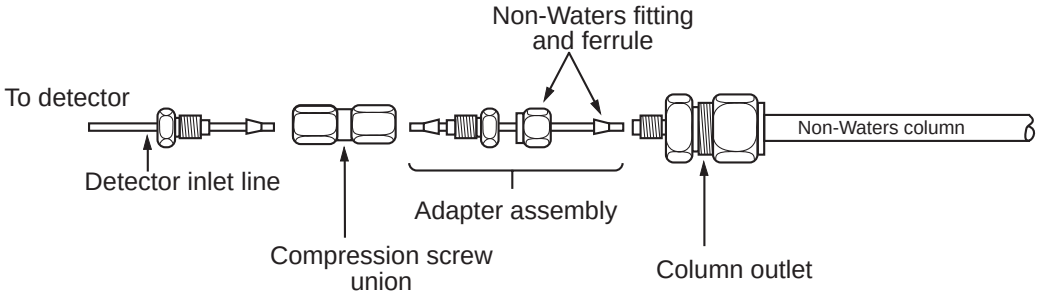


Figure 4-3 Adapter Assembly for Incompatible Fittings

When attaching an adapter assembly between different compression fittings, label each type of compression fitting (for example, Waters to Swagelok).

Installing Tubing

Most tubing in analytical LC systems have an outside diameter (OD) of 1/8-inch (3.2 mm) or 1/16-inch (1.6 mm). However, there are several sizes of tubing with varying internal diameters (ID) used in an LC system.

One source of high system backpressure is blocked or narrow tubing. Typical ID tubing sizes include:

- 0.005-inch (0.13 mm)
- 0.006-inch (0.15 mm)
- 0.007-inch (0.18 mm)
- 0.009-inch (0.23 mm)
- 0.010-inch (0.25 mm)
- 0.020-inch (0.5 mm)
- 0.030-inch (0.75 mm)
- 0.040-inch (1.0 mm)
- 0.046-inch (1.17 mm).

Each ID bore is intended for specific locations within the LC system and should not be interchanged. Refer to your column or instrument operator's manuals for tubing specifications.

When installing tubing, use the shortest length of tubing possible. This rule is especially true for the fluid path after the injector.

Do not nick, kink, or sharply bend the tubing. This may restrict flow, especially for a tubing ID of 0.020-inch (0.508 mm) or greater. Bent tubing could cause tubing failure.

After installing a piece of tubing. attach a label indicating the internal diameter of the new tubing. In addition, indicate the internal diameter of the tubing on your system map (see Section 1.3, Compare System Performance to Established Benchmarks). This avoids confusion regarding tubing size.

For the recommended procedure on cutting tubing, refer to Section 4.2.2.

Removing Tubing Blockages

Blocked tubing causes a dramatic increase in system pressure and fitting leaks. If you have a blocked piece of tubing (from particles in the solvent, sample, seal wear, precipitated buffers, and so on), isolate the location of the blockage as outlined in Section 2.2.1, System Pressure.

If the tubing is a standard size, you have the option to either attempt to remove the blockage (as outlined below) or replace the tubing. If replacement tubing is not readily available (such as an integral piece in an instrument), attempt to remove the blockage as outlined below:

To remove a tubing blockage:

1. Remove and reverse the piece of tubing. If the attached fitting is compatible with the pump, attach it to the pump outlet.
2. Place the free end of the tubing in a beaker.
3. Turn on the pump and run solvent at a flow rate of 0.3 mL/min through the tubing until the blockage is removed.

Attention: *Always observe safe laboratory practices when handling solvents.*

4. If the particle cannot be removed by this process, cut a new piece of tubing (refer to Section 4.2.2, Cutting Tubing) or contact the manufacturer.

Repairing Leaking Fittings

All compression fittings eventually wear out. When this occurs, the ferrule deforms and a leak starts. Do *not* attempt to overtighten the fitting to stop a leak. Overtightening does not improve performance and may damage the fitting threads or result in the fitting breaking off in the housing. This is especially true for polymeric fittings.

To repair a leaking fitting:

1. Stop pump flow.
2. Cut the tubing and remove the compression fitting (assuming it is not worn). The ferrule may remain on the tubing and may not be reusable.
3. If necessary, rinse the removed compression fitting with water to remove any salt crystals or debris.
4. Reassemble the compression fitting and new ferrule on the tubing.
5. If leakage still occurs, recut a new piece of tubing (using the same ID), and replace all parts.

4.2.2 Cutting Tubing

When cutting tubing, avoid angled cuts which may cause dead volume formation at the connection junction due to a poor tubing fit against the connector or part.

Stainless steel tubing with an internal diameter of less than 0.009-inch (0.23 mm) requires a special tubing cutter. When replacing this tubing, it is recommended to purchase pre-cut tubing.

To cut a length of tubing:

1. Estimate or measure the length required to connect the components. Allow slack so that the tubing is not pulled tightly around sharp corners.

   Note: *Ensure that you are using the correct ID tubing when replacing a piece of tubing.*

Cutting Stainless Steel Tubing

2. If using stainless steel tubing, use a circular tubing cutter to smoothly cut the tubing to the desired length (Figure 4-4). Rotate the cutter around the tube until it is cleanly scored.

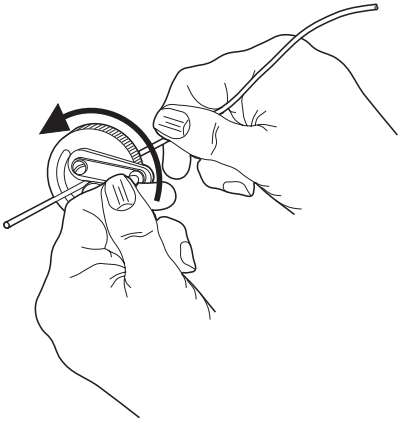


Figure 4-4 Cutting Tubing with a Circular Cutter

If you do not have a circular tubing cutter, use a knife-file to score the tube (Figure 4-5). Do not crush the tubing when scoring.

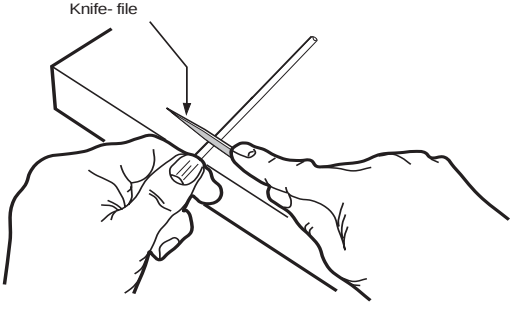


Figure 4-5 Cutting Tubing with a Sharp Object

Grip the cut tubing with two pairs of pair of smooth-jaw pliers, one on each side of the score (Figure 4-6). Gently bend back forth until the tubing cleanly snaps. This leaves the tubing bore open with minimum burrs.

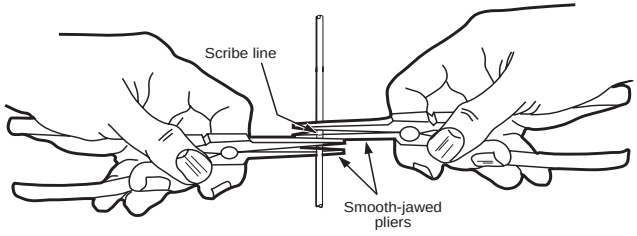


Figure 4-6 Breaking Stainless Steel Tubing

Cutting Polymeric Tubing

3. If using polymeric tubing, use a tubing cutter (Figure 4-7) or a sharp object (such as a razor blade) to square cut the tubing to the desired length. Do not crush the tubing when cutting.

   If using a polymeric tubing cutter, insert the tubing into the cutter so that the tubing extending from the metal side is the required length (see Figure 4-7). Use the proper hole to ensure a snug fit when cutting the tubing.

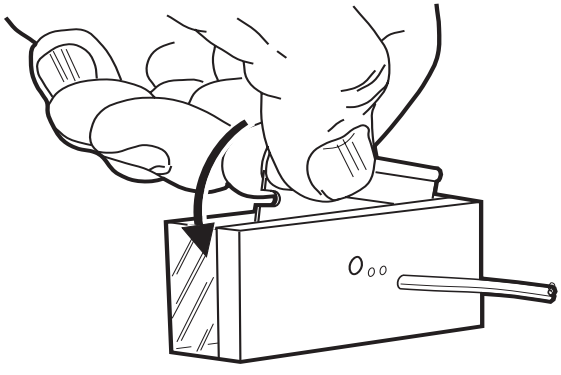


Figure 4-7 Using a Polymeric Tubing Cutter

Deburring Tubing

4. Inspect the cut for burrs or scratches and for the square of the cut. Make sure that the tubing is completely open, without any debris or burrs in the hole. If necessary, debur the hole with a very fine file or a deburring tool.

Note: *Always ensure that the tubing end is smooth, fully open, and without any remaining burrs. This allows the tubing to properly seal in the compression fitting and prevents particles from blocking interconnecting tubing.*

5. Flush the tubing with solvent prior to connection to remove any remaining particles. This prevents blockage and solvent/sample contamination.

Attaching Fittings and Ferrules

6. Attach the individual ferrules, compression fittings, and nuts as outlined in the column or instrument operator's manual.

4.3 Chromatographic Performance Tests

This section describes tests for measuring the chromatographic performance of your system.

System Performance Testing Overview

Routinely measure system performance to see if trends are occurring which may lead to future problems. In addition, when your chromatography (quality of your separation or peak shape) begins to degrade, evaluate system performance to determine if the problem is column, hardware, mobile phase, or sample-related.

System performance tests include:

- Measuring system resolution (R_s)
- Measuring capacity factor (k')
- Measuring column selectivity (α)
- Measuring column efficiency (N or column plate count)

Note: *Capacity factor (k'), selectivity (α), and column efficiency (N) are primarily for isocratic applications. If running gradients, you must rerun your original isocratic test mixture and then use k', α, and N resolution parameters to isolate the source of the problem.*

Note: *When evaluating system performance, all measurements must all be made in the same unit (minutes, milliliters, millimeters). Recommended practice is to use volume for all dimensions.*

To efficiently use system performance testing as a troubleshooting aid, you must initially have recorded system performance benchmarks in order to compare degraded system performance.

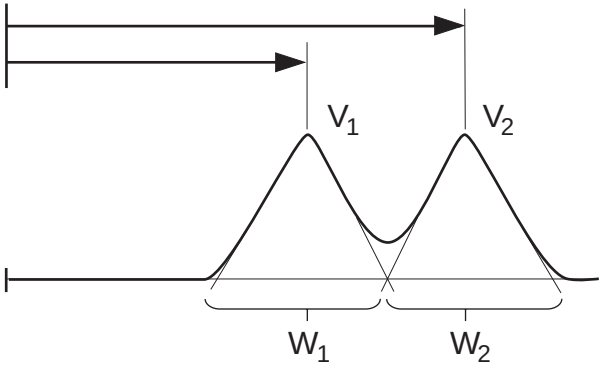
The process for measuring system bandspreading is covered in Section 4.4, Measuring System Bandspreading.

4.3.1 Measuring Resolution

Resolution is the distance between the peak centers of two component peaks divided by the average base width of the peaks.

Calculating Resolution (Rs)

The following calculation indicates the quality of a separation. Figure 4-8 illustrates how to compute resolution:



$$R_S = \frac{V_2 - V_1}{1/2(W_1 + W_2)}$$

Where:

R_s = Resolution

V_2 = Apex (retention volume) of peak 2

V_1 = Apex (retention volume) of peak 1

W_1 = Width of peak 1

W_2 = Width of peak 2

Figure 4-8 Calculating Resolution

Components of Resolution

There are three fundamental parameters that influence the resolution of a chromatographic separation:

- Capacity factor (k')
- Selectivity (α)
- Column efficiency (N)

These parameters provide you with different means to achieve better resolution, as well as defining different problem sources.

Resolution is a function of k', α, and N as shown below:

$$R_S = 1/4\left(\frac{\alpha - 1}{\alpha}\right)(\sqrt{N})\left(\frac{k'}{1 + k'}\right)$$

Where:

R_s = System resolution

α = Column selectivity

N = Column efficiency

k' = Capacity factor

Figure 4-9 illustrates the affect k', N, and α have on system resolution.

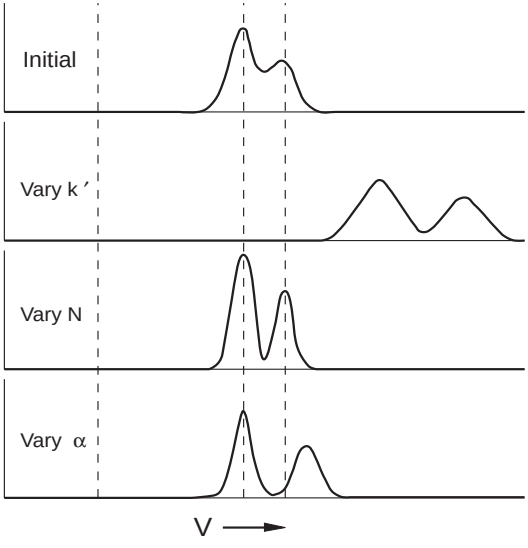


Figure 4-9 Chemical Factors that Affect System Resolution

As shown in Figure 4-9, initially, two compounds are partially separated. The resolution of these compounds can be changed in three different ways:

- If k' is increased, resolution is increased but the peaks become broader. Decreasing k' sharpens the peaks but decreases R_s.
- If N decreases, the resolution decreases because peak width broadens. However, the center-to-center (apex-to-apex) distance is constant. When N is increased, the peak width narrows.
- If α is increased, resolution is increased because one peak moves relative to another.

To measure and modify the capacity factor (k'), column selectivity (α), or column efficiency (N) parameters:

Resolution Parameter	Refer to
Capacity factor (k')	Section 4.3.2
Column selectivity (α)	Section 4.3.3
Column efficiency (N)	Section 4.3.4

4.3.2 Measuring Capacity Factor (k')

Troubleshooting Capacity Factor (k')

Capacity factor (k') is a measurement of the retention time of a sample molecule, relative to column dead volume (V_0).

Note: *V_0 is measured using a probe molecule that is unretained by the column under standard test conditions. Consult your column operator's manual for the appropriate probe molecule to use.*

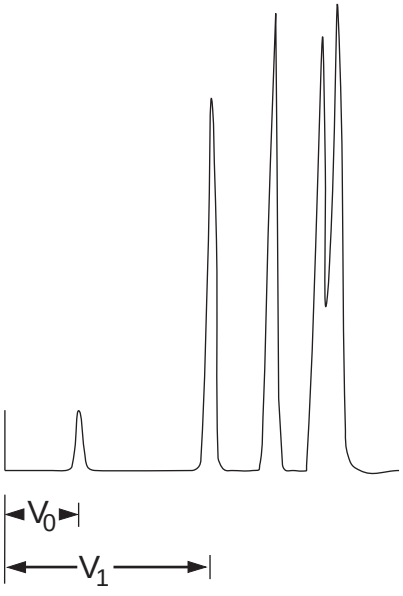
Capacity factor (k') changes are typically due to:

- Variations in mobile phase composition
- Changes in column surface chemistry (due to aging
- Changes in operating temperature.

In most chromatography modes, capacity factor (k') changes by 10 percent for a temperature change of 5° C.

Calculating Capacity Factor (k')

Use the equation in Figure 4-10 to compute capacity factor (k'):



$$k' = \frac{V_1 - V_0}{V_0}$$

Where:

k' = Capacity factor of the column

V_0 = Void volume (or dead volume) of the column (volume at which an unretained component elutes)

V_1 = Retention volume of peak 1

Figure 4-10 Calculating Capacity Factor

For example, if:

V_0 = 2.90 mL

V_1 = 9.75 mL

Then:

$$k' = \frac{9.75 - 2.90}{2.90} = 2.36$$

Adjusting Capacity Factor (k')

Good isocratic methods usually have a capacity factor (k') in the range of 2 to 10 (typically between 2 and 5.). Lower values may give inadequate resolution. Higher values are usually associated with excessively broad peaks and unacceptably long run times.

If the analytes fall outside their specified windows, run the initial column test protocol to compare the results with when the column was new.

Capacity factor (k') values are sensitive to the following change in conditions:

- Solvent strength, composition, and purity
- Temperature
- Column surface chemistry
- Sample

If the shift in capacity factor (k') value is observed with both analytes and the column test solution, the problem is most likely due to a change in the column, temperature, or mobile phase composition. This is particularly true if the shift occurred gradually over a series of runs. If, however, the test mixture runs as expected, the problem is most likely sample-related.

From these possible causes, refer to your system benchmark comparison to evaluate the origin of the problem. For example, check the following:

- Mobile phase composition (is it correct?)
- Operating temperature (has it changed?)
- Age of the column

4.3.3 Measuring Selectivity

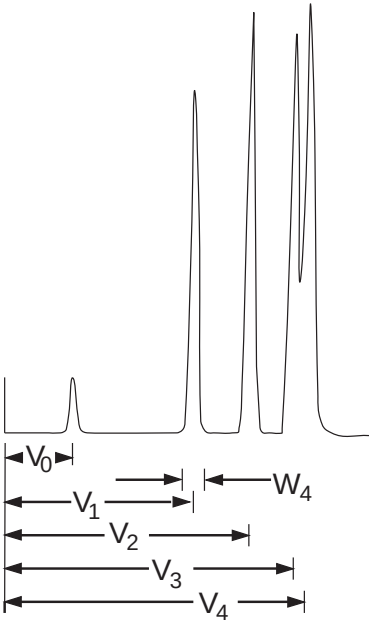
Troubleshooting Selectivity

Selectivity (α) is the relative retention of two peaks in a chromatogram (the ratio of two k' values). You can recognize problems in your separation due to selectivity (α) changes when some peaks move significantly relative to other peaks.

Usually, you can control selectivity (α) in LC by varying the "chemistry" of the system, such as mobile phase (pH, salt strength, organic solvent and composition type, or modifier type) or type of column.

Calculating Selectivity (α)

Use the equation in Figure 4-11 to compute selectivity (α).



$$\alpha = \frac{k'_2}{k'_1} = \frac{V_2 - V_0}{V_1 - V_0}$$

Where:

α = Relative retention

k'_1 = Capacity factor for V_1

k'_2 = Capacity factor for V_2

V_0 = Void volume (or dead volume) of the column (volume at which unretained component elutes)

V_1 = Retention volume of peak 1

V_2 = Retention volume of peak 2

Figure 4-11 Calculating Selectivity

For example, if:

V_1 = 9.75 mL

V_2 = 12.80 mL

V_3 = 15.20 mL

V_4 = 16.00 ml

$$\alpha V_1, V_2 = \frac{12.8 - 2.9}{9.75 - 2.9} = 1.45$$

$$\alpha V_3, V_4 = \frac{16.0 - 2.9}{15.2 - 2.9} = 1.06$$

Adjusting Selectivity (α)

When troubleshooting changes in selectivity a), the approach is similar to the approach used to troubleshoot changes in capacity factor (k').

When selectivity (α) is affected, the corrective action depends on whether the problem is mobile phase or column-related.

Be sure to compare results obtained with the test solution to those observed when the column was new. Use these results to distinquish column changes from problems with mobile phase or other operating parameters.

Selectivity (α) values are sensitive to changes in the following conditions:

- Mobile phase composition (pH, ionic strength) and purity
- Age of column
- Temperature

As outlined in Section 4.3.2, Measuring Capacity Factor (k'), rerun your test solution and compare the results. From the possible causes listed above, evaluate the origin of the problem.

4.3.4 Measuring Column Efficiency (N)

Troubleshooting Column Efficiency (N)

The column efficiency (N) (also called theoretical plate count), is a measure of the bandspreading of a peak. The smaller the band spread, the higher the number of theoretical plates, which indicates good column and system performance. The measurement of column efficiency is actually the total efficiency for the LC system and column combined.

A decline in measured efficiency may be due to:

- Age and history of the column
- Extra column band broadening (such as due to a malfunctioning injector or improper tubing ID)
- Inappropriate detector settings (for example, time constant)
- Change in flow rate and solvent viscosity

You can recognize problems in your separation due to a loss of column efficiency when the width and/or shape of all peaks are affected. Figure 4-12 illustrates low efficiency and high efficiency peak shapes.

If a loss of efficiency is the cause of your problem, repeat the column test performed when the column was new. If the test result is similar to the first test, the problem is specific to the method in use.

If the measured efficiency has degraded, either the column has degraded or system band-broadening has increased. At this point, check system bandspreading against established benchmarks (refer to Section 4.4).

Note: *If a guard column is being used, it can be a source of band-broadening and the component most likely to fail first. Always document system efficiency with and without the guard column.*

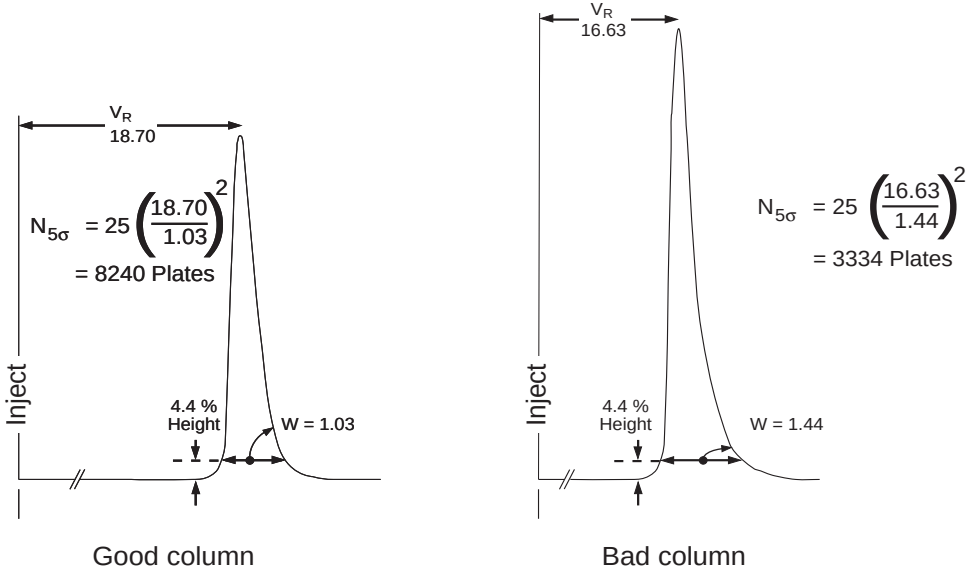


Figure 4-12 Examples of Low and High Efficiency Peaks

Methods of Measuring Column Efficiency

Methods used for the measurement and calculation of column efficiency include (in order of sensitivity to abnormal peak shape:

- Asymmetry-based (Most sensitive to tailing or fronting)
- 5 sigma
- 4 sigma
- Tangent
- 3 sigma
- 1/2 height
- 2 sigma (inflection) (Least sensitive to tailing or fronting)

Choose the method that best suits your operating requirements. It is critical that the same method always be used and executed reproducibly.

Figure 4-13 illustrates the use of the different peak widths of a Gaussian peak for the calculation of column efficiency (N).

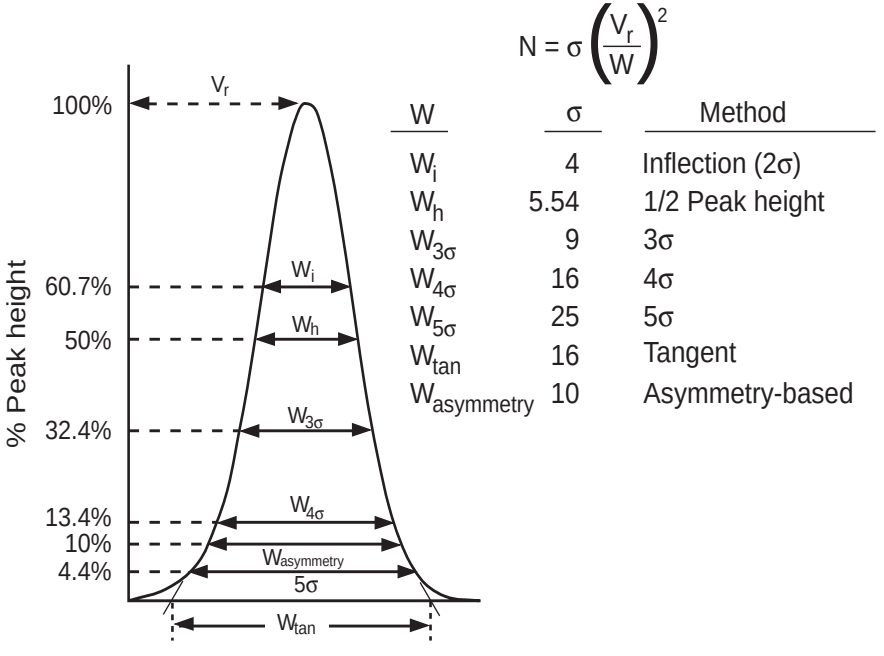
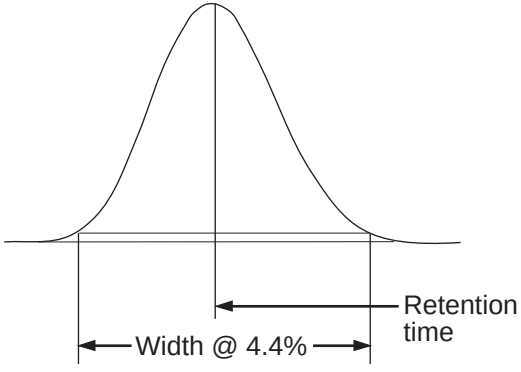


Figure 4-13 Methods of Calculating Column Efficiency

When measuring column efficiency, use test conditions identical to those used in the established benchmark performance (such as test sample, flow rate, mobile phase composition, and so on). Measure the column efficiency against the established performance.

5 Sigma

Figure 4-14 illustrates the 5 sigma method:



$$N = 25\left(\frac{Rt}{W}\right)^2$$

N = Plate count (the number of theoretical plates in a chromatographic column)

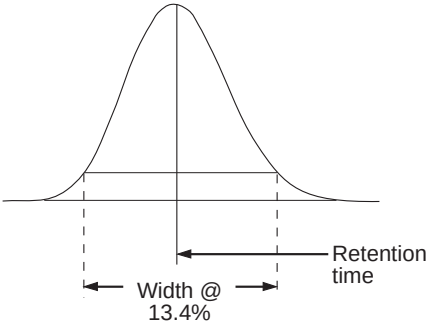
Rt = Retention time

W = Peak width at 4.4% of peak height

Figure 4-14 5 Sigma Method Calculation

4 Sigma

Figure 4-15 illustrates the 4 sigma method:



$$N = 16\left(\frac{Rt}{W}\right)^2$$

N = Plate count (the number of theoretical plates in a chromatographic column)

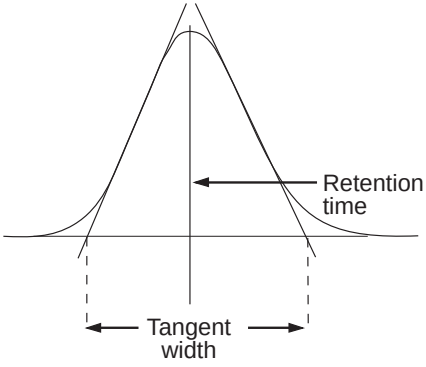
Rt = Retention time

W = Peak width at 13.4% of peak height

Figure 4-15 4 Sigma Method Calculation

Tangent

Figure 4-16 illustrates the tangent method:



$$N = 16\left(\frac{Rt}{W}\right)^2$$

N = Plate count (the number of theoretical plates in a chromatographic column)

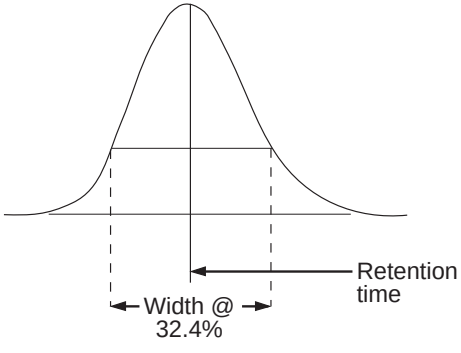
Rt = Retention time

W = Peak width at baseline determined by tangents

Figure 4-16 Tangent Method Calculation

3 Sigma

Figure 4-17 illustrates the 3 sigma method:



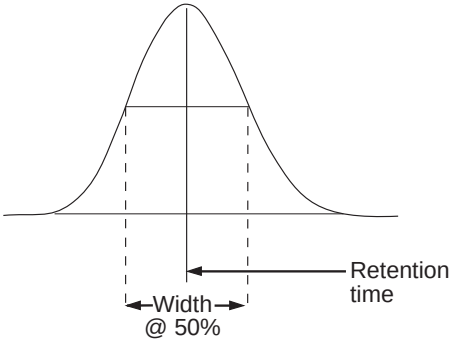
$$N = 9\left(\frac{Rt}{W}\right)^2$$

- N = Plate count (the number of theoretical plates in a chromatographic column)
- Rt = Retention time
- W = Peak width at 32.4% of peak height

Figure 4-17 3 Sigma Method Calculation

1/2 Height

Figure 4-18 illustrates the 1/2 height method:



$$N = 5.54\left(\frac{Rt}{W}\right)^2$$

N = Plate count (the number of theoretical plates in a chromatographic column)

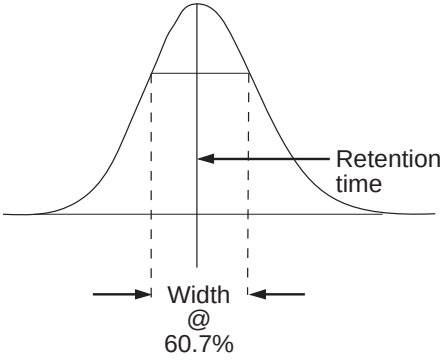
Rt = Retention time

W = Peak width at 50.0% of peak height

Figure 4-18 1/2 Height Method Calculation

2 Sigma (inflection)

Figure 4-19 illustrates the 2 sigma method:



$$N = 4\left(\frac{Rt}{W}\right)^2$$

N = Plate count (the number of theoretical plates in a chromatographic column)

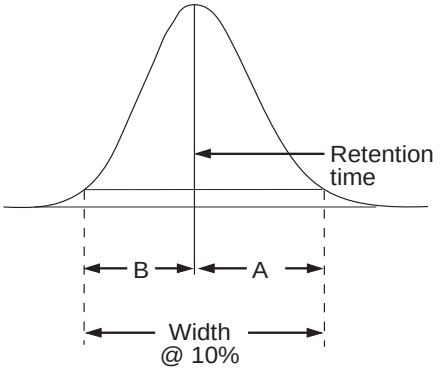
Rt = Retention time

W = Peak width at 60.7% of peak height

Figure 4-19 2 Sigma (Inflection) Method Calculation

Asymmetry-based

Figure 4-20 illustrates the asymmetry-based method:



$$N = \frac{41.7 \times \left(\frac{Rt}{W}\right)^2}{\left(\frac{A}{B} + 1.25\right)}$$

N = Plate count (the number of theoretical plates in a chromatographic column)

Rt = Retention time

W = Peak width at 10% of peak height

A = Width of line from Rt to peak end at 10.0% of peak height

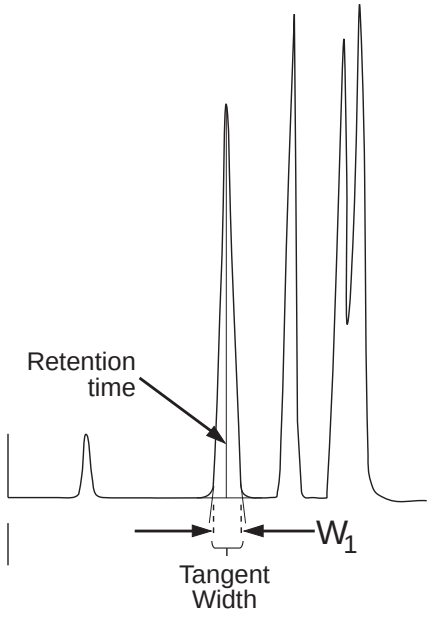
B = Width of line from peak start to Rt at 10.0% of peak height

(Foley and Dorsey, Anal. Chem., 1983, 55, 730)

Figure 4-20 Asymmetry-Based Method Calculation

Column Efficiency (N) Calculation Example (Tangent Method)

Figure 4-21 is an example of calculating column efficiency (N) using the tangent method:



$$N = 16\left(\frac{Rt}{W}\right)^2$$

$$N = 16\left(\frac{9.75}{0.6}\right)^2 = 4225 \text{plates}$$

Figure 4-21 Example of Calculating Column Efficiency (N)

Reduced Column Efficiency

If the measured column efficiency is low (less than 75 percent of the original measurement for the column), isolate the source of the problem by replacing the column with a new or known good column. Measure column efficiency for the new column.

If the column efficiency measurement for the replacement column is:

- **Normal** - The first column has degraded and may need to be replaced.
- **Low** - Investigate other areas within your system, such as incorrect tubing ID, guard column, plugged filters and frits, malfunctioning injector, or malfunctioning detector.

Perform a bandspreading measurement of the total LC system to verify system efficiency and to isolate the source of the malfunction (see Section 4.4, Measuring System Bandspreading).

4.4 Measuring System Bandspreading

Low column efficiency may not always be due to column degradation. Troubleshoot the entire system to determine the exact cause.

Perform a system bandspreading measurement to determine if low column efficiency is due to degraded system performance. If system bandspreading has increased, this allows you to start isolating the hardware problem to such areas as the injector, detector, or tubing (ID, length, and so on).

This procedure illustrates measuring system bandspreading at a peak width of 4.4 percent of the peak height (in µl).

To perform system bandspreading:

1. If you have not already done so, measure column efficiency as outlined in Section 4.3.4.
2. Remove only the column and install a union in place.

   Note: *If you require additional system backpressure (especially if using an autoinjector) during this procedure, use a piece of 0.009-inch (0.23 mm) ID tubing (or suitable restrictor) instead of a union.*

3. Configure your system to the following parameters:

Parameter	Setting
Flow Rate	1.0 ml/min
Chart Speed (using a chart recorder)	20 cm/min
Detector Sensitivity	0.5 to 1.0 AUFS
Detector time constant	0.2 or less

4. Dilute your column efficiency test mixture 1-to-10 in the mobile phase. Inject 2 to 5 µl of the solution.
5. Adjust the detector sensitivity until the peak height is between 50 and 100 percent full scale.
6. Using the 5 sigma column efficiency method, measure the peak width at 4.4 percent of the peak height.
7. Convert this value to microliters using the following equation:

$$\textit{Peak width at 4.4\% (in cm)}\left(\frac{1min}{20cm}\right)\left(\frac{1mL}{min}\right)\left(\frac{1000\mu l}{mL}\right) = bandspread(\mu l)$$

Where:

1 min/20 cm = Chart speed

1 mL/min = Flow rate

1000 µl/mL = Volume correction factor

A typical analytical system bandspread value is 100 µl (±30 µl). A larger bandspreading value may indicate a problem in the injector, detector, or in the tubing/fittings.

Note: *Certain detectors with built-in added volume (such as counter current heat exchangers) may show increased values as well.*

8. If the bandspreading value is greater than 100 µl (±30 µl), troubleshoot your LC system in the following order to isolate the cause of increased bandspreading:
   - Incorrectly installed tubing or wrong tubing ID
   - Partially plugged in-line filter
   - Injector problems
   - Detector problems
9. When the suspected problem is resolved, remeasure system bandspreading. If the bandspreading value is reduced, the problem is resolved.

   If the value is still high, continue to investigate the other areas within the LC system. Recalculate system bandspreading until the value is reduced. If the value remains high, contact your Customer Support Department.

A

Appendix A
Reference Information

This Appendix includes:

- •Solvent properties table (including information on solvent miscibility, viscosity, polarity, and boiling point)
- Refractive index of common solvents
- Solvent UV cutoffs
- Wavelength selection for chromophore detection
- Column backpressure

A.1 Solvent Properties

Before changing from one solvent to another, refer to Table A-1 to determine the miscibility and viscosity of the two solvents.

When determining miscibility using Table A-1, note:

- If two solvents are miscible, you may change directly from one solvent to the next. Changes involving two solvents that are not totally miscible (for example, chloroform to water) require an intermediate solvent (such as methanol).
- Temperature affects solvent miscibility. If operating at an elevated temperature, consider the effect of high temperature on solvent solubility.
- Buffers dissolved in water may precipitate when mixed with organic solvents. Be sure to flush your system with water *before* running this mixture.

A.1.1 Solvent Properties Table

Table A-1 lists the physical properties of a series of solvents. Use this table in conjunction with Section A.1.2, Using Miscibility Numbers (M-Numbers).

Table A-1 Physical Properties of Solvents[1]

Polarity Index	Solvent	Viscosity [n] CP, 20C	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

Table A-1 Physical Properties of Solvents[1] *(Continued)*

Polarity Index	Solvent	Viscosity [n] CP, 20C	Boiling Point° C (1 atm)	Miscibility Number (M)
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
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	Acetonirile	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	--

[1] Adapted from: Godfrey, Norman B., Solvent Selection via Miscibility Number, CHEMTECH, 359-363 (1972)

A.1.2 Using Miscibility Numbers (M-Numbers)

Miscibility numbers (M-numbers) are used to predict the miscibility of a liquid with one of the standard solvents (as listed in Table A-1).

To determine the miscibility of the two liquids, subtract the smaller 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 shows 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 solvents due to hydrogen bonding. Unusually strong hydrogen bonding is also responsible for the miscibility of long-chain alcohols or carboxylic acids with standard solvents of low M-number. Conversely, they show anomalous immiscibility with aprotic solvents of low M-number.

Some solvents prove immiscible with solvents at both ends of the lipophilicity scale. These solvents receive a dual M-number. The first number, always lower than 16, indicates the degree of miscibility with highly lipophilic solvents. The second number applies to the opposite end of the scale. A large difference between these two numbers indicates a limited range of miscibility. For example, some fluorocarbons are immiscible with all the standard solvents and have M-numbers of 0, 32. Two liquids with dual M-numbers are usually miscible with each other.

A liquid is classified in the M-number system by testing for miscibility with a sequence of standard solvents. A correction term of 15 units is then either added or subtracted from the cutoff point for miscibility.

A.2 Refractive Index of Common Solvents

Table A-2 lists the refractive index for some common chromatographic solvents. Use this table to verify that the solvent you intend to use for your analysis has different RI characteristics than the sample components.

Table A-2 Refractive Indices of Common Solvents

Solvent	RI	Solvent	RI
Fluoroalkane	1.250	Isopropyl chloride	1.378
Hexafluoro Isopropanol	1.2752	Isopropanol	1.380
Methanol	1.329	n-Propanol	1.380
Water	1.330	Methyl ethyl ketone	1.381
Acetonitrile	1.344	Diethyl amine	1.387
Ethyl ether	1.353	n-Propyl chloride	1.389
n-Pentane	1.358	Methyl-isobutylketone	1.394
Acetone	1.359	Nitromethane	1.394
Ethanol	1.361	1-Nitropropane	1.400
Methyl acetate	1.362	Octane	1.404
Isopropyl ether	1.368	Cyclopentane	1.406
Ethyl acetate	1.370	Tetrahydrofuran	1.408
1-Pentene	1.371	Amyl alcohol	1.410
Acetic acid	1.372	Diisobutylene	1.411
n-Decane	1.412	Ethylene	1.445
Amyl chloride	1.413	Carbon tetrachloride	1.466
p-Dioxane	1.422	Dimethyl sulfoxide	1.477
Ethyl bromide	1.424	Toluene	1.496

Table A-2 Refractive Indices of Common Solvents *(Continued)*

Solvent	RI	Solvent	RI
Methylene chloride	1.424	Xylene	~1.50
Cyclohexane	1.427	Benzene	1.501
Ethylene glycol	1.427	Pyridine	1.510
N,N'-Dimethyl Formamide	1.428	Chlorobenzene	1.525
N,N'-Dimethyl Acetamide	1.438	o-Chlorophenol	1.547
Ethyl sulfide	1.442	Aniline	1.586
Chloroform	1.443	Carbon disulfide	1.626

A.3 Solvent UV Cutoffs

Table A-3 lists the UV cutoff (the wavelength at which the absorbance of the solvent is equal to 1 AU) for some common chromatographic solvents. Operating at a wavelength near or below the cutoff reduces the ability to sense the compound, and increases baseline noise due to the high absorbance of the solvent.

Table A-3 UV Cutoffs for Chromatographic Solvents

Solvent	UV Cutoff	Solvent	UV Cutoff
n-Pentane	190	Methyl-isobutyl ketone	334
Octane	215	Tetrahydrofuran	230
Petroleum ether	210	Ethylene dichloride	230
Cyclohexane	200	Methyl ethyl ketone	330
Cyclopentane	200	1-Nitropropane	380

Table A-3 UV Cutoffs for Chromatographic Solvents *(Continued)*

Solvent	UV Cutoff	Solvent	UV Cutoff
Carbon disulfide	380	Acetone	330
Carbon tetrachloride	265	p-Dioxane	215
Amyl chloride	225	Ethyl acetate	256
Xylene	290	Methyl acetate	260
Isopropyl ether	220	Amyl alcohol	210
Isopropyl chloride	225	Diethyl amine	275
Toluene	285	Nitromethane	380
n-Propyl chloride	225	Acetonitrile	190
Benzene	280	Pyridine	330
Ethyl ether	220	2-Butoxyethanol	220
Ethyl sulfide	290	Isopropanol	205
Chloroform	245	n-Propanol	210
Methylene chloride	233	Ethanol	210
Methanol	205	Ethylene glycol	210

Table A-4 contains approximate wavelength cutoffs for solvents, buffers, detergents, and mobile phases.

Table A-4 Wavelength Cutoffs for Common Mobile Phases[1]

Mobile Phase	UV Cutoff	Mobile Phase	UV Cutoff
Acetic acid, 1%	230	Tween™ 20, 0.1%	190
Trifluoroacetic acid, 0.1%	205	Hydrochloric acid, 0.1%	190
Triethylamine, 1%	235	Diammonium phosphate, 50 mM	205
PIC Reagent B-6, 1 vial/liter	225	Waters PIC® Reagent A, 1 vial/liter	< 200
PIC Reagent D-4, 1 vial/liter	190	PIC Reagent B-6, low UV, 1 vial/liter	190
Ammonium bicarbonate, 10 mM	190	Ammonium acetate, 10 mM	205
HEPES, 10 mM, pH 7.6	225	EDTA, disodium, 1 mM	190
Potassium phosphate, monobasic, 10 mM dibasic,10 mM	 190 190	MES, 10 mM, pH 6.0 Sodium acetate, 10 mM	225 205
Sodium chloride, 1 M	208	Sodium citrate, 10 mM	225
Sodium formate, 10 mM	200	TRIS HCl, 20 mM, pH7.0 pH 8.0	 204 212
BRIJ 35, 0.1%	190	CHAPS, 0.1%	215
Sodium dodecyl sulfate, 0.1%	190	Triton-X™ 100, 0.1%	240

[1] From "Selection of Solvents and Mobile Phases for HPLC to Optimize Sensitivity of UV Detection", Jeanne B. Li. *Journal of Analysis and Purification*, Vol 2, No. 1 72-75 (1987).

A.4 Wavelength Selection for Chromophore Detection

Chromophores are molecular groups in a sample which absorb light. Chromophore behavior can be used to categorize the detection of sample molecules.

Table A-5 lists some common chromophores and their detection wavelengths (λ_{max}), as well as the molar absorptivity (ε_{max}) of each group. Multiple bands are listed for components that have more than one spectral maximum.

Use this information as a guide to select the optimum operating wavelength for a particular analysis. Some experimentation may be necessary to obtain the most suitable wavelengths for a particular analysis.

Table A-5 Electronic Absorption Bands for Representative Chromophores[1]

Chromo-phore	System	λmax (nm)	∈max (L/m/cm)	λmax (nm)	∈max (L/m/cm)	λmax (nm)	∈max (L/m/cm)
Ether	—O—	185	1000	---	---	---	---
Thioether	—S—	194	4600	215	1600	---	---
Amine	—NH_2	195	2800	---	---	---	---
Thiol	—SH	195	1400	---	---	---	---
Disulfide	—S—S—	194	5500	255	400	---	---
Bromide	—Br	208	300	---	---	---	---
Iodide	—I	260	400	---	---	---	---
Nitrile	—C≡N	160	---	---	---	---	---
Acetylide	—C≡C—	175-180	6000	---	---	---	---
Sulfone	—SO_2—	180	---	---	---	---	---
Oxime	—NOH	190	5000	---	---	---	---
Azidin	>C=N—	190	5000	---	---	---	---
Ethylene	—C=C—	190	8000	---	---	---	---

Table A-5 Electronic Absorption Bands for Representative Chromophores[1] *(Continued)*

Chromo-phore	System	λmax (nm)	∈max (L/m/cm)	λmax (nm)	∈max (L/m/cm)	λmax (nm)	∈max (L/m/cm)
Ketone	>C=O	195	1000	270-285	18—30	---	---
Thioketone	>C=S	205	strong	---	---	---	---
Ester	—COOR	205	50	---	---	---	---
Aldehyde	—CHO	210	strong	280-300	11—18	---	---
Carboxyl	—COOH	200-210	50-70	---	---	---	---
Sulfoxide	>S—O	210	1500	---	---	---	---
Nitro	$—NO_2$	210	strong	---	---	---	---
Nitrile	—ONO	220-230	1000-2000	300-400	10	---	---
Azo	—N=N—	285-400	3-25	---	---	---	---
Nitroso	—N=O	302	100	---	---	---	---
Nitrate	$—ONO_2$	270 (shoulder)	12	---	---	---	---
	$—(C=C)_2—$ (acyclic)	210-230	21,000	---	---	---	---
	$—(C=C)_3—$	260	35,000	---	---	---	---
Benzene	---	184	46,700	202	6,900	255	170
Diphenyl	---	246	20,000	---	---	---	---
Naphthalene	---	220	112,000	275	5,600	312	175
Anthracene	---	252	199,000	375	7,900	---	---

Table A-5 Electronic Absorption Bands for Representative Chromophores[1] *(Continued)*

Chromo-phore	System	λmax (nm)	∈max (L/m/cm)	λmax (nm)	∈max (L/m/cm)	λmax (nm)	∈max (L/m/cm)
Pyridine	---	174	80,000	195	6,000	251	1,700
Quinoline	---	227	37,000	270	3,600	314	2,750
Isoquinoline	---	218	80,000	266	4,000	317	3,500

[1] From Instrumental Methods of Analysis, 6th ed., H.H. Willard, et al. ©1981 Litton Educational Publishing, Inc. Reprinted by permission of Wadsworth Publishing Co., Belmont, California, 94002

A.5 Column Backpressure

The normal operating backpressure of a column varies considerably, and is affected by column length, flow rate, mobile phase viscosity, temperature, and particle size. Backpressure may rise significantly during the course of a gradient. This is especially evident in a water/methanol gradient.

The following equation is used to calculate the typical operating backpressure of a cartridge or column with water as the mobile phase. The sum of the equation is then multiplied by the viscosity of the mobile phase in centipoise (see Table A-1).

$$Pressure(atm)atml/minofwater = \frac{2.1 \times f \times L(cm)}{dp^2(\mu m) \times d^2(mm)} \times \eta$$

where:

L = Column length

dp = Particle diameter

d = Column diameter

f = 2000 for cartridges,
1000 for steel columns

η = Mobile phase viscosity, centipoise (see Table A-1)

Note: *A variation of ±10 to 15 percent is acceptable.*

Figure A-1 shows the variation of viscosity for several aqueous solvent mixtures.

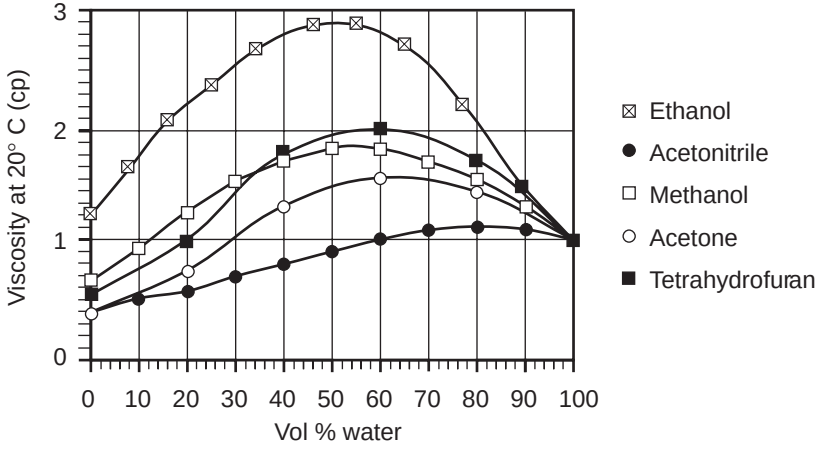


Figure A-1 Viscosity for Aqueous Solvent Mixtures