HPLC PRACTICAL GUIDE

FREE STARTER KIT

HPLC TROUBLESHOOTING STARTER KIT

WHAT TO CHECK FIRST WHEN HPLC RESULTS LOOK WRONG

A practical beginner-friendly checklist for students, junior analysts, and early-career QC laboratory professionals

INCLUDES DIAGRAMS, CHECKLISTS, TROUBLESHOOTING TABLES, AND PRACTICAL TIPS

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HPLC Practical Guide

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This free starter kit is provided for educational purposes only. It is designed to help beginners understand common HPLC troubleshooting symptoms and basic first-check logic. It is not intended to replace official laboratory training, validated analytical methods, regulatory guidelines, instrument manuals, laboratory SOPs, or professional supervision.

The chromatograms, diagrams, tables, checklists, and examples used in this starter kit are educational illustrations. They are intended to support learning and may not represent actual experimental data unless clearly stated.

About This Free Starter Kit

Many HPLC beginners feel confused when a chromatogram does not look normal. A retention time may shift, the baseline may drift, the pressure may increase, a peak may tail, or repeated injections may give poor %RSD.

The first mistake is often to change something quickly without understanding the symptom.

This starter kit is designed to help you pause, observe the problem, and decide what to check first.

It does not cover every possible HPLC problem. Instead, it gives you a simple practical framework for the most common beginner-level issues:

- Retention time shift
- Baseline noise or drift
- High pressure
- Peak tailing
- Poor injection reproducibility
- Carryover or ghost peaks

The goal is not to memorize every cause. The goal is to learn how to think more logically when HPLC results look wrong.

How to Use This Starter Kit

Read this starter kit as a quick practical reference.

Start with the HPLC troubleshooting mindset. Then review each common symptom and connect it with possible causes and first checks.

For best results:

- Do not jump directly to changing the column.
- Compare the current run with previous normal runs.
- Check what changed before the problem appeared.
- Look at pressure, baseline, retention time, peak shape, and injection order together.
- Use the final checklist before making major method changes.

By the end of this starter kit, you should be able to describe an HPLC problem more clearly and decide what to check first before taking corrective action.

Contents

1. The Beginner HPLC Troubleshooting Mindset
2. The HPLC Flow Path: Where Problems Can Start
3. Quick Symptom-to-Check Table
4. Problem 1: Retention Time Shift
5. Problem 2: Baseline Noise or Drift
6. Problem 3: High Pressure
7. Problem 4: Peak Tailing
8. Problem 5: Poor %RSD in Repeated Injections
9. Problem 6: Carryover and Ghost Peaks
10. Mini Practice Case: High Pressure After Several Injections
11. Final HPLC First-Check Checklist
12. Continue Learning with the Full Series

1. The Beginner HPLC Troubleshooting Mindset

HPLC troubleshooting starts with one important idea:

A chromatographic problem is a symptom, not a diagnosis.

For example, high pressure does not automatically mean the column is damaged. Retention time shift does not automatically mean the method is wrong. A noisy baseline does not automatically mean the detector is failing.

A good analyst first asks:

- What exactly changed?
- When did the problem start?
- Did the pressure change?
- Did the retention time change?
- Did the peak shape change?
- Did the problem appear in blank, standard, sample, or all injections?
- Was anything prepared differently today?

Troubleshooting becomes easier when you describe the problem before trying to solve it.

Practical Tip

Before changing the method, replacing the column, or adjusting integration, compare the current chromatogram with a previous acceptable chromatogram from the same method. A small difference in pressure, retention time, peak shape, or baseline behavior can guide you toward the most likely root cause.

Common Beginner Mistake

A common mistake is to blame the column too early. The column is important, but many HPLC problems begin before the column: mobile phase preparation, pump purging, air bubbles, injection issues, sample preparation, filtration, or system equilibration.

Think Like an Analyst

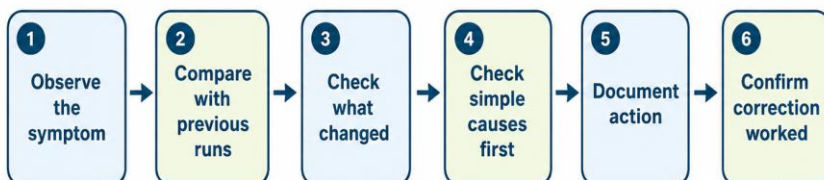
“What is wrong with the chromatogram?”

Ask:

“What evidence do I have, and what should I check first?”

Basic HPLC Troubleshooting Thinking Flow

A practical sequence for moving from symptom to corrective direction.



Core rule:
Do not change conditions randomly. Build the diagnosis from evidence, then confirm the correction.

Figure 1.1. Basic HPLC troubleshooting thinking flow.

2. The HPLC Flow Path: Where Problems Can Start

An HPLC system is a connected flow path. A problem in one part of the system can appear as a problem in the chromatogram.

Simple HPLC flow path:

Mobile Phase Reservoir → Pump → Injector / Autosampler → Column → Detector → Data System

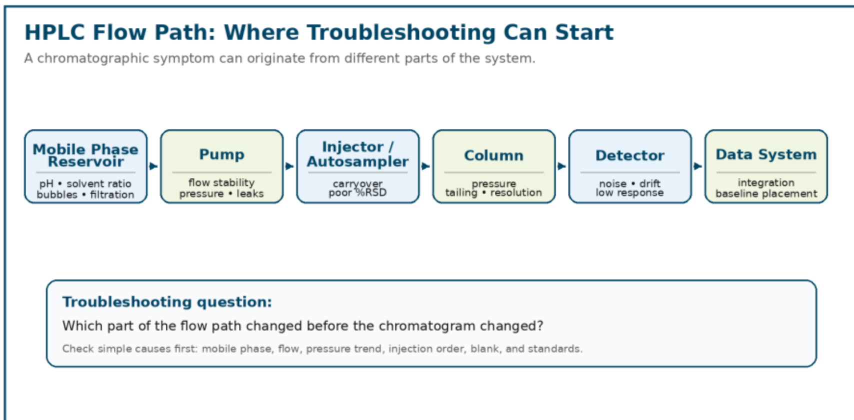


Figure 1.2. HPLC flow path and common troubleshooting sources

HPLC Area	Possible Problem	Common Result
Mobile phase	Wrong composition, pH change, poor filtration, air bubbles	Retention shift, baseline problem, pressure change
Pump	Air, leak, check valve issue, unstable flow	Retention shift, pressure fluctuation, poor reproducibility
Injector / Autosampler	Poor needle wash, vial issue, injection volume problem	Carryover, poor %RSD, extra peaks
Column	Blockage, contamination, aging, poor equilibration	High pressure, tailing, broad peaks, poor resolution
Detector	Wrong wavelength, dirty flow cell, lamp issue	Low response, noisy baseline, drift
Data system	Poor integration, wrong baseline placement	Incorrect area, wrong reported result

Table 1.1. HPLC flow path areas and common troubleshooting sources.

Practical Tip

Always look at the system as a flow path. The chromatogram is the final result of many connected steps.

3. Quick Symptom-to-Check Table

Use this table as a quick first step when you see an HPLC problem.

Symptom	Possible Causes	What to Check First
Retention time shift	Mobile phase composition, pH, flow rate, temperature, poor equilibration	Check mobile phase preparation, flow rate, pressure trend, and column equilibration.
Baseline noise	Air bubbles, poor solvent quality, wrong wavelength, dirty detector flow cell	Check degassing, solvent quality, detector wavelength, and blank injection.
Baseline drift	Gradient mismatch, temperature change, mobile phase instability, detector instability	Check mobile phase, gradient profile, equilibration, temperature, and detector condition.
High pressure	Blocked filter, guard column, column inlet, particles, viscous mobile phase	Check pressure trend, mobile phase filtration, inline filter, guard column, and sample filtration.
Peak tailing	pH issue, secondary interactions, overload, contamination, column aging	Check pH, sample concentration, column history, blank, and system suitability data.
Poor %RSD	Injection issue, vial/cap problem, air bubbles, poor preparation, integration issue	Check autosampler, vial, injection volume, standard preparation, pressure stability, and integration.
Carryover / ghost peaks	Needle wash weakness, sticky analyte, contamination, dirty vial, mobile phase impurity	Check blanks after standards/samples, needle wash, diluent, vials, and fresh mobile phase.

Table 1.2. Quick symptom-to-check table

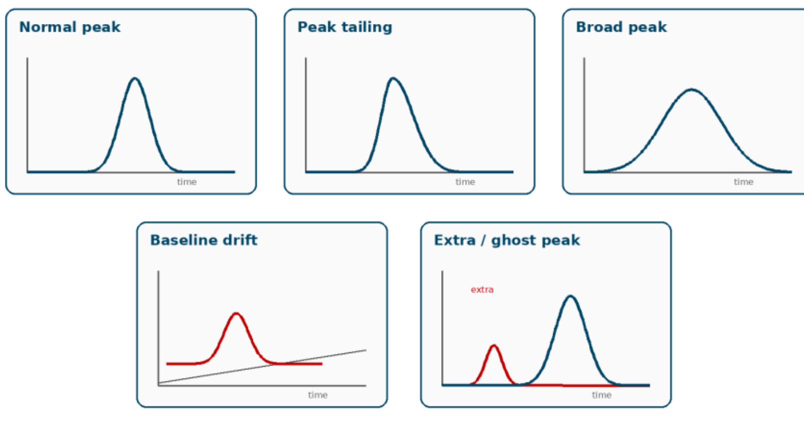


Figure 1.3. Normal peak, tailing peak, broad peak, baseline drift, and extra peak patterns.

Think Like an Analyst

One symptom can have several causes. Do not try to solve everything at once. Start with the simplest and most likely checks.

4. Problem 1: Retention Time Shift

Retention time shift means the peak appears earlier or later than expected.

This is one of the most common HPLC problems. It may be small and gradual, or sudden and obvious.

Common Signs

- The main peak appears earlier than usual.
- The main peak appears later than usual.
- All peaks shift in the same direction.

- Only some peaks shift.
- Retention time is different from system suitability expectations.

Possible Causes

Cause	How It Can Affect Retention
Organic solvent ratio	More organic solvent may reduce retention in RP-HPLC
Mobile phase pH	Ionizable compounds may retain differently when pH changes.
Flow rate	Higher flow normally shortens retention time; lower flow increases it.
Column equilibration	Poor equilibration can cause retention drift between injections.
Temperature	Temperature can affect retention and selectivity.
Column identity/history	A different or aged column may not behave exactly the same.

Table 1.3. Retention time shift checklist.

What to Check First

1. Was the mobile phase prepared correctly?
2. Was the organic solvent ratio correct?
3. Was the pH measured and adjusted correctly?
4. Was the column equilibrated long enough?
5. Is the flow rate correct?
6. Is the pressure similar to previous acceptable runs?
7. Was the same column used?

Practical Tip

If all peaks shift in the same direction, first check flow rate, mobile phase composition, temperature, and column equilibration. If only one peak shifts significantly, think more about selectivity, pH, sample chemistry, interference, or peak identification.

Common Beginner Mistake

Do not adjust the integration window immediately when retention time shifts. First, understand why the peak moved.

5. Problem 2: Baseline Noise or Drift

The baseline is the background signal of the chromatogram. A stable baseline makes peak detection and integration more reliable.

Baseline problems may appear as noise, waves, drift, spikes, or unstable signal.

Noise vs Drift: Simple Difference

Baseline Problem	Simple Description
Noise	Small rapid fluctuations in the baseline
Drift	Slow upward or downward movement over time
Spikes	Sharp sudden signal changes
Waves	Repeating baseline movement

Possible Causes of Baseline Problems

Cause	Possible Effect
Air bubbles	Noise, spikes, unstable signal
Poor solvent quality	Noise or extra peaks
Wrong wavelength	Low sensitivity or noisy response
Dirty detector flow cell	Noise, drift, unstable response
Mobile phase not equilibrated	Drift or unstable baseline
Gradient mismatch	Baseline slope or drift

What to Check First

1. Are solvents fresh and HPLC grade?
2. Was the mobile phase filtered and degassed?
3. Is the detector wavelength correct?
4. Is the flow cell clean?
5. Is the baseline stable before injection?
6. Did the problem appear in blank injections?
7. Is the gradient method properly equilibrated?

Think Like an Analyst

If a baseline problem appears in the blank injection, the cause is more likely related to the system, solvent, detector, mobile phase, or contamination in the flow path.

If the baseline problem appears only in sample injections, think more about the sample matrix, sample preparation, diluent compatibility, degradation, or interference.

6. Problem 3: High Pressure

High pressure means the system is facing more resistance to flow than expected.

Pressure should always be interpreted by comparing it with the normal pressure range for the same method, column, mobile phase, flow rate, and temperature.

Common Signs

- Pressure is higher than usual.
- Pressure increases gradually during injections.
- Pressure suddenly jumps.

- System gives overpressure error.
- Peak shape may become poor.

Possible Causes of High Pressure

Cause	Possible Explanation
Blocked inline filter	Particles collect before the column
Blocked guard column	Sample or mobile phase particles accumulate
Column blockage	Contamination or particles increase resistance
Mobile phase too viscous	Higher viscosity increases backpressure
Poor sample filtration	Particles enter the system and may block filters, guard column, or column inlet.
Precipitation	Incompatible solvents or buffers may form solids

What to Check First

1. Was the mobile phase filtered?
2. Were samples filtered or centrifuged properly?
3. Did pressure increase after specific samples?
4. Is the pressure high with the column installed?
5. Is the pressure still high without the column?
6. Is the guard column or inline filter blocked?
7. Was a buffer mixed with a strong organic solvent incorrectly?

Observation	Likely Direction
Pressure high before the column	Restriction may be before the column, such as inline filter, tubing, or pump outlet path.
Pressure normal without the column	Column, guard column, or column inlet frit may be restricted.
Pressure increased after sample injections	Sample particles, matrix, or precipitation may be contributing.
Sudden pressure jump	Possible blockage, precipitation, wrong mobile phase, or closed/blocked path.

Table 1.4. High pressure quick diagnosis.

Practical Tip

If pressure is high even without the column, the restriction may be before or after the column connection.

If pressure becomes normal after removing the column, the problem may be related to the column, guard column, or column inlet frit.

Common Beginner Mistake

Do not immediately reverse-flush a column unless the column type, manufacturer instructions, and laboratory SOP allow it.

7. Problem 4: Peak Tailing

Peak tailing means the peak is not symmetrical and has a long tail after the peak maximum.

Some tailing may be acceptable depending on the method and system suitability criteria, but excessive tailing can affect resolution, integration, and quantitation.

Common Signs

- Peak is stretched toward the right side.
- Tailing factor is higher than expected.
- Resolution between nearby peaks becomes worse.
- Integration becomes less reliable.

Possible Causes of Peak Tailing

Cause	Possible Explanation
Secondary interactions	Analyte interacts too strongly with active sites
pH not suitable	Ionization state affects peak shape
Column contamination	Active sites or retained material affect elution
Sample overload	Too much analyte causes distorted peak shape
Poor mobile phase conditions	Buffer strength or pH may be unsuitable
Column aging	Older column may show poor peak shape

What to Check First

1. Did tailing appear suddenly or gradually?
2. Is the pH correct?
3. Is the sample concentration too high?
4. Is the column properly equilibrated?
5. Is the same column used as before?
6. Is the system suitability tailing factor failing?
7. Does the blank show contamination or carryover?

Think Like an Analyst

A sudden peak shape problem may suggest contamination, preparation error, system issue, or column damage.

A gradual peak shape problem may suggest column aging, contamination buildup, or repeated exposure to harsh conditions.

8. Problem 5: Poor %RSD in Repeated Injections

Poor %RSD means repeated injections do not give consistent results.

In HPLC, poor repeatability can affect system suitability, assay results, impurity reporting, and confidence in the method.

Common Signs

- Standard injections give inconsistent peak areas.
- Retention time may be stable, but area varies.
- Peak height or shape changes between injections.
- System suitability fails for peak area precision.

Common Causes of Poor %RSD

Cause	Possible Effect
Injection volume inconsistency	Peak area changes
Autosampler needle problem	Poor reproducibility
Air bubbles	Unstable flow or response
Poor standard preparation	Area variation
Vial or cap issue	Inconsistent sample draw
Sample instability	Response changes over time

After identifying possible causes, the next step is to decide what to check first.

Possible Cause	First Check
Injection inconsistency	Check injection volume, autosampler performance, needle, and injection sequence.
Vial or cap issue	Check vial volume, cap sealing, septum condition, and sample draw position.
Standard preparation	Review weighing, dilution, mixing, potency correction, and solution stability.
Air bubbles or unstable flow	Purge pump, check pressure stability, and review solvent degassing.
Integration issue	Review baseline placement, peak identification, and integration method consistency.

Table 1.5. Poor %RSD causes and first checks.

What to Check First

1. Are standard solutions prepared correctly?
2. Is the vial volume sufficient?
3. Are vial caps properly sealed?
4. Is the autosampler needle clean?
5. Is the injection volume correct?
6. Are there air bubbles in the system?
7. Does the problem appear in all injections or only one vial?

Practical Tip

If retention time is stable but peak area is variable, look closely at injection, sample preparation, autosampler, detector response, and integration.

Common Beginner Mistake

Do not average bad injections just to pass system suitability. Investigate the cause of poor repeatability first.

9. Problem 6: Carryover and Ghost Peaks

Carryover means that material from a previous injection appears in a later injection, often in a blank.

Ghost peaks are unexpected peaks that appear without a clear expected source.

Common Signs

- A peak appears in the blank after a high-concentration standard or sample.
- Extra peaks appear at similar retention times in repeated blanks.
- The same unexpected peak appears in multiple injections.
- The blank is not clean.

Possible Causes of Carryover and Ghost Peaks

Cause	Possible Explanation
Poor needle wash	Analyte remains on the needle or injection path
Sticky analyte	Compound adsorbs to surfaces and may appear in later injections
Autosampler contamination	Residue remains in the injection system
Mobile phase contamination	Impurities may appear in every run
Vial contamination	Dirty vial, cap, septum, or insert may introduce extra peaks
Sample preparation contamination	Glassware, diluent, or filter issue

What to Check First

1. Does the peak appear in blank after standard/sample?
2. Does the peak decrease in repeated blanks?
3. Is the needle wash strong enough?
4. Is the diluent clean?
5. Are vials, caps, and filters clean?
6. Does fresh mobile phase solve the problem?
7. Does the peak appear without injection?

Observation	Possible Meaning
Peak appears after high standard or sample	Carryover is likely; check needle wash and injection path.
Peak decreases in repeated blanks	Carryover is likely and is being washed out gradually.
Peak appears in every blank from the start	Mobile phase, diluent, vial, or system contamination may be involved.
Peak appears only in sample injections	Sample matrix, preparation, degradation, or interference may be involved.

Table 1.6. Carryover and ghost peak decision table.

Think Like an Analyst

If an extra peak appears after a high-concentration injection and decreases in later blanks, carryover is likely.

If the peak appears in every blank from the beginning, contamination may be coming from the mobile phase, vial, diluent, or system.

10. Mini Practice Case: High Pressure After Several Injections

Case Scenario

A junior analyst starts an HPLC assay sequence. The first few standard injections look acceptable. After several sample injections, the system pressure gradually increases. Later, the chromatogram shows broader peaks and the system gives a high-pressure warning.

Initial Observations and What They Suggest

Observation	What It May Suggest
Pressure was normal at the start	The system may not have been blocked initially
Pressure increased after samples	Sample matrix or particles may be involved
Peaks became broader	Flow restriction or column contamination may affect performance
High-pressure warning appeared later	The flow restriction likely became worse during the sequence

Possible Causes

- Sample solutions were not filtered properly.
- Sample matrix contaminated the guard column.
- Inline filter started to block.
- Column inlet frit became restricted.

- Precipitation occurred during sample dilution or injection.
- Mobile phase and sample diluent were not fully compatible.

Troubleshooting Sequence

1. Stop the sequence safely according to laboratory procedure.
2. Check pressure trend and identify when pressure started increasing.
3. Run a blank if appropriate and allowed by SOP.
4. Check whether pressure is high with the column installed.
5. Check guard column or inline filter condition.
6. Review sample preparation and filtration.
7. Compare pressure with previous acceptable runs.
8. Do not report questionable results until the issue is understood.

Likely First Diagnosis

Because pressure increased gradually after sample injections, the first suspicion should be sample-related contamination or blockage, especially in the guard column, inline filter, or column inlet.

Key Lesson

When pressure increases during a sequence, do not only look at the last chromatogram. Review the pressure trend, injection order, sample preparation, and filtration history.

11. Final HPLC First-Check Checklist

Before making major changes, use this checklist.

A. Describe the Problem

- What is the main symptom?
- When did it start?
- Did it appear suddenly or gradually?
- Does it affect blank, standard, sample, or all injections?
- Is the problem repeatable?

B. Check Mobile Phase

- Correct solvent ratio?
- Correct pH?
- Fresh preparation?
- Filtered?
- Degassed?
- Compatible with buffer and sample diluent?

C. Check Pump and Pressure

- Stable pressure?
- Pressure higher or lower than usual?
- Air bubbles?
- Pump properly purged?
- Any leaks?
- Flow rate correct?

D. Check Injector / Autosampler

- Correct injection volume?
- Clean needle?
- Suitable needle wash?
- Vial volume sufficient?
- Caps properly sealed?
- Carryover checked with blanks?

E. Check Column

- Correct column installed?
- Proper equilibration?
- Guard column condition?
- Pressure normal with column?
- Peak shape acceptable?
- Column history reviewed?

F. Check Detector and Data System

- Correct wavelength?
- Stable baseline before injection?
- Flow cell clean?
- Correct integration method?
- Baseline placement reasonable?
- Peak identity confirmed?

Final Practical Tip

Good HPLC troubleshooting is not random. It is a step-by-step process of observing, comparing, checking, and documenting.

Checklist Area	What to Review
☐ Describe the problem	Main symptom, start time, sudden/gradual behavior, and affected injections.
☐ Check mobile phase	Solvent ratio, pH, freshness, filtration, degassing, and compatibility.
☐ Check pump and pressure	Pressure trend, air bubbles, flow rate, pump purge, and leaks.
☐ Check injector / autosampler	Injection volume, needle wash, vials, caps, and carryover blanks.
☐ Check column	Correct column, equilibration, guard column, pressure behavior, and column history.
☐ Check detector and data system	Wavelength, baseline stability, flow cell, integration, and peak identity.
☐ Document and confirm	Record the action taken and confirm that the correction worked.

Table 1.7. Final HPLC first-check checklist.

What Comes Next in the Full Series?

You now have a practical first-check framework for common HPLC problems. This starter kit helps you describe the symptom, compare it with previous normal runs, and decide what to check first.

However, real HPLC confidence comes from understanding each part of the system in more detail: mobile phase, pump, injector, column, detector, data system, system suitability, assay calculation, method development, and troubleshooting cases.

The full HPLC Practical Guide series continues this learning in a more detailed, step-by-step way.

About the Author

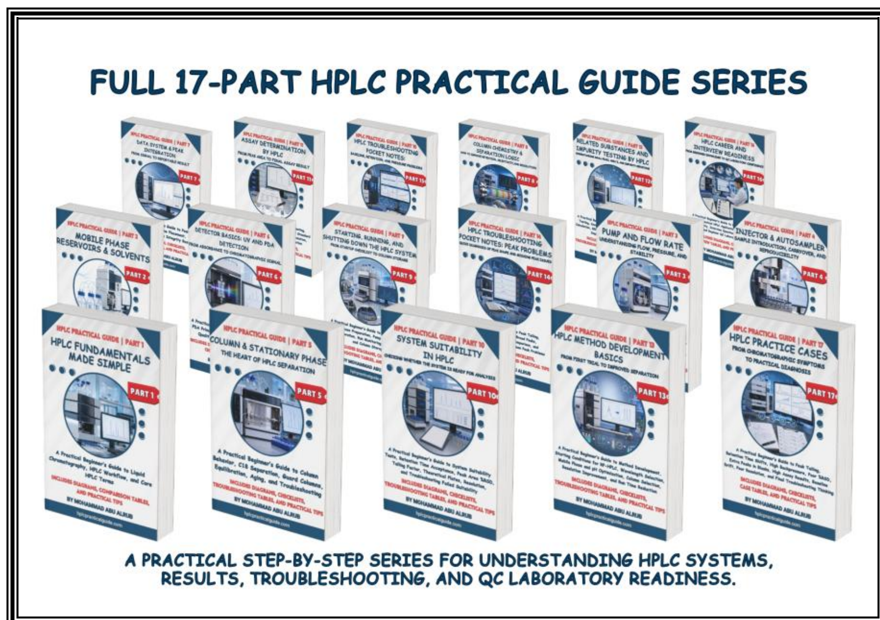
Mohammad Abu Alrub, PhD, has experience in pharmaceutical analytical chemistry, HPLC analysis, university teaching, and science education. He created the HPLC Practical Guide series to help students, fresh graduates, junior analysts, and early-career QC laboratory professionals understand HPLC in a practical, visual, and laboratory-focused way.

The goal of this free starter kit is to give beginners a clear first step before moving into the full HPLC Practical Guide series.

12. Continue Learning with the Full HPLC Practical Guide Series

This starter kit gives you a quick introduction to practical HPLC troubleshooting thinking. It helps you describe common symptoms, connect them with possible causes, and decide what to check first before making major changes.

The full HPLC Practical Guide series goes much deeper. It is designed to help beginners build practical confidence step by step, from basic HPLC concepts to system components, method operation, system suitability, assay calculation, troubleshooting, career readiness, and practice cases.



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The Full Series Includes 17 Practical PDF Parts

1. HPLC Fundamentals Made Simple
2. Mobile Phase Reservoirs and Solvents
3. Pump and Flow Rate
4. Injector and Autosampler
5. Column and Stationary Phase
6. Detector Basics: UV and PDA Detection
7. Chromatography Data System and Peak Integration
8. Column Chemistry and Separation Logic
9. Starting, Running, and Shutting Down the HPLC System
10. System Suitability in HPLC
11. Assay Determination by HPLC
12. Related Substances and Impurity Testing by HPLC
13. HPLC Method Development Basics
14. HPLC Troubleshooting Pocket Notes: Peak Problems
15. HPLC Troubleshooting Pocket Notes: Baseline, Retention, and Pressure Problems
16. HPLC Career and Interview Readiness
17. HPLC Practice Cases

Who Is the Full Series For?

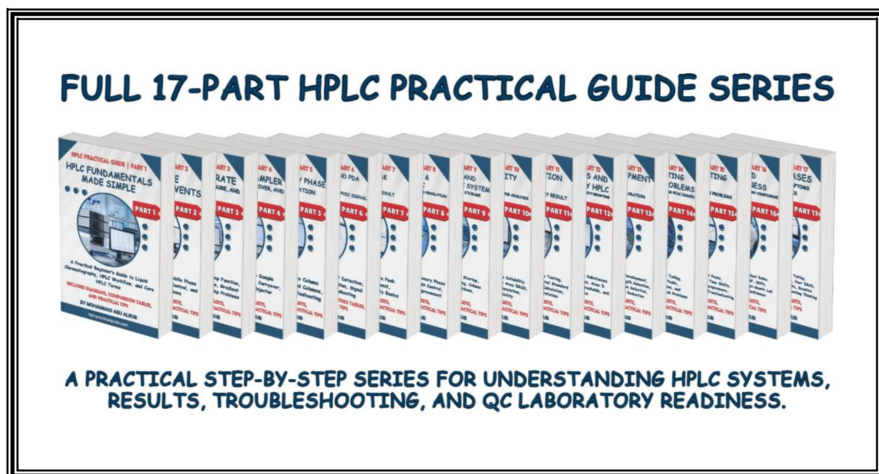
The full series is designed for chemistry and pharmacy students, new graduates, junior QC analysts, early-career laboratory professionals, and anyone who wants to understand HPLC in a practical, structured, and beginner-friendly way.

What You Will Learn

The full series helps you understand how each HPLC system component affects the chromatogram, how to prepare and run HPLC analysis more logically, how system suitability is interpreted, how assay and impurity results are approached, and how to troubleshoot common chromatographic problems with more confidence.

HPLC Practical Guide

Practical HPLC learning for students, junior analysts, and early-career QC laboratory professionals.



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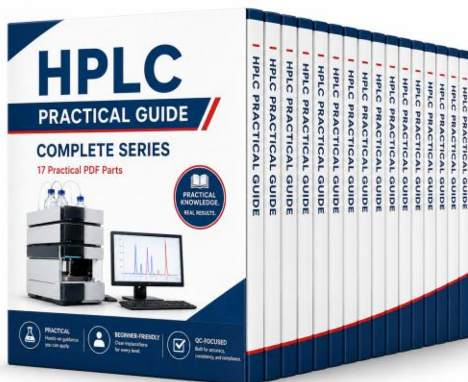

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