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Instant tips for right and effective approach to solve HPLC trouble shooting

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ABSTRACT

HPLC is one of the prominent and potent analytical tool regularly employed for the analysis of drugs in pharmaceutical formulations. Although HPLC method development has been improved by advanced innovative modifications in HPLC column technology and instrumentation day by day even though problems still arise from many sources. Generally when ever more sophisticated unit is used problems cropped up affecting overall system performance each troubled component which needs to be settled by leaps and bounds. In this review article, different trouble shooting common problems are discussed and solutions to those problems while performing method development. For easy understanding and reference the trouble shooting problems encountered with the HPLC system are organized into five major categories such as pressure abnormalities, leaks, problems with the chromatograms, injector problems and remedies and lastly other problems identified by smell, sight and sound and to be settle them and they are presented in the guide in the form of tables apart from textual matter for easy reference.

Key words: Possible causes, Solution, HPLC troubleshooting

INTRODUCTION

The practice of HPLC is now 45 years old. Certainly HPLC (Skoog DA, 2011), (Gurdeep R.Chatwal, 2002), (B.K Sharma, 2013), (Sethi P.D, 2012) is one of the most outstanding analytical techniques for identification and quantification of drugs, either in their active pharmaceutical ingredient or in their formulations throughout the process of their discovery, manufacturing and development. Its chief aim is to provide timely logical process when troubleshooting maximizes the system operation to get good chromatographic practices**.** Wherever necessary figures are inducted for understanding lucidly. This enables the guide to use quickly and efficiently by the operators with varied experience. Quick tips are included at the end of each section to resolve trouble shooting rapidly even by the less experienced HPLC users also. Analytical methods must be validated to give reliable data for regulatory submissions. These methods are essential for a number of purposes, including testing for QC release, testing of stability samples, testing of reference materials and to provide data to support specifications. Although HPLC method development (Azim Md, 2013), (Vibha Guptha, 2012), (Ravisankar P, 2014), (Ranjit singh, 2013), (Kaushal C, 2010) has been improved by advances column technology and instrumentation problems still arise. The main purpose of this guide is to utilize an easy reference tool to enable to meet suitably the trouble shooting problems (Ravisankar M, 2012), (Gupta V, 2012), Charde MS, 2014), (Runser Dj, 2001), (Christianson, 1997), (Berry VV, 1983), Dolan J.w, 1996), (Shoup R, 1989), (Dolan J.W, 1984, 1985, 1989) immediately in the day to day to run the HPLC system perfectly.

Troubleshooting strategy: Any troubleshooting strategy involves 5 steps, they are

1. Identification of the problem
2. Awareness of the causes of the problem
3. Isolation of the exact cause of the problem
4. Rectifying the problem if able
5. Returning the unit to routine use or referring the problem to your maintenance manager

Trouble shooting process: The following systematic approach should be followed logically so that the exact cause of the problem can be found.

1. To gather the facts.
2. To check the simplest things first.
3. To compare the performance obtained to the expected performance.
4. To enlist possible causes.
5. Work through the possible causes step-by-step duly checking the outcome from any changes done.

Locating and correcting the problem: A systematic approach is best to identify any problems when troubleshooting the HPLC system. This guide is organized into five major categories of symptoms to help quickly identify the source of the various problems.

- Pressure abnormalities are discussed in Table 1-8.
- Discussion on leaks shown in Table 9- 13.
- Problems with the chromatograms are shown in Table14 -36 and Figures 1 to 15.
- Discussion on Injector problems and remedies in Table 37-40.
- Discussion on other problems detected by smell, sight, and sound in Table 41- 52.

Prevention: Many liquid chromatography (LC) problems can be prevented with routine maintenance. For example, replacing pump seals at regular intervals to every pump-seal failure and its associated problems as well as preventive maintenance practices to reduce their frequency.

2. Abnormal pressure: A change in the operating pressure is a sign that there may be a problem. The solution is indicated in the last column against each relevant potential cause to rectify the problem perfectly for proper functioning of the system.

Table.1.No pressure reading, no flow

Potential cause	Solution
1. Power off	1. Turn on power
2. Fuse blown	2. Replace fuse
3. Controller setting or failure	3. a. Verify proper setting
	b. Repair or replace controller
4. Broken piston	4. Replace piston
5. Air trapped in pump head	5. Degas solvents: bleed air from
	pump, prime pump
6. Insufficient mobile phase	6. a. Replenish reservoir
	b. Replace inlet frit if blocked
7. Faulty check valve(s)	7. Replace check valve(s)
8. Major leak	8. Tighten or replace fittings

Table.2.No pressure reading, flow is normal.

Potential cause	Solution
1. Faulty meter	1. Replace meter
2. Faulty pressure transducer	2. Replace transducer

Table.3.Steady high pressure

Potential cause	Solution
1. Flow rate set too high	1. Adjust setting
2. Blocked column frit	2. a. Back flush column (if permitted)
	b. Replace frit
	c. Replace column
3. Improper mobile phase;	3. a. Use correct mobile phase
precipitated buffer	b. Wash column
4. Improper column	4. Use proper column
5. Injector blockage	5. Clear blockage or replace injector
6. Column temperature too low	6. Raise temperature
7. Controller malfunction	7. Repair or replace controller
8. Blocked guard column	8. Remove/replace guard column
9. Blocked in-line filter	9. Remove/replace in-line filter

Table.4. Steady low pressure

Potential cause	Solution
1. Flow set too low	1. Adjust flow rate
2. Leak in system	2. Locate leak and correct
3. Improper column	3. Use proper column
4. Column temperature too high	4. Lower temperature
5. Controller malfunction	5. Repair or replace controller

Table.5.Pressure climbing

Potential cause	Solution
1. Flow rate set too high	1. Adjust setting
2. Blocked column frit	2. a. Back flush column (if permitted)
	b. Replace frit
	c. Replace column
3. Improper mobile phase; precipitated buffer	3. a. Use correct mobile phase
	b. Wash column
4. Improper column	4. Use proper column
5. Injector blockage	5. Clear blockage or replace injector
6. Column temperature too low	6. Raise temperature
7. Controller malfunction	7. Repair or replace controller
8. Blocked guard column	8. Remove/replace guard column
9. Blocked in-line filter	9. Remove/replace in-line filter

Table.6.Pressure dropping to zero

Potential cause	Solution
1. Power off	1. Turn on power
2. Fuse blown	2. Replace fuse
3. Controller setting or failure	3. a. Verify proper setting
	b. Repair or replace controller
4. Broken piston	4. Replace piston
5. Air trapped in pump head	5. Degas solvents: bleed air from
	pump, prime pump
6. Insufficient mobile phase	6. a. Replenish reservoir
	b. Replace inlet frit if blocked
7. Faulty check valve(s)	7. Replace check valve(s)
8. Major leak	8. Tighten or replace fittings
9. Faulty meter	9. Replace meter
10. Faulty pressure transducer	10. Replace transducer

Table.7.Pressure dropping, but not to zero

Potential cause	Solution
1. Flow set too low	1. Adjust flow rate
2. Leak in system	2. Locate leak and correct
3. Improper column	3. Use proper column
4. Column temperature too high	4. Lower temperature
5. Controller malfunction	5. Repair or replace controller

Table.8.Pressure cycling

Potential cause	Solution
1. Air in pump	1. a. Degas solvent
	b. Bleed air from pump
2. Faulty check valve(s)	2. Replace check valve(s)
3. Pump seal failure	3. Replace pump seal
4. Insufficient degassing	4. a. Degas solvent
	b. Change degassing methods (use degasser on-line degasser)
5. Leak in system	5. Locate leak and correct
6. Using gradient elution	6. Pressure cycling is normal due to viscosity changes

3. Leaks: Leaks are usually stopped by proper suitable tightening or replacing the loose fittings. One must be aware, particularly that if metal compression fittings are over tightened may allow leaks and plastic finger tights may wear out quickly. If a fitting leak does not stop when the fitting is tightened a little, take off the fitting out and inspect the damage (e.g., distorted ferrule or particles on the sealing surface) damaged fittings should be discarded and replaced.

Table.9.Leaky fittings

Potential cause	Solution
1. Loose fitting	1. Tighten
2. Stripped fitting	2. Replace
3. Over tightened fitting	3. a. Loosen and retighten
	b. Replace
4. Dirty fitting	4. Disassemble and clean

Table.10.Leaks at pump

Potential cause	Solution
1. Loose check valves	1. a. Tighten check valve (do not over tighten)
	b. Replace check valve
2. Loose fittings	2. Tighten fittings (do not over tighten)
3. Mixer seal failure	3. a. Replace mixer seal
	b. Replace mixer
4. Pump seal failure	4. Repair or replace
5. Pressure transducer failure	5. Repair or replace
6. Pulse damper failure	6. Replace pulse damper
7. Proportioning valve failure	7. a. Check diaphragms, replace if leaky
	b. Check for fitting damage, replace
8. Purge valve	8. a. Tighten valve
	b. Replace purge valve

Table.11.Injector leaks

Potential cause	Solution
1. Rotor seal failure	1. Rebuild or replace injector
2. Blocked loop	2. Replace loop
3. Loose injection-port seal	3. Adjust
4. Improper syringe-needle diameter	4. Use correct syringe
5. Waste-line siphoning	5. Keep waste line above surface waste
6. Waste-line blockage	6. Replace waste line

Table.12.Column leaks

Potential cause	Solution
1. Loose end fitting	1. Tighten end fitting
2. Column packing in ferrule	2. Disassemble, rinse ferrule, reassemble
3. Improper frit thickness	3. Use proper frit (see Frit selection guide chart)

Table.13.Detector leaks.

Potential cause	Solution
1. Cell gasket failure	1. a. Prevent excessive backpressure
	b. Replace gasket
2. Cracked cell window(s)	2. Replace window(s)
3. Leaky fittings	3. Tighten or replace
4. Blocked waste line	4. Replace waste line
5. Blocked flow cell	5. Rebuild or replace

4. Problems with the chromatogram: Many problems in an HPLC system is sign to show the changes in the chromatograms. Some of these can be solved by replacing the non functioning components of equipment or effecting modifications to the assay procedures. Selection of the suitable column and mobile phase are key parts to obtain good chromatography.

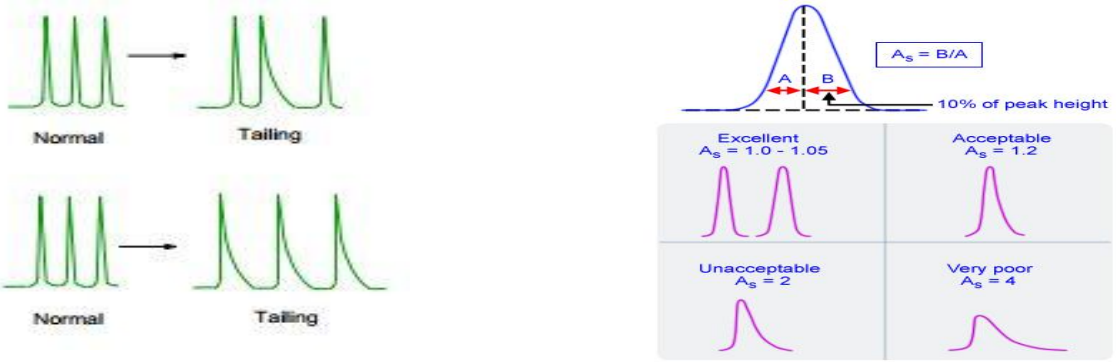


Figure 1. Peak tailing.

Due to secondary retention effects, Residual silanol interactions, and small peak elution on tail of large peak yields some irregular peaks tail.

Due to extra column effects, contamination build up on column inlet, heavy metals and bad column yields all imperfect peaks tails.

Table.14.Peak tailing

Potential cause	Solution
1. Blocked frit	1. a. Reverse flush column (if allowed)
	b. Replace inlet frit
	c. Replace column
2. Column void	2. a. Fill void
3. Interfering peak	3. a. Use longer column
	b. Change mobile-phase and/or column/ selectivity
4. Wrong mobile-phase pH	4. a. Adjust pH
	b. For basic compounds, a lower pH usually provides more symmetric peaks
5. Sample reacting with active sites	5. a. Add ion pair reagent or volatile basic
	b. Change column modifier

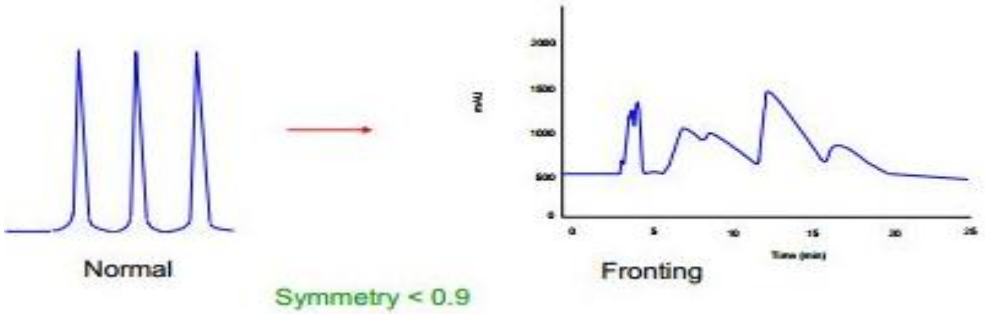


Figure 2. Peak fronting

Table.15.Peak fronting

Potential cause	Solution
1. Low temperature	1. Increase column temperature
2. Wrong sample solvent	2. Use mobile phase for injection solvent
3. Sample overload	3. Decrease sample concentration

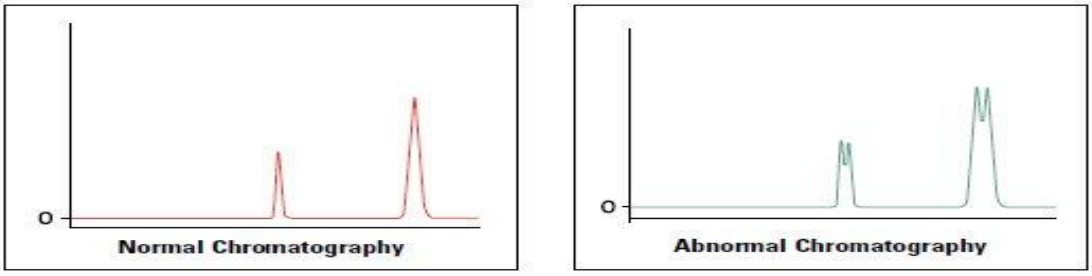


Figure.3.Split peaks

Table.16.Split peaks

Potential cause	Solution
1. Contamination on guard or analytical column inlet	1. a. Remove guard column and attempt analysis
	b. Replace guard if necessary c. If analytical column is obstructed, reverse and flush d. If problem persists, column may be fouled with strongly retained contaminations e. Use appropriate restoration procedure f. If problem persists, inlet is probably plugged g. Change frit or replace column
2. Sample solvent incompatible with mobile phase	2. a. Change solvent; whenever possible
	b. Inject samples in mobile phase

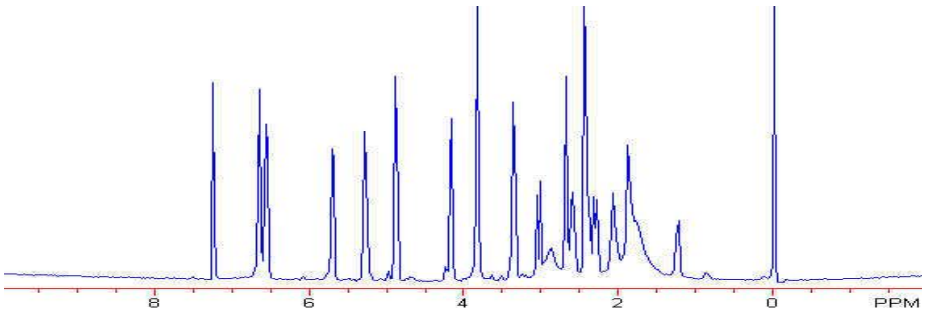


Figure.4. Distortion of larger peaks

Table.17. Distortion of larger peaks

Potential cause	Solution
1. Sample overload	1. Reduce sample size

Table.18.Distortion of early peaks

Potential cause	Solution
1. Wrong injection solvent	1. a. Reduce injection volume
	b. Use weaker injection solvent

Table.19.Tailing, early peaks more than later ones

Potential cause	Solution
1. Extra-column effects	1. a. Re plumb system (shorter, narrower tubing)
	b. Use smaller volume detector cell

Table.20.Increased tailing as k' increases

Potential cause	Solution
1. Secondary retention effects, reversed-phase mode	1. a. Add tri ethylamine (basic samples)
	b. Add acetate (acidic samples)
	c. Add salt or buffer (ionic samples)
	d. Try a different column
2. Secondary retention effects, normal-phase mode	2. a. Add tri ethylamine (basic compounds)
	b. Add acetic acid
3. Secondary retention effects, ion-pair	3. a. Add tri ethylamine (basic samples)

Table.21.Acidic or basic peaks tail

Potential cause	Solution
1. Inadequate buffering	1. a. Use 50–100 mm buffer concentration
	b. Use buffer with pKa equal to pH of mobile phase

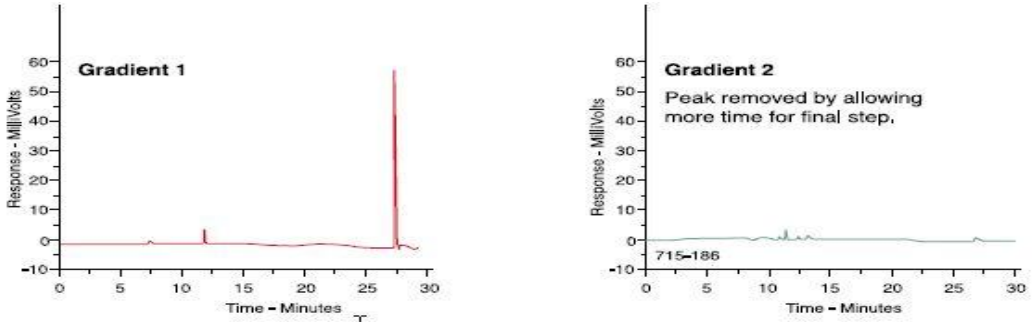


Figure 5. Extra peaks

Table 22. Extra peaks.

Potential cause	Solution
1. Other components in sample	1. Normal
2. Late-eluting peak from previous injection	2. a. Increase run time or gradient slope
	b. Increase flow rate
3. Vacancy or ghost peaks	3. a. Check purity of mobile phase
	b. Use mobile phase as injection solvent
	c. Reduce injection volume

Table.23. Retention time drifts

Potential cause	Solution
1. Poor temperature control	1. Thermostat column
2. Mobile phase changing	2. Prevent change (evaporation, reaction etc)
3. Poor column equilibration	3. Allow more time for column equilibration between runs

Table.24. Abrupt retention time changes

Potential cause	Solution
1. Flow rate change	1. Reset flow rate
2. Air bubble in pump	2. Bleed air from pump
3. Improper mobile phase	3. a. Replace with proper mobile phase
	b. Set proper mobile phase mixture on controller

Baseline irregularities:

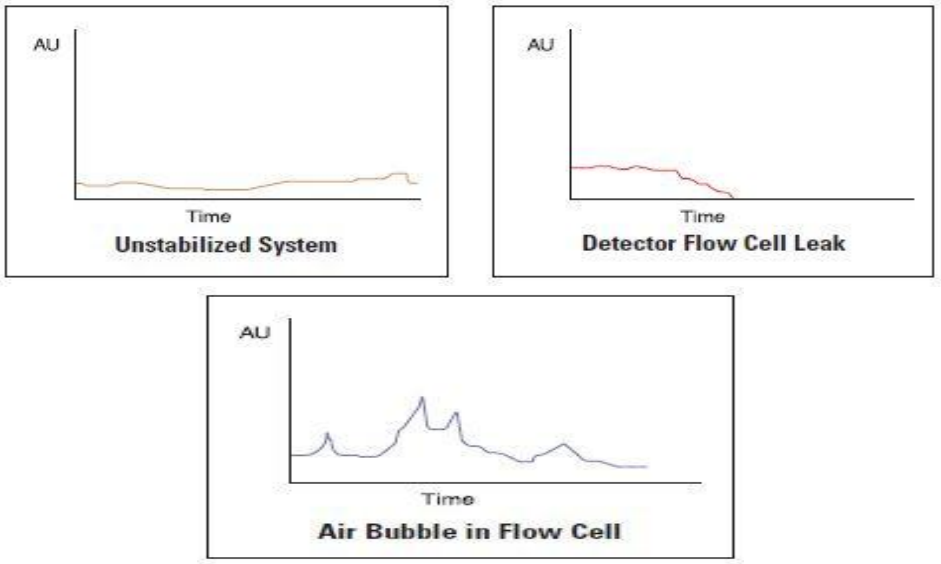


Figure6.Non-cyclic noise-fluid path problems

Table.25.Non-cyclic noise-fluid path problems

Potential cause	Solution
1. Air in mobile phase, detector cell, or pump	1. a. Degas mobile phase
	b. Flush system to remove air from detector cell or pump
2. column contamination	2. Replace with a new column
3. Air bubbles in the flow path	3. Prime the pump once again and ensure that all solvents thoroughly degassed

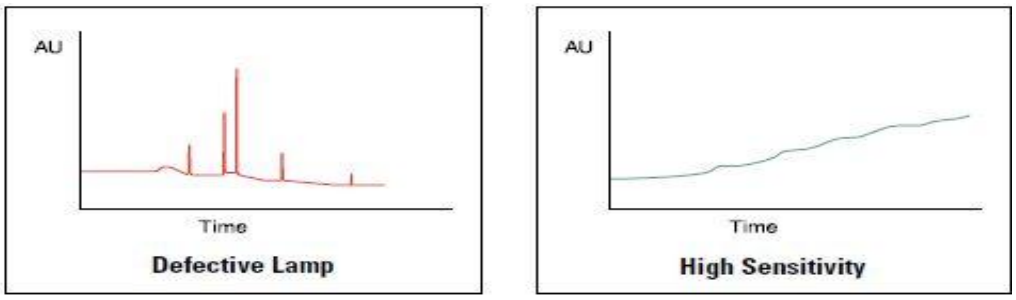


Figure.7.Non-cyclic noise-detector electronics problems

Table.26.Non-cyclic noise-detector electronics problems

Potential cause	Solution
1. Detector not stable	1. Allow sufficient time to stabilize
2. Detector lamp malfunction	2. If the lamp energy is below that recommended for normal detector operation replace the lamp
3. Contaminated/ Scratched reference electrode	3. Replace the working electrode

Figure.8.Cyclic noise-detector related problems

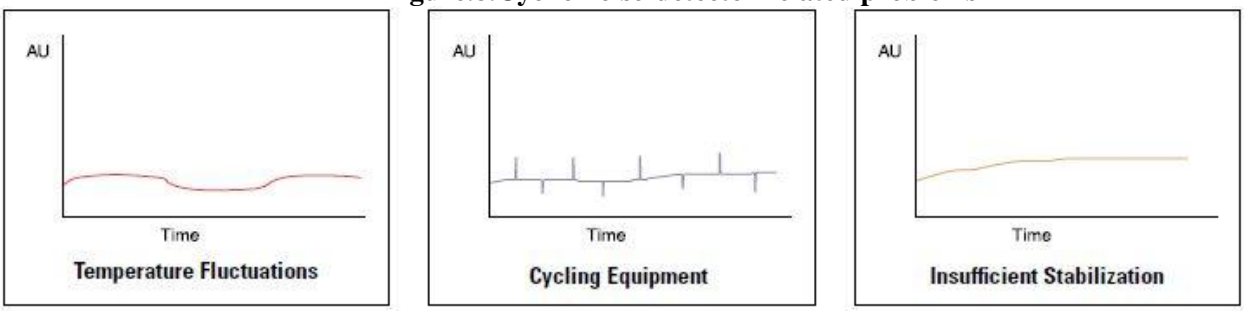


Table.27.Cyclic noise-detector related problems.

Potential cause	Solution
1.Long term detector temperature problems	1. The heater cycles on and off to maintain the detector temperature
2. Ambient temperature fluctuations	2. Stabilize the air temperature around the instrument and allow the system to return to equilibrium
3. Contaminated reference electrode	3. Replace the working electrode

Broad peaks: Due to loss of column efficiency, column void and large injection volume causes all peaks become broad which shows imperfect results.

Owing to Possible late elution from previous sample (ghost peak), high molecular weight sample- protein or polymer causes to become some peaks broad.

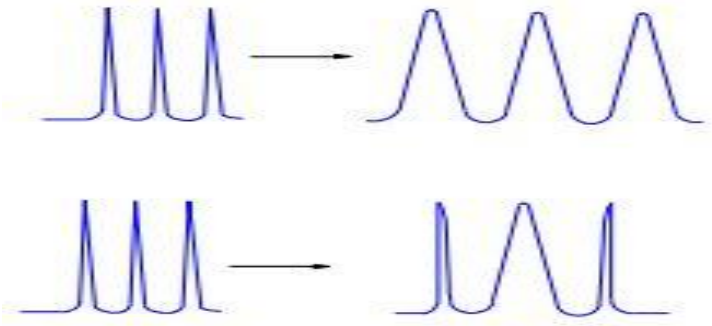


Figure.9.Broad peaks

Table.28.Broad peaks

Potential cause	Solution
1. Mobile-phase composition changed	1. Prepare new mobile phase
2. Mobile-phase flow rate too low	2. Adjust flow rate
3. Leaks (especially between column and detector)	3. a. Check system for injector leaks Check for column leaks; Check for detector leaks
	b. Check for loose fittings
	c. Check pump for leaks, salt build-up and unusual noises d. Change seals if necessary
4. Detector settings incorrect	4. Adjust settings
5. Extra-column effects:	5. a. Inject smaller column (e.g., 10 µL vs. 100 µL) or 1:10 and 1:100 dilutions of sample
a. Column overloaded	
b. Detector response time or cell volume too large	b. Reduce response time or use smaller cell
c. Tubing between column and detector too long or ID too large	c. Use as short a piece of 0.007–0.010 inch ID tubing as practical
d. Recorder response time too high	d. Reduce response time
6. Buffer concentration too low	6. Increase concentration
7. Guard column contaminated	7. Replace guard column
8. Column contaminated/worn out; low plate number	8. a. Replace column with new one of same type
	b. If new column provides symmetrical peaks, flush old column with strong solvent
9. Void at column inlet	9. Open inlet end and fill void or replace column
10. Peak represents two or more Poorly resolved solvents	10. Change column type to improve separation
11. Column temperature too low	11. Increase temperature; do not exceed 75°C unless higher temperatures are acceptable to column manufacturer
12. Detector time constant too large	12. Use smaller time constant

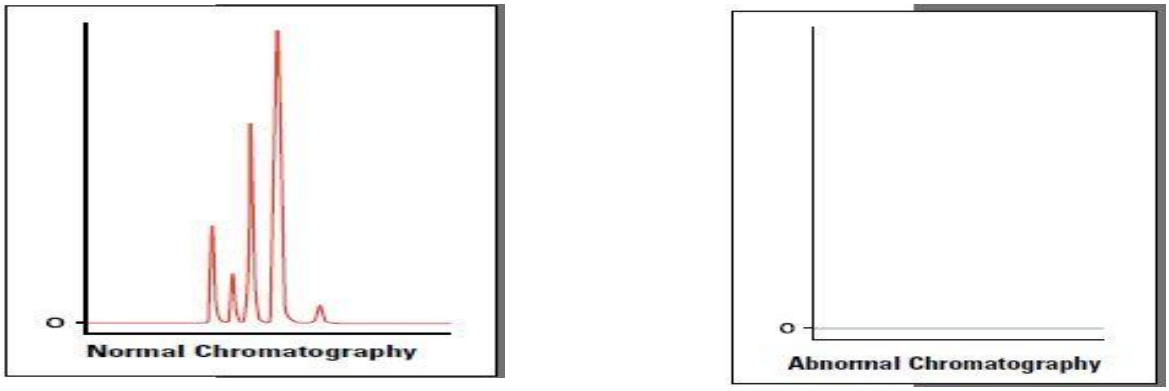


Figure.10.No peaks

Table.29. No peaks

Potential cause	Solution
1. Wrong sample being injected	1. Inject correct sample
2. The detector not being switched on (or) blockage between the injector and detector lines	2. Switch on the detector
3. Sample or mobile phase preparation has been performed correctly	3. preparation of mobile phase or sample has been performed correctly

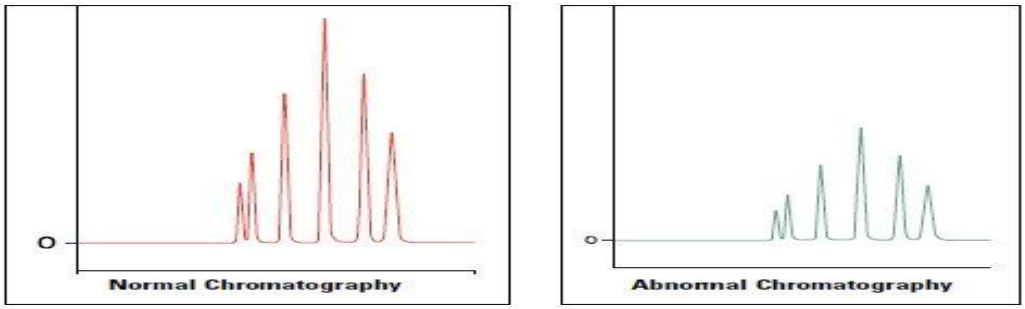


Figure.11.Smaller than expected peaks

Table.30. Smaller than expected peaks

Potential cause	Solution
1. Wrong injection volume	1. Inject the correct volume
2. Detector problem	2. Zero the detector output
3. Sample too viscous	3. Dilute the sample or decrease the rate at which the syringe draws the sample
4. Sample loop incorrect	4. Change the sample loop to the correct volume in the one in-situ is incorrect

Figure.12.Early eluting peaks broad

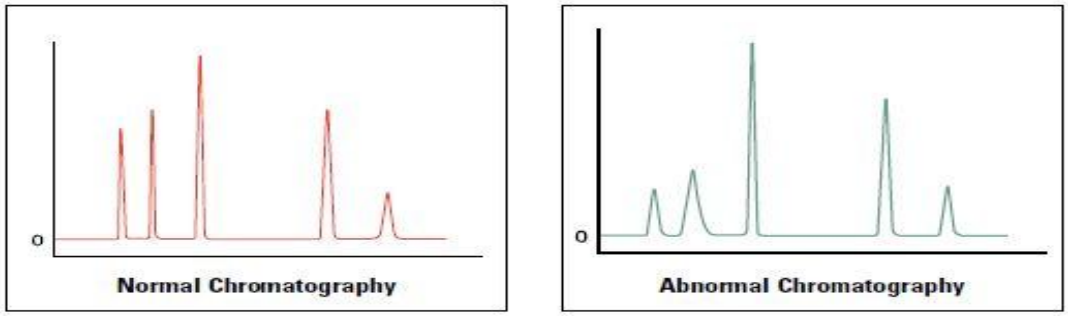
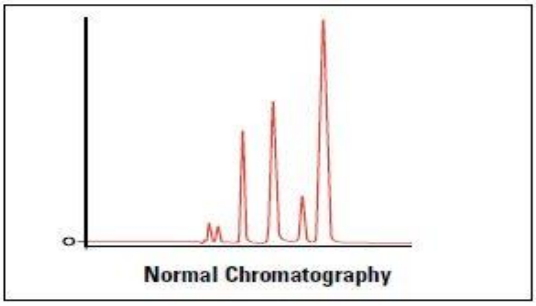


Table 31. Early eluting peaks broad.

Potential cause	Solution
1. Sample over load	1.Dilute the sample or inject a lower volume to stop equilibrium disruption
2. Detector time constant incorrect	2.Correct the detector time constant



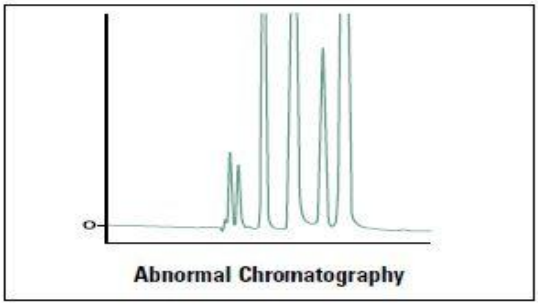
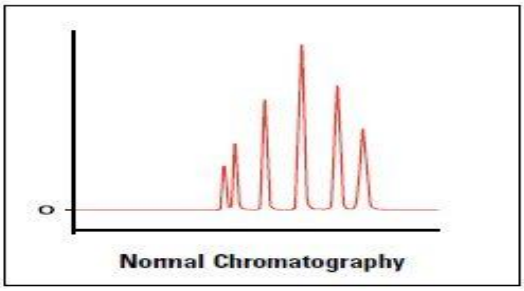


Figure.13.Flat topped peaks

Table.32.Flat topped peaks

Potential cause	Solution
1. Large injection volume of dilute sample	1. Injection of small volume of dilute sample
2. Recorder input error	2. Adjust the recorder input voltage

Figure.14.Negative peaks



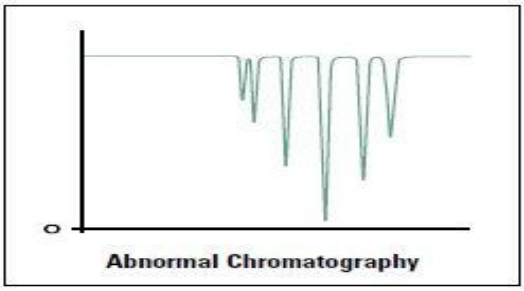


Table.33.Negative peaks

Potential cause	Solution
1. Highly adsorbing mobile phase	1. Dissolve the sample in mobile phase
2. Ion pair separation only	2. Dissolve the sample in mobile phase

Table.34.Loss of resolution

Potential cause	Solution
1. Mobile phase contaminated/ deteriorated (causing retention time to change)	1. Prepare new mobile phase
2. Obstructed guard or analytical column	2. a. Remove guard column and attempt analysis b. Replace guard if necessary c. If analytical column is obstructed, reverse and flush if problem persists, column may be fouled with strongly retained contaminants d. Use appropriate restoration procedure if problem persists, inlet is probably plugged e. Change frit or replace column

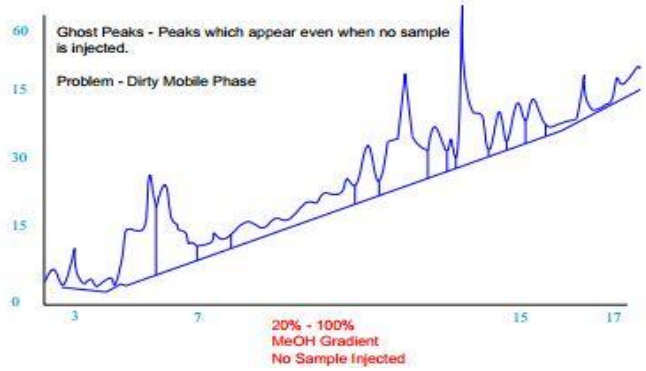
Table.35.All peaks too small

Potential cause	Solution
1. Detector attenuation too high	1. Reduce attenuation
2. Detector time constant too large	2. Use smaller time constant
3. Injection size too small	3. Use larger sample loop
4. Improper recorder connection	4. Use correct connection

Table.36.All peaks too large

Potential cause	Solution
1. Detector attenuation too low	1. Use larger attenuation
2. Injection size too large	2. Use smaller sample loop
3. Improper recorder connection	3. Use correct connection

Figure.15.Ghost Peaks



5. Problems with the injector: The problems are usually detected while using the injection valve.

Table.37.Manual injector, hard to turn

Potential cause	Solution
1. Damaged rotor seal	1. Rebuild or replace valve
2. Rotor too tight	2. Adjust rotor tension

Table.38.Manual injector, hard to load

Potential cause	Solution
1. Valve misaligned	1. Adjust alignment
2. Blocked loop	2. Replace loop
3. Dirty syringe	3. Clean or replace syringe
4. Blocked lines	4. Clear or replace lines

Table.39.Auto injector will not turn

Potential cause	Solution
1. No air pressure (or power)	1. Supply proper pressure (power)
2. Rotor too tight	2. Adjust
3. Valve misaligned	3. Adjust alignment

Table.40.Auto injector, other problems

Potential cause	Solution
1. Blockage	1. Clear or replace blocked portion
2. Jammed mechanism	2. See service manual
3. Faulty controller	3. Repair or replace controller

6. Problems detected by smell, sight and sound: All senses must be used to identify HPLC problems. Habit of taking a few minutes each day need to be cultivated to expose all senses except taste to know how far the HPLC performs properly which help to identify problems quickly. For example, often a leak can be detected by smell before it is seen. The majority of problems are identified by sight which is shown the following tables.

Table.41.Solvent smell

Potential cause	Solution
1. Leak	1. a. Check system for injector leaks
	b. Check system for loose fittings
	c. Check pump for leaks, salt build-up, unusual noises
	d. Change pump seals if necessary
2. Spill	2. a. Check for overflowing waste container
	b. Locate spill and clean up

Table.42."Hot" smell

Potential cause	Solution
1. Overheating module	1. a. Check for proper ventilation, adjust
	b. Check temperature setting, adjust
	c. Shut module off, see service manual

Table.43.Abnormal meter readings

Potential cause	Solution
1. Pressure abnormality	1. Mention in Section 2
2. Column oven problem	2. a. Check settings, adjust
	b. See service manual
3. Detector lamp failing	3. Replace lamp

Table.44.Warning lamps

Potential cause	Solution
1. Pressure limit exceeded	1. a. Check for blockage
	b. Check limit setting, adjust
2. Other warning lamps	2. See service manual

Table.45.Warning buzzers

Potential cause	Solution
1. Solvent leak/spill	1. Locate and correct
2. Other warning buzzers	2. See service manual

Table.46.Squeaks and squeals

Potential cause	Solution
1. Bearing failure	1. See service manual
2. Poor lubrication	2. Lubricate as necessary
3. Mechanical wear	3. See service manual

7. Details of Key problem areas and their preventive maintenance: The LC's operator and service manuals may have additional suggestions for preventive maintenance in addition to the causes and solutions detailed in the following tables mentioned bellow.

Table.47.Reservoir

Potential cause	Solution
1. Blocked inlet frit	1. a. Replace (3–6 months)
	b. Filter mobile phase, 0.5 µm filter
2. Gas bubbles	2. Degas mobile phase

Table.48.Pump

Potential cause	Solution
1. Air bubbles	1. Degas mobile phase
2. Pump seal failure	2. Replace (3 months)
3. Check valve failure	3. Filter mobile phase; use inlet-line frit;
	keep spare

Table.49.Injector

Potential cause	Solution
1. Rotor seal wear	1. a. Do not over tighten
	b. Filter samples

Table.50.Column

Potential cause	Solution
1. Blocked frit	1. a. Filter mobile phase
	b. Filter samples
	c. Use in-line filter and/or guard column
2. Void at head of column	2. a. Avoid mobile phase pH >8
	b. Use guard column
	c. Use pre column (saturator column)

Table.51.Detector

Potential cause	Solution
1. Lamp failure; decreased detector	1. Replace (6 months) or keep spare lamp
response; increased detector noise	
2. Bubbles in cell	2. a. Keep cell clean
	b. Use restrictor after cell
	c. Degas mobile phase

Table.52.General

Potential cause	Solution
1. Corrosive/abrasive damage	1. Flush buffer from LC and clean when not in use

CONCLUSION

HPLC is the widely utilized technique for the routine analysis of the drugs in pharmaceutical dosage forms. Several problems may occur while performing the method development by RP-HPLC. The above explained trouble shooting guidelines will render immense help to the analyst to maintain the HPLC system to overcome problems if any happened and also keep the system in smooth way of running which reduces the operation cost. The above said tips will assist to maintain the HPLC system perfectly and keeps the system out of routine problems which lessen the maintenance cost due to highest quality performance of the system. It leads to successful operation of the HPLC system if the principle "Commence with the suitable and apt questions seek the appropriate answers and ultimately the correct answers obtained will paves the way towards required solutions" is perfectly applied.

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