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AN OVERVIEW ON HPLC, UPLC AND ITS TROUBLESHOOTING

Nidhi Nk*[1], Neelam I[1], AjithaA[1], Uma Maheswar Rao V[1]

[1]Department of Pharmaceutical Analysis & Quality Assurance, CMR College of Pharmacy, JNTU (H) University, Hyderabad, Andhra Pradesh, India.

ABSTRACT

HPLC and UPLC Systems take advantage of technological strides made in particle chemistry performance, system optimization, detector design, and data processing and control. When taken together, these achievements have created a step-function improvement in chromatographic performance**.**Although HPLC method development has been improved by advances in column technology and instrumentation, problems still arise. This helps in systematic means of isolating, identifying, and correcting many typical problems. The important segments of an HPLC and UPLC system are the same, whether you use a modular system or a more sophisticated unit except the column characteristics. Problems affecting overall system performance can arise in each component. Solutions to these problems are presented in easy-to-use tables.

Keywords: Monolithic columns, Baseline drift, Shouldering peaks, Fronting peaks, Inline filter, Radio Interference, etc.

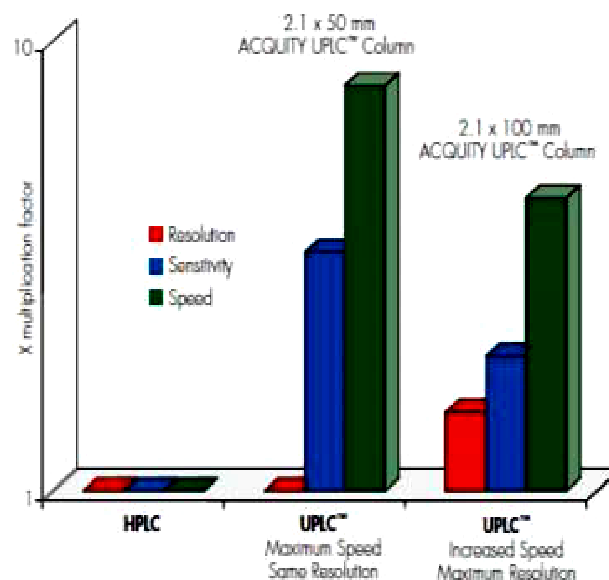
INTRODUCTION

In current trends, liquid chromatography plays a key role in the analysis of multiple API's and excipients especially HPLC and UPLC.

HPLC: High performance liquid chromatography (HPLC) is a well-known technique that has been used in laboratories worldwide from more than last 30 years.

UPLC: UPLC is a derivative of HPLC whose underlying principle is that as column packing particle size decreases, efficiency and resolution increases. If we decrease particle size less than 2 µm, the efficiency shows a significant gain by making use of the smaller particles, the speed of analysis and peak capacity i.e., number of peaks resolved per unit time, can be prolonged to the maximum values and these values are much better than the values achieved earlier by HPLC [1].

- Principles of both HPLC and UPLC are the same but not the performance.
- Differences in the speed, sensitivity and resolution are observed.



To improve the UPLC efficiency following measures need to be performed:

Corresponding Author:-N.K.Nidhi **Email: -** nidhichinnu.nk@gmail.com

1. Elevated temperature range should be employed, which will allow high flow rate of mobile phase by reducing its viscosity and thus it will significantly reduce back pressure.
2. Monolithic columns should be used, which consist of one piece of solid that possesses interconnected skeletons and interconnected flow paths (through pores).
UPLC is a technique which comprises the above mentioned features and stands better than HPLC in many ways, as it shows better chromatographic resolution, performs more sensitive analysis, consumes less time, reduces solvent consumption and has high analysis speed.

- Efficiency is three times greater with 1.7 µm particles compared to 5 µm particles and two times greater compared to 3.5 µm particles.
- Resolution is 70% higher than with 5 µm particles and 40% higher than with 3.5 µm particles.
- High speed is obtained because column length with 1.7 µm particles can be reduced by a factor of 3 compared to 5 µm particles for the same efficiency, and flow rate can be three times higher. This means separations can be nine times faster with equal resolution.
- Sensitivity increases because less band spreading occurs during migration through a column with smaller particles.

Contribution of small particles in UPLC

- UPLC required porous particles, which can withstand the high pressure in order to maintain their retention and capacity similar to that of HPLC.
- Here Silica particles possess good mechanical strength but their application was limited by narrow pH application range and generally exhibit tailing during analysis of basic analytes.
- Polymeric columns did not have any pH limitations but found to have low efficiency.

COLUMNS

In 2000, the first generation hybrid chemistry utilizes the classical sol-gel synthesis method to create durable columns that incorporated carbon in the form of methyl groups. These columns exhibit several advantages such as mechanical strength, high efficiency and are operative over an extended pH range. However, they do not possess enough mechanical stability necessitated by UPLC.

Consequently, the second generation bridged ethane hybrid (BEH) technology was developed. This technology increases the mechanical stability of 1.7 µm particles by bridging the methyl groups in the silica matrix lead to the production of the columns which can withstand high pressure and pH [2].

BEH TECHNOLOGY

In this HPT enabled XTerra columns are used with the properties of inorganic (silica) and organic (polymeric) packings are combined to produce a material that has superior mechanical strength, efficiency, high PH stability and peak shape for bases.

BEH Technology is used for detection of additional drug metabolites, for superior separation and improved spectral quality.
Examples: BEH C18, BEH C8, BEH SHIELDED RP18, BEH PHENYL, BEH AMIDE COLUMNSBEH 130, BEH 300.BEH123, BEH200, BEH450 SEC COLUMNS, BEH GLYCANCOLUMN.

HSS TECHNOLOGY

In HSS technology a mechanically tolerant, silica based material was designed to withstand UPLC pressures i.e., high strength silica particle technology
Examples: HSS T3, HSS C18, HSS C18 SB, HSS PFP, HSS CYNO COLUMNS.

CSH TECHNOLOGY

Charge Surface Hybrid (CSH) Technology is the latest advancement in hybrid materials that utilizes a controlled, low-level surface charge to provide enhanced selectivity.
Examples: CSH C18, CSH PHENYL-HEXYL, CSH FLOURO-PHENYL COLUMNS.

PUMPS: Pumps in conventional HPLC systems reach a pressure of maximum 400 bars. Pumps in UHPLC systems can reach pressures of 1000 bars and more.

DETECTOR

- TUV (Tunable Ultraviolet) detector
- PDA (Photo Diode Array) detector
- ELS (Evaporative Light Scattering) detector
- FLR (Fluorescence) detector

INTRODUCTION OF HPLC AND UPLC TROUBLE SHOOTING

Although Chromatographic method development has been improved by advances in column technology and instrumentation, problems still arise. This helps in systematic means of isolating, identifying, and correcting many typical problems. The important segments of an HPLC and UPLC system are the same, whether you use a modular system or a more sophisticated unit except the column characteristics. Problems affecting overall system performance can arise in each component. Solutions to these problems are presented in easy-to-use tables [3].

Any troubleshooting strategy in liquid chromatography involves five steps

1. Identification of the problem
2. Awareness of the cause(s) of the problem
3. Isolation of the exact cause of the problem
4. Rectifying the problem if able

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5. Returning the unit to routine use OR referring the problem to your maintenance manager.

Solving the problems

1. Gather the facts – not theories.
2. Check the simplest things first – it's easier.
3. Compare the performance obtained to the expected performance.
4. List possible causes.
5. Work through the possible causes in a step-by-step manner checking the outcome from any changes made.
6. As a last resort – get help from elsewhere, for example your instrument supplier help desk or your local technical support department.

HPLC AND UPLC TROUBLESHOOTING PARAMETERS

There are many areas in a HPLC instrument that can give rise to system and chromatographic problems which include

- Visual Inspection
- Pressure
- Baseline Irregularities
- Changes in Chromatography
- Changes in retention times
- Qualitative Results
- Quantitative Results

VISUAL INSPECTION

When a problem rises, it is advisable to perform a quick visual check of the instrument and column. This will pick up leaks, loose or disconnected tubing, breakages to the column, changes in instrument settings etc. These problems are easy to rectify and will save time [4].

PRESSURE

A) **High Pressure**: The most common causes of high pressure are blocked tubing around the injector and column inlet.

CAUSE	CORRECTIVE ACTION
change in ambient temperature	Stabilize the operating environment temperature.
If the eluent is viscous	Calculate/check the viscosity. Viscous solvents do produce higher system pressures. If possible, dilute or change to a less viscous solvent mix.
Loosen detector waste outlet fitting.	Replace blocked tubing as per the detector manual.
Loosen detector inlet fitting.	Flow cell fluid path blockage refers to the detector manual for cleaning instructions.
Loosen column outlet fitting.	Blocked outlet tubing. Replace.
Loosen fitting at guard or in-line filter.	Blocked guard or filter. Replace the disposable unit
Loosen injector outlet fitting.	Injector or connecting tubing blocked. Unblock as per injector manual instructions. Check that the vials are not coring and that samples are particulate free/soluble.
Loosen pump outlet fittings.	Outlet connecting tubing blocked. Replace tubing as per pump operating manual. Verify solvent miscibility.
Pump problem	Contact your maintenance provider

B) Low Pressure: The most common causes of no/low pressure are the solvent inlet lines not being immersed in solvent, no solvent in the reservoir and leaks.

CAUSE	CORRECTIVE ACTION
1. If the pump fuse is not in working condition.	Replace fuse and re-test
4. If the low pressure cut-off higher than the operating pressure?	Reset the low pressure cut-off to a value below the operating pressure
Does solvent flow out of the purge valve when opened?	In-line filter blocked. Clean as per the pump manual instructions
If air visible in the solvent lines?	Remove the air – check for loose connections
Are there any leaks?	Check for pools of liquid, buffer crystals Go to next question and loose connections. Clean up and stop leaks where necessary

C) **Fluctuating pressure**

CAUSE	CORRECTIVE ACTION
Improper functioning of pressure transducer functioning.	Set the flow to zero. Is the pressure Stable? Adjust the transducer.
performing a gradient analysis?	If pressure changes follow the gradient, then this may be normal, depending on the individual solvent viscosities
Using Solvents of volatile nature?	Ensure that the operating temperature is suitable for the particular solvent used. Degas thoroughly
Pump problem	Contact your maintenance provider

BASELINE DRIFT

Baseline irregularities can be non-cyclic (erratic) or cyclic (follow a pattern). They can originate from electrical interferences, detector faults, solvent impurities, column contamination etc.

To isolate the source of a baseline irregularity, it is important to determine whether the problem lies with the fluid path, detector or electrical connections.

A) NON CYCLIC NOISE: (Fluid path problems)

The most common cause of non-cyclic baseline noise related problems is air in the system. To overcome this, all solvents should be thoroughly degassed prior to use, all lines should be purged with solvent and the pump should be thoroughly primed.

CAUSE	CORRECTIVE ACTION
Air Bubble Trapped in Detector Flow Cell	To remove the air bubble, either purge the detector flow cell or apply a slight pressure to the detector waste outlet. Degass the mobile phase thoroughly. To stop air bubbles forming in the flow cell, attach a 30 to 90cm length of 0.23 mm ID/1.58 mm OD tubing to the detector water outlet. The tubing acts as a flow restrictor, increasing backpressure in the cell. When adding the tubing, please be aware of the backpressure limits of the flow cell
Column Contamination	To determine whether the column is contaminated, replace it with a new column or a column where the performance is known. Flush the column with mobile phase and monitor the baseline. A baseline free from the previous noise indicates that the original column was contaminated.
System Not Equilibrated	Allow the column, detector etc sufficient time to stabilize. If performing gradient analysis, allow sufficient time between analyses for the system to re-equilibrate.
Contaminated Mobile phase	Do not use mobile phase that is contaminated or thought to be contaminated. Ensure that no traces of detergent remain in the vessel, as this will cause spurious peaks in the baseline.
Guard/In-LineFilter Contamination	Guard cartridges and in-line filters are designed to be disposable. We do not recommend attempting to clean up these items as the costs involved in time and materials out-weights the cost of part replacement.

B) Non Cyclic Noise: (Detector electronics problems)

The most common cause of problems related to electronic baseline noise is the detector. Usually, if the detector is allowed insufficient time to equilibrate before an injection is performed, then the resultant chromatogram will contain spurious peaks and there will also be some evidence of baseline drift [5-6].

CAUSE	CORRECTIVE ACTION
Detector not stable	After turning the detector on, allow it sufficient time to stabilize. The baseline will be stable once the detector is stabilized. Different detectors and conditions will require different stabilization times. Better to refer detector manual
Detector lamp malfunction	Check that the lamp energy and reference energy are within specification limits for normal detector operation.
Contaminated Detector flow cell	Flush the system with water, followed by methanol, then water to remove any excess buffers. The detector flow cell can also be cleaned with a 50/50 v/v mixture of THF/water, then 100% THF if the system is used in normal phase.
Cables	Check that all cables are securely seated in their respective terminals. Ensure that all output switches have the correct setting are in the correct position. All cables should be well maintained and grounded where necessary.
Radio Interferance	The detector should be isolated from all sources of radio interference or cycling equipment, for example, large electric motors. The detector should be adequately grounded. If necessary, move the detector away from the source of interference or position it within a Faraday Cage.
Gain/Sensitivity Setting Too High	Re-set to a lower value on the data handling device.

CYCLIC NOISE

The most common cause of cyclic baseline noise is the detector. Usually, if the detector is allowed insufficient time to equilibrate before an injection is performed, then the resultant chromatogram will contain

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spurious peaks and there will also be some evidence of baseline drift.

CAUSE	CORRECTIVE ACTION
Sort Term Cycling Equipment Radio Interference Baseline Drift – Unstable detector Baseline Drift – Ambient Temperature Change Baseline Drift – contaminated detector flow cell Baseline Drift- Dirty Reference electrode Baseline Drift – Scratched contaminated electrode Noise spikes – detector lamp malfunction	Refer to the previous table. DRIFTNOISE(irregular) NOISE(regular)
Ambient temperature fluctuations	Stabilize the air temperature around the instrument and allow the system to return to equilibrium. If this is not possible, relocate the instrument to a laboratory position where the detector is thermally stable and/or avoid placing the instrument in direct sunlight.
Long Term Detector Temperature Problems.	The heater cycles on and off to maintain the detector temperature. Change the regularity of the on/off frequency to avoid baseline noise.

CHROMATIC IRREGULARITIES

The most common changes in chromatographic response are related to the shape and separation of peaks, their elution times and changes in established performance. There are two stages to troubleshooting unacceptable chromatography. First, the evaluation of what you have, chromatographically speaking and secondly, isolation of the source of the problem [7].

Abnormal Peak Shapes

Abnormal peak shape encompasses a range of possible peak shape problems.

- No peaks
- Fronting or tailing peaks
- Smaller than expected peaks
- Double peaks/shouldering peaks
- Broad peaks – early eluting analytes or all analytes
- Negative peaks

CAUSE	CORRECTIVE ACTION
Column problem	Typically, incorrect or missing column. Check that there is a column in the system, that it is the correct dimension and that it is packed with the correct media for the application.
Detector Problem	Ensure that the detector is set to the correct wavelength and that sensitivity and auto zeros are also correctly set. Zero the detector baseline if necessary. Check all power cables and connections between the detector and data-handling devices. Ensure that all output signal switches are in the correct position.
Fluid Path Problem	Typically, no flow or very low flow. Ensure that there is power to the pump and that it is switched on. Check that solvent comes out of the detector waste line when the pump is set running. Check that the solvent reservoirs contain solvent and that the solvent inlets are at a suitable height to draw any liquid present. Also check that the fluid inlet filters and lines are not blocked. If no solvent flow occurs, refer to the pump maintenance manual or contact your maintenance provider.
Injector Problem	No vial: Fill auto sampler position with a vial. Empty vial: Fill the vial with sample. Over full vial: Replace with a vial where there is an air gap between the top of the fluid and vial cap. Wrong vial: Check that the correct sample was injected. Insufficient sample: Inject a more concentrated sample solution or a larger volume of the same sample solution. Incorrect injection volume: Inject the correct volume of sample. Needle blocked: Remove the injector needle from its support, as per the auto sampler maintenance manual and either clean or replace it. The blockage may be due to the vial septa degrading or coring. Ideally replace all vial septa/caps with a non-coring variety. Other sources of

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	blockage include particulates in the sample. Ensure that all samples are filtered prior to injection.
Incorrect solvent/sample	Prepare new solvents, prime all lines and the pump and allow the system to reach equilibrium. Make sure that the sample injected is the correct one, that it is of the correct strength and that it has not degraded. Replace where possible. If sample preparation techniques involved an extraction or similar, ensure that the correct sample and solvents were used. Where possible, repeat the sample preparation and check that all reagents/extraction equipment are correct and within their shelf lives. *Note: Precipitation of the sample because of incompatibility with solvents will also result in no peaks being detected. There is often a rise in system backpressure accompanying this problem*
Column voiding	Using a standard mixture, solvent and analysis conditions, as close to those used to generate your column's test certificate as possible, calculate the columns efficiency (N). If N is markedly lower than is quoted on the manufacturers certificate of analysis, try cleaning the column using the procedures above mentioned. Repeat the efficiency calculation. If there is no increase in efficiency, replace the degraded column with a new one. You may wish to contact your column supplier if the degraded column is relatively new to discuss whether it was a handling error that caused the degradation
Injection Disrupting Equilibrium	Dissolve the sample in mobile phase, use a weaker diluent or make a smaller injection
Sample too viscous	Dilute the sample or decrease the rate at which the syringe draws the sample.
Vial problem	Check that the vial contained enough of the correct sample to perform the injection – if not, replace with a fresh one. Make sure that the vial seats correctly in the autosampler and that the needle is not obstructed when performing an injection.
Guard column voiding	Remove the defective guard and replace with a new one. Allow the system to reach equilibration and repeat the sample injection. If the problem persists, remove the guard and holder and perform an injection. If the problem remains, it is not related to the guard. If the problem disappears, check the connections between the guard unit and the column. Ensure that the connector adds zero dead-volume to the system and that the guard cartridges fit snugly into the holder. Ideally, use an integral guard.
Blockage/ partial blockage before the column	Check the guard, in-line filter, column inlet and all associated tubing for blockages. Replace any blocked tubing, filters or guard units. If the column inlet frit has been blocked, gently back-flush the column with a wash solvent at very low flow rate (preferably over night for best effect). Re-invert the column and equilibrate with test solvent. If this procedure has not removed the blockage, please contact your column supplier for further technical advice.
Contamination in injection or column	Flush injector between analyses (a good routine practice). If necessary, run strong solvent through column to remove late eluters. Include final wash step in gradient analyses, to remove strongly retained compounds.
Late eluting peak (usually broad) present in sample.	2. a. Check sample preparation. b. Include (step) gradient to quickly elute component

RETENTION TIME CHANGES FROM INJECTION TO INJECTION

The most common cause of peak retention time drift is an un-equilibrated system. The detector and fluid system must be stable prior to starting an analysis. Temperature changes during the analysis are another major cause of peak drift [8-10].

CAUSE	CORRECTIVE ACTION
System not equilibrated	Allow the column, detector etc. sufficient time to stabilize. If performing gradient analysis, allow sufficient time between analyses for the system to re-equilibrate. The first time ion pair reagents are used with a column, allow sufficient time and volume of solvent to adequately equilibrate the column.
Insufficient	A suitable period of time for

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equilibration-gradient analysis	equilibration must be allowed between gradient analyses. This allows the T0 mobile phase composition to be pumping through the column as the injection occurs. Insufficient equilibrium time results in erratic retention times.
Solvent bleeding problems	Check the miscibility of the solvents – if their miscibility is poor, consider changing one or all to give a miscible mix. If the solvent is mixed manually, ensure that it is filtered and thoroughly degassed before use. The solvent line and pump should be thoroughly primed with this solvent to remove all traces of previous solvents and air. The column should have a minimum of 10 column volumes of solvent passed through it to allow equilibration. Finally, a series of standard injections should be performed to ensure that the system is performing reproducibly.

CONTINUALLY INCREASING OR DECRESING RETENTION TIMES

The most common cause of peak retention time drift in one direction is poorly prepared or mixed solvents or a system leak. If you are confident that the solvents were prepared correctly, then it is very important that you determine whether they are being mixed correctly (mixing cell problems). Where solvents are mixed manually prior to pumping, ensure that the solvent flow rate is correct and constant [11-13].

CAUSE	CORRECTIVE ACTION
Column degradation	Follow Fronting treatment.
Flow rate changes	Ensure that the flow rate delivered is the same as that entered into the pump software. Also, ensure that the flow rate being used is correct for the application.
Solvent preparation	Ensure that all solvents are freshly prepared and free from microbial growth. Filter and degas thoroughly prior to use. Discard old solvents and thoroughly wash all dirty reservoirs prior to re-use. Prime the pump and solvent lines with freshly prepared solvents and allow the column/system to equilibrate.
Solvent delivery system blockage	Check the solvent lines and inlet filters for blockages. Refer to the pump maintenance manual for cleaning/replacement of blocked or dirty parts.
System leaks	Check all fittings and unions for leaks. Tighten any loose fittings, but do not be tempted to over tighten as this may damage the fitting's threads and cause leaks. Where leaks occur between tightened fittings, replace the fitting and ferrule since they may be damager or misaligned.

QUALITATIVE RESULTS

Qualitative assays do not measure exact quantities of an analyte in solution. Therefore, problems associated with these assays usually fall into one of two categories; a) missing or extra peaks and b) peak mis-identification.

The following tables will assist in tracing errors in qualitative methods.

A) Missing Peaks

Single or multiple missing peaks are usually due to the wrong sample being injected or the sample degrading. Equally likely though is a loss of resolution due to column/ solvent inconsistencies [13].

CAUSE	CORRECTIVE ACTION
Sample degradation	Perform one injection of a standard. If all the peaks are present and correct, the sample has degraded. Re-prepare the sample and re-inject.
Resolution lost	Replace the column with one where the performance is known and re-inject the sample. If the missing peak(s) re-appear, then the efficiency of your original column may be lower than that of the replacement column. Check the efficiency of the original column using a test mix and conditions similar to those used by the column manufacturer to test efficiency when the column was new. For columns that have been in use for an extended period of time, perform a column clean up. Repeat the efficiency test to determine whether this has improved column performance. You may wish to contact your column supplier if the

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	low efficiency column is relatively new to discuss whether it was a handling error that caused the problem.
Solvent flow programme inconsistencies	Verify that the analysis uses the correct proportions of solvents and that the correct gradient or isocratic path is being followed. Check that the solvent flow rate is accurate from all lines and that the correct flow rate is entered into the pump software. If a gradient is used, check that the time between analyses is sufficient to allow the system to re-equilibrate.
Column incorrect	Ensure that the correct media and dimension of column is used. Also check that the correct media particle size is used as this will have an impact on the resolution of closely eluting peaks – a 3 µm media will provide higher column efficiency than a 5 µm media.

B) Extra Peaks

Extra peaks in chromatograms are more often than not due to contamination or degradation of the sample, mobile phase or column. To check if the extra peak(s) is/are in the sample alone, perform a blank injection of sample solvent. The peak(s) should be absent Peak mis-identification occurs most often in degradation samples or those in which related substance levels are being measured. This is because the software is "calibrated" using a standard mixture with specific concentrations of each impurity. Most "real-life" samples contain impurities at very low levels so the retention time of their peaks will be slightly different to those generated by the standard mix. Identification windows should be set widely enough to take into account this time variation with respect to concentration.

C) Peaks Misidentified

Peak mis-identification occurs most often in degradation samples or those in which related substance levels are being measured. This is because the software is "calibrated" using a standard mixture with specific concentrations of each impurity. Most "real-life" samples contain impurities at very low levels so the retention time of their peaks will be slightly different to those generated by the standard mix. Identification windows should be set widely enough to take into account this time variation with respect to concentration [12].

Degraded / contaminated sample	Refer below the Precision table

CAUSE	CORRECTIVE ACTION
Data handling inaccuracies	If a data-handling system identifies your peaks then ensure that all the peak retention time variables such as peak windows, threshold, integration and retention times are correctly entered into sample and calibration tables etc. Make any necessary changes and perform a standard injection to ensure that peaks are now correctly identified. Refer to the data handling operators manual for further details.
Peak retention time variation	*Refer the changed retention time flow chart*

QUANTITATIVE RESULTS

Quantitative assays measure exact quantities of an analyte in solution. Therefore, problems associated with these assays usually fall into one of two categories;

a) Loss of precision and b) Loss of accuracy.

The most common mistake made is to assume that accuracy and precision are the same.

A) Loss Of Accuracy

Loss of Accuracy is mainly due to sample.

CAUSE	**CORRECTIVE ACTION**
Incorrect sample/ sample preparation	Check that the correct sample has been prepared and that the preparation has produced a solution that is of the correct strength. At the same time, check that any internal/external standards have been prepared correctly and are of the correct strength.
Solvent evaporation	Samples prepared in volatile solvent, such as chloroform, DCM etc. are affected by changes in ambient temperature. They will evaporate more quickly in warmer temperatures, producing more concentrated sample solutions. Ensure that the ambient temperature around the sample remains constant and that the vial cap is sufficiently tight to stop evaporation, but not tight enough to create a vacuum when injections are performed.
Peak Integration Error	Refer below the Precision table

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B) Loss Of Precision

Loss of precision is most often caused by an injector error, by a sample that is mixed poorly, or a sample that is degrading [13].

CAUSE	CORRECTIVE ACTION
Peak integration error	Ensure that the correct values of peak lift-off, touchdown, threshold etc. are entered into the data-handling device. Make any necessary changes.
Degraded / contaminated sample	Perform one injection of a standard. If all the peaks are present and correct, the sample has been contaminated or has degraded. Re prepares the sample and re-inject.
Injection error-external standard	**Manual Injection** Using a fixed-loop system: load three times the loop volume before making the injection. Using a partial fill loop: Inject less than 50% of the sample loop volume. Syringe and injection valve: Ensure that the injection technique is as constant as possible. **Automatic Injection** Check to make sure that air is not being injected, that the sample vial contains enough solvent to perform multiple injections from it and that there are no leaks in the system. Ensure that the sample loop is the correct size and that the injection system is undamaged and clean. Between injections, ensure that the injector purge is adequate to eliminate carry-over from previous injections.
Detector response Inaccuracies	Refer to the detector operator's manual for instrument specific information on troubleshooting and corrective action.

CONCLUSION

New chemistry and instrumentation technology can provide more information to fulfill the promise of higher speed, resolution and sensitivity in these techniques. Extraction of the above data as revealed that HPLC and UPLC will prove an essential and crucial tool for analyzing the pharmaceutical analysis.

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