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A REVIEW ON DEVELOPMENT AND VALIDATION OF ULTRA PERFORMANCE LIQUID CHROMATOGRAPHY FOR ESTIMATION OF DRUG IN PHARMACEUTICAL DOSAGE FORM.

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ABSTRACT

In recent years, remarkable technological advances have been made in particle chemistry performance, system optimization, detector design, and data processing and control. When brought together, the individual achievements in each discipline have created a step-function improvement in chromatographic performance ultra performance liquid chromatography (UPLC) is a modern technique which gives a new direction for liquid chromatography. UPLC which enhance mainly three areas of liquid chromatography: speed, resolution, and sensitivity. UPLC system column containing bridged ethylsiloxane / silica hybrid (BEH) structure with fine particle size (less than 1.7 m) is utilized and particle less than 2µm in diameter to acquire better resolution, speed, and sensitivity compared with HPLC. In twenty first centenary pharmaceutical industries are focusing for new ways to in economy and shorten time for development of drugs and drug product. The separation and quantification in UPLC is done under very high pressure (up to 100m pa). As contrast to HPLC, under high pressure it is observed that not any negative influence on analytical column and also other components like time and solvent consumption is less in UPLC. The commence review article is an endeavor to offer pervasive awareness around the development and Validation details about the UPLC and related techniques with the aim on practice to an estimation of medicinal Active agents in the last 10 years. The article also focused on general overview of UPLC. Method development and validation of UPLC method for estimation of drug and drug product.

KEYWORDS: Drug, UPLC, HPLC, Resolution,

1. INTRODUCTION

Analytical chemistry could be a field of study that develops strategies, instruments and methods to get data on the composition and nature of matter. It is involved with the chemical characterization of matter and therefore pharmaceutical analysis covers matter having pharmaceutical applications.[1] UPLC refers to Ultra Performance Liquid Chromatography. It improves in 3 areas: activity resolution, speed and sensitivity analysis. It uses fine particles and saves time and reduces solvent consumption.[2] The UPLC relies on the principal of use of stationary section consisting of particles but a pair of µm. The underlying principles of this progress area unit ruled by the van Demeter equation, That is associate formula that describes the connection between linear rate (flow rate) and plate height (HETP or column efficiency). UPLC comes from HPLC.[3] HPLC has been the evolution of the packing materials accustomed impact the separation.[4]

Van Deemter equation,

$H = A + B/u + Cu,$

The Van Demeter curve governed by an equation with three components shows that the usable flow range for a good efficiency with small diameter particles is much greater than for larger diameters The UPLC has involved the development of a new instrumental system for liquid chromatography, which can take advantage of the separation performance (by reducing dead volumes) and consistent with the pressures (about 8000 to 15,000 PSI, compared with 2500 to 5000 PSI in HPLC).[5] Efficiency is proportional to column length and inversely proportional to the particle size18. Therefore, the column can be shortened by the same factor as the particle size without loss of resolution.[6] The application of UPLC resulted in the detection of additional drug metabolites, superior separation and improved spectral quality.[7]

As particle size decreases to less than 2.5 m, there is a significant gain in efficiency and it's doesn't diminish at increased linear velocities or flow rates according to the common Van Deemter equation. By using smaller particles, speed and peak capacity can be expanded to new limits which is known as Ultra Performance.[8] The

typical separation method is of HPLC (High Performance Liquid Chromatography) with many advantages like robustness, ease of use, good selectivity and adjustable sensitivity.[9] Its main limitation is the want of efficiency compared to gas chromatography or the capillary electrophoresis due to low diffusion coefficients in liquid phase, involving slow diffusion of analytes in the stationary phase.[10] The Van Deemter equation shows that efficiency extent with the use of smaller size particles but this leads to a rapid increase in back pressure, while most of the HPLC system can operate only up to 378 bars.[11] That is why short columns filled with particles of about 2 m are used with these systems, to accelerate the analysis without loss of efficiency, while maintaining an acceptable loss of load.[12] To improve the efficiency of HPLC separations, the following can be done: - a) work at higher temperatures b) use of monolithic columns. UPLC presents the possibility to extend the utility of conventional HPLC, a widely used separation science. The ACQUITY UPLC System is the first instrument of its type to include Intelligent Device Management technology.[13,14]

SMALL PARTICLE CHEMISTRY

The potential of the van Deemter equation cannot be fulfilled without smaller particles than those consistently used in HPLC.[15] The design and development of sub-2 mm particles is a significant challenge, and researchers have been active in this area for some time to capitalize on their advantages. Figure 1 shows Van Deemter plot, showing the progress of particle sizes over the last three decades.[16]

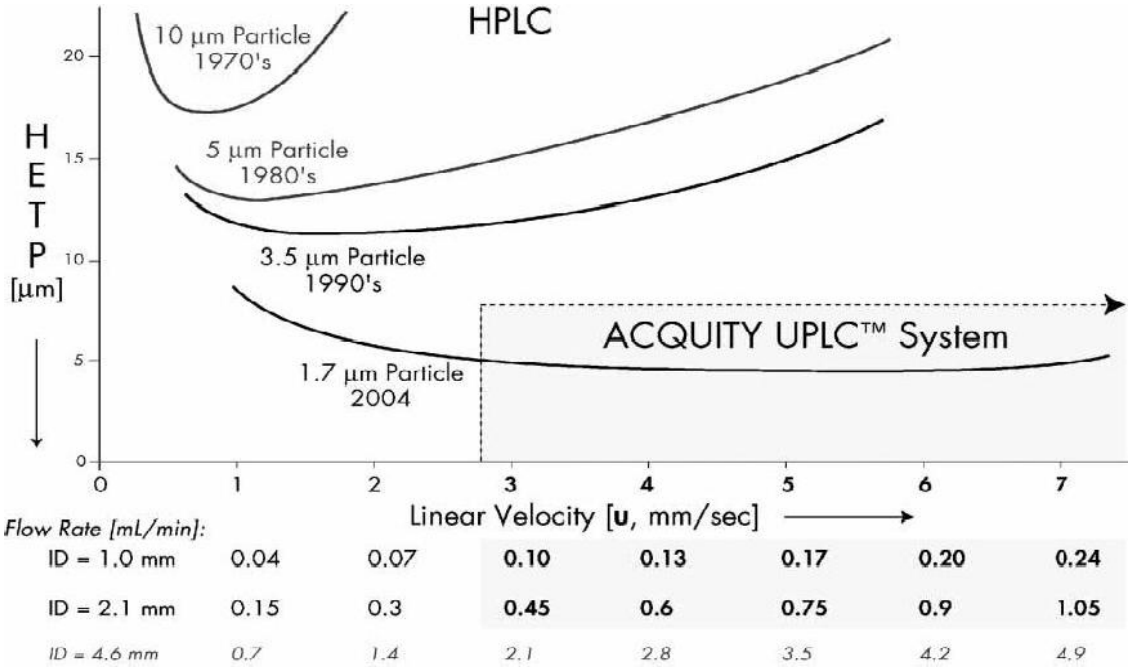

Fig 1: Van Deemter plot, illustrating the evolution of particle sizes over the last threedecades.

Although high potency, non-porous one.5 millimetre particles area unit commercially on the market, they suffer from poor loading capability and retention because of low extent. UPLC must use novel porous particles that can withstand high pressures. Silica based mostly particles have smart mechanical strength, but can suffer from a number of disadvantages, which include a limited pH range and tailing of basic analytes.[17] In 2000, a primary generation hybrid chemistry that took advantage of the simplest of each the silicon oxide and compound column worlds was introduced. Producing a classical sol-gel synthesis that includes carbon within the style of alkyl teams, these columns are mechanically strong, with high efficiency, and operate over an extended pH range.[18] But, so as to supply the sort of increased mechanical stability needed for UPLC, a second generation bridged ethane hybrid (BEH) technology was developed.

These 1.7 millimetre particles derive their increased mechanical stability by bridging the alkyl teams within the silicon oxide matrix. Packing 1.7 millimetre particles into consistent and rugged columns was additionally a challenge that required to be overcome.[19] In addition, at high pressures; frictional heating of the mobile phase can be quite significant and must be considered. With column diameters generally employed in HPLC (3.0 to 4.6 mm), a consequence of frictional heating is the loss of performance due to temperature induced non uniform flow. To minimize the effects of resistance heating, smaller diameter columns (1–2.1 mm) area unit generally used for UPLC.[20,21]

ADVANTAGES OF UPLC[22,23,24]

Various advantages of UPLC are as follows

- Decreases run time and increases sensitivity.
- Less solvent consumption

- Provides the selectivity, sensitivity, and dynamic range of LC analysis.
- UPLC's fast resolving power quickly quantifies related and unrelated compounds.
- Faster analysis through the use of a novel separation material of very fine particle size.
- Assures end-product quality, including final release testing.
- Reduces process cycle times, so that more product can be produced with existing resources.
- Delivers real-time analysis in step with manufacturing processes.

DISADVANTAGES OF UPLC

- Due to increased pressure requires more maintenance and reduces the life of the columns of these types. So far performances similar or even higher have been demonstrated by using stationary phases of size around 2 m without the adverse effects of high pressure.
- In addition, the phases of less than 2 m are generally non-re generable and thus have limited use.

INSTRUMENTATION

The schematic diagramme of UPLC and various parts of the instrument are shown in Figure1, and Figure 2, respectively.

Parts of ultra performance liquid chromatography (UPLC)

- Solvent Reservoir
- Degasser
- High Pressure Pump
- Sample Injection Port
- Column
- Detector
- Data Processing and Control
- Waste

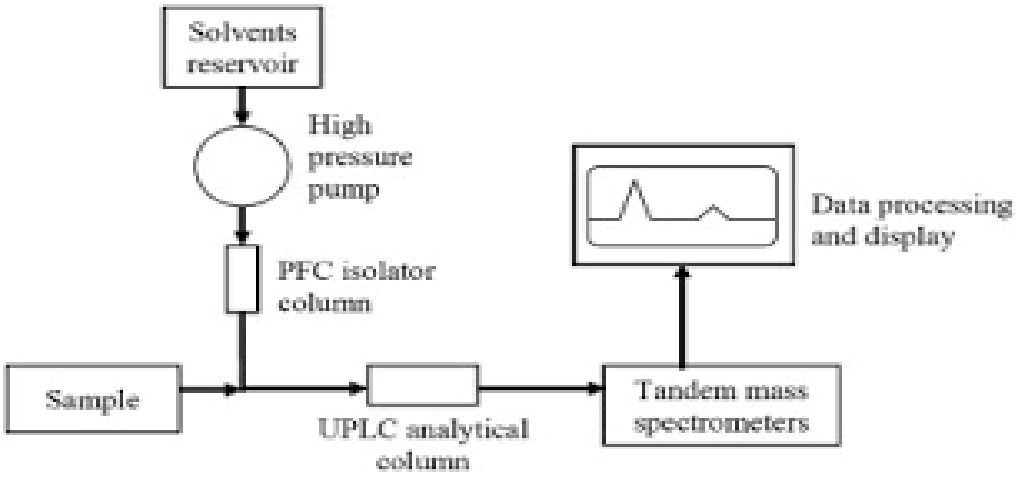

Fig 2: **Schematic diagram of UPLC.**

Fig. 3: **UPLC instrument.**

Analytical RP-UPLC method development

Analytical RP-UPLC method development and validation play significant roles in the drug discovery development and manufacture of pharmaceuticals. These

methods used to ensure the identity, purity, potency, & performance of drug products with speed, resolution, and sensitivity. There are many factors to consider when developing methods.[25] The method development start with collect the information about the analyte's physicochemical properties (pKa, log P, solubility) and detection mode would be suitable for analysis. The aim of the RP-UPLC-method is to resolve and separatly quantify the main active drug, any reaction impurities, all available synthetic inter-mediates and any degradants.[26,27]

Steps involve in RP-UPLC method development are
1. Understand the physicochemical properties of drug molecule.
2. Set up RP-UPLC conditions.
3. Preparation of sample solution for method development.
4. Method optimization.
5. Validation of method.

1) physicochemical properties of drug molecule

The Physicochemical properties of a drug molecule play an important role in method development. For Method development one has to study the physicochemical properties like molecular structure, molecular formula, solubility, polarity, pKa and pH, of the drug molecule. Polarity of analyte help, to decide the solvent and composition of the mobile phase. In a nonpolar covalent bond, the electrons are shared equally between two atoms.[28] The solubility of drug molecule help for diluents selection. It work on principle, like dissolves like i.e., materials with similar polarity are soluble in each other. Selection of diluents is based on the solubility of analyte. The analyte must be soluble in the diluents and must not react with any of the diluent components. The diluent should equivalent to the starting eluent composition of the assay to ensure that no peak deformation will occur, especially for early eluting components.[29,30]

pH and pKa plays an important role in UPLC method development. The acidity or basicity of a substance is defined most consistently by the pH value. Selecting a proper pH for ionizable analytes often leads to symmetrical and sharp peaks in UPLC. The acidity of an aqueous solution is determined by the concentration of [H3O+] ions. Thus, the pH of a solution indicates the concentration of hydrogen ions in the solution. The concentration of hydrogen ions can be indicated as [H+] or its solvated form in as [H3O+] whose value normally lies between 0 and 14. The lower the pH, the more acidic is the solution. The pH of a solution can be changed simply by adding acid or base to the solution. The pKa is characteristic of a particular compound, and it inform how readily the compound gives up a proton. If the pH is known, the ratio may be calculated. This ratio is individualistic of the analytical concentration of the acid. When the pKa and analytical concentration of the acid are known, the extent of dissociation and pH of a solution of a monoprotic acid can be easily calculated.[31]

2) UPLC Chromatographic conditions
selection UPLC Column

The heart of a UPLC system is the column. The Change in column will have the greatest effect on the resolution of analytes during method development. Generally, the nature of stationary phase has the greatest effect on capacity factor, selectivity, efficiency and elution. There are several types of matrices for support of the stationary phase, including silica and polymers. Resolution is increased in a 1.7 µm particle packed column because efficiency is better. Separation of the components of a sample requires a bonded phase that provides both retention and selectivity. The UPLC separation available in Four bonded phases. C18 and C8 (straight chain alkyl columns), Shield RP18 (embedded polar group column) and Phenyl (phenyl group tethered to the silyl functionality with a C6 alkyl). Each column chemistry provides a unique combination of property, silanol activity, hydrolytic stability and chemical interaction with analytes.[32,33]

C18 and C8 columns square measure thought-about the universal columns of selection for many UPLC separations by providing the widest hydrogen ion concentration vary. They incorporate tri practical matter bonding chemistries that manufacture superior low hydrogen ion concentration stability. This matter, combined with a similar proprietary finish capping processes because the C18 and C8 columns, provides long column lifetimes and excellent peak shape. This distinctive combination of matter and finish capping on the one.7 m BEH particle creates a replacement dimension in property permitting a fast match to the present HPLC column. An internal dimension (ID) of two.1 metric linear unit column is employed. For maximum resolution, select a one hundred metric linear unit length and for quicker analysis, and better sample turnout, choose 50 mm column. Conceptually, the sensitivity increase for UPLC detection should be 2-3 times higher than HPLC separations, depending on the detection technique. MS detection is considerably increased by UPLC; raised peak concentrations with reduced action dispersion at lower flow rates promote raised supply ionization efficiencies.[34,35]

Selection of Mobile Phase

The mobile phase is important part of liquid chromatography. The aqueous and non aqueous solvent used in mobile phase. A buffer is a partly neutralised acid which counter changes in pH. Consideration of the affect of pH on analyte retention, type of buffer to use, and its concentration, solubility in the organic modifier and its influence on detection are important in reversed-phase chromatography (RPC) method development of ionic analytes. An inappropriate choice of buffer, in terms of buffering species, ionic strength and pH, can result in poor or unrepeatable retention and tailing in

reverse-phase separation of polar and ionizable compounds.[36,37]

Buffer selection

Choice of buffer is typically governed by the desired pH. The typical pH range for reversed-phase on silica-based packing is pH 2 to 8. It is important that the buffer has a pKa close to the desired pH since buffer controls pH best at their pKa. A rule is to choose a buffer with a pKa value <2 units of the desired mobile phase pH.

Table 1: Buffer used for mobile phase.

Buffer	pKa	Useful pH Range	UV cutoff
Ammonium acetate	4.8 9.2	3.8-5.8 8.2-10.2	205(10mM)
Ammonium hydroxide/ ammonia	9.2	8.2-10.2	
KH2PO4/K2PO4	7.2	6.2-8.2	<200nm (0.1%)
KH2PO4/ phosphoric acid	2.1	1.1-3.1	<200nm (0.1%)
Potassium Acetate/ acetic acid	4.8	3.8-5.8	210nm (10mM)
Potassium formate/ formic acid	3.8	2.8-4.8	210nm (10mM)
Trifluoroacetic acid	<2	1.5-2.5	210nm (0.1%)

General considerations during buffer selection

1. Mobile phases should be degassed.
2. Phosphate is more soluble in methanol/water than in acetonitrile/water or THF/water.
3. Ammonium salts are generally more soluble in organic/water mobile phases·
4. TFA can degrade with time, is volatile, absorbs at low UV wavelengths.
5. Microbial growth can quickly occur in buffered mobile phases that contain little or no organic modifier.
6. At pH greater than 7, phosphate buffer accelerates the dissolution of silica and severely shortens the lifetime of silica-based HPLC columns. If possible, organic buffers should be used at pH greater than 7.
7. Ammonium bicarbonate buffers usually are prone to pH changes and are usually stable for only 24 to 48 hours. The pH of this mobile phase tends to become more basic due to the release of carbon dioxide.
8. After buffers are prepared, they should be filtered through a 0.2-µm filter.

Buffer concentration: Generally, a buffer concentration of 10-50 millimetre is adequate for little molecules. Generally, no quite five hundredth organic ought to be used with a buffer. This will rely on the precise buffer likewise as its concentration. Phosphoric acid and its metal or metallic element salts square measure the foremost common buffer systems for reversed-phase HPLC. Phosphonate buffers are often replaced with salt buffers once analyzing insecticide compounds.[38]

SAMPLE INJECTION

In UPLC, sample introduction is critical Conventional injection valves, either automatic or manual, don't seem to be designed and hardened to figure at extreme pressure. To protect the column from extreme pressure fluctuations, the injection method should be comparatively pulse-free and therefore the the} sweptwing volume of the device also must be stripped-down to cut back potential band spreading. A fast injection cycle time is required to completely exploit the speed afforded by UPLC, that successively needs a high sample capability. Low volume injections with stripped-down carryover also are needed to extend sensitivity. There also are direct injection approaches for biological samples.[39]

Selection of detector

Detector is a very important part of HPLC. Selection of detector depends on the chemical nature of analytes, potential interference, limit of detection needed, availableness and/or price of detector. For UPLC detection, the tunable UV/Visible detector is employed which has new physical science and code to support LAN communications at the high knowledge rates. Conventional absorbance-based optical detectors square calculate concentration sensitive detectors, and for UPLC use, the flow cell volume would ought to be reduced in normal UV/Visible detectors to keep up concentration and signal. According to Beer's Law, smaller volume conventional flow cells would also bring down the path length upon which the signal strength depends. A reduction in cross-sectional means that the sunshine path is reduced, and transmission drops with increasing noise. Therefore, if a standard HPLC flow cell were used, UPLC sensitivity would be compromised. The ACQUITY Tunable UV/Visible detector cell consists of a light guided flow cell equivalent to an optical fiber. Light is expeditiously transferred down the flow cell in an inside coefficient mode that also maintains a 10mm flow cell path length with a volume of solely 500mL.

Tubing and connections within the system square measure expeditiously routed to keep up low dispersion and to require advantage of leak detectors that act with the software system to alert the user to potential problems.

Preparation of sample solutions for method development

Sample preparation is a censorious step of method development that the analyst must explore. The drug substance being analyzed should be stable in diluent. In initial UPLC method development, preparations of the solutions in amber flasks should be performed till it is determined that the active component is stable at room temperature and does not degrade under normal laboratory conditions. The sample solution should be filtered; the use of a 0.22 or 0.45 µm pore-size filter is recommended for removal of particulates size more than 0.45 µm. Filtration is a preventive maintenance tool for HPLC analyses.[40]

The effectiveness of the syringe filters is largely determined by their ability to remove contaminants/insoluble components without leaching undesirable artifacts (i.e., extractables) into the filtrate. If any additional peaks are observed in the filtered samples, then the diluent must be filtered to determine if a leachable component is coming from the syringe filter housing/filter.

Method Validation

Validation of an analytical procedure is the process of establishing documentary evidence demonstrating that a procedure, process, or activity carried out in testing and then production maintains the desired level of compliance at all stages requirements for its intended use. It should describe in detail the steps necessary to perform each analytical test.[41] The methods validation process for analytical procedures begins with the planned and systematic collection by the applicant of the validation data to support analytical procedures. All analytical methods that are intended to be used for analyzing any drug samples will need to be validated. The validation of analytical methods is done as per ICH guidelines.[42,43]

Components of method validation

The following are typical analytical performance characterstics which may be tested during methods validation.

- System suitability determination
- Specificity
- Linearity
- Accuracy
- Precision
- ✓ Repeatability
- ✓ Intermediate precision
- Detection limit
- Quantitation limit
- Robustness

System Suitability Determination

System Suitability Determination is the evaluation of the components of an analytical system to show that the performance of a system meets the standards required by a method. These Determination can be calculated experimentally to lay out a quantitative system suitability test report in that number of theoretical plates, capacity factor, relative retention time, resolution, tailing factor, relative standard deviation (precision). These are measured on a peak or peaks of known retention time and peak width.[44,50]

Specificity

The Specificity of analytical procedure is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically these might include impurities, degradants, matrix, etc. Identification in analytical procedure to ensure the identity of an analyte. Purity Tests in analytical procedure to ensure that all the analytical procedures performed allow an accurate statement of the content of impurities of an analyte, i.e. related substances, residual solvents, heavy metals etc.[45,50]

Linearity

The linearity is definded as the ability of obtain test results which are directly proportional to the concentration of analyte in the analytical procedure. If there is a linear relationship, test results should be evaluated by appropriate statistical methods, for example, by calculation of a regression line by the method of least squares. In some cases, to obtain linearity between assays and sample concentrations, the test data may need to be subjected to a mathematical transformation prior to the regression analysis. For the establishment of linearity, a minimum of 5 concentrations is recommended. Other approaches should be justified. The correlation coefficient, y-intercept, slope of the regression line and residual sum of squares should be submitted. A plot of the data should be included. In addition, an analysis of the deviation of the actual data points from the regression line may also be helpful for evaluating linearity.[46,50]

Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the worth which is accepted either as a standard true value or an accepted reference value and the value found. The accuracy of analytical procedure to synthetic mixtures of the drug product components to which known quantities of the drug substance to be analysed are added. In cases where it's impossible to get samples of all drug product components, it's going to be acceptable either to feature known quantities of the analyte to the drug product or to match the results obtained from a second, well characterized procedure, the accuracy of which is stated and/or defined. Accuracy could also be inferred once precision, linearity and specificity are established.[47,48]

Precision

The precision of an analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of an

equivalent homogeneous sample under the prescribed conditions. Precision could also be considered at three levels: repeatability, intermediate precision and reproducibility. Precision should be investigated using homogeneous, authentic samples. However, if it's impossible to get a homogeneous sample it's going to be investigated using artificially prepared samples or a sample solution. The precision of an analytical procedure is typically expressed because the variance, variance or coefficient of variation of a series of measurements.[49,50]

i) Repeatability- Repeatability expresses the precision under an equivalent operating conditions over a brief interval your time. Repeatability is also termed intra assay precision.[50]

ii) Intermediate precision- The extent to which intermediate precision should be established depends on the circumstances under which the procedure is intended to be used. The applicant should establish the consequences of random events on the precision of the analytical procedure. Typical variations to be studied include days, analysts, equipment, etc. It is not needed to review these effects individually. The use of an experimental design is inspired.[50]

iii) Reproducibility- Reproducibility is assessed by means of an inter-laboratory trial. Reproducibility should be considered just in case of the standardisation of an analytical procedure, as an example.[50]

6) Detection limit- The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.[50]

7) Quantitation limit- The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. The quantitation limit is a parameter of quantitative assays for low levels of compounds in sample matrices, and is used particularly for the determination of impurities and/or degradation products.[50]

8) Robustness - The evaluation of robustness should be considered during the development phase and depends on the type of procedure under study. It should show the reliability of an analysis with respect to deliberate variations in method parameters. If measurements are susceptible to variations in analytical conditions, the analytical conditions should be suitably controlled or a precautionary statement should be included in the procedure. One consequence of the evaluation of robustness should be that a series of system suitability parameters (e.g., resolution test) is established to ensure that the validity of the analytical procedure is maintained whenever used.[50,51]

In the case of liquid chromatography, examples of typical variations are:
- influence of variations of pH in a mobile phase;
- influence of variations in mobile phase composition;
- different columns (different lots and/or suppliers);
- temperature;
- flow rate.

CONCLUSION

This review describes the general technique of UPLC method development and validation of analytical method. The approach for the UPLC method development for the separation of pharmaceutical compounds was discussed. UPLC by using 2 µm particle size gives increased resolution, speed and sensitivity for liquid chromatography. The UPLC method is a reduction of analysis time, along with reduced solvent consumption, high throughput analysis and reduction in cost of analysis. From the literature survey of UPLC method development and validation it can be concluded that all categories of pharmaceutical drugs can be analyzed by UPLC method within a very short period of time and with less solvent consumption. UPLC increases and expands the significance of chromatography. It is transforming lives and laboratories, creating new opportunities for business profitability, and bringing new meaning to quality. The literature survey shows that research on UPLC method development and validation, both, at national and international level have been successfully done on all categories of drugs.

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