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A REVIEW ON METHOD DEVELOPMENT AND VALIDATION OF DIFFERENT DRUGS BY RP-UPLC METHOD

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ABSTRACT
UPLC chromatographic system is designed in a special way to withstand high system backpressures. This review introduce and summarize some of the most recent research works in the UPLC field. The different mobile phases like ammonium acetate buffer: methanol, phosphate buffer: acetonitrile, water: acetonitrile, potassium dihydrogen phosphate: acetonitrile, trifluro acetic acid: acetonitrile, phosphate buffer: acetonitrile and ortho phosphoric acid: methanol were commonly used for separation in UPLC analysis. Different columns like BEH C 18, HSS-T3, SB 18, Shimpak XR ODS and BEH shield RP 18 were utilized as stationary phases for chromatographic separation. The diverse parameters like specificity, limit of detection, limit of quantification, linearity, accuracy, precision, ruggedness and robustness were analyzed. Further, the forced degradation study was also carried out under acidic, alkaline, oxidative, photolytic and thermal conditions to demonstrate the stability indicating capability of the developed UPLC method. In the result analysis, the most efficiently mobile phases are ortho phosphoric acid: methanol, water: acetonitrile and phosphate buffer: acetonitrile. BEH C18 column was one of simplest and most convenient stationary phase used for RP-UPLC. All diverse parameters were validated as per ICH guidelines and report shown within the limits. The narrow peaks produced by fast UPLC require a small detection volume and fast acquisition rate to ensure high efficiency. UPLC is a simple, precise, accurate and selective method.

KEYWORDS: Ultra performance liquid chromatography, Stability study, Method development, Drug analysis, Validation.

INTRODUCTION
Pharmaceutical analysis by definition deals with analysis of drugs, pharmaceutical substances and raw materials. It is devoted to stability testing, comparing related substances (essential similarity testing of generics), determination of impurities developing, implementing and applying active assays for the pharmaceutical industry.[1] This is mainly used for the separation of the components from the mixture and for the determination of the structure of the compounds.[2] Qualitative analysis is mainly concerned with the detection and identification of constituents of an inorganic substance or a mixture of substances. It is used in the medical and criminology fields. Medical personnel use qualitative chemical analysis in tests and procedures to treat and diagnose patients.[3] Quantitative analysis is defined as quantitative performance of suitable chemical reactions and measuring the amount of reagent needed to complete the reaction.[4] It is employed for several reasons, including measurement, performance evaluation or valuation of a financial instrument, and predicting real-world events, such as changes in a country's gross domestic product (GDP). Drug analysis is the testing of suspected controlled substance to determine its composition. It can determine the amount of the drug present and thereby provide the purity of the substance. Some of the instrumental techniques are high performance thin layer chromatography, high pressure liquid chromatography, gas chromatography, ultra performance liquid chromatography, mass spectroscopy and ultra violet spectroscopy.[5]

High performance thin layer chromatography (HPTLC) is a fast separation technique and flexible enough to analyze a wide variety of samples. This technique is advantageous as it is simple to handle and requires a short analysis time to analyze the complex or the crude sample cleanup. It evaluates the entire chromatogram with a variety of parameters without time limits. Moreover, there is simultaneous but independent development of multiple samples and standards on each plate, leading to an increased reliability of results.[6] High pressure liquid chromatography (HPLC) is a powerful separation method which resolve mixtures with a large number of similar analytes. A chromatogram provides directly both qualitative and quantitative information.

Each compound in the mixture has its own elution time (the point at which the signal appears on the screen) under a given set of conditions; and both the area and height of each signal are proportional to the amount of the corresponding substance. It yields excellent separations in a short time. The stationary phase requires very small particles and hence a high pressure is essential for forcing the mobile phase through the column.[7] Ultra performance liquid chromatography (UPLC) is a recent technique in liquid chromatography, which enables significant reductions in separation time and solvent consumption. Reducing these separation times without reducing the quality of the separation would mean that important analytical information could be generated more quickly. These particles operate at elevated mobile phase linear velocities to affect dramatic increase in resolution, sensitivity and speed of analysis.[8]

Method development and validation

Method development is the process of selecting an accurate assay procedure to determine the composition and proving that an analytical method is acceptable for use in laboratory. Analytical methods should be used within GMP and GLP environments and must be developed using the protocols and acceptance criteria set out in the ICH guidelines Q2(R1).[9] It is used during the development of drug substance and drug product in the pharmaceutical industry. Validation 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. Validation is a requirement of food, drug and pharmaceutical regulating agencies such as the US FDA and their good manufacturing practice guidelines.[10] It is used in achieving the quality and safety of the final product especially in pharmaceutical industry.[11]

Validation done by UV, HPTLC and HPLC

UV spectrophotometer shines light at various wavelengths onto the sample and investigates the degree of absorption, reflection, and transmission of the light to perform qualitative or quantitative analysis of the sample. instrument validation involves wavelength accuracy, stray light, resolution power, noise, baseline flatness, stability, photometric accuracy and linearity.[12] Validation parameters typically monitored for HPTLC method are specificity, linearity, precision, limit of detection, limit of quantification, robustness and accuracy. [13] The validation of the method was based on FDA guidelines and on standard bioanalytical method validation recommendation.[14] The goal of equipment validation is to produce constant result with minimal variation without compromising the product and performance of the equipment. parameters used in HPLC method validation are accuracy, detection limit, quantitation limit, linearity, precision (repeatability, reproducibility), robustness, sample solution stability, specificity, capacity factor, relative retention, number of theoretical plates, tailing factor and peak resolution.[15]

Validation done by UPLC

The system suitability test was used to ensure that the UPLC system and procedures are adequate for the analysis performed. It should be validated according to ICH guidelines.[16] Parameters of UPLC are precision specificity, linearity, accuracy and robustness, when these conditions were applied, satisfactory results are obtained.[17] Analysis time reduced so that more product can be produced.[18] Increases sample throughput and enables manufacturers to produce more material that consistently meet or exceeds the product specifications, potentially eliminating variability, failed batches, or the need to re-work material.[19] It Assures end product quality, including final release testing.[20] Faster analysis is possible with the use of a novel separation material which are of very fine particle size. The cost of operation is less.[21]

Analysis of different pharmaceutical formulations by RP-UPLC method

The different pharmaceutical formulations were analyzed by RP UPLC method and summarized in Table 1.

Vaijanath et al.[22] had developed and validated UPLC method for determination of primaquine phosphate and its impurities. The chromatographic separation was achieved on a Waters Acquity BEH C18, 50 × 2.1 mm, 1.7µm column within a short runtime of 5 min. Mobile phase consist of A: 0.01% aqueous trifluroacetic acid and B: acetonitrile in the ratio 75:25 (v/v) at flow rate of 1 ml/min. Quantitative parameters like specificity, precision, accuracy, linearity and robustness are validated according to the regulatory guidelines. Degradation studies were performed to demonstrate the stability indicating power of the UPLC method.

Development and validation of UPLC method for the determination of meloxicam and its impurities in active pharmaceutical ingredients was done by Louati et al[23] Chromatographic separation was achieved on Acquity UPLC HSS-T3 (2.1 × 100 mm, 1.8 µm) column. Mobile phase composed of A: potassium dihydrogenophosphate adjust (pH 6.0) with NaOH. B: methanol. The flow rate was 0.4 ml/min and injection volume was 0.8 µl. Further, oven temperature was maintained at 45°C. The developed method was validated according to the ICH guidelines for specificity, linearity, accuracy, precision, robustness, quantification limit and detection limit.

Farah et al[24] developed stress induced validated UPLC-PDA method for the analysis of eslicarbazepine acetate. In this study, mobile phase A comprises a mixture of 0.01 M potassium dihydrogen orthophosphate and acetonitrile (90:10, v/v) and B comprises a mixture of acetonitrile-water-methanol (75:5:25, v/v/v). The flow rate was 0.2 ml/min and methanol was used as a diluent. The injection amount 2 µl was used. Chromatographic separation achieved by Waters Acquity BEH 150 × 2.1 mm, 1.7 µm, C18 column. The drug show stability in acid hydrolysis, neutral hydrolysis, oxidative, thermal

and photolytic stress conditions. All validation parameters given acceptable results in terms of specificity, selectivity, linearity, precision, accuracy and robustness.

Method development, validation and stability studies for determination of bumetanide in bulk and pharmaceutical dosage form was performed by Chaitanya and Raja.[25] Chromatographic separation achieved by Acquity SB C18, 2 x 100 mm, 1.8 µm, 5µ particle size column. The solvent system, water: acetonitrile (ratio of 30:70v/v) was used as a mobile phase. The effluents were monitored at 254 nm and flow rate was 1.0 ml/min. The retention time was 0.852 min and the correlation coefficient was found to be 0.999. The % RSD for precision and accuracy of the method was found to be less than 2%. Bumetanide was subjected to stress environment of degradation in aqueous solutions including oxidation, hydrolysis, thermal and photolysis degradation. Proposed method was found to be simple, accurate, precise, and used for regular analysis.

Shihliang et al[26] developed stability-indicating UPLC method for determination of isotretinoin in bulk drug. Chromatographic separation was developed using a gradient elution in a reversed-phase system at flow rate of 0.5 ml/min with 12 min run time. The mobile phase comprises, A: contains a mixture solution of methanol/purified water/glacial acetic acid in the ratio of 30:70:0.5) and B contains a mixture of methanol/acetonitrile/purified water/glacial acetic acid in the ratio of 70:25:4.5:0.5). The method was validated for accuracy, precision, robustness, linearity and forced degradation according to ICH. The limit of detection was 0.12 µg/ml and the limit of quantification was 0.38 µg/ml. The drug was exposed to stress conditions and the results showed that all degradation products were separated from each other.

Analytical method development and validation for the analysis of verapamil hydrochloride and its related substances by using ultra perfomance liquid chromatography done by Vijayabaskar and Kalaivani.[27] A gradient elution of ammonium formate, orthophosphoric acid and acetonitrile as mobile phase. Shimpak XR ODS (75 mm × 3.0 mm, 1.7 µ particle size) was used as column. Degradation was observed only in oxidative and base hydrolysis. The method was validated for specificity, precision, linearity, accuracy and robustness.

Sahu et al[28] developed and established the inherent stability on piracetam by UPLC and validated stability indicating method. Chromatographic separation was performed on Acquity UPLC BEH C18 column (1.7 µm, 2.1 mm × 150 mm). Mobile phase was achieved by using isocratic mode acetonitrile: water and flow rate was 25:75 v/v. The r^2 value was found to be 0.999. Further, method detection limit (MDL) and method quantification limit (MQL) were found to be 0.180 µg/ml and 1.10 µg/ml for UPLC. The % RSD values for intraday and interday precision were <1.2%. The developed method was simple, fast, accurate and precise.

Development and validation of new RP-UPLC method for the determination of cefdinir in bulk and dosage form was performed by Krishnaphanisri and Raja.[29] Separation was achieved with an Acquity SB C18 (100 × 2 mm) 1.8µm column. Mobile phase contains a mixture of orthophosphoric acid and acetonitrile (60:40 v/v) and it was adjusted pH to 2.8. The flow rate was 0.3 ml/min with a column temperature of 30 °C and wavelength at 285 nm. The method was validated with respect to linearity, accuracy, precision, detection limits, robustness and specificity. The standard deviation was below 1.5%. The calibration curve was linear over a range from 25 to 150µg/ml with a correlation coefficient of 0.9993. Degradation study was carried out under acidic, alkaline, oxidative, photolytic and thermal conditions.

Novel stability-indicating UPLC method development for the determination of seven impurities in various diclofenac pharmaceutical dosage forms was performed by Azougagh et al. [30] Acquity HSS T3 (C18, 100 × 2.1 mm, 1.8 µm) column was used and mobile phase comprise of phosphoric acid, which has a pH value of 2.3 and methanol. The flow rate and the injection volume were set at 0.35 ml/min & 1 µl. It was detected at 254 nm by using photodiode array detector. Further, drug was subjected to stress conditions from acid, base, hydrolytic, thermal, oxidative and photolytic degradation. Validation was processed according to ICH with respect to specificity, limit of detection, limit of quantitation, precision, linearity, accuracy and robustness.

UPLC method for the analysis of raltegravir and lamivudine in dosage form was done by Sarif and Jacob.[31] The separation was attained with a BEH Shield RP18 column (2.1 mm × 100 mm; 1.7 µm). The mobile phase consists of buffer potassium dihydrogen orthophosphate (pH 3 adjusted) with orthophosphoric acid: methanol (30:70, %v/v). The flow rate was 0.230 ml/min. The column was maintained at an ambient temperature and examined at 254 nm using a PDA detector. The technique was exposed for forced degradation studies in five different stress conditions. The report of study was found to be selective and stability indicating. The assay of the dosage form and robustness study were obtained within the limit of ICH.

Srinivasarao et al[32] validated and developed stability-indicating RP-UPLC method for quantitative determination of bromfenac sodium and its impurities in an ophthalmic dosage form. Chromatographic separation achieved on a Waters Acquity BEH Shield RP18 (100 mm× 2.1 mm, 1.7 µm) column. The mobile phase comprise of gradient elution by using a binary mixture of potassium dihydrogen phosphate (0.01 M, pH 3.3) and acetonitrile. The flow rate was 0.5 ml/min and chromatogram was monitored at 265 nm using a

photodiode array detector (PDA). The impurities were eluted within 13 min. The parameters such as specificity, linearity, limit of detection, limit of quantification, precision, accuracy and robustness were validated as per ICH guidelines and report shown within the limits.

Stability indicating UPLC method for determination of linezolid in dosage forms was done by Alessandro et al. [33] Chromatographic separation done by C18 column. The mobile phase consists a mixture of acetonitrile, ultra-purified water and trifluoracetic acid (10:90:1). Flow rate was about 0.5 ml per min and injection time was about 6 min. In this method, linear ranges were obtained from 8.0 to 12.0 mg/ml and r^2 was 0.9985. Further, it shown good precision, accurate and robust. Linezolid submitted to alkaline degradation showed degradation and peaks with relative retention time (RRT) about 0.6. The sample submitted to oxidation showed degradation peaks with RRT about 0.4 and 0.5.

Patel et al[34] developed and validated RP-UPLC method for the estimation of amlodipine and indapamide in their combined tablet dosage form. The chromatographic separation was achieved on Acquity UPLC BEH C18 column (2.1 mm× 100 mm, 1.7µm). The mobile phase contains buffer (1% glacial acetic acid):acetonitrile (58:42, v/v) and flow rate was 0.25 ml/min. Injection volume was 2 µl and detection done at 240 nm. The retention time for amlodipine and indapamide was found to be 1.56 min and 2.58 min, respectively. In addition, range of linearity was between 10-50 µg/ml and 3-15 µg/ml for amlodipine and indapamide, respectively. The method validated according to the ICH guidelines.

Stability-indicating RP-UPLC method for the estimation of impurities in cinacalcet hydrochloride was developed and validated by Sunil reddy et al.[35] Separation was achieved on Acquity BEH Shield RP18 column. (100 × 2.1 mm). The mobile phase comprise of pH 6.6 phosphate buffer and aceto-nitrile. The flow rate was 0.3 ml/min and wavelength detected at 223 nm. The sample was subjected to acid, base, oxidative, hydrolytic, thermal, humidity and photolytic degradation. Drug was found to degrade in stress conditions and the method was validated according to ICH guidelines.

Xavier and Basavaiah[36] developed and validated metformin hydrochloride in pure drug and pharmaceutical formulations by RP-UPLC. The separation was done by using reversed-phase C-18 column (Waters Acquity BEH C18, 100 mm × 2.1 mm, 1.7 µm). The mobile phase comprises equal volumes of methanol and acetonitrile mixture (30%) and phosphate buffer with pH 3.2 (70%). The flow rate was 0.20 ml min and detected at 230 nm. Injection volume was adjusted to 2 µl and the method was validated for different parameters such as linearity, precision, accuracy, specificity and selectivity. The linear regression analysis showed a good linear correlation over the concentration range 0.1-300 µg/ml and regression coefficient r^2 was 0.9999. The LOQ and LOD were found to be 0.01 and 0.002 µg/ml. Forced degradation of the bulk sample was conducted in accordance with the ICH guidelines.

A new validated ultra performance liquid chromatographic method for the estimation of acyclovir was developed by Rao and Reddy.[37] Chromatographic separation achieved by Waters Acquity HSS T-3 (100 × 2.1mm, 1.8 µm) column and mobile phase (1.0% triethyl amine in water and acetonitrile). The flow rate was 0.4ml/min and it was detected at 254 nm. The method was found to be selective, linear, accurate and precise as per ICH guidelines. Detection and quantitation limits of drug were found to be 3 and 8 ng/ml, respectively.

Shashikant et al [38] developed and validated RP-UPLC method for the determination of iloperidone and degradation products in bulk and dosage forms. The chromatographic separation was achieved on HSS C18 (2.1 mm × 100 mm, 1.8 micron) column with a short runtime of 10 min. The mobile phase comprises a mixture of acetonitrile and methanol in the ratio of 80:20 v/v. A wavelength was detected at 225 nm over the concentration range of 0.03 - 0.15 µg/ml. In addition, the drug shows degradation under acidic, alkaline, oxidative, photolytic and thermal conditions. Hence, the method was validated according to the ICH guidelines.

Development and validation of telmisartan impurities and chlorthalidone impurities by UPLC was done by Brahmaiah and sreenivasulu.[39] The chromatographic separation was carried out by mobile phase [A consists pH 4.5 buffer & acetonitrile in the ratio 90:10 (v/v) and B consists of pH 4.5 buffer& acetonitrile in the ratio 20:80(v/v)] and column [Acquity BEH Shield-RP18, 100 x 2.1 mm, 1.7 µm]. The flow rate was 0.3ml/min with the column temperature was maintained at 25°C and detection the wavelength at 290 nm. The injection volume was 3µl. Analytical parameters like specificity, linearity, accuracy, precision, robustness and high sensitivity with detection limits and quantification limits were validated according to ICH guidelines.

Suresh et al[40] developed and validated RP-UPLC method for simultaneous determination of amlodipine and metoprol succinate in fixed dosage form. The chromatographic separation was achieved with Acquity UPLC HSS T3, 1.8 µm, 2.1×100mm analytical column at 45°C. The mobile phase, A (comprises of solution containing 5.0 gm of sodium dihydrogen phosphate monohydrate per liter of water and Acetonitrile in the ratio of 95:5) and B (acetonitrile) was used. The flow rate was 0.5ml/min and injection volume was 10µl. The detection wavelength was at 232nm and the retention times was found to be 2.8 minutes and 8.1 minutes. All the parameters were evaluated as per ICH guidelines, which remained well within acceptable limits.

Development and validation of stability-indicating RP-UPLC method for simultaneous estimation of

thiocolchicoside and aceclofenac in combined dosage form was processed by Paramasivam and Nagappan.[41] The chromatographic separation was carried out by using Thermo Scientific hypersil gold C18, column (50 x 2.1mm) particle size 1.9µm with mobile phase of 5% ammonium acetate buffer and methanol in the ratio of 40:60 (pH was adjusted to 5 with ortho phosphoric acid). The flow rate was 250µl/min and detected at 276nm. The run time was about 0.697 and 1.125 minutes. The regression equation was found to be y = 20620x-677.68 (r^2 = 0.9996) for TCC and y= 50931x-319.3 (r^2 = 0.9997) for ACF. The detection limit and quantification limit were found to be 0.076µg and 0.23µg for thiocolchicoside and 0.27µg and 0.71µg for aceclofenac. The percentage of assay was 99.50% and the method was validated according to ICH guidelines.

Hemanth et al[42] developed and validated simultaneous assessment of clomiphene citrate and n-acetyl cysteine in mixed tablet dosage form. The column C18 thermo fisher (50mm x 4.6 mm x 3µm) was used in the determination. The mobile phase consists of potassium phosphate buffer: methanol: acetonitrile in the ratio of 40:52:08 v/v/v. The flow rate and injection volume were 0.2µl - 1 and 1 µl, respectively. The detection was observed at 233nm. The retention time was about 1.7 minutes and 2.7 minutes for n-acetyl cysteine and clomiphene citrate. Total run time of 5 minutes. The linearity of the method was ranges from 96.072 to 144.048 µg/ml of n-acetyl cysteine and 8.073 to 12.01µg/ml for clomiphene citrate, respectively with a correlation of 0.9998 and 0.9999. The method was fast, simple, elegant and less time consuming.

Parvathi et al [43] performed the method development and validation of paracetamol and naproxen in mixed tablet dosage form by RP-UPLC. In this study, the column C18 thermo fisher (50mmx 4.6 mm x 3µm) was employed for determination. The mobile phase was comprised by 0.4% w/v ammonium acetate buffer: methanol: acetonitrile in the ratio of 40:40:20 v/v/v. The flow rate and injection volume were 0.2/µl and 1 µl. The detection wave length was set at 271nm and the retention time was about 1.9 minutes. Total run time was used 5 minutes and correlation coefficient was 0.9999 and 0.9997. The method was fast, simple, elegant and less time consuming method.

Nikita et al [44] developed and validated the RP-UPLC method for determination of cloxacilin sodium in its bulk form and formulation. A chromatographic separation of a drug done by Waters acquity BEH, 2.1 3 100 mm, 1.7 mm C18 column. A gradient of mobile phase, A: phosphate buffer, pH 6.8 and B :methanol: acetonitrile (75:25) was used. The detection done at wavelength 225 nm and flow rate was 0.35 ml/min with an injection volume was 10 ml. The retention time of the drug was similar to 6.9 min. The drug degrades under alkaline, acidic and oxidative conditions but was stable in temperature and light. A developed method was validated as per ICH guidelines using validation parameters such as precision, linearity, limit of quantification, specificity, assay and robustness.

Srinivasarao et al[45] performed the development and validation stability indicating RP-UPLC method for the determination of ritonavir and its related compounds. The chromatographic separation was achieved by Waters Acquity BEH Shield RP18 (100 3 2.1 mm, 1.7 mm) column thermo stated at 508ºC. The mobile phase comprise of potassium dihydrogen phosphate (0.01 M, pH 3.5) and acetonitrile at a flow rate of 0.5 ml/min which was monitored at 240 nm using a photodiode array detector. The drug was subjected to hydrolytic (acid, alkaline and water), oxidative, photolytic and thermal stress conditions. The method was validated according to the ICH guidelines.

Hasan et al[46] developed stability indicating RP-UPLC for estimation of related substances and degradants in rivaroxaban active pharmaceutical ingredient. The separation was processed by a water acquity BEH C8 column (100mm x 2.1 mm, 1.7µm) and mobile phase [A consist of 0.05M diammonium hydrogen phosphate, pH adjusted to 3.0 and acetonitrile (80:20, v/v) and B consist of mixture of acetonitrile and water (90:10, v/v)]. The column temperature was maintained at 30ºC and the detection was carried out at 254nm. The method was validated according to ICH guidelines to demonstrate precision, linearity, accuracy and robustness. The correlation coefficient values were greater than 0.999 for rivaroxaban and its five impurities. Further, detection limit and quantitation limit were found to be 0.0005µg/ml.

Stability indicating UPLC method for quantitative analysis of dronedarone in pharmaceutical dosage form was performed by Batuk et al.[47] The chromatographic separation was achieved on Acquity BEH C 18 column whereas the mobile phase comprise by 20 mm KH_2PO_4 + 1 ml triethylamine (by orthophosphoric acid)] and methanol in ratio of 40 : 60 at 30°C. The flow rate was 0.4 ml/min and validation takes place with respect to linearity, accuracy, precision, LOD, LOQ and robustness. Dronedarone was subjected to acid and alkali hydrolysis, chemical oxidation, dry heat degradation and photo degradation. The results obtained were within limits.

Mallikarjuna et al, [48] developed and validated the RP-UPLC method for estimation of amlodipine besylate and atorvastatin calcium in pharmaceutical dosage form. The chromatographic separation was achieved by Kromasil C18 column. A mobile phase contains a gradient elution of acetonitrile and 0.1% v/v triethyl amine buffer (pH 3± 0.05). The flow rate was 0.8 ml/min and detection of wavelength at 240 nm. The method was validated for different analytical parameters like accuracy, repeatability, reproducibility, robustness, linearity, limit of quantification (LOQ) and limit of detection. The

results were shown within limits.

UPLC method for determination of valsartan & hydrochlorothiazide in drug products was performed by Antil et al.[49] The method was developed using Kromasil eternity C-18 column (50 mm×2.1 mm, 3.5 µm). Triethylamine buffer (0.1% v/v) and methanol (75:25 v/v) were used as mobile phase. The flow rate was 0.6 ml/min and detection wavelength was fixed at 225 nm. The run time was within 2 min and the method was validated in terms of linearity, accuracy and reproducibility. The limit of detection and quantification values were found to be within the limits.

Table 1: Stability studies in different pharmaceutical formulation by using RP-UPLC

S. No	Drug name	Mobile phase	Stationary phase	Parameters	References
1	Primaquine phosphate	Mixture of A [0.01% aqueous trifluroacetic acid] and B [acetonitrile in the ratio 75:25 (v/v)]	BEH C18, 50 × 2.1 mm, 1.7 µm column.	Specificity, precision, accuracy, linearity, robustness, forced degradation studies and LOQ	[22]
2	Meloxicam	Mixture of A [potassium dihydrogenophosphate at 0.1% adjusted to pH 6.0 with NaOH] and B [methanol]	UPLC HSS-T3 (2.1 × 100 mm, 1.8 µm).	Specificity, linearity, accuracy, precision, robustness, quantification limit and detection limit	[23]
3	Esicarbazepine acetate	Mixture of A [0.01 M potassium dihydrogen orthophosphate and acetonitrile (90:10,V/V)] and B [acetonitrile-water-methanol (75:5:25, v/v/v)]	BEH 150 × 2.1 mm, 1.7 µm, C18 column	Acid hydrolysis, neutral hydrolysis, oxidative, thermal, photolytic stress conditions, specificity, selectivity, linearity, precision, accuracy and robustness	[24]
4	Bumetanide	Mixture of A [water] and B [acetonitrile in the ratio of (30:70 v/v)]	SB C18, 2 x 100 mm, 1.8 µmm, 5m particle size column	Oxidation, hydrolysis, thermal and photolysis degradation	[25]
5	Isotretinoin	Mixture of A [methanol/purified water/glacial acetic acid in the ratio of 30:70:0.5)] B [methanol/acetonitrile/purified water/glacial acetic acid in the ratio of 70:25:4.5:0.5)]	–	Accuracy, precision, robustness, linearity and forced degradation studies	[26]
6	Verampamil hydrochloride	Mixture of A [ammonium formate, orthophosphoric acid] and B [acetonitrile]	Shimpak XR ODS, 75 mm × 3.0 mm, 1.7 µ particle size column	Specificity, precision, linearity, accuracy and robustness	[27]
7	Piracetam	Mixture of A [acetonitrile] and B [water in the ratio of (25:75 v/v)]	UPLC BEH C18 column (1.7 µm, 2.1 mm × 150 mm	Degradation studies	[28]
8	Cefdinir	Mixture of A [orthophosphoric acid] and B [acetonitrile in ratio of (60:40 v/v)]	SB C18 (100 × 2 mm) 1.8µm column	Linearity, accuracy, precision, specificity and robustness	[29]
9	Diclofenac	Mixture of A [phosphoric acid (pH 2.3)] and B [methanol]	HSS T3 (C18, 100 × 2.1 mm, 1.8 µm)	Specificity, limit of detection, limit of quantitation, precision, linearity, accuracy and robustness	[30]
10	Raltegravir & lamivudine	Mixture of A [buffer potassiumdihydrogen orthophosphate (pH3) adjusted with orthophosphoric acid] and B [methanol (30:70 %v/v)]	BEH Shield RP18 (2.1 mm × 100 mm, 1.7 µm),	Forced degradation studies and degradation compounds detected	[31]
11	Bromfenac	Mixture of A [potassium dihydrogen	Waters Acquity	Specificity, linearity,	[32]

	sodium	phosphate (0.01 M, pH 3.3)] and B [acetonitrile]	BEH Shield RP18 (100 mm× 2.1 mm, 1.7 µm) column	limit of detection, limit of quantification, precision, accuracy, robustness, hydrolytic, oxidative, photolytic and thermal stress	
12	Linezolid	Mixture of acetonitrile, ultra-purifiedwater and trifluoracetic acid (10:90:1)	C18 column	Degradation studies, oxidation, accuracy, precision and linearity	[33]
13	Amlodipine and indapamide	Mixture of A [buffer (1% Glacial acetic acid)] and B [acetonitrile in ratio (58:42, v/v)]	Acquity UPLC BEH C18 column (2.1 mm× 100 mm, 1.7 µm)	Linearity	[34]
14	Cinacalcet hydrochloride	Mixture of A [phosphate buffer (pH 6.6)] and B [acetonitrile]	Acquity BEH Shield RP18, 100 × 2.1 mm, 1.7 µm column	Acid, base, oxidative, hydrolytic, thermal, humidity and photolytic degradation	[35]
15	Metformin hydrochloride	Mixture of A [methanol] and B [acetonitrile (30%) and phosphate buffer (pH 3.2)]	(C-18)column (Waters Acquity BEH C18, 100 mm × 2.1 mm, 1.7 µm)	Limit of quantification (LOQ) and limit of detection (LOD)	[36]
16	Acyclovir	Mixture of A [1.0% triethyl amine in water] and B [acetonitrile]	Waters Acquity HSS T-3 (100 × 2.1mm, 1.8 µm) column	Selective, linear, accurate and precise	[37]
17	Iloperidone	Mixture of A [acetonitrile] and B [methanol in the ratio of (80:20 v/v)]	Acquity UPLC® HSS C18 (2.1 mm × 100 mm, 1.8 micron), Acquity CSH Phenyl-hexyl (2.1 mm × 100 mm, 1.7 micron), and Acquity® HSS Cyano (2.1 mm × 100 mm, 1.8 micron)	Acidic, alkaline, oxidative, photolytic and thermal conditions	[38]
18	Telmisartan and chlorthalidone	Mixture of A [buffer (pH 4.5) & acetonitrile in the ratio 90:10 (v/v)] and B [buffer (pH 4.5) & acetonitrile in the ratio 20:80 (v/v)]	BEH Shield-RP18, 100 x 2.1 mm, 1.7 µm column	Specificity, linearity, accuracy, precision, robustness, detection limit and quantification limit	[39]
19	Amlodipine and Metoprol	Mixture of A [solution containing 5.0 gm of sodium dihydrogenphosphate monohydrate per liter of water and Acetonitrile in the ratio of 95:5)] and B: [Acetonitrile]	UPLC HSS T3, 1.8 µm, 2.1×100 mm analytical column at 45 °C	Specificity, linearity, accuracy and precision	[40]
20	Thiocolchicoside and aceclofenac	Mixture of A [5% ammonium acetate buffer] and B [methanol (pH 5) with ortho phosphoric acid in the ratio of 40:60)]	Thermo Scientific hypersil gold C18, (50 x 2.1mm) particle size 1.9µm	Detection limit, quantification limit and forced degradation studies	[41]
21	Clomiphene citrate and n-acetyl cysteine	Mixture of potassium phosphate buffer: methanol: acetonitrile in the ratio of (40:52:08 v/v/v)	C18 thermo fisher (50mm x 4.6 mm x 3µm)	Stability, linearity, accuracy, precision and robustness	[42]

22	Paracetmol and naproxen	Mixture of 0.4% w/v ammonium acetate buffer: methanol: acetonitrile in the ratio of 40:40:20 v/v/v	C18 thermo fisher (50mm x 4.6 mm x 3µm)	Accuracy, robustness, linearity and precision	[43]
23	Cloxacilin sodium	Mixture of A [phosphate buffer (pH 6.8)] and B [methanol: acetonitrile in ratio of (75:25)]	Waters acquity BEH, 2.1 3 100 mm, 1.7 mm C18 column	Alkaline, acidic, oxidative, thermal and photo degradation conditions	[44]
24	Ritonavir	Mixture of A [potassium dihydrogen phosphate (0.01 M, pH 3.5)] and B [acetonitrile]	Waters Acquity BEH Shield RP18 (100 3 2.1 mm, 1.7 mm) column	Tailing factor	[45]
25	Rivaroxaban	Mixture of A [0.05M diammonium hydrogen phosphate (pH 3.0) and acetonitrile in ratio (80:20, v/v)] and B [acetonitrile and water in the ratio (90:10, v/v)]	BEH C8 column (100mm x 2.1 mm, 1.7µm)	Oxidative, acid, base, hydrolytic, thermal and photolytic degradation	[46]
26	Dronedarone	Mixture of A [20 mm potassiumdihydrogen phosphate+ 1 ml Triethylamine (by orthophosphoric acid)] and B [methanol in ratio of 40 : 60)]	BEH C 18	Linearity, accuracy, precision, LOD, LOQ and robustness	[47]
27	Amlodipine besylate & Atorvastatin calcium	Mixture of A[acetonitrile] and B [0.1% v/v Triethyl amine buffer (pH 3± 0.05)]	Kromasil C18 column	Accuracy, repeatability, reproducibility, robustness, linearity, limit of quantification and limit of detection	[48]
28	Valsartan and hydrochloro thiazide	Mixture of A [Triethylamine buffer (0.1% v/v)] and B [methanol in ratio of (75:25 v/v)]	Kromasil eternity C-18 column (50 mm×2.1 mm, 3.5 µm)	Linearity, accuracy, reproducibility, Limit of detection and limit of quantification	[49]
29	Telmisartan	Mixture of A [10mm Ammonium acetate with addition of 1ml triethyl amine (pH 3.9) with ortho phosphoric acid and Acetonitrile in the ratio (90:10 (v/v)] and B [acetonitrile]	Waters Aquity BEH C18 (100 mm x 2.1 mm, 1.7 µ) column	Precision, specificity, linearity, limit of detection, limit of quantification, accuracy and robustness.	[50]
30	Glipizide	Mixture of A [phosphate buffer (pH 3.5)] and [B: acetonitrilein ratio of (60 : 40 v/v)]	Zorbax C-18 (50mm × 4.6mm × 1.8 µm) column	Limits of detection (LOD), limit of quantitation (LOQ), robustness and ruggedness	[51]

Bhavani et al [50] had developed stability indicating UPLC method for the estimation of telmisartan related substances in tablet formulation. The mobile phase comprised with mixture of 10mm ammonium acetate, 1ml triethyl amine pH (3.9) with ortho phosphoric acid and acetonitrile in the ratio 90:10 (v/v). The chromatographic separation achieved by using Waters Aquity BEH C18 (100 mm x 2.1 mm, 1.7 µm) column. The wave length for eluted compounds were monitored at 290nm and the run time was within 10 minutes. The results obtained were within the limits.

Quality design approach for the development and validation of glipizide an anti-diabetic drug by RP-UPLC method application to formulated forms and urine.[51] The method was developed using Zorbax Extend C-18 (50mm× 4.6mm × 1.8 µm) column. The mobile phase was prepared by phosphate buffer of pH 3.5 and acetonitrile (60 : 40 v/v). LOD and LOQ values were found to be 0.001 and 0.005 g/ml, respectively. The percentage relative standard deviations for robustness and ruggedness were observed within the range of 0.1 and 0.99. The calibration graph was linear in the range of 0.005–300 g/ml. The method was validated according to ICH guidelines.

CONCLUSION

It can be concluded that the most efficiently used mobile phases were ortho phosphoric acid: methanol, water: acetonitrile, phosphate buffer: acetonitrile. Similarly, BEH C18 column was one of simplest and most convenient stationary phase used for RP-UPLC. UPLC is a simple, precise, accurate and selective method. UPLC is a rising chromatographic separation technique whose

packing materials have smaller particle size lesser than 2.5µm which improves the speed, resolution and sensitivity of analysis. Important advantage is a faster run time and significant reduction in solvent use. UPLC methods are preferable than HPLC, with many associated advantages such as that UPLC operates at much higher pressure. In pharmaceutical industry, the demand of UPLC analysis is very high, because of the unique features like high resolution in chromatogram, short time analysis which make more analytical work in less time with valuable, reliable and authentic data. The separation of UPLC was performed under high pressures but it has no negative influence on analytical column or other components of chromatographic system.

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