EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 5
Issue: 12
137-147
Year: 2018

A REVIEW ON METHOD DEVELOPMENT AND VALIDATION OF STABILITY INDICATING OF VARIOUS DRUGS BY USING RP- UPLC

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Article Received on 26/09/2018 **Article Revised on 17/10/2018** **Article Accepted on 07/11/2018**

ABSTRACT
The main requirement of pharmaceutical company is to reduce the fair in the development of new drugs to enhance sensitivity, specificity, robustness, resolution for their detection. This can be achieved by UPLC which is the modified HPLC method comprising high pressure and small sized particles (less than 2µm) used in the column. The review article reveals the method development and validation of stability indicating on various drugs by RP- UPLC. The different mobile phases like potassium di hydrogen, acetonitrile, sodium phosphate, methanol, sodium di hydrogen, trifluoro aceticacid, and ammonium acetate buffer were used in RP-UPLC. The chromatographic separation was carried by different stationary phases like C18, X-terra RP18, Acquity UPLC BEHRP-18, Kromasil Eternity TM C 18, Acquity® HSS-T3, Zorbax SB-C18 which are processed by using isocratic method. The validation method was evaluated with various parameters like linearity, precision, accuracy, robustness, LOD and LOQ. The evaluation of stability of drugs was performed with various degradation parameters such as oxidation, hydrolysis, acid base hydrolysis and photolytic hydrolysis. From the review, it was observed that C18 column was efficient for the separation due to the smaller particles size provides better resolution and retention time. The different mobile phases like like potassium di hydrogen, acetonitrile, sodium phosphate, methanol, sodium di hydrogen, trifluoro acetic acid and ammonium acetate were generally favors for the effective separation and yields good retention time. All varied parameters were validated as per ICH guidelines and report shown within the limits. Similarly, it was revealed that some drugs were shown stability and mild degradation. The developed method by RP-UPLC was validated per the ICH guidelines and found to be specific, precise, accurate and linear. Hence, the review was clearly indicated that UPLC method is possible to develop a new sensitive and accurate for different pharmaceutical formulations.

KEYWORDS: Method development, Ultra pressure liquid chromatography, Column temperature, High pressure liquid chromatography, Photodiode array.

INTRODUCTION
Analytical method development and validation are the continuous and inter-dependent task associated with the research and development, quality control and quality assurance departments. Analytical procedures play a critical role in equivalence and risk assessment, management. It helps in establishment of product-specific acceptance criteria and stability of results. The Validation should demonstrate that the analytical procedure is suitable for its intended purpose.[1] Ultra performance liquid chromatography is a modern technique which gives a new direction for liquid chromatography which enhance mainly in three areas like speed, resolution and sensitivity.[1] UPLC applicable for particle less than 2µm in diameter to acquire better resolution, speed, and sensitivity compared with high-performance liquid chromatography (HPLC).[1] Ultra-pressure liquid chromatography will make the most of the separation performance (by reducing dead volumes) and according to the pressures about 8000 to 15,000 PSI compared with 2500 to 5000 PSI in HPLC[2]. Due to the use of fine particle the column length can be reduced then automatically it will give more efficiency in result. The column temperature maintained in the ultra-pressure liquid chromatography is 65°C. In every chromatographic technique detector plays major role to give the peak area results.[2] Different detectors like optical tunable ultraviolet, fluorescence, refractive index, light scattering and mass spectrometric are used in UPLC. Due to the very sharp and narrow peaks there are more number of peaks appear in short time which might facilitate in analysis of complex mixtures and which may give more information regarding the samples should be analyzed[2]. The separation and quantification in UPLC are done under very high pressure (up to 100M Pa). The UPLC was validated in terms of specificity, precision,

robustness, reproducibility, and linearity. These are validated with respective to limits.[3]

Different pharmaceutical formulations were analyzed by RP UPLC method

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

Raja Abilash et.al.[4] has developed and validated a novel and rapid RP-UPLC method for the estimation of mycophenolate in tablet formulation. Chromatographic separation was achieved on a Symmetry C18 (4.6 x 100mm, 3.5 µm, Make: XBridge) support using an isocratic method. The mobile phase was composed of potassium di potassium di-hydrogen phosphate: acetonitrile in the ratio 35:65 v/v with a flow rate of 0.2ml/min with a ambient column temperature and the detection was done at 228nm with a run rate less than 1.2min). The method was linear for mycophenolate from 10-50ug/ml and the linear regression value obtained was >0.999, precision was validated by intra-day and inter-day assays. Recovery data were in the range 99.1-102.0% with R.S.D. values < 1.5%. The short retention time of 0.612 min. The developed method was validated according to the ICH guidelines with respect to linearity, precision, accuracy, specificity and robustness.

Vishnu et.al.[5] indicates the stability-indicating ultra-performance liquid chromatographic method to develop the quantitative determination of darifenacin hydrobromide (DFN) and its related compounds in active pharmaceutical ingredient and pharmaceutical dosages. The analysis requires an Acquity UPLC BEH C18 column (100, 2.1 mm and 1.7 m) at a flow rate of 0.3 mL/min, detection was performed at 210 nm. The mobile phase A (buffer–ACN; 80:20, v/v) and mobile phase B (buffer–ACN; 15:85, v/v) was prepared. A central composite design was performed to study the main effects and interactions of the independent variables. Drugs and impurities were eluted at 13mins and with high quality recoveries of [93.8 ± 2.1 to 99.8 ± 1.5 (mean ± RSD] with a high precision. The linear regression revealed the excellent correlation between peaks and responses (R2 values of 0.9991–0.9999) for the drug and impurities. Stability indicating the capability of a method was verified by forced degradation studies and mass balance study. LC-MS revealed protonated molecular ion peaks [M + H] + at m/z 428.20, m/z 425.20 and m/z 281.30 for the acid. The m/z values of unknown degradation products were matched with the proposed structures and reported discrete fracture network metabolites. This method was capable of completely separating thirteen impurities within 15 min.

Sowjanya et.al.[6] describes the novel validated stability indicating RP-UPLC method for determination of related substances in metoclopramide bulk drugs and pharmaceutical dosage form. The separation was carried by using waters X-terra RP18 (150 4.6 mm), 3.5 mm particle size column with a gradient program. The mobile phase consisting of solvent A [30 mM monobasic sodium phosphate and 2.3 mM of pentane-1-sulphonic acid sodium salt (pH 3.0 buffer)] and solvent-B [(acetonitrile) through a flow rate of 1.2 mL/min]. The wave length detected at 273nm by UV detector at a run time of 18 mins. The drug was subjected to degradation studies like oxidative, acid & base, hydrolysis, thermal and photolytic degradation. The result of the study, it was undergone mild degradation by photolytic, hydrolysis, thermal and showed stable under acid& base, hydrolytic and humidity stress conditions. The calibration curves was obtained for the four impurities were linear over the range 0.062-3.040 mg/ml. The method was validated as per ICH guidelines.

Jebaliya et.al.[7] describes the simplest stability indicating RP- UPLC method for the determination of fluconazole in bulk and solid pharmaceutical dosage form. The chromatographic separation using BEH C18 (100 × 2.1 mm, 1.7 µm particle size) column and the mobile phase consists of methanol: water (55:45 v/v) with a flow rate of 1ml to 0.30 mL/min. The detection was done at 211nm with photodiode array detector and with a 3000 oven temperature. The injection volume was respectively 2 µL and 20 µL. Total analysis was 3.0 min. The method validation includes several parameters like accuracy, precision, linearity, robustness, specificity, degradation study, limit of detection (LOD), and limit of quantification (LOQ) and the reports were validated according to ICH guidelines.

The determination of impurities in esomeprazole magnesium gastro-resistant tablets was established by Nalwade et.al[8] and validated the stability indicating RP-UPLC method. The technique was achieved by using Acquity BEH C18, 50 mm × 2.1 mm,1.7 m column. The mobile phase consists of solvent A (0.04 molar glycine (pH 9.0) buffer) and solvent B (the mixture of acetonitrile and Milli-Q water in the ratio 90: 10 (v/v) at a flow rate of 0.21 ml/min. The wave length was detected at 305nm by PDA detector. The drug was subjected to stress conditions. Further, it was undergone mild degradation by oxidation and acid hydrolysis. The drug shown stable against base, photolytic and hydrolytic degradation. The mass balance was found to be close to 99.1%. The esomeprazole magnesium and all seven potential impurities were achieved greater than 2.0 for all pair of impurities. The developed method was validated as per ICH guidelines in terms of accuracy, precision, robustness, specificity, linearity, LOD and LOQ.

The quantitative determination of fifteen related substances in ranolazine drug substance and drug product was studied through novel RP-UPLC method by Malati et.al.[9] The process was acquired by Waters acquity BEH RP18 100 mm, 2.1mm, 1.7 mm, column and the column temperature was maintained at 270^0C.

The mobile phase A includes monobasic sodium buffer, basic organic modifier and acetonitrile. The mobile phase B was used as diluent and the absorption was monitored at a wavelength of 223 nm. The injection volume was 1.0 ml. The %RSD value of ranolazine was assessed was less than 1.0. The results of method were also capable of separating a major oxidative degradant di-N-oxide. The method was validated as per ICH guidelines in all terms of specificity, precision, accuracy, robustness, linearity, LOD and LOQ.

Trivedi and Patel.[10] developed a stability indicating RP-UPLC method for the determination of related substances in a rosuvastatin calcium tablet dosage form. The chromatographic separation was carried by Acquity BEH C18 (100 mm × 2.1 mm, 1.7 µm) column. The mobile phase containing a gradient mixture of solvent A [0.1%trifluoroacetic acid] and solvent-B [methanol]. The eluted compounds were monitored at 240 nm and the run time was 10.0 min. Degradation behavior of the rosuvastatin was studied under various degradation stress conditions. Four major unknown degradation products were found in acid stress condition and two unknown degradation products were found in oxidative stress condition. The developed method separates 6 unknown impurities, 3 known impurities and rosuvastatin substance from each other, on condition that the stability indicating power of the method. The method was developed as per ICH guidelines involves specificity, accuracy robustness, linearity selectivity.

Reddy et.al.[11] was established by stability indicating RP-UPLC method for the simultaneous determination of ibuprofen and famotidine in pharmaceutical dosage forms. The separation was done by Acquity UPLC BEH C-18, 50 mm × 2.1 mm and 1.7µm column with gradient elution. The mobile phase A (50 mM sodium acetate buffer (pH 5.5): methanol (85:15, v/v) and mobile phase B (25:75, v/v) were used with a flowrate of 0.3 ml min^{-1} and the detection wavelength was 260 nm. The limit of detection was 1.6 and 1.2µg/ml^{-1} The limit of quantification was found to be 5.1 and 4.3µg ml^{-1}. The runtime was 4min/ml within which drugs and their impurities were eluted. This method was validated as per ICH guidelines includes accuracy, precision, specificity, robustness, and linearity.

Yanamandra et.al[12] has developed and validated of a RP-UPLC method for the simultaneous estimation of bambuterol hydrochloride and montelukast sodium from tablets. The separation succeeded by BEH C18 sub-2µm acquity column. The mobile phase includes 0.025% (v/v) trifluoro acetic acid in water and acetonitrile as organic solvent in a linear gradient program. Elution time was about 6.0min and UV detection was carried at 210nm. The resolution of both drugs found to be 31. The linearity of the both the drugs was in the range of 6.25-37.5µg/ml and the percentage recovery was found to be 99.1-100.0% and 98.0-101.6%. The method was validated as per ICH guidelines.

Seshukumar et al[13] reveals the validation of stability indicating RP-UPLC method for simultaneous determination in fixed dose combination of ezetimibe and simvastatin. The separation was carried out by Kromasil Eternity TM C 18 UHPLC column (2.5) µm, 2.1 mm x 50 mm). The mobile phase, gradient elution of acetonitrile and ammonium acetate buffer (pH 6.70; 0.01 M) was used at a flow rate of 0.35ml/min with column oven temperature was of 400^0C. The UV detection was carried out using a UV-PDA detector at 235 nm. The total run time was 3.5min within which compounds and degradants were separated. The method was estimated as per ICH guidelines. the method was validated for accuracy, precision, robustness, linearity, specificity.

Xavier and Basavaiah[14] conducted and evaluated the development and validation of pioglitazone hydrochloride by RP-UPLC applied to formulated forms. The chromatographic separation was achieved by the chromatographic column Acquity UPLC BEH C-18 (100× 2.1) mm and 1.7µm particle size. The mobile phase consist of 20:80 (acetonitrile: buffer) v/v (buffer-2.2 g. potassium dihydrogen orthophosphate in 1liter water and 1 ml triethylamine. Further, P^H was adjusted to 3.2 with diluted (o-phosphoric acid) with a flow rate of 0.20ml/min^{-1}. The column temperature was adjusted to 250^0C. The injection volume 2µl was used and monitored at 220nm. The retention time of sample was 2.1 min and the method was validated as per ICH guidelines.

Goel et.al[15] developed and validated stability indicating assay method by RP- UPLC for a fixed dosage combination of atorvastatin and ezetimibe. The separation was carried by kromasil Eternity C18 UHPLC column (2.5 µm, 2.1 × 50 mm). The gradient elution of acetonitrile and ammonium acetate buffer (pH 6.70; 0.01M) was used as a mobile phase with a flow rate of 0.2ml/min and followed with column oven temperature of 400^0C. The UV detection was performed at 245nm and the total run time of about 5min. Further, the method was validated as per ICH guidelines includes accuracy, repeatability, reproducibility and robustness. linearity, limit of detection and limit of quantitation

Kumar et.al.[16] has developed and validated stability indicating the RP-UPLC method for atorvastatin calcium. The separation was carried out by C18 column and using mobile phase, buffer 0.02 M phosphoric acid and acetonitrile. The drug was subjected to stress conditions in order to study the degradation conditions. The drug shown stable towards the oxidation, and thermal degradation. Further, the result of analysis showed mild degradation to acid-hydrolysis, base-hydrolysis and peroxide hydrolysis. The developed method was validated as per ICH guidelines with respect to linearity, precision, accuracy, robustness, specificity, LOD and LOQ.

Vaghela et.al.[17] performed the development and validation of stability indicating an RP-UPLC method for the estimation of process-related impurities and degradation products of fexofenadine HCl in the pharmaceutical formulation. In this technique, Waters aquity BEH C18 (100 mm x 2.1 mm) 1.7 µm was used as a column. The mobile phase [containing a gradient mixture of solvent A (0.05% triethylamine, pH adjusted to 7.0 with ortho-phosphoric acid) and B (10:90 v/v mixture of water and acetonitrile)] was used with a flow rate of 0.4ml/min with a column temperature of 300^0C and detection wavelength at 220nm. The drug was subjected to stress conditions includes oxidation, thermal degradation, acid-hydrolysis and base-hydrolysis. In addition, the drug was shown degrading significantly by oxidative stress conditions. This method was validated as per ICH guidelines with respect to linearity, specificity, robustness, accuracy, LOD and LOQ.

Trivedi and Patel.[18] reported the stability indicating the RP-UPLC method for the rapid determination of metaxalone and its degradation products in a solid oral dosage form. The separation was done with that of Acquity® HSS-T3 (100 mm x 2.1 mm, 1.7 µm) column and maintained oven temperature at 45°C. The mobile phase, consist of mixture of water, methanol, acetonitrile and triethylamine in the ratio of 50:25:25:0.1 % v/v (pH adjusted to 6.3 with orthophosphoric acid). The elution was observed at 230nm for meta assay and 205nm for related substances. The flow rate 0.3ml/min was used. The developed method separates from its unknown and known impurities within 6.0 min. The metaxalone was subjected to stress conditions of acid hydrolysis, photolytic degradation and thermal degradation. The drug shown slight degradation in oxidative and base hydrolysis and the method was validated as per ICH guidelines.

Rao et.al.[19] reported the UPLC method for the determination of a novel validated and stability-indicating method for the estimation of lansoprazole and its impurities in bulk drug and pharmaceutical dosage forms. Waters Acquity BEH C18 column was used. The gradient program of mobile phase A (pH 7.0 phosphate buffer and methanol in the ratio of 90: 10 (v/v)) and B as (methanol and acetonitrile in the ratio of 50:50 (v/v)) was used in the process. The lansoprazole and its impurities were monitored at 250 nm. The total runtime was 11.0 minutes and the drug was subjected to stress degradation study includes oxidative, acid base hydrolysis, thermal, humidity and photolytic degradation. The degraded peaks were well resolved and method was validated as per ICH guidelines.

Nageswari et.al.[20] reported the stability indicating the RP- UPLC method for the determination of imatinib mesylate and their degradation products in active pharmaceutical ingredient and pharmaceutical dosage form. The method was achieved by Acquity UPLC BEH C-18 column (50-mm, 2.1 mm and 1.7 m) at 30°C. The mobile phase A [0.05 M ammonium acetate (pH 9.5)] and B [mixture of acetonitrile and methanol in a ratio of 40:60 (v/v)] was used with a gradient program of 9.0min. The flow rate was about 0.3 ml/min and it was monitored at 237nm. The run time was 9 min enabled the quick determination of impurities and degradation products. The resolution of imatinib and eight related compounds were found greater than 1.5. The correlation coefficients was found to be $r^2 > 0.9990$ and indicated that clear correlations between the concentrations and their peak areas for the investigated compounds. RSD was found to be 5.0%. Further, the drug was subjected to stress degradation studies like acid-base hydrolysis, photolytic degradation, thermal degradation and oxidative. The result of the study was validated as per ICH guidelines.

Sethi et.al.[21] have been initiated the validation of a stability-indicating RP-UPLC method for the quantitative analysis of nabumetone in tablet dosage form. The chromatographic separation was achieved on a waters acquity BEH column (100 mm, i.d., 2.1 mm, 1.7 mm) within a short runtime of 2 min. The mobile phase 5 MM ammonium acetate–acetonitrile (25:75, v/v) was used at a flow rate of 0.3 mL/min at an ambient temperature. The detection was done at 230nm by PDA detector over the concentration gradient of 0.05–26 mg/ml. Forced degradation study was conducted to study stability indicating parameters. The reports of the study was validated as per ICH guidelines.

The UPLC method for related substances in dorzolamide hydrochloride and timolol maleate in ophthalmic dosage form was studied by Sharma et.al.[22] The chromatographic separation was carried by using waters UPLC BEH C18 column (100 × 2.1mm, 1.7 µm). The mobile phase consist of A [phosphate buffer (0.04M), pH 2.6] and B [Milli-Q water, methanol and acetonitrile in 200:300:600, v/v/v ratios]. The elution of the compound was monitored at 254nm. The timolol maleate and its impurities were monitored at 295nm. The runtime 3.0min was used and the resolution was found to be 2.0. The drugs were subjected to stress degradation studies such as oxidative, thermal degradation, photolytic degradation and acid- base hydrolysis. The result of the study was validated as per ICH guidelines.

Trivedi and Patel[23] developed and validated the stability indicating the RP-UPLC method for determination of quetiapine in a pharmaceutical dosage form. The method was separated by Agilent Eclipse Plus C18 column [RRHD 1.8 µm, 50 mm x 2.1 mm] by using gradient elution. The mobile phase, solvent-A [0.1% aqueous triethylamine (pH 7.2)] and solvent B [80:20 v/v mixture of acetonitrile and methanol] was used for the analysis. The eluted compounds were monitored at 252 nm by UV detector. The method separates the quetiapine and its five impurities within 5 min. Further, the drug was subjected to forced degradation study in order to initiate

the stability indicating parameters. The total runtime was 5.0 minutes to enable the complete determination of drug. The report of RP-UPLC method was validated as per ICH guidelines with respect to linearity, specificity, robustness and accuracy.

Ram et.al[24] developed and validated for stability indicating UPLC method for the determination of ticlopidine hydrochloride in its tablet formulation. The separation was achieved by Zorbax SB-C18 column (50 mm, 4.6 mm, 1.81m). The mobile phase [methanol: 0.01M ammonium acetate buffer, pH 5.0 (80:20, v/v)] was used at a flow rate of 0.8 ml/ min. The injection volume 4.0 µl was used and it was monitored at 235nm using PDA detector. The drug was subjected to stress degradation studies involves oxidation, acid-base hydrolysis, thermal degradation and photolysis. The report of method was linear in the range of 62.5–375 lg/ml with the correlation coefficient of 0.9999. The precision (relative standard deviation – RSD) of six samples was found to be 1.31% for repeatability. Further, the intermediate precision among six-sample preparation was found to be 0.77% and accuracy lies between 98.80% and 101.50%. The report of method was validated as per ICH guidelines.

Karunakaran et.al.[25] explains the new RP-UPLC method for the determination of rabeprazole sodium in pharmaceutical formulation and application in dissolution studies. The separation was done by Waters Acquity BEH C18 (50 x 2.1 mm, particle size 1.7 µm) column using an isocratic method. The mobile phase containing acetonitrile and phosphate buffer (pH 7.4) in the ratio of 35:65 (v/v) and the flow rate 0.4ml/min was used. Further, the temperature was maintained at ambient and injection volume was 5µl. The detection was done at 280nm and runtime was 2 min. The linear range was found to be 0.03 - 30 µg/ml and the linear regression shown > 0.999. The RSD value was within 1.5 % and recovery data was in the range 98.0 - 101.5 %. In addition, the short retention time was found to be 1.49 min and the method was validated with respect to precision, robustness, linearity, specificity and accuracy.

Mohan et.al.[26] developed and validated of an RP-UPLC method for the assay of lacidipine and related substances. The separation was done by Acquity BEH C18 column with mobile phase (pH4.5 ammonium acetate–acetic acid buffer–methanol (70 + 30, v/v) and monitored at 240 nm. The flow rate 0.25ml/min was applied and the linearity of the drug was found to be r^2 > 0.999. The result of method was validated with various parameters such as specificity, accuracy, linearity, robustness, precision, LOD and LOQ.

The simultaneous determination of desloratadine and sodium benzoate in oral liquid pharmaceutical formulations by RP-UPLC was studied by Kumar et.al.[27] The method was separated by Acquity BEH C8 (100 mm x 2.1 mm) 1.7µm column. The mobile phase containing a gradient mixture of solvent A (0.05 M KH_2PO_4 and 0.07 M triethylamine, pH 3.0) and solvent B (50:25:25 v/v/v mixture of acetonitrile, methanol and water). The flow rate was 0.4 ml/min with a column temperature of 40°C and monitored at 272nm. The linearity ranges were formed between 0.254 µg/ml - 76.194 µg/ml for desloratadine and 1.006 µg/ml to 301.67 µg/ml for the sodium benzoate. The correlation coefficient for both drugs was more than 0.999. The method was subjected to stress degradation studies and the developed method was validated as per ICH guidelines.

Rajput et.al.[28] developed and validated of a rapid RP-UPLC method for the determination of aspirin and dipyridamole in combined capsule formulation. The chromatographic separation was carried out using a Hypersil Gold C18, column (1.9µm, 100 mm X 2.1 mm). The mobile phase composed of triethylamine phosphate buffer (pH 2.5) and methanol in the ratio 50:50% (V/V) with a flow rate of 0.5 ml/min. The wavelength was monitored at 230nm by using UV detector. The retention time for drugs was formed at 0.83 and 1.62 minute. The coefficient correlation was found to be 0.9999 and the concentration values of both drugs linearity ranges were formed between 2.509-50.190 µg/ml and 20.093- 401.860 µg/ml, respectively. The RSD values were in the range of 99.47-101.08%. Hence, the method was validated in respect of analytical parameters like precision, ruggedness, accuracy, specificity, linearity, resolution, LOD and LOQ as per ICH guidelines.

The simultaneous determination of ambroxol hydrochloride, cetirizine hydrochloride and antimicrobial preservatives in liquid formulations includes the stability indicating an RP-UPLC method was done by Trivedi RK et.al.[29]. The chromatographic separation was achieved by Agilent Eclipse plus C18 column [1.8 µm (50 x 2.1 mm] and it was eluted at 237nm.The mobile phase [A consists of a mixture of 0.01 M phosphate buffer,0.1 % triethylamine and B consist of acetonitrile] was used with a flow rate of 0.5 ml/min. The runtime was 3.5 minutes to enables for rapid determination of drugs and preservatives. The method was subjected to forced degradation study which involves separation of known and unknown impurities. The report of method was developed and validated as per ICH guidelines.

Krishnaiaha, et.al.[30] estimated and reported the stability indicating UPLC method for the determination of olanzapine and degradation products present in active pharmaceutical ingredients and pharmaceutical dosage forms. The separation was achieved by Acquity UPLC BEH (100-mm, 2.1-mm, and 1.7-µm) C-18 columns. The mobile phase, A (20mM NaH_2PO_4, H_2O and 2.0ml of triethylamine and B [buffer (pH of 6.8), acetonitrile and methanol; 50:20:30 (v/v/v)] was used. The gradient eluent time was 10.0 min and it was monitored at

250nm. The flow rate was 0.3ml/min and the column temperature was maintained at 27^0C. The resolution of drug and eight impurities were found to be 2.0. The coefficient correlation was shown $r^2 > 0.9991$. The repeatability and intermediate precision were expressed by the RSD and the values were found to be less than 2.4%. The retention time was 10.0 min to enabled rapid determination of the drug and the method was validated as per ICH guidelines with respect to specificity, linearity, robustness, accuracy, LOD, and LOQ.

Pawan et.al.[31] has developed a rapid and sensitive RP-UPLC method for the simultaneous determination of zidovudine, lamivudine, nevirapine in tablet dosage form. The separation was carried out by waters C18 column consisting high strength silica (ACQUITY UPLC HSS-T3), 100 × 2.1 mm, 1.8 mm particle size. The solvent system, 0.1%, v/v trifluoroacetic acid in water and acetonitrile was used as a.

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

S. No	Drug Name	Uses	Mobile phase	Stationary phase	Parameters	REFERENCES
1	Mycophenolate	Immuno suppressant	Mixture (potassium di-hydrogen phosphate: acetonitrile in the ratio 35:65 v/v)	Symmetry C18 column (4.6x100mm, 3.5µm, Make: XBridge)	Linearity, accuracy, precision, specificity, robustness, LOD and LOQ	[4]
2	Darifenacin hydrobromide	M3 muscarinc acetylcholine receptor	Mixture A (buffer–ACN; 80:20, v/v) and B (buffer–ACN; 15:85, v/v)	UPLC BEH C18 column (100, 2.1 mm and 1.7µm)	Linearity, accuracy, precision, specificity robustness, LOD and LOQ	[5]
3	Metoclopramide	Dopamine D_2 antagonist and antiemetic	Mixture A [30 mm monobasic sodium phosphate and 2.3mM of pentane-1- sulphonic acid sodium salt (pH 3.0 buffer)] and B (acetonitrile)]	X-terra RP18 (150 × 4.6 mm), 3.5µm particle size column	Linearity, accuracy, precision, specificity robustness, LOD and LOQ.	[6]
4	Fluconazole	Antifungal agent	Methanol:water (55: 45 v/v)	BEH C18 (100 × 2.1 mm, 1.7 µm particle size) column	Linearity, accuracy, precision, specificity, robustness, LOD and LOQ	[7]
5	Esomeprazole magnesium	Proton pump inhibitor	Mixture A [0.04 M) glycine, pH 9.0, buffer] and B [acetonitrile and milli-Q water in the ratio 90: 10 (*v/v*)]	C18, 50 mm × 2.1 mm, 1.7 µm	Linearity, accuracy, precision, specificity robustness, LOD and LOQ	[8]
6	Ranolazine	Anti anginal medication	Mixture A [acetonitrile sodium dihydrogen phosphate (pH 7.3; triethylamine 10:90: 0.1,v/v/v) and B [acetonitrile] (55:45, v/v)	Acquity UPLC BEHRP-18 100mm,2.1mm,and1.7 mm particles	Linearity, accuracy, precision, specificity robustness, LOD and LOQ	[9]
7	Rosuvastation	Antilipidemic agent	Mixture of solvent A (0.1% trifluoroacetic acid) and solvent B (methanol)	Acquity BEH C18 (100 mm × 2.1 mm, 1.7 µm) column	Linearity, accuracy, precision, specificity robustness, LOD and LOQ	[10]
8	Ibuprofen and famotidine	Nonsteroidal anti-inflammatory agent and H_2-	Mixture A [50 mM sodium acetate buffer (pH 5.5): methanol	UPLC BEH C-18,50 mm × 2.1 mm and 1.7 µm column	Linearity, accuracy, precision,	[11]

		receptor antagonist	(85:15, v/v) and B [50 mM sodium acetate buffer (pH 5.5): methanol (25:75, v/v)]		specificity robustness, LOD and LOQ	
9	Bambuterol hydrochloride and Montelukast sodium	Beta adrenoceptor agonist, used in the treatment of asthma and leukotriene receptor antagonist (LTRA)	Trifluoro acetic acid in water and acetonitrile as organic solvent in gradient elution of acetonitrile and ammonium acetate buffer (pH 6.70; 0.01M)	BEH C18 sub-2µm Acquity UPLC column using 0.025% (v/v)	Linearity, accuracy, precision, specificity and robustness, LOD and LOQ	[12]
10	Ezetimibe & Simvastatin	Lipid- lowering compound	Mixture of acetonitrile:buffer (20:80)	Kromasil Eternity TM C 18 UHPLC column (2.5 µm, 2.1 mm x 50 mm)	Linearity, accuracy, precision, specificity, and robustness, LOD and LOQ	[13]
11	Pioglitazone Hydrochloride HCL	Antihypertensive effect	Ammonium acetate buffer (pH 6.70; 0.01M)	Acquity UPLC BEH C-18 (100×2.1)mm and 1.7 µm particle size	Linearity, accuracy, precision, specificity, and robustness, LOD and LOQ	[14]
12	Atrovastatin and Ezemitide	Lipid-lowering compound	Buffer 0.02 M phosphoric acid and acetonitrile.	C18 UHPLC column (2.5 mm, 2.1 3 50 mm)	Linearity, accuracy, precision, specificity, and robustness, LOD and LOQ	[15]
13	Atrovastatin Calcium	Used in the treatment of hayfever and similar allergy symptoms	Mobile phase contains A (0.05% triethyl amine, pH adjusted to 7.0 with ortho-phosphoric acid) and B (10:90 v/v mixture of water and acetonitrile	C18 column	Linearity, accuracy, precision, specificity, and robustness, LOD and LOQ	[16]
14	Fexofenadine	Muscle relaxant	Mixture of water, methanol, acetonitrile and triethylamine in the ratio of 50:25:25:0.1 % v/v (pH adjusted to 6.3 with orthophosphoric acid)	Aquity BEH C18 (100 mm x 2.1 mm) 1.7 µm column	Linearity, accuracy, precision, specificity, robustness. LOD and LOQ	[17]
15	Metaxalone	antihistamine drug	Mixture contains A (pH 7.0 phosphate buffer and methanol in the ratio of 90: 10 (v/v), and B [methanol and acetonitrile in the ratio of 50:50 (v/v)	Acquity® HSS-T3 (100 mm x 2.1 mm, 1.7 µm) column.	Linearity, accuracy, precision, specificity, and robustness, LOD and LOQ	[18]
16	Lansoprazole	Proton pump inhibitor	Mixture contains A (0.05M ammonium acetate (pH 9.5) and B (acetonitrile and methanol, 40:60 (v/v)	Waters Acquity BEH C18 column and gradient program	Linearity, accuracy, precision, specificity, and robustness, LOD and LOQ	[19]
17	Imatinab mesylate	A small molecule kinase inhibitor	Mixture composed of 5 mm ammonium	Acquity UPLC BEH 50-mm,2.1 mm	Linearity, accuracy,	[20]

		used to treat certain types of cancer	acetate– acetonitrile (25:75, v/v)	and 1.7 m C-18 column	precision, specificity, and robustness, LOD and LOQ	
18	Nabumetone	Nonsteroidal antiinflammatory drug (NSAID)	Mixture A [phosphate buffer (0.04M), pH 2.6 buffer and B (Milli-Q water, methanol and acetonitrile in 200:300:600, v/v/v)]	Acquity BEH column	Linearity, accuracy, precision, specificity, and robustness, LOD and LOQ	[21]
19	Dorzolamine Hydrochloride and Trimolol maleate	Carbonic anhydrase (CA) inhibitor, Beta-adrenergic antagonist and used to treat migraine disorders and tremor	Mixture A [10 mM ammonium Acetate with addition of 1ml triethyl amine, pH adjusted to 3.9 with ortho phosphoric acid and acetonitrile in the ratio 90:10 (v/v) and B [acetonitrile]	Waters UPLC BEH C18, column 100 3 2.1mm,1.7 mm	Linearity, accuracy, precision, specificity robustness, LOD and LOQ	[22]
20	Quetiapine	Antipsychotic drug and has an antagonistic effect on the histamine H1.	Mixture [methanol - 0.01M ammonium acetate buffer, pH 5.0 (80:20, v/v)]	BEH C18 (100 mm x 2.1 mm, 1.7 µ) column	Linearity, accuracy, precision, specificity robustness, LOD and LOQ	[23]
21	Ticlopidine hydrochloride	An effective inhibitor of platelet aggregation.	Mixture [acetonitrile and phosphate buffer (pH 7.4) in the ratio of 35:65 (v/v)]	Zorbax SB-C18 (50 mm, 4.6 mm, 1.8l m) column	Linearity, accuracy, precision, specificity robustness, LOD and LOQ	[24]
22	Rabeprazole sodium	Antiulcer drug in the class of proton pump inhibitors	Mixture [pH 4.5 ammonium acetate–acetic acid buffer–methanol (70 + 30, v/v)	Waters Acquity BEH C18 (50 x 2.1 mm, particle size 1.7 µm) column	Linearity, accuracy, precision, specificity robustness, LOD and LOQ.	[25]
23	Lacidipine	Lipophilic dihydropyridine calcium antagonist	Mixture A [(0.05 M KH_2PO_4 and 0.07 M triethylamine, pH 3.0)] and B [50:25:25 v/v/v mixture of acetonitrile, methanol and water]	Acquity BEH C18 column	Linearity, accuracy, precision, specificity, robustness, LOD and LOQ	[26]
24	Desloratadine and sodium benzoate	Antihistamine and used as a food preservative	Mixture [triethylamine phosphate buffer (pH 2.5) and methanol in the ratio 50:50% (v/v)]	Acquity BEH C8 (100 mm x 2.1 mm) 1.7 µm column	Linearity, accuracy, precision, specificity robustness, LOD and LOQ	[27]
25	Aspirin and Dipyridamole	Analgesic, antiplatelet aggregate and phosphodiesterase inhibitor	Mixture A [0.01M phosphate buffer and 0.1% triethylamine] and B [acetonitrile]	Hypersil Gold C18, column (1.9µm, 100 mm X 2.1 mm)	Linearity, accuracy, precision, specificity robustness, LOD and LOQ.	[28]
26	Ambroxol hydrochloride and cetirizine hydrochloride	Secretolytic agent, used in the treatment of respiratory diseases and	Mixture [20 mM NaH_2PO_4, H_2O and 2.0 ml of Triethylamine buffer (pH of 6.8], acetonitrile and	Agilent Eclipse plus C18, 1.8 µm (50 x 2.1 mm) column	Linearity, accuracy, precision, specificity, robustness,	[29]

		potent second-generation histamine H1 antagonist	methanol, 50:20:30 (v/v/v)		LOD and LOQ	
27	Olanzapine	Antipsychotic	Mixture A [20mM NaH_2PO_4,H_2O and 2.0ml of triethylamine (TEA) buffer (pH of 6.8), acetonitrile, and methanol in a ratio of 50:20:30 (V/V/V)] and B [water and acetonitrile in a ratio of 10:90 (v/v)	Acquity UPLC BEH 100-mm, 2.1-mm, and 1.7-µmC-18 columns	Linearity, accuracy, precision, specificity robustness, LOD and LOQ.	[30]
28	Zidovudine, lamivudine and nevirapine	Potent inhibitor of HIV replication, reverse transcriptase inhibitor and potent non-nucleoside reverse transcriptase inhibitor	Mixture [0.1%v/v trifluro acetic acid in water and acetonitrile]	Waters C18 column, (ACQUITY UPLC HSS-T3), 100 × 2.1 mm, 1.8 mm particle size	Linearity, accuracy, precision, specificity robustness, LOD and LOQ	[31]
29	Sparfloxacin	Antibiotic	Mixture of A (0.1% aqueous OPA) and B (acetonitrile)	Waters Acquity HSS T-3 column (100 × 2.1 mm, 1.8 µm)	Linearity, accuracy, precision, specificity robustness, LOD and LOQ.	[32]
30	Desloratadine	Antihistamine	Mixture A [0.01M potassium dihydrogen ortho phosphate buffer, pH adjusted to 2.5 with ortho phosphoric acid was used as buffer. buffer, methanol and acetonitrile in the ratio 80:15:5, v/v] and B [buffer, tetrahydrofuran and acetonitrile in the ratio buffer 30:5:70, v/v/v]	Waters Aquity BEH C18 column	Linearity, accuracy, precision, specificity robustness, LOD and LOQ	[33]

Mobile phase. In this analysis, the column temperature was maintained at 50^0C with a flow rate of 0.4ml/min and monitored at 266nm by PDA detector. The linear ranges were found to be 100-500, 50-250 and 100-180 mg/mL for zidovudine, lamivudine and nevirapine, respectively. The limit of detection was found to be 0.279, 0.360 and 0.205 mg/ml for zidovudine, lamivudine and nevirapine, respectively. Similarly, limit of quantification was found to be, 0.068, 0.846 and 1.091 mg/ml for zidovudine, lamivudine and nevirapine, respectively. The method was validated as per ICH guidelines.

Himanshu et.al.[32] studied and evaluated the validation and stability indicating an RP-UPLC method for the quantitative analysis of sparfloxacin. The chromatographic separation was done by Waters Acquity HSS T-3 column (100 × 2.1 mm, 1.8 µm). The mobile phase A (mixture of 0.1% aqueous OPA on a timed gradient program: T (min)/% and B(1/10, 2/10, 3/25,4/10, and 5/10) was used with the flow rate of 0.5 ml/min and monitored at 290nm. The drug was subjected to stress degradation study and the method was validated as per ICH guidelines.

Rao et.al.[33] developed and validated stability indicating an RP-UPLC method for desloratadine and its impurities in pharmaceutical dosage forms. The chromatographic separation was carried out by using Waters Aquity BEH C18 column. The mobile phase was used as gradient mixture of solvent A (0.01M potassium dihydrogen orthophosphate buffer, pH adjusted to 2.5 with

orthophosphoric acid was used as buffer. Buffer, methanol and acetonitrile in the ratio 80:15:5, v/v/v; was used) and solvent B (buffer, tetrahydrofuran and acetonitrile in the ratio 30:5:70, v/v/v).The gradient program (T/%B) was set as 0/0, 1.5/0, 5.5/80, 6.5/80, 7.0/0 and 8.0/0. The total run time was 8 mins and it was monitored at 280 nm. The drug was subjected to stress degradation studies, it involves acid-base hydrolysis, photolytic degradation, thermal degradation and oxidation. The drug was validated as per ICH guidelines in terms of precision, accuracy, linearity, robustness, specificity, LOD and LOQ.

CONCLUSION

It can be concluded that some important mobile phases like potassium di hydrogen, acetonitrile, sodium phosphate, methanol, sodium di hydrogen, trifluoro acetic acid and ammonium acetate buffer were commonly used for method development and validation of drugs by RP-UPLC method. In stationary phase, C18 column was efficient for the separation when comparison to that of other columns. The developed method by RP-UPLC was validated per the ICH guidelines and found to be specific, precise, accurate and linear. Hence, it was clearly indicated that UPLC method is possible to develop a new sensitive and accurate for different pharmaceutical formulations. It was fulfilled that the UPLC method was simple, precise & robust and can be useful for determination of purity of many drugs in API.

ACKNOWLEDGEMENT

The authors are thankful to the management of GITAM University, Visakhapatnam, Andhra Pradesh, India, for providing necessary facilities to carry out the research work.

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