Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 7, Issue 4, 2015

Original Article

DEVELOPMENT AND VALIDATION OF A DISSOLUTION METHOD FOR FROVATRIPTAN TABLETS BY REVERSED PHASE UPLC

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Received: 08 Dec 2014 Revised and Accepted: 01 Jan 2015

ABSTRACT

Objective: The main objective of the method was to develop a simple, rapid, efficient and reproducible, stability indicating reverse phase ultra performance liquid chromatography (RP-UPLC) method for the estimation of frovatriptan in tablet dosage form.

Methods: The RP-UPLC method for estimation of frovatriptan (FRT) in their tablets was carried out on Acquity UPLC™, BEH C-18 (100 × 2.1 mm, 1.7 µm) column using 0.1% trifluroacetic acid buffer and a mixture of methanol and acetonitrile (50:50) using isocratic program. The flow rate of the mobile phase was 0.2 mL $min^{-1}$and detection wavelength was carried out at 244 nm. Total runtime is 3 minutes for chromatographic run. The method was validated in terms of specificity, linearity, accuracy, precision and robustness as per ICH guidelines.

Results: The method was found to be linear in the range of 1.41-3.67 µg mL^{-1}. Recovery was found to be in the range of 97.8-101.8%. Relative standard deviation for precision and intermediate precision was found to be less than 3%. The developed method was successfully applied for the estimation of frovatriptan in tablet formulation and average dissolution rate was found to be 93%. The results obtained from the validation experiments prove that the developed method is suitable for routine analysis.

Conclusion: The developed RP-UPLC method was simple, rapid, accurate, and precise for the estimation of dissolution rate in frovatriptan tablet dosage form.

Keywords: Frovatriptan (FRT) tablets, RP-UPLC method, Development and validation.

INTRODUCTION

Frovatriptan succinate is a white to off-white powder and soluble in water. Frovatriptan tablets contain frovatriptan succinate, a selective 5-hydroxytryptamine (5-HT_{1B}/5-HT_{1D}) receptor subtype agonist, as the active ingredient. Frovatriptan succinate is chemically designated as (+)-R-3-methylamino-6-carboxamido-1,2,3,4-tetrahydrocarbazole monosuccinate monohydrate. Frovatriptan succinate structure is shown in fig. 1. The empirical formula is $C_{14}H_{17}N_3O$. $C_4H_6O_4$. H_2O, with a molecular weight of 379.4 units. Each frovatriptan tablet for oral administration contains 3.91 mg frovatriptan succinate which is equivalent to 2.5 mg of frovatriptan base.

Frovatriptan is a 5-HT receptor agonist that binds with high affinity for 5-HT_{1B} and 5-HT_{1D} receptors. Frovatriptan has no significant effects on $GABA_A$ (γ-Aminobutyric acid) mediated channel activity and has no significant affinity for benzodiazepine binding sites. Frovatriptan is believed to act on extra cerebral, intracranial arteries and to inhibit excessive dilation of these vessels in migraine. In anaesthetized dogs and cats, intravenous administration of frovatriptan produced selective constriction of the carotid vascular bed and had no effect on blood pressure (both species) or coronary resistance (in dogs). Mean maximum blood concentrations (C_{max}) in patients are achieved approximately 2–4 hours after administration of a single oral doses of frovatriptan 2.5 mg. The absolute bioavailability of an oral dose of frovatriptan 2.5 mg in healthy subjects is about 20% in males and 30% in females. Frovatriptan is indicated for the acute treatment of migraine attacks with or without aura in adults. Frovatriptan is not intended for the prophylactic therapy of migraine or for use in the management of hemiplegic or basilar migraine. It is in the class of drugs called selective serotonin receptor agonists.

From the literature survey, it is evident that few methods were available for the estimation of frovatriptan in tablet dosage forms by using either UV-Visible spectroscopy method or by high performance liquid chromatography (HPLC) method. None of the methods was developed by ultra performance liquid chromatography (UPLC) for the estimation of frovatriptan in tablet dosage forms. UPLC is specially designed to withstand higher system pressures during chromatographic analysis so that it enables a significant decrease in separation time and solvent consumption. The UPLC columns packed with 1.7 µm sized particles provides not only increased efficiency but also the ability to work at an increased linear velocity without loss of efficiency, providing both resolution and speed. Using advantages of UPLC, a number of applications in different fields, including pharmacy [1-3] clinical analysis, pesticide analysis [4] and tetracyclines in human urine [5] have been reported.

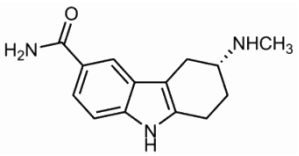

Fig. 1: Structure of frovatriptan

Stability indicating assay method for the frovatriptan succinate monohydrate was developed by Hitesh Verma et al., by UV spectrophotometric technique [6]. Raju et al., developed a method for estimation of frovatriptan succinate in tablet dosage forms by RP-HPLC [7]. Usha Rani et al., developed RP-HPLC and spectrophotometry method for the analysis of frovatriptan in formulations [8]. Kumara Swamy et al., developed a method for development and validation of RP-HPLC method for the estimation of frovatriptan succinate in bulk and tablet dosage forms [9]. As per the literature review, no method was published for the estimation of frovatriptan in finished dosage forms by using UPLC and in addition to that, no pharmacopoeial methods are available for the estimation of the frovatriptan dissolution rate in tablet dosage forms.

The present work describes a specific and stability-indicating UPLC method for the determination of frovatriptan in frovatriptan tablets.

The developed UPLC method was validated with respect to specificity, linearity, precision, accuracy and robustness as per ICH guidelines.

MATERIALS AND METHODS

Chemicals and reagents

Frovatriptan was obtained as a gift sample from Glenmark Pharmaceuticals Ltd (India). HPLC grade methanol (MeOH) and acetonitrile (ACN) were obtained from Rankem (Mumbai, India). Potassium dihydrogen orthophosphate (KH_2PO_4), trifluoroacetic acid (TFA) and sodium hydroxide (NaOH) were purchased from Merck specialties Pvt. Ltd (Worli, Mumbai).

Instrumentation

The Waters UPLC system equipped with a diode array detector was used for method development and validation studies. The output signal was monitored and processed using Empower software. Dissolution test was carried out using Electrolab dissolution apparatus, system (EDT-08Lx).

Chromatographic conditions

The chromatographic separation was achieved on Acquity UPLC™, BEH C-18 (100 × 2.1 mm, 1.7 µm) column using premix of mobile phase-A composed of 0.1% trifluoroacetic acid buffer filtered through 0.2 µm nylon filter and mobile phase-B was 50:50 of ACN and MeOH. An isocratic program used for chromatographic separation was a premix of degassed mobile phase-A and mobile phase-B in the ratio of 80:20. Flow rate was set to 0.2 mL min^{-1} with a column temperature of 30°C and detection wavelength was carried out at 244 nm. The injection volume was 1 µl (Partial loop with needle overfill). Water and methanol in the ratio of 20:80 filtered through 0.2 µm nylon filter used as a strong needle wash. Water and methanol in the ratio of 90:10 filtered through 0.2 µm nylon filter used as a weak needle wash and seal wash.

Preparation of dissolution media:

Accurately weighed and transferred 68.0 g of KH_2PO_4 and dissolved in 10 L of demineralized water and mixed well. The pH of the dissolution medium adjusted to 5.5±0.05 with 0.2 M sodium hydroxide solution.

Dissolution parameters

Medium: pH 5.5 Phosphate buffer

Volume: 900 ml

Apparatus: USP Type-II (Paddle)

RPM: 50

Temperature: 37°C ± 0.5°C

Time: 30 minutes.

Preparation of standard and sample solutions

Standard solution of frovatriptan

Accurately weighed and transferred 28 mg of frovatriptan succinate working standard into a 100 mL clean dry volumetric flask. To this added 70 mL of dissolution medium, sonicated for 5 minutes and make up to the final volume with the dissolution medium. A 5 mL of this stock solution was transferred into a 50 mL volumetric flask and made up to 50 mL with dissolution medium. Further 5 mL of the above standard solution was taken into a 50 mL volumetric flask and made up to 50 mL with the dissolution medium. Standard solution was filtered through a 0.2 µm nylon filter by discarding the first 5 mL filtrate.

Preparation of sample solution

One tablet was dropped into each of the six dissolution vessels containing preheated dissolution media. 10 mL of the aliquot was withdrawn from each dissolution vessel at the specified time interval and filtered through 0.2 µm nylon filter after discarding the first 5 mL of filtrate.

System suitability test

The system suitability test is an integral part of chromatographic methods and used to verify that the reproducibility of the chromatographic system is adequate for the analysis to be performed. Retention time (R_t), the number of theoretical plates (N), peak tailing factor (T) and %RSD of five injections were evaluated for frovatriptan standard at working concentration. The sample peaks from the chromatograms were identified by comparing the retention time with the standard peaks.

Method validation

Specificity

The specificity of the method was established to prove the absence of interference from placebo (excipients) which take part in the pharmaceutical preparation. Specificity was performed by adding placebo powder equivalent to 1 tablet dosage unit into each dissolution vessel containing preheated dissolution media and analyzing the placebo sample as per the analytical method to evaluate the placebo interference at the retention time of an active drug.

Linearity

Linearity is the method's ability to obtain results which are either directly, or after mathematical transformation proportional to the concentration of the analyte within a given range. The linearity of response for frovatriptan was determined in the range from 50% to 130% of the working concentration. A graph was plotted between the peak areas versus concentration to obtain the calibration curve. The five concentrations of active drug component were subjected to regression analysis by least-squares method to calculate correlation co-efficient and calibration equation. The method of linear regression was used for the data evaluation. Peak areas of standard compound were plotted against the respective concentrations.

Precision

Precision is a measure of the reproducibility of the whole analytical method under normal operating circumstances. The precision was expressed as the relative standard deviation (RSD).

% RSD = (Standard deviation/ average) x 100

The precision of the developed method was determined by preparing the test solution at 100% concentration level for six tablet dosage units and calculated the % RSD for frovatriptan drug release.

Accuracy

Accuracy was determined by applying the method to samples in which known amounts of analyte have been added. These should be analyzed against standard solutions to ensure that the sample solution accuracy results are comparable. The accuracy was calculated from the test results as a percentage of the analyte recovered by the assay. Accuracy of the present method was carried out by injecting sample solutions at three different concentration levels of 70%, 100% and 130% in triplicate preparations. The % recovery was calculated from the main active drug component. The mean of percentage recovery was designed.

Robustness

Robustness of the method indicates the reliability of an analysis with respect to deliberate variations in method parameters. It was performed by injecting the 100% test solution by changing several parameters including a different batch of the same column, mobile phase composition, and flow rate and column temperature.

Solution stability

The solutions of sample and standard containing frovatriptan were prepared as per the test procedure. All these solutions were kept at room temperature (25°C). The freshly prepared solutions and the solutions which were stored at room temperature up to 24 hours were injected at different time intervals. The % difference in different time interval against the freshly prepared solutions was compared.

RESULTS AND DISCUSSION

Optimization of chromatographic conditions

The main purpose of the current chromatographic method was to develop a fast liquid chromatography method for the estimation and quantification of frovatriptan in frovatriptan tablets at a level of %100 test concentrations. From the structure of frovatriptan, it was observed that frovatriptan has a pKa value of 9.93. In reversed-phase liquid chromatographic separations, pH of selected buffer should have the pH±1.5 units of the pKa values of the analytes [10]. The selection of buffer with proper pH leads to ionization of analytes which consequences the sharp and symmetric peak shapes and reproducible retention times (R_t). Silica dissolves slowly and results in the inconsistent retention times and results due to increase in pH of the mobile phase, hence the volatile buffer at lower pH was selected for mobile phase preparation [10]. Mobile phase consisting of 50 mM ammonium acetate and a mixture of ACN: MeOH (50:50) in the ratio of 85:15 was used for initial method development trials. Different mobile phase compositions with flow rate changes were performed with ammonium acetate buffer, however, more peak tailing was observed in all the trials performed. 0.1% trifluoroacetic acid buffer and mixture of ACN: MeOH (50:50) as solvent was selected for final mobile phase composition based on repeatability and reproducibility of analytical results.

Different trials for the estimation of frovatriptan were carried on different HPLC columns. The screening studies were performed on variety of columns to cover a wide range of stationary phase properties including carbon chain length, carbon loading, surface area, temperature and pH range. Each of these selected columns was screened with different mobile phase ratios, different type of organic solvents including MeOH and ACN. Acquity UPLC™, BEH C-18 (100 × 2.1 mm, 1.7 µm) column was selected for the final method after several method development trials.

Method validation

The described UPLC method has been extensively validated for estimation of dissolution rate as per ICH guidelines [11]. Method validation [12-20] was performed after completion of method development [21-23] to ensure that the developed method was capable of giving reproducible and reliable results when used by different operators employed in the same equipment of the same lab or of different laboratories. The developed UPLC method was validated for estimation of drug content of frovatriptan in their tablet dosage forms by determining the parameters including specificity, linearity, accuracy, precision and robustness according to the ICH guidelines [11].

Specificity

The specificity of the developed method was performed by injecting a blank solution, placebo solution and test solutions. The specificity study was carried out using the samples which include tablet powder containing frovatriptan and placebo powder without active drug frovatriptan.

No interference was observed either from blank solution or from the placebo solution at the retention time of frovatriptan. Blank and placebo chromatograms are shown in fig. 2 and fig. 3. Standard and sample chromatograms of frovatriptan are shown in fig. 4 and fig. 5.

Linearity

The response obtained for frovatriptan was found to be linear from 50 to 130% of standard concentration. Correlation co-efficient for frovatriptan compound was not less than 0.99. Linearity statistical values of frovatriptan compound were shown in table 1 and the linearity plot is shown in Fig.6. The results demonstrate that an excellent correlation between the peak areas against their respective concentrations of frovatriptan shows that the response of the method is linear.

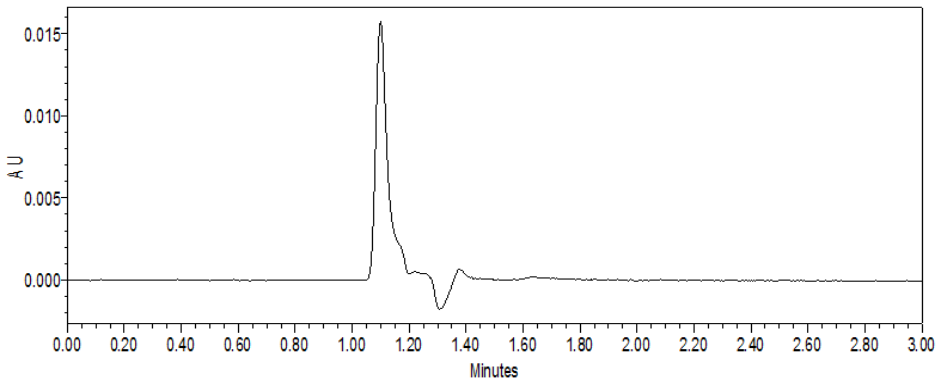

Fig. 2: Chromatogram of blank

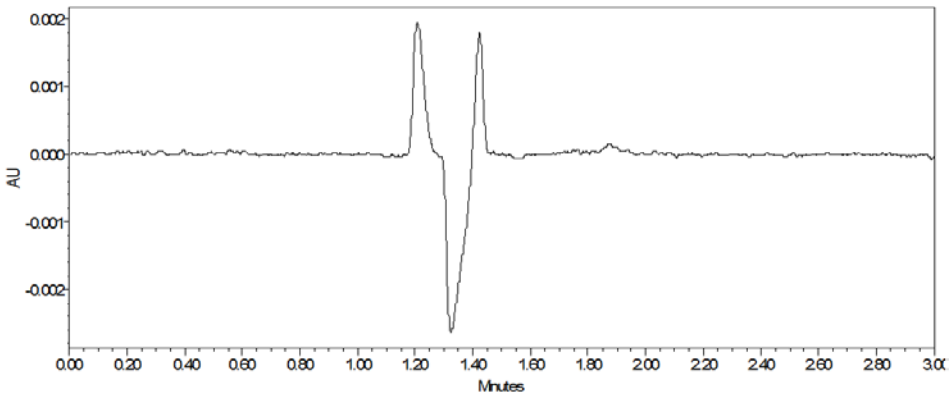

Fig. 3: Chromatogram of placebo solution

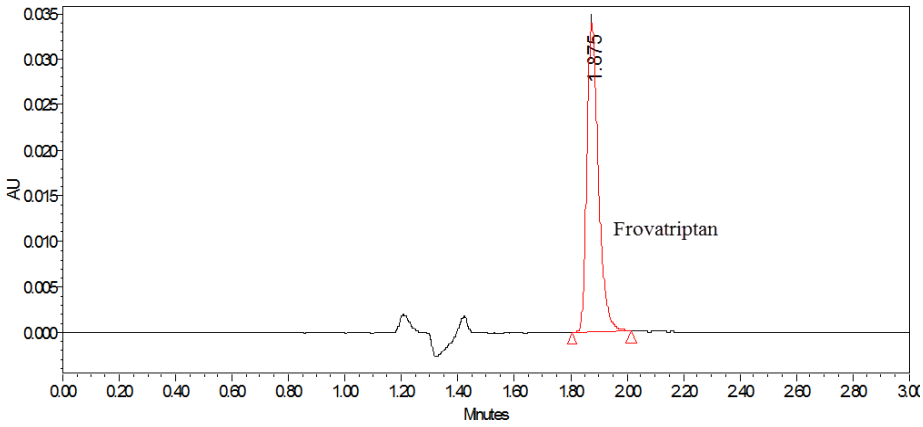

Fig. 4: Chromatogram of FRT standard solution

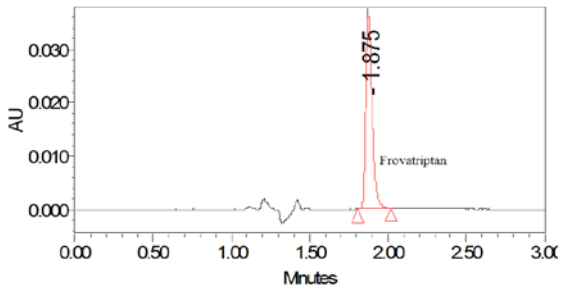

Fig. 5: Chromatogram of FRT sample solution

Precision

System precision was determined by injecting the standard solution for five injections and the observed value was reported in table 4. The % RSD for the area of frovatriptan standard solution for five injections was not more than 0.5%. Method precision (Intra-day precision) and intermediate precision (Inter-day precision) of the method are studied by injecting the frovatriptan sample solution of six test solution preparations performed at the100% level and the values were shown in table 2. Difference in results between two analysts found to be less indicating that the method is precise and has good intermediate precision.

Table 1: Linearity study results of frovatriptan

Regression statistics parameter	Frovatriptan
Correlation coefficient (R)	0.9997
Coefficient of determination (R^2)	0.9994
Slope of regression line	34931
Intercept	-892.27
Residual sum of squares	2216479.82
Residual standard deviation (RSD)	859.55

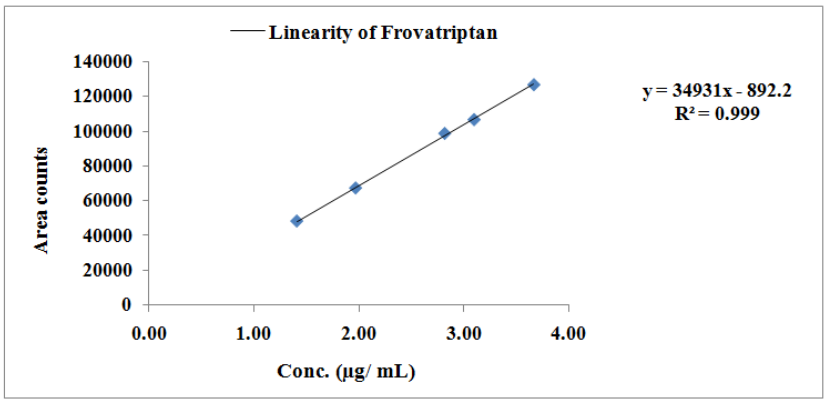

Fig. 6: Linearity of frovatriptan

Table 2: Results of precision of the frovatriptan test method

	% Drug dissolved in 30 minutes			
Parameter	**Intra-day precision**		**Inter-day precision**	
	% w/w	**Reported**	**% w/w**	**Reported**
Unit-1	95.9	96	92.8	93
Unit-2	95.3	95	97.0	97
Unit-3	92.0	92	91.1	91
Unit-4	91.1	91	95.5	96
Unit-5	89.9	90	94.6	95
Unit-6	92.4	92	91.1	91
Mean	92.8	93	93.7	94
SD	2.37	2.4	2.43	2.4
%RSD	2.55	2.6	2.59	2.6

Table 3: Results of recovery study

Level of recovery (%)	% Recovery range for triplicate preparations		
	% Dissolution (Min-Max)	% Average of dissolution release	%RSD
70%	98.9-101.8	100.1	1.5
100%	97.8-100.4	98.7	1.4
130%	98.5-101.0	99.6	1.3

Accuracy

The percentage recovery results of frovatriptan were varied from 97.8-101.8%at three different concentration levels of 70%, 100% and 130% and the results were shown in table 3. Based on the % recovery data, developed method is capable for the estimation of frovatriptan in frovatriptan tablet dosage forms and is adequate for routine analysis.

System suitability test

The system suitability test is an integral part of chromatographic methods and used to verify that the reproducibility of the chromatographic system is adequate for the analysis to be performed. Retention time (Rt), the number of theoretical plates (N), peak Tailing factor (T) and %RSD of five injections were evaluated for frovatriptan standard at working concentration. The results shown in table 4 are within the acceptable limits. The sample peaks from the chromatograms are identified by comparing the retention time with the standard peaks.

Robustness

In all the robust conditions, including flow rate, column temperature, minor component change in mobile phase composition, theoretical plates and tailing factor, %RSD results are well within the acceptable limits and consistent with the initial results. The peak shape for frovatriptan was found to be good and also the tailing factor for frovatriptan peak is not more than 1.5. %RSD was not more than 2% for replicate injections of frovatriptan during all the robustness conditions. Results are shown in table 4 for all the robustness conditions performed.

Solution stability

Frovatriptan percentage difference was determined for solutions stored at room temperature condition (25°C) at different time intervals up to 24 hours. Frovatriptan sample and standard solutions were found to be stable up to 24 hours at room temperature (25°C). Results are shown in table 5 for all the solution stability conditions performed.

Table 4: Results of robustness study

Robustness parameters	Retention time in minutes	Theoretical plates	Tailing factor	%RSD
Control (System precision)	1.87	10807	1.39	0.5
Flow (0.22 mL/min)	1.71	10736	1.41	0.3
Flow (0.18 mL/min)	2.07	10815	1.40	0.0
Temperature (35°C)	1.80	10680	1.41	0.2
Temperature (25°C)	1.95	10641	1.39	0.2
Minor component (M. P-A: M. P-B: :74:26)	1.71	9840	1.43	0.4
Minor component (M. P-A: M. P-B: :86:14)	2.11	11850	1.36	0.3

Table 5: Results of solution stability

Time	% Assay of standard preparation	% Difference	% Dissolution of test preparation	% Difference
Initial	99.5	Not applicable	95.9	Not applicable
After 12 h	99.3	0.2	95.3	0.6
After 24 h	98.6	0.9	94.8	1.1

CONCLUSION

A novel reverse phase UPLC method was developed and validated for the quantification of frovatriptan in their pharmaceutical dosage forms for dissolution testing. The developed method was precise, accurate and robust to the fast liquid chromatographic conditions. The developed method can be used in routine analysis for the quantification of frovatriptan in frovatriptan tablets.

ACKNOWLEDGEMENT

The authors would like to thank Glenmark Pharmaceuticals Ltd for providing free gift samples for research work and Department of Inorganic and Analytical Chemistry, Andhra University, Vishakhapatnam, A. P, India for their encouragement.

CONFLICT OF INTERESTS

Declared None

REFERENCES

1. Jerkovich AD, Lo Brutto R, Vivilecchia RV. LC-GC North. Am Suppl 2005;(23):15.
2. Ram Reddy GV, Reddy BV, Haque SW, Gautam HD, Poonam K, Praveen Kumar A, *et al*. Development and validation of a stability-indicating UPLC method for rosuvastatin and its related impurities in pharmaceutical dosage forms. Quim Nova 2011;34(2):250-5.
3. Sridhar NY, Rajasekhar E, Ram Reddy GV, Praveen Kumar A, Gopi KKB, Srinivasulu A. Dissolution method development of lamivudine, nevirapine and stavudine in tablets by UPLC. Int J Pharm Pharm Sci 2012;4(l3):113-7.
4. Leandro CC, Hancock P, Fussel RJ, Keely BJ. Comparison of ultra-performance liquid chromatography and high-performance liquid chromatography for the determination of priority pesticides in baby foods by tandem quadrupole mass spectrometry. J Chromatogr A 2006;1103(1):94-101.
5. Jin H, Praveen Kumar A, Paik DH, Ha KC, Yoo YJ, Lee YI. Trace analysis of tetracycline antibiotics in human urine using UPLC–QToF mass spectrometry. Microchem J 2010;94(2):139-47.
6. Hitesh V, Surajpal V, Harman Preet S. A stability indicating assay method development and validation for the Frovatriptan succinate monohydrate by using UV spectrophotometric technique. ISRN Spectroscopy 2013;1-6.

7. Gadi RRR, Raju VVK, Prasad GS. Estimation of Frovatriptan succinate in tablet dosage form by RP-HPLC. Der Pharm Lett 2013;5(5):151-7.
8. Usharani N, Sreenivasa RR, Saraswathi K, Murthy TEGK. RP-HPLC and spectrophotometry method for the analysis of Frovatriptan in formulations. IJSID 2011;1(1):53-61.
9. Kumara SG, Kumar JMR, Sheshagiri RJVLN, Vinay KD. Development and validation of RP-HPLC method for the estimation of Frovatriptan succinate in bulk and tablet dosage form. J Pharm Res 2012;5(2):894-5.
10. Lloyd RS, Joseph JK, Joseph LG. A book on practical HPLC method development. 2nd Edition. Wiley-Blackwell; 1997.
11. ICH, Validation of analytical procedures: Text on Analytical methodology Q2(R1). International Conference on Harmonization, Geneva, Switzerland; 2005.
12. US Food and Drug Administration Guidance for industry: analytical procedures and methods validation: chemistry, manufacturing, and controls documentation. Draft Guidance. US Food and Drug Administration; 2001.
13. Bakshi M, Singh S. Development of validated stability indicating assay methods-critical review. J Pharm Biomed Anal 2002;28:1011–40.
14. Ghulam AS. Validation of high performance liquid chromatography methods for pharmaceutical analysis: Understanding the differences and similarities between validation requirements of the US Food and Drug Administration, the US Pharmacopeia and the International Conference on Harmonization. J Chromatography A 2003;987(1–2):57–66.
15. Dennis RJ. Chromatographic method validation: A review of current practices and procedures I. General concepts and guidelines. J Liq Chromatogr Relat Technol 1996;19(5):719-36.
16. Dennis RJ. Chromatographic method validation: A review of current practices and procedures. II. Guidelines for primary validation parameters. J Liq Chromatogr Relat Technol 1996;19(5):737-57.
17. Dennis RJ. Chromatographic method validation: A review of current practices and procedures. III. Ruggedness, re-validation and system suitability. J Liq Chromatogr Relat Technol 1996;19(12):1873-91.
18. Ghulam AS, John LW, Shafique AA, Tony KB. Evaluation and application of best practice in analytical method validation. J Liq Chromatogr Relat Technol 2007;30(3):311-33.
19. Reddy BV, Praveen Kumar A, Ram Reddy GV, Sahaia M, Sreeramulu J, Park JH. Stability indicating reverse phase high performance liquid chromatography method for determination of impurities in Ofloxacin tablet formulations. Anal Lett 2010;43:2653–62.
20. Ram Reddy GV, Praveen Kumar A, Reddy BV, Kumar P, Gauttam HD. Identification of degradation products in Aripiprazole tablets by LC-QToF mass spectrometry. Eur J Chem 2010;1(1):20-7.
21. General chapter 1092. The dissolution procedure: Development and validation, United States Pharmacopeia, 37th revision, National Formulary, 32nd edition, Rockville, MD, The United States Pharmacopeial Convention, Inc; 2014.
22. General chapter 5171. European Pharmacopoeia, 8.0. Recommendation on dissolution testing; 2014.
23. General chapter 293. European Pharmacopoeia. 8.0. Dissolution test for solid dosage forms; 2014.