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Estimation of Frovatriptan Succinate in Tablet Dosage Form by RP-HPLC

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ABSTRACT

A simple, precise, rapid and accurate RP- HPLC method was developed for the estimation of Frovatriptan Succinate (FTP) in tablet dosage forms. An XTerra RP-C_{18} Column, 250x4.6 mm, column with 5 µm particle sizes and the Mobile Phase consisting of 0.02M Potassium Dihydrogen Phosphate pH: 3.2 adjusted with Methanol & Acetonitrile in ratio of 70:30 v/v & water: Acetonitrile (50:50 v/v) was used as a diluent in the gradient mode. The flow rate was 0.8 ml/min and the effluents were monitored at 242 nm. The retention time was 3.066 min and the detector response was linear in the concentration range of 10-120 µg/mL for FTP successively. The respective linear regression equation being Y= 65647.112x + 45974.1647 for FTP. The Limit of Detection (LOD) & The Limit of Quantification (LOQ) was found to be 1.00 & 3.00 µg/mL respectively for FTP. The percentage assay of FTP was 98.17%. The method was validated by determining its accuracy, precision and system suitability. The results of the study showed that the proposed RP-HPLC method is simple, rapid, precise and accurate, which is useful for the routine determination of FTP in bulk drug and in its pharmaceutical dosage forms.

Key Words: Frovatriptan Succinate (FTP), RP-HPLC, Validation and Tablets.

INTRODUCTION

Chemically, Frovatriptan Succinate is 3-methylamino-6-carboxamido-1, 2, 3, 4-tetrahydrocarbazole Succinate **(Figure 1)**. Molecular weight is 243.304 g/mol. It is a potent and selective agonist of 5-HT_{1B} and 5-HT_{1D} receptors. By acting on 5-HT_{1B} receptor, it causes constriction of dilated arteriovenous anastomoses. The action on 5-HT_{1D} causes the inhibition of Substance P and calcitonin gene-related peptide (CGRP) release. Among all the triptans, it has the longest elimination half-life ($_{1/2}$) of 26 hours. It has all the ideal characteristics to be used as a first line drug for menstruation migraine **[1-5]**. Literature survey reveals a few chromatographic methods to determine the FTP in tablet dosage form and also in biological fluids. So far, few assay methods by liquid chromatography **[6-11]** were reported for the estimation of FTP in pharmaceutical dosage forms at the time of commencement of these investigations. As in pharmaceutical industry, time and expense plays a very crucial role. Pharmaceutical industries always admire simple and more sensitive methods for routine usage. Therefore, a need of simple, reliable, inexpensive, and accurate stability indicating method for analysis of FTP as bulk or as pharmaceutical dosage forms has always been felt. The availability of an HPLC method with high sensitivity and selectivity will be very useful for the determination of FTP in pharmaceutical formulations. A detailed account of all analytical methods existing for the drug is made to avoid duplication of the method developed. The authors had made some humble attempts, hoping to fulfill and bridge this gap, in succeeding the developing analytical methods, by using HPLC System. The results of this labor of love are set forth by developing a simple, precise and accurate reverse-phase HPLC method for the estimation of FTP in bulk drug samples and in pharmaceutical dosage forms.

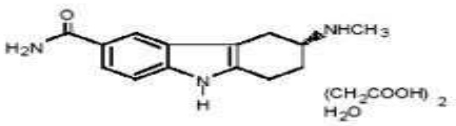

Figure 1: Frovatriptan Succinate (FTP)

MATERIALS AND METHODS

2.1 Materials / Chemicals and Reagents: Frovatriptan succinate was generous gift sample from Azakem Labs Pvt Ltd, Hyderabad. Acetonitrile, Methanol & Water used were of HPLC grade (Qualigens). Potassium Dihydrogen Phosphate was obtained from SDFCL, Mumbai. Commercially available tablets were procured from local market.

2.2 Chromatography Instrument: Quantitative HPLC was performed on liquid chromatography, Waters separation 2996, PDA detector module equipped with automatic injector with injection volume 20 µl, and 2693 pump. An XTerra RP-C_{18} Column, (250x4.6 mm i.d; particle size 5 µ) was used. The HPLC system was equipped with Empower 2 Software. The column was maintained at 40^{o} C and eluted under isocratic conditions over 30.0 min at a flow rate of 0.8 ml/min.

2.3 HPLC Conditions: The contents of the Mobile Phase consisting of 0.02M Potassium Dihydrogen Phosphate pH: 3.2 adjusted with Methanol & Acetonitrile in ratio of 70:30 v/v & Water: Acetonitrile (50:50 v/v) was used as a diluent in the gradient mode. They were filtered before use, through a 0.45 µm membrane filter, and pumped from the respective solvent reservoirs to the column at a flow rate of 0.8 ml/min. The run time was set at 30.0 min and the column temperature was ambient. Prior to the injection (20 µl) of the drug solution, the column was equilibrated for at least 30 min with the mobile phase flowing through the system. The eluents were monitored at 242 nm.

2.4 Preparation of the Primary Standard/Stock Drug Solution: A standard stock solution of the drug was prepared by dissolving 100 mg of FTP in 100 ml volumetric flask containing 30 ml of diluent (Water: Acetonitrile 50:50 v/v), sonicated for about 15 min and then made up to 100 ml with Methanol & Acetonitrile in ratio of 70:30 v/v to get standard stock solution of 1 mg/ml of FTP.

2.5 Preparation of the Working Standard Drug Solution: 5ml of the above stock solution was taken in 50 ml volumetric flask and made up to 50 ml with diluent Water: Acetonitrile 50:50 v/v to get a concentration of each 100 µg/mL of FTP respectively.

2.6 Preparation of Sample solution: Twenty tablets (Frova® - Endo Pharmaceuticals Inc) were weighed, and then powdered. A sample of the powdered tablets, equivalent to mixture containing concentration of each 100 mg/mL of FTP active ingredients, was mixed with 30 ml of Water: Acetonitrile 50:50 v/v as diluent in 50 ml volumetric flask. The mixture was allowed to stand for 1 hr with intermittent sonication to ensure complete solubility of the drug, and then filtered through a 0.45 µm membrane filter, followed by adding methanol up to 100 ml to obtain a stock solution each of 1 mg/ml of FTP. 5ml of the above sample stock solution was taken in 50 ml volumetric flask and made up to 50 ml with diluent to get a concentration of each 100 µg/mL of FTP respectively.

2.7 Linearity: Aliquots of primary standard FTP stock solution was taken in different 10 ml volumetric flasks and diluted up to the mark with the mobile phase such that the final concentrations of FTP was in the range of 5-150 µg/mL respectively. Each of these drug solutions (20 µL) was injected three times into the column, and the peak areas and retention times were recorded. Evaluation was performed with PDA detector at 242 nm and the calibration graph was obtained by plotting peak area versus concentration of FTP **(Figure: 4)**. The plot of peak area of each sample against respective concentration of FTP was found to be linear in the range of 10-120 µg/mL with correlation coefficient of 0.9998. Linear regression least square fit data obtained from the measurements are given in **Table 1**. The respective linear regression equation being Y= 65647.112x + 45974.1647 for FTP. The regression characteristics, such as slope, intercept, and %RSD were calculated for this method and given in **Table 1.**

2.8 Accuracy: Accuracy was evaluated in triplicate by addition of three different amounts of FTP to a previously analyzed sample and comparing the amounts of analytes recovered with the amounts added. The amounts added

were equivalent to 80, 100, and 120% of the amount originally present. %Recovery and RSD (%) were calculated for amount added. From the data obtained, it is obvious that the method is remarkably accurate, which ensures that this method produces reliable results as depicted in **Table: 2**.

2.9 Precision: The precision of the method was ascertained, separately from the peak area obtained by actual determination of six replicas of a fixed amount of the drug and formulation.

The HPLC systems were set up, describing chromatographic conditions, mentioned as above and following the system equilibration of the working standard solution containing 100 µg/mL of FTP was injected six times and the response peak areas were recorded. The precision was repeated with the formulated sample for the same concentrations by injecting the working sample solutions containing 100 µg/mL of FTP. The sample was processed six times for the response of peak area. The % Relative Standard Deviation (RSD) and % range of error (at 0.05 and 0.01 confidence levels) were calculated and presented in **Tables: 3& 4** respectively.

2.10 Limits of Detection and Quantitation: Limit of Detection (LOD) of the method was determined as the lowest concentrations of active pharmaceutical ingredients producing a signal-to-noise (S/N) ratio of about 3. The Limit of Quantitation (LOQ) was determined as the lowest concentrations of active pharmaceutical ingredients capable of being quantified with acceptable accuracy and precision producing signal-to-noise (S/N) ratio of about 10.

2.11 Method Applicability: The present developed method was evaluated by applying to Pharmaceutical dosage forms for the estimation of FTP by our research group.

2.12 Assay: 20 µL of sample solution was injected into the injector of liquid chromatography. The retention time was found to be 3.066 min for FTP successively. The amount of drug present per tablet was calculated by comparing the peak area of the sample solution with that of the standard solution. The data are presented in **Table 2**.

2.13 Recovery Studies: Accuracy was determined by recovery studies of FTP; known amount of standard was added to the pre-analyzed sample and subjected to the proposed HPLC analysis. Results of recovery studies are shown in **Table 2**. The study was done at three different concentration levels.

RESULTS AND DISCUSSION

3.1 HPLC Method Development and Optimization [12]: In response to lack of simple, reliable and easy-to-use method for the determination of FTP concentrations in pharmaceutical matrices, an isocratic RP- HPLC method was developed for quantification of above mentioned, API. We examined several HPLC method variables with respect to their corresponding effects on the result of analysis. To optimize the chromatographic conditions, different combinations of Methanol-Water, and Acetonitrile-Water and Acetonitrile-Potassium Dihydrogen Phosphate buffer (0.02M) & Methanol- Acetonitrile were tested. Methanol- Acetonitrile with 0.02M Potassium Dihydrogen Phosphate [pH- 3.2] was promisingly preferred, because it resulted in greater resolution of API after several preliminary investigatory runs, compared with other mobile phases. The other parameters in this factorial design were temperature, flow rate, detection wavelength and volume of injection. Buffer molarity was changed and optimum buffer strength was selected as 0.02M on the basis of theoretical plate number. At 242 nm, UV responses of all three active pharmaceutical analytes were good and free form interferences. Under these conditions, the analyte peaks were well defined and free from tailing. Considering the whole body of the data obtained from this extensive study, the set of conditions indicated earlier in this article was selected for further validation. Typical chromatogram of FTP (Standard and Working Sample) has been shown in **Figure: 5 & 6.**The system suitability tests were carried out on freshly prepared standard stock solutions of FTP. Parameters that were studied to evaluate the suitability of the system were discussed and presented in **Table 5**.

3.2 Method Validation Tests: Recommended method validation characteristics including method precision (RSD, %), method accuracy (Recovery % and RSD, %), linear range (Correlation Coefficient), and LOD & LOQ, were investigated systematically.

3.3 Linearity: The plot of peak areas of each sample against respective concentrations were found to be linear, in the range of 10-120 µg/mL for FTP with correlation coefficient of 0.9998 (**Table: 1)**. Linear regression least square fit data obtained from the measurements are given in **Table: 1.** The respective linear regression equation being Y=

65647.112x + 45974.1647 for FTP. The regression characteristics, such as slope, intercept, and %RSD were calculated for this method and given in **Table I.** These results show that there was an excellent correlation between peak areas and analyte concentration.

3.4 Accuracy: Recovery of the individual substances at 80%, 100%, and 120% of specified concentrations were between 101.16% -108.75%, which proves the accuracy of the method. From these data, RSD was always less than 1%, which indicates it is obvious that the method is remarkably accurate, produces reliable results (**Table: 2**)

3.5 Precision: The low value (<1%) of RSD indicates the repeatability of the method. These data indicate a considerable degree of precision and reproducibility for the method both during one analytical run and between different runs (**Table: 3**).

3.6 Robustness: Robustness was studied out to evaluate the effect of small but deliberate variations in the chromatographic conditions at three different levels, i.e. –2, 0, +2. To determine the robustness of this method, the experimental conditions were deliberately altered at three different levels and retention time and chromatographic response were evaluated. One factor at a time was changed to study the effect. Variation of the columns by ±2 nm (240 nm and 244 nm), mobile phase Methanol to Acetonitrile ratio (68:32 and 72:28, *v*/*v*), mobile phase pH by ±0.2 units (pH 3.0 and 3.4), and mobile phase flow rate by 0.8 mL min^{-1} (0.6 and 1.0 mL min^{-1}) had no significant effect on the retention time and chromatographic response of the method, indicating that the method was robust. The results are shown in **Table: V**.

3.7 Limit of Detection (LOD) and Limit of Quantification (LOQ): The Limit of Detection (LOD) & The Limit of Quantification (LOQ) analyzed were found to be 1.0 & 3.0 µg/mL for FTP respectively. These values reflect the high sensitivity of the method, which is of great importance in most studies and also indicating the method can be used for detection and quantification of analytes in a very wide concentration range.

3.8 Specificity: No evidence of signals, in the corresponding times of the chromatogram were monitored as a sign of potential interfering peaks, were found when the pharmaceutical dosage formulations was tested. Hence, this method can be used reliably for the estimation of respected active pharmaceutical ingredients in a variety of dosage forms.

Table 1: Linear Regression Data of Calibration Curve

Parameter	Frovatriptan Succinate (FTP)
Concentration range (µg/mL)	10-120
Slope (m)	65647.112
Intercept (Y)	-105593.8824
Standard error of estimate (c)	45974.1647
Correlation coefficient (r)	0.9998
Linear regression (r^2)	0.99
%RSD	0.20

Table 2: Assay & Accuracy Recovery Studies of Frovatriptan Succinate (Ftp) In Tablet Dosage Forms

Tablet formulation	Amount claim (mg/tablet)	Amount Obtained (mg)* by proposed method		** % Recovery by the Proposed method	
	FTP	FTP		FTP	
1). 120%	2.5	2.44		108.75	
2).100%	2.5	2.38		101.16	
3). 80%	2.5	2.21		107.00	
Average Mean	2.5	2.34		105.63	

**Average of three determinations*
*** after spiking the sample*

Table: 3: Precision of Recommended Procedure Using API & Sample (Frova®): Frovatriptan Succinate (FTP)

Sr. No	Inj. No	Name of the Drug & Conc. (500 µg/mL)	Standard Drug		Sample Drug	
			Retention time in minutes	Peak Area	Retention time in minutes	Peak Area
1	1	FTP	3.050	6684222	3.059	6501761
2	2	FTP	3.054	6682902	3.054	6567481
3	3	FTP	3.046	6657722	3.055	6623565
4	4	FTP	3.046	6691130	3.048	6531119
5	5	FTP	3.047	6682450	3.056	6522685
6	6	FTP	3.055	6670410	3.057	6486938
7	Mean		3.049	6678139.4	3.055	6538925
8	Standard Deviation		0.004	12030.2	0.004	49775.1
9	% RSD		0.13	0.2	0.12	0.8

Table 4: Validation Summary / System Suitability:

PARAMETER	FROVATRIPTAN SUCCINATE (FTP)
Theoretical Plates(N)	2087.14
Tailing factor	1.19
Retention time(min)	3.066
Resolution	----
Area	5171267
% Peak Area	99.22
LOD (µg/mL)	1
LOQ (µg/mL)	3

Table 5: Results from testing of the Robustness of the method (n=3, 100% of the Working Standard Solution & Sample solution contains: 100 µg/mL of Frovatriptan Succinate (FTP)

Condition Studied in Robustness	Modification In OFAT analysis	Mean Peak Area ± S.D		% RSD (Peak Area)	Mean Retention Time (in min)± S.D	% RSD (Retention time)
		Parameter Fixation	FTP	FTP	FTP	FTP
Column(s) (XTerra RP- C_{18})	Hypersil &, Hypurity C_{18}	Std	6663256 ± 18087.3	0.4	3.051 ± 0.819	0.2
		Sample	6562279 ± 16211.5	0.9	3.023 ± 0.304	0.9
Flow rate (0.8 ml/min)	0.6 ml/min & 1.0 ml/min	Std – Increase	6875781.7 ± 10258.1	0.7	3.058 ± 0.230	0.7
		Std- Decrease	6769867.3 ± 17434.1	0.3	3.031 ± 0.785	0.8
		Sample- Increase	6519372.0 ± 16413.9	0.9	3.070 ± 0.538	0.5
		Sample- Decrease	646886.1 ± 14037.9	0.1	3.049 ± 0.407	0.3
pH (3.2)	3.0 & 3.4	Std - Increase	6599213.2 ± 12496.7	0.3	3.057 ± 0.304	0.4
		Std- Decrease	6499733.5 ± 11903.3	0.4	3.034 ± 0.305	0.8
		Sample - Increase	6850466.8 ± 16153.6	0.1	3.056 ± 0.311	0.5
		Sample - Decrease	6705739.6 ± 15360.8	0.8	3.044 ± 0.274	0.6

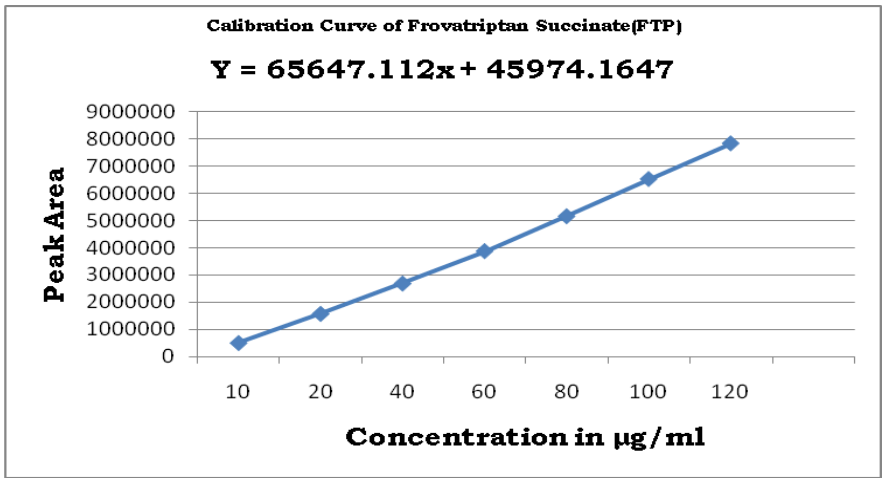

Figure 4: Calibration Curve of the Frovatriptan Succinate (FTP) by RP-HPLC

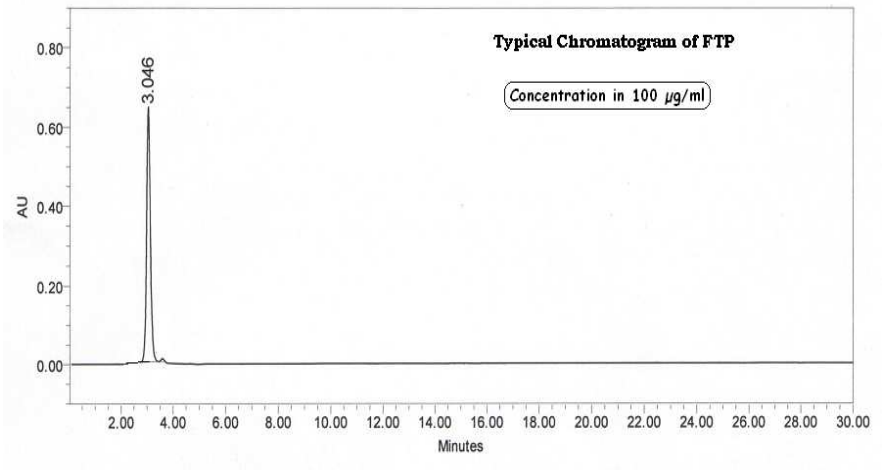

Figure 5: Typical Chromatogram of standard Frovatriptan Succinate (FTP) by RP-HPLC

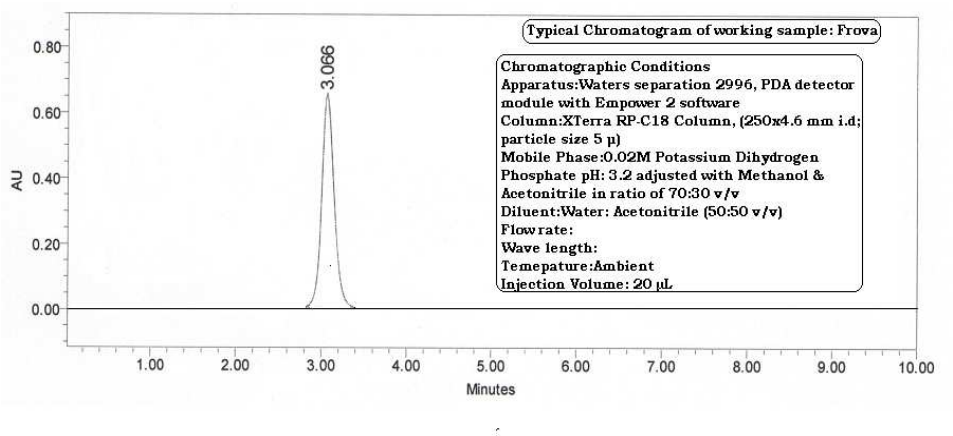

Figure 6: Typical Chromatogram of standard Frovatriptan Succinate (FTP) Working Sample by RP-HPLC

CONCLUSION

A simple and easily available HPLC method was developed in this study for the quantification of FTP in pharmaceutical matrices. The main advantages of this method are its considerably shorter run times, easy-to-use and its simplicity. All of these properties are very important in practice, particularly when a large number of samples are to be analyzed. The absence of additional peaks in the chromatogram indicates non-interference of the common excipients used in the tablets. The results of validation tests were, collectively, indicative for a method with a relatively wide linear range, acceptable precision and accuracy and practically reliable sensitivity. The method enables simple, selective, sensitive, and specific analysis of FTP can be used for routine analysis in pharmaceutical quality control within a short time.

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