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Validated spectrophotometric methods for determination of Frovatriptan Succinate Monohydrate in pharmaceutical dosage forms

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ABSTRACT

UV, first derivative, second derivative and AUC-spectrophotometric methods for the determination of Frovatriptan Succinate Monohydrate (FSM) in pharmaceutical formulations have been developed. For the first method, UV-spectrophotometry, standard solutions were measured at 279 nm. The linearity ranges were found to be 1–80 µgml^{-1} in 0.1N HCl and the regression equation was A=1.8866×10^{-2}C-2.3397×10^{-3} (r=0.9992). For the second method, first derivative spectrophotometry, the response (dA/dλ) of standard solutions was measured at 292 nm. Calibration curve was constructed by plotting dA/dλ values against concentrations, 2.5–80 µgml^{-1} of FSM standards in 0.1N HCl. Regression equation of linear calibration graph was calculated as D$_1$= -6.36×10^{-4}C-1.73×10^{-4}(r=0.9997). For the third method, second derivative spectrophotometry, the response (d^{2}A/dλ^2) of standard solutions was measured at 281 nm. Calibration curve was constructed by plotting d^{2}A/dλ^2 values against concentrations, 10–80 µg ml^{-1} of FSM standards in 0.1N HCl. Regression equation of linear calibration graph was calculated as D$_2$= -9.7×10^{-5}C – 6.1×10^{-5} (r=0.9995). The fourth method was based on calculation of Area under Curve (AUC) for analysis of FSM in the wavelength range of 274–284 nm. Calibration curve was constructed by plotting AUC values against concentrations, 1–80 µg ml^{-1}of FSM standards in 0.1N HCl. Regression equation of linear calibration graph was calculated as AUC=0.1855C-0.0246 (r=0.9992). The methods were validated by following the analytical performance parameters suggested by the International Conference on Harmonization. All validation parameters were within the acceptable range. The developed methods were successfully applied to estimate the amount of FSM in pharmaceutical formulations.

Keywords: Frovatriptan Succinate Monohydrate; UV-spectrophotometry; Derivative-spectrophotometry; AUC- spectrophotometry.

INTRODUCTION

Frovatriptan Succinate Monohydrate (FSM) chemically [1], (3R)-2,3,4,9-Tetrahydro-3-(methylamino)-1H-carbazole-6-carboxamide Butanedioic Acid Monohydrate (Figure 1). It is a selective 5-hydroxy-tryptamine (5-$HT_{1B/1D}$) receptor subtype agonist which is used in treatment of migraine headaches, in particular those associated with menstruation. Frovatriptan [2] reverses cerebral vasodilation by activating 5-HT_{1B}, and it prevents neurogenic inflammation by activating 5-HT_{1D}.

A survey of the literature has not revealed any analytical method for determination of FSM in pharmaceutical formulation or biological fluids. Therefore the objective of the present study was to develop four simple, precise, accurate, validated, economic analytical methods, in accordance with International Conference on Harmonization (ICH), for quantification of FSM in bulk and pharmaceutical formulations.

MATERIALS AND METHODS

Chemicals and Reagents

FSM working standard was obtained from Alembic Ltd., (Vadodara, India). A commercial tablet formulation was purchased from the local market. Hydrochloric acid (0.1N) of analytical grade solution was prepared in double distilled water.

Instrument

A double beam UV-VIS spectrophotometer (UV-1800, Shimadzu, Japan) connected to computer loaded with spectra manager software UV Probe with 1cm quartz cells was used. The spectra were obtained with the instrumental parameters as follows: wavelength range: 200-400 nm; scan speed: medium; sampling interval: 1 nm; derivative mode: ^1D (first order derivative, $dA/d\lambda$) and ^2D (second order derivative, $d^2A/d\lambda^2$); band width ($\Delta\lambda$): for ^1D and ^2D, 10 nm; spectral slit width: 1nm. All weights were taken on electronic balance (Denver, Germany).

Preparation of standard stock solution

The standard solution of FSM was prepared by dissolving accurately weighed 10 mg of the drug in 0.1N HCl and diluted to 100 ml with 0.1N HCl to obtain a final concentration of 100 $\mu g ml^{-1}$. This stock solution was used to prepare further dilutions of standard solutions.

Method I: UV- Spectrophotometry

Series dilutions of the stock solution were made by pipetting out 0.1, 0.25, 0.5, 1, 2, 3, 4, 6 and 8 ml stock solution into separate 10 ml volumetric flasks and diluting to volume with 0.1N HCl to produce the concentrations ranging from 1-80 $\mu g ml^{-1}$. The above solutions were scanned over the range of 400 nm to 200nm against blank. The λ_{max} was found to be at 279 nm**.** The calibration curve was constructed by plotting concentration (1-80 $\mu g ml^{-1}$) versus absorbance at 279 nm.

Method II: First- derivative spectrophotometry
The spectrums obtained in method I was derivatised to get first order derivative spectra and the response ($dA/d\lambda$) of the spectra were measured at 292 nm and then calibration curve was constructed by plotting concentration (2.5-80 $\mu g ml^{-1}$) versus response ($dA/d\lambda$) at 292 nm.

Method III: Second- derivative spectrometry
The spectrums obtained in method I was derivatised to get second order derivative spectra and the response ($d^2A/d\lambda^2$) of the spectra were measured at 281 nm and then calibration curve was constructed by plotting concentration (10-80 $\mu g ml^{-1}$) versus response ($d^2A/d\lambda^2$) at 281nm.

Method IV: Area Under Curve
The AUC (area under curve) method is applicable where there is no sharp peak or when broad spectra are obtained. It involves the calculation of integrated value of absorbance with respect to the wavelength between the two selected wavelengths λ_1 and λ_2. Area calculation processing item calculates the area bound by the curve and the horizontal axis. The horizontal axis is selected by entering the wavelength range over which area has to be calculated. This wavelength range is selected on the basis of repeated observation so as to get the linearity between area under curve and concentration. The spectrums obtained in method I was used to calculate AUC**.** The calibration curve was constructed by plotting concentration (1-80 $\mu g ml^{-1}$) versus AUC**.**

Estimation of Frovatriptan Succinate monohydrate in tablets
For the analysis of the pharmaceutical dosage form, a total of twenty tablets were weighed and finely powdered. A portion of the powder, equivalent to about 10 mg FSM was weighed accurately and transferred into 100ml volumetric flask and 50 ml 0.1N HCl was added. After ultrasonic vibration for 30 min, the mixture was diluted to volume with 0.1N HCl and filtered through Whatman filter paper (No. 41). Appropriate dilution was made into 20 $\mu g ml^{-1}$ with 0.1N HCl from the stock solution for all the methods and the amounts of FSM were determined. Percent labeled claim and Standard Deviation (S.D) was calculated.

Validation of Methods

Linearity: For all the methods, 6-point calibration curves were prepared on 3 different days. The results obtained were used to calculate the equation of the line by using linear regression by the least-squares regression method.

Precision: The intraday and interday precisions of the proposed spectrophotometric methods were determined by estimating the corresponding response 3 times on the same day and on 3 different days over a period of 1 week for 3 different concentrations of FSM (10, 20, and 40 $\mu g ml^{-1}$) and the results are reported in terms relative standard deviation.

Accuracy: This parameter was evaluated by the percent recovery studies at concentration levels of 80, 100, and 120%, which consisted of adding known amount of FSM reference material to a prequantified sample solution. Aliquot of sample solution containing FSM at 20 $\mu g\ ml^{-1}$ was transferred to three 10ml volumetric flasks containing, respectively, 16, 20, and 24 $\mu g\ ml^{-1}$ FSM reference solutions. The contents were mixed and diluted to volume in order to obtain final concentrations of 36, 40 and 44 $\mu g ml^{-1}$ FSM. The recoveries were verified by estimation of drugs

in triplicate preparations at each specified concentration level. The spectrums were recorded in the UV range and then analyzed. The results are reported in terms of % recovery.

Specificity: Results of tablet solution showed that there is no interference of excipients when compared with the working standard solution. Thus, the methods were said to be specific.

Robustness: The robustness of the proposed methods was tested by changing parameters such as wavelength range and slit width. None of these variables significantly affected the absorbance of the drugs indicating that the proposed methods could be considered as robust.

Ruggedness: Ruggedness of the proposed methods was determined by analyzing aliquots from homogenous slot (20 μgml^{-1}) in different laboratories by different analyst using similar operational and environmental conditions. The results are reported in terms of % RSD.

RESULTS AND DISCUSSION

Figure 2, 3 and 4 show overlaid UV-spectrophotometric (1-80 μgml^{-1}), first-derivative (2.5-80 μgml^{-1}) and second-derivative (10-80 μgml^{-1}) absorption spectra of FSM respectively and the spectra were found to be similar in nature and overlapping. Figure 5 shows the absorption spectrum of FSM (20 μgml^{-1}) in 0.1N HCl for the method IV. Optical characteristics of FSM were calculated by the proposed methods and presented in table1.

From the calibration curve (Graph 1), it was observed that with the increase in FSM concentration, the responses are increased. In Method I, the λ_{max} was found to be at 281 and 245 nm (Figure 2). But study was carried out at 281 nm because at this wavelength the Beer-Lambert's law was following properly. Derivative spectrophotometry is an analytical technique for the enhancement of sensitivity and specificity in qualitative and quantitative analysis of various compounds including pharmaceuticals. Hence method II and III were carried out for FSM. For Method II (Figure 3), 292 nm is selected because at 225 nm and 259 nm peaks are distorted and maximum wavelength of the peaks as well as zero crossing point are not remaining constant. At 273 nm, good linearity range was not obtained; hence this wavelength was also not selected for Method II. For Method III (Figure 4), the wavelength 281 nm is selected because; zero crossing point and maximum wavelength are not remaining constant for each concentration at other wavelengths i.e 231, 254 and 264 nm. In Method IV (Figure 5), study was carried out at two wavelength ranges i.e 274-284 nm and 269-289 nm, but good linearity range was obtained at the wavelength range of 274-284 nm.

Tablets were analyzed and amount of the drug determined by proposed methods; it was in good agreement with the label claim (Table 2). It was also observed that there was no significant difference in the content of FSM obtained by using the different proposed spectrophotometric methods.

The recoveries of FSM which was evaluated by the percent recovery studies at concentration levels of 80, 100, and 120% were found to be in the acceptable range (Table 4). Excipients used in the formulation did not interfere with response of the drug at its analytical wavelengths. Also, no significant change in response of FSM was observed by changing parameters such as

wavelength range and slit width. The intra-day and inter-day precision values (%RSD) were calculated (Table 3) and lying in the acceptable range for FSM. Ruggedness of proposed methods were determined with the help of two different analysts and results were evaluated by calculating the %RSD value and lying within the range (Table 5).Hence, the proposed methods are precise, specific, accurate, ruggedness and robust for estimation of FSM in bulk and pharmaceutical formulations.

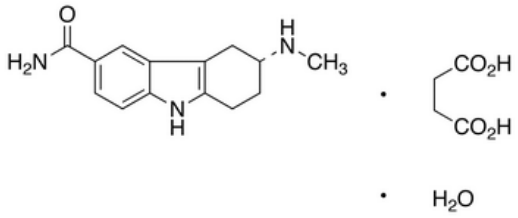


Figure 1: Chemical structure of Frovatriptan Succinate Monohydrate

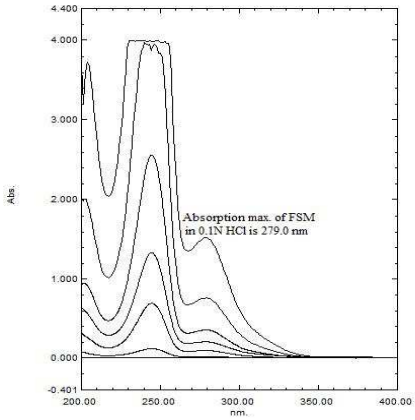
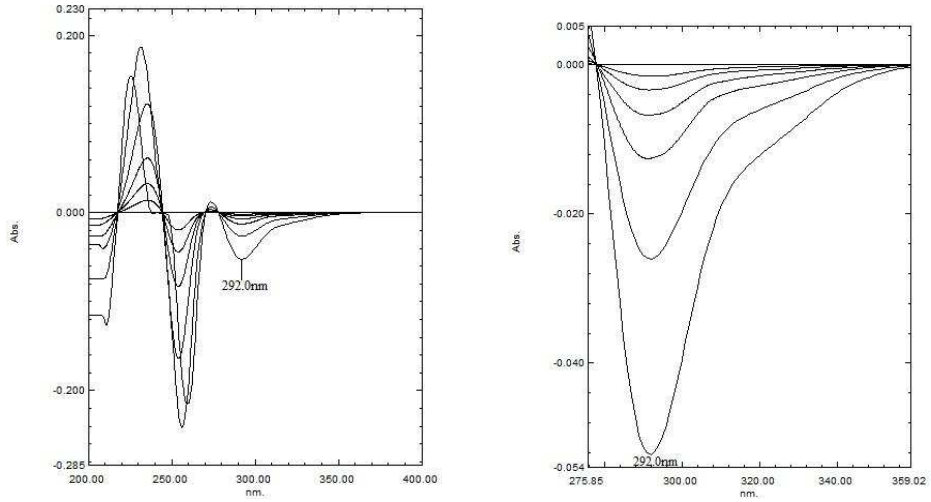
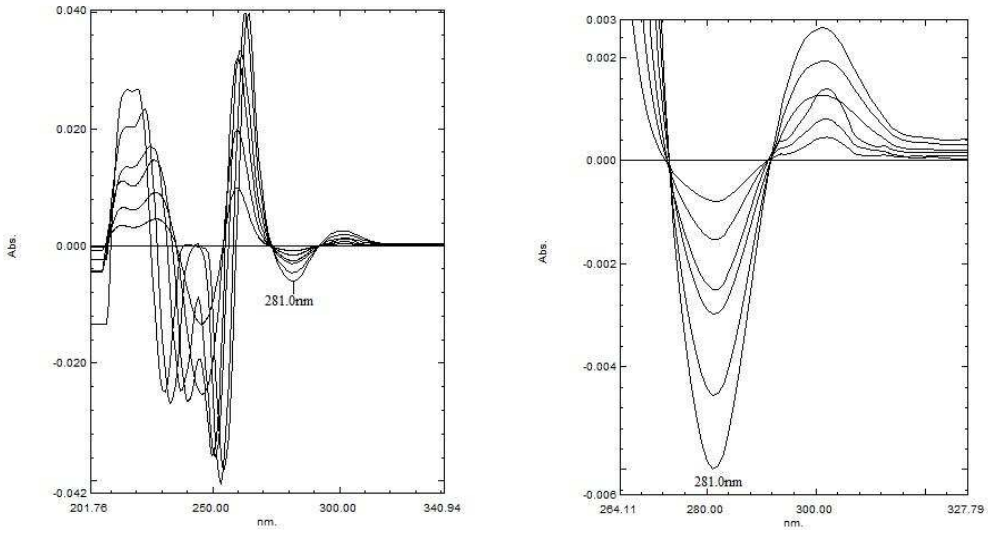


Figure 2. Absorption spectrum of FSM in 0.1N HCl (1-80 µg ml^{-1})



(A) **(B)**

Figure 3. First- derivative absorption spectrum of FSM in 0.1N HCl (2.5-80 µgml^{-1}): (A) Normal View, (B) Large View



(A) **(B)**

Figure 4. Second- derivative absorption spectrum of FSM in 0.1N HCl (10-80 µgml^{-1}): (A) Normal View, (B) Large View

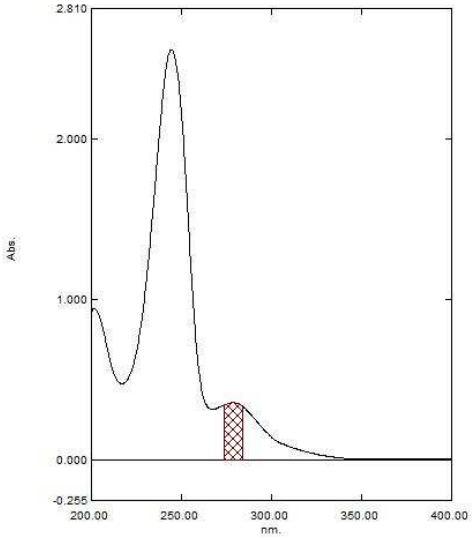
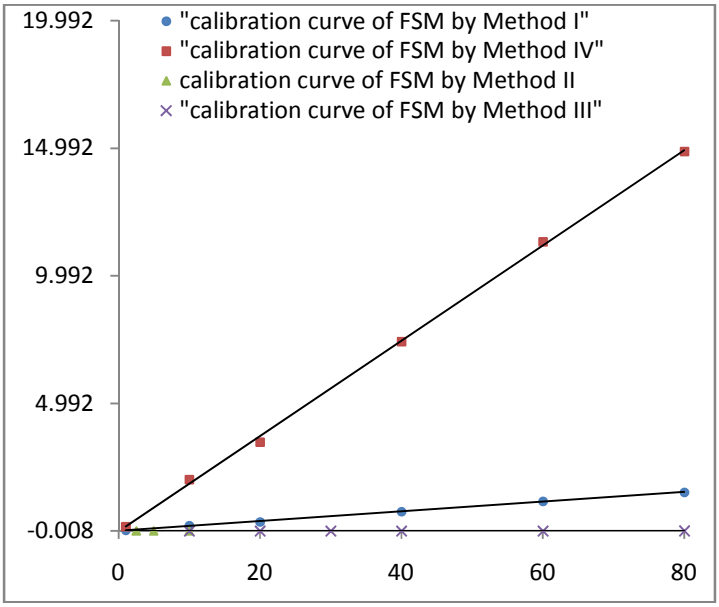


Figure 5. Absorption spectrum of FSM in 0.1N HCl (20 µgml^{-1}) [274-284 nm range was selected for Method-IV]



Graph 1: Calibration curves of FSM in 0.1N HCl (Method I, II, III and IV)

Table 1. Optical characteristics of FSM by the proposed methods

Parameters	Method I	Method II	Method III	Method IV
Beer-Lambert's range($\mu g ml^{-1}$)	1-80	2.5-80	10-80	1-80
λ_{max}(nm)/ wave length range (nm)	279	292	281	274-284
Molar absorptivity±SD (l/mol.cm)	7145.292 ± 366.92435	-256.102 ± 26.14903	-37.7829 ± 0.3872337	69888.9 ± 4102.7381
Sandell sensitivity ($\mu g\ cm^{-2}$/0.001 A)	0.053214	-	-	-
Slope	0.01886667	-0.000636	-0.0000970	0.18553333
Standard deviation of slope	0.000351	0.000012	0.000001	0.001150
%RSD of slope	1.861423	-1.90635	-1.03093	0.62003
Intercept	0.0023397	0.000173	0.000061	0.024600

Standard deviation of intercept	0.000042	0.00000208	0.000001	0.000361
%RSD of intercept	1.814024	1.200961	1.63934	1.465671
Correlation coefficient	0.999173	0.999722	0.999465	0.999237
%RSD of Correlation coefficient	0.059818	0.016676	0.010784	0.079069
Limit of detection($\mu g ml^{-1}$)	0.007424	-0.0108	-0.03402	0.006413
Limit of quantitation($\mu g ml^{-1}$)	0.022496	-0.03273	-0.10309	0.019433

Table2. Assay results of FSM in pharmaceutical dosage form (Tablet-2.5mg) by using the proposed spectrophotometric methods.

Label Claim (mg/tab)	% Label Claimed ±SD(n=5)				%RSD			
	Method I	Method II	Method III	Method IV	Method I	Method II	Method III	Method IV
2.5	99.988± 0.1353	99.854± 0.889	100.646± 0.942	100.432± 0.859	0.1354	0.8913	0.9360	0.8555

Table3: Results for Precision studies of FSM by proposed spectrophotometric methods.

method	Intraday (n=3); (RSD, %)			Interday (n=3); (RSD, %)		
	Drug Conc. taken ($\mu g\ ml^{-1}$)			Drug Conc. taken ($\mu g\ ml^{-1}$)		
	10	20	40	10	20	40
I	0.38	0.44	0.57	0.36	0.46	0.59
II	0.49	0.42	0.38	0.51	0.91	0.46
III	0.67	0.76	0.54	0.38	0.67	0.74
IV	0.54	0.45	0.38	0.87	0.68	0.76

Table4: Results for Accuracy studies of FSM by proposed spectrophotometric methods

method	Accuracy (% recovery*± SD)		
	80% (20+16 µgml^{-1})	100% (20+20 µgml^{-1})	120% (20+24µgml^{-1})
I	99.85±0.28	100.33±0.21	99.73±0.14
II	99.88±0.08	100.21±0.10	99.95±0.29
III	99.63±0.56	100.14±0.17	100.46±0.66
IV	100.27±0.51	100.10±0.12	100.19±0.89

* Mean of three determinations

Table 5: Ruggedness data of FSM (20 µgml$^{-1)}$ by proposed methods

Analyst I, %RSD				Analyst II, %RSD			
Method I	method II	method III	method IV	Method I	method II	method III	Method IV
0.44	0.54	0.38	0.57	0.46	0.52	0.41	0.61

CONCLUSIONS

Four methods that were developed for the determination of FSM are based on different analytical techniques, zero-derivative, first-derivative, second-derivative spectrophotometry and AUC method. All the methods were validated and found to be simple, sensitive, accurate and precise. Hence, all the methods can be used successfully for routine analysis of pharmaceutical dosage forms of FSM.

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REFERENCES

[1] The Merck Index, An Encyclopedia Of Chemical, Drug's and Biologicals, Maryadele J.O. Neil.Eds, Published by Merck Research Lab, Division of Merck and co. Inc., Whitehouse Station, NJ: **2006**,14th, 733.
[2] Comer M.B., *J. Head Face Pain*, **2001**, Vol 42 (2), S47.