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Research Article

SPECTROPHOTOMETRIC METHODS FOR THE DETERMINATION OF FROVATRIPTAN SUCCINATE MONOHYDRATE IN BULK AND PHARMACEUTICAL DOSAGE FORMS

Sasmita Kumari Acharjya*, Priyambada Mallick, Pinakini Panda and M.Mathrusri Annapurna
Roland Institute of Pharmaceutical Sciences, Department of Pharmaceutical Analysis and Quality Assurance, Berhampur, Orissa, India, 760010.
Email: *acharjya2009@gmail.com*

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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 280.2 nm. The linearity ranges were found to be 1.0–80.0 µg ml^{-1} in 0.1N NaOH and the regression equation was $A=2.3214\times10^{-2}C-1.1543\times10^{-2}$ (r=0.9995). For the second method, first derivative spectrophotometry, the response ($dA/d\lambda$) of standard solutions was measured at 294.0 nm. Calibration curve was constructed by plotting $dA/d\lambda$ values against concentrations, 2.5–80.0µgml^{-1} of FSM standards in 0.1N NaOH. Regression equation of linear calibration graph was calculated as $D_1= -8.71\times10^{-4}C+5.17\times10^{-4}$(r=0.9995). For the third method, second derivative spectrophotometry, the response ($d^2A/d\lambda^2$) of standard solutions was measured at 283.2 nm. Calibration curve was constructed by plotting $d^2A/d\lambda^2$ values against concentrations, 10.0–80.0µg ml^{-1} of FSM standards in 0.1N NaOH. Regression equation of linear calibration graph was calculated as $D_2= -9.8\times10^{-5}C - 6.1\times10^{-5}$ (r=0.9995). The fourth method was based on calculation of Area under Curve (AUC) for analysis of FSM in the wavelength range of 275.0–285.0 nm. Calibration curve was constructed by plotting AUC values against concentrations, 1.0–80.0µg $ml^{-1}$of FSM standards in 0.1N NaOH. Regression

equation of linear calibration graph was calculated as AUC=0.2287C-0.1229 (r=0.9995). 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}.

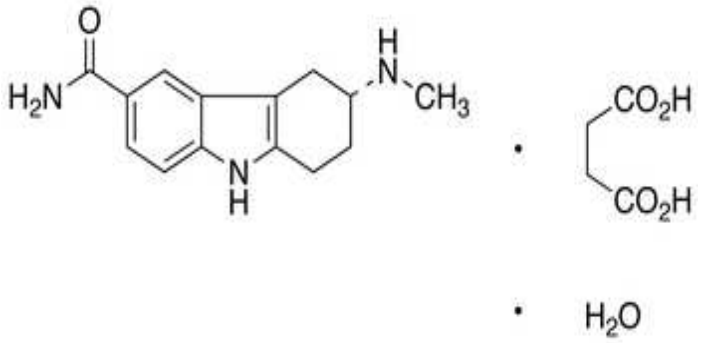


Figure 1: Chemical structure of Frovatriptan Succinate Monohydrate

On detailed literature survey, it was found that till now no method is reported for the determination FSM as an active pharmaceutical ingredient (API) and in its pharmaceutical formulations. The aim of the present work is to develop and validate new spectrophotometric methods for the estimation of FSM in bulk and pharmaceutical formulations.

EXPERIMENTAL:

CHEMICALS AND REAGENTS

FSM working standard was kindly provided by Alembic Ltd., (Vadodara, India) and was used as received. A commercial tablet formulation was purchased from the local market. Sodium hydroxide (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 1.0 cm quartz cells was used. The spectra were obtained with the instrumental parameters as follows: wavelength range: 200-400 nm; scan speed: medium; sampling interval: 0.2 nm; derivative mode: ^{1}D (first order derivative, $dA/d\lambda$) and ^{2}D (second order derivative, $d^2A/d\lambda^2$); band width ($\Delta\lambda$): for ^{1}D and ^{2}D, 8.0 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 10mg of the drug in 0.1N NaOH and diluted to 100 ml with 0.1N NaOH to obtain a final concentration of 100µg 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, 1.0, 2.0, 3.0, 4.0, 6.0 and 8.0ml stock solution into separate 10ml volumetric flasks and diluting to volume with 0.1N NaOH to produce the concentrations ranging from 1.0-80.0 µg 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 280.2 nm**.** The calibration curve was constructed by plotting concentration (1.0-80.0µgml^{-1}) versus absorbance at 280.2 nm.

METHOD II

FIRST- DERIVATIVE SPECTROPHOTOMETRY

The spectrums obtained in method I was derivatised to get first order derivative spectra and the response (dA/dλ) of the spectra were measured at 294.0 nm and then calibration curve was constructed by plotting concentration (2.5-80.0µgml^{-1}) versus response (dA/dλ) at 294.0nm.

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 283.2 nm and then calibration curve was constructed by plotting concentration (10.0-80.0µgml^{-1}) versus response ($d^2A/d\lambda^2$) at 283.2nm.

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 (1.0-80.0µgml^{-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 NaOH was added. After ultrasonic vibration for 30 min, the mixture was diluted to volume with 0.1N NaOH and filtered through Whatman filter paper (No. 41).

Appropriate dilution was made into 20.0µgml^{-1} with 0.1N NaOH 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 (10.0, 20.0, and 40.0 µg/ml) 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 20.0 µg ml^{-1} was transferred to three 10ml volumetric flasks containing, respectively, 16.0, 20.0, and 24.0 µg ml^{-1} FSM reference solutions. The contents were mixed and diluted to volume in order to obtain final concentrations of 36.0, 40.0 and 44.0 µg 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 (20.0µgml^{-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 (1.0-80.0µgml^{-1}), first-derivative (2.5-80.0µgml^{-1}) and second-derivative (10.0-80.0µgml^{-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 (20.0µgml^{-1}) in 0.1N NaOH for the method IV. Optical characteristics of FSM were calculated by the proposed methods and presented in table1.

Table 1. Optical characteristics of FSM by the proposed methods.

Parameters	Method I	Method II	Method III	Method IV
Beer-Lambert's range(µg ml^{-1})	1.0-80.0	2.5-80.0	10.0-80.0	1.0-80.0
λ_{max}(nm)/ wave length range (nm)	280.2	294.0	283.2	275.0-285.0
Molar absorptivity±SD (l/mol.cm)	8557.013± 347.785	-312.223± 13.553	-37.783± 0.387	83718± 3418.509
Sandell sensitivity (µg cm^{-2}/0.001 A)	0.0444	-	-	-
Slope	0.023144	-0.00087	0.000097	0.228365
Standard deviation of slope	0.000061	0.000001	0.000001	0.000577
%RSD of slope	0.264804	-0.11494	-1.03093	0.252819
Intercept	-0.011539	0.000516	0.000061	0.122842

Standard deviation of intercept	0.000018	0.00000153	0.000001	0.000047
%RSD of intercept	0.15413	0.296223	1.63934	0.038287
Correlation coefficient	0.999518	0.999529	0.999465	0.999461
%RSD of Correlation coefficient	0.000755	0.002853	0.010784	0.001151
Limit of detection	0.002536	-0.00579	-0.03402	0.00068
Limit of quantitation	0.007685	-0.01756	-0.10309	0.00206

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 280.2 and 246.8nm (Figure 2). But study was carried out at 280.2 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), 294.0nm is selected because at 225.4nm and 262.8nm peaks are distorted and maximum wavelength of the peaks as well as zero crossing point are not remaining constant. At 275.2 nm, good linearity range was not obtained; hence this wavelength was also not selected for Method II. For Method III (Figure 4), the wavelength 283.2nm is selected because, zero crossing point and maximum wavelength are not remaining constant for each concentration at other wavelengths i.e 219.8, 230.4 258.6 and 266.4nm. In Method IV (Figure 5), study was carried out at two wavelength ranges i.e 275.0-285.0nm and 270.0-290.0nm, but good linearity range was obtained at the wavelength range of 275.0-285.0nm.

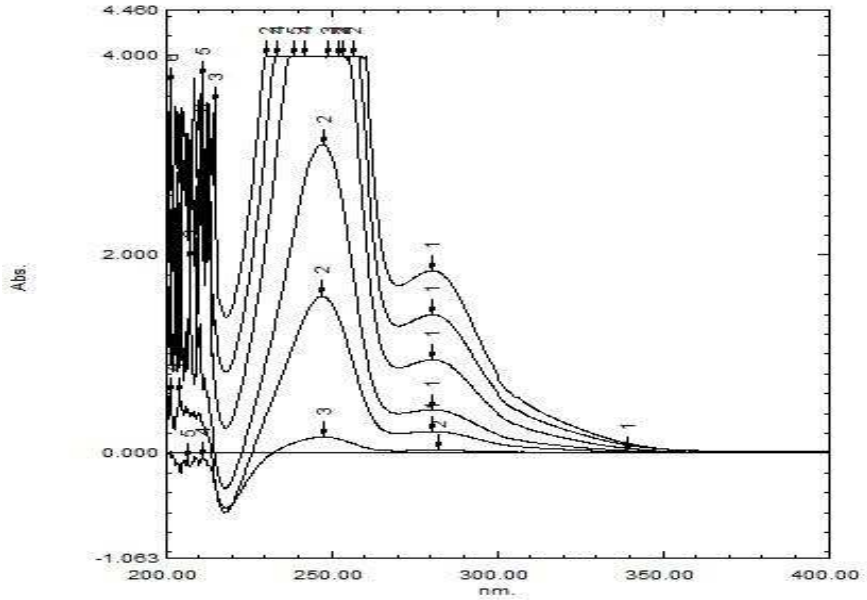
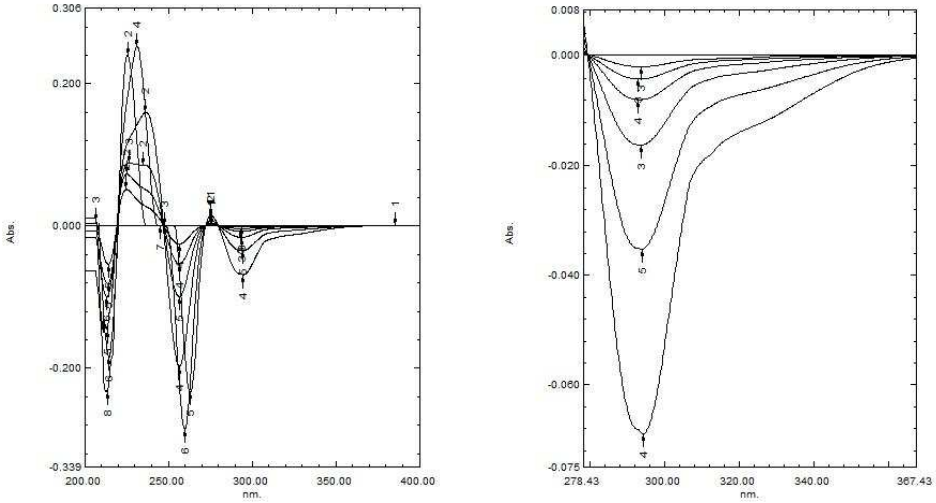
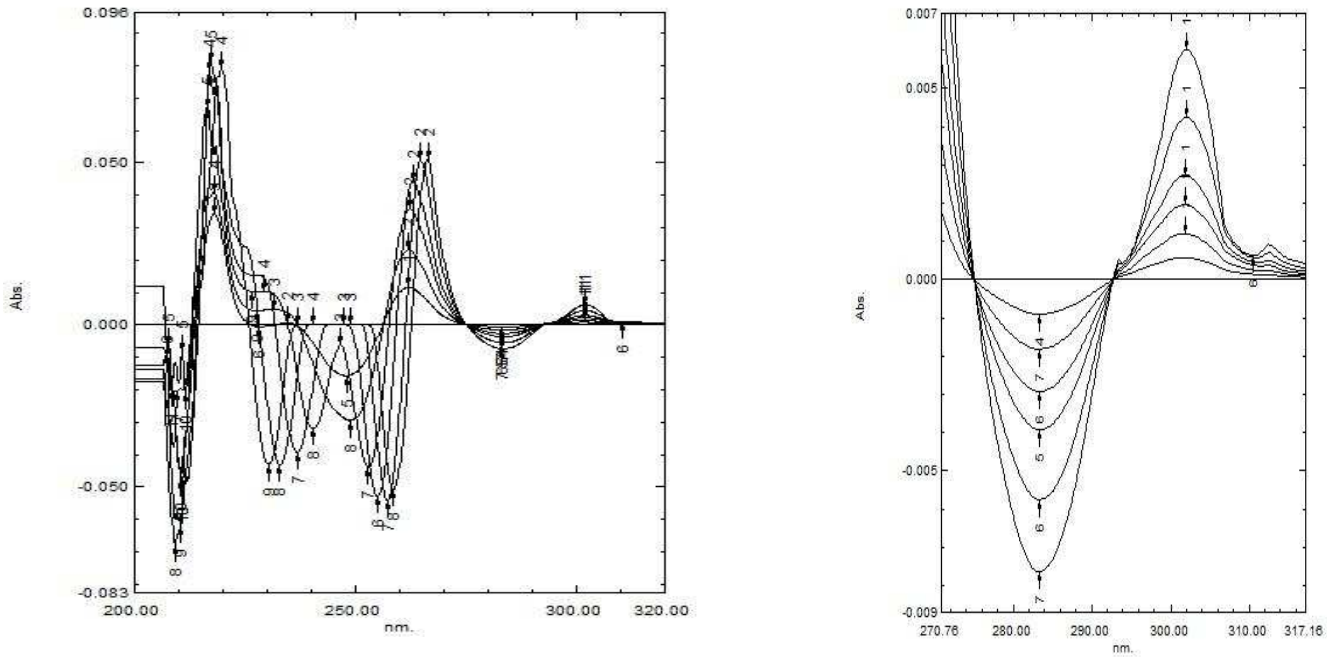


Figure 2. Absorption spectrum of FSM in 0.1N NaOH (1.0-80.0 µg ml^{-1})



(A) **(B)**

Figure 3. First- derivative absorption spectrum of FSM in 0.1N NaOH (2.5-80.0 µg ml^{-1}): (A) Normal View, (B) Large View



(A) **(B)**

Figure 4. Second- derivative absorption spectrum of FSM in 0.1N NaOH (10.0-80.0 µg ml^{-1}): (A) Normal View, (B) Large View

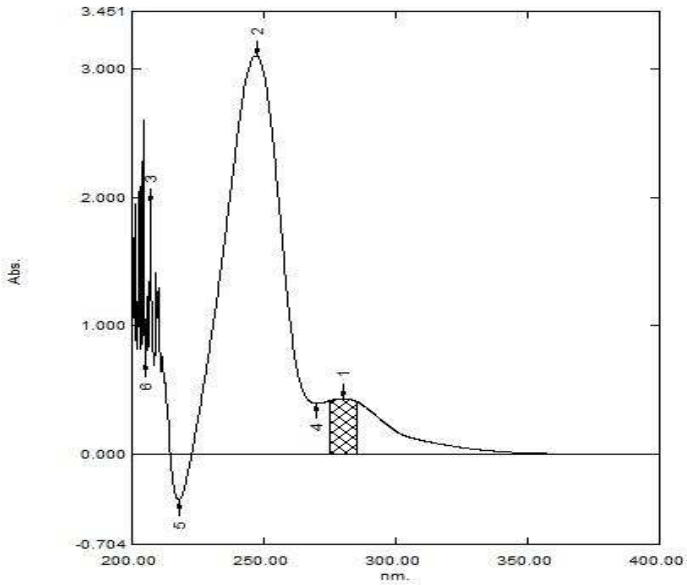
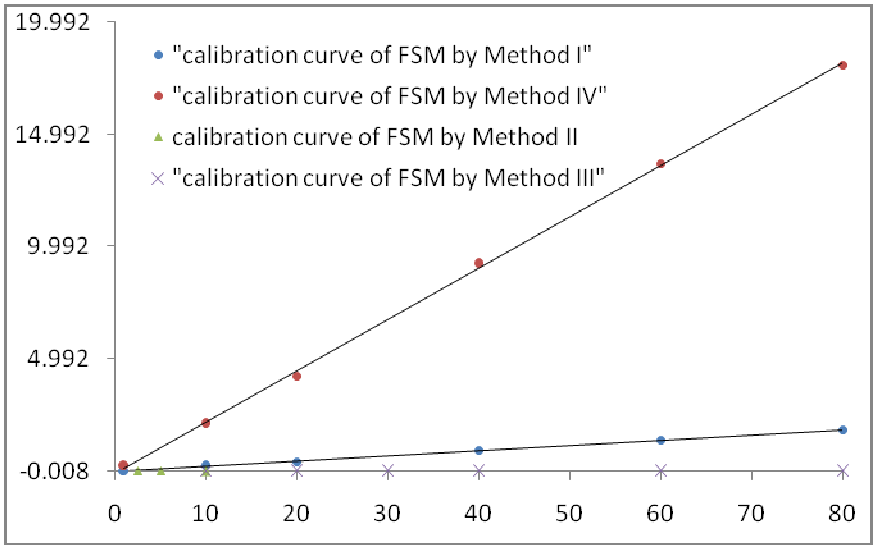


Figure 5. Absorption spectrum of FSM in 0.1N NaOH (20.0 µg ml^{-1})

[275.0-285.0nm range was selected for Method-IV]



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

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.

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

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.

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

method	Intraday (n=3); (RSD, %)			Interday (n=3); (RSD, %)		
	Drug Conc. taken (µg ml^{-1})			Drug Conc. taken (µg ml^{-1})		
	10.0	**20.0**	**40.0**	**10.0**	**20.0**	**40.0**
Method I	0.34	0.44	0.57	0.36	0.46	0.59
Method II	0.42	0.42	0.35	0.59	0.91	0.46
Method III	0.67	0.76	0.54	0.38	0.67	0.74
Method IV	0.54	0.52	0.38	0.87	0.68	0.76

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

method	Accuracy (% recovery*± SD)		
	80% (20.0+16.0 µg ml^{-1})	**100% (20.0+20.0 µg ml^{-1})**	**120% (20.0+24.0µg ml^{-1})**
Method I	99.85±0.28	100.33±0.21	99.73±0.14
Method II	99.88±0.08	100.22±0.10	99.95±0.29
Method III	99.61±0.56	100.16±0.17	100.46±0.66
Method IV	100.28±0.51	100.10±0.12	100.19±0.89

* Mean of three determinations

Table 5: Ruggedness data of FSM (20.0µg $ml^{-1)}$ by proposed methods

Analyst I, %RSD				Analyst II, %RSD			
Method I	**methodII**	**methodIII**	**methodIV**	**Method I**	**methodII**	**methodIII**	**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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***Corresponding author**

Sasmita Kumari Acharjya*,
Roland Institute of Pharmaceutical Sciences,
Department of Pharmaceutical Analysis and Quality Assurance,
Berhampur, Orissa, India, 760010.
Email: acharjya2009@gmail.com