Research Article

A Stability Indicating Assay Method Development and Validation for the Frovatriptan Succinate Monohydrate by Using UV: A Spectrophotometric Technique

Hitesh Verma, Surajpal Verma, and Harmanpreet Singh

*School of Pharmaceutical Sciences, Lovely Faculty of Applied Medical Sciences, Lovely Professional University,
Phagwara, Punjab 144411, India*

Correspondence should be addressed to Hitesh Verma; verma2908@gmail.com

A new simple, reliable, inexpensive, and accurate method was developed for the quantification of Frovatriptan Succinate Monohydrate in different physiological media at 244 nm in bulk and in tablet dosage forms. The developed method is an attempt to surpass the disadvantages associated with the reported methods, namely, less sensitive and tedious in usage for routine purposes. Beer's law was followed over the range of 1.0 μg/mL to 4.5 μg/mL. Stability indicating assay method was developed and validated as per the ICH guidelines using various parameters, for example, accuracy, precision, limit of quantification, limit of detection, robustness, ruggedness, solution stability, recovery, forced degradation (hydrolysis, photo degradation, thermal degradation, and oxidation), and so forth. Percent relative standard deviation associated with all the parameters was less than 2, showing compliance with the acceptance criteria of ICH guidelines. The developed method was very sensitive as limit of quantification and limit of detection were found to be 0.025 μg/mL and 0.00625 μg/mL, respectively. Forced degradation studies of drug reveal good stability under the chosen experimental conditions.

1. Introduction

Chemically, the Frovatriptan Succinate Monohydrate (FSM) is 3-methylamino-6-carboxamido-1,2,3,4-tetrahydrocarbazole succinate monohydrate (Figure 1). 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 ($t_{1/2}$) of 26 hours. This carabzole derivative was approved by FDA in 2001. It has all the ideal characteristics to be used as a first line drug for menstruation migraine [1–3].

Despite tremendous utility of FSM in migraine, there was no simple, sensitive, and reliable stability indicating that UV spectroscopic method has been reported for its analysis in the vast reviewed literature. Only two UV spectroscopic methods have been mentioned in the literature for the analysis of FSM in 0.1 N NaOH and 0.1 N HCl at 280.2 nm and 279 nm, but these methods are less sensitive and tedious to be used for routine purposes because they involve first-order derivatization, second-order derivatization, and area-under-the-curve (AUC) analysis in order to make their method sensitive [4, 5]. HPLC methods were reported in the literature for their analysis in dosage forms and in blood samples [6–8]. Till now, no forced degradation data is available in the literature for FSM.

As in pharmaceutical industry, time and expense play 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 FSM as bulk or as pharmaceutical dosage forms has always been felt.

The present study was aimed to develop and validate analytical methods for the analysis of FSM in distilled water, phosphate buffer (pH 6.7), and acetate buffer (pH 5.5) at two different storage conditions, that is, room temperature
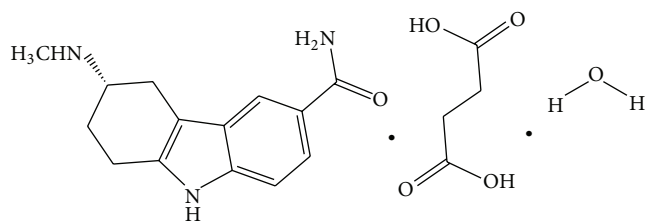
Figure 1: Structure of Frovatriptan Succinate Monohydrate (FSM).

($25°C$) and freeze temperature ($5°C$) at 244 nm. Forced degradation studies were carried out using aqueous solution of FSM ($2.5\,\mu$g/mL). Test solution was exposed to various types of ICH prescribed stress conditions, like hydrolysis (neutral, acid catalyzed, and base catalysed), photolysis, thermal degradation, and oxidation, so as to check its stability. Developed methods were validated as per ICH guidelines and validation data was evaluated with the aid of various statistical parameters [9].

2. Materials and Methods

2.1. Chemicals and Reagents. FSM was procured as a gift sample from Azakem Labs Pvt. Ltd., India. Potassium dihydrogen orthophosphate, sodium hydroxide, and sodium acetate trihydrate were obtained from Himedia Ltd., India. Hydrogen peroxide (30% w/v) was obtained from Moly chem., India. All other chemicals used were of analytical grade.

2.2. Instruments. Double beam UV-visible spectrophotometer (Shimadzu, UV-1800, Japan) in connection with computer having UV Probe 2.34 software and equipped with matched quartz cells of 1.0 cm each was used.

2.3. Construction of Calibration Plot. Different stock solutions were prepared in distilled water, phosphate buffer (pH 6.7), and acetate buffer (pH 5.5) by accurately weighing about 156.4 mg of FSM (equivalent to 100 mg of frovatriptan) in a volumetric flask of 100 mL capacity. Volume was made up to the mark using suitable media. Different dilutions were made in the range of $1\,\mu$g/mL–$3.5\,\mu$g/mL and analysed.

2.4. Analytical Method Validation

2.4.1. Accuracy. Accuracy of the developed method was determined with the aid of three different concentration levels, namely, low concentration (LC) $2.0\,\mu$g/mL, intermediate concentration (IC) $2.5\,\mu$g/mL, and a higher concentration (HC) $3.0\,\mu$g/mL. LC, IC, and HC were prepared in all the three media from independent stock solutions, in duplicate, and were analysed ($n = 3$). Accuracy of the method was determined with the help of standard deviation (SD), percent relative standard deviation (RSD), and recovery at each level.

2.4.2. Precision. Repeatability and intermediate precision studies were determined for developed methods using same concentration levels as were taken in accuracy studies. Percent RSD and recovery were taken as precision measure.

2.4.3. Linearity. Linearity was determined by preparing a series of dilutions in three media and then measuring the absorbance at 244 nm ($n = 3$), so as to find out the range over which Beer's law followed. Linear regression coefficient value (R^2) was taken as linearity measure.

2.4.4. Limit of Quantification (LOQ) and Limit of Detection (LOD). LOD and LOQ measurements were determined by subjecting minimum concentration of calibration plot to series of dilutions. Absorbance was measured in replicate of six for each case, till RSD (%) was came as $\geq 10\%$ and $\geq 30\%$, which signify LOQ and LOD of the developed method.

2.4.5. Robustness. Robustness was determined by taking similar concentration levels as were taken in accuracy studies and storing them at two different temperatures ($5°C$ and $25°C$) and by varying the pH of the media by ± 0.1 units.

2.4.6. Forced Degradation Studies. Test solution ($2.5\,\mu$g/mL) was exposed to different strengths of hydrochloric acid (0.1 N to 1.0 N), sodium hydroxide (0.1 N to 1.0 N), and hydrogen peroxide solutions (from 3% w/v to 30% w/v), and the extent of degradation was analysed with respect to time and volume of exposure. Test solution was also exposed separately to the radiations of 300–800 nm and to $70°C$ temperature to evaluate the extent of photolytic and thermal degradation, respectively [10, 11].

3. Results

3.1. Calibration Curve. Least square regression equation of FSM in different media at two different temperatures had shown R^2 value close to 1 which ensured high degree of positive correlation between the two variables, namely, absorbance and concentration.

3.2. Analytical Method Validation

3.2.1. Accuracy. Accuracy data associated with LC, IC, and HC is mentioned in Table 1. In each case, RSD (%) was less than 2, which shows compliance with ICH guidelines. Recovery as in accuracy studies were found to be 100%–104% for distilled water, 97%–100% for phosphate buffer (pH 6.7), and 96%–100% for acetate buffer (pH 5.5), respectively.

3.2.2. Precision. The RSD (%) data associated with repeatability studies ranges from 0.432 to 1.139 and 0.332 to 1.392 for distilled water at freeze temperature and room temperature, respectively. The % RSD data associated with repeatability studies in phosphate buffer (pH 6.7) ranges from 0.460–1.283 and 0.666–1.119 at freeze temperature and room temperature, respectively. The % RSD data associated with repeatability studies in acetate buffer (pH 5.5) ranges from 0.706 to 1.722 and 0.275 to 1.004 at freeze temperature and room temperature, respectively. In intermediate precision, values of RSD (%) varies from 0.563 to 1.254 and 0.578 to 1.164 for distilled water at freeze temperature and room temperature, respectively. The phosphate buffer (pH 6.7) values of RSD

Table 1: Accuracy data of developed method for analysis of FSM in various media ($n = 3$).

Media	Levels	Freeze (5°C ± 0.5°C)			Room (25°C ± 0.5°C)		
		Absorbance (±SD)	RSD (%)	Recovery (%)	Absorbance (±SD)	RSD (%)	Recovery (%)
Distilled water	LC	0.351 ± 0.001	0.285	103.931	0.352 ± 0.003	0.714	104.320
	IC	0.441 ± 0.003	0.600	104.112	0.437 ± 0.004	0.804	103.102
	HC	0.514 ± 0.003	0.625	100.997	0.520 ± 0.003	0.484	102.161
Phosphate buffer (pH 6.7)	LC	0.388 ± 0.002	0.536	100.139	0.382 ± 0.004	0.944	97.460
	IC	0.475 ± 0.004	0.740	98.154	0.475 ± 0.004	0.740	97.580
	HC	0.578 ± 0.003	0.556	99.849	0.578 ± 0.003	0.612	97.425
Acetate buffer (pH 5.5)	LC	0.374 ± 0.003	0.706	98.289	0.364 ± 0.001	0.275	96.313
	IC	0.480 ± 0.006	1.163	99.404	0.471 ± 0.004	0.925	99.756
	HC	0.554 ± 0.007	1.212	98.275	0.567 ± 0.006	1.003	100.047

Table 2: Linearity data for developed method of FSM.

Media	Freeze (5°C ± 0.5°C)			Room (25°C ± 0.5°C)		
	Slope	Intercept	R^2	Slope	Intercept	R^2
Distilled water	0.1716	−0.0058	0.9984	0.1716	−0.0058	0.9984
Phosphate buffer (pH 6.7)	0.1889	+0.0138	0.9983	0.1885	+0.0009	0.9995
Acetate buffer (pH 5.5)	0.1914	+0.005	0.9999	0.1901	+0.0013	0.9996

Table 3: LOQ and LOD data for developed method of FSM ($n = 6$).

Concentration (μg/mL)	Absorbance (±SD)	RSD (%)
0.025	0.028 ± 0.003	11.208
0.0125	0.013 ± 0.003	27.200
0.006	0.009 ± 0.003	30.051

Table 4: Effect of change in pH of media by ±0.1 unit on the sensitivity of developed method of FSM ($n = 6$).

Concentration (μg/mL)	Absorbance (±SD)	RSD (%)
2	0.346 ± 0.001	0.371
2.5	0.436 ± 0.002	0.479
3	0.520 ± 0.003	0.289

(%) vary from 1.020 to 1.397 and 1.089 to 1.371 at freeze temperature and room temperature, respectively. The acetate buffer (pH 5.5) values of RSD (%) vary from 0.629 to 1.344 and 0.684 to 1.100 at freeze temperature and room temperature, respectively. Recovery ranges from 96% to 104.9% in both types of precision study.

3.2.3. Linearity. Beer's law follows over the range of 1.0 μg/mL–4.5 μg/mL in all the media at both storage conditions. The values of slope, intercept, and regression coefficient (R^2) associated with linearity plots are listed in Table 2. Values of regression coefficients are close to 1 which shows that there is a directed positive correlation between the two variables, namely, absorbance and concentration (Figure 2).

3.2.4. LOQ and LOD. LOQ and LOD for FSM were found to be 0.025 μg/mL and 0.00625 μg/mL, respectively. Data associated with LOD and LOQ analysis is mentioned in Table 3.

3.2.5. Robustness. Variation in pH of media by ±0.1 unit and storage conditions do not seem to have effect on the absorption values and $\lambda_{\max}$ (Table 4).

When paired Student's t-test (two tailed) was applied on the accessed data, calculated value (0.9073) was much less than that of the table value at 95% confidence level, which ensures the robustness of the experiment. Similar results were obtained when prepared dilutions were subjected to two different storage conditions (freeze and room) for 24 hours.

3.2.6. Forced Degradation Studies. Test solution was found to undergo photolysis after 12 hours of exposure to radiations ranging from 300 to 800 nm. Extent of degradation after 12 hours was found to be 31.34%. Plot constructed between amount remaining to degrade (y-axis) and time (x-axis) yields a straight line curve with a negative slope R^2 value of 0.9421 (Table 5).

Storage of test solution at 70°C for 7 days yields no significant degradation (Table 6).

When constant volume (1.0 mL) of various strengths HCl from (0.1 N to 1.0 N) was added individually to 10 mL of test solution, it was found to be stable in 0.1 N, 0.2 N, 0.3 N, and 0.4 N HCl for 6 hours without heating. Addition of other strengths of HCl yields significant degradation of more than 31% within 6 hours of exposure (Table 7 and Figure 3).
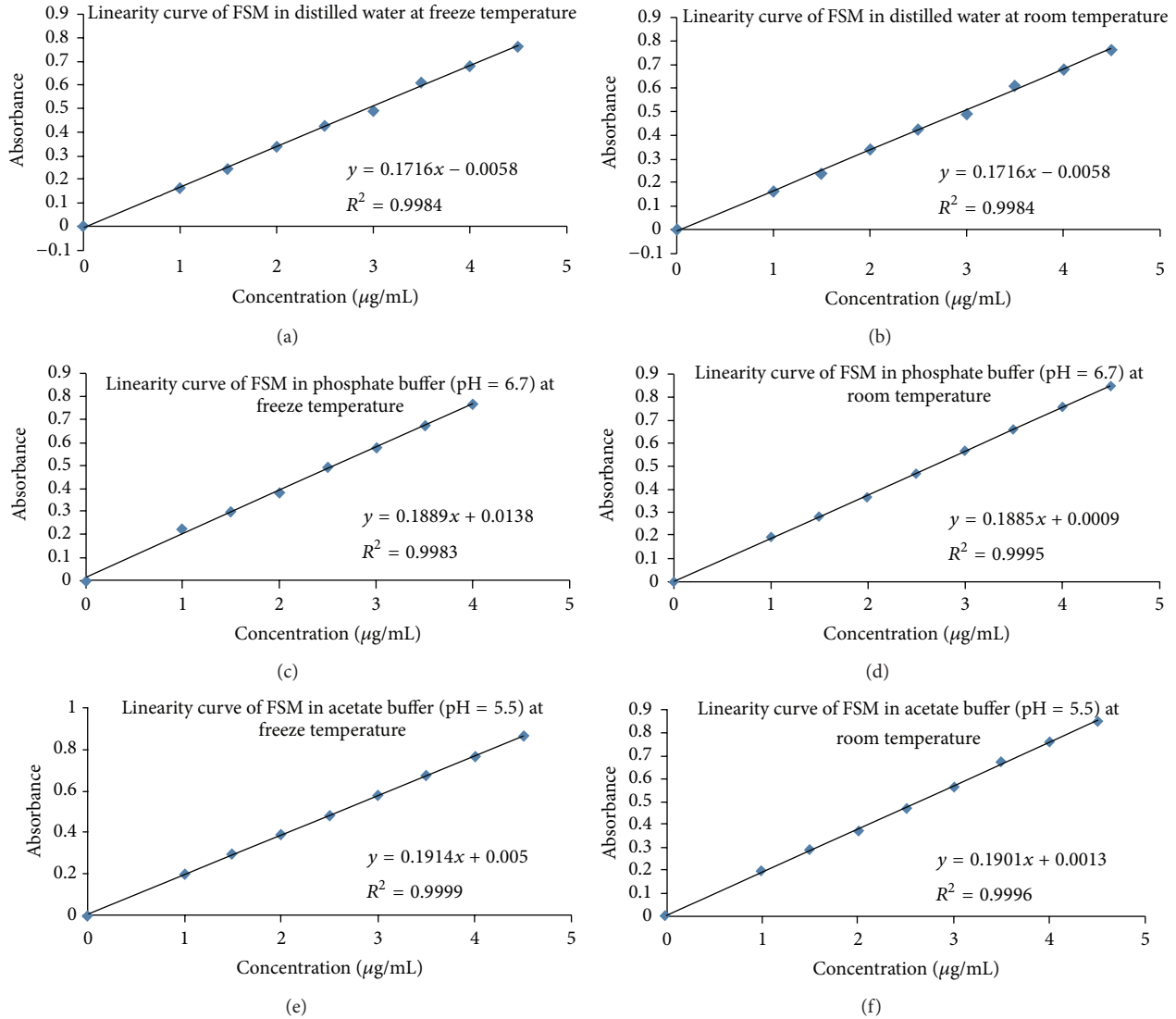
(a)

(b)

(c)

(d)

(e)

(f)

FIGURE 2: Linearity plots of FSM in different media. (a) Linearity curve of FSM in distilled water at freeze temperature, (b) linearity curve of FSM in distilled water at room temperature, (c) linearity curve of FSM in phosphate buffer (pH = 6.7) at freeze temperature, (d) linearity curve of FSM in phosphate buffer (pH = 6.7) at room temperature, (e) linearity curve of FSM in acetate buffer (pH = 5.5) at freeze temperature, and (f) linearity curve of FSM in acetate buffer (pH = 5.5) at freeze temperature.

TABLE 5: Photolytic degradation of FSM ($n = 6$).

Time (hours)	Concentration remaining (μg/mL ± SD)	RSD (%)	Degradation (%)
0	2.415 ± 0.003	0.554	3.532
0.5	2.391 ± 0.002	0.338	4.498
1	2.393 ± 0.001	0.220	4.424
2	2.390 ± 0.000	0.000	4.572
3	2.219 ± 0.001	0.136	11.636
6	2.160 ± 0.003	0.697	14.089
9	2.060 ± 0.002	0.396	18.253
12	1.744 ± 0.001	0.126	31.338

TABLE 6: Thermal stability data of FSM ($n = 6$).

Time (days)	Concentration remaining (μg/mL ± SD)	RSD (%)
1	2.498 ± 0.002	0.449
2	2.493 ± 0.002	0.330
3	2.489 ± 0.002	0.331
4	2.485 ± 0.001	0.217
5	2.489 ± 0.002	0.331
6	2.493 ± 0.002	0.330
7	2.489 ± 0.001	0.125

TABLE 7: Acid catalysed hydrolysis of FSM ($n = 6$).

Strength (N)	Amount remaining to degrade (μg/mL)		
	Time (t) (hours)		
	0	3	6
0.1	2.437	2.437	2.383
0.2	2.344	2.153	2.114
0.3	2.129	2.011	2.015
0.4	2.108	1.966	1.959
0.5	1.957	1.645	1.613
0.6	1.862	1.568	1.508
0.7	1.839	1.469	1.428
0.8	1.791	1.378	1.325
0.9	1.563	1.282	1.114
1	1.417	1.271	0.817

TABLE 8: Base catalysed hydrolysis of FSM ($n = 6$).

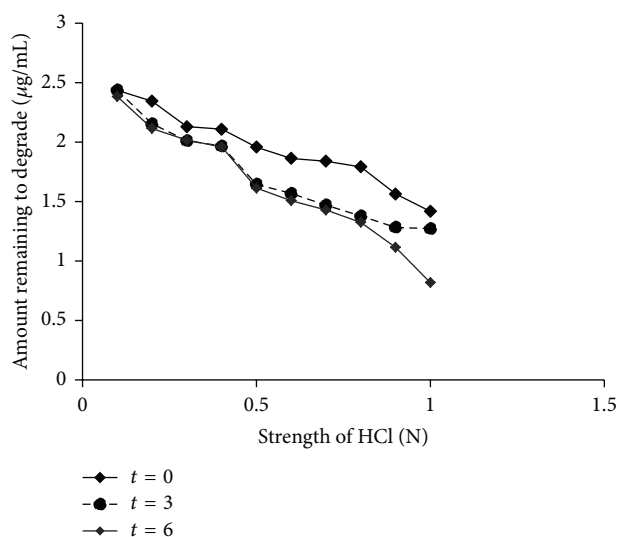| Strength (N)	Amount remaining to degrade (μg/mL)		
	Time (t) (hours)		
	0	3	6
0.1	2.486	2.461	2.451
0.2	1.727	1.693	1.535
0.3	1.697	1.669	1.472
0.4	1.644	1.588	1.423
0.5	1.558	1.549	1.388
0.6	1.443	1.434	1.362
0.7	1.414	1.389	1.346
0.8	1.347	1.339	1.294
0.9	1.283	1.240	1.160
1	1.153	1.056	0.955

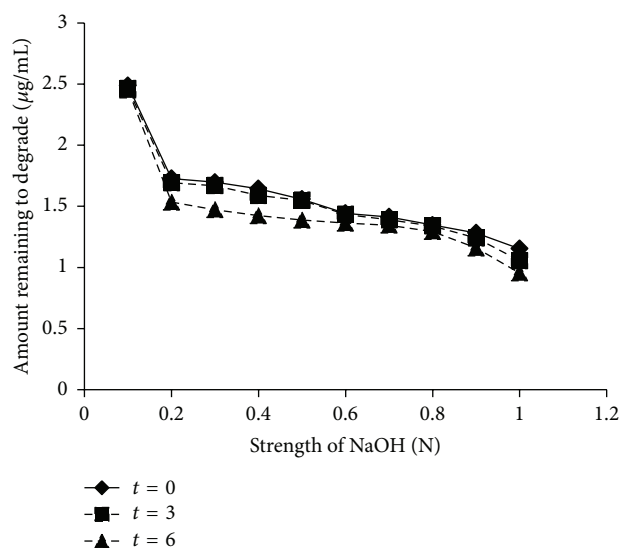FIGURE 3: Forced degradation (acid catalysed hydrolysis) plots of FSM.

FIGURE 4: Forced degradation (base catalysed hydrolysis) plots of FSM.

Exposure of test solution to various strength of NaOH from (0.1 N to 1.0 N) without heating over 6 hours showed its stability in 0.1 N only (Table 8 and Figure 4). In the rest of the sample solutions, significant degradation occurs within 6 hours of exposure (>30%).

In oxidative degradation studies, FSM was found to be stable on the exposure of ≤0.2 mL volume of 3% w/v solution of hydrogen peroxide per 10 mL of test solution without heating. Addition of more volume (>0.2 mL) or solution containing higher concentration of hydrogen peroxide causes immediate degradation of test samples (Table 9 and Figure 5).

Test solution was found to be stable for 90 days on storage at room temperature (25°C ± 0.5°C).

4. Discussion

Different media for analytical method development were selected based on the solubility of drug, economy, and applicability of the developed method. Values of R^2 in calibration plots and during linearity analysis were near to 1 in each case, which show direct positive correlation between the concentration of the solution and the absorption of UV light. Analyses of accuracy and precision data show that the method is accurate and reliable. LOD and LOQ data analysis reflect that the developed method is very sensitive and selective. Forced degradation studies were performed as per ICH guidelines, and it was found that FSM was stable in these stressed conditions.

5. Conclusion

FSM is a potent and selective agonist of 5-HT$_{1B}$ and 5-HT$_{1D}$ receptors. Among all the triptans, it has the longest elimination half-life of 26 hours, even after 11 years of its existence in pharmaceutical market, till now no simple, sensitive, and economical UV-spectroscopy method for the analysis of FSM has been reported. Hence, an attempt had been taken to the developed method in distilled water, phosphate buffer (pH

TABLE 9: Oxidative stability data of FSM.

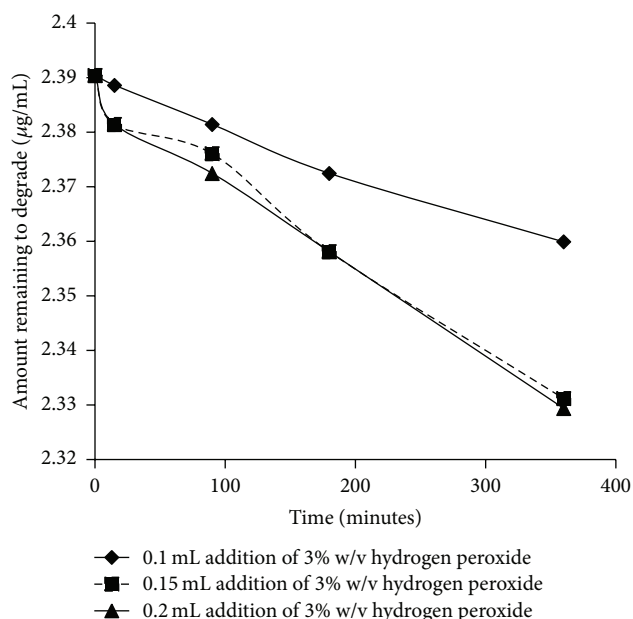| Time (minutes)	Volume of 3% w/v solution added per 10 mL of drug solution					
	0.1 mL Concentration (μg/mL)	0.15 mL. Concentration (μg/mL)	0.2 mL Concentration (μg/mL)	0.1 mL Degradation (%)	0.15 mL Degradation (%)	0.2 mL Degradation (%)
0	2.390	2.390	2.390	4.385	4.385	4.385
15	2.389	2.381	2.381	4.456	4.743	4.743
90	2.381	2.376	2.372	4.743	4.959	5.102
180	2.372	2.358	2.358	5.102	5.676	5.676
360	2.360	2.331	2.329	5.605	6.753	6.825

FIGURE 5: Oxidative degradation plots of FSM using hydrogen peroxide as oxidizing agent.

6.7), and acetate buffer (pH 5.5). The developed method was found to be reliable and accurate. Irrespective to media, FSM shows the maximum absorbance at the wavelength of 244 nm. Forced degradation studies show stability of FSM test solution under the chosen experimental conditions.

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