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Research Article

Stress Degradation Studies on Flupirtine Maleate Using Stability-Indicating RP-HPLC Method

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With the objective of developing an advanced method for rapid separation with shorter runtime, a simple, precise, and accurate stability-indicating isocratic RP-LC method coupled with PDA detector was developed for the quantitative determination of flupirtine maleate in bulk and in capsule dosage form. Good resolution between the peaks for degradation products and the analyte was achieved on a Waters Agilent XDB C_{18} (150 × 4.6 mm, 5 μm) column using mobile phase containing a mixture of phosphate buffer pH 3.36 and acetonitrile in the ratio of 65 : 35. The eluted compounds were monitored at 344 nm and the flow rate employed for the present investigation was 1 mL/min. The newly developed method was validated as per ICH guidelines with respect to specificity, linearity, limit of detection, limit of quantification, accuracy, precision, and robustness. The method may be employed for the assay determination of flupirtine maleate in pharmaceutical dosage forms.

1. Introduction

Flupirtine maleate is a nonopioid centrally acting, structurally dissimilar from other analgesics. It is amino pyridine derivative [1] with the chemical name ethyl 2-amino-6-(4-flurobenzylamino) 3-pyridyl carbamate maleate (Figure 1). The spectrum of action of flupirtine includes analgesia, muscle relaxation, and neuroprotection. The analgesic effect of flupirtine does not appear to be associated with any central opioid effect. Flupirtine does not appear to act on the usual binding sites of the N-methyl-D-aspartate receptor (NMDA) such as glycine site or polyamine site or the magnesium site. There is evidence to show that flupirtine may suppress the opening of the NMDA channel by acting as an oxidizing agent at the redox site of the NMDA receptor. Jakob and Krieglstein found an activation of G protein-regulated inwardly rectifying K^+ channels (GIRKs) by flupirtine in therapeutically relevant concentration ranges [2]. The opening of these channels inhibits exaggerated neuronal action potential generation and controls neuronal excitability [3].

Flupirtine is used for the treatment of acute and chronic pain, that is, for painful increased muscle tone of the posture and motor muscles, primary headache, tumor pain, dysmenorrhea, and pain after orthopedic operations and injuries. Some neuroprotective effects due to NMDA receptor antagonistic properties of flupirtine may also be used in the treatment of Creutzfeldt-Jakob disease, Alzheimer's disease, and multiple sclerosis [4].

Stability testing forms an important part of the process of drug product development. The purpose of stability testing is to provide evidence on how the quality of a drug substance varies with time under the influence of a variety of environmental factors such as temperature, humidity, and light which enables recommendation of storage conditions, retest periods, and establishing shelf life. An extensive literature survey revealed the availability of few analytical methods which includes spectrophotometric, chromatographic and bioanalytical methods [5–9]. Till now no stability-indicating analytical method for the determination of flupirtine maleate was found by computer assisted literature survey. So the ultimate aim of the present investigation was to develop a simple, precise, and stability-indicating HPLC method which resolves all the degradation products formed during the stress degradation studies and to validate the method according

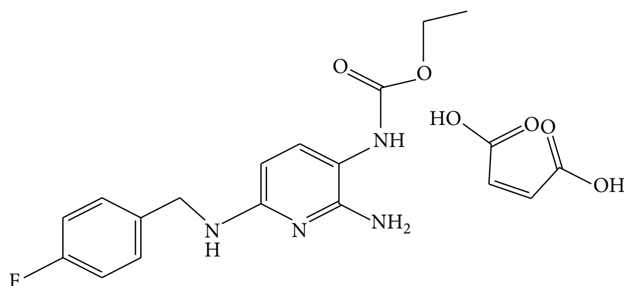

Figure 1: Structure of flupirtine maleate.

to the present ICH guidelines [10]. The optimization of the method separation, stability-indicating property, evaluation, and quantification of flupirtine maleate are reported in the following sections.

2. Experimental

2.1. Materials and Reagents. Authentic sample of flupirtine maleate was kindly supplied by Spectrum Pharma Research Solutions (Andhra Pradesh, India). Flupirtine maleate capsules (Retense-Sun pharmaceuticals Ltd.) containing 100 mg flupirtine maleate as per label claim were purchased from local pharmacy. HPLC grade water, methanol, acetonitrile, and triethylamine were procured from S.D. Fine-chem Ltd. (New Delhi, India). High purity water was prepared by using Millipore Milli-Q plus water purification system, Bedford, MA, USA.

2.2. HPLC Instrumentation. The HPLC consists of a Waters 2695 series with photodiode array detector and Empower 2 software. The chromatographic separation was performed using Agilent XDB C_{18}, 5 μm, 150 × 4.6 mm i.d. column. Separation was achieved using a mobile phase consisting of 0.01 M potassium dihydrogen orthophosphate buffer adjusted to pH 3.36 with dil. orthophosphoric acid : acetonitrile (65 : 35% v/v) solution at a flow rate of 1 mL/min. The eluent was monitored using PDA detection at a wavelength of 344 nm. The column was maintained at ambient temperature and injection volume of 10 μL was used. The mobile phase was filtered through 0.45 μ membrane micron filter prior to use.

2.3. Preparation of Standard Solution. A stock solution of flupirtine (1 mg/mL) was prepared by accurately weighing 100 mg of flupirtine in 100 mL volumetric flask then it was dissolved in methanol and was made up to the volume with diluent (methanol). Aliquots of the standard stock solutions of flupirtine were transferred using A-grade bulb pipettes to 10 mL volumetric flask and solutions were made up to the volume with the diluent to give the final concentrations of 20, 50, 70, 100, 120, and 150 μg/mL, respectively.

2.4. Preparation of Sample Solution. To determine the content of flupirtine maleate in capsules (label claim: 100 mg flupirtine maleate), 20 capsules were opened and the contents were weighed and mixed. An aliquot of powder equivalent to the weight of one capsule was accurately weighed and transferred to 100 mL volumetric flask and was dissolved in diluent and made up to the volume with diluent. The volumetric flask was sonicated for 30 minutes to affect complete dissolution. The solutions were filtered through a 0.45 μm nylon filter. Suitable aliquots of the filtered solution were added to a volumetric flask and made up to the volume with diluent to yield the concentration of 100 μg/mL. A 10 μL volume of each sample solution was injected into HPLC six times, under the conditions described above. The peak areas were measured at 344 nm and concentrations in the sample were determined by comparing the area of sample with that of the standard.

2.5. Method Validation

2.5.1. Linearity. Linearity was established by least squares linear regression analysis of the calibration curve. The linearity of response for flupirtine assay method was determined by preparing and injecting solutions with concentrations of about 20, 50, 70, 100, 120, and 150 μg/mL of flupirtine. Peak areas of flupirtine were plotted against their respective concentrations and linear regression analysis was performed on the resultant curve.

2.5.2. Precision. Precision was measured in terms of repeatability of application and measurement. The system precision was carried out using six replicate injections of standard concentration. The method precision was carried out using six replicate injections of sample concentration and the %RSD was calculated.

2.5.3. Accuracy. Accuracy of the method was determined by standard addition method. A known amount of standard drug was added to the fixed amount of preanalyzed sample solution. The standard addition method was performed at 50%, 100%, and 150% levels of sample solution. The resulting solutions were analyzed in triplicate at each level as per the ICH guidelines.

2.5.4. LOD and LOQ. Limit of detection is defined as lowest concentration of analyte that can be detected, but not necessarily quantified, by the analytical method. Limit of detection is determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be reliably detected.

Limit of quantification is the concentration that can be quantitated reliably with a specified level of accuracy and precision. Consider the following:

$$\text{Limit of Detection} = \frac{\sigma \times 3.3}{S},$$
$$\text{Limit of Quantitation} = \frac{\sigma \times 10}{S}, \quad (1)$$

where σ = the standard deviation of the response. S = the slope of the calibration curve (of the analyte).

Table 1: Linearity regression data of flupirtine maleate ($n = 3$).

Parameter	Flupirtine maleate
Linearity	20–150 μg/mL
Correlation coefficient	0.9996
Slope	30296
Intercept	15084

Table 2: System precision of flupirtine maleate ($n = 3$).

S. no.	Area of flupirtine maleate	t_R (min)
(1)	1703662	3.107
(2)	1705406	3.132
(3)	1708279	3.137
(4)	1706769	3.142
(5)	1705713	3.159
(6)	1704132	3.163
Mean	1705660	3.14
S.D	1702	0.0202
%RSD	0.1	0.64

2.5.5. Robustness. The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate, variations in method parameters and provides an indication of its reliability during normal usage. Robustness of the developed method was demonstrated by evaluating the influences of small changes in the experimental conditions like temperature, mobile phase, and flow rate. Robustness of method was carried out with variation of flow rate ±0.1 mL/min, mobile phase ±5%, and temperature ±5°C.

2.5.6. Specificity. Specificity is the ability of the analytical method to measure the analyte free from interference due to other components. Specificity was determined by comparing test results obtained from analysis of sample solution containing ingredients with the test results obtained from standard drug.

2.5.7. Forced Degradation Studies [11]. To determine whether the analytical method and assay were stability-indicating, flupirtine standard drug was stressed under various conditions to conduct forced degradation studies. Intentional degradation was attempted to stress conditions of photolytic degradation, acid hydrolysis (using 1N HCl), base hydrolysis (1N NaOH), oxidative degradation (20% H_2O_2), and thermal treatment (heated at 100°C for 5 hrs) to evaluate the ability of the proposed method to separate flupirtine from its degradation products.

2.5.8. Acid and Alkaline Degradation. Forced degradation in acidic media was performed by taking 100 mg of flupirtine pure drug into 100 mL volumetric flask followed by the addition of 1N HCl and 70 mL diluent and the mixture was heated under reflux for 2 hrs at 60°C, and the volume was made up to the mark with diluent and filtered. The resultant solution was diluted to obtain 100 μg/mL solutions and 10 μL was injected into the system and the chromatograms were recorded to assess the stability of sample. Similarly, forced degradation in alkaline medium was performed using 1 N NaOH.

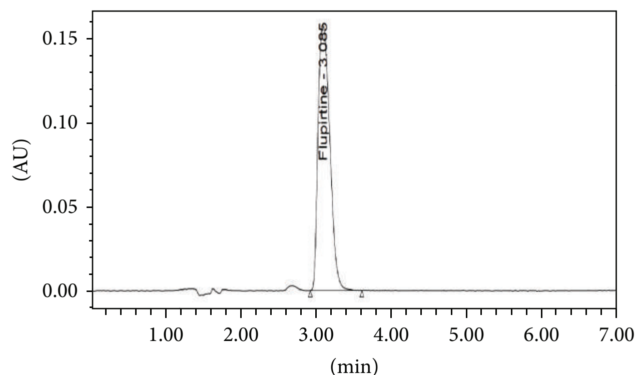

Figure 2: Typical chromatogram of flupirtine maleate standard.

2.5.9. Oxidative Degradation. Oxidative degradation was performed by taking 100 mg of flupirtine pure drug in 100 mL volumetric flask then 1 mL of 20% H_2O_2 and 70 mL of diluent were added and the mixture was heated under reflux for 2 hrs at 60°C, and the volume was made up to the mark with diluent. Appropriate aliquot was taken from the above solution and diluted with diluent to obtain a final concentration of 100 μg/mL. The chromatogram was recorded with the help of HPLC.

2.5.10. Photostability. Photostability was performed by exposing the pure drug to sunlight for 7 days and it was transferred to 100 mL volumetric flask and the volume was made up to the mark with diluent. Appropriate aliquot was taken from the above solution and diluted to obtain a final concentration of 100 μg/mL. The chromatogram was recorded to assess the stability of sample.

2.5.11. Thermal Degradation. The standard drug was placed in an oven at 100°C for 5 hrs to study dry heat degradation. For HPLC study, the resultant solution was diluted to 100 μg/mL solution and 10 μL was injected into the system and the chromatograms were recorded to assess the stability of the sample.

3. Results and Discussions

3.1. Method Development. The main target of the chromatographic method is to achieve the separation of flupirtine maleate along with the degradation products. The maximum absorption wavelength of the reference drug substance and the forcibly degraded drug solution is 344 nm; hence this wavelength was selected as the detection wavelength for LC analysis. Pure drug along with its degraded products was injected and run in different solvent systems. Initially acetonitrile and water in different ratios were tried. Since acetonitrile : water was not able to give good peak symmetry

Table 3: Method precision of flupirtine maleate ($n = 3$).

S. no.	Area of flupirtine maleate	t_R (min)
(1)	1729347	3.067
(2)	1707363	3.085
(3)	1734438	3.089
(4)	1721039	3.160
(5)	1707799	3.165
(6)	1724954	3.165
Mean	1720823	3.121
S.D	11188	0.0461
%RSD	0.65	1.47

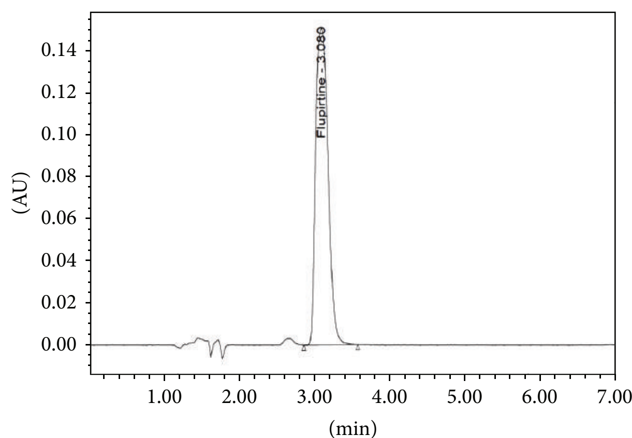

Figure 3: Sample chromatogram of flupirtine maleate.

with acceptable retention time, an attempt to improve peak symmetry was made by adding phosphate buffer to the mobile phase resulting in excellent overall chromatography with appropriate peak symmetry and complete base line resolution. Finally the mobile phase consisting of potassium dihydrogen orthophosphate buffer and acetonitrile (65 : 35 v/v) with pH 3.36 adjusted with orthophosphoric acid was selected for validation purpose and stability studies. Several preliminary chromatographic runs were performed to investigate the suitability for drug content estimation and cost because of the increasing importance of rapid economic analysis in pharmaceutical analysis to increase the throughput. By using the developed analytical method, system suitability parameters, USP tailing factor, resolution of flupirtine maleate, and the degradation products were calculated and were found to be in the specified range. The method was also validated with respect to parameters including linearity, limit of detection (LOD), and limit of quantitation (LOQ), recovery, precision, accuracy, robustness, and specificity.

3.2. Linearity. Calibration curves were obtained for flupirtine from which the linear regression equation was computed and found to be $Y = 30296\,x - 15084$ and correlation coefficient (R^2) was found to be 0.9996. The linear regression data values are shown in Table 1.

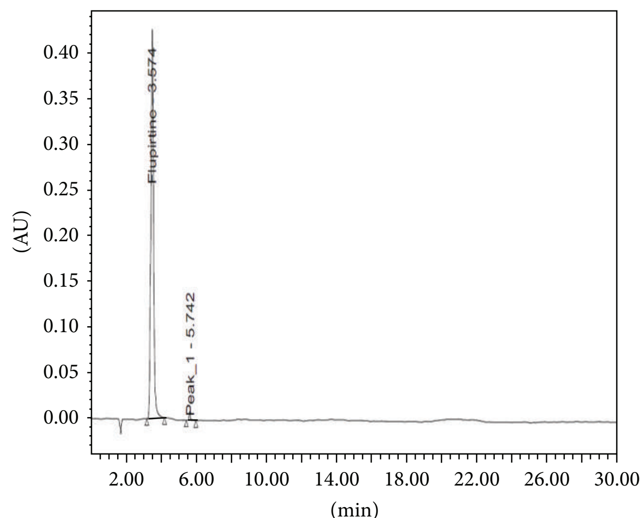

Figure 4: Typical chromatogram obtained after acidic degradation of flupirtine maleate (peak 1 degradation product showing R_t at 5.742 min).

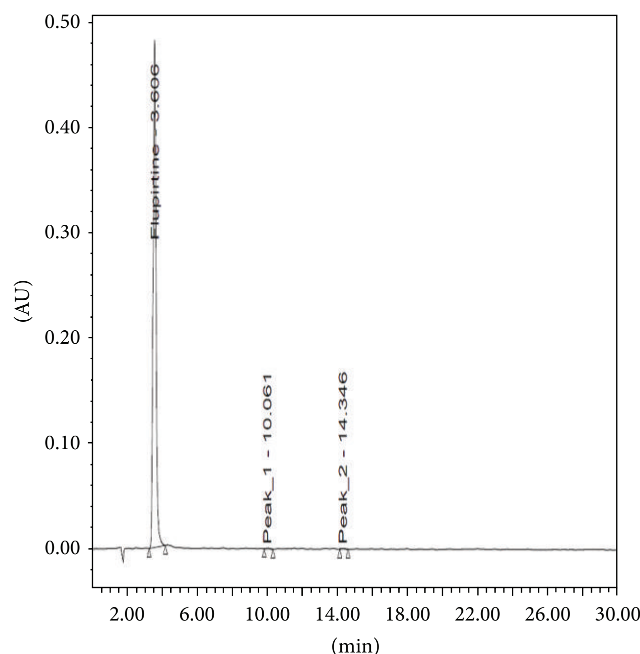

Figure 5: Chromatogram of alkali stressed sample of flupirtine maleate showing degradation peaks at 10.061 and 14.346 min, respectively.

3.3. Precision. System precision was carried out by injecting standard flupirtine maleate six times and the method precision was carried out by injecting the sample flupirtine maleate for six times. The %RSD for repeatability of both standard and sample solutions was found to be 0.1 and 0.56, respectively. This shows that precision of the method is satisfactory as % relative standard deviation is not more than 2.0%. The results are depicted in Tables 2 and 3, respectively.

Table 4: Recovery data of flupirtine maleate ($n = 3$).

Concentration level (%)	Spiked concentration (μg/mL)	Concentration obtained (μg/mL)	% Recovery	Mean % recovery
50	50	49.7	99.40	99.83
	50	50.04	100.09	
	50	50.00	100.01	
100	100	99.95	99.95	100.03
	100	99.98	99.98	
	100	100.17	100.17	
150	150	150.49	100.33	100.28
	150	150.65	100.43	
	150	150.13	100.09	

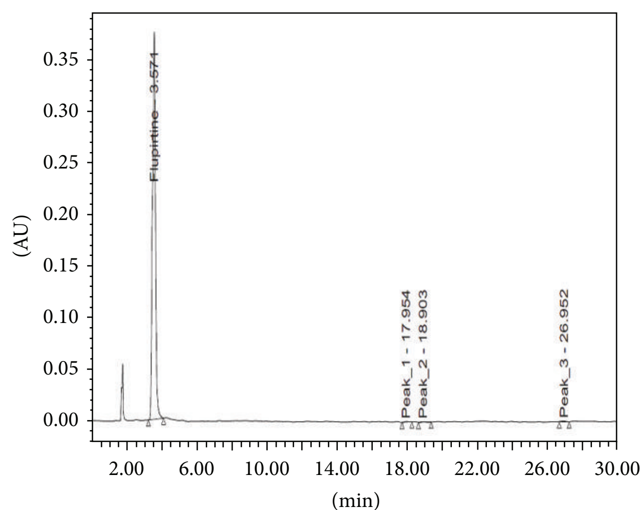

Figure 6: Chromatogram of oxidative stressed sample of flupirtine maleate. (peaks 1, 2, and 3 are degradation products having R_t at 17.954 min, 18.903 min, and 26.952 min, resp.)

Table 5: Robustness of the method ($n = 3$).

S. No	Parameter	Modification	Retention time (min)	%RSD
(1)	Flow rate	0.9 mL/min	3.16	0.86
		1.1 mL/min	2.84	0.97
(2)	Mobile phase	−5%	2.97	0.72
		+5%	3.05	0.89
(3)	Temperature	−5°C	3.11	0.40
		+5°C	3.14	0.48

3.4. Accuracy. The accuracy of the method was established by recovery studies. The recovery of flupirtine maleate by proposed method is satisfactory and means recovery was found to be in the range of 99.44–100.28%. The results are shown in Table 4.

3.5. LOD & LOQ. LOD and LOQ were determined by the standard deviation method and were found to be 1.64 and 4.97 μg/mL, respectively.

Table 6: Summary of forced degradation results.

S. No	Stress condition	t_R in min	Peak area	% purity	% degradation
(1)	Acid degradation	3.574	1669932	97.21	2.79
(2)	Base degradation	3.606	1648315	95.95	4.05
(3)	Oxidative degradation	3.571	1597637	93.00	7.0
(4)	Photolytic degradation	3.640	1546934	90.05	9.95
(5)	Thermal degradation	3.533	1629303	94.84	5.16

Table 7: Determination of flupirtine maleate in capsule dosage form.

Parameter	Value
Label claim mg/capsule	100 mg
Drug content % ± SD*1	99.68 ± 0.62
%RSD*2	0.86

*1Standard deviation. *2Relative standard deviation.

3.6. Robustness. Under all the deliberately altered chromatographic conditions (flow rate, mobile phase, and temperature), all peaks were adequately resolved and elution orders remained unchanged which indicate that the method is robust. The results are summarized in Table 5.

3.7. Specificity. The method was declared specific, as there were no interfering peaks at the retention time of flupirtine and the flupirtine peak was well resolved from the peaks of all possible degradation products. A typical chromatogram of standard and sample flupirtine for specificity study is shown in Figures 2 and 3, respectively.

3.8. Forced Degradation Studies. The results of stress testing studies indicated a high degree of selectivity of the method. Typical chromatograms obtained from stressed samples are shown in Figures 4, 5, 6, 7, and 8, respectively. The drug was unstable under acid stress conditions when kept for 2 hrs at 60°C. The drug was degraded approximately to 2.79%. Also it

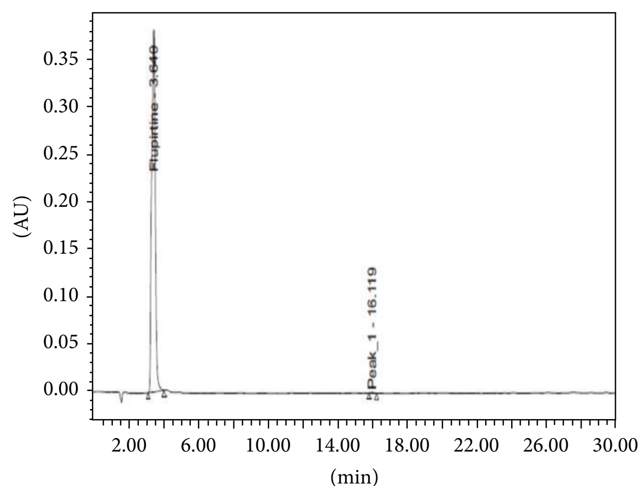

Figure 7: Typical chromatogram of photolytic stressed sample showing degradation peak at 16.119 min.

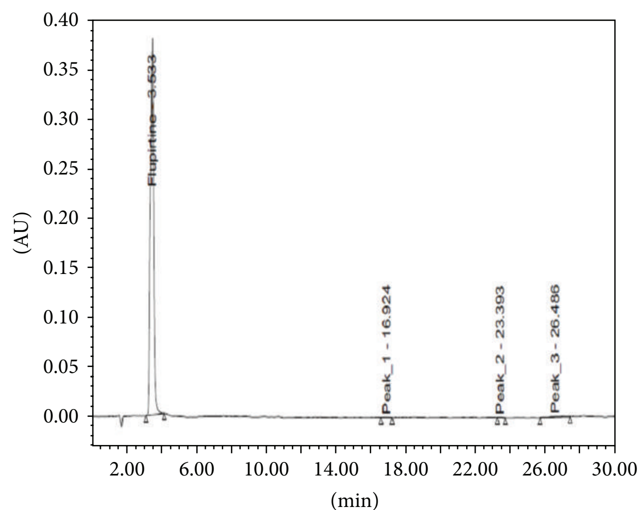

Figure 8: Typical chromatogram for thermal stressed degradation of flupirtine. 1, degradation product R_t: 16.924 min; 2, degradation product R_t: 23.39 min; and 3, degradation product R_t: 26.486 min.

was unstable in basic conditions when kept for 2 hrs at 60°C. The drug was degraded approximately to 4.05%. The drug was found to be degraded around 7% when kept under oxidative stress conditions with 20% H_2O_2 for 2 hrs at 60°C. When the solid drug powder was exposed to light for 7days, the drug underwent 9.95% degradation. The drug was unstable under thermal stress conditions when kept for 5 hrs at 100°C and the amount of degradation was found to be 5.16% (Table 6). The proposed method was applied to the determination of flupirtine maleate in retense capsules. The result of this assay yielded 99.68 ± 0.6 (%RSD = 0.86) of label claim of the capsules. The results of the assay indicate that the method is selective for the assay of flupirtine without interference of the excipients used in these capsules (Table 7).

4. Conclusions

The developed HPLC technique is precise, specific, accurate, and stability-indicating. Statistical analysis proves that the method is reproducible and selective for the analysis of flupirtine in pharmaceutical dosage form. The method can be used to determine the purity of the drug available from various sources. As the method separates the drug from its degradation products, it can be employed as stability-indicating.

Acknowledgment

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References

[1] T. P. Aneesh and D. Amal, “Method Development and Validation for the estimation of flupirtne maleate in bulk and pharmaceutical dosage form by UV- Visible spectrophotometry,” *International Research Journal of Pharmacy*, vol. 2, no. 12, pp. 179–182, 2011.

[2] R. Jakob and J. Krieglstein, “Influence of flupirtine on a G-protein coupled inwardly rectifying potassium current in hippocampal neurones,” *British Journal of Pharmacology*, vol. 122, no. 7, pp. 1333–1338, 1997.

[3] J. Devulder, “Flupirtine in pain management: pharmacological properties and clinical use,” *CNS Drugs*, vol. 24, no. 10, pp. 867–881, 2010.

[4] C. Klawe and M. Maschke, “Flupirtine: pharmacology and clinical applications of a nonopioid analgesic and potentially neuroprotective compound,” *Expert Opinion on Pharmacotherapy*, vol. 10, no. 9, pp. 1495–1500, 2009.

[5] W. Xue-yan, S. Yi, C. Xiu-feng, L. Xiao-wei, and C. Ruo-bing, “Determination of dissociation constant for Flupirtine by UV,” *West China Journal of Pharmaceutical Sciences*, vol. 25, no. 3, 2010.

[6] W. Hai-yang, Z. Yan-ling, and N. Lei, “Determination of flupirtine maleate capsules by RP-HPLC,” *Qilu Pharmaceutical Affairs*, vol. 29, no. 10, 2010.

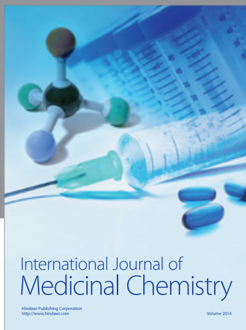
[7] X. Chen, D. Zhong, H. Xu, B. Schug, and H. Blume, “Simultaneous determination of flupirtine and its major active metabolite in human plasma by liquid chromatography-tandem mass spectrometry,” *Journal of Chromatography B*, vol. 755, no. 1-2, pp. 195–202, 2001.

[8] L. Long-xing, X. Dong-ya, and G. Tao, “Determination of the concentration of flupirtine in human plasma by RP-HPLC,” *Journal of Shenyang Pharmaceutical University*, vol. 27, no. 07, pp. 559–562, 2010.

[9] K. Kandasamy, V. S. Gowdra, H. Nammalvar, and A. K. K. S. Govindarajan, “Bioanalytical method development, validation and quantification of flupirtine maleate in rat plasma by liquid chromatography-tandem mass spectrometry,” *Arzneimittel-Forschung/Drug Research*, vol. 61, no. 12, pp. 693–699, 2011.

[10] ICH Topic Q2 (R1), *Validation of Analytical Procedures: Methodology*, The European Agency for the Evaluation of Medicinal Products, Geneva, Switzerland, 2005.

[11] ICH (QIB), “Harmonized tripartite guideline, stability testing: photostability testing of new drug substances and products,” in *Proceedings of the International Conference on Harmonization*, Geneva, Switzerland, November 1996.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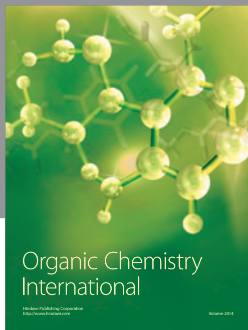
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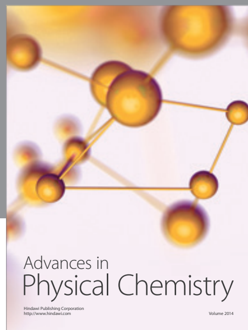
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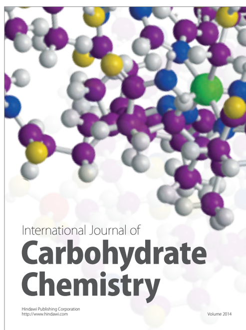
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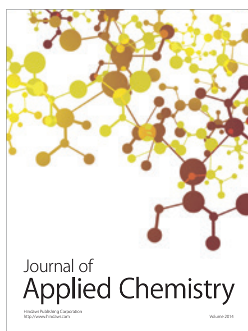
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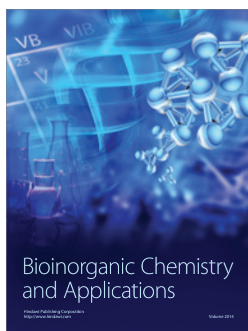
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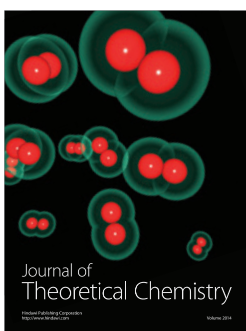
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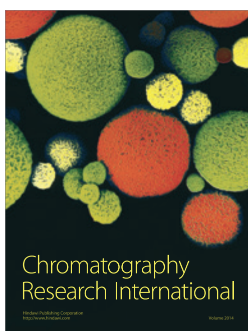
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