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Research Article

A NOVEL STABILITY-INDICATING RP-HPLC METHOD FOR THE ESTIMATION OF FLUPIRTINE IN PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

A stability-indicating RP-HPLC method was developed and validated for the estimation of Flupirtine in pharmaceutical dosage forms. An Inertsil ODS C18 column (150 mm x 4.6 mm), 5 µ particle size was used as stationary phase with mobile phase consisting of 0.01M potassium dihydrogen orthophosphate buffer having pH 3.6 adjusted with orthophosphoric acid and acetonitrile in the ratio of 75:25 V/V. The flow rate was maintained at 1 mL/min and effluents were monitored at 246 nm. The retention time was 4.12 min. The stress studies were performed as per ICH guidelines under acidic, alakiline, neutral, oxidative, UV and thermal conditions. The linearity of the method was observed in the concentration range of 100-600 µg/mL with a correlation coefficient of 0.999. The percentage assay of Flupirtine was found to be 99.84 %. The method was validated for its accuracy, precision and system suitability studies. The results obtained in the study were within the limits of ICH guidelines and hence this method can be used for the estimation of Flupirtine in pharmaceutical dosage forms.

Keywords: Flupirtine, RP-HPLC, Degradation, Validation.

INTRODUCTION

Flupirtine is an amino pyridine derivative with non-opioid analgesic activity[1]. Flupirtine is used as an analgesic for acute and chronic pain, in moderate to severe cases[2]. Chemically it is ethyl{2-amino-6-[(4-fluorobenzyl)amino]pyridin-3-yl} carbamate (Fig. 1)[3]. Flupirtine is a selective neuronal potassium channel opener that also has NMDA receptor antagonist properties[4-6].

Literature survey revealed few analytical methods such as UV spectrophotometric[7,8], HPLC[9-13], UPLC[14] and LC-MS[15-17] methods have been reported for the estimation of Flupirtine and hence we tried to develop and validate a new HPLC method[18] as per ICH guidelines[19,20] for the estimation of Flupirtine in bulk and pharmaceutical dosage forms.

MATERIALS AND METHODS

Instrumentation

To develop a high pressure liquid chromatographic method for quantitative estimation of Flupirtine using Waters HPLC system an Inertsil ODS C18 column (150 mm x 4.6 mm, 5µ) was used. The instrument is equipped with an auto sampler and DAD or UV detector. A 20 µL rheodyne injector port was used for injecting the samples. Data was analyzed by using Empower 2 software.

Chemicals and solvents

The working standard of Flupirtine was provided as gift sample from Spectrum Labs, Hyderabad, India. The market formulation LUPIRTIN tablets (Flupirtine 100 mg) were procured from local market. HPLC grade water, methanol and

acetonitrile were purchased from E.Merck (India) Ltd, Mumbai, India. Potassium dihydrogen orthophosphate, triethylamine and orthophosphoric acid of AR grade were obtained from S.D. Fine Chemicals Ltd, Mumbai, India.

Chromatographic conditions

0.01M Potassium dihydrogen orthophosphate buffer pH 3.6 adjusted with orthophosphoric acid and acetonitrile in the ratio of 75:25 V/V was found to be the most suitable mobile phase for ideal chromatographic separation of Flupirtine. The solvent mixture was filtered through a 0.45 µ membrane filter and sonicated before use. It was pumped through the column at a flow rate of 1.0 mL/min. Injection volume was 20 µL and the column was maintained at ambient temperature. The column was equilibrated by pumping the mobile phase through the column for at least 30 minutes prior to the injection of the drug solution. The detection of the drug was monitored at 246 nm. The run time was set as 8 min.

Preparation of phosphate buffer pH 3.6

Accurately weighed and transferred 1.36 gm of potassium dihydrogen orthophosphate in a 1000 mL of volumetric flask and about 900 mL of milli-Q water added. Then 1 mL of triethylamine was added and sonicated to degas and finally made upto the volume with water, then adjusted to pH to 3.6 with dilute ortho phosphoric acid solution.

Preparation of mobile phase and diluent

750 mL of the 0.01M potassium dihydrogen orthophosphate buffer was mixed with 250 mL of acetonitrile. The solution was degassed in an ultrasonic water bath for 5 minutes and filtered through 0.45 µm filter under vacuum. The methanol was used as diluent.

Preparation of standard solution

40 mg of Flupirtine was accurately weighed, transferred to 10 mL volumetric flask and is dissolved in 7 mL of the diluent. The solution was sonicated for few minutes to dissolve the drug completely. Then it is filtered through 0.45 µ filter and the volume is made up to 10 mL with diluent to get a concentration of 4 mg/mL stock solution. Further pipetted 1 mL of the above stock solution into a 10 mL volumetric flask and diluted upto the mark with diluent to obtain required concentrations.

Preparation of sample solution

Ten commercial tablet contents were weighed to obtain the average tablet content weight and the contents were mixed. A sample of the mixed tablet content of the powder equivalent to 100 mg of Flupirtine was accurately weighed, transferred to 10 mL volumetric flask and is dissolved in 7 mL of the diluent. The solution was sonicated for few minutes to dissolve the drug completely. Then it is filtered through 0.45 µ filter and the volume is made up to 10 mL with diluent to get a concentration of 10 mg/mL stock solution. Further pipetted 0.4 mL of the above stock solution into a 10 mL volumetric flask and diluted up to the mark with diluent to obtain required concentrations of Flupirtine in pharmaceutical dosage form.

Degradation Studies

Acid degradation studies

To 1 mL of s tock solution of Flupiritine, 1mL of 2N hydrochloric acid was added and refluxed for 30 min at 600C. The resultant solution was diluted to obtain 400 µg/mL and 10 µL were injected into the system and the chromatograms were recorded to assess the stability of sample.

Alkali degradation studies

To 1 mL of stock solution of Flupiritine, 1 mL of 2 N sodium hydroxide was added and refluxed for 30 min at 60^0C. The resultant solution was diluted to obtain 400 µg/mL solution and 10 µL were injected into the system and the chromatograms were recorded to assess the stability of sample.

Oxidation studies

To 1 mL of stock solution of Flupiritine, 1 mL of 20% hydrogen peroxide (H_2O_2) was added separately. The solutions were kept for 30 min at 60^0C. For HPLC study, the resultant solution was diluted to obtain 400 µg/mL solution and 10 µL were injected into the system and the chromatograms were recorded to assess the stability of sample.

Dry heat degradation studies

The standard drug solution was placed in oven at 105^0C for 6 hours to study dry heat degradation. For HPLC study, the resultant solution was diluted to 400 µg/mL solution and 10 µL were injected into the system and the chromatograms were recorded to assess the stability of the sample.

Photostability studies
The photochemical stability of the drug was also studied by exposing the 400 µg/mL solution to UV Light by keeping the beaker in UV Chamber for 7 days or 200 Watt hours/m^2 in photo stability chamber. For HPLC study, the resultant solution was diluted to obtain 400 µg/mL solution and 10 µL were injected into the system and the chromatograms were recorded to assess the stability of sample.

Neutral degradation studies
Stress testing under neutral conditions was studied by refluxing the drug in water for 6 hours at a temperature of 60ºC. For HPLC study, the resultant solution was diluted to 400 µg/mL solution and 10 µL were injected into the system and the chromatograms were recorded to assess the stability of the sample.

Method Validation
Linearity
Several aliquots of standard solution of Flupirtine was taken in different 10 mL volumetric flasks and diluted up to the mark with diluent such that the final concentrations of Flupirtine were in the range of 100 to 600 µg/mL. Evaluation of the drug was performed with UV detector at 246 nm and peak area was recorded for all the peaks. The correlation coefficient value of Flupirtine was 0.999. The results show that an excellent correlation exists between peak area and concentration of drug within the concentration range indicated.

Limit of detection and limit of quantification
The limit of detection (LOD) and limit of quantification (LOQ) of the developed method were determined by injecting progressively low concentrations of the standard solution using the developed HPLC method. The LOD for Flupirtine was found to be 0.47 µg/mL. The LOQ for Flupirtine was found to be 1.45 µg/mL.

System suitability
System suitability parameters like retention time, resolution, theoretical plates and tailing factor were calculated and compared with standard values.

Accuracy
The accuracy of the method was assessed by recovery studies of Flupirtine in the dosage form at three concentration levels. A fixed amount of pre-analyzed sample was taken and standard drug was added at 50%, 100% and 150% levels. Each level was repeated three times. The content of Flupirtine per tablet was calculated. The mean recovery of Flupirtine was in the range of 99.84 % that shows there is no interference from excipients and the lower values of RSD of assay indicate the method is accurate.

Precision
The precision was determined for Flupirtine in terms of intra-day and inter-day precision. For intra-day precision evaluation, a standard solution of fixed concentration was injected at various time intervals and % RSD for Flupirtine was 0.81% (limit %RSD < 2.0%). In addition, the inter-day precision was studied by injecting the same concentration of standard solution on consecutive days and the % RSD for Flupirtine was 0.65% (limit %RSD < 2.0%).

Ruggedness and robustness
The ruggedness of the method was determined by carrying out the experiment on different instruments by different operators using different columns of similar types. Robustness of the method was determined by making slight changes in the chromatographic conditions. It was observed that there were no marked changes in the chromatograms, which demonstrated that the HPLC method so developed is rugged and robust.

Assay
20 µL of each standard and sample solution were injected and from the peak area of Flupirtine, amount of each drug in samples were computed. The result of assay undertaken yielded 100.30 % of label claim of Flupirtine.

RESULTS AND DISCUSSION
The stability-indicating RP-HPLC procedure was optimized with a view to develop an accurate method in tablet dosage form using Inertsil ODS C18 column (150 x 4.6 mm, 5 µ) in isocratic mode with mobile phase composition of 0.01M Potassium dihydrogen orthophosphate buffer pH 3.6 adjusted with orthophosphoric acid and acetonitrile in the ratio of 75:25 V/V. The use of phosphate buffer and acetonitrile in the ratio of 75:25 V/V resulted in peak with good shape and resolution. The flow rate was 1.0 mL/min and the drug component was measured with UV detector at 246 nm. The results of optimized HPLC conditions were shown in Table 1. Typical chromatograms of drug Flupirtine standard & sample were shown in Fig. 2 & Fig. 3.

The method was linear in the range of 100 to 600 µg/mL for Flupirtine with correlation coefficient of 0.999. The linearity results were shown in Table 2 and the linearity curve was shown in Fig. 4. The % recovery was found to be 99.84 % for Flupirtine, which indicate the method is accurate. The results of recovery studies were shown in Table 3. The % RSD for intra-day precision and inter-day precision for Flupirtine were found to be 0.81 and 0.65, which indicate the method is precise. The results of precision studies were shown in Table 4.

The % assay was found to be 100.30% for Flupirtine and the assay results were shown in Table 5. The retention time of Flupirtine was 4.124 min. The number of theoretical plates was 4760 and tailing factor was 1.40 for Flupirtine, which indicates efficient performance of the column. The limit of detection and limit of quantification for Flupirtine were found to be 0.47 µg/mL and 1.45 µg/mL, which indicate the sensitivity of the method. The summary of system suitability parameters and validation parameters were shown in Table 6. Validated method was applied for the determination of Flupirtine in commercial formulations. During the degradation studies, Flupirtine was investigated under stress conditions i.e., the reaction to acid (0.1 M HCl), base (0.1 M NaOH), hydrogen peroxide (10 % H_2O_2), thermal, UV light and neutral conditions. The chromatograms showing degraded peaks give evidence of degraded products which are evident in Table 7. It was observed that Flupirtine was fairly unstable in almost all stress conditions except under neutral conditions. Five degraded products were released by the drug under acidic conditions which corresponds to five degraded peaks in the chromatogram shown in Fig. 5. Similarly, five degraded products were released by the drug under basic conditions which corresponds to five degraded peaks in the chromatogram shown in Fig. 6. Four degraded products were released by the drug under peroxide conditions which corresponds to four degraded peaks in the chromatogram shown in Fig. 7. But, only two degraded products were released by the drug under thermal conditions and UV which corresponds to two degraded peaks in the chromatograms shown in Fig. 8 & Fig. 9. One degraded peak is released under neutral condition and the chromatogram shown in Fig. 10.

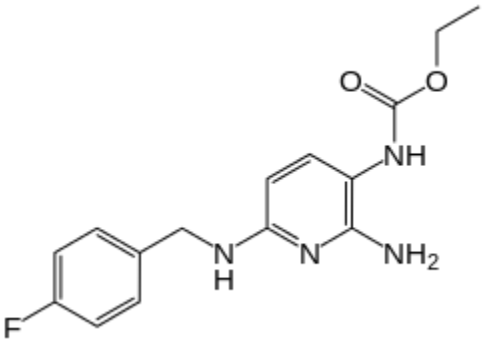

Fig. 1: Chemical structure of Flupirtine

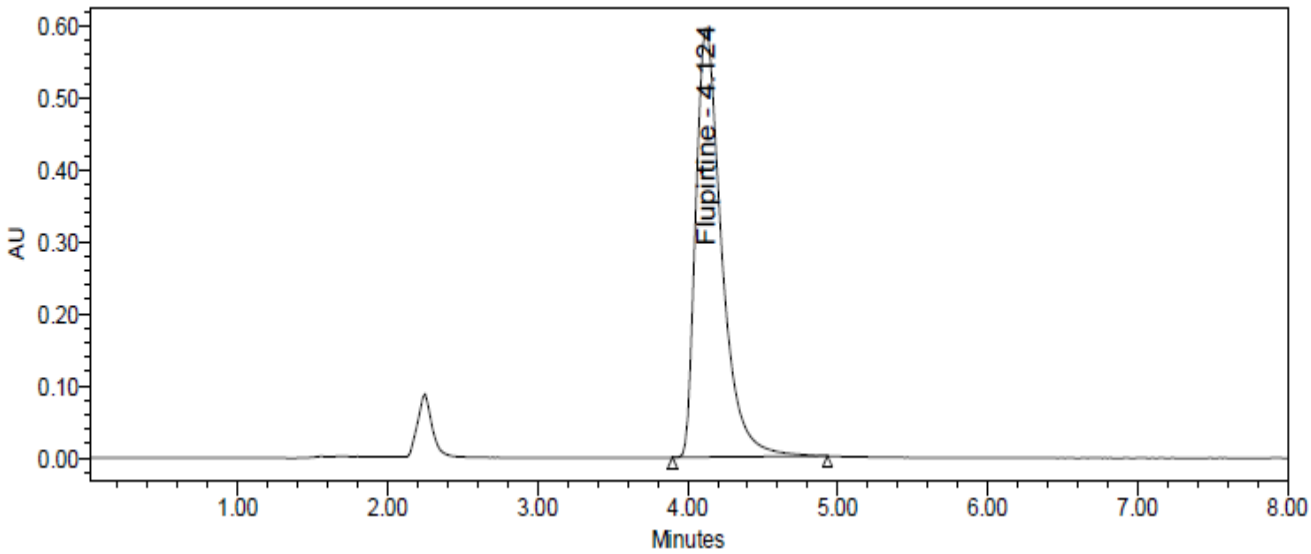

Fig. 2: Typical chromatogram of Flupirtine standard

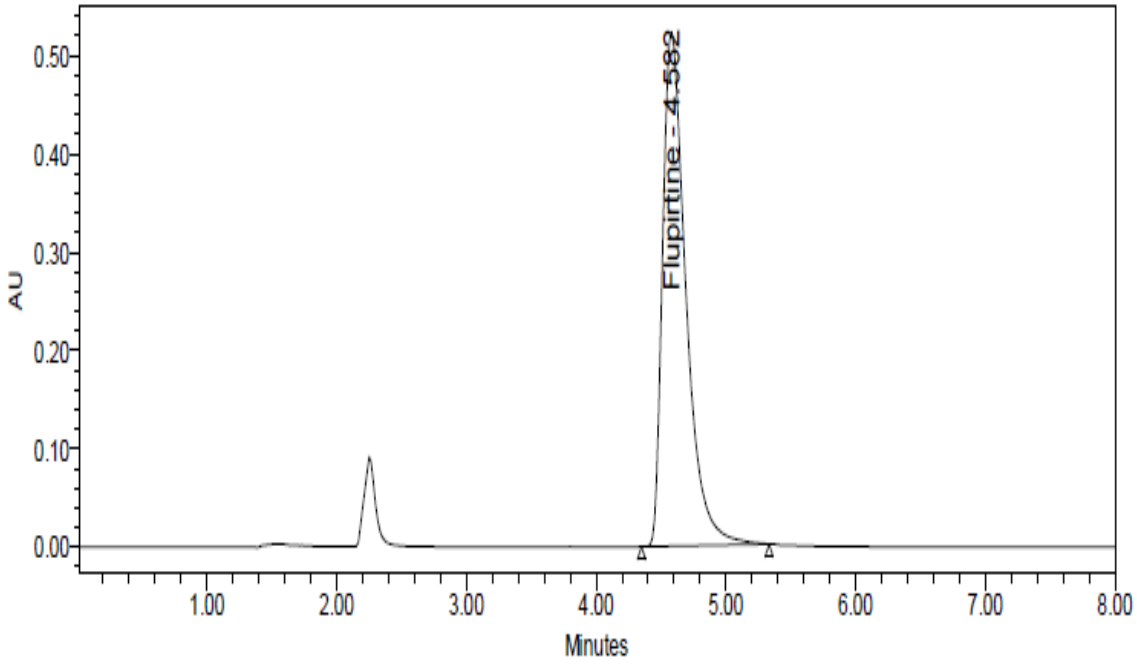

Fig. 3: Typical chromatogram of Flupirtine sample

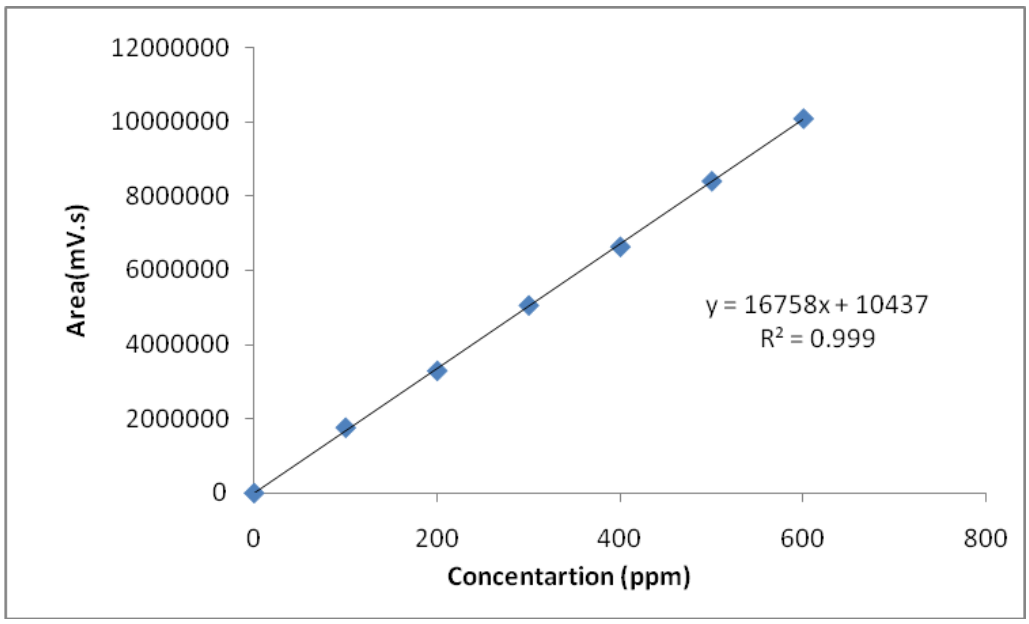

Fig. 4: Linearity curve of Flupirtine

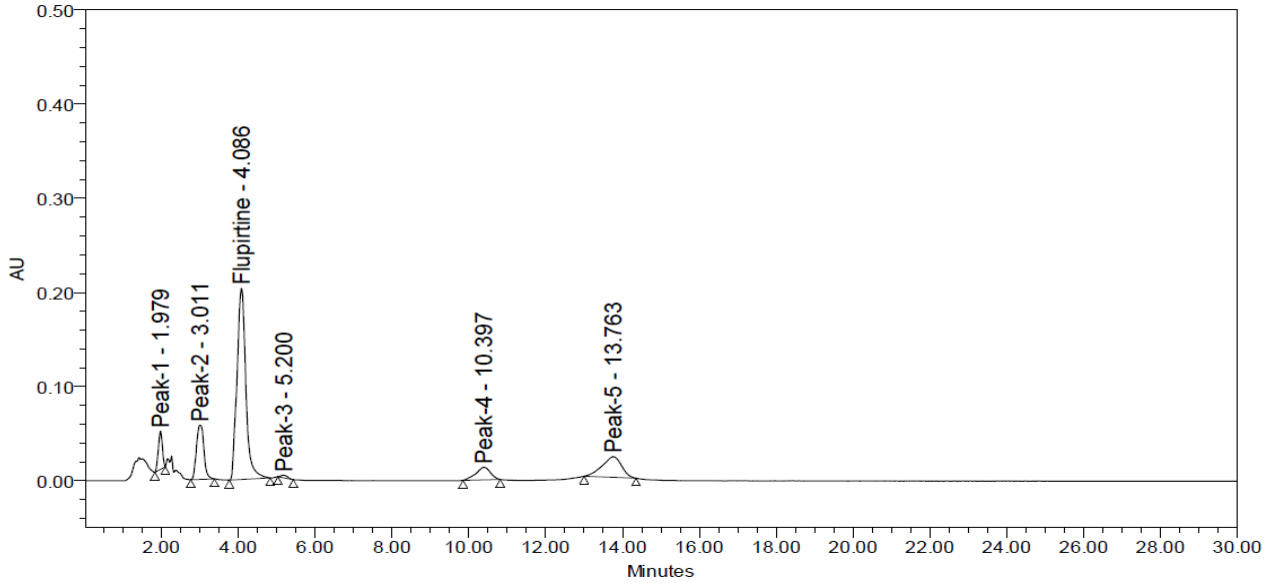

Fig. 5: Chromatogram showing degraded peaks under acidic conditions

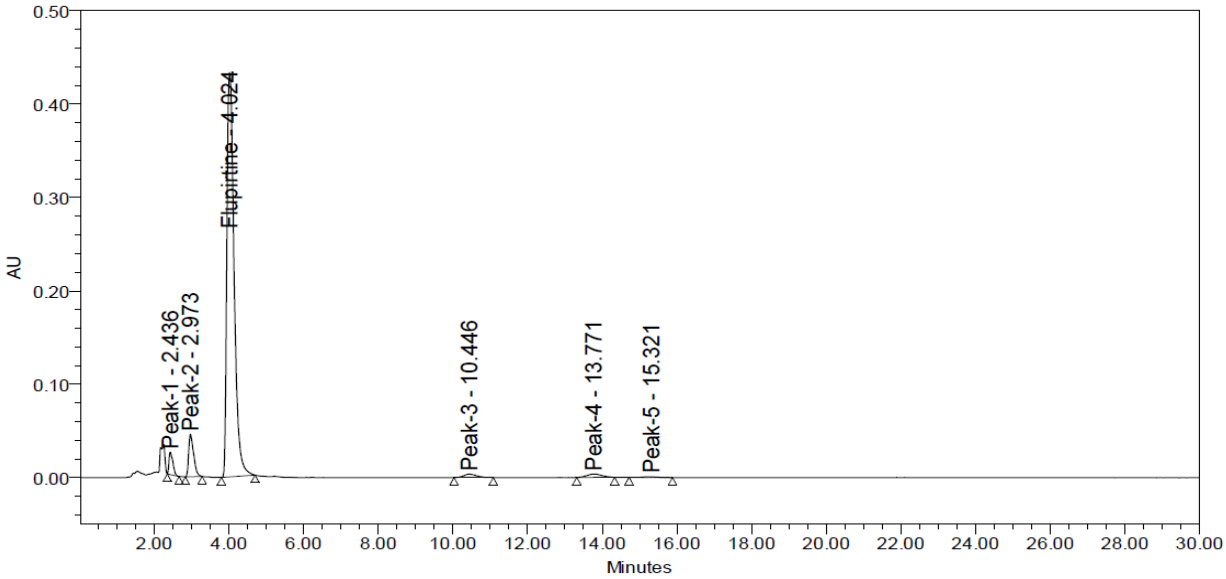

Fig. 6: Chromatogram showing degraded peaks under basic conditions

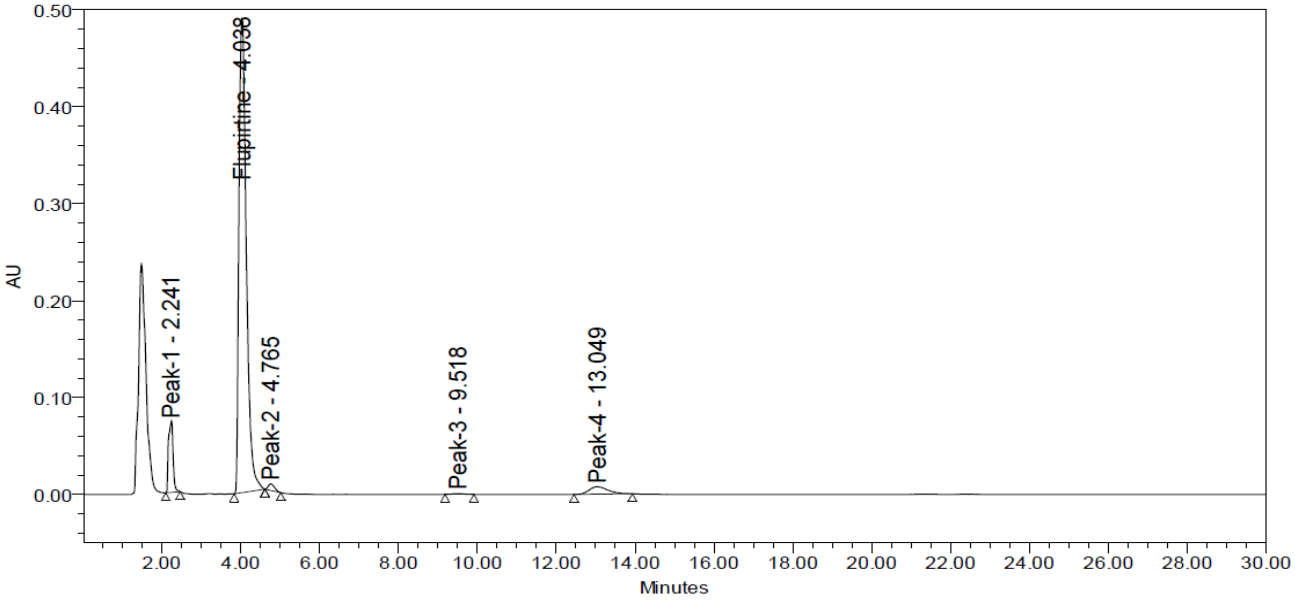

Fig. 7: Chromatogram showing degraded peaks under peroxide conditions

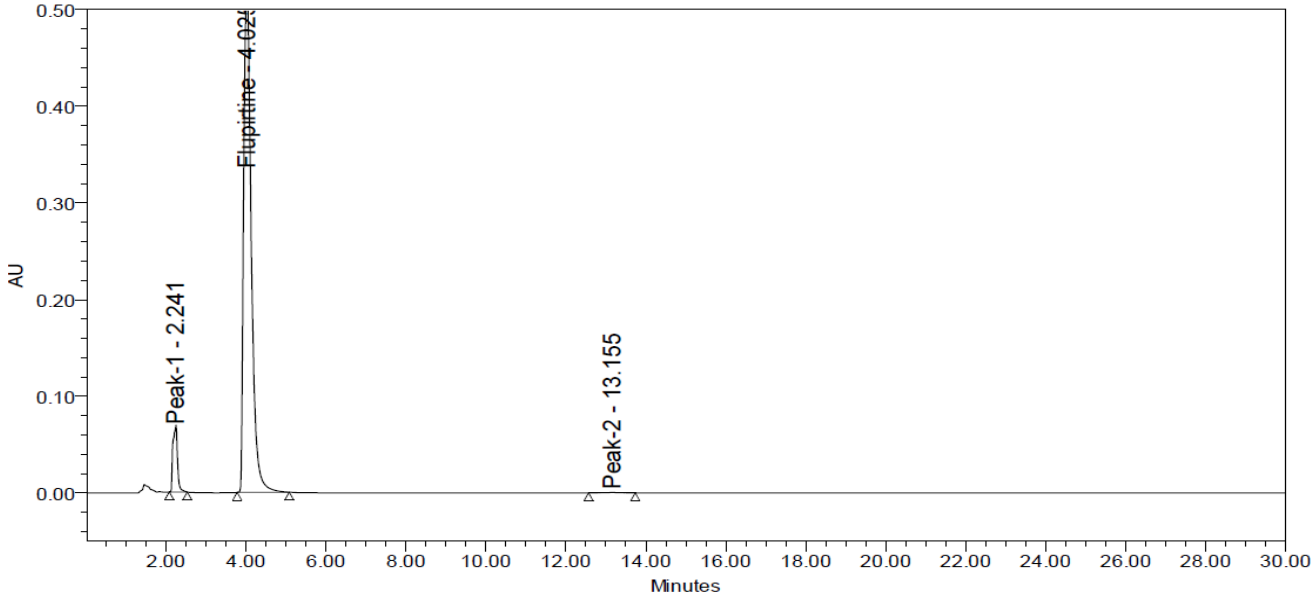

Fig. 8: Chromatogram showing degraded peaks under thermal conditions

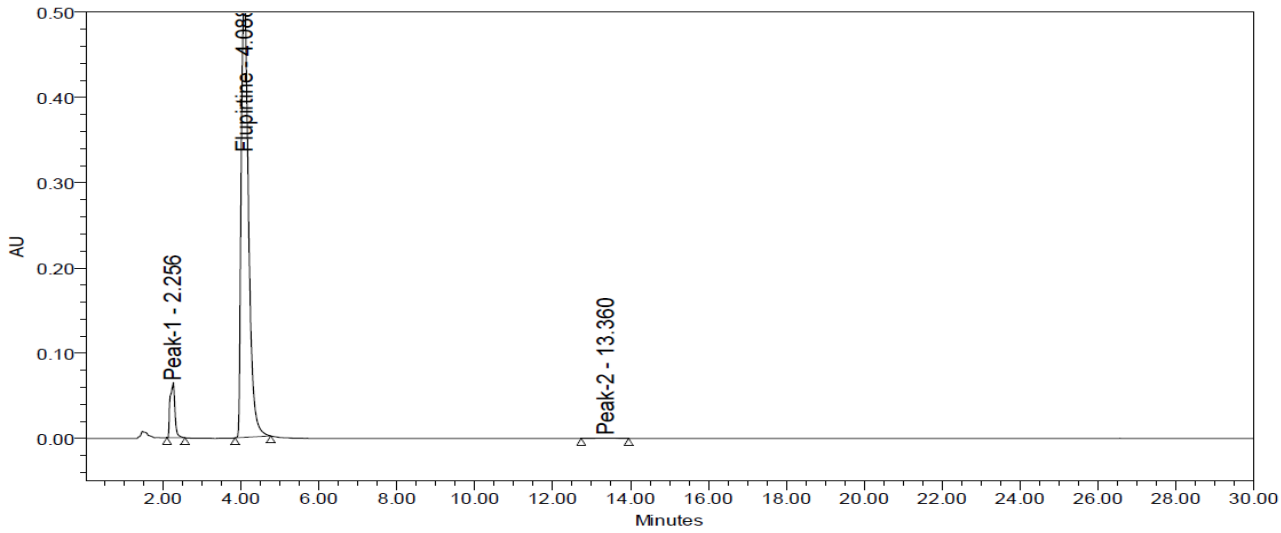

Fig. 9: Chromatogram showing degraded peaks under UV conditions

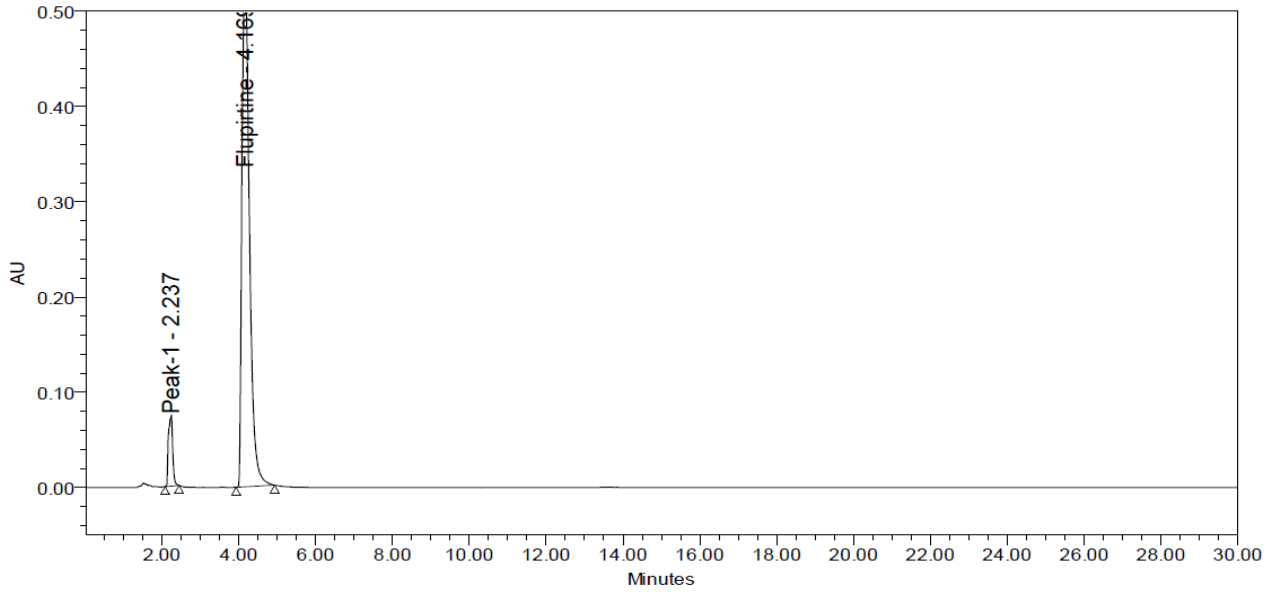

Fig. 10: Chromatogram showing degraded peak under neutral conditions

Table 1: Optimized chromatographic conditions of Flupirtine

S. No.	Parameter	Condition
1	Mobile phase	Buffer:acetonitrile (75:25% V/V)
2	pH	3.6
3	Diluent	Methanol
4	Column	Inertsil ODS
5	Column temperature	30^{0}C
6	Wave length	246 nm
7	Injection volume	10 µL
8	Flow rate	1.0 mL/min
9	Run time	8 mins

Table 2: Linearity results

S. No.	Concentration in µg/mL	Area
1	100	1750139
2	200	3328893
3	300	5051971
4	400	6639646
5	500	8403272
6	600	10095489

Table 3: Recovery results of Flupirtine

Level	Amount added(µg/mL)	Amount found(µg/mL)	% Recovery	Mean recovery
50%	200	199.61	99.80	99.84 %
100%	400	398.41	99.60	
150%	600	600.67	100.11	

Table 4: Precision studies of Flupirtine

Concentration (µg/mL)	Intra-day precision (%RSD)	Inter-day precision (%RSD)
30	0.81	0.65

Table 5: Assay results of Flupirtine

S. No.	Formulation	Label claim	Amount found	%Assay
1	LUPIRTIN	100 mg	99.5 mg	100.3%

Table 6: Summary of validation parameters

S. No.	System suitability	Results
1	Linearity range (µg/mL)	100-600 µg/mL
2	Correlation coefficient	0.999
3	Theoretical plates (N)	4760
4	Tailing factor	1.40
5	LOD (µg/mL)	0.47 µg/mL
6	LOQ (µg/mL)	1.45 µg/mL
7	Regression Equation	Y=16758+10437

Table 7: Degradation data of Flupiritine

Stress conditions	Peak area	Degradation of drug (%)	Active drug (%) after degradation
Standard	7141453	0.00	100
Acid	6373084	10.78	89.22
Base	6588271	7.76	92.24
Peroxide	6651260	6.88	93.12
Thermal	6972835	2.38	97.62
UV	6912094	3.23	96.77
Neutral	7103781	0.55	99.45

CONCLUSION

A stability-indicating, simple, precise, selective and sensitive RP-HPLC method with UV detection for Flupirtine was developed and validated. This method will be useful for the easy and quick estimation of Flupirtine in bulk and pharmaceutical dosage forms.

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