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RESEARCH ARTICLE

Analytical Method for Development and Validation of Flupirtine Maleate by Reverse-phase High-performance Liquid Chromatography

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ABSTRACT

Background: Flupirtine is a non-opioid analgesic. Flupirtine acts like a N-Methyl-D-aspartate receptor antagonist, yet does not bind to the receptor. **Objective:** A novel reversed-phase high-performance liquid chromatography (HPLC) method was established to the analysis of flupirtine in raw material and finished products. The purpose of the present research work was to find quantitation of flupirtine by reversed-phase HPLC (RP-HPLC) in tablet dose form. The proposed strategy is approved according to the ICH rules. **Materials and Methods:** Orthophosphoric acid, HPLC grade methanol, triethylamine, methanol, and chloroform were used. HPLC Column C18 (150 mm × 25.4 mm) with a mobile phase methanol:water (90:10), flow rate of 1 ml/min, was used injection volume 20 µl in run time 20 min. Then we performed method development and its subsequent validation, accuracy and analyte of Robust. **Results:** All the system suitability parameters were within the limit and a sharp peak with better resolution and purity was obtained with the developed method. Recovery studies are between the ranges of 98.0% and 120% with a relative standard deviation at each level of <2.0%, which proves that the method is accurate for the estimation of flupirtine maleate over the range 50%–150% of target concentration. **Conclusion:** The developed and validated RP-HPLC analysis method is, therefore, recommended to use for routine analysis.

Keywords: Accuracy, photodiode array detector, precision, reversed-phase high-performance liquid chromatography, system suitability, validation

INTRODUCTION

Flupirtine is a pyridine derivative that is in clinical use as a nonopioid pain relieving.[1] It was approved for the treatment of pain in 1984 in Europe. It is not affirmed for use in the U.S. or then again Canada, yet is as of now in Stage II preliminaries for the treatment of fibromyalgia. Flupirtine up manages Bcl-2, increased glutathione levels, actuates an internally amending potassium channel, and postpones loss of intermitochondrial layer calcium maintenance limit.[1-13] Flupirtine acts like a N-Methyl-D-aspartate receptor antagonist, yet does not bind to the receptor. One investigation inferred that the discriminative impacts of flupirtine are neither of opioid nor of alpha-1 adrenergic type, yet are essentially interceded through alpha-2 adrenergic systems[13] The Molecular formula of Flupritine is $C_{15}H_{17}FN_4O_2$ and the structure of Flupritine is given in Figure 1. Literature review uncovers that strategies have been accounted for examination of chlorpheniramine maleate, phenylpropanolamine hydrochloride, and paracetamol either alone or in a blend with different medications.[14] In case of chlorpheniramine maleate, UV spectrophoto metric technique, High-performance liquid chromatography strategy, dependability showing HPLC technique in mix with different medications are accounted.[15] So also, HPLC strategy has been accounted for phenylpropanolamine hydrochloride alone and HPLC technique in the mix with different medications.[16] Up till now, there have been no distributed reports about the quantitation of flupirtine maleate, by chromatographic strategy

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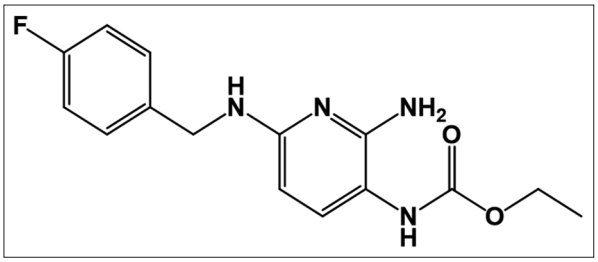

Figure 1: Structure of flupirtine

in tablet dose structure. This present investigation reports for the first time quantitation of the drug by RP-HPLC in tablet dose form. The proposed strategy is approved according to the ICH rules.[17-20]

MATERIALS AND METHODS

Method development involves the evaluation and optimization of various stages of sample preparation, chromatographic separation, and detection. Optimization of various parameters was performed to develop a selective and sensitive method for analysis of flupirtine maleate on RP-HPLC using photodiode array detector (PDAD).[21-36]

Materials

Orthophosphoric acid, HPLC grade methanol, triethylamine, methanol, and chloroform were purchased from Rankem New Delhi, India. All the chemicals and solvents of HPLC grade were used without any additional purification. All the solutions were arranged in HPLC grade water. Market formulation of Ketoflam SR (LUPIN Ltd. Kartholi, Bari Brahmana, in Jammu, India) was procured from the local drug store. Specified all solutions were filtered through 0.22 µ.

Instrumentation

It is the binary LC system 515 equipped with W2998 PDA detector. 20 µl Rheodyne loop injector and Empower 2 data station software were used. Separation was performed on C18 column. Chromatographic data were processed using EMPOWER 2 Software. Methanol:water (90:10) was selected as the mobile phase for dissolution.

Flupirtine maleate standard preparation

About 25 mg of working standard of flupirtine maleate was weighed accurately and transferred it into 100 ml dry volumetric flask. The drug was dissolved completely into 25 ml of methanol and make up the volume.

Sample preparation

Tablets were accurately weighed and calculated for average weight. The tablets were crushed to powder form and weighed immediately with the help of weighing balance and transferred into a 100 ml dry volumetric flask. Then 25 ml of methanol were added into a 100 ml volumetric flask and shaken for 10 min and made up the volume up to the mark with methanol.

Chromatographic parameters

Column: C18 (150 mm × 25.4 mm)
Flow rate: 1 ml/min
Injection volume: 20 µl
Run time: 20 min
Mobile phase: Methanol:Water (90:10)

VALIDATION OF PROPOSED METHOD

System suitability

System suitability analysis is an important part of many analytical procedures. The tests are created on the idea that the equipment, electronics, analytical operations, and samples to be analyzed constitute an integral system that can be evaluated as such. System suitability test parameters to be established for a particular procedure depend on the type of procedure being validated.[37-44] System suitability was demonstrated by preparation of standard solution, which was included in ICH guideline. Here chromatographs were same for HPLC system for all 5 replicates; the peak area of analyte was recorded for these replicated injections. The tailing factor and percentage relative standard deviation (RSD) were evaluated for analyte peaks. The results for the above development method are tabulated in Table 1.

Method precision

Method precision was confirmed by preparing six samples as per the test method representing a single batch. The assay and precision of the methods were determined. The precision of the method was evaluated by computing the percentage RSD of

Table 1: Linearity study of flupirtine maleate

Linearity level (%)	Concentration of flupirtine maleate in mcg/ml (ppm)	Peak area of flupirtine maleate
60	150	332,190
80	200	453,216
100	250	620,176
120	300	753,150
140	350	920,570

Table 2: System suitability data

Injection number	Peak areas FlupirtineMeleate
1	620,357
2	622,535
3	621,375
4	623,012
5	621,032
Average	621,662
%RSD	0.17
Tailing factor	0.5

%RSD: Percentage relative standard deviation

Table 3: Intraday precision data

Serial number	Precision	Percentage assay of FlupirtineMeleate
1	Precision set – 1	98.6
2	Precision set – 2	100.2
3	Precision set – 3	99.4
4	Precision set – 4	100.4
5	Precision set – 5	98.9
6	Precision set – 6	99.7
Average		99.5
%RSD		0.8

%RSD: Percentage relative standard deviation

the assay result.[45-50] The result precision studies are tabulated in Tables 2 and 3. Intermediate precision (Ruggedness): The ruggedness of an tampering method is the stratum of reproducibility of test results obtained by the wringer of the same samples under a variety of conditions. Intraday and interday precision for the ripened method was measured in terms of percentage RSD. The experiments were repeated 3 times a day for intraday precision and on three variegated days for interday precision. The concentration value for both intraday and interday precision was calculated. Finally, the midpoint of percentage RSD is calculated.

Linearity

Linearity should be calculated by graphical inspection of a plot of signals as a function of analyte concentration or content. If there is a linear relationship, test results should be evaluated by appropriate statistical methods, for example, by calculation of a regression line by the method of least squares in addition, an analysis of the deviation of the actual data points from the regression line may also be helpful for evaluating linearity. Linearity is the ability of the method to obtain the test results which are directly proportional to the concentration of analyte in the sample. The linearity of detector response for flupirtine maleate was demonstrated by preparing solution of working standard as per the method over the range of 50%–150% of target concentration. These solutions were injected into the system and the peak area of analyte was recorded. A graph of concentration versus peak area of analyte was plotted.

Accuracy

Accuracy has to be described as percent regaining by the assay of known added amount of analyte in the sample or as the difference between the mean and the accepted true value together with the confidence intervals.[11,12] The parameters provide information about the regaining of the drug from sample and effect of matrix, as regaining are likely to be excessive as well as incomplete. The accuracy of test method was demonstrated by preparing recovery samples (i.e., spiking placebo with known quantities of levels of 50%, 100%, and 150%), the recovery samples were prepared in triplicate at each level. The above samples were chromatographed and the percentage recovery for the amount added was estimated.

Robustness

The evaluation of robustness should be considered during the development phase and depends on the type of procedure under study. If measurements are susceptible to variations in analytical conditions, the analytical conditions should be suitably controlled, or a precautionary statement should be included in the procedure.[13-16] The robustness of the method was determined by performing the assay in triplicate by deliberately alternating parameters. Here, the peak area of analyte in sample solution and standard solution should not differ by more than 2.0% from initial peak area

for the accepted storage time. Hence sample and standard were stable for 36 hours

RESULTS AND DISCUSSION

Identification tests

Physical appearance

The physical appearance of flupirtine maleate was noted by visual observation. It appeared as white colored powder and off-white.

Melting point

The melting point of flupirtine maleate was found to be 176–179°C which complies with literature.

Solubility

Flupirtine maleate was found to slightly soluble in water, freely soluble in methanol, and sparingly soluble in ethanol.

Analytical method development by RP-HPLC method

Method development

The chromatographic separation was achieved on C18 (150 mm × 25.4 mm) analytical column with the mobile phase consisting ratio mixture of methanol and OPA0.2% (90:10) at a flow rate of 1 ml/min when injection volume 20 µl and run time 20 min at detector wavelength of 254 nm.

Table 4: Observation

Observation number	RT	Area	Percentage area	Height
1	9.061	604,001	100.00	18,836
2	9.060	603,876	100.00	18,832
3	9.061	603,783	100.00	18,830

RT: Retention time

Trial 1 and Trial 2

In trial first, chromatographic separation was achieved on C18 (150 mm × 25.4 mm). Analytical column with the mobile phase of methanol and OPA 0.2% (80:20) at a flow rate of 1ml/min at a detection wavelength of 256 mm. Peak was broad and not symmetrical. In trial second, chromatographic separation was achieved on C18 (150 mm × 25.4 mm). Analytical column with the mobile phase of methanol and OPA 0.2% (70:30) at a flow rate of 1 ml/min at the detection wavelength of 256 mm. Peak was not symmetrical. In trial second, chromatographic separation was achieved on C18 (150 mm × 25.4 mm). Analytical column with the mobile phase of methanol and OPA 0.2% (90:10) at a flow rate of 1 ml/min at the detection wavelength of 256 nm. Peak was symmetrical.

The observations are presented in Table 4, Figures 2-4.

Optimization of chromatographic conditions

The effect of chromatographic conditions on the instrument response creates a situation where one has to compromise between different experimental variables to achieve the best chromatographic separations. The resolution and sensitivity of the method were obtained at 254 nm, and the mobile phase flow rate was 1 ml/min. The retention time of flupirtine maleate was 9.061 when PH at 3.

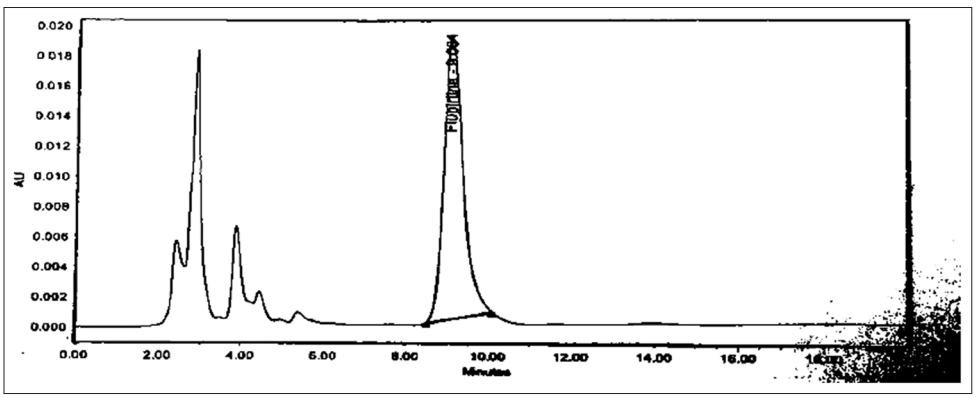

Figure 2: Spectra 1 of flupirtine maleate

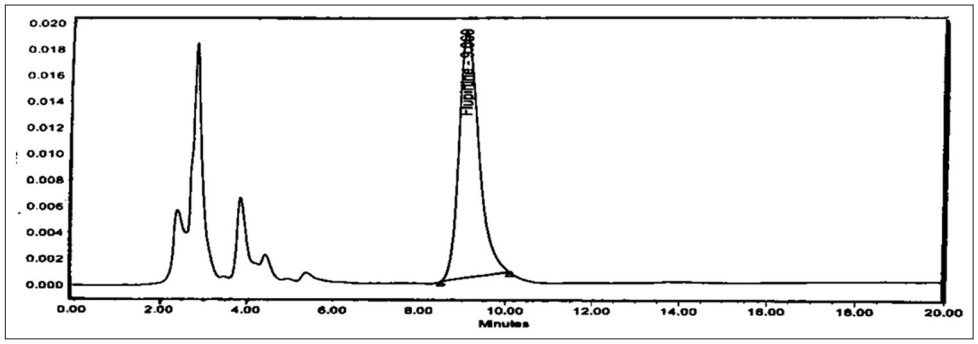

Figure 3: Spectra 2 of flupirtine maleate

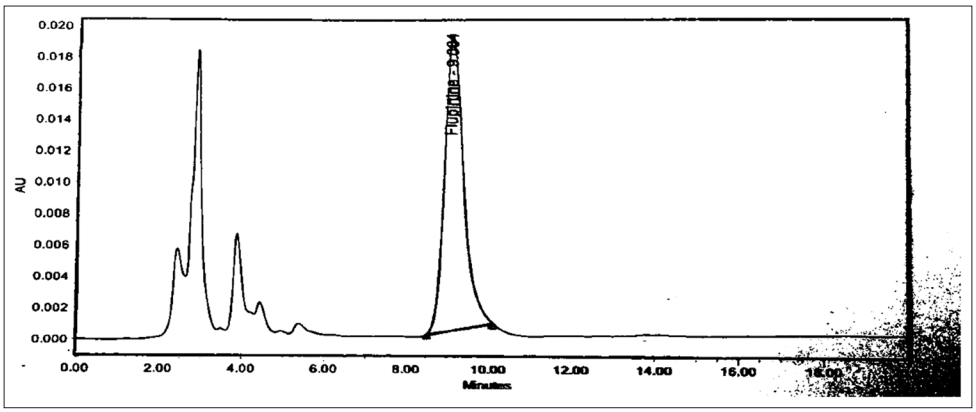

Figure 4: Spectra 3 of flupirtine maleate

System suitability

System suitability was demonstrated by preparation of standard solution, which was included in ICH guideline. Here chromatographs were same for HPLC system for all 5 replicates; the peak area of analyte was recorded for these replicated injections. The tailing factor and RSD were evaluated for the analytes peak.

Acceptance criteria

Tailing factor for analyte peak should not be >2.0. Percentage RSD of five replicate standard injections should not be >2.0. The results are shown in Table 2.

Method precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at three levels: Repeatability, intermediate precision, and reproducibility. Method precision was demonstrated by preparing six samples as per the test method representing a single batch. The assay of these samples was determined, and the precision of the method was evaluated by computing the percentage RSD of the assay results. The results of the precision study are tabulated in Table 3.

Acceptance criteria

Percentage RSD for assay of six preparations should not be >2.0.

Intermediate precision (ruggedness)

The ruggedness of test method was demonstrated by carrying out a precision study in six replicates of the assay on a single batch sample on two different days, using two different columns [Tables 1,5,6], and on two different instruments, as per the matrix tabulated in Table 7-9. Linearity is the ability of the method to obtain the test results which are directly proportional to the concentration of analyte in the sample. The linearity of detector response for flupirtine meleate was demonstrated by preparing the solution of flupirtine maleate working standard over the range 50%–150% of target concentration [Tables 1, 8-10, and Figure 5]. These solutions were injected into the system and the peak area of analyte was recorded. A graph of concentration versus peak area

Table 5: Interday precision data

Serial number	Precision	Percentage assay of Flupirtine Maleate
1	Precision set – 1	99.3
2	Precision set – 2	100.2
3	Precision set – 3	98.7
4	Precision set – 4	99.5
5	Precision set – 5	100.5
6	Precision set – 6	100.6
Average		99.8
%RSD		0.7

%RSD: Percentage relative standard deviation

Table 6: Comparison between interday and intraday precision

Parameters	Percentage assay of flupirtine maleate
Intraday precision	99.5
Interday precision	99.8
Difference	0.3
%RSD	0.2

%RSD: Percentage relative standard deviation

Table 7: Validation parameters of linearity by reversed-phase high-performance liquid chromatography method for flupirtine maleate

Parameters	Flupirtine Maleate
Linearity range	150–350 ppm
Regression equation	115486 + 2933 X
Slope	2933
Intercept	115,486
Correlation coefficient	0.999

Table 8: Recovery at 50% level

Sample	Amount of flupirtine maleate spiked (mg)	Amount of flupirtine maleate recovered (mg)	Percentage recovery
1	12.35	12.50	98.8
2	12.35	12.45	99.1
3	12.35	12.55	98.4
Average			98.7
%RSD			0.3

%RSD: Percentage relative standard deviation

Table 9: Recovery at 100% level

Sample	Amount of flupirtine maleate spiked (mg)	Amount of flupirtine maleate recovered (mg)	Percentage recovery
1	Equivalent to 25	25.45	98.2
2	Equivalent to 25	25.25	99.0
3	Equivalent to 25	25.50	98.0
Average			98.4
%RSD			0.4

%RSD: Percentage relative standard deviation

Table 10: Recovery at 150% level

Sample	Amount of flupirtine maleate spiked (mg)	Amount of flupirtine maleate recovered (mg)	Percentage recovery
1	38.40	38.75	99.0
2	38.40	38.55	99.6
3	38.40	38.70	99.2
Average			99.2
%RSD			0.8

%RSD: Percentage relative standard deviation

Table 11: Robustness data for standard solution

Time (in h)	Peak area of flupirtine maleate	Percentage deviation from the initial area
00	621,032	-
6	609,945	−1.7
12	630,335	1.4
18	619,025	−0.3
24	622,110	0.2
30	619,530	−0.2
36	610,120	−1.8
%RSD	1.1	

%RSD: Percentage relative standard deviation

Table 12: Robustness data for sample solution

Time (in h)	Peak area of flupirtine maleate	Percentage deviation from initial area
00	604,530	-
6	613,025	1.4
12	600,253	−0.7
18	603,975	0.1
24	600,290	−0.7
30	613,876	1.5
36	602,520	−0.3
%RSD	1.9	

%RSD: Percentage relative standard deviation

of analyte was plotted [Table 11]. The correlation coefficient between concentration and peak area and y-intercept of the correlation plot were evaluated. The observations are tabulated in Table 12.

CONCLUSION

It is concluded that RP-HPLC method is successfully utilized for the estimation of flupirtine maleate. The validation data obtained in the developed method indicated that proposed developed RP-HPLC method with PDA detection is simple, sensitive, accurate, more precise,

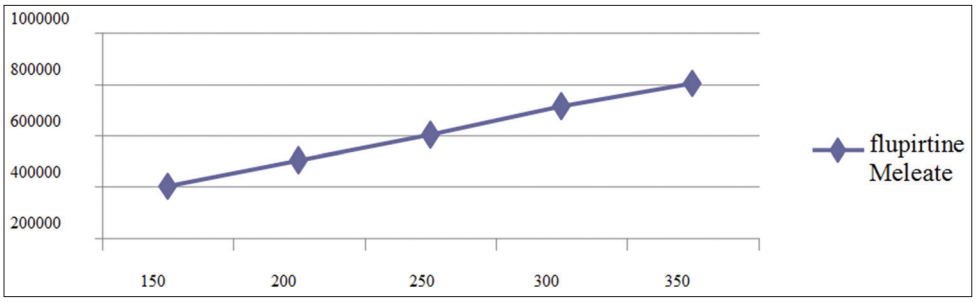

Figure 5: Linearity plot of flupirtine maleate

and less time consuming and can be usefulfor routine determination of flupirtine maleate. Method development involves the evaluation and optimization of various stages of sample preparation, chromatographic separation, and detection. Optimization of various parameters was performed to develop a selective and sensitive method for analysis of flupirtine maleate on RP-HPLC using PDAD. All the system suitability parameters were within the limit, and a sharp peak with better resolution and purity was obtained with the developed method. Recovery studies are between the ranges of 98.0% and 120% with a RSD at each level of >2.0%, which proves that the method is accurate for the estimation of flupirtine maleate over the range 50%–150% of target concentration. In precision studies, the low percentage RSD has been observed on the assay value which indicates that method is precise while the intraday precision studies, assay result obtained on two different analysts, on two different days were found to be within an acceptable limit, which shows that the test method is rugged. The above HPLC analysis method is, therefore, recommended to use for routine analysis.

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