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Development and validation of spectrofluorimetric method for the estimation of Flupirtine maleate in bulk drug and pharmaceutical dosage form

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ABSTRACT

Spectrofluorimetric analysis for the quantification of Flupirtine maleate belongs to centrally acting non-opioid analgesic in pure and pharmaceutical dosage form is described. The calibration curves were found linear between fluorescence intensity and drug concentration in the range of 50-300 ng/ml with coefficients of determination above 0.9992 for all the analytes. Fluorescence was monitored at 388 nm emission wavelength while excited at 320 nm. The method recoveries were higher than 99%. The % RSD value of intra- and interday variation coefficients were observed less than 2%. The limits of detection (LOD) and limit of quantification (LOQ) were found in the range of 6.216 ng/ml and 18.839 ng/ml, respectively. Thus, the method will be applied successfully to the determination of the cited drugs in pure and pharmaceutical dosage with good accuracy and precision. These methods are simple, accurate and rapid; those require no preliminary separation and therefore can be used for routine analysis of Flupirtine maleate in quality control laboratories.

KEYWORDS

Flupirtine maleate;
Spectrofluorimetric;
Fluorescence;
Excitation;
Emission;
ICH guidelines.

INTRODUCTION

Flupirtine maleate, ethyl (2-amino-6-[(4-fluorobenzyl) amino] pyridine-3-yl) carbamate (Figure 1), is an aminopyridine derivative, it used as a centrally acting non-opioid analgesic. Its muscle relaxant properties make it popular for back pain and other orthopaedic uses[1]. Flupirtine also indirectly act as N- methyl-D-aspartate (NMDA) antagonist[2].

Various methods have been reported for estimation of Flupirtine maleate in bulk and pharmaceutical formulations: the examples of which being. UV Spectrophotometric method[3], determination of Flupirtine maleate in human plasma by RP-HPLC[4], Bioanalytical method for quantification of Flupirtine maleate in rat plasma by liquid chromatography-tandem mass spectrometry[5] were developed in individually. Among the various methods are available for the determination of Flupirtine maleate but spectrofluorimetry continues to be very popular because of their simplicity, specificity, and sensitivity and economic. Therefore, the need to develop fast, economic and selective method for the routine quality control analysis of pharmaceutical formulations containing Flupirtine maleate.

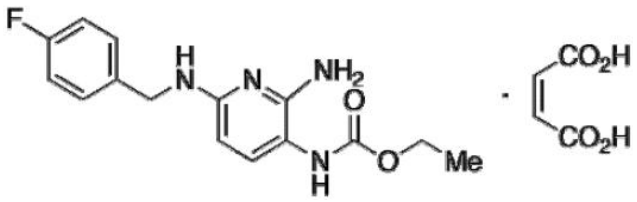

Figure 1 : Chemical structure of Flupirtine maleate

EXPERIMENTAL PRODEDURE

Reagents and apparatus

Pharmaceutical grades of Flupirtine maleate was kindly gifted by Lupin pharma Ltd (Vadodara, India) and certified to contain > 99% of Flupirtine maleate. A tablet formulation was purchased from the local market (Snepdol containing Flupirtine maleate 100 mg). All the reagents used in this method were of analytical grade and Spectroflourometric analysis was performed on Perkin Elmer LS 55 Fluroscence Spectrofluorometer with xenon discharge lamp (20KW), two automatic monochromators, Photomultiplier tube as detector; Software (FL WinLAB) and quartz cuvette was used. All weights were taken on Shimadzu electronic balance AUX 220.

Preparation of stock solutions

The standard solution of Flupirtine maleate was prepared by weighing 10 mg of the drug, dissolved in 100 ml volumetric flask containing of methanol (100 µg/ml) and further diluted 1 ml solution in 100 ml volumetric flask to get a concentration of 1000 ng/ml. The concentration of 500ng/ml was used for optimization of instrumental parameters.

Preparation of solution for calibration curve

From the above solution pipette out 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 ml stock solution in 10 ml volumetric flask and make up the volume up to 10 ml with methanol. Then take the spectra of above 6 solutions and plot the calibration curve of concentration on X axis versus fluorescence intensity on the Y axis and the r^2 value was found to be 0.9992.

Selection of wavelength

The first step involved in fluorimetric analysis was the selection of excitation and emission wavelength. The 500 ng/ml concentration solution was scanned in the region of 200 - 800 nm. Spectra of excitation and emission of Flupirtine maleate were recorded. Keeping the emission wavelength constant, the excitation spectrum of Flupirtine maleate was measured in the spectral measurement mode of the instrument. Similarly, the emission spectrum was again measured with the fixed excitation wavelength. The found constant excitation and emission wavelength for Flupirtine maleate was 320 nm and 388 nm respectively (Figure 2).

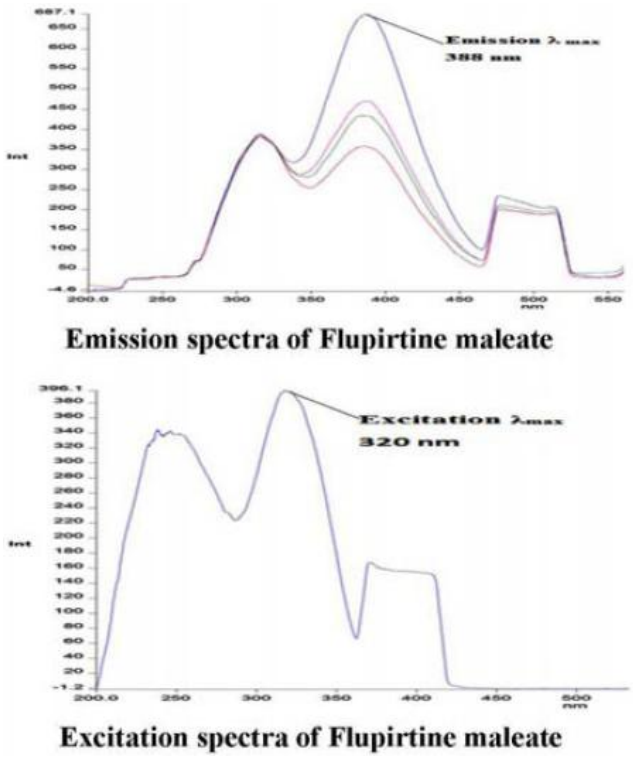

Figure 2 : Emission & Excitation spectra of Flupirtine maleate

Validation of spectrofluorimetric method

Validation is a process of establishing documented evidence which provides a high degree of assurance that specific activity will consistently produce the desired result or product which meeting its predetermined specification and quality attributes[6]. The method was validated for different parameters like linearity, accuracy, precision, specificity, repeatability, limit of detection (LOD), limit of quantification (LOQ) and robustness.

Linearity

Under proposed experimental conditions, the relationship between the relative fluorescence intensity and

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the concentration of Flupirtine maleate was studied. The calibration curve was plotted between concentrations versus fluorescence intensity by the prepared concentration of 50 - 300 ng/ml of stock solution and the fluorescence intensity were measured at the fixed excitation and emission wavelength of 320 and 388 nm. (Figure 3)

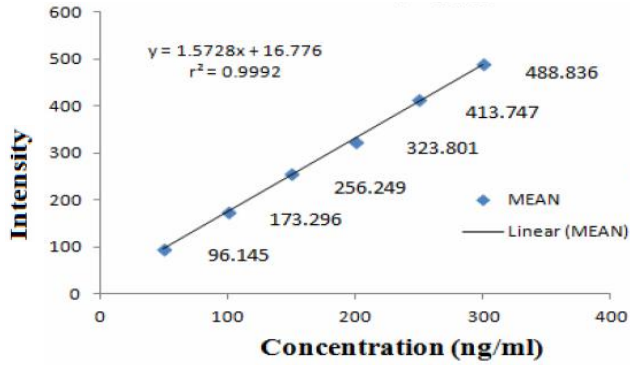

Figure 3 : Calibration curve of Flupirtine maleate

Repeatability

The six replicate of prepared 250 ng/ml solution of Flupirtine maleate taken from different stock solution and scanned at 200-800 nm for fluorescence intensity. The relative standard deviation (%RSD) was found to be less than 2 %, which indicates that the proposed method is repeatable.

Precision

The Intraday and interday precision were carried out through replicating analysis (n=3) for 3 concentrations (50, 150 and 300 ng/ml). For interday precision assay, the analysis was carried out for three consecutive days at the same concentration level as used in intraday precision. And the intraday precision was carried out by using three concentrations at different time interval in a day. The fluorescence intensity recorded as %RSD (TABLE 1)[7].

Specificity

The prepared standard, sample solutions and the blank solution were scanned from 200-600 nm using the optimized spectrofluorimetric conditions and checked for the change in emission at respective wavelengths.

Analysis of marketed formulation

Twenty tablets (Snepdol 100 mg of Flupirtine maleate per tablet) were accurately weighed and finely powdered. A quantity of the powder equivalent to 10 mg of Flupirtine maleate was extracted by shaking with 20 ml of methanol, followed by another two extractions each with 10 ml of methanol. It was filtered on whatmann filter paper no. 42 to remove insoluble materials. The volume of filtrate was diluted to 100 ml with methanol (100µg/ml). It was further diluted according to the need and then analyzed following the proposed procedures. The nominal content of the tablets was calculated either from the previously plotted calibration graphs or using regression equations (TABLE 2).

TABLE 1 : Intraday and interday precision data

Intraday Precision Data			
Conc.(n=3)	**50 ng/ml**	**150 ng/ml**	**300 ng/ml**
Mean	94.297	255.026	485.22
SD	0.814	1.763	5.954
%RSD	0.863	0.691	1.227
Interday Precision Data			
Conc.(n=3)	**50 ng/ml**	**150 ng/ml**	**300 ng/ml**
Mean	97.993	257.473	492.453
SD	1.735	2.835	7.226
%RSD	1.771	1.101	1.467

TABLE 2 : Assay of marketed formulation

Name of drug	Label claim	Concentration prepared	Amount found ± SD	%Assay
Flupirtine maleate	100mg	100 ng/ml	100.975 ± 0.916	100.975

Accuracy

To find the accuracy of the method, the recovery experiment was carried out using the standard addition method. For the previously analyzed sample (100 ng/ml), a known amount of standard drug was added at 50%, 100% and 150 % level. The contents were re-analyzed with the above described procedure (TABLE 3).

LOD and LOQ

LOD and LOQ were determined using mathematical equations.

LOD = 3.3 X σ/S and LOQ = 10 X σ/S

Where, σ = Standard deviation of the response; S =

Slope of the calibration curve.

Robustness

The robustness of a method is its ability to remain unaffected by small changes in parameters like changes in emission wavelength. With this change in emission wavelength ± 2 nm. Take the spectra at 386 nm and 390 nm.

TABLE 3 : Accuracy data

Sr. No	Level	Sample Conc. (ng/ml)	Amt. of std. Added (ng/ml)	Total Conc. (ng/ml)	Found Conc. (ng/ml)	Mean Intensity	% RSD	% Recovery
1	50	100	50	150	150.626	255.452	0.625	101.168
2		100	50	150	152.599			
3		100	50	150	152.032			
4	100	100	100	200	198.067	329.580	0.411	99.441
5		100	100	200	198.799			
6		100	100	200	199.785			
7	150	100	150	250	253.815	415.313	0.339	101.357
8		100	150	250	253.973			
9		100	150	250	252.391			

TABLE 4 : Summary of validation parameters

Parameters	Flupirtine maleate
Range (ng/ml)	20 - 600
Linearity (ng/ml)	50 - 300
Regression equation	Y= 1.5728x + 16.776
r^2 Value	0.9992
Intraday precision (n=3)	0.691 – 1.227
Interday precision (n=3)	1.101 – 1.771
% Recovery	99.441 – 101.357
LOD (ng/ml)	6.216
LOQ (ng/ml)	18.839
Robustness	Robust

RESULTS AND DISCUSSION

Linearity was assessed for Flupirtine maleate by plotting calibration curves of the Intensity versus the concentration over the concentration range 50-300 ng/ml for Flupirtine maleate. The correlation coefficient (r2) for Flupirtine maleate was found to be 0.9992 (TABLE 4). The following equations for straight line were obtained for Flupirtine maleate.

Linear equation for Flupirtine maleate, y = 1.5728x + 16.776

The % recoveries were found to be in the range of 99.44-101.35 % for Flupirtine maleate (TABLE 3). The precision of method was determined by intraday and inter-day precision and was expressed as the %RSD (TABLE 1), which indicate good method precision. The Limit of detection for Flupirtine maleate was found to be 6.21 ng/ml. Limit of quantification for Flupirtine maleate was found to be 18.83 ng/ml (TABLE 4).

CONCLUSION

Overall advantages of the developed spectrofluorimetric method are its simple, sensitive, rapid and cost effective in comparison to reported methods. It does not suffer from any interference due to common excipients of tablets (glucose, starch, talc, lactose, and sucrose). It does not contain any stringent experimental variables which affect the reliability of the results. This method can be applicable in different dosage forms in comparison to official method and other time consuming techniques such as GC and HPLC. Therefore, it is evident from the results that the developed method will be highly useful for routine analysis of Flupirtine maleate in bulk drug and pharmaceutical dosage form.

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