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Research Article

METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF FLUPIRTINE MALEATE IN BULK AND PHARMACEUTICAL DOSAGE FORMS USING UV-VIS SPECTROPHOTOMETRY

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ABSTRACT
The present study describes a simple, accurate, precise and cost effective UV-VIS Spectrophotometric method for the estimation of Flupirtine an analgesic drug in bulk and pharmaceutical dosage form. The solvent used is methanol and the λ max or the absorption maxima of the drug was found to be 250nm. A linear response was observed in the range of 5-50µg/ml with regression coefficient of 0.997 and linear equation of y = 0.045x+ 0.004. The method was then validated for different parameters as per ICH guidelines. This method can be used for the determination of Flupirtine in quality control of formulation without interference of the excipient

KEYWORDS: Flupirtine maleate, ICH, UV-VIS Spectroscopy.

INTRODUCTION

Flupirtine maleate is a non opioid centrally – acting, structurally dissimilar from other analgesics. It is pyridine derivative with the chemical name of ethyl 2amino 6(4-flurobenzylamino) 3-pyridylcarbamate maleate Fig (1). Flupirtine is a unique centrally-acting, non opioid analgesic with muscle relaxant and neuro protective properties[1]. The analgesic effect of flupirtine is dose related. It appears to work by suppressing pain perception transmission to the brain and spinal cord through stimulation of descending monoaminergic pathways. It is a prototype of a class of agents known as selective neuronal potassium channel opener (SNEPCO).Activates G- protein- coupled inwards acting K+ channels of the nerve cell. Due to the selective opening of the voltage-independent k+ channel resting potential of the nerve cell stabilizes, so the activation of the nerve cell membrane reduced. Flupirtine also indirectly acts as N-methyl-D-aspartate (NMDA) – antagonist[2].

Flupirtine is indicated for the treatment of acute and chronic pain, for painful increased muscle tone of the posture and motor muscle, primary head ace, tumor pain dysmenorrheal and pain after traumatologic/ orthopedic operations and injuries. No UV-VIS spectrophotometric method was proposed for the estimation of flupirtine in bulk and pharmaceutical dosage forms. The aim of the work is to develop and validate an analytical method using UV-VIS spetrophotometry for the estimation of flupirtine maleate in bulk and pharmaceutical dosage forms.

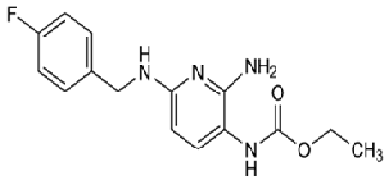

FIGURE 1. Structure of flupirtine maleate

MATERIALS AND METHODS

Flupirtine maleate was obtained as a gift sample. The solvent used was methanol. The instrument used was a double beam UV-VIS spectrophotometer (uv-1800, shimadzu).

METHOD DEVELOPMENT

Solubility Test: solubility test for the drug flupirtine maleate was performed by various solvents. The solvents include Water, methanol, DMSO, methanol, ethanol and chloroform. However methanol was chosen as a solvent for developing the method.

Determination of λmax

Preparation of stock solution: Standard stock solution of flupirtine maleate prepared by dissolving 10mg of flupirtine maleate in methanol to produce a concentration of 1000µg/ml. 1ml of this stock solution was taken then diluted up to 10ml by using methanol to produce a concentration of 100µg/ml which is the standard stock solution.

From the above stock solution, 2ml was pipette out into a 10ml volumetric flask and the volume was made up to the mark with methanol to prepare a concentration of 20µg/ml. Then the sample was scanned in UV-VIS Spectrophotometer in the range 400-200nm using methanol as a blank and the wave length corresponding to maximum absorbance (λmax) was found to be 250nm (Fig 2).

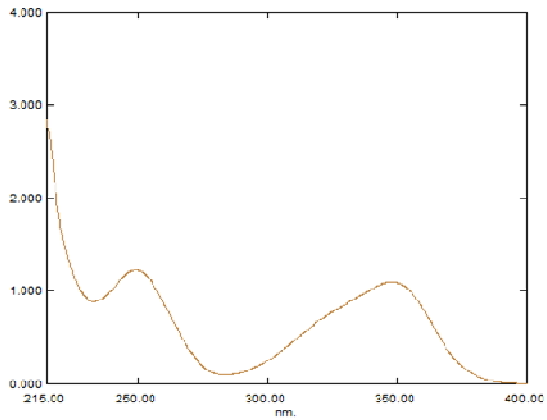

FIGURE 2. λmax of flupirtine maleate

Preparation of calibration curve: 0.5ml, 1ml, 2ml, 3ml, 4ml, 5ml of 100ug/ml solution was diluted to 10ml using methanol to produce 5µg/ml, 10µg/ml, 20µg/ml, 30µg/ml, 40µg/ml, 50µg/ml solutions respectively. Then the construction of calibration curve was done by taking the above prepared solutions of different concentration ranging from 5-50µg/ml. Calibration curve was plotted by taking concentration on x-axis and absorbance on y-axis. The curve showed linearity in the concentration range of 5-50µg/ml, the correlation coefficient was found to be 0.997 (Fig 3).

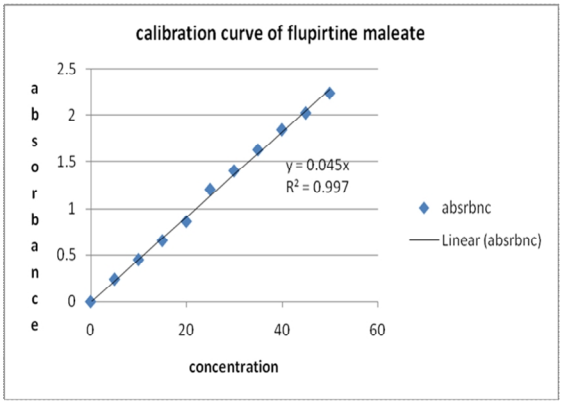

FIGURE 3: Calibration curve of flupirtine maleate

Table 1: Linearity of Flupirtine maleate

Concentration (µg /ml)	Absorbance
5	0.242
10	0.449
15	0.652
20	0.861
25	1.203
30	1.402
35	1.631
40	1.845
45	2.023
50	2.234

Table 2 Optical Characteristics

Beer's Law limit (µg/ml)	5-50
Correlation coefficient	0.997
Regression equation	Y=0.045 + 0.004
Slope (a)	0.045
Intercept (b)	0.004

METHOD VALIDATION

Validation is a process of establishing documented evidence, which provides a high degree of assurance that a specific activity will consistently produce desired result or product meeting its predetermined specifications and quality characteristics[3].

The method was validated for different parameters like Linearity, Accuracy, Precision, Robustness, Ruggedness, Limit of quantification (LOQ), and Limit of Detection (LOD).

Linearity: The method was validated according to ICH Q2B guidelines for validation of analytical procedure in order to determine the linearity, sensitivity and accuracy of the analyte[3,4]. A calibration curve was generated with appropriate volumes of the working standard solutions for UV methods. The linearity found between the 10-50ug/ml. (Table1).

Accuracy: The accuracy of the method was determined by preparing solutions of different concentrations that is 80%, 100%, 120% in which the amount of marketed formulation (RETENSE - 100mg) was kept constant (10mg) and the amount of pure drug was varied that is 8mg, 10mg, 12mg for 80%, 100%, and 120% respectively. The solutions were prepared in triplicates and the accuracy was indicated by % recovery (Table 3) .

Table 3: accuracy reading of flupirtine maleate

Observation/Result						
No. of preparations	Concentration (µg/ml)		% Recovery	Statistical Result		
	Formulation	Pure drug		Mean	SD	%RSD
80%	10	8	100.1			
80%	10	8	99.9	100.4	0.7	0.69
80%	10	8	101.2			
100%	10	10	101.2			
100%	10	10	99.9	100	1.15325	1.15
100%	10	10	98.9			
120%	10	12	100.9			
120%	10	12	99.9	100.9	1	0.99
120%	10	12	101.9			

Precision: Precision is the degree of repeatability of an analytical method under normal operational conditions. The precision of the assay was determined by repeatability (intraday) and intermediate precision (inter-day). The intraday precision study of different solutions of same concentrations were prepared and analyzed three times in a day i.e. morning, afternoon and evening, absorbance noted and result was reported as RSD %[4]. The inter-day precision was studied by comparing the assays on three different days and the results are documented as the standard deviation and RSD % (Table 4, 5&6).

Table 4: precision result showing repeatability of flupirtine maleate

Concentration (µg/ml)	Absorbance	Statistical Analysis
20	0.861	
20	0.862	
20	0.860	Mean =0.861
20	0.861	SD =0.001187
20	0.862	%RSD =0.14%
20	0.861	
20	0.862	
20	0.864	

Table 5: intra assay precision

Concentration (µg/ml)	Absorbance 1 (Morning)	Absorbance 2 (Afternoon)	Absorbance3 (Evening)	Average% RSD
20	0.861	0.861	0.861	
20	0.860	0.862	0.859	
20	0.861	0.860	0.861	
20	0.861	0.859	0.862	
20	0.863	0.862	0.861	
20	0.862	0.861	0.862	
20	0.860	0.864	0.861	
20	0.863	0.863	0.859	
20	0.863	0.864	0.864	
%RSD	0.14	0.20	0.18	0.16

Table 6: inter-assay precision

Concentration(µg/ml)	%RSD			Average % RSD
	Day 1	Day 2	Day 3	
20	0.13	0.15	0.18	0.16

Specificity: 10mg of flupirtine maleate was spiked with 50% (5mg), 100% (10mg), 150% (15mg) of excipient mix (magnesium stearate) and the sample was analyzed for % recovery (Table 7).

Table 7: Test for specificity showing no effect of excipients

Sample No	Excipient Conc. (%)	Flupirtine Input (mg)	Flupirtine recovered (mg)	Flupirtine recovered (%)	Mean Recovered (%)	SD	%RSD
1	100	10	9.81	98.1			
2	50	10	10.05	100.5	100.1%	1.877	1.87%
3	150	10	10.18	101.8			

Ruggedness: Analysis was carried out by different analyst in order to determine the ruggedness and the respective absorbance was noted and the result was indicated as% RSD (Table 8).

Table 8: Result showing Ruggedness

Analyst -1		
Conc.(µg/ml)	Absorbance	Statistical analysis
20	0.861	
20	0.861	Mean – 0.8606
20	0.862	SD – 0.00103
20	0.859	%RSD – 0.12%
20	0.860	
20	0.861	
Analyst -2		
Conc.(µg/ml)	Absorbance	Statistical analysis
20	0.861	
20	0.862	Mean – 0.860
20	0.861	SD – 0.0009
20	0.859	%RSD – 0.11%
20	0.861	
20	0.861	

Robustness: Analysis was carried out at two different temperatures ie, room temperature and 18°c to determine the robustness and the respective absorbance were noted and the result were indicated as% RSD (Table9).

Table 9: Result showing Robustness

Room temperature		
Conc.(µg/ml)	Absorbance	Statistical analysis
20	0.861	
20	0.863	Mean – 0.8616
20	0.862	SD – 0.00136
20	0.859	%RSD – 0.16%
20	0.862	
20	0.861	
Temperature 18°c		
20	0.861	
20	0.862	Mean -0.861
20	0.863	SD-0.0015
20	0.859	%RSD-0.17%
20	0.862	
20	0.861	

LOD and LOQ: The limit of detection (LOD) is defined as the lowest concentration of an analyte in the sample that can be detected. The limit of quantification (LOQ) is defined as the lowest concentration of the standard curve that can be measured with an acceptable accuracy, precision and variability[4,5].

In this study, LOD and LOQ were based on the standard deviation of the response and the slope of the corresponding curve using the following equations-

$$LOD = 3\ s/m;\ LOQ = 10\ s/m$$

Where s, the noise of estimate, is the standard deviation of the absorbance of the sample and m is the slope of the related calibrations graphs.

The values of LOD and LOQ are found to be 0.5µg/ml and 1.55µg/ml respectively (Table 10).

RESULT AND DISCUSSION

The development of a simple, rapid, sensitive and accurate analytical method for the quantitative determination of samples will reduce unnecessary tedious sample preparations and the cost of materials and labor. Flupirtine is a UV absorbing molecule with specific chromophores in the structure that absorb at a particular wavelength. The λmax of the drug for analysis was determined by scaning of the drug sample solutions in the entire UV region. It was found to be that maximum absorbance observed at the wavelength of 250nm (fig 2). The developed method was found to be precise as the %RSD values for the intra-day and inter-day was found to be less than 2%. Recoveries of the drug were obtained at 98.1%-101.8%, indicating the method was accurate and specific. The LOD and LOQ were found to be in sub microgram level indicating the sensitivity of the method. The method was also found to be rugged and robust as indicated by the %RSD values which are less than 2%. The summary of validation parameters of proposed method is shown in table 10.

Table 10:Summary of validation

Parameters	Values
Linearity Range	10-50
Precision(%)	0.16%
Accuracy(%)	98.9-101.9%
LOD(µg/ml)	0.5
LOQ(µg/ml)	1.55
Stability (h)	2
Std deviation(SD)	0.6314
%RSD	55.13

CONCLUSION

All the above factors lead to the conclusion that the proposed method is accurate, precise, simple, sensitive, robust and cost effective and can be applied successfully for the estimation of flupirtine maleate in bulk and pharmaceutical dosage formulations.

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