Original Research

DEVELOPMENT AND VALIDATION OF ULTRAVIOLET-VISIBLE SPECTROPHOTOMETRIC METHOD FOR THE ESTIMATION OF DRUG FLUPIRTINE MALEATE IN BULK DRUG AND PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT: An economical, simple and accurate UV-Visible spectrophotometric method for the determination of the drug Flupirtine maleate in bulk drug and pharmaceutical dosage form has been developed. Flupirtine maleate is non steroidal, non NSAID and non opiod drug which is used in neuromuscular pain, back pain, dysmenorrheal etc. The absortion maxima of the drug was observed at 245nm. The linearity range was found to be 2-16µg/ml with regression coefficient value 0.9994. Validation of the proposed method was done in accordance with the ICH guidelines.

KEYWORDS: Flupirtine maleate, ICH, UV-Visible spectrophotometer, Bulk drugs

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INTRODUCTION: Flupirtine maleate is an (Z)-but-2-enedioic acid; ethyl {2-amino-6-[(4- fluorobenzyl) amino] pyridin-3-yl} carbamateethyl, an aminopyridine that functions as a centrally acting non-opioid analgesic. The mechanism of action of the drug is to selectively open the neuronal potassium channel and it also work as NMDA receptor antagonist[6]. Flupirtine maleate is not official in IP, BP and USP also literature survey reveals that there is only one method for the estimation of the drug through UV-Visible spectrophotometry using methanol as a solvent[3,4,5]. So the objective of present study is to develop a UV- Visible spectrophotometry method using a different solvent, which is Acetonitrile.[1,2]

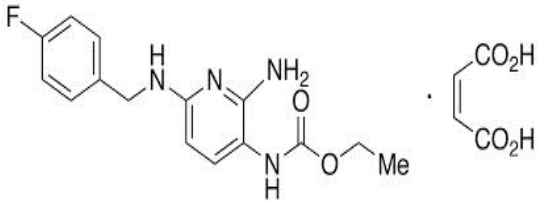

Figure 1. Structure of flupirtine maleate

EXPERIMENTALS:

Materials and reagents: AR grade acetonitrile, methanol were procured from Loba Chemie Pvt. Ltd. (India). Authentic gift sample of Flupirtine maleate was supplied by Lupin laboratories ltd, Mumbai. Capsules of Flupirtine maleate(Retense-Sun Pharma Ltd.) was purchased from local pharmacy. Instrument used for the work was the Perkin Elmer, Lambda 45.

Preparation of standard stock solution: Stock solution of concentration 1mg/ml was prepared by dissolving 50mg of flupirtine maleate in 50ml of acetonitrile in 50ml volumetric flask.

Preparation of working solutions: Using the stock solution of the drug flupirtine maleate various dilution ranging from 2-16µg/ml was prepared in 10ml volumetric flask.

Preparation of sample solution: Marketed formulation (Retense-Sun Pharma Ltd.) was procured from the local pharmacy.For the preparation of sample solution, 20 capsules were taken and weighed accurately using an electric balance (w_1). Now the shells of capsules were removed & empty shells were weighed (w_2). The weight of the capsule contents was calculated by subtracting the empty shells weight from capsules weight $w_3 = (w_1 - w_2)$. The contents were crushed into powder form. Then powdered weight equivalent to one capsule i.e. 100mg of flupirtine maleate was taken into a 100mL volumetric flask. 50mL of acetonitrile was added to it & sonicated for 10min. The solution was then filtered through Whatman filter paper (0.22µm) and diluted up to the mark to prepare solution of 1mg/ml. From the above solution 20µl was pippetted into a 10mL volumetric flask & diluted with acetonitrile to obtain final concentrations of 2µg/mL.

Validation of developed methods:

Linearity: A stock solution was prepared by dissolving 50mg of the drug in 50ml of mobile phase. Then from this stock solution dilutions of various concentration from 2µg/ml to 16µg/ml were prepared.3 Each dilution was analysed in series to construct the calibration curve. Absorbance of each dilution was noted and plotted against the concentration of each dilution.

Accuracy: Accuracy was determined by calculating recovery % of flupirtine maleate by standard addition method. The pre- analyzed sample solutions (2 µg ml^{-1}) were spiked with standard drug solutions at three different levels- 80, 100, 120 %. The resulting mixtures were reanalyzed using the proposed method. The experiment was conducted in triplicates .Accuracy was reported as % recovery.

Precision: Precision of the proposed method was calculated by conducting intermediate precision.

1. Repeatability or Intra Day Precision: The intraday precision was determined by estimating the corresponding absorbance of three different concentrations- 2, 4, 6µg ml^{-1} (in triplicates) three times on the same day.

2. Intermediate or Inter Day Precision: Intermediate precision (inter day) was established by analysing the

three different concentrations- 2, 4, 6µg ml^{-1} (in triplicates) on three different days.

The standard deviation, % relative standard deviation and estimated concentrations based on standard curve were reported for each set of data.

Robustness: Robustness of the developed method was determined by injecting three different concentration (2, 4, 6µg/ml) in triplicates by varying the wavelength (±2). Robustness is reported in %RSD.

Table 1. Linearity results

Regression equation	y = 0.025x + 0.045
Regression coefficient(R^2)	0.9994
Slope	0.025
Range	2-16µg/ml
Intercept	0.045
LOD	0.132 µg/ml
LOQ	0.4 µg/ml

LOD and LOQ: Detection limit and Quantitation limit of the drug is calculated by using the calibration curve standards. DL and QLwas calculated from the equation 3.3σ/S and 10σ/S respectively, where σ is the standard devition of y-intercept and S is the slope of the calibration curve.

Specificity: The specificity of the developed method was seen by analyzing solutions containing excipients (lactose, microcrystalline cellulose, talc, PEG 8000 and magnesium stearate) and pure drug and demonstrating that the result is unaffected by the presence of the excipients present in it (by using two tailed student paired t-test).

Application of the validated method on the marketed formulation(RETENSE®): 20 capsules were taken and weighed accurately. Then the capsule shells were removed and empty shells were weighed. The weight of capsule contents was calculated by subtracting the empty shells weight from capsules weight. The contents were crushed into powder and powdered weight equivalent to one capsule i.e. 100mg of flupirtine maleate were taken into 100ml volumetric flask. 50ml of acetonitrile was added to it and soicated for 10mins and diluted upto the mark to prepare 1mg/ml solution. The resultant solution was filtered through 0.22µm syringe driven filter unit. From this solution a dilution of 10µg/ml of the drug was prepared and its absorbance was taken.

RESULTS AND DISCUSSION:

Method validation:

Linearity: The calibration curve drawn by using the proposed method was found to be linear in the range (2-16µg/ml). Table 1 shows the calibration data with regression coefficient (0.9994) and %RSD was found to be less than 2.

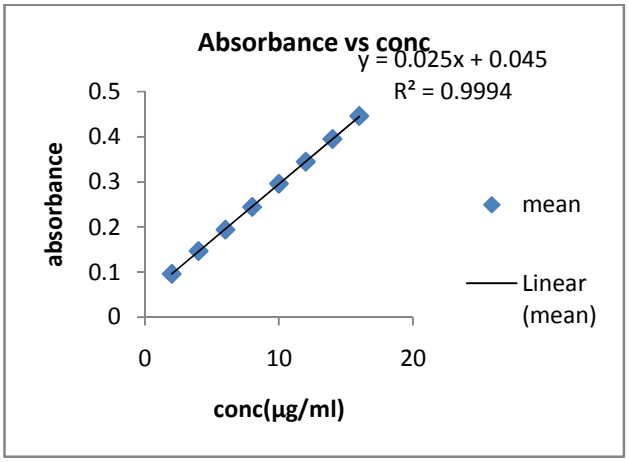

Figure 2. Calibration curve of flupirtine maleate

Accuracy (recovery): Accuracy of the method was determined by using standard addition method and % recovery was found in the range 98-102% and %RSD was within the range. Table 2 shows the results of accuracy studies.

Conc. (µg/ml)	**Excess Drug added to the analyte (%)**	**Theortical Content \ (µg/ml)**	**Mean absorbance**	**SD**	**RSD %**	**Recovery %**
2	80	3.6	0.131	0.0006	0.442	95.27
2	100	4	0.142	0.0006	0.406	97.25
2	120	4.4	0.152	0.0006	0.381	97.04

Table 2. Results of accuracy studies

Precision: Repeatability was assessed by analyzing three different concentrations. The method passed the test as the %RSD was found to be less than 2. Intermediate precision was assessed by analyzing three different concentrations for three different days. The method passed the test as the %RSD was found to be less than 2. Results are shown in table 3 and table 4.

Conc.	Observed absorbance			Mean	SD	RSD%
2	0.093	0.094	0.093	0.093333	0.000577	0.619
4	0.145	0.146	0.144	0.145	0.001	0.690
6	0.195	0.194	0.195	0.195	0.0006	0.297

Table 3. Results of intraday studies

	Conc.	Observed absorbance			Mean	SD	RSD%
	2	0.094	0.095	0.094	0.094	0.0006	0.612
	4	0.144	0.143	0.144	0.144	0.0006	0.402
DAY 1	6	0.193	0.192	0.193	0.193	0.0006	0.300
	2	0.092	0.093	0.092	0.092	0.0006	0.625
	4	0.142	0.144	0.142	0.143	0.0012	0.809
DAY 2	6	0.194	0.193	0.194	0.194	0.0006	0.298
	2	0.093	0.093	0.092	0.093	0.0006	0.623
	4	0.143	0.144	0.144	0.144	0.0006	0.402
DAY 3	6	0.195	0.194	0.193	0.194	0.0010	0.515

Table 4. Results of interday studies

Robustness: Robustness studies were performed by varying the detection wavelength (±2). The method was found to be robust. Results are shown in table 5.

λ_{max} (±2 nm)	Conc.	Observed absorbance			Mean	SD	RSD%
245+2	2	0.094	0.095	0.094	0.094	0.0006	0.612
245-2	2	0.093	0.092	0.093	0.093	0.0006	0.623

Table 5. Results of robustness studies

RESULT OF SPECIFICITY

Specificity studies were performed by spiking the drug with the exipients like magnesium stearate, talc, lactose using two tailed unpaired t-test. Table7 shows the results of specificity studies.

Conc (µg/ml)	Absorbance			mean	SD	p-value	Significantly different? (P < 0.05)
2	0.093	0.092	0.093	0.093	0.000577		
2*	0.092	0.091	0.092	0.092	0.000577	0.1012	No
4	0.143	0.143	0.145	0.144	0.001155		
4*	0.142	0.145	0.143	0.143	0.001528	0.7791	No
6	0.195	0.194	0.194	0.194	0.000577		
6*	0.194	0.193	0.192	0.193	0.001000	0.1336	No

Note: 2, 4, 6 are unspiked/std drug, while 2*, 4*, 6* are spiked with talc, magnesium stearate & lactose.

Table 6. Specificity studies

Conc	Observed concentration			Mean	SD	RSD %	Recovery %
2	1.92	1.96	1.92	1.93	0.0231	1.195	96.5

Table 7. Application of the Method for the Assay of Marketed Formulations (RETENSE®)

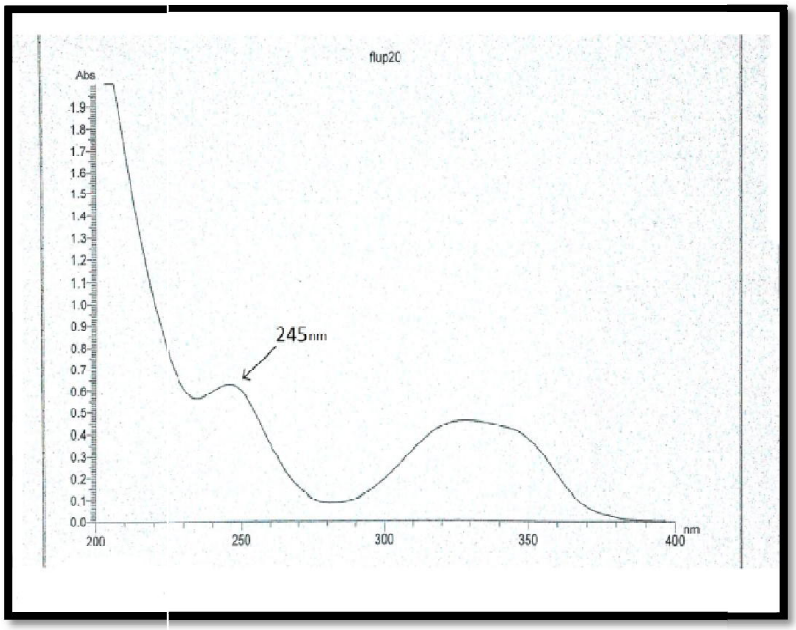

Figure 3. UV Scan of Flupirtine maleate

Conclusion: From the present study it can be concluded that the proposed UV-Visible spectrophotometric method is accurate, precise, cost effective and reproducible. The method can be successfully applied in the pharmaceutical industries for the estimation of the drug Flupirtine maleate in bulk drugs and pharmaceutical dosage form.

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AUTHOR'S CONTRIBUTIONS

MS, Dharti Patel has carried out entire research work i.e. procurement of drug, its identification and the UV-Visible method development and validation.

Dr. Sharad Wakode has given his guidance and valuable suggestions for carrying out the project work.

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ABBREVIATIONS USED:

etc	et cetera
i.e	that is
µg	micogram
nm	nanometer
mg	milligram
LOD	limit of detection
LOQ	limit of quantification
Conc	concentration
SD	standard deviation
RSD	relative standard deviation
%	percentage

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