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Development and Validation of Stability Indicating UV and RP-HPLC Method for The Estimation of *Flupritine maleate* in Bulk and Formulations

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ABSTRACT

The objective of the study was to develop stability indicating UV spectroscopy and RP-HPLC method for the estimation of flupritine maleate in bulk and marketed tablets. Chromatographic separation was achieved on Agilent Eclipse C18, 150 x 4.6 mm, 3µ with mobile phase consisting of Buffer(1ml OPA and 1ml TEA in 1000 ml water and pH was adjusted to 3 with dilute OPA) and Methanol are taken in 50:50%v/v. The effluent was monitored at 246 nm. A sharp peak was observed at 4.0 min. UV Spectrophotometric method was performed at 250 nm using methanol as the solvent. R^2=0.999 for HPLC method and R^2=0.998 for UV Spectrophotometric method. The method was validated as per ICH guidelines with linearity, precision, accuracy, robustness, ruggedness and specificity.Statistical analysis showed that both the methods were precise, accurate, sensitive, and Stability indicating can be used for the routine analysis of flupritine economically in bulk and commercial formulations.

Key words: Flupritine maleate, RP-HPLC, UV-Spectrophotometric, Method development and validation, Degradation, ICH guidelines.

INTRODUCTION

Flupritine acts as a centrally acting non opioid analgesic[1]. Chemically it is amino pyridine derivative with the chemical name of ethyl 2-amino-6-[(4 fluorobenzyl) amino] pyridin-3-yl carbamate maleat[2]. Flupritine was noted for its neuroprotective properties and also investigated for use in Creutzfeldt–Jakob disease, Alzheimer's disease, and multiple sclerosis. Literature review reveals that various analytical methods like UVSpectrophotometry[3-4], HPLC[5-7] have been

developed However nostability indicating UV methods are reported but one stability indicating HPLC method[8] was reported for the estimation of flupritine maleate Hence, an attempt was madeto develop simple, accurate and precise stability indicating UV and RP-HPLC methods for the estimation of flupritine maleate in pure and their marketed formulations.

EXPERIMENTAL

Materials and methods

LABINDIA-3000+ UV-Visible double beam spectrophotometer with a fixed slit width 1nm and 1cm matched quartz cells was used for all the spectral measurements.Alliance WATERS e 2695 HPLC with UV, VISIBLE-2489 & PDA-2998 Detector. All reagents used were of analytical reagent (AR) grade. HPLC grade methanol and water was used throughout analysis. Flupritine sample was kindly provided by Sun pharmaceuticals Ltd.

Chromatogrphic conditions and mobile phase in HPLC

Chromatographic separation was achieved on Agilent Eclipse C18, 150 x 4.6 mm, 3µ. Flow rate was maintained at 1.0 ml/min with 30°c column temperature.The detection was monitored at 246 nm and the run time was 8 minutes. The volume of injection loop was 10 µl prior to injection of the drug solution. To 1000ml of HPLC grade water 1ml of orthophosphoric acid and 1ml of TEA were added and the pH adjusted to 3 with dilute OPA solution. The mobile contains Buffer and Methanol in the ratio of 60:40%v/v, filtered through 0.45µ membrane filter and sonicated for 20 minutes to degas. Water and Methanol(50:50) was used as diluent.

Optimized conditions in UV

Methanol is used as diluent. 250 nm was selected as detection wavelength.

Preparation of solutions in HPLC

Preparation of standard solution

Accurately Weighed and transferred 10mg of flupritine maleate working Standard into a 10 ml clean dry volumetric flask, and 7 ml of HPLC grade Water was added, sonicated for 5 minutes and make up to the final volume with diluent. (Fig.2)

Preparation of sample solution

20 tablets were weighed and crushed into powder, weight equivalent to 1 tablet was calculated. 5 tablets powder weight was transferred into a 200 mL volumetric flask, 150mL of diluent added and sonicated for 25 min, further the volume made up with diluent and filtered. From the filtered solution 4.0ml was pipetted out into a 100 ml volumetric flask and made up to 100ml with diluent. The % assay was found to be 100%.

Preparation of solutions in UV

Preparation of standard solution

Accurately weigh and transfer 10 mg of flupritine Working standard into a 10 mL volumetric flask add about 7 mL of Diluent and sonicated to dissolve it completely and madethe volume up to the mark with the same solvent (Stock solution).Further pipette 0.3ml of the flupritine stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. (Fig.3)

Preparation of Sample preparation

Accurately weigh and transfer equivalent to10 mg of Tablet powder Fpmt Working standard into a10 mL volumetric flask add about 7 mL of Diluent and sonicated to dissolve it completely and made volume up to the mark with the same solvent (Stock solution).Further pipette 0.3ml of the flupritine stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. The % assay was found to be 100%.

Validation of the method

Validation of the optimized method was performed according to the ICH Q2 (B) guidelines[9-10].

System suitability studies

The system suitability test was carried out on freshly prepared stock solution of flupritine to check various parameters such as column efficiency, tailing factor and number of theoretical and presented in Table 1. The values obtained were demonstrated the suitability of the system for the analysis of the drug. System suitability parameter may fall within 2 % standard deviation range during routine performance of the method.

Linearity

For the HPLC methodfor the determination of linearity, prepared standard solution at five different concentration levels. The method was found to be linear in the range of 50 to 150% of target assay concentration i.e. 200 to 600 µg/ml. peak areas were recorded and calibration curve was plotted by plotting peak areas on y axis and concentration on x axis. The calibration curve was shown in fig.3and the results are presented in table 2.In UV the method was found to be linear in the range of 50 to 175 % of target assay concentration. I.e. 10 to 35 µg/ml measured the absorbances of the above levels at 250nm Plotted a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient.The calibration curve was shown in fig.4 and the results are presented in table 3.

Method precision (intra and inter day)

The repeatability of the analytical method under normal operational conditions was verified by injecting assay preparationssix times individuallyand calculated the % RSD. The same was performed on different day also. And the % RSD values for intraday and interday precision were based on the mean and standard deviations. The % RSD was found to be less than 1% by UV and less than 2 % by HPLC, indicating that the method was sufficiently precise; the results are shown in **table 4**.

Accuracy or recovery

Accuracy is the percent of the analyte recovered by assay from a known added amount. This study is mainly useful in order to check the accuracy of the developed method and to study the interference of the formulation additives. For the measurement of accuracy data from nine determinations over three concentration levels i.e. 50%, 100% and 150 % were determined. The results are presented in table 5.

Robustness

Robustness of the method was verified by altering individually the chromatographic conditions like mobile phase composition (10%) , flow rate (±0.2 ml), column temperature (±5°C), etc.it was observed that no much variation in system suitability parameters even after these small delibral changes.These results proved that the developed method was robust.In UV method the wave length was varied at 248 nm to 252nm. On evaluation of the above results, it can be concluded that the variation in wave length affected the method significantly. Hence it indicates that the method is robust even by change in the wave length.

Forced degradation studies

The International Conference on Harmonization (ICH) guideline entitled stability testing of new drug substances and products requires that stress testing be carried out to elucidate the inherent stability characteristics of the active substance. The aim of this work was to perform the stress degradation studies on theflupritine using the proposed methodForced degradation of the flupritine maleate drug substance was performed under acid, alkaline, peroxide, thermal, photolytic and hydrolysis stress conditions. The procedure and results were summarized in the table 6& 7.

RESULTS AND DISCUSSION

The developed methods for estimation of flupritine maleate were found to be accurate, simple and precise. The simplicity and ease of the developed methods lie in using Methanol as solvent.

The linearity of both HPLC and UV methods showed excellent correlation coefficient of 0.999.

In precision studythe% RSD was found to be less than 1% by UV and less than 2 % by HPLC, indicating that the method was sufficiently precise. Both methods showed good recovery and the recovery values are found between 98 to 101 % and % RSD was found to be less than 1% indicating accuracy of the method. The method was found to be robust because system suitability was passed even though deliberal changes were made in the developed HPLC and UV methods. In degradation studies it was observed that purity threshold value greater than the purity angle in each condition value it indicates the noninterference of the excipients, degradants with the analyte peak.

Table 1: System suitability

Injection	RT	Peak Area	USP plate count	USP Tailing
1	4.986	5229276	3832	1.7
2	4.956	5245400	3837	1.7
3	4.912	5236608	3842	1.7
4	4.863	2546067	3839	1.7
5	4.833	5197315	3762	1.7
6	4.802	5010547	3962	1.7
Mean	4.892	5194202	3845.6	1.683
SD	0.071769	91737.14	-	-
%SD	1.467	1.766	-	-

Table 2: Results of Linearity by HPLC

Linearity level	Concentration	Area
L1-50%	200 µg/ml	2707744
L2-7575%	300 µg/ml	4120178
L3-100%	400 µg/ml	5664215
L4-125%	500 µg/ml	7052773
L5-150%	600 µg/ml	8632105

Table 3: Results of Linearity by UV spectroscopy

S. No	Linearity Level	Concentration	Absorbance
1	I (50%)	10µg/ml	0.326
2	II (70%)	15µg/ml	0.456
3	III (100%)	20µg/ml	0.6
4	IV (125%)	25µg/ml	0.719
5	V (150%)	30µg/ml	0.883
6	VI (175%)	35µg/ml	1.01
Correlation coefficient	0.999		

Table 4: Results of Precision by HPLC and UV

Sample No.	HPLC		UV spectroscopy	
	Intraday % Assay	Interday % Assay	Intraday % Assay	Interday % Assay
1	100.3	100.2	99.4	99.6
2	100.6	98.8	99.4	99.6
3	100.4	100.7	99.4	99.6
4	100.6	99.1	99.4	99.6
5	99.7	101.0	99.6	99.8
6	96.1	100.6	99.4	99.8
Mean	99.6	100.1	99.4	99.7
RSD (%)	1.76	0.89	0.1	0.1

Table 5: Results of Recovery by HPLC and UV

S. No	Level of Recovery (%)	Recovery (%) N=3	%R.S.D. N = 3	Level of Recovery (%)	Recovery (%) N=3	%R.S.D N = 3
1	50%	99.4%	0.3	50%	101.3%	0.3
2	100%	100.1%	0.3	100%	99.6%	0.2
3	150%	98.6%	0.2	150%	98.7	0.1

Table 6: Summary of degradation studies byHPLC

Mode of degradation	Conditions	Assay	Flupritine maleate		
		(mg/ tablet)	%Degradation w.r.t. control	Purity angle	Purity Threshold
Control	No treatment	398.2	-	-	-
Acid degradation 5N HCl	40°C/5min	369.2	7.30	0.131	0.289
Alkali degradation 1N NaOH	80°C/1hr	360.76	9.40	0.153	0.293
Peroxide degradation 30%W/V H_2O_2	80°C/10min	293.76	26.20	0.209	0.296
Thermal degradation	105°C/72hrs	376.32	5.50	0.195	0.306
Photolytic degradation	UV/72hrs	331.56	16.70	0.176	0.304
Humidity degradation	25°C/90%RH/72 hrs	395.08	0.80	0.146	0.289

Table 7: Summary of degradation studies by UV

Type	Conditions	Sample Absorbance	% Assay	% DEG	Observation
Acid degradation 5N HCl	40°C/5 min	0.513	85.2	14.80	degraded
Alkali degradation 1 N NaOH	80°C/1hr	0.511	84.7	15.30	degraded
Peroxide degradation 30 % W/V H2O2	80°C/10 mins	0.538	89.3	11%	degraded
Thermal degradation	105°C/72 hrs	0.565	93.9	7%	degraded
Photolytic degradation	UV/72 hrs	0.506	84	16%	degraded
Humidity degradation	25°C/90%RH/72hrs	0.567	94	6%	degraded

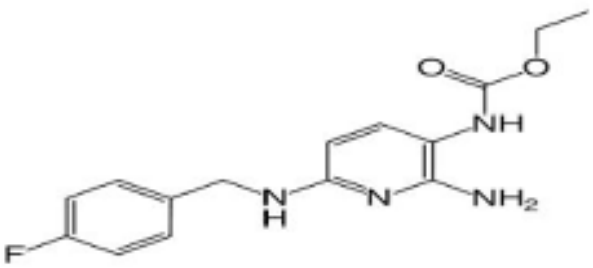


Fig. 1: Chemical structure of flupritine maleate

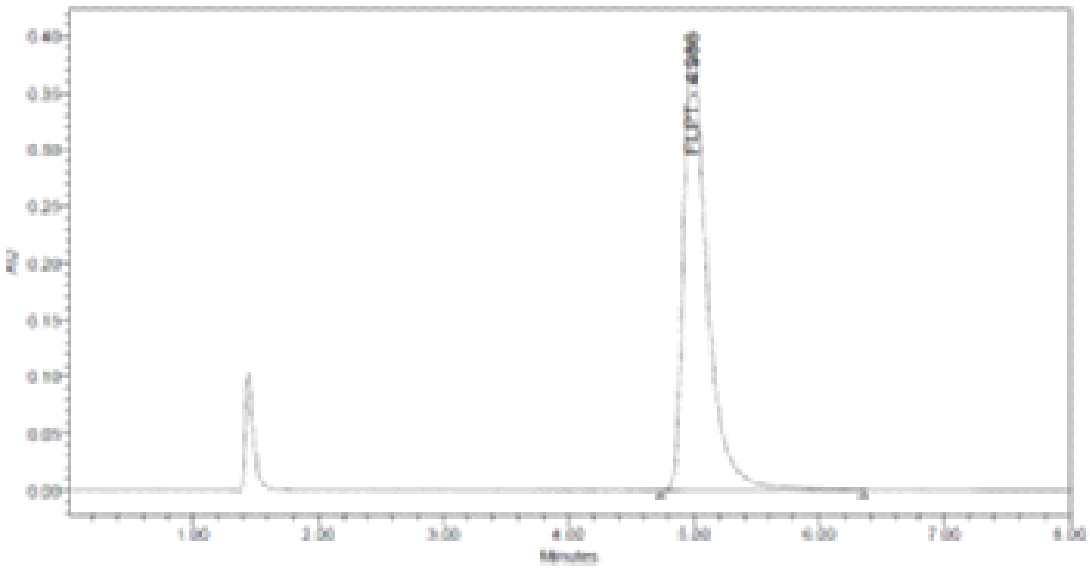
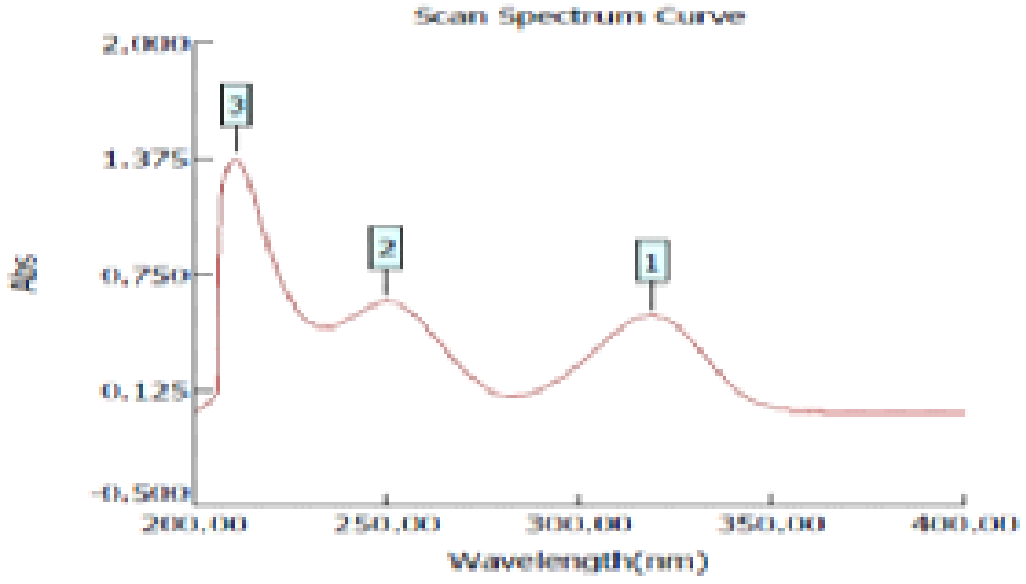


Fig. 2: Standard chromatogram of Flupritine maleate



1	Peak	319.00	0.526
2	Peak	250.00	0.602
3	Peak	211.00	1.374

Fig. 3: Tpyical chromatogram of standard

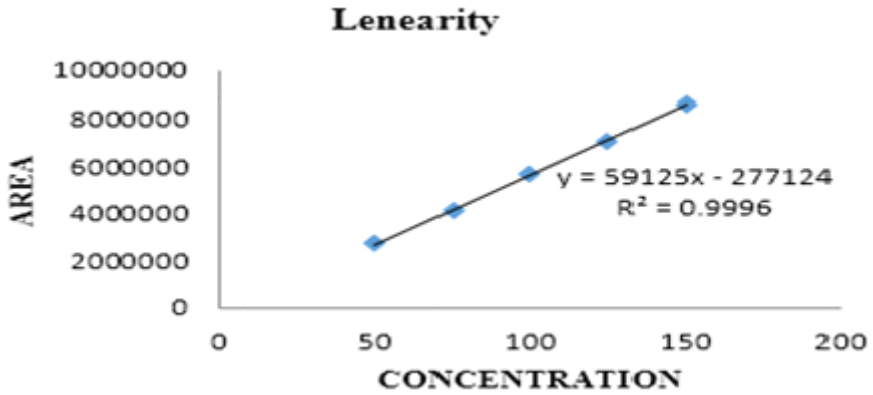


Fig. 4: Calibration curve by HPLC

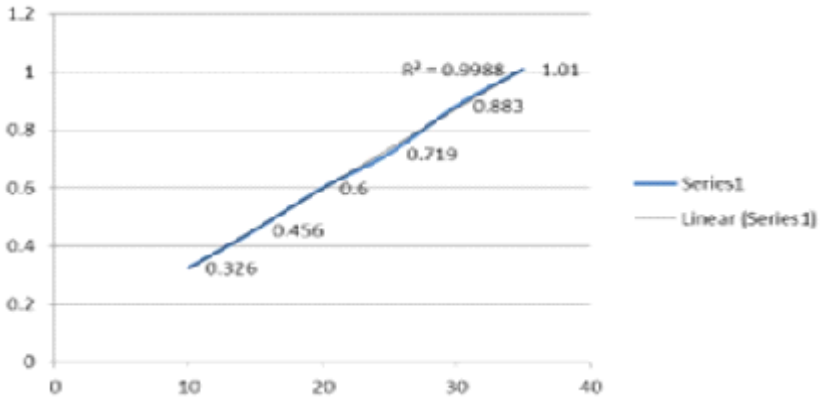


Fig. 5: Calibration curve by UV

CONCLUSION

The developed methods are able to analyze the commercial formulations economically.

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