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DEVELOPMENT AND VALIDATION OF TLC-DENSITOMETRIC METHOD FOR ESTIMATION OF FLUPIRTINE MALEATE AND ITS FORMULATIONS

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Keywords:

Flupirtine Maleate, Spectrophotometry, ICH, HPTLC, Capsules

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ABSTRACT: A simple, precise, accurate, and reliable high-performance thin-layer chromatographic method has been developed and validated for the analysis of flupirtine maleate in capsule dosage form. Identification and analysis were performed on 10 cm × 10 cm layer thickness 0.2 mm, precoated silica gel G60-F254 aluminum sheet, prewashed with methanol, and dried in an oven at 50 °C for 5 min. Ethyl acetate: Chloroform (60 : 40) (v/v) was used as mobile phase. A calibration plot was established, showing the dependence of response (peak area) on the amount chromatographed. The validated calibration range was found to be 50-400 ng/spot and for flupirtine maleate with correlation coefficient (r^2) 0.999. The average % recovery was between 99.85-100.25%. The limit of detection and limit of quantification for flupirtine maleate were found to be 7.17 and 21.7 ng/spot, respectively. The spots were scanned with a densitometer at λ_{max} 326nm in a reflectance mode. In this method, the drug was achieved R_f value of 0.46±0.02. The proposed method was validated as per ICH guidelines and successfully applied to the estimation of flupirtine maleate in various pharmaceutical dosage forms.

INTRODUCTION: Flupirtine maleate (FPM) is a pyridine derivative with the chemical name of N-[2-Amino-6-[[(4-fluorophenyl) methyl] amino]-3-pyridinyl] carbamic Acid Ethyl Ester (2Z)-2-Butenedioate; 2-Amino-6-[(p-fluorobenzyl) amino] -3pyridine carbamic Acid Ethyl Ester Maleate **Fig. 1**. It is a non-opioid analgesic with muscle relaxant and neuroprotective properties, hydrolyzed to form the active agent, Flupirtine [1].

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Literature survey reveals that only UV [2], HPLC [3-9] & LC-MS/MS [10, 11] methods are available for analytical estimation of FPM alone or combination with other drugs. Nowadays, high-performance thin-layer chromatography (HPTLC) has become a routine analytical technique due to its advantages of reliability in quantization, analysis at microgram and even in nanogram levels and cost-effectiveness. This method is advantageous since a large number of samples can be simultaneously subjected to analysis. The amount of solvent required in comparison to HPLC is very less. This reduces the time and cost of analysis and possibilities of pollution of the environment. HPTLC also facilitates repeated detection (scanning) of the chromatogram with the same or different parameters.

Simultaneous assay of several components in a multicomponent formulation is also possible [12, 13]. To the best of our knowledge, there is no published HPTLC method for this drug. So, the present paper describes a simple, accurate, and precise method for the estimation of flupirtine maleate (FPM) in capsule dosage form by the HPTLC method. The developed method was validated in accordance with ICH guidelines [14] and successfully employed for the assay of flupirtine maleate in various dosage forms.

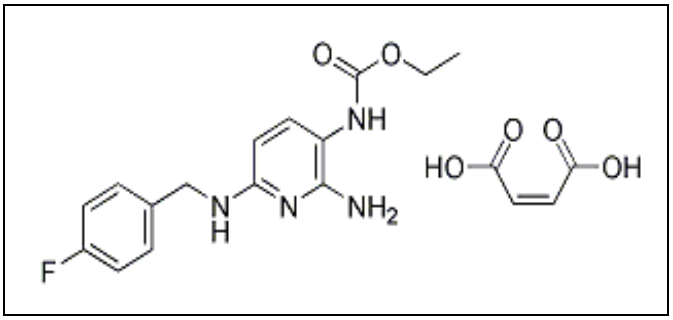

FIG. 1: FLUPIRTINE MALEATE STRUCTURE

MATERIALS AND METHODS:

Reagents and Chemicals: Analytically pure flupirtine maleate (FPM) was kindly provided by Sun Pharmaceuticals Ltd., Hyderabad, India as gift sample. Analytical grade methanol, ethyl acetate, and chloroform were purchased from MERCK Ltd., Mumbai, India. Capsules of flupirtine maleate (RETENSE-100 mg) were procured from the local market.

Instrumentation and Conditions: Chromatography was performed on 10cm × 10cm on pre-coated silica gel G60- F254 aluminum sheet (E. Merck, Mumbai, India). Before use the plates were washed with methanol then dried at room temperature. Samples were applied as 6mm bands by means of a Camag Linomat V (Muttenz, Switzerland) sample applicator equipped with 10 μL syringe; operated with settings of band length, 6 mm; distance between bands, 5 mm; distance from the plate edge, 10mm; and distance from the bottom of the plate, 10 mm. The constant application rate was 15 s/μL, and a nitrogen aspirator was used. Ascending development of plate, migration distance 8.5cm was performed at ambient temperature with Ethyl acetate: Chloroform (60: 40) (v/v), as mobile phase in a 10 cm × 10 cm Camag twin-trough chamber previously saturated for 15 min. After development, the plates were dried with a hot-hair dryer and viewed in a CAMAG UV cabinet. Densitometric scanning at λ_{max} 326 nm was then performed with a Camag TLC Scanner 3 equipped with WINCAT 3.2.1 software. The scanning rate was 20mm/s. The source of radiation used was the deuterium lamp.

Standard Stock Solution: Flupirtine maleate standard drug about 50 mg was weighed and transferred to a 50 mL volumetric flask and dissolved in methanol. The flask was sonicated, and then the volume was made up to the mark with methanol to give a solution containing 1 mg/mL. One mL of this aliquot was transferred to 10 mL volumetric flask, and the volume was made up to the mark to get containing 100 µg/mL by using same solvent.

Calibration Curve for Flupirtine Maleate: Semi-automatic spotter was used containing a syringe having a capacity of 10 μL. Standard stock solution of FPM was filled in the syringe, and under nitrogen stream, it was applied in the form of a band of desired concentration range for each spot on a single plate having a concentration of 50 to 400 ng/spot. The plate was developed using the above-mentioned conditions.

Analysis of Marketed Formulation: Twenty capsules were weighed accurately, and a quantity equivalent to 50 mg FPM was transferred to 50 mL volumetric flask. A sufficient amount of methanol was transferred to this volumetric flask and sonicated for 10 min to dissolve the drug & it was filtered through 0.45 μ Whatman filter paper; then, the volume was made up to the mark with the same solvent. Then the resulting solution was further diluted within the calibration range & it was analyzed photometrically. From the peak area obtained in the densitogram, the amount of the drug was calculated.

Validation of the Method:

Accuracy: The accuracy of this method find out by known amounts of a standard solution of FPM (160, 200, and 240 ng/spot) were added to the pre marketed sample solution.

Precision: The precision of the proposed HPTLC method was determined by analyzing the standard solution of FPM at three different concentrations

(150, 200, and 250 ng/spot) in triplicate on the same day and on three different days. The results were reported in terms of coefficient of variance (CV).

Limit of Detection and Limit of Quantification: The limit of detection (LOD) and the limit of quantitation (LOQ) of the drug was derived by using the following equations as per International Conference on Harmonization (ICH) guidelines.

$$\text{Limit of Detection} = 3.3\times\sigma/S$$
$$\text{Limit of Quantitation} = 10\times\sigma/S$$

Where σ is the standard deviation of y-intercepts of regression lines and S is the slope of calibration curve.

RESULTS AND DISCUSSION: There are several spectroscopic and chromatographic methods reported for assay of flupirtine maleate, but HPTLC method has not been reported so far. Hence the author made an attempt to develop and validate the HPTLC method for analysis of flupirtine maleate. A suitable solvent system for the development of chromatogram was optimized by testing different solvent mixtures of varying polarity. The best results were obtained by using ethyl acetate: chloroform (60:40, v/v). Under optimized conditions, both standard and sample densitograms of flupirtine maleate were recorded and shown in **Fig. 2** and **3**. There was no interference from the formulation component, and R_f value of flupirtine maleate standard and the sample was found to be the same, thereby confirming the method's selectivity. Densitometric scanning of all the tracks at 326 nm shown that the flupirtine maleate with R_f value was 0.46±0.02. Three-dimensional overlay spectra of flupirtine maleate were shown in **Fig. 4**. The calibration curve was found to be linear in the concentration range of 50–400 ng/spot with a good correlation coefficient **Fig. 5**.

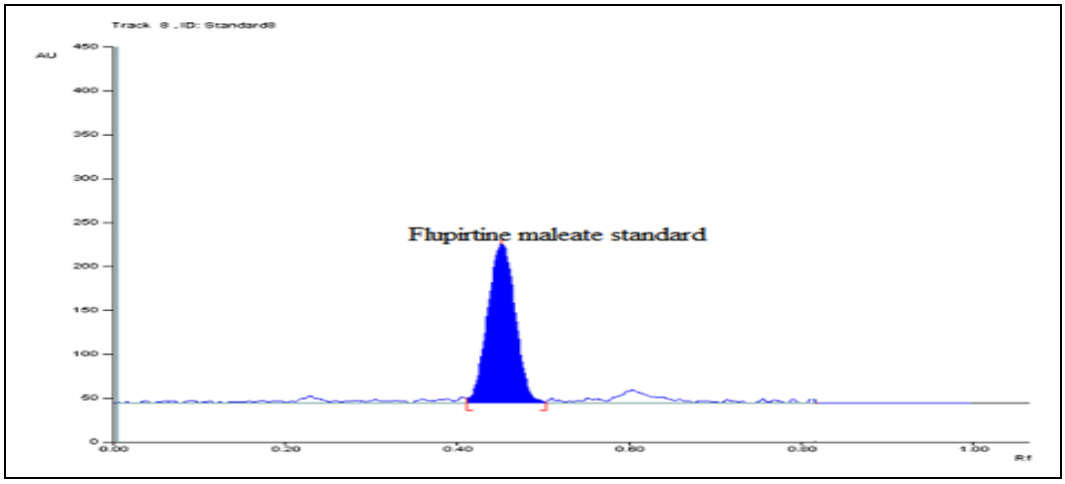

FIG. 2: STANDARD DENSITOGRAM OF FLUPIRTINE MALEATE

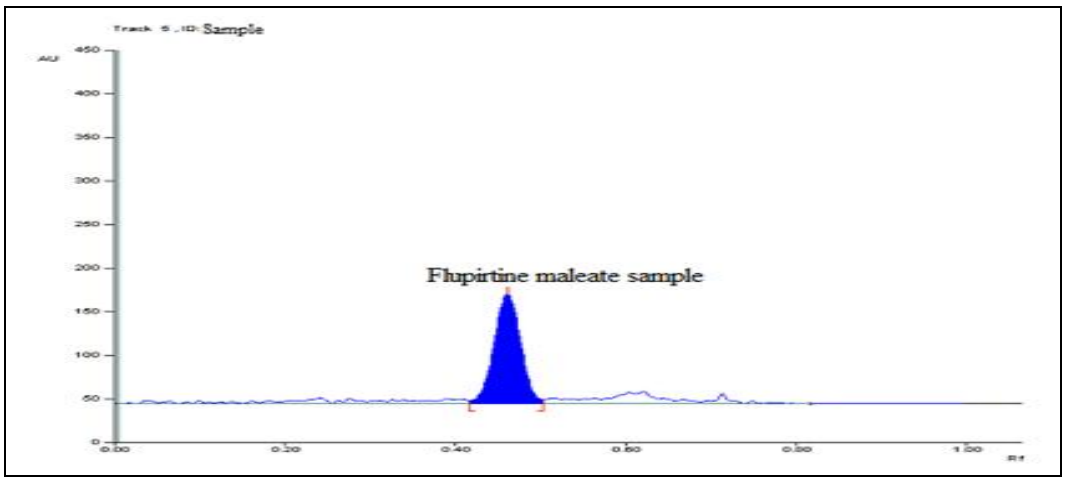

FIG. 3: SAMPLE DENSITOGRAM OF FLUPIRTINE MALEATE

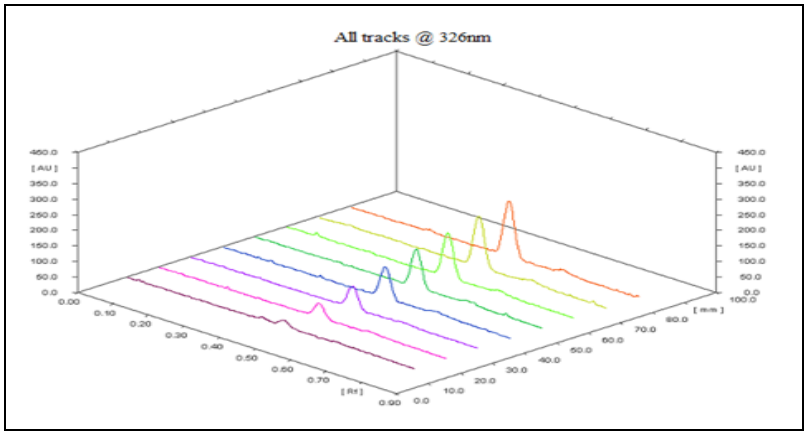

FIG. 4: THREE DIMENSIONAL OVERLAY SPECTRUM OF STANDARD FLUPIRTINE MALEATE

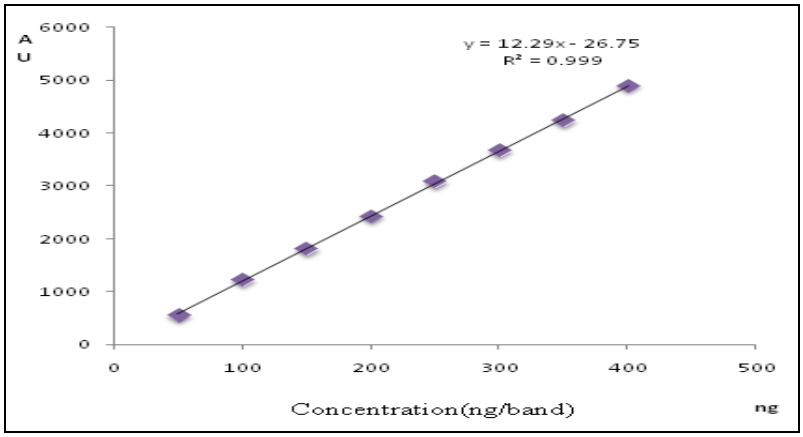

FIG. 5: CALIBRATION CURVE OF FLUPIRTINE MALEATE

The accuracy of the method was checked by adding standard solution into the pre-analyzed sample, and the % recovery of flupirtine maleate ranged from 99.85% to 100.25%, which indicates that the method is more accurate. The results were incorporated in **Table 1**.

TABLE 1: ACCURACY OF FLUPIRTINE MALEATE

Ingredient	Level of addition (%)	Amount added (ng)	Amount recovered (ng)	%Recovery*
Flupirtine maleate	80	160	159.86	99.85
	100	200	200.25	100.12
	120	240	240.42	100.25

*Average of three determinations

The precision of the method was tested by using different aliquots of a sample solution containing 150, 200, and 250 ng of flupirtine maleate. In order to control the scanner parameters, one spot was analyzed several times. The repeatability and intermediate precision studies of flupirtine maleate at different levels were given in **Table 2**. The percentage RSD was found in the range of 0.53 to 0.85%, which confirms the good precision.

TABLE 2: PRECISION OF FLUPIRTINE MALEATE

Ingredient	Concentration (ng/mL)	Intraday (%RSD)*	Interday (%RSD)*
Flupirtine maleate	150	0.64	0.85
	200	0.72	0.82
	250	0.83	0.63

*Average of six determinations

The developed assay method was applied for the estimation of flupirtine maleate from RETENSE-100mg capsule formulation. The % assay of flupirtine maleate was calculated and the results were mentioned in **Table 3**.

TABLE 3: ASSAY OF FLUPIRTINE MALEATE

Brand Name	Lable claim	Amount found	% Assay *	%RSD
RETENSE-100mg capsules	100 mg	99.98 mg	99.98	0.79

*Average of six determinations

The optical characteristics are recorded, and the results are summarized in **Table 4**. Rigorous analysis of the results shows that the presence of excipients in capsules did not interfere with the determination of the active component of FPM.

TABLE 4: OPTICAL CHARACTERISTICS OF FLUPIRTINE MALEATE

Parameters	Flupirtine maleate
R_f value	0.46±0.02
Linearity (ng/mL)	50-400
Regression equation (y=mx+c)	y = 12.29x - 26.75
Intraday Precision (%RSD)	0.73
Interday Precision (%RSD)	0.76
LOD (ng/mL)	7.17
LOQ (ng/mL)	21.78
Correlation coefficient	0.999

Statistical data analysis proves that the method is precise and reproducible for the analysis of the flupirtine maleate. The system is economical can be employed for the routine estimation of the drug in pharmaceutical formulations as well as in bulk drug analysis.

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CONFLICTS OF INTEREST: The author has no conflicts of interest in the present article.

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