Simultaneous Determination and Validation of Flupirtine maleate and Paracetamol in combined dosage form by a Chromatographic technique

ABSTRACT

Background: Flupirtine maleate is a centrally acting non-opioid analgesic used in acute and chronic pain and act by antagonize NMDA. Paracetamol is widely used as an analgesics and antipyretics and act by inhibiting cyclooxygenase enzyme, which is responsible for the synthesis of prostaglandin.

Objective: The present research work focused on developing a validated high-performance thin layer chromatographic (HPTLC) method for analysis of Flupirtine maleate and Paracetamol in combined dosage form.

Method: The detection was executed at absorption wavelengths of 286 nm for Paracetamol and Flupirtine maleate using a mobile phase Ethyl acetate: Chloroform (7:5 v/v). This method was validated according to the guidelines of the International Conference on Harmonization (ICH).

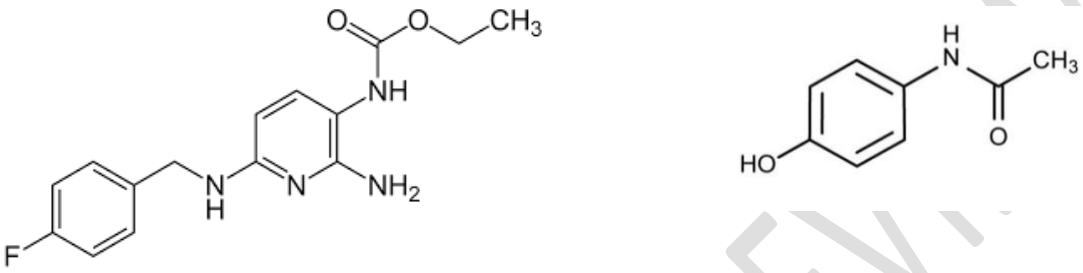
Result: In this standard procedure the R_f value for Paracetamol was detected 0.31 and 0.52 for Flupirtine maleate. The linearity was found to be in the range of 3250-6500 ng/band for Paracetamol and 1000-2000 ng/band for Flupirtine maleate, Accuracy of the method was determined by recovery studies and showed % recovery between 98 to 102%. In Precision study the percentage RSD was found to be less than 2 and assay result was within the limit for both the drugs.

Conclusion: The proposed method can be used for routine analysis and quality control assay of Flupirtine maleate and Paracetamol in combined dosage form.

Keywords: Flupirtine maleate, Paracetamol, HPTLC, Lupirtin-P

1. INTRODUCTION

Flupirtine maleate is a centrally acting non-opioid analgesic used in acute and chronic pain. Flupirtine maleate (Figure 1A), chemically named as Ethyl-2-amino-6(4-fluorobenzylamino)3- pyridylcabamate maleate acts as N-methyl-D-aspartate (NMDA) antagonist, hence neuronal excitation is inhibited [1-3]. Paracetamol is widely used as an analgesics and antipyretics. Paracetamol (Figure 1B), chemically named as N-(4-hydroxyphenyl)acetamide act by inhibiting cyclooxygenase enzyme, which is responsible for the synthesis of prostaglandin [4-6].



A **B**

Figure 1: (A) Chemical structure of A. Flupirtine maleate and B. Paracetamol

The review of the literature revealed that various analytical methods involving spectrophotometry, HPLC and stability study have been reported for Flupirtine maleate alone and in combination with other drugs [7-10]. Analytical methods like spectrophotometry, HPLC, UPLC, LC-MS and Stability study have been reported for Paracetamol with other combinations [11-19]. In the literature, various spectrophotometric, chromatographic and stability indicating methods were found for the determination of Flupirtine maleate and Paracetamol in combined dosage form [20-25]. The existing study describes a HPTLC method for the estimation of Flupirtine maleate and Paracetamol in combined dosage form. The prospective method is validated as per ICH guidelines [26].

2. MATERIALS AND METHODS:

2.1 Materials:

Reference standard of Flupirtine maleate was obtained from Datt Chemicals, Gujarat and Paracetamol was obtained from Yarrow Chem Laboratories, Maharashtra. The marketed formulation of Flupirtine maleate and Paracetamol in combination (Lupirtin-P) was procured from local pharmacy. Methanol, Ethyl acetate, Chloroform and distilled water were used throughout the study are AR grade.

2.2 Chromatographic conditions:

The HPTLC system consist of TLC Aluminum Sheet precoated with Silica Gel 60 F_{254} as stationary Phase, Win- CATS software, 100 µl Hamilton syringe with CAMAG Linomat 5 applicator and 286 nm was selected as the wavelength for measurement of Paracetamol and Flupirtine maleate. On the basis of optimum resolution and R_f value, the mobile phase was selected as a mixture containing Ethyl acetate: Chloroform (7:5 v/v).

2.3 Preparation of standard stock solution of Flupirtine maleate and Paracetamol:

Accurately weighed quantity of Flupirtine maleate and Paracetamol (50 mg) was taken in a 50 ml volumetric flask separately, dissolved and volume made up to mark with methanol (1000 µg/ml). From the stock solutions 1 ml of Flupirtine maleate and 3.25 ml of Paracetamol were taken in a 10 ml volumetric flask, mixed and volume made up with methanol to get a solution containing 100 µg/ml of Flupirtine maleate and 325 µg/ml of Paracetamol, respectively. Each microlitre (µl) of resulting solution contains 100 ng of Flupirtine maleate and 325 ng of Paracetamol.

2.4 Selection of wavelength:

Standard stock solutions of Paracetamol (3250 ng/band) and Flupirtine maleate (1000 ng/band) were prepared and applied on the precoated TLC plate and scanned using CAMAG HPTLC scanner III. It was observed that Paracetamol and Flupirtine maleate showed considerable absorbance at 286 nm. So, 286 nm was selected as the wavelength for measurement of Paracetamol and Flupirtine maleate throughout the method.

2.5 Preparation of calibration curve:

Different concentrations of standard Flupirtine maleate solution ranging from 1000-2000 ng/band and Paracetamol solution ranging from 3250-6500 ng/band were prepared by taken 10, 12, 14, 16, 18 and 20 µl from mixed standard stock solution, applied to the plate for the calibration curve of these drugs. Peak area of the spots was measured at 286 nm in the absorbance mode with CAMAG TLC scanner.

2.6 Method Validation: [26-28]

2.6.1 Linearity and Range:

The linearity of response was determined in concentration range of 1000-2000 ng/band for Flupirtine maleate and 3250-6500 for ng/band Paracetamol. The calibration curve was plotted using peak areas vs. concentrations to get correlation-coefficient and regression line equations for Flupirtine maleate and Paracetamol. Linearity is expressed in terms of correlation co-efficient of linear regression line.

2.6.2 Precision:

2.6.2.1 ***Repeatability:***

Mixed standard solution containing Flupirtine maleate (1600 ng/band) and Paracetamol (5200 ng/band) was applied six times, scanned, peak area was measured and % RSD was calculated.

2.6.2.2 ***Intra-day precision:***

Variation of results within same day is called Intra-day precision. The Intra-day precision was determined for standard solution of Flupirtine maleate and Paracetamol for the three different concentrations three times on the same day. Peak areas was measured and % RSD was calculated.

2.6.2.3 ***Inter-day precision:***

The Inter day precision was determined for standard solution of Flupirtine maleate and Paracetamol for the three different concentrations were analyzed 3 times on the three different days. The % RSD was calculated.

2.6.3 Accuracy:

1600 ng/band and 5200 ng/band drug solution of Flupirtine maleate and Paracetamol was taken in three different flask labelled as A, B and C respectively. Spiked 80%, 100%, 120% of standards solution in it and diluted up to 10 ml. The peak area of each solution was measured at 286 nm. The amount of Flupirtine maleate and Paracetamol was calculated at each level and % recoveries were computed.

2.6.4 Limit of detection and limit of quantification:

In order to determine the detection and quantification limits, following equations designated by International Conference of Harmonization (ICH) guidelines.

$$LOD = 3.3 \times (SD / Slope)$$

$$LOQ = 10 \times (SD / Slope)$$

2.6.5 Estimation of Flupirtine Maleate and Paracetamol in formulation:

20 tablets crushed and powdered. Powder equivalent to 50 mg of Flupirtine Maleate and 162.5 mg of Paracetamol was taken in a 50 ml volumetric flask, volume made up with methanol and sonicated for 30 minutes, filtered the solution, which contains 1000 µg/ml of Flupirtine maleate and 3250 µg/ml of Paracetamol. From the solution 1 ml was taken in a 10 ml volumetric flask and volume made up to mark with methanol, to get 100 µg/ml of Flupirtine maleate and 325 µg/ml of Paracetamol. The solution was injected 16 µl. The areas of resulting peak were measured at 286 nm.

4. RESULTS AND DISCUSSION:

4.1 Mobile phase optimisation:

Different solvent systems were tried for separation of paracetamol and flupiritine maleate. Separation was achived in mobile phase Ethyl acetate: Chloroform (7:5 v/v). The Rf values were found to be 0.31 for paracetamol and 0.52 for flupiritine maleate. Chromatogram of paracetamol and flupiritine maleate shown in Figure 2.

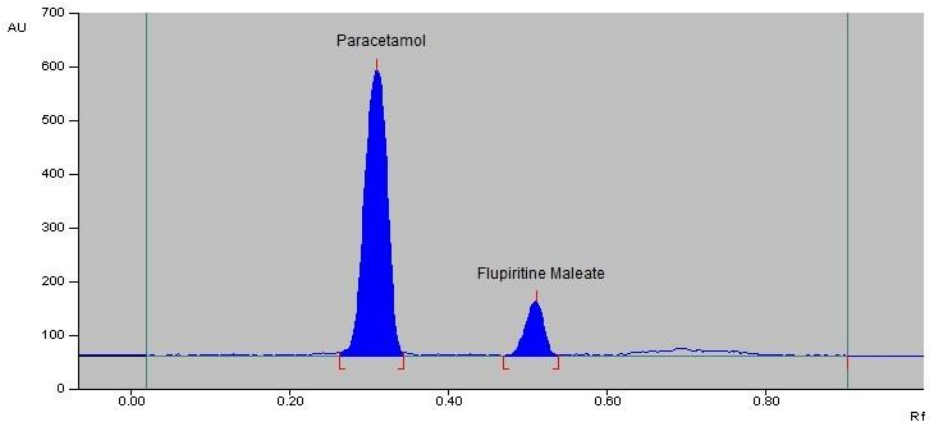


Figure 2: Chromatogram of standard mixture, peak 1: Paracetamol (Rf: 0.31) and peak 2: Flupiritine maleate (Rf: 0.52)

4.2 Method Validation:

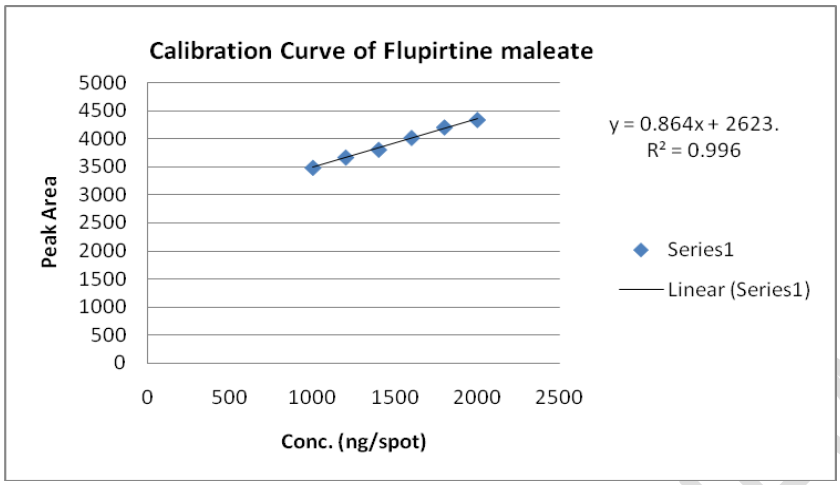
4.2.1 Linearity and Range:

The linearity was found in the concentration range of 1000-2000 ng/band for Flupirtine maleate and 3250-6500 ng/band for Paracetamol (Table 1). Correlation co-efficient for the calibration curve of Flupirtine maleate and Paracetamol were found to be 0.996 and 0.994 respectively. The calibration curve of Flupirtine maleate and Paracetamol were shown in Figure 3. The regression line equation was Y=0.864x+2623 for Flupirtine maleate and Y=1.049x+8071 for Paracetamol. The linearity chromatogram of Flupirtine maleate and Paracetamol were shown in Figure 4.

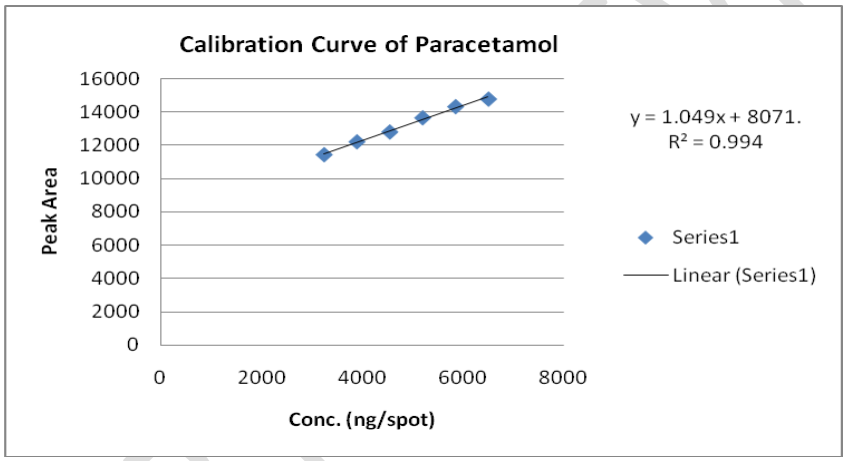
Table 1: Calibration data for Flupirtine maleate and Paracetamol:

Flupirtine maleate		Paracetamol	
Conc. (ng/spot)	**Peak area (n=3)**	**Conc. (ng/spot)**	**Peak area (n=3)**
1000	3487.78	3250	11424.75
1200	3669.91	3900	12198.69
1400	3807.57	4550	12786.39
1600	4017.08	5200	13640.07
1800	4203.72	5850	14309.40
2000	4336.33	6500	14761.85

n= number of determinations



(A)



(B)

Figure 3: Calibration curve of (A) Flupirtine maleate and (B) Paracetamol

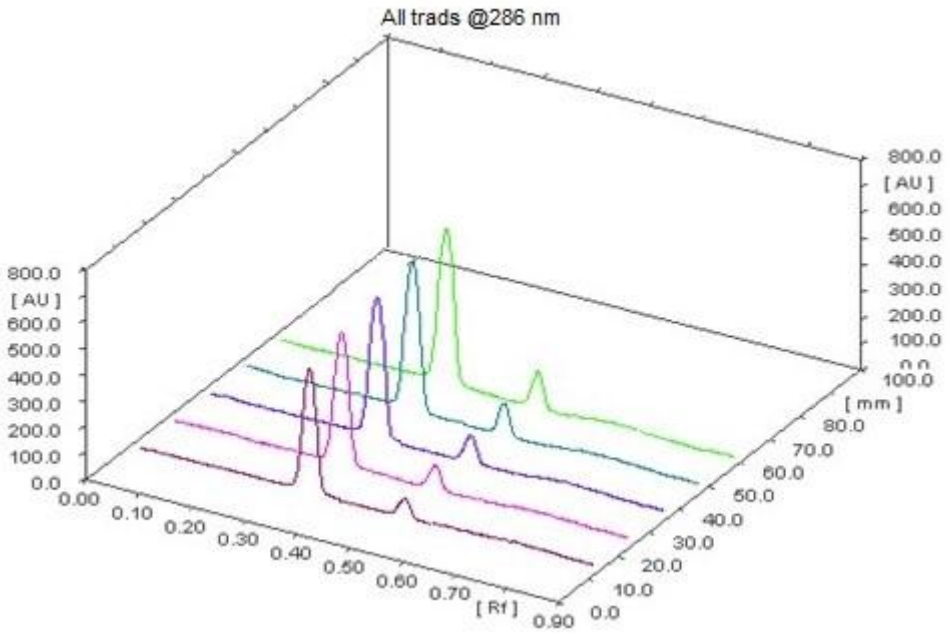


Figure 4: 3D linearity chromatogram of Paracetamol (Rf: 0.31 ± 0.02) and Flupirtine maleate (Rf: 0.52 ± 0.02)

4.2.2 Precision:

The precision of the proposed method was estimated in terms of repeatability, interday precision and intraday precision. In Repeatability study same concentration analyzed six times. In intraday precision, the method was analyzed three times on the same day and in interday precision, the method was analyzed three times on the different days. The results shown that the percentage RSD is less than 2% at each level, clearly indicates that the proposed method is precise enough for the analysis of drug (Table 2).

4.2.3 LOD and LOQ:

LOD and LOQ of the proposed method were found to be 300.38 and 910.24 for Flupirtine maleate, 1042.90 and 3160.32 for Paracetamol respectively (Table-2).

4.2.4 Accuracy:

Accuracy of the analytical procedure was established by percentage recovery study from marketed formulation at three level of standard addition. Values of recovery in the range of 98 – 102 % indicate that proposed method is accurate for the analysis of drug (Table 2).

Table 2: Summary of Validation Parameters for the HPTLC method

Parameters	Flupirtine maleate	Paracetamol
Linearity Range (ng/spot)	1000-2000	3250-6500
Correlation coefficient	0.996	0.994
Regression equation	Y=0.864x+2623	Y=1.049x+8071
LOD (ng/spot)	300.38	1042.90
LOQ (ng/spot)	910.24	3160.32
Precision (%RSD)		
Repeatability (n=6)	0.02293	0.0055
Intraday (n=3)	0.7995	0.7999
Interday (n=3)	0.9110	0.6890
Accuracy		
80% (n=3)	101.06	99.07
100% (n=3)	100.52	100.31
120% (n=3)	100.16	99.70

n= number of determinations

4.3 Analysis of marketed formulation:

Applicability of the proposed method was tested by analyzing the commercially available Tablet formulation Lupirtin-P. Their results are shown in Table 3.

Table 3: Assay of marketed formulation by proposed HPTLC method

Drugs	Labeled amount (mg)	Amount found (mg)	Amount found (%)*	% RSD
Flupirtine maleate	100	99.30	99.30 ± 0.832	0.837
Paracetamol	325	321.29	98.86 ± 1.323	1.338

* mean ± SD

5. CONCLUSION:

The proposed HPTLC method provides unique quantitative analysis for the determination of Flupirtine maleate and Paracetamol in combined dosage form. According to ICH guidelines this method was validated in terms of linearity, accuracy, precision, limits of detection (LOD) and limits of quantification (LOQ). The proposed method can be used for routine analysis and quality control assay of Flupirtine maleate and Paracetamol in combined dosage form.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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