Research Article

Development of HPTLC Method for Simultaneous Estimation of Paracetamol and Flupirtine Maleate in their Combined Tablet Dosage Form

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ABSTRACT

A simple, accurate and precise high-performance thin layer chromatographic method for simultaneous estimation of paracetamol and flupirtine maleate in their combined tablet dosage form has been developed. The method employed thin layer chromatographic aluminium plates pre-coated with silica gel 60F254 as the stationary phase and toluene: acetone: triethylamine (6:4:0.5 v/v/v) as mobile phase. Chromatographic analysis was carried out in the reflectance/absorbance mode at 250 nm. The method was validated with respect to linearity, specificity, accuracy, precision, limit of detection and limit of quantitation and applied for analysis of paracetamol and flupirtine maleate in combined tablet dosage form. The Rf values were found to be 0.27 ± 0.02 and 0.47 ± 0.02 for paracetamol and flupirtine maleate, respectively. The linear regression analysis data for the calibration plots showed a linear relationship in the concentration range 975 - 2275 ng/band with correlation coefficient 0.9990 for paracetamol and 300 - 700 ng/band with correlation coefficient 0.9963 for flupirtine maleate. The proposed method can be applied for routine analysis of tablets containing flupirtine maleate and paracetamol in combination.

Keywords : Paracetamol, Flupirtine maleate

1. INTRODUCTION

Paracetamol, chemically N-(4-hydroxyphenyl)acetamide, is a p-aminophenol derivative which is widely used as analgesic and antipyretic (Figure 1 a). It is used for the relief of fever, headaches, and other minor aches and pains [1-3]. Flupirtine maleate, chemically ethyl{2-amino-6-[(4-fluorobenzyl)amino]pyridin-3-yl}carbamate maleate, is a centrally acting, non-opioid analgesic, used for the treatment of a variety of pain states (Figure 1 b). Its muscle relaxant properties makes it popular for back pain and other orthopaedic uses [2-4]. Combined tablet dosage form of paracetamol and flupirtine maleate is available in the Indian market for treatment of acute and chronic pain. Various HPLC, HPTLC, fluorimetric, UV-Visible spectrophotometric [5-10] methods have been reported in literature for estimation of paracetamol individually and in combination with other drugs. Techniques such as UPLC, HPLC, LC-MS, fluorimetry and UV-Visible spectrophotometry [11-15] have been reported in literature for estimation of flupirtine maleate. Techniques such as HPLC [16] and spectrophotometry [17-18] for simultaneous estimation of paracetamol and flupirtine maleate have been reported in literature. No HPTLC method for simultaneous estimation of

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paracetamol and flupirtine maleate has been reported in literature. HPTLC is a simple, less solvent consuming and less time consuming technique. The present work describes development and validation of HPTLC method for simultaneous estimation of paracetamol and flupirtine maleate in their combined tablet dosage form.

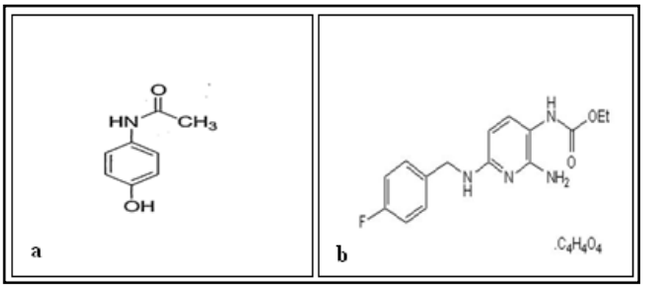

Figure 1 Chemical structure of (a) paracetamol and (b) flupirtine maleate

2. MATERIALS AND METHODS

2.1. Instruments

The HPTLC system (Camag, Switzerland) consisting of Linomat V semiautomatic spotting device, a TLC Scanner IV (Camag, Switzerland), twin-trough developing chamber (10 x10 cm), a UV cabinet with dual wavelength UV lamps, win-CATS software, syringe (100 µl, Hamilton) were used for chromatographic study. UV- visible spectrophotometer (Shimadzu UV 1800) and electronic analytical balance (Shimadzu AUX-220) were used during the study.

2.2. Materials

Paracetamol was purchased from Yarrow Chem Products, Mumbai, India and flupirtine maleate was received as a gift sample from Lupin Pharmaceuticals Ltd., Baroda, India. All chemicals and reagents (analytical grade) were purchased from S.D. Fine-Chem Limited, Mumbai, India. Tablets (Lupirtin P, Lupin Ltd.) containing paracetamol 325 mg and flupirtine maleate 100 mg were procured from the local market.

2.3. Standard solutions of paracetamol and flupirtine maleate

Accurately weighed 65 mg of standard paracetamol was transferred to a 10 ml volumetric flask, dissolved and diluted to mark with methanol to get standard stock solution having strength 6500 µg/ml. Aliquot 5 ml was further diluted to 10 ml with methanol to obtain solution of strength 3250 µg/ml.

Accurately weighed 25 mg standard flupirtine maleate was transferred to a 25 ml volumetric flask, dissolved and diluted to mark with methanol to get solution of strength 1000 µg/ml.

An accurately measured volume of 0.5 ml of each stock solution was transferred to a 10 ml volumetric flask and diluted to mark with methanol to get concentration 162.5 µg/ml for paracetamol and 50 µg/ml for flupirtine maleate.

2.4. Selection of wavelength

Standard solutions of paracetamol (13.0 µg/ml) and flupirtine maleate (4.0 µg/ml) were prepared in methanol and scanned between 200-400 nm against methanol as blank using UV-visible spectrophotometer. Wavelength for scanning was determined from overlay spectra of paracetamol and flupirtine maleate.

2.5. Chromatographic conditions

Chromatographic separation was performed on 10 × 10 cm aluminium backed plates pre-coated with 100 µm layer of silica gel 60 F_{254} (E. Merck, Darmstadt, Germany). The TLC plates were pre-washed with methanol and activated at 80°C for 5 min prior to sample application. The samples were applied on TLC plate 15 mm from the bottom edge by Linomat V semi-automatic applicator using following parameters: band width, 6 mm; track distance, 11.6 mm and application rate, 0.1 µl/s. The TLC plate was developed in twin trough chamber using toluene: acetone: triethylamine (6: 4: 0.5 v/v/v) as mobile phase at temperature, 25 ± 2˚C; relative humidity, 35 ± 5%; chamber saturation time, 20 min and migration distance, 75 mm. The TLC plates were dried, scanned and

analysed by TLC Scanner IV and Win-CATS software using: slit dimension, 4 × 0.30 mm; scanning speed, 20 mm/sec and detection wavelength, 250 nm.

2.6. Method validation

2.6.1. Linearity

The linearity range was determined by analysing 5 independent levels of calibration curve in the range of 975-2275 ng/band for paracetamol and 300-700 ng/band for flupirtine maleate. A volume of 6, 8, 10, 12 and 14 µl of combined working standard solution (162.5 µg/ml paracetamol and 50 µg/ml flupirtine maleate) were applied to TLC plate, developed and analysed as per the proposed method.The calibration curve was prepared by plotting peak area vs. respective concentration and correlation coefficient was calculated.

2.6.2. Specificity

The specificity of the method was ascertained by analysing standard drug and sample. The bands of paracetamol and flupirtine maleate in samples were confirmed by comparing the R_f and UV spectra of the bands with those obtained from respective standards. The peak purity was assessed in spectrum mode by comparing spectra acquired at three different positions on the band, i.e. peak start (s), peak apex (m), and peak end (e) for each peak in sample.

2.6.3. Precision

Precision was studied as repeatability and intermediate precision. Repeatability of area measurement was determined by applying combined working standard solution (10 µl) on the TLC plate. Plate was developed and analysed as per the proposed method. The band was scanned seven times without changing plate position and %RSD of peak area was calculated for both drugs. Repeatability of sample application was determined by applying combined working standard solution (10 µl) seven times on the same TLC plate. Plate was developed and analysed as per the proposed method. The area of seven bands was measured and the %RSD of peak area was calculated for both drugs. Intraday precision was determined by analyzing combined standard solution over entire calibration range three times on same day and expressed as % RSD. Interday precision was determined by analyzing combined standard solution over entire calibration range on three different days and expressed as % RSD.

2.6.4. Limit of detection (LOD) and limit of quantitation (LOQ)

The LOD was determined experimentally by spotting gradually low concentrations of combined working standard solution till the drugs could not be detected and the LOQ was determined by establishing the minimum level at which the drugs could be quantified with acceptable accuracy and precision.

2.6.5. Accuracy

The accuracy of the method was evaluated by recovery study. It was performed in triplicate by standard addition method at 80%, 100% and 120% levels. Known amounts of standard paracetamol and flupirtine maleate were added to pre-analyzed samples and were analyzed by the proposed method.

2.7. Solution stability

The freshly prepared combined working standard solution of paracetamol and flupirtine maleate was analysed as per the proposed method and stored at 25±2°C. The solution was analysed after 6, 12 and 24 hrs. The stability of the solution was determined by comparing peak areas of paracetamol (1625 ng/band) and flupirtine maleate (500ng/band) with their respective initial peak areas.

2.8. Analysis of marketed formulations by proposed method

Twenty tablets were weighed, powdered and mixed. Powder equivalent to 25 mg of flupirtine maleate (or 81.25 mg of paracetamol) was transferred to a 25 ml volumetric flask, about 15 ml methanol was added, sonicated for 15 min, diluted with methanol up to the mark, mixed well and filtered through Whatman filter paper no. 42.

Filtrate was suitably diluted to obtain concentration 50 µg/ml flupirtine maleate and 162.5 µg/ml paracetamol. A volume of 10 µl of solution was applied on the TLC plate and analyzed. Amount of drugs in tablets was calculated using respective straight line equation of standard curve.

3. RESULTS AND DISCUSSION

3.1. Selection of wavelength

The overlain UV spectra of paracetamol and flupirtine maleate showed reasonable absorbance at 250 nm wavelength. Therefore, 250 nm was selected as wavelength for detection of the two drugs.

3.2. Mobile phase optimization

Different solvent systems were tried for separation of paracetamol and flupirtine maleate. Separation was achieved in mobile phase toluene: acetone: triethylamine (4:6:0.5 v/v/v), saturated for 30 min. The R_f values were found to be 0.27 for paracetamol and 0.47 for flupirtine maleate. Chromatogram of paracetamol and flupirtine maleate is shown in Figure 2.

3.3. Method validation

3.3.1. Linearity

A linear relationship over the concentration range 975 - 2275 ng per band for paracetamol and 300 - 700 ng per band for flupirtine maleate was observed. From the calibration curves, the orrelation coefficient was found to be 0.9990 for paracetamol and 0.9963 for flupirtine maleate. Regression line equations were y = 3.06 x + 7205.12 and y = 3.13 x + 500.63 for paracetamol and flupirtine maleate respectively. The 3D chromatogram of calibration concentrations for paracetamol and flupirtine maleate is shown in Figure 3.

3.3.2. Specificity

Comparison of chromatograms of standard and sample solutions showed identical R_f values, i.e 0.27±0.02 for paracetamol and 0.47±0.02 for flupirtine. Absorbance/reflectance spectra scanned at peak start (s), peak apex (m) and peak end (e) positions of bands showed a high degree of correlation r (s,m) 0.9998 and r (m,e) 0.9993 for paracetamol; and r (s,m) 0.9998 and r (m,e) 0.9995 for flupirtine which confirmed the purity of the two bands. The spectra of paracetamol and flupirtine maleate in sample were also compared with their respective standard spectra (Figure 4).

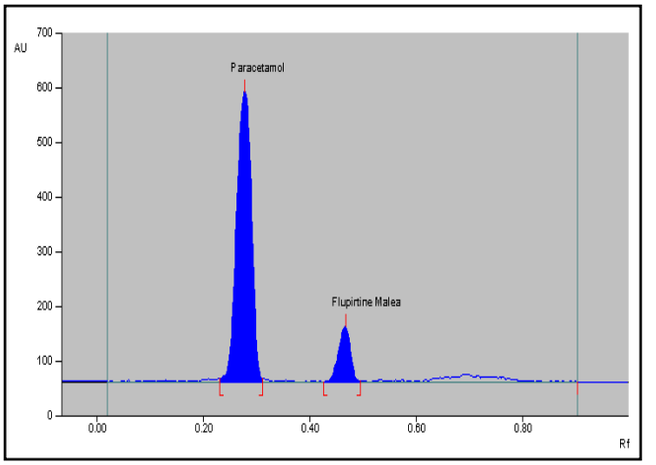

Figure 2. Chromatogram of standard mixture, peak 1: paracetamol (R_f: 0.27) and peak 2: flupirtine maleate (R_f: 0.47)

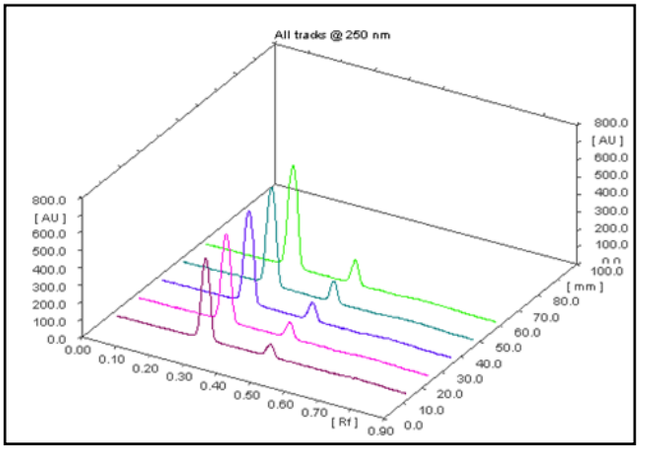

Figure 3 3D Chromatogram of paracetamol (975 - 2275 ng/band) (R_f: 0.27± 0.02) and flupirtine maleate (300 - 700 ng/band) (R_f: 0.47± 0.02)

3.3.3. Repeatability

The %RSD for repeatability of area measurement and sample application were found to be 0.66 and 0.82 for paracetamol and 0.64 and 0.80 for flupirtine respectively.

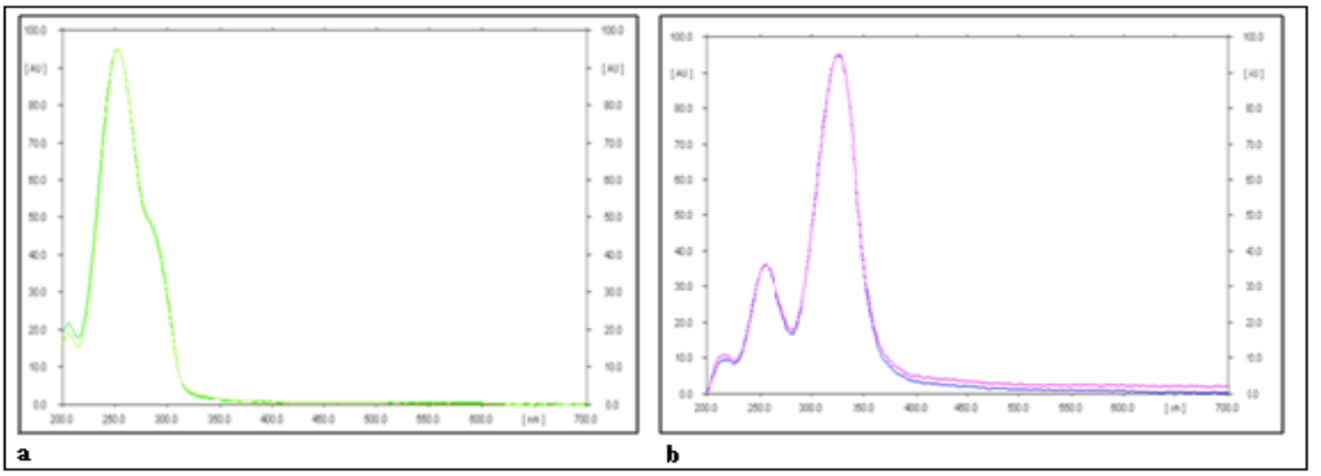

Figure 4. Overlain spectra of standard and sample of (a) paracetamol and (b) flupirtine maleate

Table 1 Results for intraday precision

Paracetamol			Flupirtine maleate		
Amount (ng/band)	**Mean area ± SD (n=3)**	**RSD (%)**	**Amount (ng/band)**	**Mean area ± SD (n=3)**	**RSD (%)**
975	10187.87 ± 125.96	1.23	300	1439.88 ± 19.72	1.36
1300	11194.86 ± 121.34	1.08	400	1789.25 ± 16.99	0.95
1625	12182.36 ± 133.48	1.09	500	2040.58 ± 25.00	1.22
1950	13080.27 ± 192.21	1.47	600	2341.53 ± 26.90	1.15
2275	14205.92 ± 177.07	1.24	700	2709.56 ± 29.17	1.07

Table 2 Results for interday precision

Paracetamol			Flupirtine maleate		
Amount (ng/band)	**Mean area ± SD (n=3)**	**RSD (%)**	**Amount (ng/band)**	**Area ± SD (n=3)**	**RSD (%)**
975	10212.45 ± 201.24	1.97	300	1439.61 ± 21.09	1.46
1300	11112.26 ± 145.63	1.31	400	1768.06 ± 34.61	1.95
1625	12284.18 ± 158.36	1.29	500	2042.13 ± 26.11	1.28
1950	13135.95 ± 197.40	1.50	600	2339.83 ± 39.64	1.64
2275	14194.53 ± 215.17	1.50	700	2791.76 ± 39.07	1.42

Table 3 Summary of validation parameters

Parameter	Paracetamol	Flupirtine
Linearity Range (ng/band)	975-2275	300-700
Correlation coefficient (R^2)	0.9990	0.9963
Precision (% RSD)		
Repeatability of measurement (n=7)	0.66	0.64
Repeatability of application (n=7)	0.82	0.80
Intraday precision(n=3)	1.08 - 1.47	0.95 - 1.36
Interday precision(n=3)	1.29 - 1.97	1.28 - 1.95
Accuracy (% recovery)	98.28 - 99.03	98.07 - 99.81
LOD (ng/band)	292	90
LOQ (ng/band)	975	300

3.3.4. Intraday and interday precision

The percent RSD for intraday precision was found to be 1.08 - 1.47 for paracetamol and 0.95 -1.36 for flupirtine maleate and interday precision, 1.29 -1.97% for paracetamol and 1.28 -1.95% for flupirtine maleate. The results for intraday and interday precision are presented in Table 1 and 2, respectively.

3.3.5. Accuracy

The accuracy of the method was determined by recovery study at three levels 80%, 100% and 120%. The recovery values were found to be 98.28 - 99.03% for paracetamol and 98.07 - 99.81% for flupirtine.

The results of validation parameters are summarized in Table 3.

3.4. Solution stability

The combined working standard solution was found to be stable for 24 hrs at 25 ± 2°C as there was no significant difference in peak area.

3.5. Analysis of marketed formulation

The amount of paracetamol and flupirtine maleate in tablets expressed as % of labelled amount calculated from the calibration curves is shown in Table 4.

Table 4 Analysis of marketed formulation

Drug	Label claim (mg)	Mean amount recovered per tablet(mg)	Assay (% of label claim) ± S.D (n=3)
Paracetamol	325	319.80	98.40 ± 1.39
Flupirtine maleate	100	98.13	98.13 ± 0.65

4. CONCLUSION

A specific, accurate and precise HPTLC method was developed for simultaneous estimation of paracetamol and flupirtine maleate in tablet dosage form. The developed method was applied for assay of tablet formulation and results were found to be in agreement with the label claim. The proposed method can be applied for routine analysis of tablet formulation.

5. ACKNOWLEDGEMENTS

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