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Simultaneous Determination of Paracetamol and Piroxicam in Tablets by Thin Layer Chromatography Combined with Densitometry

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Abstract

A simple, rapid and accurate High-performance thin-layer chromatography (HPTLC) method has been established and validated for the simultaneous determination of paracetamol and piroxicam in tablets. The method is based on HPTLC separation of the two drugs followed by densitometric measurements of their spots at 288 nm. The separation was carried out on Merck TLC aluminium sheets of silica gel 60F-254 using n-Dichloroethane: methanol: triethylamine (10:2.5:1, *v/v*) as a mobile phase. Calibration curves were linear in range of 1.625 - 14.625 µg/spot and 0.1 - 0.9 µg/spot for paracetamol and piroxicam, respectively. Method was successively applied to tablet formulation. No chromatographic interferences from the tablet excipients were found. The method was validated in accordance with the requirements of ICH guidelines.

Key words: Paracetamol; Piroxicam; High-performance thin-layer chromatography; Validation

1. Introduction

Paracetamol, N-(4-hydroxyphenyl) acetamide is antipyretic agent and piroxicam, 4-Hydroxy-2-methyl-N-(pyridin-2yl)-2H-1, 2-benzothiazine-3-carboxamide 1, 1- dioxide is analgesic and anti-inflammatory agent [1-5]. Both these drugs are official in Indian Pharmacopoeia [6], British Pharmacopoeia [7], European Pharmacopoeia [8] and United States Pharmacopeia [9]. The structures of drugs are shown in Fig 1.

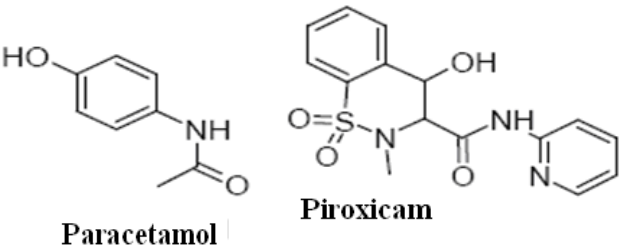

Fig.1: Structure of paracetamol and piroxicam

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Literature survey revealed many spectrophotometric and chromatographic methods for determination of paracetamol alone or in combinations with other drugs from pharmaceutical formulations and biological fluids [10-23]. Several spectrophotometric and chromatographic methods have also been reported for determination of piroxicam from pharmaceutical formulations and biological fluids [24-33]. But none of these methods demonstrate the simultaneous determination of these two drugs in tablet dosage form.

The present paper describes a reliable, rapid and accurate HPTLC method for determination of paracetamol and piroxicam using HPTLC densitometry.

2. Experimental

2.1 Materials and Reagents

Paracetamol and piroxicam were kindly supplied as a gift sample by Liva Pharmaceuticals Ltd., Nashik. n-Dichloroethane, methanol and triethylamine were used as solvents to prepare the mobile phase. All the reagents used were of analytical reagent grade (S.D. Fine Chemicals, Mumbai, India) and used without further purification.

2.2 Instrumentation and chromatographic conditions

The samples were spotted in the form of bands of width 6 mm with 100 µL sample syringe on precoated silica gel aluminium plate 60F-254 (20 cm × 10 cm) with 250 µm thickness; (E MERCK, Darmstadt, Germany) using a Camag Linomat V (Switzerland). The plates were prewashed with methanol and activated at 110^0C for 5 min, prior to chromatography. A constant application rate of 150 nL /sec was employed and space between two bands was 11.6 mm. The slit dimension was kept at 6 mm × 0.45 mm. The mobile phase consists of n-Dichloroethane: methanol: triethylamine (10:2.5:1, v/*v*). Linear ascending development was carried out in 20 cm x 10 cm twin trough glass chamber (Camag, Muttenz, Switzerland). The optimized chamber saturation time for mobile phase was 35 min, at temperature (25^0C ± 2); the relative humidity (60% ± 5%); the length of chromatogram run was 8 cm and TLC plates were air dried. Densitometric scanning was performed on Camag TLC Scanner 3 equipped with winCATS software version 1.3.0 at 288 nm. The source of radiation utilized was deuterium lamp. Evaluation was performed using linear regression analysis *via* peak areas.

2.3 Standard solutions and calibration graphs

Combined standard stock solution containing 1.625 µg μL^{-1} of paracetamol and 0.1 µg μL^{-1} of piroxicam was prepared in methanol. Calibration was done by applying mix

standard solutions ranging from 1 - 9 µL by Hamilton syringe with the help of automatic sample applicator Linomat V on TLC plate that gave concentration 1.625 -14.625 µg $spot^{-1}$ of paracetamol and 0.1 - 0.9 µg $spot^{-1}$ of piroxicam, respectively. Each concentration was spotted six times on the TLC plates. The plates were developed using previously described mobile phase. The calibration graph was plotted as peak areas *versus* corresponding concentrations.

2.4 Method Validation [34, 35]

2.4.1 Optimization of HPTLC method

Initially, n-Dichloroethane and methanol in the ratio of 1:1 (*v/v*) was tried for both drugs simultaneously. The spots were not developed properly and dragging was observed. Then, n-Dichloroethane and methanol in the ratio of 4:1 (*v/v*) was tried. The developed spots were diffused. To the above mobile phase, 0.5 mL triethylamine was added. Both the peaks were symmetrical in nature and tailing was observed. To improve resolution, the volume of triethylamine was increased to 1 mL. Ultimately, mobile phase consisting of n-Dichloroethane: methanol: triethylamine (10:2.5:1 v*/v*) gave good resolution. Both the peaks were symmetrical in nature and no tailing was observed when plate was scanned at 288 nm. The chamber was saturated with the mobile phase for 35 min at room temperature and plates were activated at 110^0 C for 5 min to obtain well-defined spots.

2.4.2 Linearity

Linearity responses for paracetamol and piroxicam were assessed in the concentration range 1.625 - 14.625 µg $spot^{-1}$ and 0.1 -0.9 µg $spot^{-1}$, respectively.

The linear equations for the calibration plots were Y = 2.553 X - 251.63 and Y = 11.015 X + 44.17, with correlation coefficient (r) being 0.9990 and 0.9998 for paracetamol and piroxicam, respectively. Range was established with five replicate readings of each concentration.

2.4.3 Precision

Precision of the method was determined in the terms of intra-day and inter-day variation (%RSD). Intra-day precision (%RSD) was assessed by analyzing standard drug solutions within the calibration range, three times on the same day.

Inter-day precision (%RSD) was assessed by analyzing drug solutions within the calibration range on three different days over a period of a week. The results are shown in Table 1.

Table 1: Result of Precision Study

Drug	Amount Applied ng/spot	Intraday Precision [%RSD, n=3]	Inter-day precision [%RSD, n=3]
Paracetamol	6500	0.014	0.014
	8125	0.004	0.007
	9750	0.070	0.008
Piroxicam	400	0.046	0.046
	500	0.022	0.022
	600	0.024	0.023

2.4.4 Sensitivity

The sensitivity of measurement of paracetamol and piroxicam by the use of proposed method was estimated in terms of Limit of Quantitation (LOQ) and Limit of Detection (LOD). The LOQ and LOD were calculated by use of the equation LOD = 3 X N/B and LOQ = 10 X N/B, where N/B is noise to base ratio and 'N' is standard deviation of the peak areas of the drugs (n = 3), taken as a measure of noise, and 'B' is the slope of the corresponding calibration curve. The LOQ and LOD for paracetamol were 224.91 ng and 104.71 ng. For piroxicam, LOQ and LOD were found to be 90.46 ng and 45.57 ng, respectively.

2.4.5 Accuracy

To the pre-analysed sample a known amount of standard solution of pure drug (paracetamol and piroxicam) was added at three different levels. These solutions were subjected to re-analysis by the proposed method; results of the same are shown in Table 2.

Table 2: Results of Recovery Studies

Component	Label claim (mg/tablet)	Amount of Standard Drug added (%)	% Drug Recovered *	% RSD
Paracetamol	325	0	99.39	0.88
		80	99.79	0.53
		100	99.00	0.69
		120	99.66	1.21
Piroxicam	20	0	99.65	0.84
		80	100.84	0.70
		100	99.77	0.83
		120	100.98	0.81

* mean of three estimations at each level

2.4.6 Specificity

Specificity of the method was ascertained by analysing standard drug and sample. The mobile phase resolved both the drugs very efficiently, as shown in Fig.2. The spot for paracetamol and piroxicam was confirmed by comparing the R_f and spectra of the spot with that of standard. Typical absorption overlain spectrum of paracetamol and piroxicam is shown in Fig.3. The wavelength 288 nm for detecting peak purity of paracetamol and piroxicam was assessed by comparing the spectra at three different levels, i.e., peak start (S), peak apex (M) and peak end (E) positions of the spot.

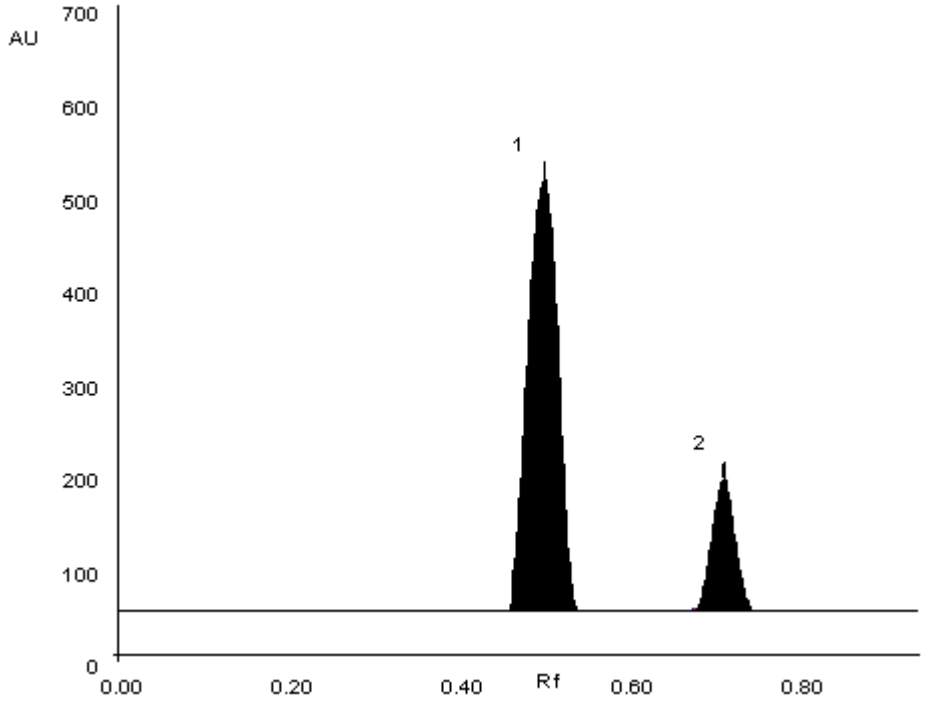

Fig.2: Densitogram of standard paracetamol (1) and piroxicam (2)

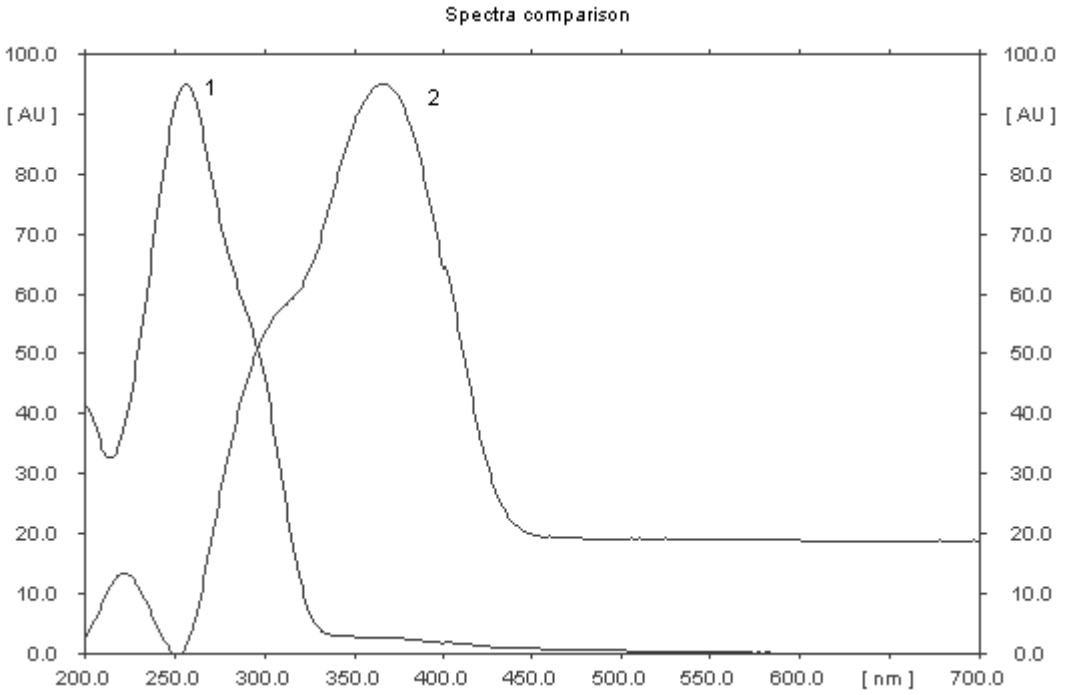

Fig.3: Typical overlain spectra of standard paracetamol (1) and piroxicam (2) drug solutions at λ =288 nm

2.4.7 Robustness and Ruggedness

Robustness is a measure of the capacity of a method to remain unaffected by small but deliberate variations in the method conditions, and is an indication of the reliability of the method.

Robustness was assessed by changing the migration distance of the solvent system.

The results are shown in Table 3.

The ruggedness of the proposed method was evaluated by two different analysts. The results for paracetamol and piroxicam were found to be 99.45 %, 99.76 % and 99.65 %, 99.47 %, respectively.

Table 3: Results from Robustness Studies

Development distance	Paracetamol (%)	Piroxicam (%)
7.0	100.25	99.28
7.5	99.15	100.14
8.0	99.62	99.49

2.4.8 Repeatability

Repeatability of sample application was assessed by spotting (0.1 µg μL^{-1}) of drug solution seven times on a TLC, followed by development of plate and recording the peak area for seven spots. The % RSD for peak area values of paracetamol and piroxicam was found to be 0.06 and 0.56, respectively.

2.5 Analysis of paracetamol and piroxicam in marketed formulation

To determine the content of paracetamol and piroxicam simultaneously in conventional tablets (label claim 325 mg paracetamol and 20 mg piroxicam); twenty tablets were accurately weighed, average weight determined and ground to fine powder. A quantity of powder equivalent to 10 mg (piroxicam) and 162.5 mg (paracetamol) was transferred into 100 mL volumetric flask containing 50 mL methanol, sonicated for 30 min and diluted to mark with same solvent. The resulting solution was filtered using 0.45 µm filter (Millifilter, MA). 1µL of the above solution applied on TLC plate followed by development and scanning as described in section 2.2.The analysis was repeated for six times. Paracetamol and piroxicam gave sharp and well defined peaks at R_f 0.48 and 0.71, respectively, when scanned at 288 nm. The results are shown in Table 4 indicate that there was no interferences from the excipients commonly present in the tablets.

Table 4: Assay of Tablet Formulation

Component	Label Claim (mg)	% Label Claim*	% RSD
Paracetamol	325	99.75	0.78
Piroxicam	20	99.67	1.12

*mean of six estimations

3. Conclusion

The developed HPTLC method is simple, precise, accurate and reproducible and can be used for simultaneous determination of paracetamol and piroxicam in tablets. The method was validated as per ICH guidelines.

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