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Der Pharma Chemica, 2012, 4(5):1798-1802
(http://derpharmachemica.com/archive.html)

ISSN 0975-413X
CODEN (USA): PCHHAX

Simultaneous Determination of Paracetamol, Lornoxicam and Chlorzoxazone in Tablets by High Performance Thin Layer Chromatography

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ABSTRACT

A simple, specific, accurate and precise HPTLC method for simultaneous estimation of paracetamol, lornoxicam and chlorzoxazone as the bulk drug and in tablet dosage form. Chromatographic separation of the drugs was performed on aluminum plates precoated with silica gel 60 F_{254} as the stationary phase and the solvent system consisted of chloroform: toluene: methanol: glacial acetic acid 6: 3: 1: 0.04 (v/v/v/v). Densitometric evaluation of the separated zones was performed at 275 nm. The three drugs were satisfactorily resolved with R_F values 0.26 ± 0.02, 0.45 ± 0.03 and 0.53 ± 0.01 for paracetamol, lornoxicam and chlorzoxazone respectively. The method can be used for estimation of combination of these drugs in tablets. The method was validated as per ICH guidelines. The linearity of developed method was achieved in the range of 10 – 50 ng/spot for each of paracetamol, lornoxicam and chlorzoxazone and recoveries from tablets were between 99.27 ± 0.70, 99.37 ± 0.06 and 99.10 ± 0.30 %. Due to these attributes, the proposed method could be used for routine quality control analysis of these drugs in combined dosage forms.

Keywords: Simultaneous Estimation, HPTLC, Paracetamol, Lornoxicam and Chlorzoxazone

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INTRODUCTION

Paracetamol, N-(4-hydroxyphenyl) ethanamide (Fig.1a) is a widely used analgesic and antipyretic for the relief of fever, headaches, and other minor aches and pains, and is a major ingredient in numerous cold and flu remedies. In combination with non-steroidal anti-inflammatory drugs (NSAIDs) and opioid analgesics, paracetamol is used also in the management of severe pain (such as postoperative pain). Paracetamol is official in I.P[1], B.P[2] and USP[3]. Lornoxicam (LOX) is 6-chloro-4-hydroxy-2-methyl-N-2-pyridinyl-2H-thieno-[2,3-e]-1,2- thiazine-3-carboxamide 1,1-dioxide (Fig. 1b) is a novel non-steroidal anti-inflammatory drug (NSAID) with marked analgesic properties. Lornoxicam is not official in any Pharmacopoeia, but listed in the Merck Index[4]. Chlorzoxazone (5-chloro-2(3H)-benzoxazolone) is a compound with skeletal muscle relaxant property (Fig.1c). It is used to decrease muscle tone and tension and thus to relieve spasm and pain associated with musculoskeletal disorders[5]. Literature survey reveals many analytical methods for determination of paracetamol such as UV Spectrophotometry[6], HPLC[7-12], and Capillary electrophoresis[13] methods from pharmaceutical preparations. Few analytical methods for determination of lornoxicam using UV Spectroscopy[14], HPLC[15-17], HPTLC [18-19] and polarography[20] in plasma and pharmaceutical formulation have been reported. Few analytical methods for determination of chlorzoxazone using UV Spectroscopy[21], HPLC[22-23]. But no HPTLC method was reported for simultaneous estimation of paracetamol, lornoxicam and chlorzoxazone in combined dosage forms. The review of literature prompted us to develop an accurate, selective and precise simultaneous method for the estimation of Paracetamol, lornoxicam and chlorzoxazone in combined dosage forms. The present paper describes a reliable, rapid and accurate HPTLC method for determination of paracetamol, lornoxicam and chlorzoxazone using HPTLC densitometry. The proposed method was optimized and validated in accordance with International Conference on Harmonization (ICH) guidelines[24].

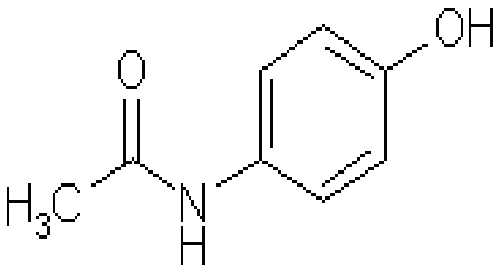

Fig.1a Structure of Paracetamol.

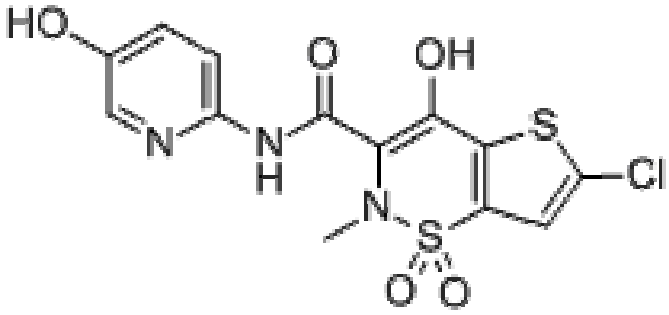

Fig.1b Structure of Lornoxicam

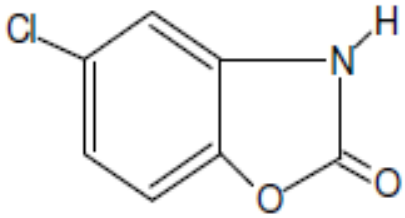

Fig.1c Structure of Chlorzoxazone

MATERIALS AND METHODS

Materials:

Paracetamol, lornoxicam, chlorzoxazone were kindly supplied by Itros Pharmaceuticals ltd (Pune), India as gift samples. Chloroform, toluene, methanol, glacial acetic acid used was of analytical grade from Merck Chemicals, India. Marketed formulation (Lornidol-CX Tablet) is procured from local market.

Preparation of Standard and Sample Solutions:

A standard mixed stock solution of paracetamol, lornoxicam and chlorzoxazone was prepared by accurately weighing paracetamol (10 mg), lornoxicam (10 mg) and chlorzoxazone (10 mg) into a 10-mL volumetric flask. The drugs were dissolved in methanol and the solution was diluted to volume.

Twenty tablets of the pharmaceutical formulation Lornidol-CX (containing 500 mg Paracetamol, 8 mg lornoxicam and 500 mg chlorzoxazone) were assayed. They were crushed to a fine powder and an amount of the powder corresponding to approximately 500 mg Paracetamol, 8 mg lornoxicam and 500 mg chlorzoxazone was weighed in a 100-mL volumetric flask. After addition of 50 mL methanol and sonication (20–30 min) the solution was diluted to volume with methanol and filtered through a Whatman no. 41 filter paper.

Chromatography:

TLC was performed on aluminium foil plates coated with 0.2-mm layers of silica gel 60F254 (Merck). Before use plates were prewashed with methanol then dried and activated. Samples were applied to the plates, as 6-mm bands by means of a Camag Linomat 5 sample applicator used. Plates were developed with chloroform: toluene: methanol: glacial acetic acid 6: 3: 1: 0.04 (v/v/v/v) as mobile phase in a Camag twin-trough chamber previously saturated with mobile phase vapour for 30 min at room temperature (25 ± 2°C). The development distance was approximately 80 mm. After development the plates were scanned in absorbance mode at 275 nm by use of a Camag TLC Scanner 3 controlled by winCATS software. The slit dimensions were 5 mm × 0.45 mm and the source of radiation was a deuterium lamp emitting a continuous UV spectrum in the range 190–400 nm.

Method Validation

Precision:

The 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.

Limits of Detection and Quantitation:

To determine the limits of detection (LOD) and quantitation (LOQ), solutions of concentration in the lower part of the linear range of the calibration plot were used. LOD and LOQ were calculated using the equations LOD = 3.3 × *N*/*B* and LOQ = 10 × *N*/*B*, where *N* is the 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 plot.

Specificity:

The specificity of the method was ascertained by analysis of drug standards and samples. The mobile phase resolved both the drugs very efficiently, as shown in Fig. 2.

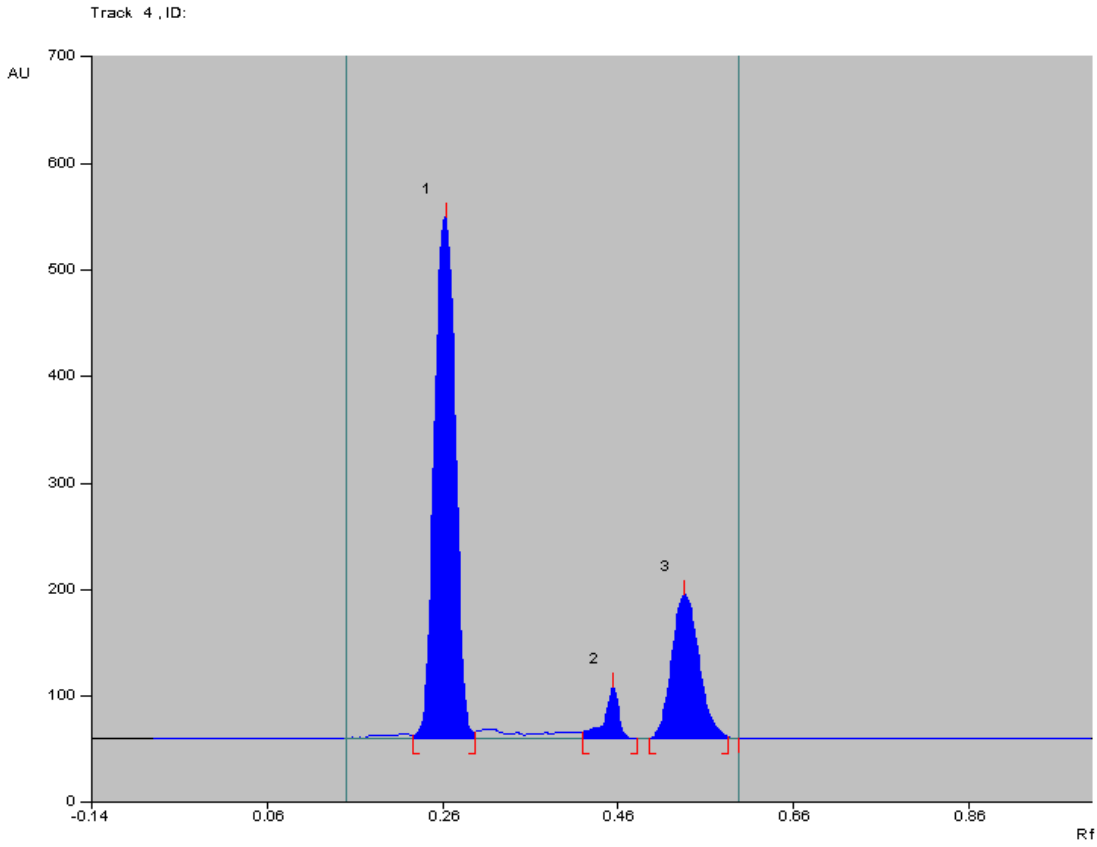

Fig. 2: Densitogram of paracetamol (R_F 0.26), lornoxicam (R_F 0.45) and chlorzoxazone (R_F 0.53) of formulation (LORNIDOL-CX tablet).

Accuracy:

Analysed samples were overapplied with an extra 80, 100, and 120% of the drugs from standard solutions of paracetamol, lornoxicam and chlorzoxazone and the mixtures were reanalyzed by use of the method. The experiment was conducted in triplicate. This was done to check for the recovery of the drug at different levels in the formulation.

Robustness:

Robustness was assessed by deliberately changing the chromatographic conditions and studying the effects on the results obtained.

RESULTS AND DISCUSSION

Method Development:

The TLC procedure was optimized for simultaneous determination of paracetamol, lornoxicam and chlorzoxazone. The mobile phase chloroform: toluene: methanol: glacial acetic acid 6: 3: 1: 0.04 (v/v/v/v) resulted in good resolution, and sharp and symmetrical peaks of R_F 0.26 paracetamol, 0.45 lornoxicam and 0.53 for chlorzoxazone. It was observed that prewashing of TLC plates with methanol (followed by drying and activation) and pre-saturation of TLC chamber with mobile phase for 30 min (optimum chamber saturation time) ensured good reproducibility and peak shape of three drugs.

VALIDATION OF THE METHOD

Linearity:

Linear regression data for the calibration plots revealed good linear relationships between response and concentration over the ranges 10-50 ng per spot for each paracetamol, lornoxicam and chlorzoxazone. Each concentration was applied in triplicate on the HPTLC plate (Table 1).

Table 1: Linear regression data for the calibration curves

Parameters	Paracetamol	Lornoxicam	Chlorzoxazone
Linearity range	10-50ng/spot	10-50ng/spot	10-50ng/spot
correlation coefficient (r^2)	0.999	0.997	0.997
Slope	150.02	10.59	66.62
Intercept	9522	1094	1679

LOD and LOQ:

The limits of detection and quantitation were found to be 2 and 8 ng/spot, respectively, for paracetamol, 1 and 4 ng/spot for lornoxicam and 3 and 10 ng/spot for chlorzoxazone. This indicates the method is sufficiently sensitive.

Precision:

The precision of the method was expressed as relative standard deviation (RSD, %). The results listed in Table 2 reveal the high precision of the method.

Table 2: Precision study

Drug	Conc. (ng/spot)	Intra-day precision (n=3)		Interday precision (n=3)	
		Measured Conc. (ng/spot)	% RSD	Measured Conc. (ng/spot)	% RSD
Paracetamol	10	9.82	0.10	9.85	0.30
	30	29.56	0.06	29.58	0.10
	50	48.99	0.20	49.10	0.40
Lornoxicam	10	9.92	0.30	9.94	0.39
	30	29.90	0.23	29.92	0.92
	50	49.22	0.80	49.37	0.70
Chlorzoxazone	10	9.87	0.20	9.90	0.34
	30	29.62	0.16	29.68	0.28
	50	49.20	0.65	49.60	0.73

Accuracy:

When the method was used for extraction and subsequent analysis of three drugs from the pharmaceutical dosage forms, and the extract was overapplied with 80, 100, and 120% of additional drug, the recovery was 98–100%, as listed in Table 3.

Table 3: Recovery studies

Drug	Label claim mg/tablet	Standard amount added %	Total amount mg	Amount recoverd mg (%RSD)[a]	% Recovery
Paraceatmol	500	80	900	899.8 (0.10)	99.97
		100	1000	993 (0.34)	99.30
		120	1100	1084 (0.47)	98.54
Lornoxicam	8	80	14.4	14.3 (0.38)	99.31
		100	16.0	15.9 (0.26)	99.38
		120	17.6	17.5 (0.41)	99.43
Chlorzoxazone	500	80	900	894.6 (0.26)	99.4
		100	1000	991.2 (0.53)	99.10
		120	1100	1087 (0.27)	98.81

[a]*Mean from three estimates at each level*

Robustness:
The relative standard deviation of peak areas was less than 2%. The RSD shown in Table 4 indicate the robustness of the method.

Table 4: Robustness of the method[a]

Parameters	% RSD for Paracetamol	% RSD for Lornoxicam	% RSD for Chlorzoxazone
Mobile phase composition (± 0.1 ml)	0.05	0.01	0.41
Amount of mobile phase (± 0.5 %)	0.04	0.75	0.25
Time from spotting to chromatography (20 min)	0.18	1.09	1.3
Time from chromatography to scanning	0.98	1.03	0.96

[a]*Mean from three estimates*

Analysis of marketed formulation:
When the Lornidol-CX tablets were analysed, sharp and well defined peaks for atenolol and lercanidipine hydrochloride were obtained. The amount of the label claim measured were 99.29 % for paracetamol, 98.75% for lornoxicam and 99.70 % for chlorzoxazone.

CONCLUSION

The method proves to be specific accurate and precise. Hence the developed HPTLC method can be used for the simultaneous analysis of paracetamol, lornoxicam and chlorzoxazone in bulk drugs and in pharmaceutical dosage forms without any interference from the excipients.

Acknowledgment
The authors are thankful to Poona College of Pharmacy, Pune (M.S.), India, for providing necessary facilities and author also thankful to Itros Pharmaceutical Ltd. Pune, for providing drug samples.

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