

DEVELOPMENT OF STABILITY INDICATING HPTLC METHOD FOR SIMULTANEOUS ESTIMATION OF PARACETAMOL AND DEXIBUPROFEN

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ABSTRACT

The present work describes a stability-indicating HPTLC method for analysis of paracetamol and dexibuprofen in bulk and pharmaceutical dosage form. Precoated silica gel 60 F₂₅₄ plate was used as stationary phase. The separation was carried out using n-hexane: ethyl acetate: glacial acetic acid (5: 5: 0.2 %v/v/v) as mobile phase. The densitometric scanning was carried out at 223 nm. The linearity was obtained in the range 10–50 µg/band for paracetamol and 6–30 µg/band for dexibuprofen with correlation coefficients (r^2) 0.9915 and 0.9969 for paracetamol and dexibuprofen respectively. The method was validated as per ICH guidelines. The combination was subjected to forced degradation by acid, alkali, oxidation and dry heat. The degradation products were well resolved from the pure drug with significantly different R_f values.

KEY WORDS: Paracetamol, Dexibuprofen, HPTLC, Validation, Stability Studies.

1. INTRODUCTION

Chemically, Paracetamol (PCM) is 4'-Hydroxyacetanilide. It is antipyretic and analgesic (Budavari, 1996). Paracetamol alone or in combination with other drugs is reported to be estimated by spectrophotometric method (Nogowska, 1999; Dinc, 2002), HPLC (Zarapkar, 1999), TLC (Liang, 2006), HPTLC (Argekar, Sawant., 1999), LC-MS (Godejohann, 2004), FT-IR (Ekgasit, 2007), Amperometric determination (Prabakar and Narayanan, 2007), Fluorimetry (Llorent, 2007) and Micellar electrokinetic chromatographic method (Emre and Ozaltin, 2007).

Chemically, Dexibuprofen (DEX) the S+ enantiomer of Ibuprofen is (S-2-(4-Isobutylphenyl)-propionic acid). It is a widely used non-steroidal anti-inflammatory drug. (Bonabello, 2003). The reported methods of analysis include DRIFT spectroscopy (Agatonovic, 2000), HPLC in plasma (Mandal, 2008), TLC-densitometric method for analysis of DEX in pharmaceutical formulation along with other drugs (Starek, 2008) and separation of ibuprofen and its enantiomer by HPLC (Zheng, 2008). Since no HPTLC method is reported, a successful attempt has been made to estimate both these drugs simultaneously by simple HPTLC method.

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Journal of Chemical and Pharmaceutical Sciences.

2. MATERIALS AND METHODS

Chemicals: Pure drug samples of PCM and DEX were obtained as gift samples from Emcure Pharmaceuticals Ltd., Pune, India. All chemicals and reagents used were of HPLC/AR grade.

Preparation of the standard solutions: Stock solutions were prepared by dissolving 10 mg of paracetamol and dexibuprofen in 10 ml methanol separately, from the stock solutions of 1 µg/µl, the standard solutions were applied to reach a concentration range 10–50 µg/band and 6–30 µg/band for PCM and DEX respectively. The plate was developed on previously described mobile phase and well resolved bands of drug were scanned at 223 nm with scanner. The peak areas were plotted against the corresponding concentrations to obtain the calibration curve.

Preparation of the sample solutions: Twenty tablets were weighed, finely powdered and powder equivalent to 500 mg of PCM and 300 mg of DEX was dissolved in methanol and final dilutions containing 5 and 3 µg/µl of PCM and DEX were prepared respectively. The sample solutions were applied six times on TLC plate to give spot concentration 20 and 12 µg/band of PCM and DEX respectively. The plate was developed in the previously described chromatographic conditions. The peak area of the spots was measured at 223 nm and concentrations in the samples were determined using multilevel calibration.

Chromatographic conditions: The standard solutions of PCM and DEX were applied on precoated silica gel

60 F₂₅₄ plate in the form of bands with 100µl sample syringe using automatic sample applicator LINOMAT V. It was developed in a twin trough glass chamber which was already saturated for 30 min. with the mobile phase. The mobile phase consisted of n-hexane: ethyl acetate: glacial acetic acid (5: 5: 0.2% v/v/v). After development, plate was immediately dried with the help of dryer and was observed under UV chamber. The well resolved bands of drugs were scanned at 223 nm with Camag TLC scanner III densitometer controlled by WINCAT's software version 4.

Method validation: The methods were validated according to ICH guidelines (ICH Q2A, 1994; ICH Q2B, 1996; ICH Guidance, 2002) for validation of analytical procedures.

Forced Degradation Studies: In order to ensure that the analytical method was stability indicating, stress studies were performed.

Acid degradation studies: 1 ml of 0.1N hydrochloric acid was added to 9 ml of drug solution to get the final concentration of 20 and 12 µg/ml of PCM and DEX respectively. This solution was allowed to stand for 24 hrs, 15 days and 1 month.

Alkali degradation studies: 1 ml of 0.1N sodium hydroxide was added to 9 ml of drug solution to get the final concentration of 20 and 12 µg/ml of PCM and DEX respectively. This solution was allowed to stand for 24 hrs, 15 days and 1 month.

Oxidation studies: 1 ml of a 3% hydrogen peroxide solution was added to 9 ml of drug solution to get the final concentration of 20 and 12 µg/ml of PCM and DEX respectively. This solution was allowed to stand for 24 hrs, 15 days and 1 month.

Temperature stress studies: A drug solution containing 20 and 12 µg/ml of PCM and DEX respectively was exposed to 50°C for 24 hrs, 15 days and 1 month.

3.RESULTS

Optimization of procedure: Different proportions of n-hexane, ethyl acetate, glacial acetic acid were tried for mobile phase selection. Ultimately n-hexane: ethyl acetate: glacial acetic acid (5: 5: 0.2%v/v/v) was finalized as mobile phase. The spots developed were dense, compact and typical peak of PCM and DEX was obtained as shown in fig 1. Peaks were symmetrical in nature and no tailing was observed when plates were scanned at 223 nm.

Linearity: The analytical concentration ranges over which the drugs obeyed Beer Lambert's law was found to be 10-50 and 6-30 µg/band with r² 0.9915 and 0.9969 for PCM and DEX respectively.

Analysis of marketed formulation: The spots at R_f 0.22 and 0.79 were observed in the densitogram of the drug samples extracted from tablets. There was no interference from the excipients commonly present in the tablets. The PCM and DEX content was found to be close to 100% and the results are summarized in table 1. The low %RSD value indicated the suitability of this method for routine analysis.

Specificity: Good correlation was obtained between standard and sample spectra of combination.

Precision: Precision was evaluated by carrying out six independent sample preparation of a single lot of formulation. Percentage relative standard deviation (%RSD) was found to be less than 2% for within a day and day to day variations, which proves that method is precise.

Recovery studies: To check the degree of accuracy of the method, recovery studies were performed in triplicate by standard addition method at 80%, 100% and 120%. The values range from 98.60 to 101.67 for PCM and 98.57 to 101.88 for DEX.

Robustness: To evaluate the robustness of the developed HPTLC method, small deliberate variations in the optimized method parameters were done like in mobile phase composition (± 2%) in terms of n-hexane and ethyl acetate, chamber saturation period (± 10%), development distance (± 10%), time from application to development (0, 10, 20, 30 min), time from development to scanning (0, 10, 20, 30 min). One factor at a time was changed at a concentration level of 20 and 12 µg/band of PCM and DEX respectively, to study the effect on the peak area of the drugs. The method was found to be unaffected by small changes.

Stability Studies: Studies of the samples obtained during the stress testing of combination under different conditions using n-hexane: ethyl acetate: glacial acetic acid (5: 5: 0.2%v/v/v) as the mobile phase suggested the following degradation behavior.

Acid-induced degradation: The drugs were degraded in acidic condition and degradation products appeared at R_f 0.41, 0.67 and 0.92 in 24 hrs 0.33, 0.64, 0.68, 0.88 and 0.91 in 15 days 0.01, 0.34, 0.65, 0.67, 0.69, 0.89 and 0.91 in 1 month.

Alkali-induced degradation: The drugs were degraded in alkaline condition and degradation products appeared at R_f 0.04, 0.09, 0.38, 0.49, 0.52, 0.59 and 0.69 in 24 hrs 0.03, 0.06, 0.39, 0.52, 0.61, 0.87 and 0.89 in 15 days 0.02, 0.04, 0.38, 0.51, 0.58, 0.89 and 0.90 in 1 month.

Hydrogen peroxide-induced degradation: The drugs were degraded in hydrogen peroxide (3%) at room temperature and degradation products appeared at R_f 0.01, 0.12, 0.73, 0.89 and 0.91 in 24 hrs 0.01, 0.04, 0.12, 0.54, 0.59, 0.66, 0.73 and 0.88 in 15 days 0.01, 0.03, 0.10, 0.52, 0.55, 0.58, 0.69, 0.89 and 0.90 in 1 month.

Temperature-induced degradation: The drugs when subjected to temperature (50°C) were degraded and degradation products appeared at R_f 0.08, 0.56, 0.59, 0.87, 0.89 and 0.91 in 24 hrs 0.03, 0.09, 0.19, 0.65, 0.87 and 0.89 in 15 days 0.01, 0.03, 0.09, 0.61, 0.89 and 0.90 in 1 month.

DISCUSSION

The proposed HPTLC method was validated as per ICH guidelines. The standard deviation, %RSD and standard error calculated for the method are low, indicating high degree of precision of the methods. The results of the recovery studies performed show the high degree of accuracy of the proposed methods. The results of forced degradation studies indicate that DEX degraded more than PCM over the period of exposure to stress conditions. The degradation products were well resolved from the pure drug with significantly different R_f values even after one month indicating the suitability of the method for degradation studies. The studies also indicate the specificity of the method. Hence, it can be concluded that the developed HPTLC method is accurate, precise and selective and can be employed successfully for the estimation of PCM and DEX in marketed formulation.

4. AKNOWLEDGEMENTS

The authors are thankful to Director, Dr. Avinash D. Deshpande, Padm. Dr. D. Y. Patil Institute of Pharmaceutical Sciences and Research, Pimpri, Pune for providing necessary facilities and Emcure pharmaceuticals Ltd, Pune (India) for providing gift sample of pure drug.

Table 1: Results of marketed formulation: Brutek-P (Zuventus)

Marketed formulation	Drug	Label Claim (mg)	Amt. Taken ($\mu\text{g}/\text{band}$)	Area* of densito-gram	Amt. of drug estimated (mg) \pm S.D*	% Label claim* \pm S.D*
Brutek-P (Zuventus)	PCM	500	20	34406.40	503.50 \pm 3.77	100.70 \pm 0.75
	DEX	300	12	24531.47	297.58 \pm 2.42	99.19 \pm 0.81

*Average of six determinations

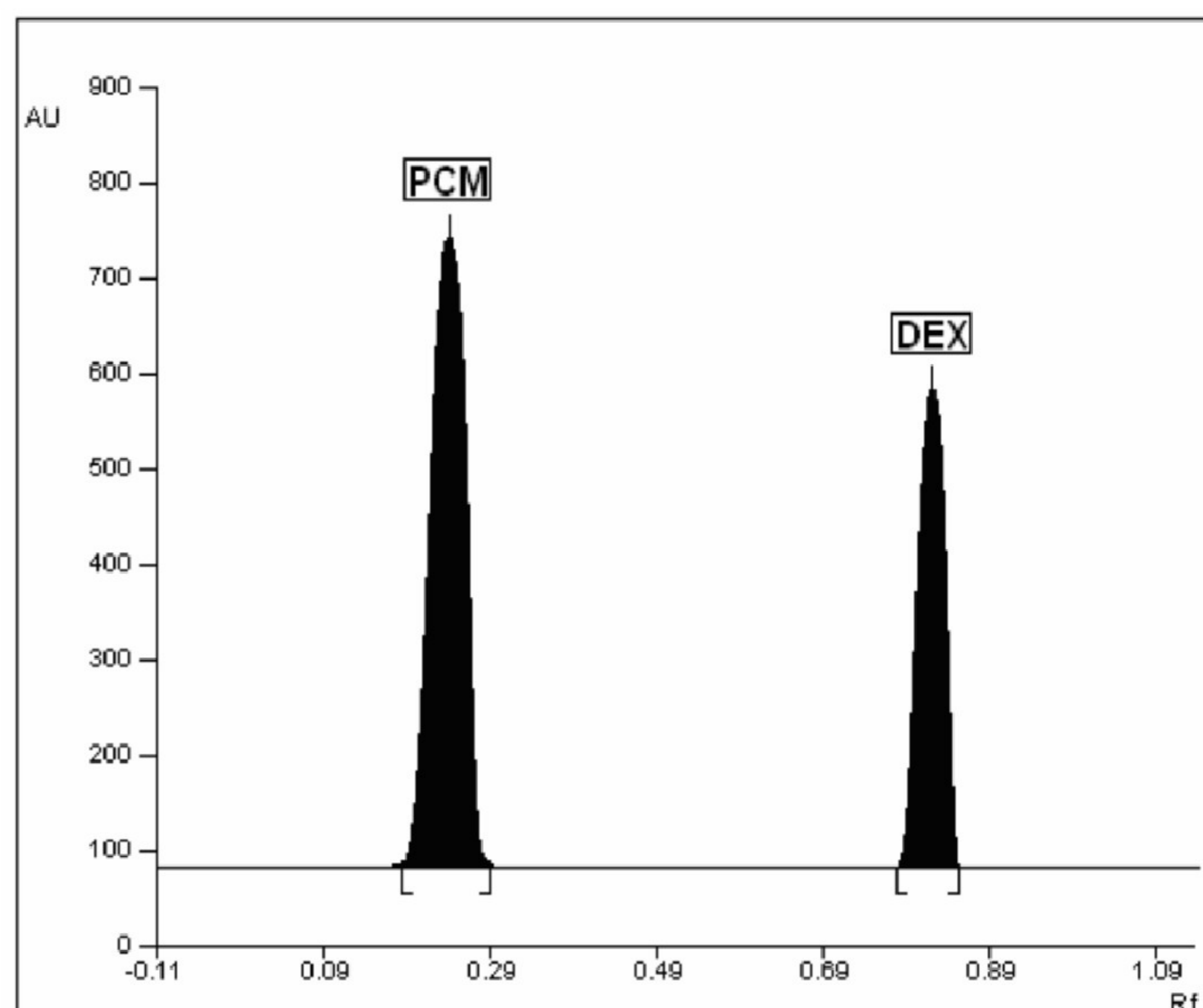


Fig. 1. Densitogram of PCM and DEX in mixture

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