

HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHIC DETERMINATION OF DROTAVERINE HYDROCHLORIDE AND ACECLOFENAC IN COMBINED TABLET DOSAGE FORM

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ABSTRACT

A new simple high performance thin layer chromatographic method for determination of Drotaverine hydrochloride and Aceclofenac in combined tablet dosage form has been developed and validated. The separation was carried out on Merck aluminum plates precoated with silica gel 60 F₂₅₄, using Ethyl acetate: Methanol: Triethylamine (7.5: 2: 0.5, v/v/v) as mobile phase. Quantitative determination of spots was done by densitometric scanning at 284 nm. The method was validated with respect to linearity, accuracy, precision and robustness. The calibration curve was linear over a range of 100-500 ng band⁻¹ for both the drugs. The method has been successfully applied for the analysis of drugs in pharmaceutical formulation.

KEYWORDS: Drotaverine hydrochloride; Aceclofenac; High performance thin layer chromatography.

INTRODUCTION

Drotaverine hydrochloride (DRO) Chemically 1-[(3, 4-[diethoxyphenyl) methylene]-6,7-Diethoxy-1, 2,3,4-tetrahydroisoquinoline is an papaver analogue mainly used as an antispasmodic and smooth-muscle relaxant in pain associated with gastrointestinal colic, biliary colic and postsurgical spasms¹. Aceclofenac, (ACE) chemically, 2-[(2',6-dichlorophenyl) amino] phenylacetoxyacetic acid, is a phenylacetic acid derivative with potent analgesic and anti-inflammatory properties. It is official in *Indian Pharmacopoeia*².

Literature survey reveals several high performance liquid chromatographic (HPLC) methods for determination of DRO in human plasma and in pharmaceutical formulations either as single and in combination with other drugs³⁻⁶. Spectrophotometric methods for simultaneous estimation of DRO with other drugs have also been reported⁷⁻⁹. HPLC methods have been reported for the determination of ACE either in single or in combination with other drugs¹⁰⁻¹³. HPTLC methods have been reported for determination of ACE in single or in combination with other drugs^{14,15}. Spectrophotometric methods for simultaneous estimation of ACE with other drugs also reported^{16,17}.

To best of our knowledge no reports were found for the simultaneous estimation of the DRO and ACE in combined tablet dosage form by HPTLC method. This paper describes a simple, accurate and validated HPTLC method for simultaneous quantification of these compounds as the bulk drug and in combined tablet dosage forms. The proposed method is optimized and validated as per the International Conference on Harmonization (ICH) guidelines¹⁸.

MATERIALS AND METHODS

Reagents and Chemicals

Analytically pure samples of DRO and ACE were provided as gift sample by Akums Drugs & Pharmaceuticals Ltd. (Haridwar, India). The pharmaceutical dosage form used in this study was Canefo-D tablets (Medopharm, India) labeled to contain 80 mg of DRO and 100 mg of ACE per tablet were procured from local market. Methanol, Ethyl acetate, Triethylamine (all AR grade) were obtained from Sisco Research Laboratories (Mumbai, India).

Instrumentation and Chromatographic Conditions

The samples were spotted in the form of bands of width of 6 mm with space between bands of 5 mm, with a 100 μ L sample syringe (Hamilton, Bonaduz, Switzerland) on precoated silica gel aluminium plate 60 F₂₅₄ (20 cm $\times$ 10 cm) with 250 μ m thickness (E. MERCK, Darmstadt, Germany) using a CAMAG Linomat 5 sample applicator (Switzerland). The slit dimensions 5 mm $\times$ 0.45 mm and scanning speed of 20 mm/sec was employed.

The linear ascending development was carried out in 20 cm $\times$ 10 cm twin trough glass chamber (CAMAG, Muttenz, Switzerland) using Ethyl acetate: Methanol: Triethylamine (7.5: 2: 0.5, v/v/v) as mobile phase. The optimized chamber saturation time for mobile phase was 25 min. The length of chromatogram run was 9 cm and development time was approximately 20 min. TLC plates were dried in a current of air with the help of a hair drier. Densitometric scanning was performed on CAMAG thin layer chromatography scanner at 284 nm for all developments operated by Wincats software version 1.4.2. The source of radiation utilized was deuterium lamp emitting a continuous UV spectrum between 200 to 400 nm.

Preparation of Standard Stock Solutions

Standard stock solution of DRO and ACE was prepared by dissolving 10 mg of each drug in 10 mL of methanol separately to get concentration of 1 mg mL⁻¹ from which 0.5 mL was further diluted to 10 mL to get stock solution of 50 ng/ μ L of each drug.

Selection of Detection Wavelength

After chromatographic development bands were scanned over the range of 200-400 nm and the spectra were overlain. It was observed that both drugs showed considerable absorbance at 284 nm, So 284 nm was selected as the wavelength for detection as shown in Figure 1.

Preparation of Calibration Curves

The standard stock solutions of DRO and ACE (50 ng/ μ L each) were applied by overspotting on TLC plate in range of 2, 4, 6, 8 and 10 μ L with the help of CAMAG 100 μ L sample syringe, using Linomat 5 sample applicator. The plate was developed and scanned under above established chromatographic conditions. Each standard in five replicates was analyzed and peak areas were recorded. Calibration curves of DRO and ACE were plotted separately of peak area vs respective concentration of DRO and ACE.

Analysis of Tablet Formulation

Twenty tablets were weighed accurately and a quantity of tablet powder equivalent to 10 mg of ACE was weighed and transferred to a 10 mL volumetric flask containing approximately 7 mL of methanol, ultrasonicated for 5 min and volume was made up to the mark with the methanol. The solution was filtered through Whatman filter paper No. 41 and 0.5 mL of filtrate was further diluted to 10 mL with methanol. Four μ L volume was applied to a TLC plate to furnish final concentration of 160 ng band⁻¹ for DRO and 200 ng band⁻¹ for ACE. After chromatographic development the peak areas of the bands were measured at 284 nm and the amount of each drug present per tablet was estimated from the respective calibration curves. Procedure was repeated six times for the analysis of homogenous sample.

Robustness Studies

In the robustness study, the influence of small, deliberate variations of the analytical parameters on peak area of the drugs were examined. Factors varied were development distance ($\pm$ 5 %), time from application to development (0, 10, 20, and 30 min) and from development to scanning (0, 30, 60, and 90 min). One factor at a time was changed to estimate the effect. Robustness of the method was checked at a concentration level of 160 ng band⁻¹ for DRO and 200 ng band⁻¹ for ACE. The results of robustness data obtained are given in Table 1.

Recovery Studies

To check the accuracy of the method, recovery studies were carried out by addition of standard drug solution to pre-analyzed sample solution at three different levels 50, 100 and 150 %. The percentages of recoveries were calculated, results of which are represented in Table 2.

RESULTS AND DISCUSSION

Different mobile phases containing various ratios of ethyl acetate, methanol, triethylamine and toluene were examined (data not shown). Finally the mobile phase containing Ethyl acetate: Methanol: Triethylamine (7.5: 2: 0.5, v/v/v) was selected as optimal for obtaining well defined and resolved peaks. The optimum wavelength for detection and quantitation used was 284 nm. The retention factors for DRO and ACE were found to be 0.76 ± 0.03 and 0.29 ± 0.03 respectively. Representative densitogram of mixed standard solution of DRO and ACE is shown in Figure 2.

Straight-line calibration graphs were obtained for DRO and ACE in the concentration range 100-500 ng band⁻¹ for both the drugs with high correlation coefficient > 0.998 . The proposed method was also evaluated by the assay of commercially available tablets containing DRO and ACE. The % assay (Mean $\pm$ S.D.) was found to be 99.89 ± 1.15 for DRO and 101.10 ± 0.71 for ACE. Robustness of the method checked after deliberate alterations of the analytical parameters showed that areas of peaks of interest remained unaffected by small changes of the operational parameters (% RSD < 2), which demonstrated that the RP-HPLC method developed is robust.

For DRO, the recovery study results ranged from 98.99 to 100.50 % with % RSD values ranging from 0.58 to 0.85. For ACE, the recovery results ranged from 99.30 to 100.85 % with % RSD values ranging from 0.52 to 1.77. The method was found to be accurate and precise, as indicated by recovery studies and % RSD not more than 2.

CONCLUSION

The validated HPTLC method employed here proved to be simple, fast, accurate, precise and robust, thus can be used for routine analysis of DRO and ACE in combined tablet dosage form.

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Table 1: Robustness Data in Terms of Peak Area (% RSD)

Sr. No.	Parameter Varied	DRO	ACE
1	Development distance	0.91	1.07
2	Time from application to development (Mins.)	0.87	0.98
3	Time from development to scanning (Mins.)	1.15	1.33

Table 2: Recovery studies of DRO and ACE

Level of % Recovery	% Mean Recovery*		Standard Deviation*		% R.S.D.*	
	DRO	ACE	DRO	ACE	DRO	ACE
50	100.50	100.85	0.70	1.78	0.69	1.77
100	99.29	99.30	0.57	0.52	0.58	0.52
150	98.99	99.82	1.00	0.73	0.85	1.01

*Average of three Determinations

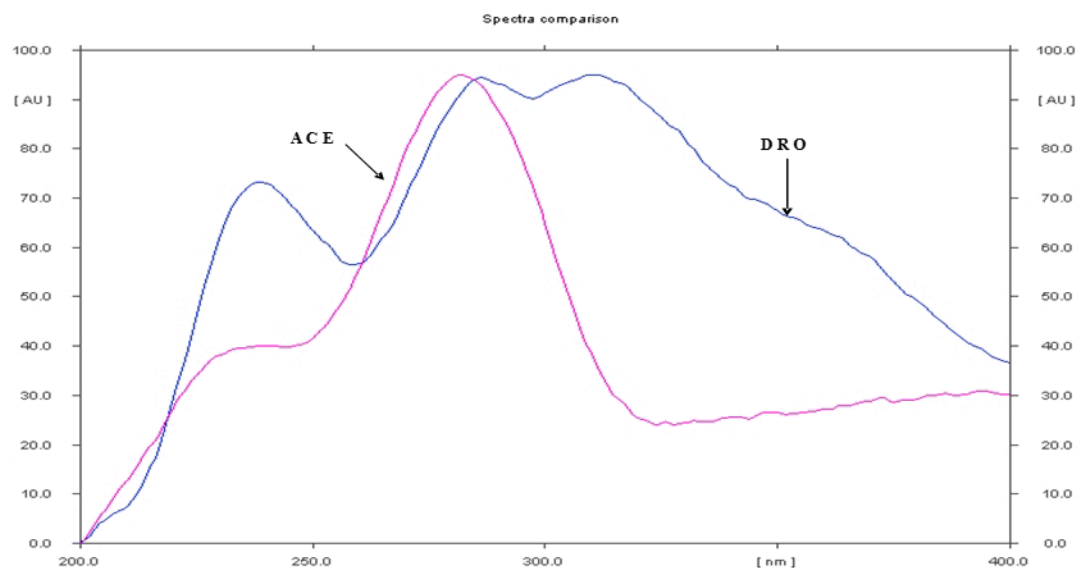


Figure 1: Overlay spectra of DRO and ACE from 200 to 400 nm

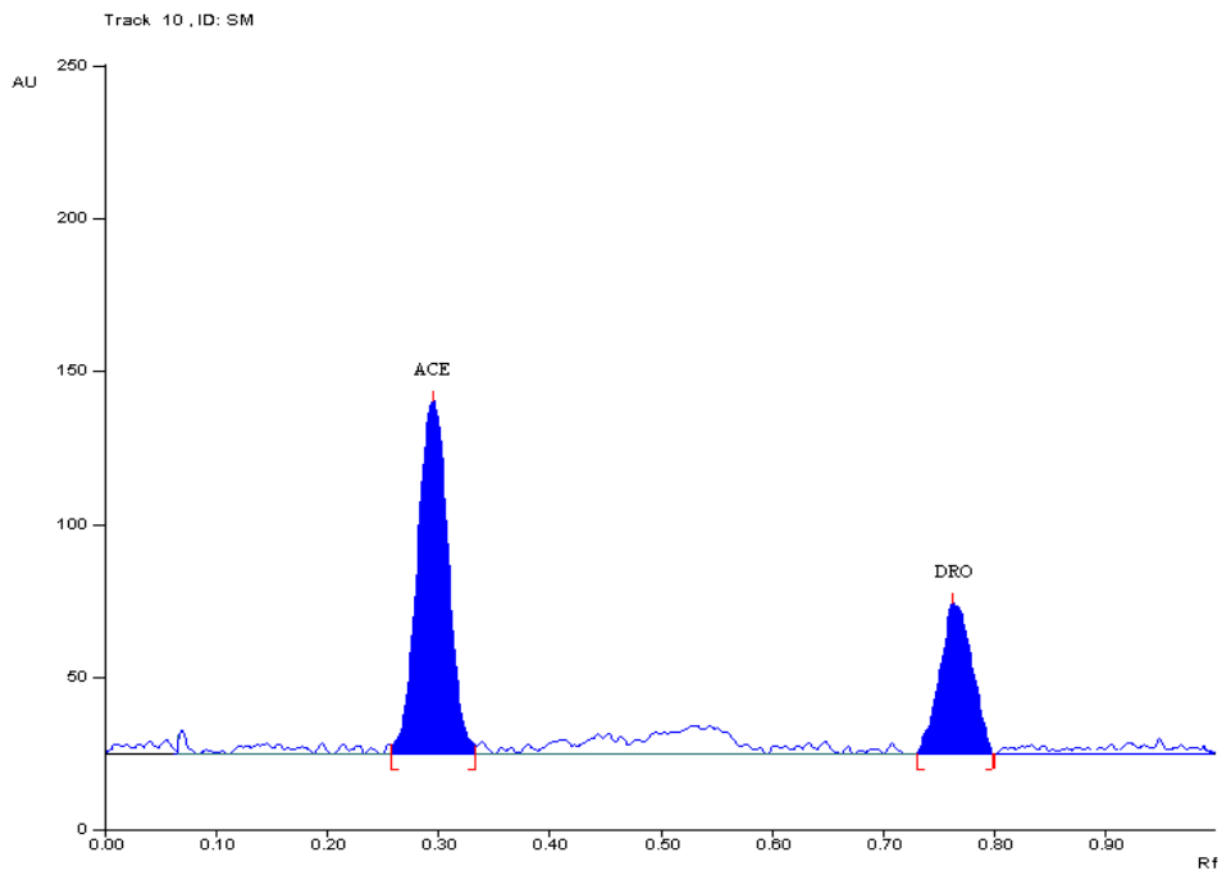


Figure 2: Representative densitogram obtained from a mixed standard solution of DRO (160 ng band⁻¹, $R_F = 0.76 \pm 0.03$) and ACE (200 ng band⁻¹, $R_F = 0.29 \pm 0.03$).

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