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Simultaneous densitometric analysis of Drotaverine and Aceclofenac by HPTLC method

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Abstract

A simple, precise, accurate and rapid high performance thin layer chromatographic method has been developed and validated for the simultaneous estimation of drotaverine and aceclofenac in combined dosage forms. The stationary phase and mobile phase used were pre coated silica gel 60F_{254} and a mixture of methanol-ethyl acetate-glacial acetic acid (1:9:0.01v/v/v). The detection of spots was carried out at 300 nm. The calibration was found to be linear between 80 to 560 ng/spot for drotaverine and 100 to 700 ng/spot for aceclofenac. The limit of detection and quantification for drotaverine were 24 and 20 ng/spot and aceclofenac 80 and 100 ng/spot, respectively. Statistical analysis proved that the method was precise, accurate and reproducible. Hence, the method is suitable for routine analysis of drotaverine and aceclofenac in combined dosage form.

Key Words: Drotaverine, Aceclofenac, HPTLC, Quantitative Analysis.

INTRODUCTION

Fixed dose combination of drotaverine and aceclofenac tablet contains 80 mg drotaverine and 100 mg aceclofenac. Drotaverine hydrochloride (DRO) chemically 1-[(3, 4-[diethoxyphenyl) methylene] - 6, 7-diethoxy-1, 2, 3, 4-tetrahydroisoquinolene is mainly used as an antispasmodic and smooth-muscle relaxant [1]. Aceclofenac, (ACE) chemically, 2-[(2, 6- dichlorophenyl) amino] phenylacetoxyacectic acid, is a phenylacetic acid derivative with potent analgesic and anti-inflammatory properties. It is official in *Indian Pharmacopoeia* [2].

Literature survey revealed RP-HPLC simultaneous determination of DRO in presence Nifuroxazide [3] as well as Omeprazole [4] in pharmaceutical samples. Spectrophotometric [3, 5, 6] and HPTLC [7, 8] methods have been reported for the estimation of DRO in combination with other drugs. Spectrofluorimetric [9] measurement of DRO also has been reported. HPLC method has been reported for estimation of ACE in formulations in combination with other drugs [10].

Few spectrophotometric [11, 12, 13], and HPTLC [14, 15] methods are also reported. Extensive literature survey reveals that no chromatographic methods have been reported for simultaneous determination of Drotaverine hydrochloride and Aceclofenac in tablet dosage form. Therefore the aim of present work was to develop simple, precise, accurate and economical HPTLC method for simultaneous determination of binary drug formulation without prior physical separation from tablets.

MATERIALS AND METHODS

Chemicals

Drotaverine and aceclofenac were supplied as gift samples by AD Pharmaceuticals Ltd, India. Tablets containing drotaverine [80mg] and aceclofenac [100mg] were procured locally. Methanol, ethyl acetate and glacial acetic acid of A.R. grade were from S.D Fine chemicals, India.

Standard solution

A standard stock solution containing drotaverine (80mcg mL^{-1}) and aceclofenac (100mcg $mL^{-1)}$ were prepared by dissolving accurately weighed drotaverine [8mg] and aceclofenac [10mg] in 100ml methanol. This solution was used for calibration.

Sample preparation

For analysis of drotaverine and aceclofenac in the pharmaceutical preparation, not less than 20 tablets each containing 80 mg of drotaverine and 100mg aceclofenac were finely powdered. A quantity of powder equivalent to 8 mg of drotaverine was extracted with methanol and filtered. The filtrate was transferred to a 100 ml volumetric flask and diluted to volume with methanol, so that the final concentration of drotaverine and aceclofenac were 80 mcg mL^{-1} and 100mcg $mL^{-1,}$ respectively. The amounts of the drug were computed by use of external standard quantification method. Repeatability studies were performed on data from six independent analyses.

Chromatography

TLC was performed on 10cm x 10 cm aluminium foil HPTLC plates coated with 0.2 mm layers of silica gel 60 GF_{254} (Merck, Germany). All plates were prewashed with methanol to ensure a clean background, and dried at room temperature in desiccators. After spotting with an appropriate volume of solution (1-7µL), plates were saturated with mobile phase vapour by equilibration for 10 min, then developed to a distance of 85 mm, at ambient temperature (25°c), with methanol - ethyl acetate- -glacial acetic acid, 1:9:0.01 (v/v/v) as mobile phase in a glass twin- trough chamber. Each separation was performed with 10 ml mobile phase. After development, plates were dried in air at room temperature. Evaluation of developed plates was performed densitometrically by the use of a Camag scanning densitometer controlled by Wincats software. Absorbance was measured at 300nm, using a deuterium lamp. The slit dimensions were 5 mm x 0.45 mm and the scan speed was 20 mm s^{-1}.

RESULTS AND DISCUSSION

Use of high – performance thin layer chromatography requires optimization of the mobile phase not only to enable satisfactory separation of the components of the investigation mixture but also to obtain symmetrical well shaped spots. Several mobile phases were investigated for the separation of drugs. Use of methanol-ethyl acetate-glacial acetic acid 1:9:0.01 (v/v/v) gave good separation for the two drugs. The R_f values were 0.18±0.02and 0.51±0.02, respectively, for

drotaverine and aceclofenac. The excipients in the tablets analyzed were not detected under these chromatographic conditions. The densitogram obtained is presented in fig 1.

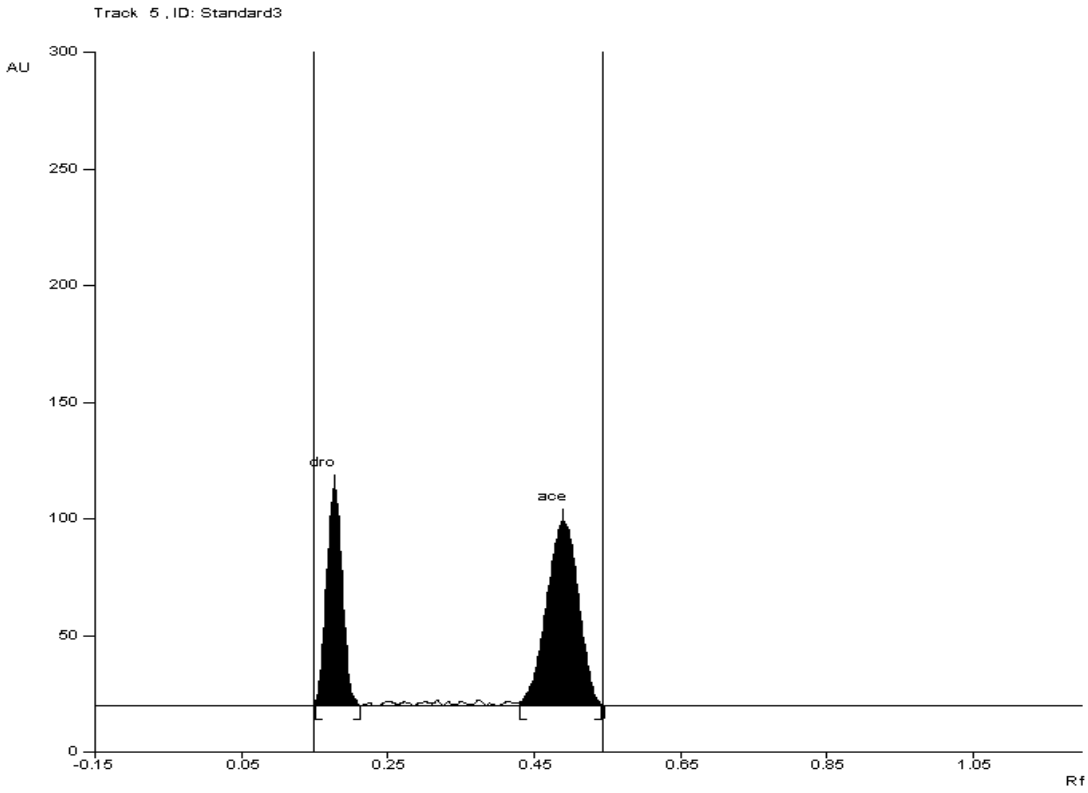

Fig 1: Chromatogram of drotaverine and aceclofenac

Response was a linear function of amount in the ranges 80-560 ng per spot for drotaverine and 100 to 700 ng per spot for aceclofenac. Quantification was achieved by use of calibration plots generated by plotting analyte peak area against concentration. For construction of each plot calibration standard solutions were prepared five times. The regression equations for the calibration plots were:

-peak area =282.28+6.732 x concentration (r =0.99757) for drotaverine; and
-peak area = 8.4286+5.0864 x concentration (r =0.9984) for aceclofenac.

Intra –day and inter-day precisions were measured for three different concentrations and three determinations were performed at each concentration. Instrument precision was studied by replicate application of the same sample on a TLC plate by use of a Linomat applicator and replicate measurement (scanning) of the plate six times without changing the position.

On each occasion peak area and R_f values were noted and relative standard deviation (n=6) was calculated for each. The average RSD was 0.3155% for drotaverine and 1.1733% for aceclofenac, indicating the precision of quantification was acceptable.

Recovery experiment was performed by adding known amount of the drugs to real samples containing known (previously determined) concentration of drotaverine and aceclofenac. Recovery of each added standard was determined at two levels (50 and 100%) and repeated three

times. The average accuracy was 99.65±0.1732% for drotaverine and 99.82±0.2167% for aceclofenac.

Solution containing decreasing concentration of the drugs were prepared and processed as described above to study the detection limit. Minimum detection limits were 24ng per spot for drotaverine and 20ng per spot for aceclofenac. Quantitation limits were 80ng and 100ng per spot for drotaverine and aceclofenac, respectively. In replicate analysis (n=6) of the samples, the average amount of drotaverine and aceclofenac per tablet found by the proposed method were close to the label claim. Statistical data pertaining to the accuracy, precision and recovery of the method are also summarized in Table1.

Table 1: Validation Parameters

Parameter	**Drotaverine**	**Aceclofenac**
Mean amount found (mg/tablet)	79.82	100.21
Assay (%)±SD*	99.65±0.1732	99.82±0.2167
Precision(%RSD)		
Intra-day	0.3828	0.7629
Inter-day		
1st day	0.3828	0.7629
2nd day	0.2481	1.5836
Repeatability of application	0.2845	0.5487
Repeatability of measurement	0.2759	0.3826

CONCLUSION

The proposed HPTLC method is suitable for quantification of drotaverine and aceclofenac in bulk and tablets. All the standards and samples can be analyzed simultaneously by the current HPTLC method in nanogram level. Hence, the HPTLC method reported here can be used for the routine quality-control testing of tablets containing both drotaverine and aceclofenac.

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