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Simultaneous HPTLC Determination of Aceclofenac and Drotaverine Hydrochloride in Tablet Dosage Form

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Abstract: *A normal-phase simple, rapid and precise high performance thin – layer chromatographic method has been developed for simultaneous quantitative determination of Aceclofenac and Drotaverine hydrochloride in a tablet dosage form. The analysis was performed on Silica gel 60F_{254} on aluminum plates with acetonitrile – ethyl acetate – triethylamine, 2 : 7.6 : 0.4 v/v as a mobile phase. Detection and quantitation were performed densitometrically at wavelength 282nm. The developed method was validated for linearity, precision, solution stability, accuracy and robustness parameters. The linearity of Aceclofenac and Drotaverine hydrochloride were in the range of 50-150 µg/mL and 40-120µg/mL respectively. The correlation coefficient of Aceclofenac and Drotaverine hydrochloride were observed 0.9992 and 0.9995 respectively. Accuracy was checked by performing recovery studies from the pharmaceutical preparation. The average was found to be 99.48 ± 1.62% for Aceclofenac and 99.32 ± 1.52% for Drotaverine hydrochloride. The proposed HPTLC method was found to be accurate, precise and rapid for the simultaneous determination of Aceclofenac and Drotaverine hydrochloride in tablet dosage form.*

Keywords: Drotaverine hydrochloride, Aceclofeanc, HPTLC

I. INTRODUCTION

Aceclofenac which has molecular formula $C_{16}H_{13}Cl_2NO_4$, molecular weight 354.19. Aceclofenac (ACF), {[2-(2',6'-dichlorophenyl) amino] phenyl acetoxyacetic acid } is a new phenyl acetic acid [1] derivative with potent analgesic and anti-inflammatory properties with improved gastric tolerance. Drotaverine is an antispasmodic drug that works by inhibiting phosphodiesterase-4 (PDE4).5 It is a benzylisoquinoline derivative that is structurally related to papaverine, although it displays more potent antispasmodic activities than papaverine. Drotaverine Hydrochloride (DTV) chemically 1-[3, 4-(diethoxyphenyl)methylene]-6,7-Diethoxy-1,2,3,4-tetrahydroisoquinoline is an papaver analogue mainly used as antispasmodic and smooth muscle relaxant.[1]

Drotaverine has been used in the symptomatic treatment of various spastic conditions, such as gastrointestinal diseases, biliary dyskinesia, and vasomotor diseases associated with smooth muscle spasms. Literature survey revealed that various methods have been reported for the simultaneous determination of Aceclofenac and Drotaverine hydrochloride in pharmaceutical formulations,[1,3] viz, HPLC, UV and HPTLC [4-12]

II. EXPERIMENTAL

2.1 Working Standards and Chemicals

DTV and ACE working standard were obtained from ZESTICA Pharma (India). Tablet containing DTV(80mg) and ACE(100mg) were obtained from local market, AR grade methanol , acetonitrile and triethylamine were purchased from Merck India.

2.2 Instrumentation and Chromatographic Condition

The samples were spotted in the form of bands of width 5 mm with a desaga 100 µL sample syringe on silica gel precoated Al plate 60 F_{254} , with 200 µm thickness . These bands were applied with the help of Desaga AS 30- sample applicator at a distance of 10mm from X axis and 15mm from Y axis at the edge of the HPTLC plate with the speed of 150nl/sec for

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methanol The plates were prewashed by methanol and activated at 110^0C for 5min prior to chromatography. The space between two bands was kept at 10mm. The slit dimension was kept at 4 x 3 mm and 4.0mm/s scanning speed was employed. The monochromator bandwidth was set at 10nm, each track was scanned thrice and base line correction was used. The mobile phase composed of acetonitrile – ethyl acetate – triethyl amine, in the ratio 2 : 7.6 : 0.4 v/v.

Linear ascending development was carried out in a twin trough glass chamber saturated with the mobile phase. The optimized chamber saturation time for mobile phase was 30min at room temperature (25^0c ± 2) at relative humidity of 55% ± 5. TLC plates were dried in current of air with the help of air dryer. Detection and quantification was performed in the absorbance mode using Desaga TLC scanner with Pro-Quant software. During the method development the spots on the TLC plate were visualized in a UV chamber equipped with a UV lamp at wavelength 254nm. The developed TLC plate was scanned between 200nm and 400nm wavelength using CD- 60 Densitometer. The wavelength selected for further quantification was 270nm.

2.3 Preparation of Standard Solution

100mg of ACE and 80 mg of DTV was accurately weighed and transferred to a 100mL standard flask. It was dissolved in a methanol and then diluted up to the mark with methanol.

The concentration of the solution obtained was 1000µg/mL for Aceclofenac and 800µg/mL for Drotaverine hydrochloride (solution A). 5 mL of this solution A was diluted to 50mL in a volumetric flask with methanol. The concentration of the solution obtained was 100µg/mL for ACE and 80µg/mL for DTV.

2.4 Preparation of Sample Solution

Twenty tablets were weighed and the average weight was calculated. These tablets were powdered and a weight equivalent to one tablet was taken in a 100mL volumetric flask and dissolved in minimum amount of methanol and was sonicated for about 30 minutes then diluted upto the mark with methanol. This solution was filtered through syringe filter. Then 5 mL of the sample solution was taken in a 50mL dilution flask and was diluted upto the mark with methanol (solution A). This is the sample solution of 100% level.

III. Validation of the Method

The method was validated for linearity, precision, accuracy, specificity and solution stability.

Standard plots were constructed for both ACE and DTH in the range of 50-150µg/mL. The experiment was repeated thrice on the same day and additionally on two consecutive days to determine intra- and inter-day precision, respectively.

The intermediate precision (ruggedness) of the method was determined by repeating the experiment on two different instruments. Accuracy was determined by recovery studies. It was carried out by standard addition method by spiking 10%, 20% and 30% of the standard drugs in the 100 % sample solution. Three determinations were performed at each level.
Further specificity of the method was tested by study of the resolution factor of the drug peaks from nearest resolving peaks. Robustness of the method was carried out by small changes in the mobile phase composition (± 0.1 mL for each component) and the effects on the results were studied. Time from spotting to chromatography and from chromatography to scanning was varied by ± 15 minutes.

3.1 Analysis of Marked Formulation

The developed method can be applied in determination of ACE and DTV in a tablet DROTANEC SPAS (ZESTICA Pharma) which is marketed oral solid dosage formulation. To determine the contents of Aceclofenac and Drotaverine hydrochloride (label claim : 100mg ACE and 80mg DTV per tablet), the contents of the tablet were emptied and weighed. The drug from the powder was extracted with 15mL of methanol. To ensure complete extraction of the analytes, it was sonicated for 30 min. The resulting solution was allowed to settle for about an hour and the supernatant was suitably diluted to give desired concentration. 10µL of the solution was applied on TLC plate followed by development, visualization and scanned. The analysis was repeated in triplicate. The possibility of excipients interference in the analysis was studied.

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IV. Results and Discussions

4.1 Optimization of the Chromatographic Conditions

In order to develop a normal phase HPTLC method for the determination of Aceclofenac and Drotaverine hydrochloride in combined dosage form the chromatographic conditions were optimized. For better separation and resolution, the mixtures of different solvents of varying polarity were tried.

The different compositions of mobile phases were changed for getting better separation of analytes. Initially, chloroform –ethyl acetate 4 : 6 (v/v) and acetonitrile, toluene 5 : 5 (v/v) were used. The best results were obtained by the use of acetonitrile, ethyl acetate and triethylamine in the ratio of (2 : 7.6 : 0.4v/v/v). This mobile phase showed good resolution and separation of ACE and DTV peak from other formulation components or excipients tested as seen in fig 2.

Densitometric scanning of all the tracks showed compound with Rf value 0.38 ± 0.05 for Aceclofenac and 0.59 ± 0.04 for Drotavereine HCL. As the separation was takes place in a short time period the proposed method is quicker as compare to reported method.

Parameters	Chromatographic conditions
Development chamber	Twin trough chamber
Stationary phase	Silica gel
Mobile Phase	Acetonitrile : Ethyl acetate : Triethylamine (2 : 7.6 : 0.4v/v/v)
Chamber saturation	15 min
Sample applicator	AS 30 - SAMPLE APPLICATOR
Band	8mm
Space	12mm
Scanning speed	20mm/sec
Development distance	8 cm
Drying of plate	Room temperature
Densitometric scanner	CD 60 - DENSITOMETER / SCANNER
Lamp	Deuterium
Wavelength	282 nm
Volume	10µl

Table 1: Optimized chromatographic conditions

4.2 Method Validation

A. Linearity and Range

Linearity was observed over the concentration range of 50 - 150µg/mL for ACE and 37.5 – 112.5µg/mL for DTV (Table 2). The linearity was confirmed by the high value of the correlation coefficients of r^2= 0.9992 for ACE and 0.9995 for DTV

Table 2. Linear regression data

Drug	Linearity range	Correlation coefficient (r^2)	Slope	Intercept
Aceclofenac	50 - 150µg/mL	0.9992	3.250	-6.485
Drotaverine HCl	40 - 120 µg/mL	0.9995	7.846	-12.75

B. Precision

The developed method was validated for system precision and method precision. The precision study of the proposed method gave the results in the prescribed limits of relative standard deviation. This is less than 2 % for both analytes. The low value of RSD showed that the proposed method was reliable and reproducible.

Table 3: Precision study for Paracetamol and Mefenamic acid

Obs No	Aceclofenac		Drotaverine HCl	
	Peak Area	% Assay	Peak Area	% Assay
1	2450	101.61	1051	101.50
2	2475	101.50	1075	99.82

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3	2445	101.05	1085	98.92
4	2499	100.80	1045	99.34
5	2442	98.98	1048	97.94
6	2482	99.78	1105	99.80
	Mean	100.62	Mean	99.55
	S.D	1.036	S.D	1.180
	%R.S.D	1.029	%R.S.D	1.186

C. Solution Stability

Stability of a sample solution was checked by using sample prepared in the precision study. The sample solution was stored at room temperature for 24 hrs then it was withdrawn at the intervals of 2 hr, 4 hrs , 12 hrs and then applied on the chromatographic plate stored at room temperature for 24 hours, withdrawn at the intervals of 2hrs, 4 hrs, 12 hrs and 24 hrs and then applied on the chromatoplate.

After development, the chromatogram was evaluated for additional spots if any. There was no indication of compound instability in the sample solution. The results shows that the solutions were stable for 24 hrs at room temperature.

D. Specificity

An investigation specificity was conducted during the validation of identification tests, the determination of impurities and the assay. Demonstration of specificity requires that there should not be any interference of impurities and excipients. In practice this was done by taking the chromatogram of sample solution and the assay result was unaffected by the extraneous material. It has been found that there was no interference of the diluents, placebo at the Rf value of the analytes.

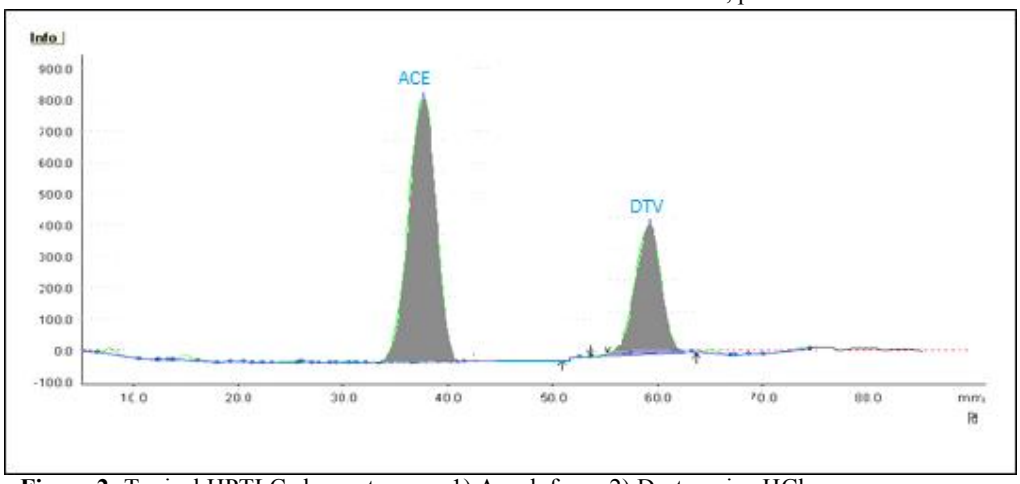

Figure 2: Typical HPTLC chromatogram 1) Aceclofenac 2) Drotaverine HCl

E. System Suitability Test

A system suitability test should be carried out to see if the HPTLC system is performing properly. System suitability tests were carried out as per the USP to confirm the suitability and the reproducibility of the system. The experiment was carried out using 100% level mixed standard solution of ACE and DTV. This solution was spotted five times on the chromatographic plate under the optimized chromatographic conditions. % RSD of the peak area shows that (Table 4) the proposed method was suitable for the system.

Table 4: System suitability for Aceclofenac and Drotaverine HCl

Obs No	100% Level			
	Aceclofenac		Drotaverine Hydrochloride	
	Peak area	Rf value	Peak area	Rf value
1	2433	0.38	1051	0.59

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2	2450	0.39	1102	0.60
3	2451	0.39	1086	0.59
4	2459	0.39	1075	0.61
5	2425	0.38	1015	0.60
Mean	2443	0.386	1065	0.598
S.D	14.06	0.0054	33.92	0.0083
% RSD	0.5755	1.418	3.18	1.399

4.3 Accuracy (Recovery Experiment)

The accuracy of the method was determined by the standard addition method at three different levels. The sample solution of 100% level was considered as a zero level and 10% , 20% and 30% of the standard drug of ACE and DTV were added respectively. Each determination was performed in triplicates. The accuracy was then calculated as the percentage of the standard drug recovered by the recovery study. Mean recoveries for ACE and DTV from the sample solution are shown in Table 5 and 6. The results are within the acceptance limit and hence the method is accurate.

Table 5: % Recovery of ACE

Amount of Aceclofenac in ppm								
Sr. No	% Added	Original amount	Added amount	Total amount	Mean (n = 5)	% Recovery	S.D	% RSD
1	10	100	10.40	110.40	109.98	99.61	0.3881	0.3886
2	20	100	20.25	120.25	120.05	99.83	0.4922	0.4996
3	30	100	30.10	130.10	130.78	100.52	0.645	0.651

Table 6: % Recovery of ACF

Amount of Aceclofenac in ppm								
Sr. No	% Added	Original amount	Added amount	Total amount	Mean (n = 5)	% Recovery	S.D	% RSD
1	10	80	10.35	90.35	90.41	100.06	0.042	0.044
2	20	80	20.12	100.12	99.94	99.82	0.127	0.128
3	30	80	30.16	110.16	109.92	99.78	0.169	0.172

V. CONCLUSION

The HPTLC method for the determination of Aceclofenac and Drotaverine HCL from their tablet dosage form was found to be accurate, precise, specific and rapid. The results of the recovery studies show the high degree of accuracy of the proposed method. The advantage of the proposed method is that it require less time and cost effective method. Solvent consumption during the analysis is less. Therefore the proposed method can be applied successfully in routine analysis.

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