Asian Journal of Chemistry Vol. 19, No. 2 (2007), 1487-1493

Simultaneous HPTLC Estimation of Ambroxol HCl and Cetirizine HCl in Their Combined Dose Tablet

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A simple, rapid, reproducible and economical high performance thin layer chromatographic method for simultaneous estimation of ambroxol HCl and cetirizine HCl in tablet form has been developed. It was performed on silica gel 60 GF_{254} thin layer chromatographic plates using mobile phase comprising of methanol: ethyl acetate: toluene: ammonia (4:1:5:0.5:6: 1 drop) v/v and the detection was carried out at 231 nm showing R_f value 0.78 for ambroxol HCl and 0.40 for cetirizine HCl. The calibration curve response was observed between 4-10 µg for ambroxol HCl and 0.4-0.8 µg for cetirizine HCl by height and by area. The per cent drug estimated for ambroxol HCl and cetirizine HCl from marketed formulation was found to be 99.86 ± 1.72, 100.18 ± 2.0 by height and 100.81 ± 1.54, 99.13 ± 1.76 by area, respectively. The per cent recovery by height and by area was found to be 102.66 ± 1.53 and 100.04 ± 1.79 for ambroxol HCl and 99.31 ± 1.25 and 98.06 ± 1.45 for cetirizine HCl. The method was validated with the determination of accuracy, precision, specificity, linearity detector response and ruggedness.

Key Words: Ambroxol HCl , Cetirizine HCl, HPTLC, Validation.

INTRODUCTION

Ambroxol HCl (AMB) is an expectorant and mucolytic agent, chemically it is 4-[(2-amino-3,5-dibromobenzyl)amino] cyclohexanol hydrochloride[1]. Cetirizine HCl (CTZ) is histamine H_1-receptor antagonist, chemically it is 2-[2-[4(4-chlorophenyl) phenyl methyl]piperazin-1-yl] ethoxy] acetic acid dihydrochloride[2]. Literature survey reveals that several methods such as UV-spectrophotometric[3,4] and chromatographic[5-10] have been reported for estimation of AMB in pharmaceutical formulation. However several methods like spectrophotometric[11-14] and HPLC[15-19] were reported for estimation of CTZ.

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There is no HPTLC method reported for simultaneous estimation of the AMB and CTZ in combined dosage form. In present experiment the efforts were done to develop method which will show good resolution, separation and estimation of AMB and CTZ. The method was validated to show the sensitivity and reproducibility.

EXPERIMENTAL

The instrument used in present study was CAMAG-HPTLC system comprises CAMAG LINOMAT IV automatic sample applicator, CAMAG TLC SCANNER III with CATS 4.0 software, CAMAG Twin trough glass chamber.

The chemicals and reagents used throughout the work were AR grade. The chemicals used were methanol, toluene, ethyl acetate and ammonia solution.

Standard solution: Standard solution of AMB (1.2 mg/mL) and CTZ (0.1 mg/mL) were prepared in water : methanol (1:9 v/v).

Mixed standard solution (sample solution): Solution containing both drugs *i.e.*, AMB and CTZ - 1.2 mg/mL and 0.1 mg/mL, prepared in water : methanol (1:9 v/v).

Experimental chromatographic conditions: Standard experimental conditions were followed during the present experimental study. Stationary phase-silica gel 60 GF_{254} TLC precoated aluminium foiled plates, mobile phase-methanol: ethyl acetate: toluene: ammonia (4:1:5:0.5:6:1 drop) v/v, saturation time-15 min, thickness of plate-200 μm, sample application - 6 mm band, separation technique-ascending, temperature-20 ± 5ºC, relative humidity - 50-60%, migration distance-70 mm, scanning mode-absorbance/reflectance, detection wavelength-231 nm, the detection wavelength was selected from overlain spectra of both the drugs in methanol (Fig.1).

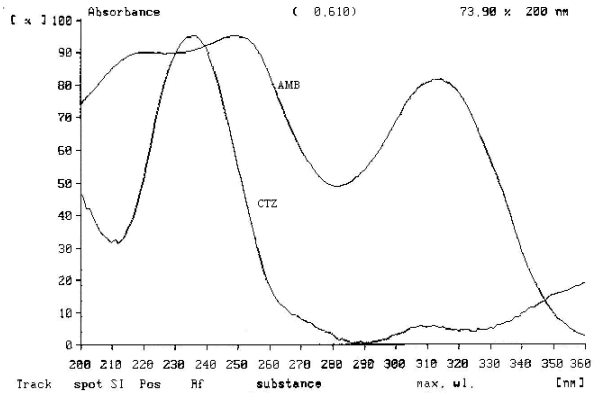

Fig. 1. Selection wavelength for densitometric evaluation of ambroxol HCl and cetirizine HCl

Calibration curve response: AMB (1.2 µg/mL) and CTZ (0.1 µg/mL) solution ranging from 6-12 µL were applied on TLC plate by microliter syringe with the help of automatic sample applicator. The plates were developed, dried and densitometrically scanned at 231 nm. Peak height and areas were recorded for each concentration of drugs and curves (concentration *vs* peak height/area) were constructed (Fig. 2).

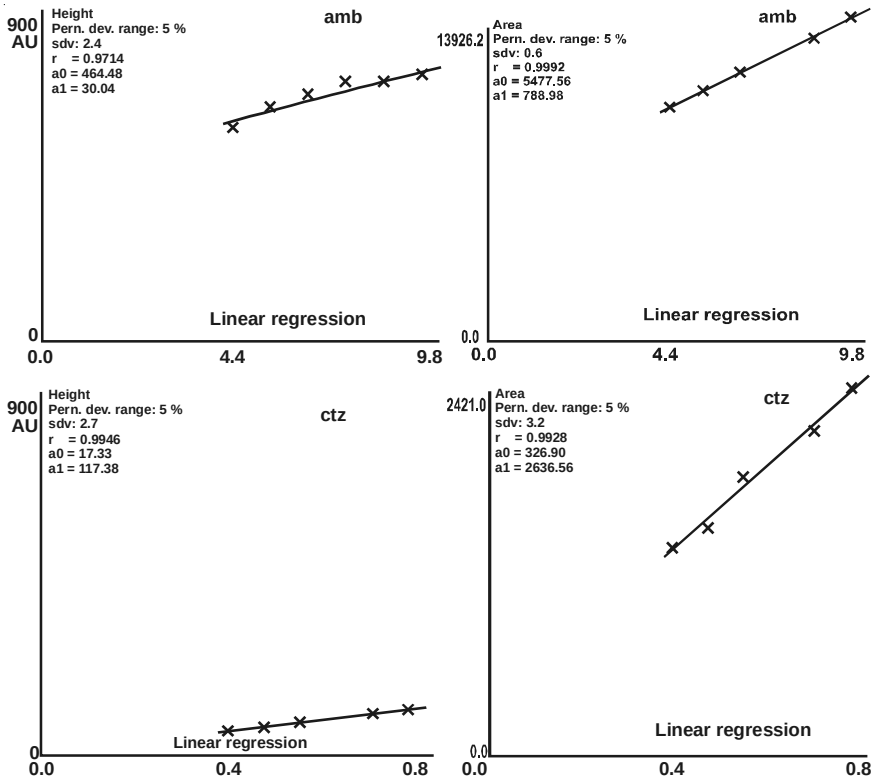

Fig. 2. Linearity range of ambroxol HCl cetirizine HCl by height and area

System suitability test: The system suitability test was performed by applying 6.0 µL of mixed standard solution containing (1.2 µg/mL) of AMB and (0.1 µg/mL) of CTZ. The mean value, standard deviation and coefficient of variance were calculated for peak height and peak area.

Standard laboratory mixtures: Different laboratory mixtures were prepared in the same manner as that of a standard preparation to get the final concentration of AMB 1.2 mg/mL and CTZ 0.1 mg/mL. A 6.0 µL of each mixed standard solution (duplicate) and laboratory mixture (quadruplet) were applied on TLC plate in the form of 6.0 mm band. Plates were then developed in presaturated twin trough chamber with mobile phase. After development the plates were dried with the help of hot air dryer and evaluated desitometrically at a wavelength of 231 nm (Fig. 3).

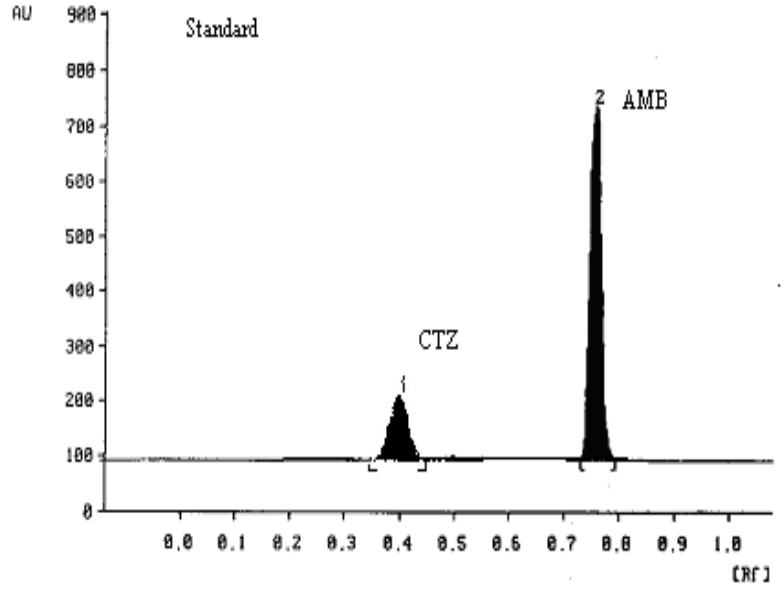

Fig. 3. Densitogram of ambroxol HCl and cetirizine for laboratory mixture

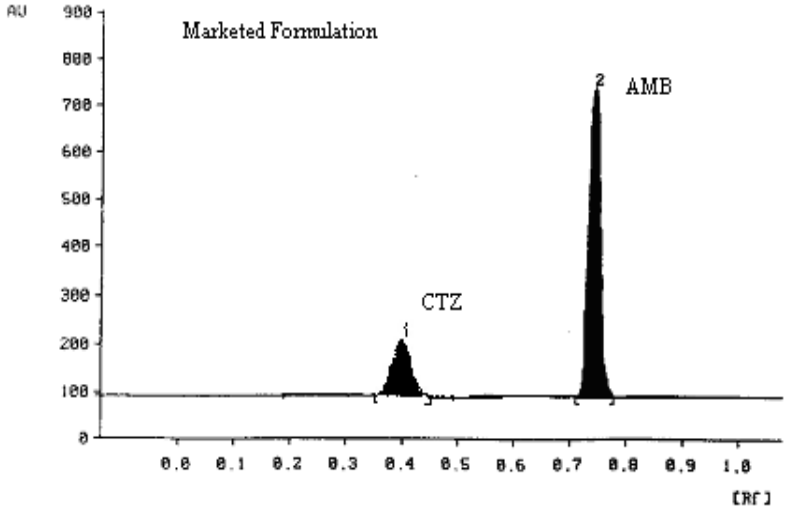

Fig. 4. Densitogram of ambroxol HCl and cetirizine for marketed formulation

Validation of proposed method: The proposed method was validating by considering following parameters.

Accuracy: The accuracy of proposed method was ascertained by carrying out recovery studies by standard addition method. The recovery study was performed to determine if there are positive or negative interference from excipients present in formulation. The method was ascertained on the basis of recovery study by standard addition method to preanalyzed sample (Table-1).

TABLE-1
PER CENT ESTIMATION OF DRUG FROM LABORATORY MIXTURE, MARKETED FORMULATION RECOVERY STUDY

Sample	% Estimation of labeled claim*				% Recovery			
	AMB		CTZ		AMB		CTZ	
	By Height	By Area	By Height	By Area	By Height	By Area	By Height	By Area
Standard Lab. Mixture	100.42	100.4	101	100.1	–	–	–	–
	0.91	0.91	1.41	1.84	–	–	–	–
	1.55	1.809	1.47	0.936	–	–	–	–
Marketed Formulation	99.86	100.1	100.81	99.13	102.66	100.0	99.31	98.06
	1.72	2.0	1.54	1.76	1.53	1.79	1.25	1.45
	1.62	0.72	1.00	1.19	1.62	1.922	1.78	1.98

*Mean, S.D. and C.V. of four observations

Precision: Precision of an analytical method is expressed as S.D. or R.S.D. of series of measurement. It was ascertained by replicate estimation of both the drugs by proposed method (Table-1).

Specificity: The specificity of the method was ascertained by analyzing standard drug and sample. The spot for AMB and CTZ in sample was confirmed by comparing the R_f and spectra of the spot with that of standard. The peak purity of both the drugs was assessed by comparing the spectra at three different levels *i.e.*, peak start (S), peak apex (M) and peak end (E) positions of the spot.

Linearity detector response: The study was performed by application of different volume of mixed standard and response was obtained densitometrically (Table-2, Fig. 2).

TABLE-2
RESULTS OF LINEARITY DETECTOR RESPONSE

Drug	Linearity range		Coefficient of correlation		Slope		Y-intercept	
	By Height	By Area	By Height	By Area	By Height	By Area	By Height	By Area
AMB	4.4-9.8	4.4-9.8	0.9714	0.9946	30.04	788.98	464.480	6477.56
CTZ	0.4-0.8	0.4-0.8	0.9946	0.9928	17.33	326.90	117.382	2636.56

Ruggedness: Ruggedness was carried out under the different conditions *i.e.* different days and different analysts (Table-3).

RESULTS AND DISCUSSION

Before preceding to the experiment both the drug were standardized by the official methods.

Various pure solvent and mixtures in varying proportion were tried as mobile phase. However, mobile phase consisting of methanol: ethyl acetate: toluene: ammonia (1:5:0.5:6:1) v/v were found to be more suitable

for better separation of AMB and CTZ with R_f value 0.78 and 0.40, respectively with saturation period of 15 min at 231 nm. The selection of wavelength was based on nearly equal absorbance by both the component of mixture for optimum sensitivity (Fig. 1). The calibration curves were drawn with peak height and peak area for each concentration of drugs (Fig. 2).

TABLE-3
RESULTS OF RUGGEDNESS STUDY

Different Days

Days	% Labeled Claim			
	AMB		CTZ	
	By Height	By Area	By Height	By Area
Day-1	98.310	100.100	99.510	100.450
Day-4	100.410	98.520	100.640	100.990
Day-7	100.150	100.010	100.240	100.300
Mean	99.620	99.540	100.130	100.250
± S.D.	1.436	0.456	0.964	1.564
C.V.	1.441	0.458	0.962	1.560

*Mean of four observations

Different Analyst

Days	% Labeled Claim			
	AMB		CTZ	
	By Height	By Area	By Height	By Area
Analyst-1	98.130	100.660	99.880	100.640
Analyst-2	99.480	100.070	100.360	100.190
Analyst-3	100.990	101.280	99.880	100.160
Mean	99.530	100.670	100.040	100.330
± S.D.	0.432	0.864	1.030	0.770
C.V.	0.435	0.858	1.037	0.767

*Mean of four observations

The above-observed results evidenced that the proposed method is simple, accurate, specific, rapid and can be used for simultaneous estimation of AMB and CTZ in combined dosages form. The value within the limit of standard deviation and coefficient of variance signifies high precision of method. The proposed method can be used for the routine analysis of AMB and CTZ in their combined dosage form.

ACKNOWLEDGEMENTS

The authors are thankful to Head of the Department for providing laboratory facilities at Department of Pharmaceutical Sciences, R.T.M. Nagpur University, Nagpur. Authors are also thankful to M/S Modimundi Pharma, New Delhi, for providing gift sample of drugs.

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(*Received*: 23 January 2006; *Accepted*: 20 September 2006) AJC-5119