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Simultaneous Estimation of Chlordiazepoxide and Clidinium Bromide in Combined Dose Tablet by High Performance Thin Layer Chromatography

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A simple, accurate, precise and economical high performance thin layer chromatographic method for simultaneous estimation of chlordiazepoxide and clidinium bromide 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 : acetonitrile : water : glacial acetic acid in the ratio 2.0 : 6.5 : 1.0 : 0.5 and the detection was carried out at 217 nm. The R_f values were 0.83 for chlordiazepoxide and 0.30 for clidinium bromide. The linear response was observed over 2.2–12.2 g of chlordiazepoxide and 1.2–8.8 μg of clidinium bromide both by height and by area. The recovery of the drugs from tablet carried out by standard addition method was found to be 99.31 ± 1.25 (by height) and 98.06 ± 1.45 (by area) for chlordiazepoxide and 102.66 ± 1.53 (by height) and 100.04 ± 1.79 (by area) for clidinium bromide. The method was validated with respect to its accuracy, precision, specificity and ruggedness.

Key Words: Chlordiazepoxide, Clidinium bromide, HPTLC.

INTRODUCTION

Chlordiazepoxide (CH) is a tranquillizer and anxiolytic. It is official in IP[1], BP[2] and USP/NF[3]. Chemically, it is 7-chloro-2-methyl amino-5-phenyl-3H-1,4-benzodiazepine-4-oxide. Clidinium bromide (CL) is an anticholinergic agent and it is official in USP/NF[4]. Chemically, it is 3-[(hydroxydiphenyl acetyl)-oxy]-1-methyl-1-azoniabicyclo[2,2,2]octane bromide.

Thin layer chromatographic and UV-spectrophotometric[5], differential spectrophotometric[6,7], polarographic[8], atomic absorption spectrophotometric[9], colorimetric[10], GC[11], derivative spectrophotometric[12–14], HPLC and first derivative spectroscopic[15] methods have been reported for estimation of chlordiazepoxide alone and in combination with other drugs in pharmaceutical formulations. Capillary electrophoresis[16], LC-MS[17], potentiometric[18] and TLC, RP-HPLC, GLC[19] methods have been reported for estimation of clidinium bromide in pharmaceutical formulations. The methods reported for simultaneous estimation

of chlordiazepoxide and clidinium bromide are derivative spectrophotometric[20, 21], ratio spectra derivative spectrophotometric[22] and HPLC[23, 24].

The present paper describes a simple high performance thin layer chromatography (HPTLC) method for simultaneous estimation of chlordiazepoxide and clidinium bromide in tablet formulations.

EXPERIMENTAL

Camag-HPTLC system comprising of Camag Linomat-IV automatic sample applicator, Camag TLC Scanner-III with CATS 4.0 version software and Camag Twin trough glass chamber were used. The chemicals and reagents used were of AR and HPLC grade. Mixed standard solution containing 1.0 mg/mL of chlordiazepoxide and 0.5 mg/mL of clidinium bromide was prepared in methanol.

Chromatographic conditions: Stationary phase: Merck silica gel 60 GF_{254} TLC precoated aluminium plates, 200 μm layer thickness. Mobile phase : methanol : acetonitrile : water : acetic acid in the ratio of 2.0 : 6.5 : 1.0 : 0.5.

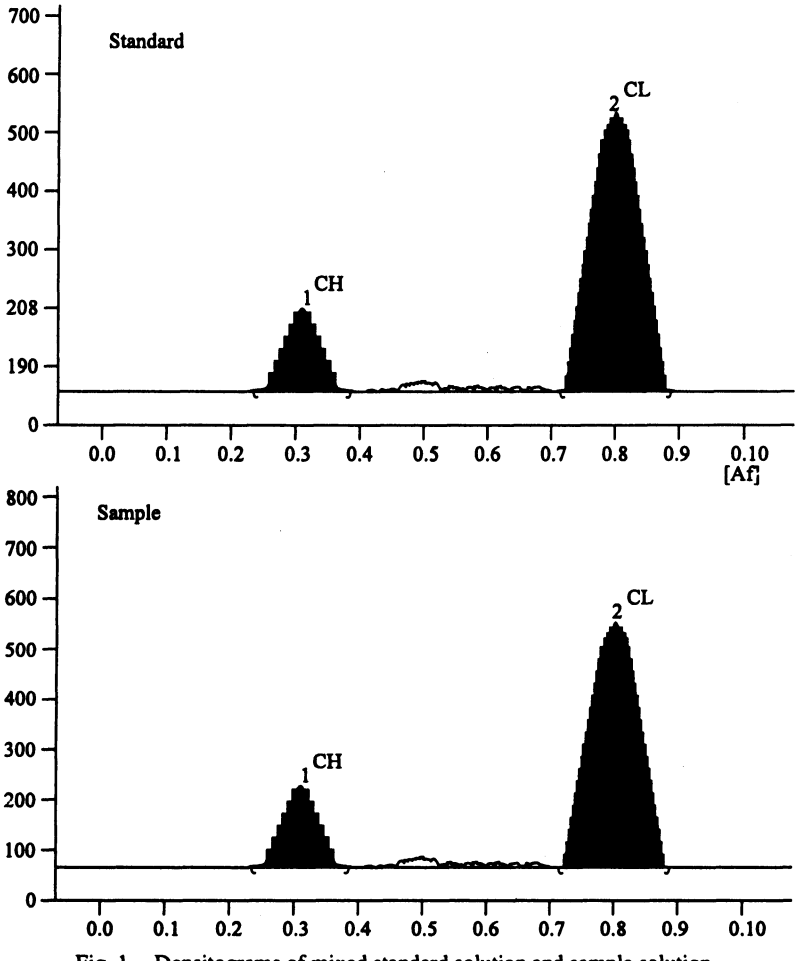

Fig. 1. Densitograms of mixed standard solution and sample solution

Chamber saturation time: 10 min. Sample application: 6 mm band. Separation technique: ascending. Temperature: 20 ± 5°C. Relative humidity: 50–60%. Migration distance: 70 mm. Scanning mode: Absorbance/reflectance. Detection wavelength: 217 nm. The parameters were optimized on the basis of rigorous experimental work.

Calibration curve: Mixed standard solutions ranging between 2–14 μg of chlordiazepoxide were applied on TLC plate by a micro-litre syringe with the help of automatic sample applicator. The plates were developed, dried and densitometrically scanned at 217 nm. Peak heights and areas were recorded for each drug and curves (concentration *vs.* peak height/area) were plotted (Fig. 2).

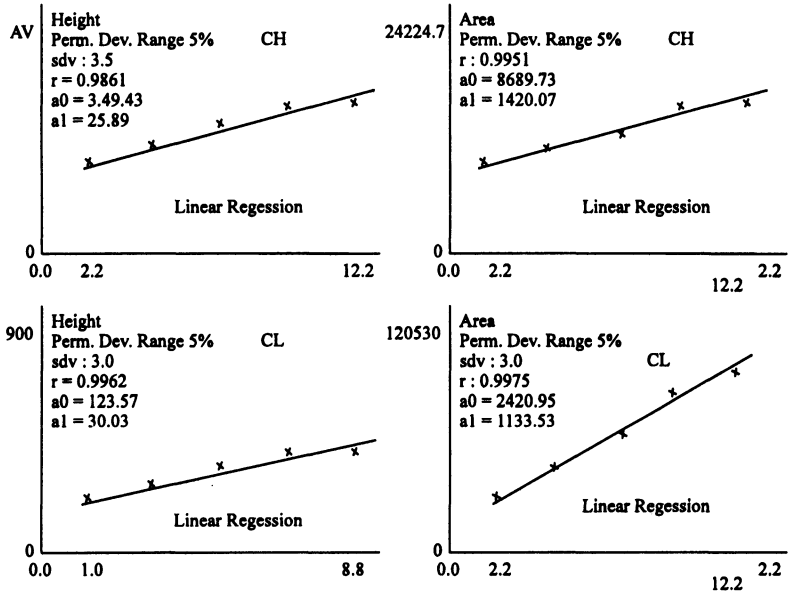

Fig. 2. Linearity range of chlordiazepoxide and clidinium bromide by height and area.

Analysis of standard laboratory mixtures: Different laboratory mixtures were prepared in the same manner as that of a standard solution to get the final concentration of accurately about CH 1.0 mg/mL and CL 0.5 mg/mL. 4.0 μL each of mixed standard solution (duplicate) and laboratory mixture (quadruplicate) were applied on TLC plate in the form of 6.0 mm band. Plates were developed in presaturated twin trough chamber with mobile phase. After development the plates were dried with the help of hot air dryer and evaluated densitometrically at 217 nm.

The per cent estimation of drug in laboratory mixture was calculated by using the formula:

$$\% \text{ estimated} = \frac{\text{Amount estimated}}{\text{Amount applied}} \times 100 \quad (1)$$

Assay: Twenty tablets (Librax), each labelled to contain CH 5.0 mg and CL 2.5 mg per tablet, were weighed and finely powdered. An accurately weighed

quantity of tablet powder equivalent to 5.0 mg of CH was shaken with 20.0 mL of methanol for about 15 min and volume was made up to 25.0 mL. The solution was then filtered through Whatmann No. 1 filter paper and the filtrate was used as sample solution. Procedure as detailed under analysis of standard laboratory mixture was followed using sample solution instead of laboratory mixture.

The contents of drugs per tablet (as per cent of labelled claim) were calculated using the formula

$$\% \text{ of labelled claim} = \frac{\text{Amount estimated}}{\text{Amount applied on labelled claim basis}} \times 100 \quad (2)$$

Validation of Proposed Method

The proposed method was validated for the following parameters:

Accuracy: The accuracy of the proposed method was ascertained by analyzing the standard laboratory mixture and also on the basis of recovery studies performed by standard addition method (Table-1).

TABLE-1
RESULT OF ESTIMATION OF DRUGS IN TABLET AND RECOVERY STUDIES

S. No.	Sample	% Estimation of labelled claim*				% Recovery*			
		Chlordiazepoxide		Clidinium bromide		Chlordiazepoxide		Clidinium bromide	
		By height	By area	By height	By area	By height	By area	By height	By area
1.	Standard laboratory mix.	101.0 (±1.41) (1.396)	100.13 (±1.84) (1.837)	100.42 (±0.91) (0.906)	100.48 (±0.91) (0.906)	— —	— —	— —	— —
2.	Marketed preparation	100.81 (±1.54) (1.527)	99.12 (±1.76) (1.775)	99.86 (±1.72) (1.722)	100.16 (±2.00) (1.996)	99.31 (±1.25) (1.259)	98.06 (±1.45) (1.479)	102.66 (±1.53) (1.490)	100.04 (±1.79) (1.789)

*Each reading is the mean of four observations.

Precision: It was ascertained by replicate estimation of drugs in tablet by proposed method and evaluating S.D. or R.S.D. of series of measurements (Table-1).

Specificity: Accurately weighed quantities of tablet powder, each equivalent to about 5.0 mg of CH, were expressed in various forced degradation conditions like 1.0 mL of 0.1 N HCl at 50°C, 1.0 mL of 0.1 N NaOH at 50°C, 3% of H_2O_2 (oxidation) at 50°C in UV-cabinet at 265 nm and at 60°C. After 24 h the contents in the flasks were shaken with methanol for 15 min and volumes were made up to 25.0 mL. The solutions were filtered and the filtrates were analyzed by the proposed method (Table-2).

TABLE-2
RESULTS OF SPECIFICITY STUDY

S. No.	Sample	% Labelled claim*			
		Chlordiazepoxide		Clidinium bromide	
		By height	By area	By height	By area
1.	Normal	99.60	98.45	101.93	101.32
2.	Acid	28.05	26.87	113.15	129.18
3.	Alkali	100.32	97.06	43.95	41.71
4.	Oxide	94.24	93.79	86.45	86.80
5.	Heat	101.04	102.64	99.47	100.16
6.	U.V.	92.01	91.99	83.80	81.48

*Results are average of two observations.

Ruggedness: Ruggedness studies were carried out by different analysts (Table-3).

TABLE-3
RESULTS OF RUGGEDNESS STUDY

S. No.	Days	% Labelled claim			
		Chlordiazepoxide		Clidinium bromide	
		By height	By area	By height	By area
1.	Analyst-1	101.60	99.90	99.81	99.05
2.	Analyst-2	98.64	97.38	101.39	99.81
3.	Analyst-3	98.55	98.52	99.08	99.47
		99.59	98.60	100.00	99.44
		(±1.735)	(±1.2619)	(±1.1807)	(±0.3807)
		(1.742)	(1.2798)	(1.1797)	(0.3828)

RESULTS AND DISCUSSION

In an attempt to develop HPTLC method for simultaneous estimation of chlordiazepoxide and clidinium bromide in fixed dose combination tablet, a satisfactory resolution of components was achieved on silica gel GF_{254} thin layer plate using Methanol : Acetonitrile : water : glacial acetic acid in the ratio of 2.0 : 6.5 : 1.0 : 0.5 (v/v) as mobile phase. The R_f values were 0.30 and 0.83 for CL and CH respectively (Fig. 1). The wavelength, 217 nm, was selected for densitometric quantitation in reflectance mode due to high absorptivity of both the components; hence higher sensitivity (Fig. 3). Other conditions as detailed under experimental part were rigorously optimized to achieve reproducible separation. The drugs had shown linearity of concentration *vs.* peak height/peak area over the concentration range 2.2–12.2 g of CH and 1.2–8.8 g of CL with correlation coefficient well above 0.99 by height and by area (Fig. 2).

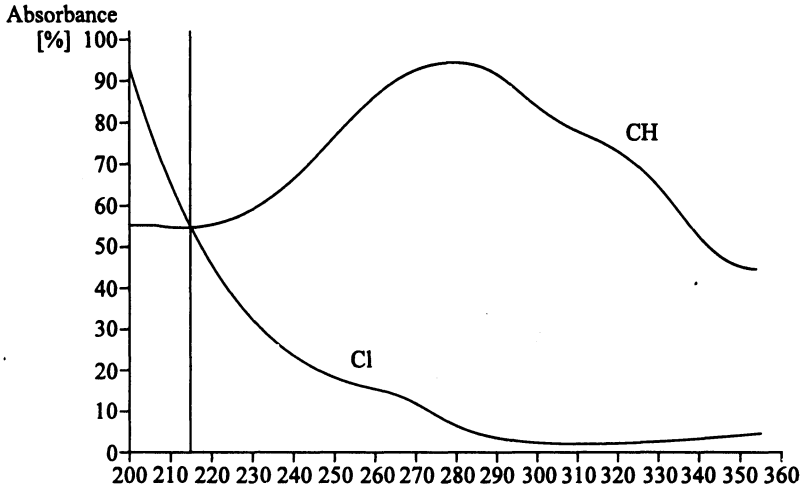

Fig. 3. *In-situ* UV-spectra of chlordiazepoxide and clidinium bromide in reflectance mode of TLC scanner.

The results of estimation of the drugs in marketed tablet formulation (Table-1) had been concurrent and repeatable with R.S.D. value less than 2% indicating precision of the proposed method. The results of estimations in standard laboratory mixtures and also the recovery studies performed by standard addition method, being close to 100% (Table-1), are indicative of the accuracy of the method and non-interference of excipients present in formulation.

The specificity of the method evaluated on the basis of forced degradation by exposing the sample to various stress conditions has shown reasonable specificity of the proposed method. In case of estimation of chlordiazepoxide, the results of the sample treated with an acid were much on the lower side along with appearance of additional peaks indicating that the sample has undergone degradation and the method is capable of estimating chlordiazepoxide specifically in presence of its acid degradation products. In addition to this, the results were also low by about 5–8% in samples treated with hydrogen peroxide and UV radiations (Table-2) compared to the normal samples. In case of clidinium bromide the sample treated with alkali had yielded the results of the order of about 43.0% indicating that the sample has undergone some degradation and the method is capable of detecting it. In case of UV irradiated and hydrogen peroxide treated sample the results were low by about 15%. In case of all other treated samples, the results of estimation of chlordiazepoxide and clidinium bromide were close to normal samples indicating that either there is no degradation or otherwise the proposed method is incapable of specifically detecting it.

The ruggedness studies performed by carrying out the estimations by different analysts (Table-3) have indicated the reproducibility of the method.

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