DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF CLIDINIUM BROMIDE, CHLORDIAZEPOXIDE AND DICYCLOMINE HYDROCHLORIDE IN TABLET DOSAGE FORM

ABSTRACT

A simple, specific, accurate reversed phase high performance liquid chromatographic method was developed for the simultaneous estimation of clidinium bromide, chlordiazepoxide and dicyclomine hydrochloride. Chromatographic separation of the three drugs was performed on a Chromatopak C-18 column (25 cm x 4.6 i.d. x 5µm) as the stationary phase with a mobile phase composed of 0.1 % triethylamine in water pH adjusted by 5 % o-phosphoric acid and acetonitrile in the ratio 30:70 at a flow rate of 0.8mL/min, Detection was carried out at 210 nm. The retention times of clidinium bromide, chlordiazepoxide and, dicyclomine hydrochloride were found to be 3.9 min, 5.4 min, and 6.8 min, respectively. The proposed method was validated for linearity, accuracy, precision, LOD and LOQ.

Keywords: RP-HPLC, clidinium bromide, chlordiazepoxide, dicyclomine HCl, acetonitrile

INTRODUCTION

Chlordiazepoxide (7-chloro-*N*-methyl-5-phenyl-3*H*-1,4-benzodiazepin-2-amina-4-oxide) is a sedative-hypnotic drug widely used as a tranquilizer and antidepressant[1]. Clidinium bromide (3-[(hydroxydiphenylacetyl)-oxy]-1-methyl -1 -azoniabicyclo-[2.2.2] octane bromide) is used in the treatment of anxiety-related conditions including spastic colon[2]. Dicyclomine hydrchloride is chemically 2-diethylaminoethyl- bicyclohexyl-1-carboxylate hydrochloride and is employed as an antispasmodic agent[3]. Literature survey reveals that some analytical methods have been used for the estimation of chlordiazepoxide, clidinium bromide and dicyclomine hydrochloride individually or in combination with other drugs. Few methods for the determination of clidinium bromide and chlordiazepoxide in combined dosage form reportedly exist, including RP-HPLC[4-8] and derivative spectroscopy[9-10]. Chlordiazepoxide has been determined alone or with other compounds in pharmaceutical formulations using first-derivative spectrophotometry[11], High-Performance Liquid Chromatography[12-14], HPTLC [15-16],voltammetry[17] and stability indicating HPLC[18]. The method described here is simple, fast, precise and accurate for simultaneous determination of clidinium bromide, chlordiazepoxide, and dicyclomine hydrochloride from pharmaceutical preparation.

MATERIALS AND METHODS

Pure samples of clidinium bromide, chlordiazepoxide, and dicyclomine hydrochloride were obtained from CIPLA Ltd., Vikhroli West Mumbai, India. The tablet dosage form, CIBIS manufactured by Cadila Pharmaceuticals Limited Ahmedabad, Gujarat. (label claim: 2.5mg clidinium bromide, 5mg chlordiazepoxide and 10mg dicyclomine hydrochloride) was procured from the local market.

Experimental

To develop a suitable LC method for the analysis of clidinium bromide, chlordiazepoxide and dicyclomine hydrochloride in their combined dosage form, different mobile phases were tried. The criteria employed for selecting the mobile phase for the analyses of the drugs were cost involved, time required for the analysis and better separation of drugs. The chromatographic system consisted of pump (Shimadzu LC 10AT VP) with universal loop injector (Rheodyne 7725 i) of injection capacity 20 µL. Detector consisted of photodiode array detector (PDA) SPD-10 AVP UV-Visible detector and column used was Chromatopak C18 (25cm×4.6mm i.d.×5µm). The equipment was controlled by a PC work station equipped with software CLASS M 10-VP software (Shimadzu, Kyoto, Japan).

Preparation of standard stock solutions

The equivalents of 10 mg each of CLD, CDPZ and DCM were accurately weighed and place in 100 mL volumetric flasks separately and dissolved in 25 mL of water: acetonitrile in 1:1 ratio to prepare standard stock solutions. After the immediate dissolution, the volume was made up to the mark with solvent system. These standard stock solutions were observed to contain 100 µg. mL^{-1} of CLD, CDPZ and DCM.

Analysis of tablet dosage form

For the preparation of the stock solution of tablet dosage form, 20 tablets of CIBIS were taken, their average weight was determined and they were crushed to fine powder. Then, powder equivalent to 10mg was

taken in 100mL volumetric flask and dissolved in 30mL of solvent (acetonitrile and water in the ratio of 1:1) with vigorous shaking for 5-10 minutes. The supernatant liquid was transferred to 100mL of volumetric flask through a Whatman #41 filter paper. The residue was washed twice with solvent and the combined filtrate was made up to 100mL mark. After that, 10 mL of the above solution was diluted up to 100 mL with solvent. From the study it was found that best result was obtained in a quality separation in terms of peak symmetry, resolution, reasonable run time and other parameters by use of acetonitrile and 0.1 % triethyl amine in water pH 3.5 adjusted by 5 % o-phosphoric acid as mobile phase in the ratio of 30:70 (V/V) at a flow rate of 0.8 mL min^{-1}. Wavelength for detection was 210nm.

Validation

Selectivity of the method towards the drugs was established through study of resolution factor between the drug peaks. Under the proposed chromatographic conditions, all the three drugs were completely separated from each other with a resolution of 6.45 between CLD and CDPZ and 4.73 between CDPZ and DCM. It indicates that the method is suitable for simultaneous estimation. Specificity was assessed by comparing the chromatograms of tablet solution with the placebo solution and also with the chromatograms obtained from standard drugs.

For analysis of sample, six replicates of sample solutions were prepared and 20µL of each replicate was injected into the system and their chromatograms were recorded (Fig.1). From the chromatograms, it was observed that CLD, CDPZ and DCM eluted at 3.9 min, 5.4 and 6.8 min, respectively with a resolution of 6.45 between CLD and CDPZ and 4.73 between CDPZ and DCM. Accuracy was confirmed by doing recovery study as per ICH norms, where to a reanalyzed sample solution standard solutions of drugs were added equivalent to 80 %, 100 % and 120 % of its drug content. The percentages of drug recoveries for DCM in 80 %, 100 % and 120 % were 100.16, 100.23 and 100.29, for CDPZ 100.66, 100.52 and 101.06 and for CLD 101.08, 101.03 and 101.12 respectively. As all the statistical results were within the range of acceptance i.e. % COV< 2.0 and S.D. < 1.0, the method was considered to be accurate for simultaneous quantitative estimation of all the three drugs DCM, CDPZ and CLD. The results are reported in Table I. Intermediate precision study was carried out by doing intra-day and inter-day precision study. Here, six replicates of sample solutions were prepared from the stock solution. For intra-day precision study, concentrations of all the three drugs were calculated for six times on the same day at intervals of 1 h. In inter-day study the concentration of drug contents were calculated on three different days 1st, 2nd and 3rd day. LOD and LOQ values were calculated to check the detection limit and quantitation limit by injecting progressively low concentration of the standard solutions with the optimized chromatographic conditions. For linearity study, from the standard stock solution of CLD CDPZ and DCM different dilutions were prepared for each drug. Then, 20µL of these solutions were injected into the LC system with the help of Hamilton syringe. Then, the chromatograms were recorded at 210 nm. Calibration curve was plotted between the areas against their respective concentrations. From the calibration curve, it was clear that DCM has linearity range between 60–180 µg mL^{-1}, CDPZ in 20-100 µg. $mL^{-1,}$ and CLD in 10-50 µg. mL^{-1}. The system suitability was checked by injecting the standard solution and the results were found to be within the range. The retention times obtained

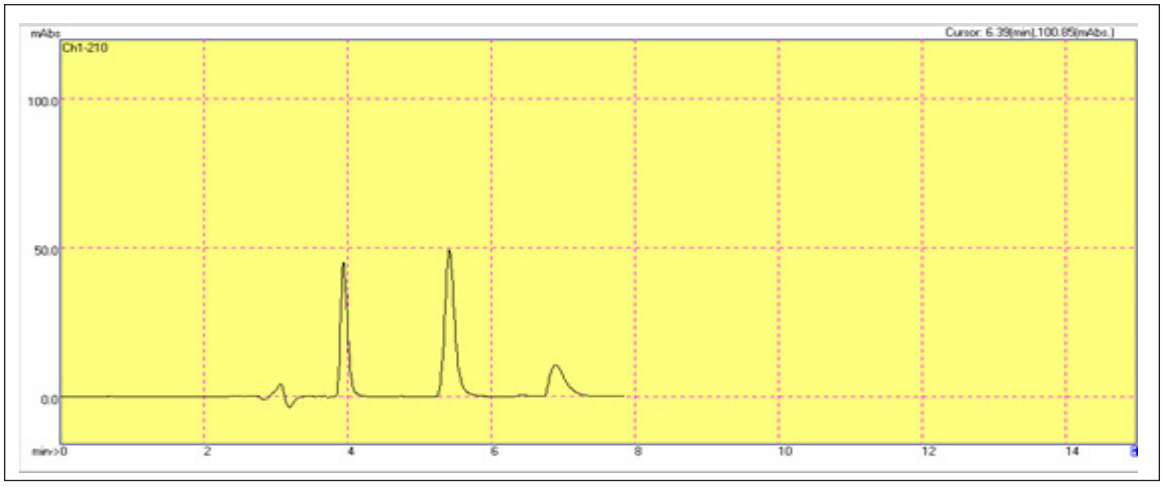

Fig.1: Chromatogram of tablet dosage form (CIBIS) at 210 nm showing the retention time for clidinium bromide (3.9 min), chlordiazepoxide (5.4 min) and dicyclomine hydrochloride (6.8 min)

Table I: Results of recovery studies

Replicate	Amount taken(μg/mL)			Amount added (μg/mL)				% Recovery		
	DCM	CDPZ	CLD	%	DCM	CDPZ	CLD	DCM	CDPZ	CLD
1	80	40	20	80 %	64	32	16	100.4	101.4	101.0
2	80	40	20		64	32	16	100.4	100.2	101.7
3	80	40	20		64	32	16	99.7	100.4	100.5
1	80	40	20	100 %	80	40	20	100.2	100.3	100.7
2	80	40	20		80	40	20	100.4	101.5	101.0
3	80	40	20		80	40	20	100.1	99.7	100.2
1	80	40	20	120 %	96	48	24	100.3	101.5	101.0
2	80	40	20		96	48	24	100.8	100.5	101.1
3	80	40	20		96	48	24	99.7	101.2	101.2

Table II: Result from system suitability study

Property	CLD	CDPZ	DCM
R^t	3.9	5.4	6.8
T_f	1.4	1.3	1.7
K'	0.3	0.8	1.28
N	6769	6693	6279
R_s	-	6.45	4.73

for CLD, CDPZ and DCM were 3.9, 5.4, and 6.8 min. respectively. The resolution is 6.45 between CLD and CDPZ and 4.73 between CDPZ and DCM. The results of capacity factor, tailing factor, theoretical plate number are reported in Table II.

RESULTS AND DISCUSSION

From the chromatogram, it was found that CLD, CDPZ and DCM eluted at retention time of 3.9, 5.4 and 6.8 min. respectively, with a resolution of 6.45 between CLD and CDPZ and 4.73 between CDPZ and DCM. The mean percentage of drug content in tablet dosage form was performed by doing replicate study. From the result, total contents of CLD, CDPZ and DCM were found to be 99.17 %, 99.94 % and 100.84 %, with a standard deviation of 0.880, 0.943 and 0.770, respectively. The percentage of drug recoveries for DCM in 80 %, 100 % and 120 % were 100.16, 100.23 and 100.29, for CDPZ 100.66, 100.52 and 101.06 and for CLD 101.08, 101.03 and 101.12, respectively. As all the statistical results were within the range of acceptance i.e. % COV< 2.0 and S.D. < 1.0, the method was considered to be accurate for simultaneous quantitative estimation of all the three drugs CLD, CDPZ and DCM. In intra-day study, the sample solutions were analyzed on the same day at an interval of 1 h for 3 h and the total drug content in it measured. From the results it was observed that DCM has drug content of 100.17 %, 99.48 % and 99.68 %, CDPZ 99.68 %, 99.54 % and 99.99 %, CLD 99.73 %, 99.16 % and 99.4 %, in first, second and third h respectively. In inter-day study, the sample solutions were analyzed on 1st, 2nd and 3rd day. From the results, it was found that DCM has drug content of 99.28 %, 99.23 % and 99.66 %, CDPZ 99.41 %, 98.95 % and 99.13 % and CLD 99.37 %, 99.66 % and 98.38 % on first, second and third day, respectively. Both intra and inter day accuracies were within acceptability criteria. For precision study, all the statistical results were within the range of acceptance i.e. % COV< 2.0 or COV ≤ 0.02 and S.D. < 1.0, the % COV was within acceptability criteria indicating that the method was precise for quantitative estimation of all three drugs.

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