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DETERMINATION OF CHLORDIAZEPOXIDE, ITS HYDROCHLORIDE AND RELATED IMPURITIES IN PHARMACEUTICAL FORMULATIONS BY REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

A quantitative high-performance liquid chromatographic method using an octadecylsilane column and a methanol–water mobile phase was employed for the determination of chlordiazepoxide, chlordiazepoxide · HCl and related impurities in capsule and tablet preparations. Each component is well separated and directly detected by 254 nm absorption. For chlordiazepoxide and chlordiazepoxide · HCl the coefficient of variation for replicated injections was below 1%. Recovery of authentic samples ranged from 98.4 to 101.6% for both capsule and tablet formulations.

INTRODUCTION

The determination of the sedative chlordiazepoxide [7-chloro-2-(methylamino)-5-phenyl-3H-1,4-diazepine 4-oxide] (CDE) and its hydrochloride salt in pharmaceutical formulations has received considerable attention in recent years. The current pharmaceutical specifications[1] include limits tests for two related compounds, 7-chloro-1,3-dihydro-5-phenyl-2H-1,4-benzodiazepin-2-one 4-oxide (CBO) and 2-amino-5-chlorobenzophenone (ACB). The structure of each compound is shown in Fig. 1.

The USP specifications[1] set limits of 0.1% and 0.01% for CBO and ACB, respectively in both CDE and chlordiazepoxide hydrochloride (CDE · HCl) bulk powder. In CDE tablets the limits for CBO and ACB are 4% and 0.1%, respectively, while in CDE · HCl capsules the limits for CBO and ACB are 3% and 0.1%, respectively.

The intent of the present study was to develop and validate a single analysis procedure which could be used to assay both the bulk powder and dosage preparations, and perform the limit tests on the related impurities. The current USP XX procedures[1] employ non-aqueous titrations to assay the bulk powders, ultraviolet

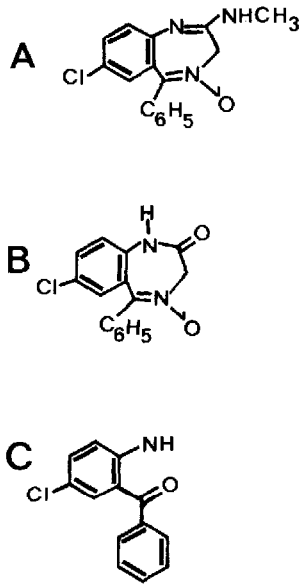

Fig. 1. Structure of the chemical substances. (A) CDE, Chlordiazepoxide; (B) CBO, 7-chloro-1,3-dihydro-5-phenyl-2*H*-1,4-benzodiazepin-2-one 4 oxide; (C) ACB, 2-amino-5-chlorobenzophenone.

(UV) absorption to assay the dosage forms, and thin-layer chromatography (TLC) to test for the related impurities.

The UV method lacks sufficient specificity, while the TLC procedure is unreliable and lacks in quantitative reproducibility and sensitivity. Separation by gas chromatography has been applied to these compounds[2], but this was found to be unacceptable for various reasons. Most liquid chromatographic methods either focused on separating mixtures of drugs rather than impurities[3], employed two mobile phases[4], or were applicable to only one of the two impurities[5]. One previous study[6] employed reversed-phase high-performance liquid chromatographic (HPLC) to separate the impurities from the drug, but the precision for replicate analysis was unacceptably low.

This paper describes the separation and quantitation of CDE, ACB and CBO using a single, simple isocratic mobile phase, and a standard octadecylsilane reversed-phase HPLC column.

EXPERIMENTAL

Materials

The water used was deionized-distilled, suitable for HPLC. The methanol (J. T. Baker, Phillipsburg, NJ, U.S.A.) was HPLC-grade. The drug working standard bulk powders and formulations were from commercial sources. The purities of the drug standards were determined by USP methods and were found to be 100.2 and 100.0% for CDE · HCl and CDE, respectively. The related compounds ACB and CBO were obtained from the USP and assumed to be 100.0% purity. The commercial tablets and capsules were declared to contain 5, 10 and 25 mg of CDE (tablets) or

CDE · HCl (capsules). Authentic samples were also prepared simulating several of the commercial preparations and were used to validate the procedures.

Instrumentation

The HPLC system consisted of a dual-piston, positive-displacement pump (Model M6000, Waters Assoc., Milford, MA, U.S.A.) an automatic injector (WISP, Waters Assoc.) an UV absorption detector operated at 254 nm (Model 440, Waters Assoc.) and a 10-mV recorder (Omni-Scribe B-5000, Houston Instrument, Austin, TX, U.S.A.). The HPLC column was a commercially packed 30 cm × 3.9 mm I.D. chemically bonded octadecylsilane reversed-phase material (μBondapak C_{18}, 10 μm; Waters Assoc.). The mobile phase was filtered through a 0.45-μm polymeric membrane filter (Nylon-66, Rainin, Woburn, MA, U.S.A.).

Standard preparations

Accurately weigh approximately 2 mg of CDE or CDE · HCl and transfer to a 10-ml volumetric flask. Dilute to volume with mobile phase and mix. This solution must be prepared fresh daily.

Transfer about 2 mg of CBO, accurately weighed, to a 100-ml volumetric flask, add 50 ml of mobile phase, sonicate for 5 min, if necessary, until solution is complete, dilute to volume with mobile phase, and mix. Pipet 10 ml of this solution into a 100-ml volumetric flask, dilute to volume with mobile phase and mix.

Transfer about 2 mg of ACB, accurately weighed, to a 100-ml volumetric flask, add 50 ml of mobile phase solvent, sonicate for 5 min, if necessary, until solution is complete, dilute to volume with mobile phase and mix. Pipet 1 ml of this solution into a 100-ml volumetric flask, dilute to volume with mobile phase, and mix.

Sample preparations

Prepare composite samples by grinding 20 tablets to a fine powder, or by combining the contents of 20 capsules, and pass through a 60-mesh sieve. Weigh a portion of the composite equivalent to 5 mg, transfer to a 25-ml volumetric flask and dilute to volume with mobile phase. Sonicate this mixture for 5 min, mix and then filter through a 0.45 μm membrane filter.

Mobile phase

Prepare the mobile phase by mixing 650 ml methanol with 350 ml water. Filter and vacuum degas.

Procedure

The HPLC flow-rate is maintained at 1 ml/min with the mobile phase at ambient temperature. Inject a 5-μl volume of the standard and sample solutions and adjust the sensitivity so that the CDE · HCl peak response is about 0.5 full scale (approx. 0.5 a.u.f.s.). To assay the related impurities, increase the sensitivity to approx. 0.02 a.u.f.s., and separately inject 5 μl of the ACB standard and sample solution and 50 μl of the CBO standard and sample solution. The CBO standard peak response should be about 0.5 full scale and the ACB should be about 0.05 full scale.

The precision of the system was determined by replicate injections of the major component CDE · HCl and the coefficient of variation (C.V.) of peak height was

TABLE I

RETENTION DATA FOR THREE SUBSTANCES

Compound	*Retention time (min)*	*Capacity factor, k'*
CBO	5.5	1.7
CDE	9.2	2.9
ACB	16.4	5.1

calculated. Recovery was determined by preparing authentic mixtures with a known amount of standard and taking the mixtures through the analysis method.

RESULTS AND DISCUSSION

Chromatographic performance

Results of the direct quantitation of CDE in tablets and the hydrochloride in capsules are reported. Retention information for CDE and the two known impurities studied is shown in Table I. Each peak is observed to be sharp and symmetrical (Fig. 2). A suitably high resolution column, exhibiting 12,000 plates per meter is needed to resolve the ACB peak at trace levels from the tail of the CDE peak (Fig. 3). The chromatographic conditions selected represent a compromise which facilitates the determination of both the major drug component and the trace impurities using the same HPLC separation. A stronger mobile phase could be used for faster analysis of CDE, but increased resolution was needed to separate the ACB peak from the much higher levels of CDE.

Quantitation of CDE · HCl

The linearity of this method for the determination of CDE and CDE · HCl was evaluated by triplicate injection of standard solutions of CDE or CDE · HCl over the range of 0.4 to 1.6 μg/injection (Fig. 4). A correlation coefficient of 0.9993 was observed for both compounds. The precision of the method was measured by ten replicate injections of a standard solution. The coefficient of variation (relative standard deviation) was found to be 0.85% for CDE · HCl and 0.53% for CDE.

The accuracy of the method was demonstrated by analyzing synthetic mixtures of CDE and CDE · HCl simulating capsule formulations. For each mixture five assays were performed with two injections per assay. Six synthetic mixtures were prepared. Each mixture was assayed by both the USP titrimetric method and the HPLC method. The results obtained (Table II) demonstrate a high degree of correlation between the two procedures.

Analysis of commercial CDE · HCl and CDE bulk substances

Six samples of CDE · HCl and one sample of CDE bulk substance from six manufacturers were analyzed by both the USP titrimetric method and the HPLC procedure. The results obtained (Table III) clearly indicate the quantitative acceptability of the HPLC analysis.

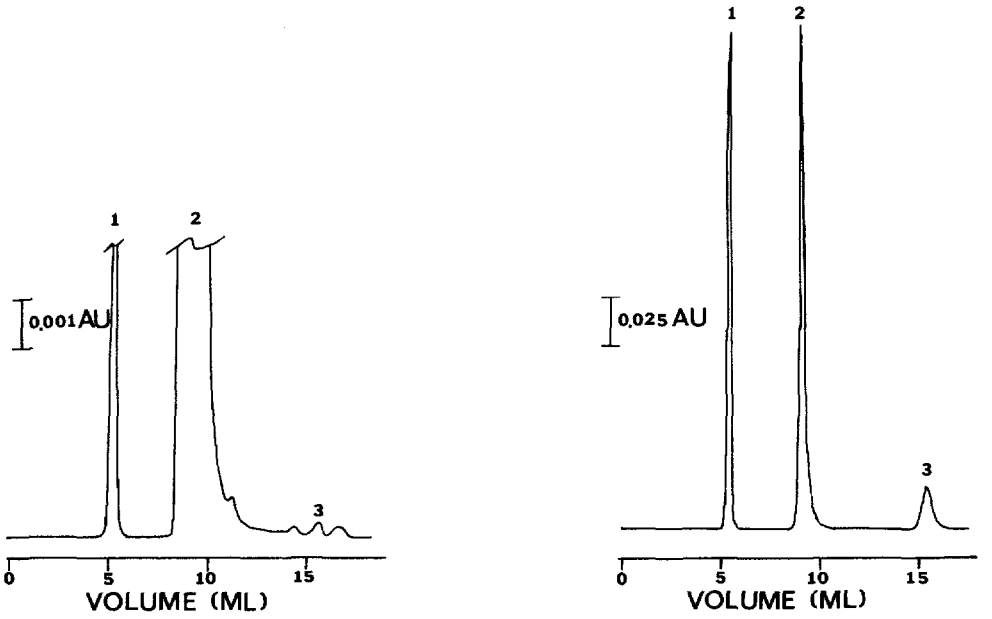

Fig. 2. Chromatogram of the standard materials. Peaks: 1 = CBO, 2 = CDE, 3 = ACB.

Fig. 3. Determination of ACB in a sample prepared from 5-mg CDE tablets. Peaks: 1 = CBO, 2 = CDE, 3 = ACB.

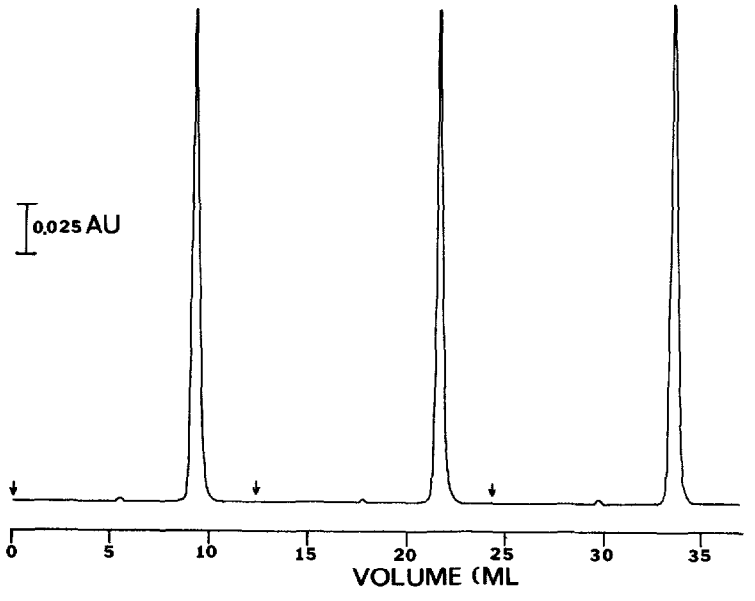

Fig. 4. Triplicate injections of a sample from a composite of 5-mg CDE tablets.

TABLE II

ANALYSIS OF SYNTHETIC MIXTURES OF CDE SIMULATING CAPSULE AND TABLET FORMULATIONS

Five assays per sample, two injections per assay.

Sample	*Type*	*Assay by titration (C.V.)*	*Assay by HPLC (C.V.)*
1	capsule	100.7 (1.08)	100.5 (0.22)
2	capsule	100.3 (0.43)	99.0 (0.50)
3	capsule	100.7 (0.44)	100.1 (1.21)
4	capsule	100.0 (0.47)	99.6 (0.36)
5	capsule	101.4 (0.58)	100.1 (0.50)

Determination of CDE · HCl in commercial capsules and tablets

Six capsule or tablet samples from six manufacturers were analyzed by both USP titrimetric and HPLC (Table IV). Again excellent agreement between the two methods is observed.

TABLE III

ANALYSIS OF COMMERCIAL CDE BULK SUBSTANCES

Source	*Sample*	*Purity (%)*	
		HPLC Method	*USP Method*
1	CDE · HCl	98.8	99.4
2	CDE · HCl	99.9	99.6
3	CDE · HCl	99.3	99.4
4	CDE · HCl	99.3	99.4
5	CDE · HCl	99.3	99.7
6	CDE · HCl	100.4	99.5
7	CDE	99.3	99.5

TABLE IV

ANALYSIS OF COMMERCIAL CDE CAPSULES AND TABLETS

Source	*Type*	*Assay by USP titration*	*Assay by HPLC (C.V.)*
1	5-mg capsule	103.4	101.8 (1.03)
2	5-mg capsule	99.6	98.8 (1.70)
3	5-mg capsule	105.6	105.9 (1.54)
4	5-mg capsule	99.8	100.1 (1.04)
5	10-mg capsule	103.3	103.1 (0.86)
6	5-mg tablets	102.8	101.9 (0.78)

Quantitation of the related impurities

Quantitative analysis of the two related impurities present at the compendial limit[1] was studied. Averaged triplicate injections of CBO standards over the range of 6 to 42 ng/injection produced a correlation coefficient of 0.9998. A typical chromatogram for the determination of CBO is shown in (Fig. 5). The CBO content of these tablets is observed to be 0.6%. A CDE sample fortified to contain CBO at the USP impurity limit of 3% is shown in (Fig. 6). Triplicate injections of ACB standards in the range of 2–14 ng produced a correlation coefficient of 0.9978. A chromatogram for the determination of ACB in a 5-mg CDE tablet is shown in Fig. 3. The ACB level is calculated to be 0.03%. The chromatogram for a CDE sample fortified to contain ACB at the USP limit of 0.1% is shown in (Fig. 7).

The reproducibility for quantitating each impurity was determined by studying ten replicate injections. For a 30-ng per injection CBO standard a C.V. of 0.59% was obtained. For a 10-ng per injection ACB standard a C.V. of 0.72% was achieved.

Since it was not possible to prepare reliable authentic mixtures of the impurities in CDE at the compendial limit, the quantitative recovery of this method was evaluated by standard addition analysis. Two injections were made for each impurity. For

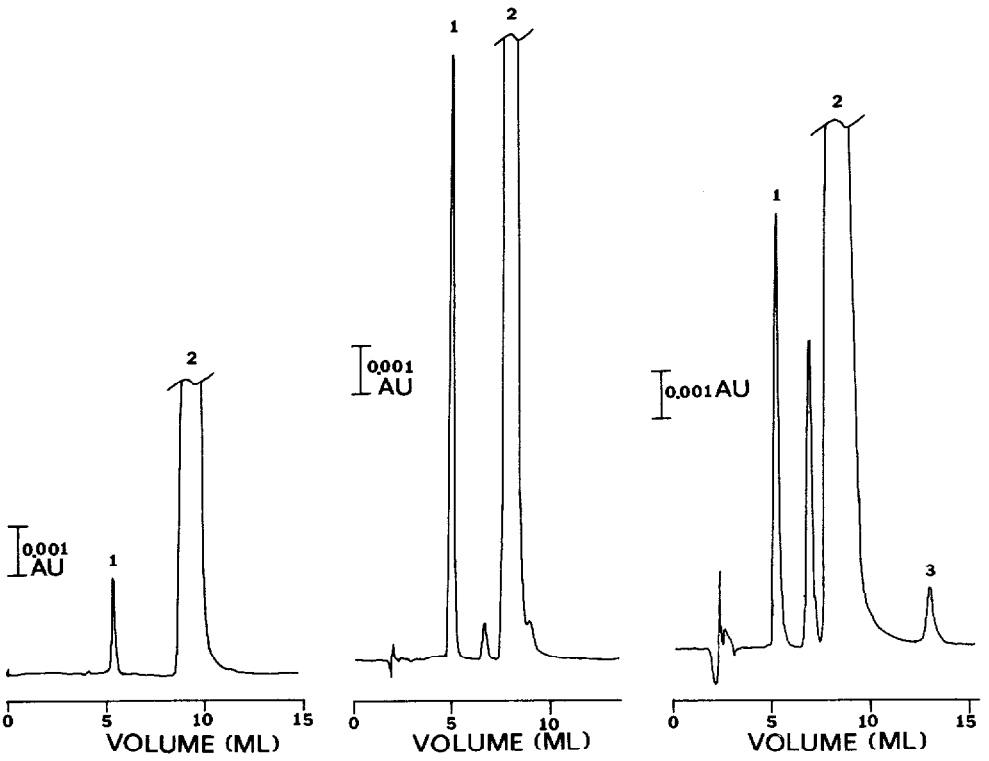

Fig. 5. Determination of CBO in sample prepared from 5-mg CDE tablets. Peaks: 1 = CBO, 2 = CDE.

Fig. 6. Chromatogram for a CDE sample fortified with 3% CBO. Peaks: 1 = CBO, 2 = CDE.

Fig. 7. Chromatogram for a CDE sample fortified with 0.1% ACB. Peaks: 1 = CBO, 2 = CDE, 3 = ACB.

TABLE V

DETERMINATION OF THE RELATED IMPURITIES IN A VARIETY OF COMMERCIAL PREPARATIONS

Source	*Sample type*	*CBO (%)*		*ACB (%)*	
		HPLC	*USP*	*HPLC*	*USP*
1	5 mg CDE · HCl capsule	0.32	<3	none	none
1	5 mg CDE · HCl bulk	0.13	none	none	<0.01
1	5 mg CDE · HCl bulk	0.16			
2	5 mg CDE · HCl capsule	1.45	<3	none	none
2	5 mg CDE · HCl bulk	0.15	none	none	<0.01
2		0.17			
3	5 mg CDE · HCl capsule	0.83	<3	none	<0.01
3	5 mg CDE · HCl bulk	0.20	none	none	<0.01
5	5 mg CDE · HCl bulk	0.23			
4	5 mg CDE · HCl capsule	0.37	<3	none	none
4	5 mg CDE · HCl bulk	0.14	none	none	<0.01
4	5 mg CDE · HCl bulk	0.17			
5	10 mg CDE · HCl capsule	0.22	<3	none	none
5	10 mg CDE · HCl bulk	0.18	none	0.003	<0.01
5	10 mg CDE · HCl bulk	0.23			
6	5 mg CDE tablet	0.60	<4	0.034	none
6	CDE · HCl Sterile Inj.	0.23	none	0.013	none
		0.24		0.015	
		0.26		0.013	
		0.20			
6	CDE · HCl bulk	0.18	none	none	<0.01
6	CDE · HCl bulk	0.22			
6	CDE bulk	0.02	none	none	none

a CDE sample, the recovery of CBO and ACB were seen to be 97.2% and 97.4%, respectively. For a CDE · HCl sample, the recovery observed for CBO and ACB were 98.2% and 99.8%, respectively.

Pharmaceutical preparations from six manufacturers were analyzed for the two impurities by both the HPLC and USP TLC methods. The results are reported in Table V. The results of the two methods are in agreement, however, the TLC procedure is only used as a limits test. The HPLC method can be used for quantitative analysis.

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