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HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC SEPARATION AND DETERMINATION OF CHLORDIAZEPOXIDE HYDROCHLORIDE AND TWO OF ITS DECOMPOSITION PRODUCTS

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SUMMARY

Chlordiazepoxide hydrochloride and two of its decomposition products, 7-chloro-1,3-dihydro-5-phenyl-2H-1,4-benzodiazepin-2-one-4-oxide (I) and 2-amino-5-chlorobenzophenone (II) were analyzed through high performance liquid chromatography in a commercial drug substance, tablet-mass and dragees. The analysis was performed on an adsorption and a reversed-phase column under isocratic conditions with a single mobile phase in each case. Three preparations analyzed contained these decomposition products from undetectable amounts to 2.14%.

INTRODUCTION

Chlordiazepoxide (7-2-(methylamino)-5-phenyl-3H-1,4-benzodiazepine-4-oxide) and its hydrochloride is an extensively used tranquilizer in therapy. The two decomposition products, 7-chloro-1,3-dihydro-5-phenyl-2H-1,4-benzodiazepin-2-one-4-oxide (I) and 2-amino-5-chlorobenzophenone (II) are generally found as impurities in chlordiazepoxide. USP XIX, NF XIV and BP 73 specifications allow a maximum of 0.1% for I and in the range of 0.10–0.05% for II in the chlordiazepoxide drug substance. The limit of these impurities is increased in formulations such as capsules to 3% for I and 0.1% for II (USP XIX).

HPLC has recently been used for the separation of the two related compounds I and II from the main drug on a reversed phase column with aqueous mobile phase and on an adsorption column with two different organic mobile phases (Butterfield et al., 1977; Zagar et al., 1978). In the present work, separation and quantitation of the two impurities, I and II, from chlordiazepoxide hydrochoride was carried out in silica gel adsorption and chemically bonded reversed-phase columns with organic and aqueous single mobile phases under isocratic conditions. The compounds were determined by

chromatographing samples and appropriately diluted standards at different detector sensitivities for chlordiazepoxide hydrochloride and the compounds I and II. The estimation was carried out in a pure drug sample, in tablet-mass and in a pharmaceutical formulation (dragees).

MATERIALS AND METHODS

Apparatus and reagents

Perkin-Elmer liquid chromatograph 1220 and serie 2, each with a variable wavelength detector LC 55 with a cell volume of 8 µl, connected with separate recorders (Perkin-Elmer 123) were used. A Hewlett-Packard laboratory data system 3352 C was connected to both liquid chromatographs through A/D convertors; the chromatographs were operated at ambient temperature. The detector wavelength in both cases was adjusted at 260 nm.

Analytical columns and mobile phases

Adsorption chromatography: silica A columns (Perkin-Elmer, Norfolk, Conn.), stainless steel tube, 25 cm × 2.6 mm i.d., packed with silica gel; particle size: 13 µm; mobile phase: cyclohexane + methanol + chloroform + glacial acetic acid (60 + 5 + 35 + 0.5); flow rate: 0.5 ml/min.

Reversed-phase chromatography: Perkin-Elmer reversed-phase column, 25 cm × 2.6 mm i.d., packed with silica gel SI 100, 10 µm, coated with octadecylsilane chemically bonded organic phase; mobile phase acetonitrile + water + potassium bromide (65 ml + 35 ml + 100 mg); acetonitrile was redistilled; flow-rate: 1 ml/min.

Standards

Chlordiazepoxide hydrochloride and the compounds I and II were obtained as reference standards from British Pharmacopoeia Commission Laboratory.

Standard solutions: fresh solutions of chlordiazepoxide hydrochloride and the compounds I and II were prepared in methanol. 25 mg of chlordiazepoxide hydrochloride and 2–3 mg of compounds I and II were each weighed accurately, dissolved in methanol and diluted further to obtain solutions of 100 µg/ml chlordiazepoxide hydrochloride and 25 µg/ml each of compounds I and II. A total of 5 µl were injected, 4–5 times each on both columns into the liquid chromatographs and the integrated peak areas obtained from the computer were used for further calculations.

Chlordiazepoxide hydrochloride commercial drug substance

100 mg substance were weighed accurately and dissolved in 10 ml methanol. The determination of the compounds I and II was performed by injecting 10 µl of this solution directly into the liquid chromatograph. The estimation of chlordiazepoxide hydrochloride itself was done from a diluted solution containing 100 µg/ml.

Chlordiazepoxide hydrochloride tablet-mass and dragees

An amount of tablet mass or dragees containing 100–200 mg of chlordiazepoxide hydrochloride was accurately weighed, extracted with 5–10 ml methanol in a volumetric

flask in ultrasonic bath for 10 min, centrifuged and the supernatant liquid was injected into the liquid chromatograph directly for the determination of the decomposition compounds I and II. Chlordiazepoxide hydrochloride was then evaluated from a diluted solution containing 100 μg/ml.

RESULTS AND DISCUSSION

It was could be demonstrated that the separation of the two decomposition products, I and II, from chlordiazepoxide hydrochloride could be accomplished on an adsorption and a reversed-phased column under isocratic conditions using only one mobile phase in each case (Figs. 1 and 2). The k′-values for chlordiazepoxide hydrochloride and the compounds I and II on an adsorption column are 1.28, 2.53 and 0.44, respectively, whereas on a C_{18}-reversed-phase column, 0.58, 0.06 and 1.42 were obtained as k′-values for the 3 compounds. The composition of the mobile phase was carefully adjusted after several trials with varying amounts of chloroform, cyclohexane, ethyl acetate, methanol and acetic acid. The mobile phase thus chosen for this work on an adsorption column brings about an optimum separation of all components in a reasonable time. The detection wavelength of 260 nm was chosen, as all 3 substances have an absorption maximum near this wavelength.

The reversed-phase column brought about the separation of all 3 components only after the addition of potassium bromide to the mobile phase. Neither could the major component, chlordiazepoxide hydrochloride, be eluted nor the two decomposition products, I and II, be separated with only mixtures of acetonitrile–water of varying compositions. The separation mechanism could here be attributed to an ion-pair-mechanism where potassium bromide functions as an ion-pair reagent. It is interesting to note that the same effect could be obtained by replacing potassium bromide with other salts such as ammonium chloride, sodium potassium tartarate and ammonium nitrate. The addition of an organic ion-pair reagent such as tetradecyl trimethyl ammonium bromide also separates all 3 compounds but the peaks are broadly shaped and not well formed.

It is interesting to note that the order of elution of decomposition products I and II is exactly reversed on an adsorption column in comparison to a reversed-phase column. 7-Chloro-1,3-dihydro-5-phenyl-2H-1,4-benzodiazepin-2-one-4-oxide (I) is eluted before chlordiazepoxide hydrochloride on a reversed-phase column and after it on the adsorption column. 2-Amino-5-chlorobenzophenone (II) behaves exactly in the opposite manner (Figs. 1 and 2). Four concentrations of chlordiazepoxide hydrochloride, 20, 50, 100 and 200 μg/ml, injected into the liquid chromatograph (5 μl), showed a linear relationship between concentration and peak area. Linearity was also found for the compounds I and II in the applied concentration range of 10–100 μg/ml. The detection limit for all 3 compounds lies at about 1 μg/ml for an injected volume of 5 μl. The sensitivity could be further enhanced by increasing the injection volumina. The relative standard deviation for chlordiazepoxide hydrochloride (100 μg/ml; n = 7) as an external standard was found to be ±1.8%. The decomposition products I and II showed a relative standard deviation (10 μg/ml; n = 5) of ±4.1%.

A commercial drug substance analyzed, chlordiazepoxide hydrochloride, contained 0.1% I and compound II was not detected. Tablet-mass and a dragee preparation analyzed

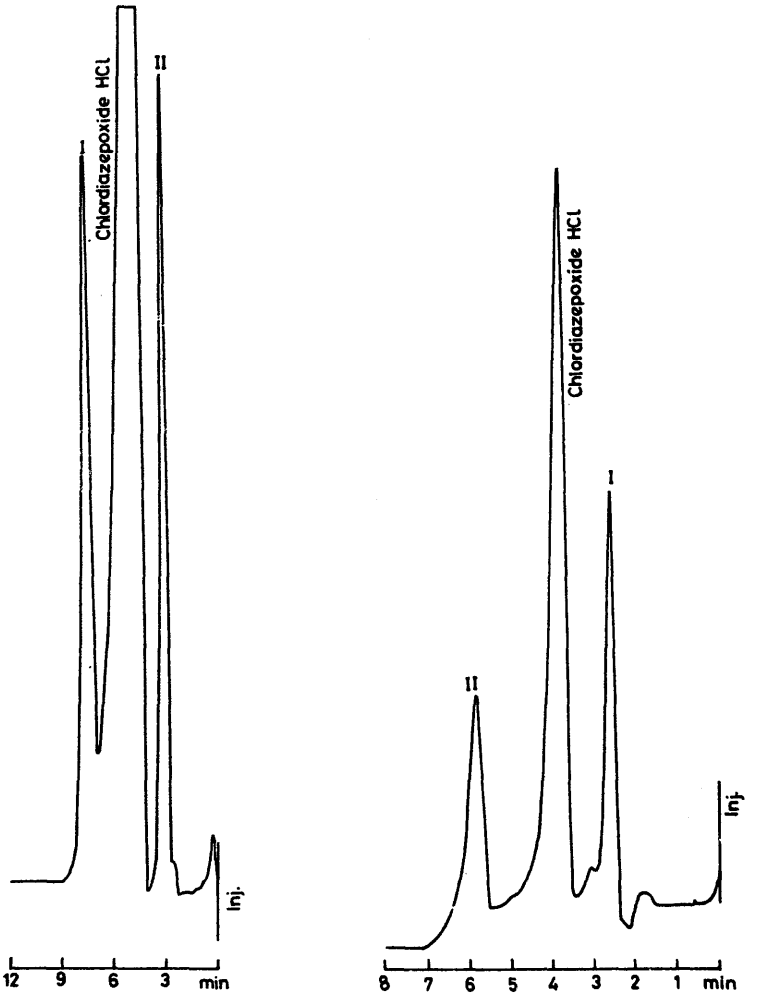

Fig. 1. Separation of chlordiazepoxide hydrochloride and the decomposition products I and II on a Perkin-Elmer silica A adsorption column; mobile phase: cyclohexane + methanol + chloroform + glacial acetic acid (60 + 5 + 35 + 0.5).

Fig. 2. Separation of chlordiazepoxide hydrochloride and the decomposition products I and II on a Perkin-Elmer reversed-phase C_{18}-column; mobile phase: acetonitrile water + potassium bromide (65 ml + 35 ml + 100 mg).

showed compound I to be 1.15 and 2.14% and compound II to be 0.05 and 0.1%, respectively.

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