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DETERMINATION OF N-DESMETHYLDIAZEPAM IN PLASMA BY GAS CHROMATOGRAPHY WITH AN INTERNAL STANDARD

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SUMMARY

N-Desmethyldiazepam in plasma has been determined by gas chromatography with electron capture detection, using N-desmethyltetrazepam as internal standard and a column filled with Poly-A 103 on Gas-Chrom Q. No prior transformation of benzodiazepines is necessary. The sensitivity is *ca.* 1 ng/ml and the recovery is $\geqslant 70\%$. The method is particularly suitable for the pharmacokinetic study of dipotassium clorazepate: the amount of unchanged clorazepate may be estimated from the difference between the amount of N-desmethyldiazepam present initially and the total amount of N-desmethyldiazepam obtained after acidic treatment of the sample; the method is also useful for therapeutic control and for analytical toxicology.

INTRODUCTION

N-Desmethyldiazepam (DDZ) is a metabolite of several benzodiazepines, such as diazepam[1,2] or dipotassium clorazepate (PC)[3–5], or even prazepam[6] or demoxepam[7]. The usual methods for its determination in biological fluids include an intermediate stage of acid hydrolysis to 2-amino-5-chlorobenzophenone (ACB) (Fig. 1); the ACB is then determined either by colorimetry after diazotization[3] or by gas–liquid chromatography (GLC)[8–11]. The GLC technique gives good sensitivity, especially if electron capture detection is used, but it is time consuming and needs extremely pure reagents and perfectly clean glassware. Moreover, the appropriate temperature and acidity conditions for hydrolysis must be strictly maintained. DDZ can also be determined directly in plasma (without hydrolysis), by gas chromatography and electron-capture detection, but the methods proposed seem to lack sensitivity[11]. Nevertheless, in cerebrospinal fluid, Hendel[12] obtained a sensitivity of 1 ng/ml of

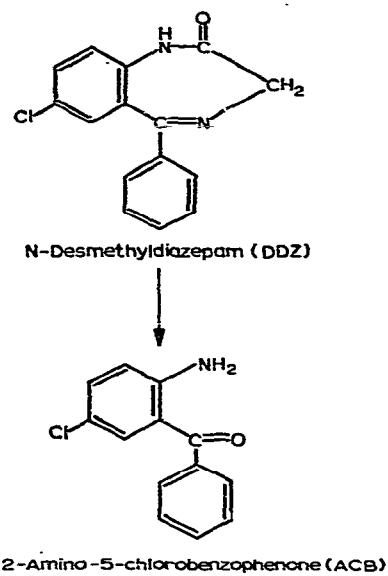

Fig. 1. 2-Amino-5-chlorobenzophenone (ACB), the hydrolysis product of N-desmethyldiazepam (DDZ).

analyzed sample: griseofulvin is used as internal standard; it is added to the extraction residue just before injection into the chromatograph.

On the basis of our previous research[13–16] concerned especially with the GC determination of bromazepam, clonazepam, flunitrazepam and flurazepam, we could have determined DDZ by using the procedure recommended by De Silva[10] and modified by Hoffman and Chon[9], *i.e.*, after hydrolysis to ACB. We preferred, however, as in the case of flurazepam, to develop a technique for determining DDZ without altering its structure. Thus we retained N-desmethyltetrazepam (DTZ) as internal standard and used a ^{63}Ni ECD for the terminal analysis. The main stages of the experimental procedure are given in Fig. 2.

EXPERIMENTAL

Reagents and standards

4 *N* Hydrochloric acid was obtained by dilution of concentrated HCl ("suprapur"). 4 *N* Sodium hydroxide was prepared from very pure sodium hydroxide pellets ("pro analysi"). Buffer (pH 9): sodium hydroxide–potassium chloride–boric acid. Bromothymol blue, 0.1% solution in 50% ethanol. Solvents purified by S.D.S. (Peypin, France) were diethyl ether, hexane and acetone (all "pestipur" grade).

Standard solutions of N-desmethyldiazepam and of N-desmethyltetrazepam were prepared as follows. Approximately 20 mg of each compound was weighed exactly and dissolved in 10 ml of absolute ethanol; the volume was made up to 20 ml with hexane–acetone (4:1). Three successive ten-fold dilutions with the same solvent led to a solution containing 1 μg/ml, *i.e.*, 1 ng/μl.

All the reagents must be extremely pure. Each new reagent must be tested. The

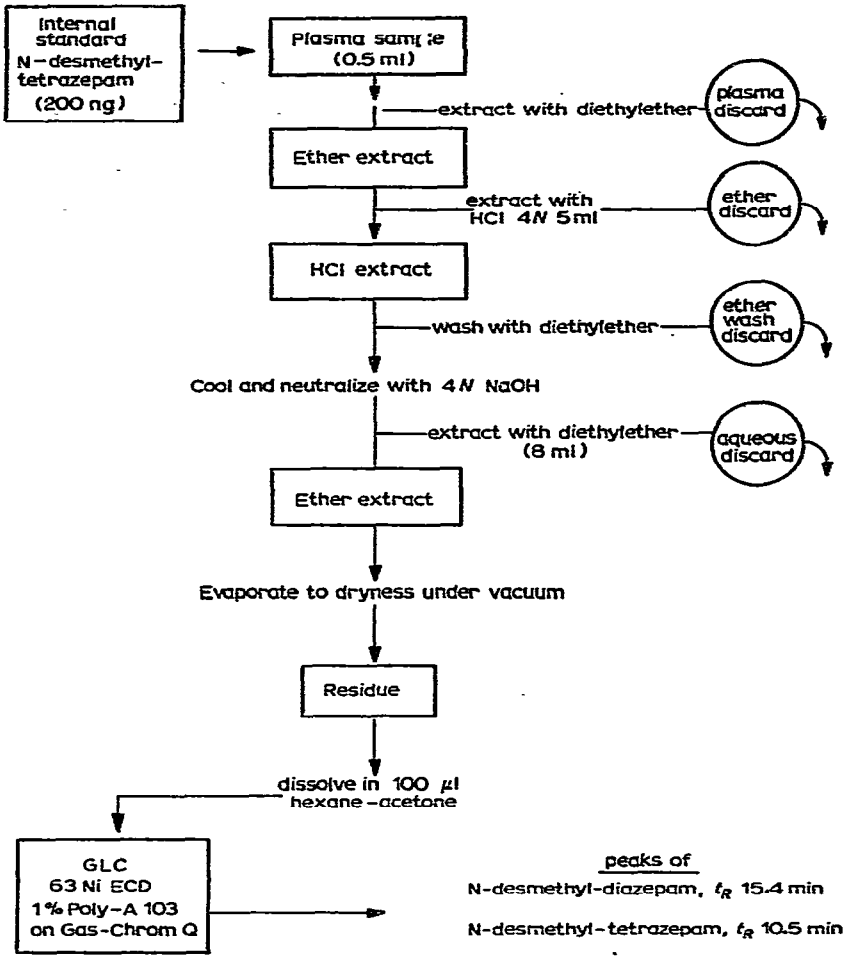

Fig. 2. Analysis scheme for N-desmethyldiazepam in plasma.

hydrochloric acid solutions were washed with diethyl ether; they were prepared each day, as were the sodium hydroxide solutions. The aqueous solutions were made from twice distilled water. The glassware must be perfectly clean, washed with R.B.S. 25 (T.C.S., Lambersart, France), placed in 10% hydrochloric acid and then in sulphuric acid–chromic acid, rinsed with twice distilled water and dried for 24 h in an oven at 70°.

Samples

The blood samples were taken with potassium oxalate as anticoagulant and immediately stored at 4°. Plasmas, separated as soon as possible, were treated at once or frozen at −25° until analyzed.

Procedure

The plasma, thawed rapidly if necessary, was shaken in a Vortex mixer for 30 sec. Two hundred µl of the 1 ng/µl DTZ solution were placed in a 30-ml cylindro-