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Note

Determination of meprobamate as an *n*-butylboronate ester derivative in serum by gas—liquid chromatography

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Meprobamate, 2-methyl-2-propyl-1,3-propanediol dicarbamate, has been used for many years as a tranquillizer and a muscle relaxant. Previously described methods for the analysis of meprobamate in biological material are based on colorimetry [1, 2] and gas—liquid chromatography (GLC) [3, 4]. Colorimetric methods lack both specificity and sensitivity for measurements of therapeutic concentrations. The GLC methods available involve either direct injection of the sample with the problem of thermal decomposition, or a silylation step with limited reproducibility.

To improve the chromatographic properties of such polar bifunctional compounds as 1,2- and 1,3-diols, the formation of cyclic esters with boronic acids has been recommended and used for the separation of diols from organic natural products [5, 6] and for the quantitation of ethylene glycol in blood [7]. We have applied this reaction in the determination of serum concentrations of meprobamate. The procedure is based on alkaline hydrolysis of meprobamate to the corresponding propanediol, followed by esterification by *n*-butylboronic acid to form 2-methyl-2-propyl-1,3-propanediol *n*-butylboronate.

EXPERIMENTAL

Materials

Meprobamate was of pharmaceutical purity. The internal standard (2-methyl-2-ethyl-1,3-propanediol) was obtained from K & K Labs. (Plainview, NY, U.S.A.), and *n*-butylboronic acid from Fluka (Buchs, Switzerland). All

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other chemicals used were of analytical-reagent grade and supplied by E. Merck (Darmstadt, F.R.G.).

Apparatus

A Varian 3700 gas chromatograph equipped with a flame ionization detector was used. The glass column (180 × 0.2 cm I.D.) was packed with 10% OV-17 on Chromosorb W HP, 100—120 mesh. The oven temperature was set at 140°C, with the injector operating at 190°C and the detector at 210°C. Nitrogen (25 ml/min) was used as carrier gas.

Methods

To evaluate the reaction time, two 1-ml serum samples, containing 25 and 250 μmol/l meprobamate, respectively, were each mixed with 1 ml of 12.5 *M* sodium hydroxide. The two serum samples were hydrolysed and extracted as described below. At different reaction times (5—60 min) aliquots of 1 μl were injected onto the chromatograph.

Determination of meprobamate in serum was carried out as follows. A 1-ml serum sample or standard sample (25—250 μmol/l) was hydrolysed by addition of 1 ml of 12.5 *M* sodium hydroxide and kept at 100°C for 10 min. To this mixture were added 5 ml of chloroform containing the internal standard (24 μmol/l). The tube was shaken for 10 min, and after centrifugation at 2500 *g* for another 10 min 4 ml of the organic phase were transferred to a clean test-tube, dried over anhydrous sodium sulphate, and evaporated to dryness under nitrogen. The residue was suspended in 25 μl of 50 m*M* *n*-butylboronic acid in dimethylformamide, and 1 μl of the organic phase was injected onto the chromatograph.

RESULTS AND DISCUSSION

In our investigation we used the quantitative hydrolysis and extraction procedures of Martis and Levy [4], and our results were in agreement with theirs. Esterification of the meprobamate hydrolysis product and the internal standard was completed in 15 min at room temperature. Both boronate ester derivatives were stable for more than 24 h under the reaction conditions used. As internal standard 2-methyl-2-ethyl-1,3-propanediol was selected owing to its structural similarity to meprobamate. Typical gas chromatograms of a serum sample, a blank serum and a serum sample with addition of meprobamate are shown in Fig. 1.

For the determination of concentrations in serum, 1 μl of the reaction mixture was injected directly into the chromatograph without further purification or concentration. A linear standard graph between peak height ratios and meprobamate concentration was obtained ($r = 0.998$) for serum concentrations ranging from 25 to 250 μmol/l. The precision of the method was tested at two concentrations, 25 and 200 μmol/l. Mean values of 26.4 ± 0.9 ($n = 10$) and 204.6 ± 8.2 ($n = 10$) were found, corresponding to a coefficient of variation of 3.6% and 4.0%, respectively. The mean recovery of meprobamate after addition of 25 and 200 μmol/l of pure drug to human serum was found to be 95% and 94%, respectively, in comparison with the

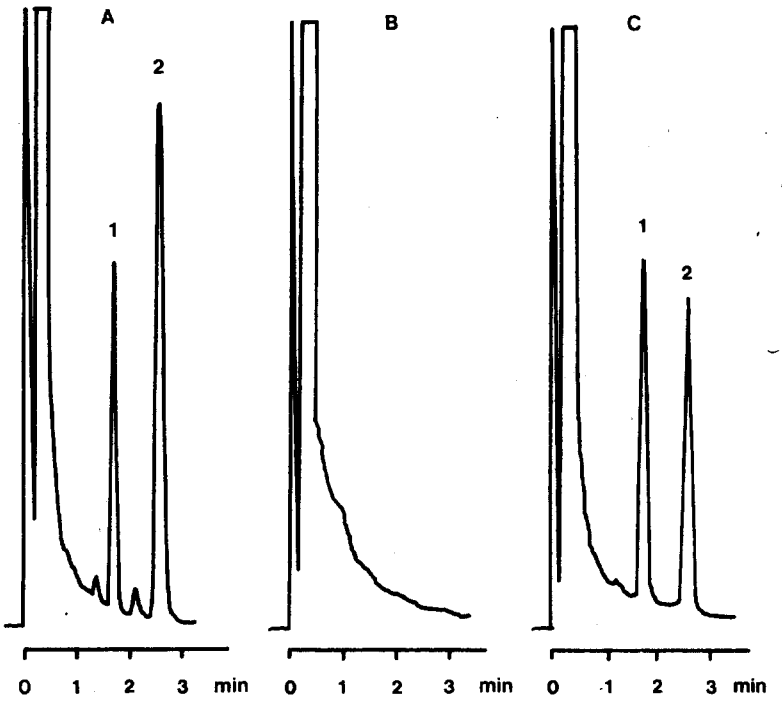

Fig. 1. Gas chromatograms from the analysis of (A) a patient's serum containing meprobamate (380 μmol/l), (B) a blank serum and (C) a patient's serum containing meprobamate (75 μmol/l) with pure substance added (100 μmol/l) (175 μmol/l meprobamate in total). Peaks: 1 = 2-methyl-2-ethyl-1,3-propanediol *n*-butylboronate (internal standard) (120 μmol/l); 2 = meprobamate (2-methyl-2-propyl-1,3-propanediol *n*-butylboronate).

yield found in methanolic standard solutions. With the proposed method, meprobamate was detected in serum at concentrations down to 1.0 μmol/l.

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