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DETERMINATION OF VALPROIC ACID (DI-*N*-PROPYL ACETIC ACID) IN PLASMA BY GAS-LIQUID CHROMATOGRAPHY WITH PRE-COLUMN BUTYLATION

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Summary

A gas-liquid chromatographic procedure for the determination of the anticonvulsant drug valproic acid in plasma or serum is described. Valproic acid is extracted from acidified plasma (or serum) into toluene containing octanoic acid as the internal standard. The toluene layer is back extracted with a methanolic solution of tetramethylammonium hydroxide; an aliquot of the methanolic layer is mixed after phase separation with *N*,*N*-dimethylacetamide and 1-iodobutane. After centrifugation the clear supernatant (which is stable for at least 24 h) is analysed by gas-liquid chromatography, on a 3% OV17 column. The procedure is rapid and sensitive, and the back-extraction step with methanolic tetramethylammonium hydroxide results in clean chromatograms obtained from blank plasma. Sample volumes of 50 μl suffice for the determination of therapeutic levels of VPA.

Introduction

The determination of underivatized valproic acid (VPA) in plasma or serum by gas-liquid chromatography (GLC) has been the subject of several recent reports [1—4].

When developing a GLC method for VPA a number of problems have to be solved. In order to prevent severe tailing of the VPA peak in the chromatograms, careful de-activation of the column support material is essential. Consequently, highly polar stationary phases have been used [1—4], which cannot serve as the liquid phase in multi-purpose columns.

After direct extraction of the plasma sample and injection of an aliquot of

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the extract into the gas chromatograph, peaks interfering with the VPA peak may well show up in the chromatogram. Some investigators solved this problem by applying a double extraction technique [1,3]; others have used a very apolar and therefore selective extracting solvent, although even when extracting the plasma with hexane an interfering peak may occur unless special precautions are taken [2]. Finally, increasing the sensitivity of the method by concentration of the solution through evaporation of the solvent after extraction is hazardous due to the volatility of VPA.

We have recently developed a simple and rapid clean-up and derivatisation procedure for the GLC analysis of acidic drugs in plasma, which is applicable to compounds belonging to several different chemical classes [5,6]. In this procedure, in its simplest form, the plasma sample is acidified and extracted with toluene.

After phase separation the toluene layer is back extracted with a small volume of 2 mol/l tetramethylammonium hydroxide solution in methanol (TMAH). The acidic drug in an aliquot of the TMAH layer is then butylated by adding *N,N*-dimethylacetamide (DMA) and 1-iodobutane [7]; the butyl ester of the compound is chromatographed on a 3% OV17 column.

In the present investigation the usefulness of this method was tested for the determination of VPA.

Materials and methods

1. Reagents and glassware

VPA solution, 7 mmol/l in water. An aliquot of an aqueous sodium valproate solution, 1.80 mol/l was diluted with water (Labaz, Maassluis, The Netherlands).

Hydrochloric acid, 4 mol/l, was prepared by diluting 12 mol/l hydrochloric acid, p.a. (Merck, Darmstadt, F.R.G.).

Toluene, p.a. (Merck).

Internal standard solution; octanoic acid, puriss. (Fluka A.G., Basel, Switzerland) in toluene, 0.28 mmol/l.

N,N-Dimethylacetamide (British Drug Houses, Poole, England).

Tetramethylammonium hydroxide, 2 mol/l in methanol (Aldrich-Europe, Beerse, Belgium).

1-Iodobutane, purum (Fluka A.G.) was distilled before use.

Pooled human plasma.

Centrifuge tubes 7 ml capacity, with glass stoppers and the conical ends drawn to a fine point.

Glass capillary tubes, 5 cm × 3 mm i.d.

2. Extraction procedure

To 1.0 plasma in a centrifuge tube add 5—100 µl VPA solution (35—700 nmol VPA) and water, to a final volume of 1.1 ml.

Add 50 µl 4 mol/l hydrochloric acid and mix by vortex for a few seconds. Add 2.00 ml internal standard solution, vortex for 30 sec and centrifuge at 2500 × *g* for 3 min. Transfer as much as possible of the upper toluene layer to another centrifuge tube, add 20 µl TMAH and vortex for 30 sec. Centrifuge at 2500 × *g* for 3 min and transfer 10 µl of the bottom TMAH layer to a glass

capillary tube. Add 30 μl DMA, vortex for a few sec, add 10 μl 1-iodobutane and again mix by vortexing for a few sec. After 10 min, centrifuge at 2500 × *g* for 5 min.

3. Gas-liquid chromatography

GLC was performed on a Packard-Becker model 419 gas chromatograph (Becker, Delft, The Netherlands), equipped with flame ionisation detectors and fitted with a 1.5 m × 2 mm i.d. glass column packed with 3% OV17 on Chromosorb WHP 100—120 (Chrompack, Middelburg, The Netherlands). The oven temperature was maintained at 115° C, the injector block and the detector at 230° C. Carrier gas (nitrogen), hydrogen and air flow rates were 20, 30 and 300 ml/min, respectively. 1—2 μl of the clear supernatant obtained after the extraction procedure was injected. Under the above conditions the retention time for butylated VPA was 2.8 min and for butylated octanoic acid 5.3 min.

4. Determination of absolute recovery of VPA

a. To 1.0 ml plasma samples 100 μl VPA solution and 50 μl 4 mol/l hydrochloric acid were added. After extraction with toluene and back extraction of the toluene layer with TMAH, as described above, 10 μl of the TMAH layer was transferred to a glass capillary tube and vortexed with 30 μl DMA containing 375 nmol octanoic acid. After derivatisation with 1-iodobutane (see above) 1 μl of the supernatant was injected into the gas chromatograph.

b. To a glass capillary tube 30 μl DMA containing 350 nmol VPA and 375 nmol octanoic acid was transferred and vortexed with 10 μl TMAH. After derivatisation with 10 μl 1-iodobutane, 1 μl of the supernatant was injected into the gas chromatograph.

Results

Specificity

Both VPA and the internal standard, octanoic acid, gave 1 peak in the chromatogram after butylation (Fig. 1). No disturbing peaks from endogenous compounds in the plasma showed up in the chromatogram. The butyl esters of other frequently prescribed acidic drugs have much longer retention times than VPA and octanoic acid [5].

Precision

The mean peak-height ratio VPA/internal standard was calculated at 5 different VPA concentrations in plasma, ranging from 35 to 700 μmol/l, as well as the coefficients of variation. The results are summarized in Table I. A calibration plot was constructed, using plasma samples spiked with VPA, by plotting the peak-height ratio against the VPA concentration. The calibration plot proved to be a straight line through the origin, as is clear from the constant value (within the experimental error) of the ratio of the peak-height ratio and the VPA concentration.

Sensitivity

Plasma samples (1 ml) were spiked with 3.5 nmol VPA and processed as

TABLE I

PEAK HEIGHT RATIOS OF VPA TO INTERNAL STANDARD (OCTANOIC ACID) AND COEFFICIENTS OF VARIATION (C.V.), OBTAINED WITH PLASMA SAMPLES (1 ml) CONTAINING 35—700 μmol/l VPA

Concentration (μmol/l)	PHR *	PHR/Concentration	C.V. (%)
35	0.109	2.18	5.0
175	0.549	2.20	3.1
350	1.097	2.19	3.3
525	1.613	2.15	3.4
700	2.164	2.16	2.9

* PHR = Peak-height ratio. Each ratio is the mean value of 6 determinations.

described above using a 10-fold dilution (with toluene) of the internal standard solution as the extracting solvent. The mean peak-height ratio was found to be 0.104 ($N = 6$, C.V. = 12%), indicating that 3 μmol/l VPA in plasma can be determined with acceptable precision in 1 ml plasma samples.

Absolute recovery

Peak-height ratios of VPA/octanoic acid were calculated for plasma samples (6) and for standard solutions as described above. When calculating the absolute recovery of VPA from plasma, a correction was made for the 25% reduction in the TMAH volume during the back extraction [6]. Following the proposed procedure 65 ± 3.4% (mean ± S.D.) of the VPA was recovered from plasma.

Stability

After injection into the gas chromatograph the derivatisation mixture was

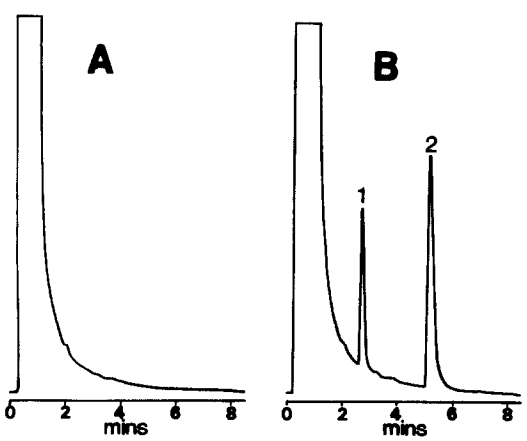

Fig. 1. GLC of plasma samples. Chromatogram A was obtained from blank plasma, chromatogram B from blank plasma spiked with 210 μmol/l VPA (1, VPA; 2, octanoic acid).

occasionally stored in the dark at room temperature for 24 h, and injected again. The peak-height ratios before and after storage were always the same within the experimental error.

Discussion

The proposed method is suitable for the determination of VPA in plasma or serum. Its precision and sensitivity are sufficient for the determination of therapeutic levels of VPA (70—1100 μmol/l) [8] in plasma samples no larger than 50 μl; the method is therefore also applicable if the available sample volume is severely limited. Apart from its rapidity the extraction procedure described above has the advantage that virtually no peaks due to the endogenous compounds in the plasma appear in the chromatograms [5,6]; the TMAH back extraction is a clean-up step as well as a concentration step. A common multi-purpose column material (3% OV17 as the stationary phase) can be used in the analysis of the butylated VPA.

Acidic drugs, such as many of the anti-epileptics, are frequently derivatized by flash-heater methylation upon injection into the gas chromatograph [9]. Methylation of VPA with 0.2 mol/l trimethylanilinium hydroxide has been reported recently [10], but proved to be impossible under our conditions because of interference of the reagent peak with the peak belonging to the methyl ester of VPA.

The resulting solution after butylation as described here is stable, thus allowing automation and the processing of large numbers of samples after transferring the clear supernatants into suitable vials.

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