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Note

Use of formic acid in carrier gas: a rapid method to quantitate dipropylacetate in plasma by gas—liquid chromatography

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A rapid and sensitive method to quantitate drugs in biological fluids is most helpful in pharmacokinetic studies or in drug therapy, particularly when biological drug levels vary with rapid elimination and/or with individual variations, as is noticed with dipropylacetate (DPA) [1].

Dipropylacetic acid (valproic acid) is a branched chain fatty acid whose sodium salt is recognized to be effective in the treatment of petit mal epilepsy and, in association with other drugs in cases of grand mal epilepsy. As far as we know the methods already described for DPA determination require microdiffusion [2] or solvent extraction, often dessication and sometimes derivatisation [3—7]. Moreover, most of these methods need large samples and therefore are time consuming and not suitable for pediatric requirements or for pharmacokinetic studies stretching over 48 h.

In the direct procedure described below, no pre-chromatographic manipulations are necessary. This method is sensitive enough for quantitation to be carried out on small plasma samples. The biological sample is directly injected into a gas chromatograph, DPA is displaced from its plasmatic salts by a formic acid gas current saturating the carrier gas, and detected in approx. 10 min up to a minimum level of 2.5 ng.

MATERIALS AND METHODS

Reagents

Sodium dipropylacetate was used in the form of the commercial 20% solution (Depakine, Labaz, 33440 Ambares, France). Octanoic acid was purchased

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from Prolabo (Paris, France) and formic acid came from Baker (Deventer, The Netherlands) and chromatographic reagents from Varian Aerograph (Walnut Creek, Calif., U.S.A.). All volumes were measured with Hamilton syringes.

Gas chromatography (GC)

An Intersmat IGC 120 DFL bi-column gas chromatograph equipped with a flame ionisation detector (FID) was used without modification.

Both FID and injector were maintained at 160°. The injector was equipped with a glass pre-column, 100 × 2 mm I.D. (Insert tube Intersmat), carefully acid washed, rinsed out, then heated overnight at 160° in the injection port before use. This pre-column was used for up to ca. 20 injections with protein-containing samples (100 µl injected).

The metal column (2 m × 2 mm I.D.) was coated with 5% Carbowax 20 M on 80—100 mesh, Chromosorb P and was operated at 150° after a conditioning of 48 h at 160° under a stream of nitrogen, then one night at 100° without the carrier gas (ref. 8 slightly modified). The glass wool occluding the column was treated with phosphoric acid (85%) to avoid adsorption of DPA fractions on the column head.

Just before gas entry, the nitrogen flow (45 ml/min) was saturated with acid by flowing, without bubbling, over 1 ml of pure formic acid contained in a 5-ml screw cap vial equipped with an O-ring (for more details see [9]). The use of pure anhydrous formic acid was necessary to avoid the appearance of interfering peaks due to volatile compounds eventually present in the acid, and also the premature wear of the chromatograph. In such conditions formic acid vapors were able to displace DPA from its plasmatic salts.

Procedure

A 2.5-µl aliquot of internal standard (octanoic acid 12.6 mmole/l in aqueous alkaline solution, stored at −20° in a 1-ml vial until utilization) was added to 100 µl of heparinized plasma, possibly obtained from blood drawn from the finger tip, and 5 µl of the resulting solution were injected into the chromatograph. The volume of heparinized plasma is only limited by the injection volume and can be reduced to 20 µl.

Between injections of biological samples, 10 µl of 50% (v/v) formic acid were injected into the pre-column (5 µl to pre-column head, half a syringe needle and 5 µl to pre-column end), and the oven heated to 160° for 10 min in order to clean the pre-column of residual compounds eventually adsorbed on proteins denatured by injector heat.

RESULTS AND DISCUSSION

A typical chromatogram of DPA in patient's plasma is shown in Fig. 1. DPA and the internal standard were well separated and no interfering endogenous compounds were noticed. The DPA calibration graph indicates a linear relationship between added DPA in normal plasma and detector response, from 2.5 µg/ml to 170 µg/ml (minimum detectable amount 0.5 µg/ml; Fig. 2). This concentration range allows a wide margin for DPA therapeutic concentrations, (from 40 to 120 µg/ml). It seems suitable for drug concentration

TABLE I

PERCENTAGE RECOVERY OF ADDED DPA IN PLASMA

Internal standard: same concentrations in aqueous and plasma samples. Each value is the mean of three determinations and is measured at least one hour after plasma addition to allow proteic fixation of DPA and of internal standard.

Concentration of added DPA in water and plasma	$\frac{\text{Absolute DPA peak height (plasma)}}{\text{Absolute DPA peak height (water)}} \times 100(\%)$	$\frac{\text{DPA relative to internal standard peak height (plasma)}}{\text{DPA relative to internal standard peak height (water)}} \times 100(\%)$
43 $\mu g\ ml^{-1}$	92	108*
172 $\mu g\ ml^{-1}$	80	105*
344 $\mu g\ ml^{-1}$	83	100

*An increase in percentage recovery seems due to a lower recovery of octanoic acid in plasma (recovery of octanoic acid is 80% for ten determinations in water and plasma at internal standard concentrations).

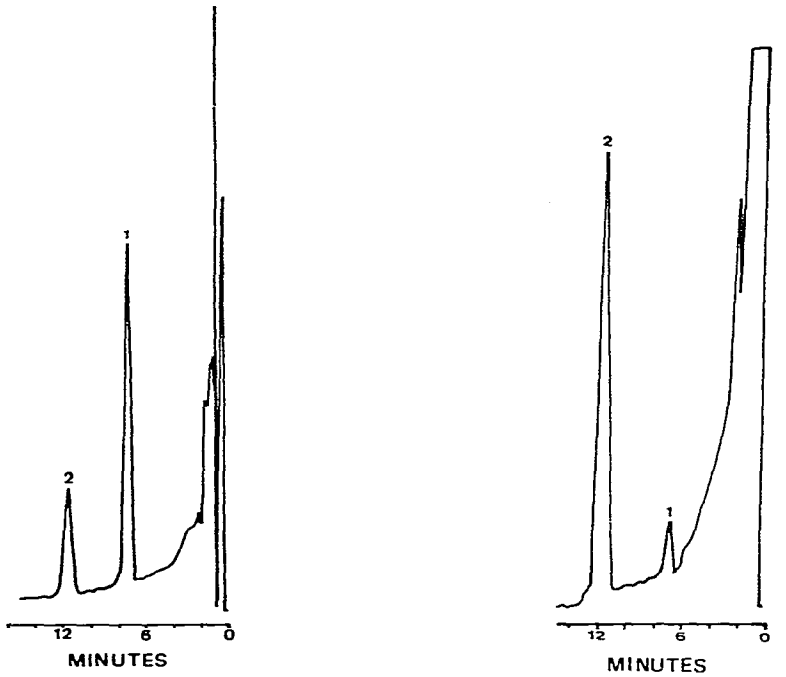

Fig. 1. Gas chromatogram for children's plasma one hour after ingestion of DPA 200 mg (Depakine). (1) DPA, 65 $\mu g \cdot ml^{-1}$; (2) internal standard octanoic acid, 47 $\mu g \cdot ml^{-1}$. For gas chromatographic details see text (5-μl injected).

Fig. 2. Gas chromatogram for plasma with added DPA near minimum detectable amount (5-μl injected). (1) DPA, 2.5 $\mu g \cdot ml^{-1}$; (2) internal standard, octanoic acid, 47 $\mu g \cdot ml^{-1}$. For gas chromatographic details see text.

studies in saliva or spinal fluid or for pharmacokinetic studies for long periods after drug administration [5].

The reproducibility of the method has been studied with plasma samples corresponding to the lowest therapeutic level (40 μg/ml). For the first ten injections, the standard deviation was found to be equal to 3.5% and for the following ten equal to 6%. This seems due to a slight adsorption of plasma DPA on protein residues in the pre-column. However, this reproducibility fell within acceptable limits for clinical purposes.

Choice of technical conditions

Since the original publication of James and Martin, many authors have noticed a column adsorption of free volatile acids analysed by GC. Adsorption generally gives rise to tailing peaks, irregular shaped peaks and ghosting. This phenomenon seems avoidable by heating the column without the carrier gas and by using formic acid to saturate the carrier gas. Poorly detectable by flame ionization, formic acid is more polar than DPA, thus strongly bound to adsorption sites, and so it prevents tailing peaks and ghosting. Moreover, formic acid is able to displace DPA from its plasmatic salts, rendering all previous sample treatments useless. In spite of the use of an acid carrier gas, no premature wear of the chromatograph could be noticed after several months of constant use

(certainly because of the pure anhydrous formic acid used). Utilization of such formic acid (the 5-ml vial was filled with 1 ml twice a week) allows the quantitation of DPA on very small blood samples.

A residual adsorption of plasmatic DPA by denaturated proteins in the pre-column was not completely prevented, as is shown by the percentage recovery studied in Table I. The percentage recovery of added plasma DPA compared to an aqueous DPA solution has been tested on three DPA concentrations, including the lowest therapeutic level. Taking into account the residual adsorption of DPA, it was obvious that the use of an internal standard was necessary, so, one chemically related to DPA was chosen. This residual adsorption justifies the injection of 10 μl of formic acid between two samples; by sweeping up waste from the pre-column, it allows repeated injections of biological samples. The pre-column precents column pollution by proteins.

The Carbowax phase was compared with the neopentylglycolsuccinate phase (NPGS) 10% on Chromosorb G. Carbowax 20 M was chosen since it gives a better resolution under the conditions used in spite of a longer retention time. Remesy and Demigne [8] had already shown the possibility of using Carbowax 20 M to assay free volatile acids, and they pointed out that eddy peaks are noticed over 130° due to the thermal decomposition of this phase. Such a phenomenon was not observed despite the use of a temperature of 150°. This could be due to the low coating that had been chosen in order to allow a rapid elution (absolute retention time for DPA is 7 min), i.e. mild conditions which avoid thermal decomposition yielding eddy peaks and premature wear of the column.

The use of formic acid in the carrier gas prevents most of the problems already described in volatile acid analysis by GC. It allows a simple procedure for DPA determination in plasma by direct injection of the biological sample and so makes this method particularly adaptable for pediatric requirements.

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