*Journal of Chromatography*, 233 (1982) 371—374
*Biomedical Applications*
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROMBIO. 1413

## Note

# Quantitative determination of *n*-dipropylacetamide in the plasma of epileptic patients by gas—liquid chromatography with nitrogen-selective detection

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(First received April 2nd, 1982; revised manuscript received July 1st, 1982)

*n*-Dipropylacetamide (DPM) is the primary amide of valproic acid (VPA), a widely used antiepileptic drug. DPM is commercially available for therapeutic use and its antiepileptic properties have been reported [1—5]. After ingestion, it is converted almost completely to VPA [6,7] so that its therapeutic dose in epilepsy is considered to be the same as that of VPA [6,7]. Similar doses of DPM or VPA during chronic therapy produce similar VPA plasma concentrations [6]; moreover, VPA plasma concentrations are used to adjust the therapy in patients taking DPM.

Gas chromatographic methods for the determination of plasma DPM concentrations have already been published [8,9] but they are very time-consuming [8] or have unsatisfactory sensitivity [9] for clinical use.

The plasma concentrations of DPM after normal therapeutic doses in epileptic patients were always found to be below 1 μg/ml, which is the detection limit of the analytical procedure used [6]. Even if the plasma concentrations of DPM are so low, their measurement may be important to investigate the metabolism and kinetics of this drug in patients during acute and chronic therapies.

The aim of this work is to indicate a more sensitive gas chromatographic method for the measurement of DPM in plasma of epileptic patients.

## MATERIALS AND METHODS

### *Reagents and standards*

*n*-Dipropylacetamide and *n*-tripropylacetamide (TPM, employed as an internal standard) were obtained from Sigma-Tau (Pomezia, Italy). All chemicals were of analytical grade.

Stock solutions of DPM and TPM were prepared in acetone to give a concentration of 1 mg/ml for each compound.

0378-4347/82/0000—0000/$02.75 

Calibration samples were prepared at the time of the analysis by adding 0.05, 0.1, 0.25 and 0.5 μg of DPM in acetone to a series of test-tubes, which were then evaporated to dryness under vacuum at room temperature and thus redissolved into 0.5 ml of drug-free plasma.

*Apparatus*

A Carlo-Erba Fractovap 2150 gas chromatograph equipped with a nitrogen-selective detector and a Hewlett-Packard 3380 A recorder—integrator were used. The glass column, 1.5 m × 3 mm I.D., was packed with 10% diethyleneglycol-succinate—phosphate (DEGS-PS) on 80—100 mesh Supelcoport (Supelco, Bellefonte, PA, U.S.A.). The following flow-rates were used: hydrogen 36 ml/min; air 250 ml/min; carrier gas (nitrogen) 35 ml/min. The temperature of the column was 190°C; the temperature of the injector was 225°C. The sensitivity of the detector was checked daily.

*Extraction procedure*

Twenty-five microlitres of the diluted internal standard solution (10 μg/ml in acetone), 0.5 ml of 0.5 *M* $H_3PO_4$ and 8 ml of diethyl ether were added to 0.5 ml of plasma (calibration samples or patients' samples). The test-tubes were shaken mechanically for 10 min, then centrifuged for 10 min at 2000 *g*; 6.5 ml of the organic phase were transferred to a second test-tube and evaporated to dryness under vacuum at room temperature.

The residues were dissolved in 100 μl of *n*-hexane and 1—2 μl of this solution were injected into the gas chromatograph.

Calibration graphs were constructed of the peak-area ratio of DPM to TPM versus concentration of DPM. For each series of analyses a new calibration graph had to be prepared.

*Recovery*

Various amounts (0.05, 0.1, 0.25, 0.5 μg) of DPM were dissolved in 0.5 ml of drug-free plasma. The samples were extracted as described above but without adding TPM. The residues were dissolved in 100 μl of *n*-hexane containing 0.25 μg of TPM. A second series of standards was prepared simultaneously by extracting 0.5 ml of drug-free plasma and then adding both DPM and TPM to the dried extract at the concentrations above indicated. The recovery was calculated by comparing the peak-area ratios of the extracted standards to the ratios obtained from the standards to which DPM had been added after extraction. Thus this value was corrected by a factor representing the ratio between the diethyl ether volume added to the plasma and the diethyl ether volume subsequently removed and evaporated during the extraction procedure.

Due to the partial solubility of water in the diethyl ether, this value of recovery may be different from the actual value of absolute recovery; the difference should, however, be slight.

*Linearity*

The linearity was calculated by using the results obtained from the calibration graphs (from 0.1 to 1 μg/ml).

*Interference from other antiepileptic drugs*

To determine the potential usefulness of the procedure, we checked for possible interferences from other antiepileptic drugs (phenobarbital, mephobarbital, primidone, carbamazepine, phenytoin, ethosuximide and valproic acid) by chromatographing extracts from plasma of epileptic patients taking the above drugs and pure standards at normal therapeutic concentrations.

*Plasma of patients*

Samples of blood from patients receiving DPM orally thrice daily (at 8 a.m., 2 p.m., 8 p.m.) together with other antiepileptic drugs were taken in heparinized test-tubes just before the morning dose. The plasma was separated as soon as possible, immediately frozen and stored at —20°C until taken for analysis.

## RESULTS AND DISCUSSION

Under our gas chromatographic conditions, only two of the antiepileptic drugs tested were seen on the chromatograms; valproic acid, which eluted in the front of the solvent, and ethosuximide, which had a retention time of 11.5 min. Since DPM and TPM had a retention time of about 2.6 min and 3.4 min, respectively, we can conclude that no antiepileptic drug tested (at normal

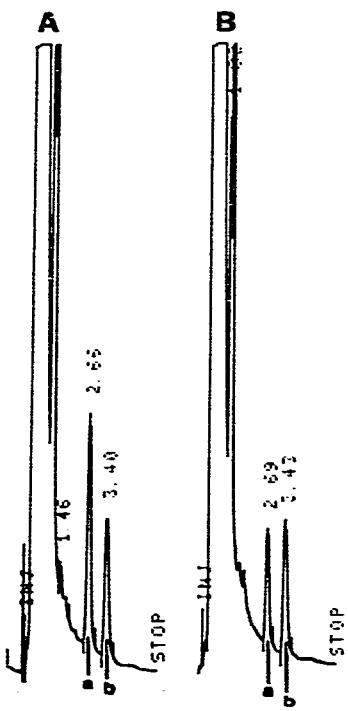


**Fig. 1. Gas chromatographic response of two extracted plasma samples. (A) Extract from a calibration sample (0.5 μg of DPM per 1 ml of plasma). (B) Extract from plasma of a patient taking DPM (16 mg/kg per day), phenytoin and carbamazepine (DPM plasma level = 0.21 μg/ml); a = DPM; b = TPM.**

therapeutic concentrations) interfered with the analysis. Moreover, no interference from endogenous plasma compounds was seen on the chromatograms of drug-free plasma samples.

Typical chromatograms of extracts from a plasma calibration sample and a plasma sample of a patient taking DPM, phenytoin and carbamazepine are shown in Fig. 1.

Calibration curves from extracted plasma showed a linear correlation between concentrations and respective readings: $Y = 1.79\ X + 0.009$, $r = 0.997$. To calculate these curves, a least-square linear regression method was used. The minimal amount of DPM detectable by the described procedure is about 20 ng/ml of plasma. The mean ± S.D. recovery from four analyses of plasma samples containing four different DPM concentrations (0.1, 0.25, 0.5, 1 μg/ml) was 90.2 ± 3.5% ($n = 16$).

In a series of 13 patients, with a DPM dosage of 10—40 mg/kg per day (mean ± S.D. = 27.1 ± 9.5) we found plasma concentrations of 0.03—3 μg/ml (mean ± S.D. = 0.668 ± 0.840) regardless of the co-medications. In the same samples, the concentrations of VPA, measured by gas—liquid chromatography [10], were 30—80 times higher than DPM concentrations.

The proposed method is, to the best of our knowledge, the most sensitive available to determine the concentrations of DPM in human plasma and it is suitable to study the pharmacokinetics and metabolism of DPM in human subjects.

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