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Note

Rapid quantitation of N-dipropylacetamide in human plasma by gas–liquid chromatography

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Several clinical investigations have provided evidence that N-dipropylacetamide (DPM) is active in various forms of epilepsy and during the last few years there has been a progressive increase in its use[1–8]. Although a gas chromatographic method for DPM assay has already been described[9], it is time consuming and therefore unsatisfactory for routine use.

The aim of this work was to develop a more rapid and sensitive gas chromatographic method for the routine assay of DPM in plasma.

MATERIALS AND METHODS

Reagents

N-Dipropylacetamide was obtained from Sigma-Tau (Rome, Italy), α,α-dimethyl-β-methylsuccinimide from Aldrich-Europe (Beerse, Belgium; Cat. No. 16350) and chloroform and hydrochloric acid from Carlo Erba (Milan, Italy).

Apparatus

A Carlo Erba Fractovap Model GV gas chromatograph equipped with a flame-ionization detector was used. The glass column (1 m × 3 mm I.D.) was pretreated with dimethyldichlorosilane (Merck, Darmstadt, G.F.R.) and packed with 10% DEGS-PS on 80–100-mesh Supelcoport (Supelco, Bellefonte, Pa., U.S.A.).

The injection temperature was 220°, column temperature 175°, detector temperature 220°, nitrogen flow-rate 1.7 ml/min, hydrogen flow-rate 1.7 ml/min, air flow-rate 2.5 ml/min and attenuation ×10 and ×32.

Sample for analysis

To study recovery and accuracy, a standard drug solution in chloroform (1 mg/ml) was prepared and aliquots were added to drug-free human plasma (1 ml per sample).

Extraction procedure

Volumes of 1 ml of human plasma, 0.5 ml of 1 *N* hydrochloric acid and 0.5 ml of a 100 μg/ml solution of α,α-dimethyl-β-methylsuccinimide in chloroform were placed in

a centrifuge tube, mixed for 15 min with a rotary mixer and centrifuged for 10 min at 1430 *g*. The aqueous phase was discarded and a 1–2 μl sample of the remaining organic phase was injected into the gas chromatograph.

RESULTS AND DISCUSSION

Under the experimental conditions used the drug and internal standard peaks appeared within 3.5 min (Fig. 1). A calibration graph was constructed using plasma containing drug concentrations of 5, 10, 25, 50, 100, 150 and 200 μg/ml, the plot of peak-height ratio of DPM to internal standard versus the amount of DPM added being linear.

The recovery and reproducibility of the method at three different concentrations are shown in Table I.

Several drug-free plasma samples and several plasma samples from patients treated with antiepileptics and other drugs (*e.g.*, psychoactive and antiphlogistic agents) were also examined in order to check the specificity of the method. No interfering peaks were observed.

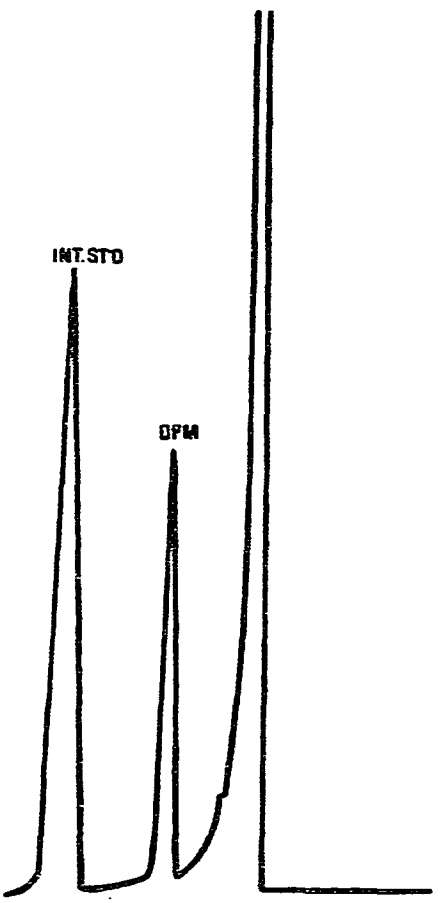

Fig. 1. Gas–liquid chromatogram of DPM (15 μg/ml). Internal standard = α,α-dimethylsuccinimide.

TABLE I

RECOVERY AND REPRODUCIBILITY OF THE METHOD

Parameter	*Amount of DPM (μg)*		
	5	*15*	*25*
Mean* recovery (%)	78.8	81.2	80.6
Reproducibility*	0.7 ± 0.01**	2.1 ± 0.04**	3.5 ± 0.02**

* 20 analyses.

** Mean (±S.D.) values of the peak-area ratio of DPM to internal standard.

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REFERENCES

1 S. Borselli, *Premiers Essais Cliniques de l'Amide Primaire de l'Acide Di-n-propylacétique ou Depamide: Activité Antiépileptique et Neuropsychotrope, Thèse Méd.*, Grenoble, 1967.
2 G. Carraz, M. Darbon, S. Lebreton and H. Beriel, *Thérapie*, (1964) 468.
3 G. Carraz, S. Lebreton, M. Bottard, S. Borselli and J. Bonnin, *Encéphale*, 54 (1965) 458.
4 P. Favel, J. Cartier, J. P. Gratadou and D. Gratadou, *Epilepsia*, 14 (1973) 329.
5 S. Garrel, G. Carraz, S. Borselli, C. Baudrand and J. Dekaunay, *C.R. Congr. Psychiat. Neurol. Langue Fr., 64ème Session, Grenoble, 12–17 September, 1966*, p. 1030.
6 P. Giovanardi-Rossi, P. Martinelli and M. Boschi, *Neuropsichiatr. Infant.*, 151–152 (1974) 105.
7 J. Pougetoux, S. Garrel and S. Borselli, *J. Med. Lyon*, 1108 (1966) 1595.
8 R. Musolino, G. Gallitto, L. Morgante, F. Pisani, G. Nistico' and R. Di Perri, *Depamide: a Preliminary Report on its Therapeutic Efficacy in Epilepsy, Second European Regional Conference on Epilepsy, Warsaw, October 5–7, 1978.*
9 J. Alary, D. Contin, A. Coeur and G. Carraz, *Bull. Trav. Soc. Pharm. Lyon*, 16 (1973) 53.