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Note

Rapid gas chromatographic assay for monitoring valnoctamide in plasma

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Valnoctamide (valmethamide, or 2-ethyl-3-methylpentamide) has been used as a tranquillizer and in the treatment of anxiety and tension [1—3], and as an antiepileptic drug [4]. In the current literature, there is not a single report on an assay for monitoring plasma levels of valnoctamide.

The aim of this work was to develop a rapid and sensitive gas chromatographic method for routine assay of valnoctamide in plasma. Pharmacokinetic application of the new gas—liquid chromatographic (GLC) method is presented by plasma monitoring of valnoctamide after oral administration of a commercially available tablet of the drug (2 × 200 mg of Nirvanil®; Clin Midy, France) to a dog.

EXPERIMENTAL

Reagents and standards

Valnoctamide was obtained from Clin Midy (Paris, France), *p-tert.*-butylphenol was obtained from Fluka (Switzerland), chloroform (AnalaR) from BDH (U.K.) and hydrochloric acid from Frutarom (Israel). An organic stock solution of valnoctamide was prepared by dissolving the drug in chloroform. An aqueous stock solution was prepared by dissolving valnoctamide in water. The concentration of each stock solution was 0.5 mg/ml. *p-tert.*-Butylphenol was used as an internal standard and was dissolved in chloroform at a concentration of 0.25 mg/ml. Stock solutions were stored at —20°C.

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Apparatus and conditions

A Packard Model 437 gas chromatograph equipped with a flame-ionization detector and a recorder (Unicorder 225; Kyoto, Japan) was used. The glass column was 180 cm × 2 mm I.D. and was packed with 5% free fatty acid phase (FFAP; Applied Science Labs., State College, PA, U.S.A.) on 80—100 mesh Gas-Chrom Q. Flow-rates were as follows: hydrogen 25 ml/min; air 250 ml/min; carrier gas (nitrogen) 28 ml/min. The temperatures were: column 185°C; injector 205°C; detector 220°C. The same conditions were used in the work with the gas chromatograph—mass spectrometer (LKB Model 2091) which was operated with an ionization electron beam energy of 70 eV.

Extraction procedure

To 0.5 ml of plasma spiked with an appropriate aliquot of valnoctamide (or taken from the dog), were added 0.2 ml of chloroform, 50 μl of internal standard solution and 0.25 ml of 1 *M* hydrochloric acid. The sample was vortexed for 15 min, shaken for 30 min and centrifuged at 3000 *g* for 15 min. Of the organic phase, 3 μl were injected into the gas chromatograph.

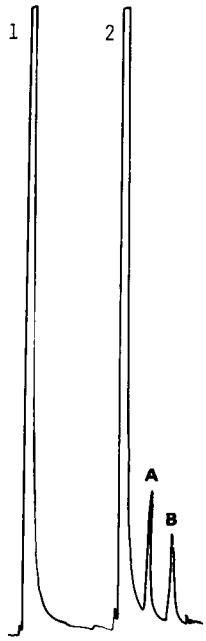
In order to determine the precision of the assay, 5 ml of human plasma were spiked with appropriate aliquots of the aqueous stock solution of valnoctamide and were stored at −20°C during the two months of the precision study. On different days, 0.5 ml was taken from the various stored samples and analysed against a fresh calibration curve made according to the extraction procedure on the same day.

RESULTS AND DISCUSSION

Typical chromatograms of a plasma extract and drug-free plasma are presented in Fig. 1. Under the assay conditions, the following retention times were obtained: valnoctamide 3.0 min; internal standard 3.6 min. The identification of peak A (Fig. 1) as valnoctamide was confirmed by GLC—mass spectrometric (MS) analysis. There was no interference from endogenous plasma components.

Calibration curves from plasma extracts showed a linear correlation between peak height ratio (y) (valnoctamide against internal standard) and plasma concentration of the drug (x). The linear calibration equation was $y = 0.138x + 0.0015$. To calculate this curve, a least-squares linear regression method was used. The minimal detectable concentration was 0.3 μg/ml of plasma. The linearity range of the assay was between 0.3 and 200 μg/ml.

Analytical recoveries of the drug were established as follows. Various amounts (10, 20, 30, 40, 50, 60, 80 and 100 μg) were taken from the aqueous stock solution of valnoctamide, and the volume was made up to 1 ml with drug-free plasma. After acidification, plasma samples were extracted into 0.20 ml of chloroform, which contained 50 μl of internal standard solution. A series of external standards were prepared by adding 50 μl of internal standard solution to 0.2 ml of chloroform containing the various amounts taken from the organic stock solution (10, 20, 30, 40, 50, 60, 70, 80 μg) of valnoctamide. Analytical recoveries were calculated by comparing peak height ratios of the extracted standard to the external standards (Table I). The standard deviation



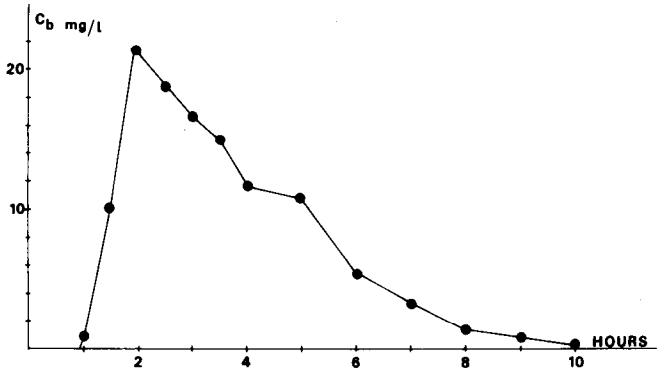


Fig. 1. Examples of chromatograms: (1) human plasma blank; (2) valnoctamide (A) 10 μg/ml (t_R 3.0 min), and internal standard (B) 50 μg/ml (t_R 3.6 min) in human plasma.

Fig. 2. Plasma levels of valnoctamide (Nirvanil) to a dog.

TABLE I

RECOVERY AND REPRODUCIBILITY OF VALNOCTAMIDE IN HUMAN PLASMA

Concentration (μg/ml)	Recovery* (%)	S.D.	C.V. (%)
10	90.6	5.5	6.1
20	89.0	4.3	4.8
30	87.4	3.7	4.2
40	87.6	3.2	3.6
50	89.6	2.3	2.6
60	88.1	2.2	2.5
80	87.6	2.7	3.1
100	90.9	5.8	6.4
Mean	88.9	1.4	1.6

*Mean of eight determinations.

of the analytical recoveries can serve as a good estimate of reproducibility (Table I).

Precision or accuracy of the assay was determined by performing eight replicate analyses of five control samples containing 10, 20, 30, 50 and 80 μg/ml of the drug on different days over a two-month period. The results are shown in Table II. The mean (± S.D.) percentage recovery of valnoctamide, as presented in Table I, is 88.9% ± 1.4 (n = 8). The observed values of the

TABLE II

PRECISION OF THE ASSAY FOR VALNOCTAMIDE IN HUMAN PLASMA

Concentration (μg/ml)	Conc. found* (μg/ml)	S.D.	C.V. (%)
10	10.47	0.41	3.92
20	20.43	0.81	3.96
30	29.35	1.22	4.16
50	48.61	1.88	3.87
80	78.84	2.41	3.06

*Mean of eight determinations.

various valnoctamide concentrations (Table II) were not different statistically from the added concentrations ($P > 0.05$) [5].

A biomedical application of the new GLC method is presented in a preliminary pharmacokinetic study. In this study, valnoctamide (400 mg) was administered orally to a dog (mongrel, 20 kg). The plasma levels of valnoctamide obtained are presented in Fig. 2 (mean of three replicates). The coefficient of variation among the three replicates at each data point was 5%. GLC—MS analysis of peak A (Fig. 1) in various samples obtained from the dog after oral administration of valnoctamide gave an identical MS fragmentation to that of a valnoctamide standard.

Although the retention time of valnoctamide in this assay was significantly different from those of related drugs such as valpromide and valproic acid, in humans there is a possibility of interference by other endogenous substances normally present in plasma or by other prescribed drugs which might be co-administered with valnoctamide.

The proposed method describes for the first time a very rapid, convenient and specific assay for valnoctamide. This assay is very useful in any pharmacokinetic study or therapeutic plasma monitoring of valnoctamide.

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