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Note

Determination of gabapentin in plasma and urine by capillary column gas chromatography

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Gabapentin [1-(aminomethyl)cyclohexaneacetic acid] (Fig. 1) is an analogue of γ-aminobutyric acid (GABA) which is currently undergoing clinical development as an anticonvulsant [1]. Gabapentin has GABA-mimetic properties as an inhibitory neurotransmitter, is well absorbed following oral administration and, unlike GABA, readily crosses the blood–brain barrier [2]. The drug has very low toxicity in man. It is not metabolized, not appreciably bound to plasma proteins and is largely eliminated by renal clearance.

Most of the pharmacokinetic studies published to date [2,3] have used a high-performance liquid chromatographic (HPLC) assay involving derivatization of gabapentin with 2,4,6-trinitrobenzenesulphonic acid to provide a chromophore for UV detection [4]. The structural similarity of gabapentin to the essential amino acids suggested that it should, like them, be amenable to analysis by gas chromatography (GC) [5].

We describe here a capillary column GC method for the quantification of gabapentin in plasma and urine, which has been successfully applied in clinical pharmacokinetic studies with the drug [6].

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EXPERIMENTAL

Reagents and standards

Gabapentin and the structural analogue which served as the internal standard (I.S.) [1-(aminomethyl)cycloheptaneacetic acid; Gö 3609A] (Fig. 1) were obtained from the Gödecke Research Institute (Freiburg, F.R.G.) through Parke Davis (Sydney, Australia). Acetonitrile (ChromAR) and chloroform (nanograde) were from Mallinckrodt (Clayton, Australia). Hydrochloric acid (36%, w/v) and ammonia solution (28–30%, w/v) were analytical-reagent grade, supplied by Ajax Chemicals (Auburn, Australia). Ion-exchange resin (Dowex 50-AG 50W-X8, 100–120 mesh) was obtained from Bio-Rad Australia (Hornsby, Australia). Methanolic hydrogen chloride was obtained in kit-form (Cat. No. 18053) from Alltech Assoc. (Deerfield, IL, U.S.A.) and trifluoroacetic anhydride (>99% purity) was from Pierce (Rockford, IL, U.S.A.).

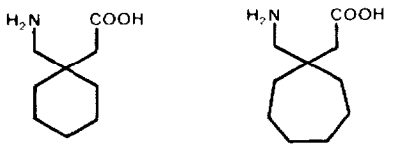
A stock standard solution of gabapentin (1.0 mg/ml) was prepared by dissolving 10.0 mg gabapentin in 10 ml water. Diluted aqueous standard solutions were prepared as required, and plasma and urine standards were obtained by adding appropriate aliquots of aqueous standards to drug-free human plasma or urine. Plasma standards ranged in concentration from 0.2 to 20.0 μg/ml and urine standards from 5 to 500 μg/ml.

The concentration of the aqueous I.S. solution was 0.5 mg/ml.

Aqueous solutions were stored at 4°C and were stable for at least several months. Plasma and urine solutions were stored at −20°C and were also extremely stable.

Equipment

The GC systems used in these studies were a Tracor Model 560 and Tracor Model 565. Minor modifications were made to the injectors by installing SGE (Ringwood, Australia) column adaptors (No. 1034610) to permit direct on-column injections into megabore capillary columns (Megabore DB-1, 30 m×0.53 mm I.D., with 1.5-μm coating of liquid phase, obtained from J & W Scientific, Folsom, CA, U.S.A.). Manual injections were performed with an SGE Type A microlitre syringe. The carrier gas was helium at a flow-rate of approximately 8 ml/min. Make-up gas (nitrogen, approximately 40 ml/min)



Gabapentin **Gö 3609A**

Fig. 1. Chemical structures of gabapentin and the internal standard (Gö 3609A).

was added through a T-fitting at the exit end of the column. The flame ionization detector had conventional supplies of hydrogen (30 ml/min) and air (300 ml/min). The injector and detector temperatures were maintained at 285°C, and the column oven was programmed from 160 to 260°C at 15°C/min following a 4-min initial hold. The detector output was monitored using a Shimadzu C-R4A integrator equipped with floppy disk drives and CRT display. Quantification was achieved using peak-area ratios.

Sample preparation

An aliquot of I.S. solution (0.1 ml containing 50 μg of I.S.) was added to a plasma sample (1.0 ml) or plasma standard prepared as above, in a 10-ml capped polypropylene centrifuge tube. After mixing, acetonitrile (1.5 ml) was added, the tube shaken vigorously by hand (1 min) and centrifuged at approximately 1000 *g* for 2 min. As much as possible of the supernatant was transferred by pasteur pipette to a 10-ml polypropylene centrifuge tube containing approximately 0.5 g ion-exchange resin (as moist slurry). Hydrochloric acid (1.0 *M*; 0.2 ml) was added, the tube capped, shaken for 1 min and centrifuged (2 min). The clear supernatant was aspirated to waste with a water pump, and the resin washed with two 10-ml portions of water using the same procedure. The amino acids were then eluted from the resin with ammonium hydroxide solution (12 *M*; 2.0 ml), which was transferred to a 5-ml Reacti-Vial (Pierce). The ammonia solution was evaporated to dryness using a vacuum centrifuge (Savant Model SVC 200H). Alternatively the evaporation could be satisfactorily performed under a stream of air with heating of the Reacti-Vial to approximately 70°C.

The dried residue was derivatized as follows. Methyl esters of the carboxylic acids were formed by adding methanolic hydrogen chloride (100 μl) and heating the sealed Reacti-Vial at 60°C for 30 min. The solvent was then removed under an air stream. The amine functions were then derivatized by addition of trifluoroacetic anhydride (100 μl) and standing at room temperature for 30 min. The reagent was again removed by evaporation, and the residue dissolved in 50–100 μl of chloroform. This was gently concentrated to 1–2 μl, and 0.2–0.5 μl was injected onto the GC column.

Urine samples (0.1-ml aliquots) were processed in an identical manner.

Assay validation

Linearity of the assay was assessed on three separate occasions approximately one week apart, over the concentration ranges noted above. Within-batch precision and accuracy were assessed by replicate assays ($n=6$) on pooled plasma or urinary standards at three different concentration values for each fluid. Between-batch (overall) precision and accuracy were assessed by repeating this exercise three times. Recoveries of gabapentin from plasma and urine were assessed by comparing peak-area ratios (gabapentin/I.S.) obtained

after extracting gabapentin from spiked plasma or urine standards with the ratios obtained when identical quantities of the drug were dispensed and derivatized without prior extraction. In the extraction studies, the I.S. was added after extraction of gabapentin to permit direct comparison with the results for non-extracted samples. Minimum quantifiable concentration was determined as three times the standard deviation of the back-calculated concentration values of the 0.2 μg/ml standards ($n=9$) for plasma or the 5.0 μg/ml standards ($n=7$) for urine. Specificity of the procedure was evaluated by assaying a range of drug-free plasma and urine samples and samples from patients taking drugs other than gabapentin (in particular the common anticonvulsants).

RESULTS AND DISCUSSION

The major difficulty in the analysis of gabapentin is that its zwitterionic character renders it extremely difficult to extract from an aqueous medium. Furthermore its structural similarity to the endogenous amino acids ensures that its analysis by any conventional analytical technique will be susceptible to interference by at least the twenty or so amino acids present in varying quantities in plasma samples. Hengy and Kolle [4] overcame these problems by derivatizing gabapentin (and the other amino acids) in deproteinised plasma with 2,4,6-trinitrobenzenesulphonic acid, extracting the derivatives with toluene, and separating them on reversed-phase HPLC. We have elected to adapt a GC method for amino acids analysis which uses a cation-exchange resin to remove the amino acids from deproteinized plasma, followed by formation of the methyl ester and N-trifluoroacetyl derivatives for capillary column GC.

Typical chromatograms for analysis of blank plasma, a plasma standard spiked with gabapentin and a plasma sample from a volunteer who took gabapentin are shown in Fig. 2. Despite the many peaks in the chromatograms, the gabapentin and I.S. were always well resolved from potentially interfering compounds, using the megabore capillary columns. At an early stage in the development of this method we anticipated obtaining optimal sensitivity with a nitrogen-selective thermionic detector. When evaluated, however, this proved inferior to the conventional flame ionization detector because of increased background and interfering signals.

Representative chromatograms for urine analyses are shown in Fig. 3. Again the peaks of interest were well separated from potential interferences. We elected for convenience to use the same procedure for urine as for plasma, although there is no necessity to deproteinize urine with acetonitrile. It is possible that the acetonitrile addition could be omitted for urine specimens, though it is recommended for the GC analysis of amino acids in plasma [5].

The procedure has been formally validated, and its performance characteristics are summarized briefly. For both plasma (0.2–20.0 μg/ml) and urine (5–500 μg/ml) the linearity was excellent over the clinically relevant concentra-

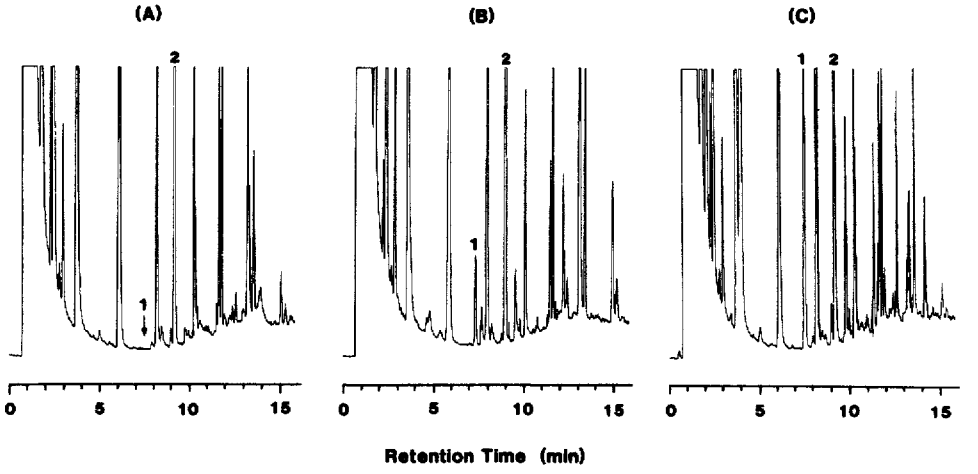


Fig. 2. Chromatograms obtained after analysis of (A) blank plasma to which internal standard (peak 2) was added, (B) plasma standard spiked with gabapentin, 1.0 μg/ml (peak 1) and (C) plasma sample (0.5 h post-dose) from a volunteer who took gabapentin, 300 mg.

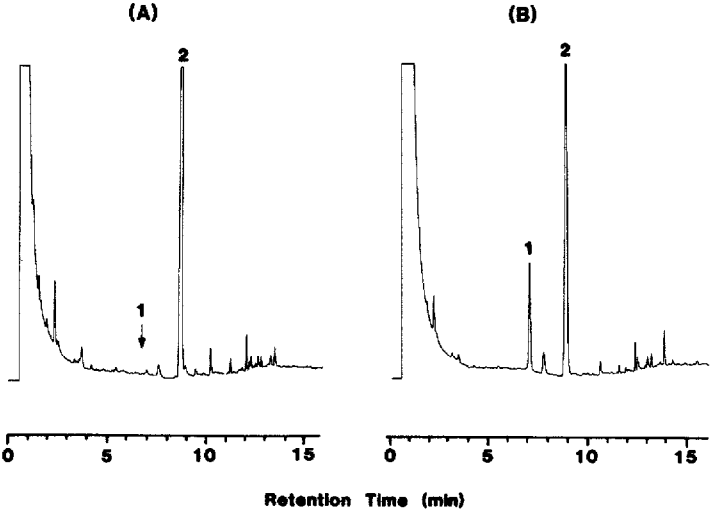


Fig. 3. Chromatograms obtained after analysis of (A) blank urine to which internal standard (peak 2) was added and (B) urine sample (0–8 h post-dose) from a volunteer who took gabapentin (peak 1), 300 mg.

tion ranges, with coefficients of determination usually approximately 0.999. For plasma, overall precision (R.S.D.) ranged from 6.2% at 0.5 μg/ml to 2.3% at 10 μg/ml ($n=18$ obtained by pooling results from six replicates on three separate occasions). Overall accuracy for the same specimens was 4.8% at 0.5 μg/ml and 2.8% at 10 μg/ml. Analogous data for urine assays showed precision

of 4.0% at 20 μg/ml and 6.8% at 500 μg/ml, with accuracy of 12.2 and 5.6% at the same two concentrations.

The minimum quantifiable concentration (MQC) for plasma was determined to be 0.2 μg/ml (at which concentration the precision was 20%) and for urine 5 μg/ml. Limits of detection were somewhat lower; a recognizable GC peak was obtained for 0.1 μg/ml in plasma, but quantification was unreliable. Recovery from plasma or urine was approximately 33%, throughout the concentration ranges studied. Despite this relatively low recovery the reproducibility of the procedure was very high. The major penalty was that the MQC would clearly have been lower if the recovery had been higher. The previously published HPLC method achieved a somewhat lower MQC, but the sensitivity of the present GC method has proven adequate for clinical applications.

As a further check on the performance of the assay, coded plasma and urine samples which had been assayed in the F.R.G. by the HPLC method [4] were sent to Brisbane by Dr. H. Hengy (Gödecke Research Institute, Freiburg, F.R.G.) and reassayed by GC. The comparison of results for plasma is given in Table I, and the correspondence is exceptionally good. Two urine samples

TABLE I

COMPARISON OF RESULTS FOR ASSAY OF GABAPENTIN IN PLASMA BY GC AND BY HPLC

Sample No.	Gabapentin concentration (μg/ml)	
	Brisbane (GC)	Freiburg (HPLC)
1	1.22	1.17
2	1.19	1.17
3	1.18	1.01
4	1.48	1.47
5	2.59	2.55
6	3.16	3.16
7	3.20	3.02
8	2.87	2.73
9	2.53	2.72
10	2.00	1.88
11	1.46	1.42
12	1.12	0.99
13	0.72	0.70
14	0.30	0.28
15	0.17	0.14
16	I.P.[a]	0.13
17	I.P.[a]	N.P.[b]
18	N.P.[b]	N.P.[b]

[a]Identifiable peak, but concentration < MQC

[b]No peak greater than baseline noise.

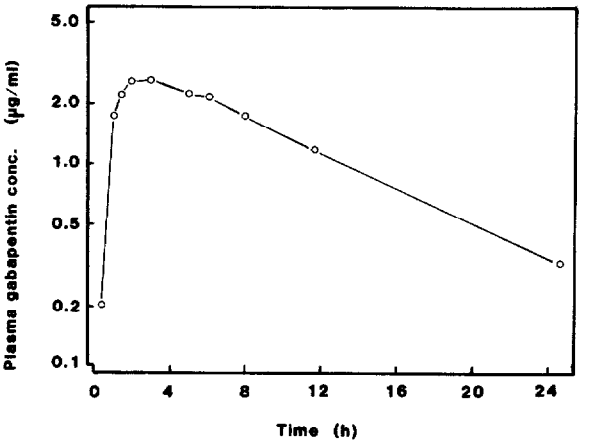


Fig. 4. Plasma gabapentin concentration–time profile for a volunteer who took a single oral dose of gabapentin, 300 mg.

gave values of 50.9 and 46.7 μg/ml (GC) and 46.1 and 46.2 μg/ml (HPLC), respectively.

The adequacy of the method for pharmacokinetic studies has been demonstrated. A typical plasma concentration–time curve following a single dose of gabapentin to a volunteer is shown in Fig. 4. A full report of pharmacokinetic studies has been presented elsewhere [6]. The assay is also currently being applied to monitoring plasma gabapentin concentrations in patients participating in clinical trials. Although the procedure is somewhat laborious, an analyst can easily process twenty specimens plus standards in a day, for chromatographing on the following day.

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