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Note

Determination of gabapentin in plasma and urine by high-performance liquid chromatography and pre-column labelling for ultraviolet detection

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Gabapentin [1-(aminomethyl)cyclohexaneacetic acid] (Fig. 1) is a new γ-aminobutyric acid (GABA) analogous substance [1] with penetration of the blood brain barrier and anticonvulsant activities, mainly in seizures elicited by interference with gabaergic transmission [2] or in seizures provoked by excitatory amino acids [3]; for a review, see ref. 4. It is well absorbed and excreted completely unchanged by humans [5] and shows no protein binding.

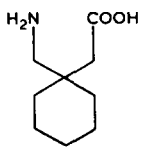

Fig. 1. Chemical structure of gabapentin.

For pharmacokinetic investigations, an assay method for gabapentin in biological fluids was required. This paper reports the sensitive and selective determination of gabapentin in the submicrogram and nanogram range in plasma and urine by means of high-performance liquid chromatography (HPLC). The method is based on the detection of amino acids by pre-column derivatization with 2,4,6-trinitrobenzenesulphonic acid (TNBS) [6, 7] utilising UV photometric detection. The applicability of the method to pharmacokinetic studies is demonstrated.

EXPERIMENTAL

Chromatography

A Series 2/2 high-performance liquid chromatograph equipped with a Rheo-

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dyne 7125 injection valve (Perkin-Elmer, Überlingen, F.R.G.) was used in combination with a Model SF 773 UV detector with a 12-μl flow cell (Kratos, Karlsruhe, F.R.G.). The analyses were performed on a 25 cm x 4 mm I.D. stainless-steel column packed with 10-μm LiChrosorb RP-18 (Knauer, Berlin, F.R.G.).

Chromatograms were recorded on a 0.001—10 V recorder (Hitachi—Perkin-Elmer, Überlingen, F.R.G.). Peak areas were determined by an Autolab System I computing integrator (Spectra-Physics, Darmstadt, F.R.G.).

All analyses were performed using a mobile phase consisting of 58% acetonitrile in water containing 0.5% acetic acid. The flow-rate was 1.0 ml/min. The chromatography was carried out at ambient temperature.

Reagents and standards

Gabapentin and the internal standard, 1-(aminomethyl)cycloheptaneacetic acid (Gö 3609), were obtained from the Chemistry Department, Gödecke Research Institute, Freiburg, F.R.G.

All chemicals were of the highest grade commercially available. They were purchased from E. Merck (Darmstadt, F.R.G.), except for TNBS, which was supplied by Serva (Heidelberg, F.R.G.).

For aqueous solutions, water was purified by reverse osmosis and additionally passed through a water purification system for adsorption of organic substances (Millipore, Neu Isenburg, F.R.G.).

Crystalline forms of 2,4,6-trinitrophenylgabapentin and 2,4,6-trinitro-1-(aminomethyl)cycloheptaneacetic acid were prepared by a procedure similar to that of Caudill et al. [7].

A stock standard solution of gabapentin was prepared by dissolving an appropriate amount of gabapentin in water. Working standards were prepared freshly in drug-free plasma from the stock solution to yield concentrations from 50 ng/ml to 10 μg/ml.

Sample preparation

The derivatization procedure is a modified version of that described by Caudill et al. [7] for the determination of GABA. A 0.5-ml aliquot of standard, control or patient plasma was placed in a 1.5-ml Eppendorf centrifuge tube and an appropriate amount of the internal standard, dissolved in 10 μl water, was added. This was followed by the addition of five drops of 2 *M* perchloric acid to deproteinize the sample. The tube was vortexed vigorously for a few seconds and then centrifuged for 2 min in an Eppendorf microcentrifuge at 15 000 *g* to precipitate the proteins. The supernatant was collected in stoppered conical glass tubes (100 x 14 mm) and 0.5 ml of 1 *M* sodium hydrogen carbonate and 50 μl of a 2 *M* aqueous solution of the derivatizing agent TNBS were added. The pH was adjusted to 8.5 with 0.1 *M* sodium hydroxide solution and the reaction was allowed to progress for 30 min at room temperature. The reaction was quenched by the addition of two drops of 25% hydrochloric acid. Toluene (3 ml) was added to the acidified sample and the mixture shaken for 10 min, followed by centrifugation for 2 min at 5000 *g*. The upper organic phase was transferred into a 5-ml tapered flask and evaporated to dryness on a rotary evaporator at 40°C. The residue was reconstituted with 100 μl of 0.2 *M* sodium

borate buffer (pH 8.5) and washed (vortexing for 1 min) with 1 ml of cyclohexane containing 10% of toluene. A volume of 10—50 μl of the sodium borate buffer solution containing the TNP derivatives was injected directly on to the HPLC column.

When analysing urine samples for their gabapentin content, 10—100 μl of urine were fortified with 2 μg of internal standard dissolved in 10 μl of water, and processed as described above for the plasma samples after the deproteinization step.

Stability studies were also conducted using a pool of plasma spiked with known amounts of gabapentin. Aliquots of these samples were frozen at −18°C and analysed over a period of six months. No significant changes in the gabapentin content were seen.

The within-run precision was evaluated by assaying a prepared gabapentin plasma pool.

RESULTS AND DISCUSSION

The direct isolation of gabapentin from an aqueous matrix was hampered by its hydrophilic nature. Therefore, derivatization was carried out in order to facilitate the extraction of the substance and to aid its sensitive detection by attachment of a chromophoric group.

TNBS proved to be a suitable derivatizing reagent, resulting in the formation of the corresponding HPLC-detectable TNP derivatives of gabapentin and the

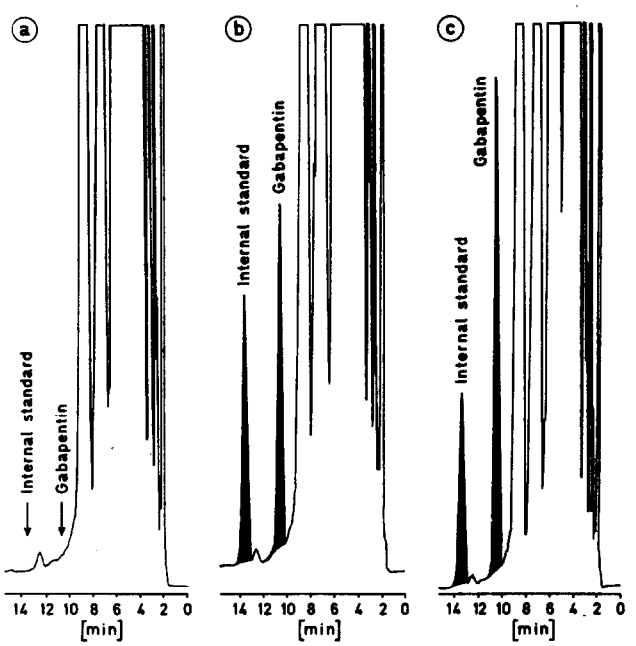

Fig. 2. Chromatograms of human plasma samples obtained after derivatization with 2,4,6-trinitrobenzenesulphonic acid. (a) Blank plasma (0.5 ml). (b) Plasma sample, supplemented with 500 ng of gabapentin and 400 ng of internal standard per 0.5 ml. (c) Plasma sample, 10 h after oral administration of 200 mg of gabapentin to a human volunteer (amount equal to 1844 ng/ml gabapentin).

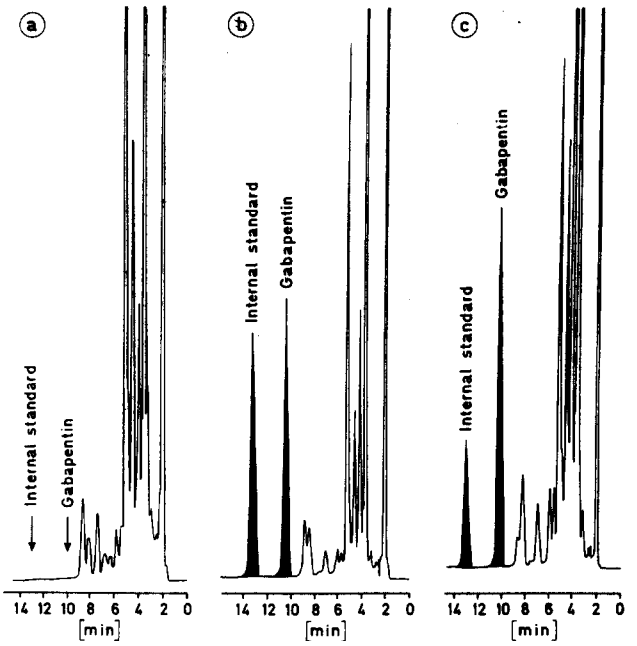

Fig. 3. Chromatograms of human urine samples obtained after derivatization with 2,4,6-trinitrobenzenesulphonic acid. (a) Blank urine (50 μl). (b) Urine sample, fortified with 2 μg of gabapentin and 2 μg of internal standard per 50 μl. (c) Urine sample, 12—15 h fraction after oral administration of 200 mg of gabapentin to a human volunteer (amount equal to 4.8 μg of gabapentin per 50 μl).

internal standard. Amino acids or biogenic amines, present in large amounts in plasma and urine, also reacted with TNBS, forming intensely yellow-coloured compounds. However, these reaction products appeared at the beginning of the chromatogram, were well separated and did not interfere with the compounds of interest.

Typical chromatograms of plasma and urine samples are shown in Figs. 2 and 3. The retention times for 2,4,6-trinitrophenylgabapentin and 2,4,6-trinitrophenyl-1-(aminomethyl)cycloheptaneacetic acid (internal standard) were 10.3 and 13.2 min, respectively. The recovery and derivatization yield of gabapentin and the internal standard with the above method were ca. 90% when comparing the peak heights with directly injected TNP-gabapentin and internal standard derivatives. The minimum detectable concentrations were determined to be about 10 ng/ml in plasma. The peak-area ratios for gabapentin and the internal standard were linearly related ($r = 0.999$) to the amount of gabapentin added to blank plasma over a range from 20 ng/ml to 10 μg/ml.

The reproducibility of the calibration graphs was assessed by assaying triplicate plasma standards over a one-day period. The relative standard deviations of the peak-area ratios between the lowest and highest concentrations were found to range from 8.6% to 0.5%, respectively. These data are summarized in Table I. To enhance the precision when analysing lower concentrations of gabapentin (below 400 ng/ml in plasma), only 150 ng of internal standard were used.

TABLE I

WITHIN-DAY PRECISION AND ACCURACY

Peak-height ratios of gabapentin versus internal standard after three calibration runs with spiked human plasma ($n = 3$).

Amount (ng per 0.5 ml)	Amount of internal standard used (ng)	Mean peak-height ratio	Coefficient of variation (%)
10	150	0.113	8.67
20		0.207	4.73
50		0.503	9.80
100		0.987	0.58
200		2.037	1.58
500	1200	0.4730	2.43
1000		0.9270	0.81
2000		1.8900	2.01
5000		4.6200	0.43

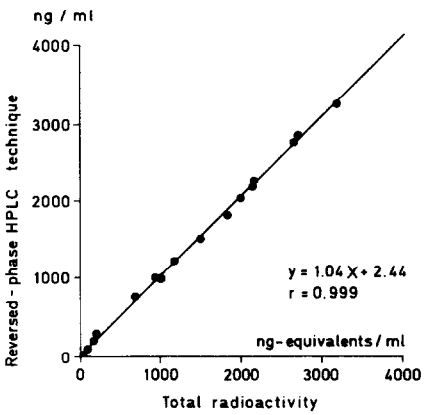

Fig. 4. Comparison of HPLC data and total radioactivity. Gabapentin concentrations in a series of human plasma samples following an oral dose of 200 mg of ^{14}C-labelled compound, determined both by HPLC and by total radioactivity monitoring.

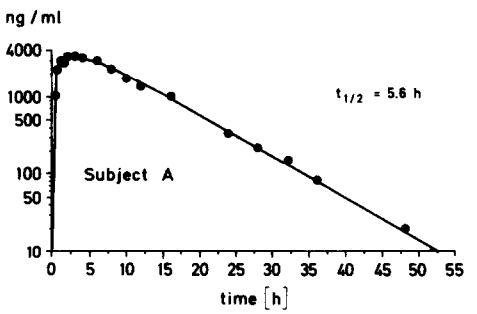

Fig. 5. Plasma levels of gabapentin following an oral dose of 200 mg to a human volunteer, obtained by HPLC.

Plasma and urine samples, obtained in the course of a metabolic study in human volunteers using ^{14}C-labelled gabapentin [5], were assayed by HPLC. These samples had been analysed by liquid scintillation counting on a previous occasion [5]. As gabapentin does not undergo metabolic transformation in humans, the total ^{14}C radioactivity data correspond to the unchanged compound in plasma. The results of both the HPLC and radioactivity detection are presented in Fig. 4 and exhibit an excellent correlation (slope = 1.04, $r = 0.999$) between the methods over a concentration range from 20 ng/ml to 3.5 μg/ml.

Fig. 5 shows a characteristic plasma level versus time profile following oral administration of 200 mg of gabapentin to a male volunteer.

REFERENCES

1 G. Satzinger, J. Hartenstein, M. Herrmann and W. Heldt, Ger. Offen., DE 2,460,891, 1974.
2 G.D. Bartoszyk, E. Fritschi, M. Herrmann and G. Satzinger, Naunyn-Schmiedeberg's Arch. Pharmacol., 322 (1983) R94.
3 G.D. Bartoszyk, Naunyn-Schmiedeberg's Arch. Pharmacol., 324 (1983) R24.
4 Anonymous, Drugs Future, 9 (1984) 418.
5 A. von Hodenberg and K.-O. Vollmer, Naunyn-Schmiedeberg's Arch. Pharmacol., 324 (1983) R74.
6 D.J. Edwards, in K. Blau and G.S. King (Editors), Handbook of Derivatives for Chromatography, Heyden, London, 1977, p. 395.
7 W.L. Caudill, G.P. Houck and R.M. Wightman, J. Chromatogr., 227 (1982) 331.