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Gas chromatographic assay of vigabatrin enantiomers in plasma

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ABSTRACT

A rapid and specific gas chromatographic method has been developed for the determination of plasma *R*(−)- and *S*(+)-vigabatrin concentrations. The method involves a double derivatisation step, chromatography on a megabore Chirasil-Val capillary column and thermionic specific detection. Concentrations in the range 1.0–200 μg/ml can be measured for *R*(−)-vigabatrin, and 0.5–100 μg/ml for *S*(+)-vigabatrin, using 250 μl of plasma. The assay is suitable for pharmacokinetic studies and routine therapeutic drug monitoring in humans.

INTRODUCTION

Vigabatrin (γ-vinyl-γ-aminobutryic acid, GVG; Fig. 1) is a structural analogue of GABA (γ-aminobutyric acid) and inhibits the enzyme GABA transaminase irreversibly [1]. This inhibition prevents the physiological degradation of GABA, thus causing the increased brain levels of this inhibitory neurotransmitter which are believed responsible for the drug's antiepileptic action. GVG is chiral and is marketed as a racemate, but only the *S*(+)-enantiomer is pharmacologically active [2]. *R*(−)-GVG does not undergo chiral inversion and does not interfere with the action of *S*(+)-GVG. The pharmacokinctic profiles of the two enantiomers in human volunteers differ in certain regards [1]. The enantiomers have been separated by high-performance liquid chromatography [3] and by gas chromatography (GC) [4] but the only published method for their measurement in plasma involves the use of gas chromatography–mass spectrometry (GC–MS) [5]. The GC method described below was developed as a simpler alternative to GC–MS, and should facilitate further pharmacokinetic studies and permit assessment of the clinical utility of monitoring plasma *S*(+)-GVG concentrations.

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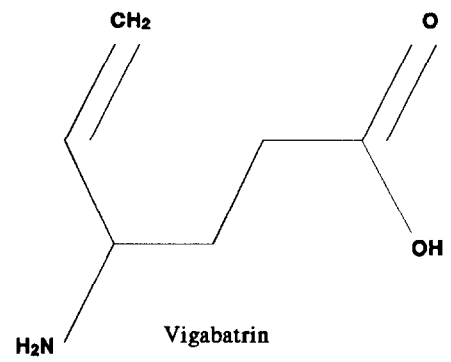

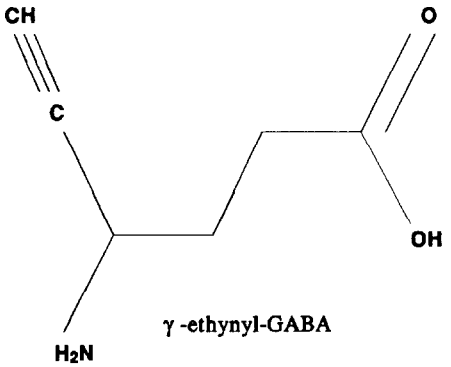

Fig. 1. Chemical structures for vigabatrin and γ-ethynyl-GABA.

EXPERIMENTAL

Reagents

Methanol (ChromAR grade) and butanol (nanograde) were obtained from Mallinckrodt (Clayton, Australia), toluene (AR grade) from Ajax (Auburn, Australia), trifluoroacetic anhydride (TFAA) (>99% purity) from Tokyo Kasei (Tokyo, Japan) and acetyl chloride (Analar grade) from BDH (London, UK). Butanolic hydrogen chloride was prepared, as required, by the addition of 1.25 ml of acetyl chloride to 25 ml of butanol.

Merrell Dow Pharmaceuticals (Sydney, Australia) supplied samples of the vigabatrin racemate (*rac.*-GVG), the individual isomers, *R*(−)-GVG and *S*(+)-GVG, and also *S*-γ-ethynyl-GABA (GEG; Fig. 1), which served as the internal standard.

Standards and internal standard solutions

Stock aqueous solutions of *R*(−)-GVG (4 mg/ml) and *S*(+)-GVG (2 mg/ml) were prepared separately. Appropriate mixtures and dilutions yielded a series of standard solutions containing both *R*(−)-GVG and *S*(+)-GVG. The concentration range was intended to be clinically relevant and the solutions were prepared as required. Plasma standards were obtained by adding 25 μl of each of the standard solutions to 250 μl of drug-free human plasma to yield final concentrations of 200 and 100, 100 and 50, 50 and 20, 20 and 10, 10 and 5, 5 and 2, 2 and 1, and 1 and 0.5 μg of *R*(−)-GVG and *S*(+)-GVG, respectively, per ml of plasma. Plasma seeded controls were prepared at three concentrations: 5.0 μg/ml *R*(−)-GVG with 2.0 μg/ml *S*(+)-GVG, 20.0 μg/ml *R*(−)-GVG with 10.0 μg/ml *S*(+)-GVG, and 100.0 μg/ml *R*(−)-GVG with 50.0 μg/ml *S*(+)-GVG. These controls were prepared on one occasion and stored at −20°C until required.

A stock internal standard solution (1 mg/ml) was prepared by dissolving 10 mg of GEG in 10 ml of water, and a working solution (400 μg/ml) was made from the stock solution. Aqueous solutions were stored at 4°C and showed no evidence of instablity at four weeks.

Sample preparation

Venous blood (1–2 ml) from epileptic patients treated with *rac.*-GVG was collected into tubes containing 125 I.U. of lithium heparin. Following centrifugation, the plasma was transferred to 5-ml plastic tubes and stored at −20°C until analysed.

Aliquots (25 μl) of working internal standard solution were added to patient plasma samples, plasma standards (as described above) and plasma seeded controls, contained in 15-ml glass assay tubes. Water (25 μl) was added to patient plasma samples and plasma seeded controls to give a total volume (275 μl), equal to that of the

plasma standards. After mixing, methanol (1 ml) was added and the tubes were shaken by hand (3 min), then centrifuged at approximately 1000 *g* (5 min). The supernatants were transferred by Pasteur pipette to 5-ml Reacti-vials (Pierce, Rockford, IL, U.S.A) and evaporated to dryness under a stream of air. The butyl esters of the carboxylic acid moieties were formed by adding butanolic hydrogen chloride (100 μl) to the dried residue, then sealing and heating at 65°C for 30 min. The reagents were then removed under a stream of air and the amine functional groups acetylated by adding TFAA (100 μl) to the dried residue, sealing and standing at room temperature for 30 min. The excess reagent was then removed under a stream of air and the residue reconstituted in toluene (30 μl). This volume was transferred to an injection vial and 0.2 μl was injected onto the GC column.

Equipment and chromatographic conditions

The GC system consisted of a Varian 3400 gas chromatograph with a universal on-column injector and a Varian 8100 autosampler. The injector was set at 150°C. The Varian thermionic specific detector output was monitored using a Shimadzu CR4A integrator equipped with floppy disk drives and a CRT display. The detector was set at 300°C with the bead gases, air and hydrogen, at flow-rates of 175 and 4.6 ml/min, respectively.

A megabore Chirasil-Val non-packed 20 m × 0.53 mm I.D. capillary column with film thickness 0.125 μm from Alltech (Deerfield, IL, USA) was used to separate the enantiomers. The carrier gas was helium (2.0 ml/min) and the make-up gas nitrogen (20 ml/min). The column oven temperature was programmed in three stages, as follows: initial 80°C (hold 1 min); then ramp at 20°C/min to 129°C (hold 2 min); then ramp at 1°C/min to 135°C (hold 20 min); then ramp at 20°C/min to 200°C (hold 10 min).

The compounds of interest, *R*(−)-GVG, *S*(+)-GVG and GEG, eluted at 21.5, 22.2 and 23.2 min, respectively. Quantitation was achieved using peak-area ratios.

Specificity

Specificity of the procedure was evaluated by assaying a range of drug-free plasma samples and samples from patients taking medications other than GVG. Of particular interest were the anticonvulsants that were likely to be coadministered, such as phenytoin, phenobarbitone, clonazepam, carbamazepine, valproate and primidone.

Recovery

Recoveries of GVG enantiomers from plasma were assessed by comparing peak-area ratios [*R*(−)-GVG/GEG and *S*(+)-GVG/GEG] obtained after extracting the GVG enantiomers from spiked plasma standards with the ratios obtained when identical quantities were dispensed and derivatised without extraction. In the extraction studies, GEG was added after the extraction of GVG to permit direct comparison with the results for the non-extracted samples.

Assay validation

Linearity of the assay was assessed on three occasions approximately one week apart. Each assessment comprised of three standard curves, each involving eight concentrations points (as described previously) and six plasma seeded controls at the three different concentrations. Intra-assay precision and accuracy were assessed by replicate samples ($n = 6$) of the plasma seeded controls from occasion 1. Overall precision and accuracy were obtained using the data from all three occasions ($n = 18$).

RESULTS AND DISCUSSION

A successful GC assay for the extraction from plasma of an amino acid analogue, gabapentin, involving a double derivatisation step had previously been developed in the authors' laboratory [6]. Therefore GVG, extracted from deproteinized plasma, was also derivatised by esterification (butylation) of the carboxy function and N-trifluoroacetylation of the amino function, prior to chromatography.

Previously, GABA analogues as the N-penta-

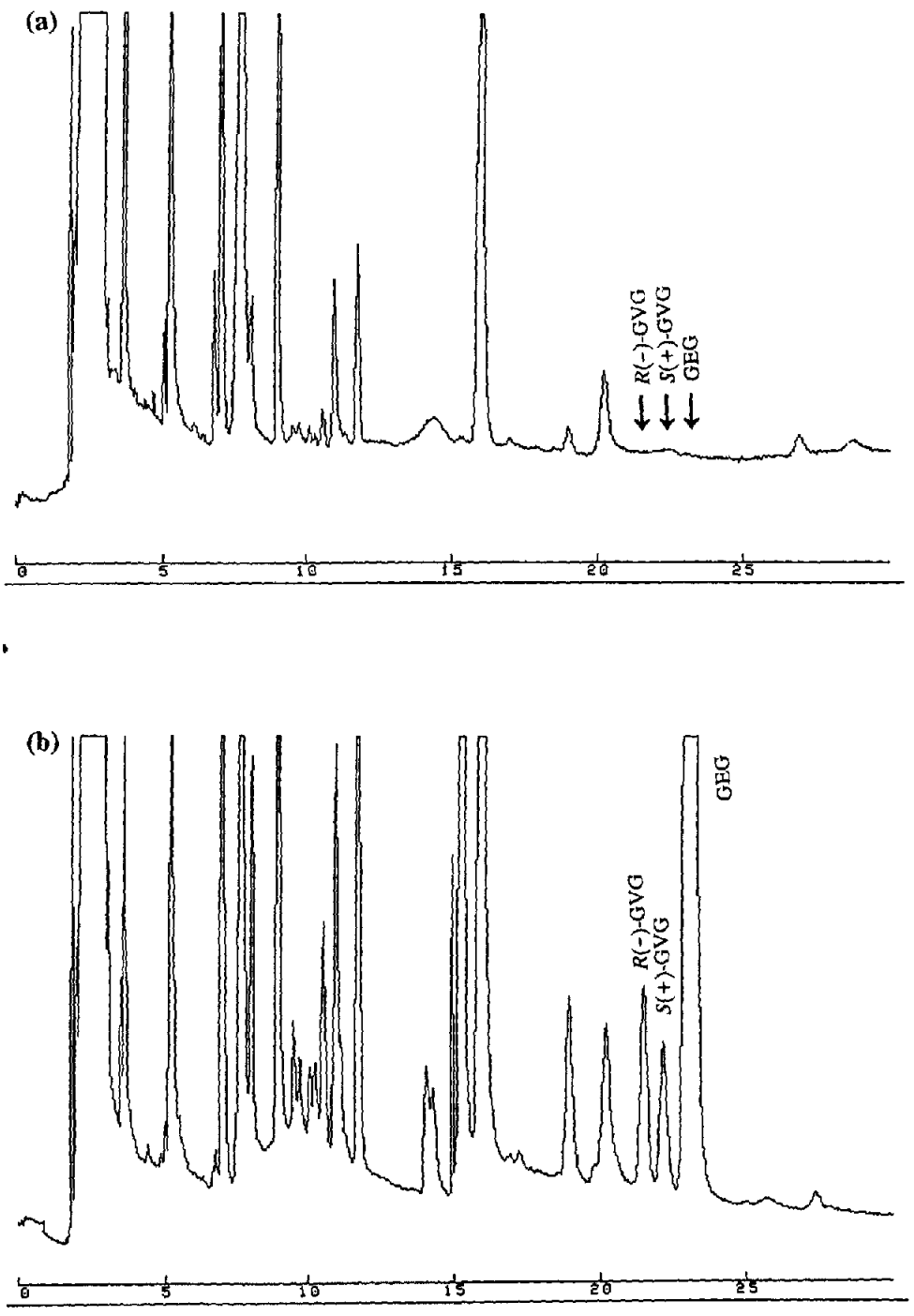

Fig. 2. Typical chromatograms for (a) blank plasma and (b) plasma from a patient receiving vigabatrin whose vigabatrin levels were 1.82 μg/ml *R*(−)-GVG and 1.36 μg/ml *S*(+)-GVG.

fluoropropionyl ethyl esters have been separated on Chirasil-Val by GC using flame ionization detection (FID) [5], and N-trifluoroacetic esters of γ-amino acids using FID on other stationary phases [7,8]. However conventional, FID failed to provide adequate specificity in the case of GVG, since an unidentified endogenous compound interfered with the quantitation of *R*(−)-GVG. Thermionic specific detection was then tried and yielded adequate sensitivity and specificity.

Typical chromatograms for the analysis of blank plasma and plasma from a subject who took GVG are shown in Fig. 2. The internal standard and the two isomer peaks occur in an area of the chromatogram that is free of interfering endogenous compounds.

Dead time (t_m) was 1.85 min while k_R and k_S values were 10.7 and 11.1, respectively. The separation coefficient, α, was 1.04 where $\alpha = (t_S - t_m)/(t_R - t_m)$. The peak resolution, R_S, is defined as $R_S = 1.18\ [(t_{R(S)} - t_{R(R)})/(W_{h(R)} + W_{h(S)})]$, where W_h is the width at half-height. The R_S was equal to 1.31.

The method has been used to evaluate steady-state GVG enantiomer plasma levels in a group of epileptic patients participating in a clinical trial of the drug. The relation between the two enantiomer concentrations appeared linear, the mean ratio of *R*(−)-GVG to *S*(+)-GVG being 1.3:1 (Fig. 3).

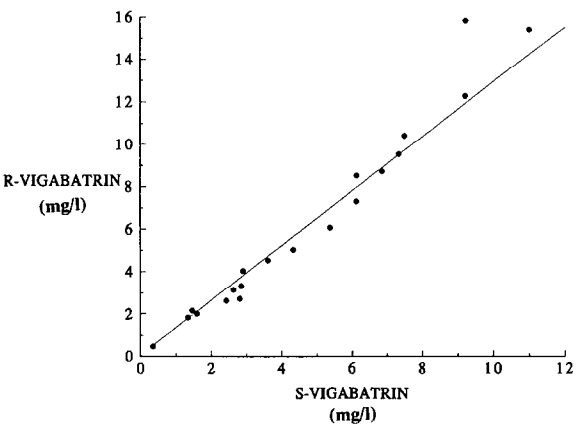

Fig. 3. Plot of simultaneous concentrations of *R*(−)-GVG *versus* *S*(+)-GVG showing the linear relationship between the two.

Specificity

No interference in the assay was observed due to commonly used anticonvulsants likely to be coadministered with GVG.

Recovery

Recoveries for *R*(−)-GVG, *S*(+)-GVG and GEG were 98.5, 95.0 and 95.8%, respectively.

Assay validation

The assay procedure was formally validated and its performance characteristics are summarised briefly. The linearity was good with coefficients of determination of approximately 0.997 for *R*(−)-GVG and 0.995 for *S*(+)-GVG (n = 24). The plasma seeded controls, stored at −20°C, showed no evidence of instability at four months.

Precision

For *R*(−)-GVG, overall precision (R.S.D.) ranged from 6.4% at 5 μg/ml to 4.6% at 100.0 μg/ml and for *S*(+)-GVG from 7.4% at 2.0 μg/ml to 7.0% at 50.0 μg/ml (n = 18).

TABLE I

PRECISION AND ACCURACY OF THE GC METHOD FOR THE QUANTITATION OF VIGABATRIN ENANTIOMERS

Mean of six measurements of vigabatrin (GVG) concentrations obtained on one occasion (intra-assay) and of eighteen measurements obtained on three separate occasions (overall); values in parentheses are precision (relative coefficient of variation) and accuracy (mean relative errors).

True concentration (μg/ml)	Measured concentration (μg/ml)	
	Intra-assay	Overall
R(−)-GVG		
5.0	4.85 (3.3, 4.0)	4.74 (6.4, 6.3)
20.0	19.0 (3.4, 5.0)	18.9 (5.2, 6.9)
100.0	102 (4.6, 4.4)	99.9 (4.6, 4.0)
S(+)-GVG		
2.0	1.88 (4.5, 6.5)	1.87 (7.4, 7.9)
10.0	9.60 (6.6, 6.1)	9.62 (6.4, 5.7)
50.0	50.8 (7.0, 5.8)	49.9 (7.0, 5.5)

Accuracy

The overall accuracy for *R*(−)-GVG was 6.3% at 5.0 μg/ml and 4.0% at 100.0 μg/ml and for *S*(+)-GVG 7.9% at 2.0 μg/ml and 5.5% at 50.0 μg/ml (Table I).

CONCLUSION

The method described is rapid, specific, relatively inexpensive and employs equipment generally available in clinical pathology laboratories. It appears suitable for pharmacokinetic studies and the routine monitoring of plasma GVG enantiomer levels in patients treated with the racemic drug.

REFERENCES

1 S. M. Grant and R. C. Heel, *Drugs*, 41 (1991) 889.
2 E. Rey, G. Pons, M. O. Richard, F. Vauzelle, P. H. D'Athis, C. Chiron, O. Dulac, D. Beaumont and G. Olive, *Br. J. Clin. Pharmacol.*, 30 (1990) 253.
3 T. Chen and J. J. Contario, *J Chromatogr.*, 314 (1984) 495.
4 J. Wagner, Z. E. Wolf, B. Heintzelmann and C. Gaget, *J. Chromatogr.*, 392 (1987) 211.
5 K. D. Haegele, J. Schoun, R. G. Aiken and N. D. Hucbert, *J. Chromatogr.*, 274 (1983) 103.
6 W. D. Hooper, M. C. Kavanagh and R. G. Dickinson, *J. Chromatogr.*, 529 (1990) 167.
7 R. Charles and E. Gil-Av, *J. Chromatogr.*, 195 (1980) 317.
8 R. Charles and K. Watabe, *J. Chromatogr.*, 298 (1984) 253.