ELSEVIER

Journal of Chromatography B, 716 (1998) 233–238

JOURNAL OF CHROMATOGRAPHY B

High-performance liquid chromatographic analysis of vigabatrin enantiomers in human serum by precolumn derivatization with *o*-phthaldialdehyde–*N*-acetyl-L-cysteine and fluorescence detection

T.A.C. Vermeij, P.M. Edelbroek*

Laboratory of Clinical Chemistry and Clinical Pharmacology, Instituut voor Epilepsiebestrijding, P.O. Box 21, 2100 AA Heemstede, The Netherlands

Received 30 December 1997; received in revised form 4 May 1998; accepted 2 June 1998

Abstract

A rapid and simple method is presented for the determination of vigabatrin enantiomers in human serum by high-performance liquid chromatography. Serum is deproteinized with trichloroacetic acid and aliquots of the supernatant are precolumn derivatized with *o*-phthaldialdehyde and *N*-acetyl-L-cysteine, resulting in the formation of diastereomeric isoindoles. Separation was achieved on a Spherisorb 3ODS2 column using a gradient solvent program and the column eluent is monitored using fluorescence detection. L-Homoarginine was used as an internal standard. Within-day precisions (C.V.; $n=8$) were 2.8 and 1.1%, respectively, for the (*R*)-(−)- and (*S*)-(+)-enantiomer in serum containing 15.4 mg/l (*RS*)-vigabatrin. The method was linear in the 0–45 mg/l range for both enantiomers and the minimum quantitation limit was 0.20 mg/l for (*R*)-(−)-vigabatrin and 0.14 mg/l for (*S*)-(+)-vigabatrin. No interferences were found from commonly co-administered antiepileptic drugs and from endogenous amino acids. The method is suitable for routine therapeutic drug monitoring and for pharmacokinetic studies.

Keywords: Enantiomer separation; Vigabatrin

1. Introduction

The new anticonvulsant drug vigabatrin (γ-vinyl-GABA) is a structural analogue of γ-aminobutyric acid (GABA) and its action is attributed to the irreversible inhibition of the enzyme GABA-transaminase, thus preventing the physiological degradation of GABA in the brain; a secondary mechanism of a blockade for GABA uptake is also suggested [1]. Vigabatrin is supplied as a racemic mixture of the enantiomers, but only the (*S*)-(+)-enantiomer is pharmacologically active [2].

Determination of vigabatrin as the racemate can be performed by high-performance liquid chromatography (HPLC) and fluorescence detection after rapid and simple derivatization using *o*-phthaldialdehyde (OPA) and 2-mercaptoethanol [3,4]. For the analysis of vigabatrin enantiomers in human serum or plasma, the only methods reported make use of gas chromatography–mass spectrometry (GC–MS) [5] or gas–liquid chromatography (GLC) [6]. Both assays imply expensive chiral capillary columns and time-consuming and laborious sample preparation. Only one

*Corresponding author.

0378-4347/98/$19.00
PII: S0378-4347(98)00269-2

method has been reported for HPLC separation of the vigabatrin enantiomers using the formation of diastereometric derivatives by reaction with *tert*.-butoxy-carbonyl-L-leucine *N*-hydroxysuccinimide ester [7], but this method was not applied to biological materials.

It has been shown that the enantioseparation of mixtures of DL-amino acids in a food matrix can be performed by HPLC after converting them to the diastereomeric isoindolyl derivatives using OPA and chiral *N*-acylated cysteines [8]. To study the pharmacokinetics of vigabatrin, we applied this approach to develop a rapid and simple HPLC method for the separation and quantification of both optical isomers in serum. After precipitation of serum proteins with trichloroacetic acid, an aliquot of the supernatant was derivatized with OPA and *N*-acetyl-L-cysteine under alkaline conditions. The diastereomers formed are then separated by reversed-phase HPLC using a gradient solvent program and fluorescence detection.

2. Experimental

2.1. Reagents and standards

(*RS*)-γ-Vinyl-γ-aminobutyric acid (vigabatrin, MRI-71754), (*R*)-(−)-γ-vinyl-γ-aminobutyric acid (MDL-71894) and (*S*)-(+)-γ-vinyl-γ-aminobutyric acid (MDL-71890) were gifts from the Merrell Dow Research Institute (Merrell Dow Pharmaceuticals, Egham, UK). The internal standard L-homoarginine·HCl was obtained from Koch-Light Laboratories (Coinbrook, Bucks, UK). Methanol (Lichrosolv), 85% ortho-phosphoric acid, boric acid, sodium hydroxide, sodium acetate and trichloroacetic acid (all p.a.) were from Merck (Darmstadt, Germany). Acetonitrile (Ultra Gradient Grade) and acetic acid (HPLC reagent) were from J.T. Baker (Phillipsburg, NJ, USA). The derivatizing reagent *ortho*-phthaldialdehyde (OPA) was purchased from Sigma (St. Louis, MO, USA) and *N*-acetyl-L-cysteine (AcCys) was from OPG Farma (Utrecht, The Netherlands). Ultrapure water was prepared using the Millipore-Q-plus water purification system (Millipore, Bedford, MA, USA).

Borate buffer (0.1 *M*, pH 10.4) was prepared by dissolving 3.1 g boric acid in 490 ml water. After addition of 3.0 ml 12 *M* NaOH the pH was adjusted to 10.4 with 2–3 ml 2 *M* NaOH.

Stock solution of OPA was prepared by dissolving 125 mg OPA in 1.0 ml methanol. The derivatizing reagent working solution was prepared by adding 50 μl OPA stock solution to 1.0 ml 0.1 *M* borate buffer (pH 10.4) and then dissolving 50 mg *N*-acetyl-L-cysteine in the solution. The storage life of the stock solution is at least 4 weeks at 4°C; the working solution must be prepared daily.

Stock solutions of the racemic (*RS*)-vigabatrin, of both enantiomers and of the internal standard (all 1 mg/ml) were prepared in water and stored at 4°C. The working internal standard solution was prepared by diluting the stock solution with 25 volumes of water prior to use. Calibration serum was prepared by spiking blank bovine serum with racemic (*RS*)-vigabatrin in three concentrations: 8, 16 and 24 mg/l.

2.2. Instrumentation

HPLC analysis was carried out on a P4000 quarternary solvent delivery system equipped with an AS3500 autosampler (Thermo Separation Products, San Jose, CA, USA). Integration and system parameters were controlled by Spectrasystem PC1000 software (Thermo Separation Products). Separation was performed on a home-made reversed-phase analytical column (15×0.46 cm) packed with Spherisorb 3ODS2 (Phase Separations, Deeside, UK) maintained at room temperature. The column eluate was monitored by a FP920 fluorescence detector (Jasco, Tokyo, Japan) set at an excitation wavelength of 330 nm and an emission wavelength of 450 nm. Detector gain was set at 100. The mobile phase consisted of a mixture of acetonitrile (A) and 55 m*M* sodium acetate in water, pH adjusted to 7.60 with 1% acetic acid solution in water (B) and was delivered as a linear gradient at a flow-rate of 1.0 ml/min: 5% A (0 min), 17.5% A (20 min), 35% A (21 min), 35% A (24 min), 5% A (25 min), 5% A (27 min).

2.3. Sample preparation

To 200 μl of serum was added 50 μl of the internal standard working solution and 100 μl of a

20% solution of trichloroacetic acid in water. The mixture was vortexed for 15 s and then centrifuged for 10 min at 2600*g*. Twenty microliters of supernatant was transferred to an autosampler microvial (300 μl), 100 μl of borate buffer was added and the capped vial was placed in the autosampler. Through an automatic sample preparation method with the autosampler, 10 μl OPA–*N*-acetyl-L-cysteine working reagent was added and after 1 min 5 μl of the reaction product was injected onto the HPLC system.

2.4. Patients

Serum samples from 34 adult epileptic patients treated with vigabatrin racemate, almost always in combination with one or more conventional anticonvulsants (carbamazepine, phenytoin, phenobarbital, primidone, valproate, acetazolamide, clobazam and flunarizin), were analyzed. The group included 18 females and 16 males with a mean (±SD; range) age of 36.7 (±9.4; 19–53) and 35.9 (±12.8; 17–62) years, respectively. The mean (range) vigabatrin dose was 1600 mg (500–2500 mg) for the females and 1900 mg (500–4000 mg) for the males, given as the racemate.

Furthermore, from one adult patient on phenytoin (425 mg), clobazam (20 mg) and vigabatrin (2500 mg) serum samples were collected before and 30, 60, 120, 240 and 360 min after receiving his morning dose of 1000 mg vigabatrin racemate.

3. Results and discussion

3.1. Derivatization

OPA reacts with all primary amino acids under alkaline conditions and in the presence of a thiol. The reaction is complete within 1–2 min and occurs at room temperature [9]. The fluorescence of the formed isoindole starts to decrease after 10–20 min. Fig. 1 shows the reaction of vigabatrin with OPA and *N*-acetyl-L-cysteine. The asymmetric *N*-acetyl-L-cysteine results in the formation of diastereomeric derivatives of amino acids, having different physical and chemical properties and thus separable by means of chromatography.

3.2. Chromatography

Fig. 2 shows chromatograms of (a) a blank patient sample (patient not on vigabatrine treatment), (b) a patient sample during vigabatrin therapy and (c) a calibration sample in bovine serum. OPA derivatives of the internal standard L-homoarginine (retention time 13.1 min), the (*R*)-(−)-enantiomer (retention time 14.2 min) and the (*S*)-(+)-enantiomer (retention time 16.4 min) of vigabatrin are well separated from the endogenous amino acids. The critical point is the separation of (*R*)-(−)-vigabatrin and the next eluting amino acid derivative which is strongly influenced by the pH of the mobile phase. Retention times of OPA derivatives of both enantiomers were deter-

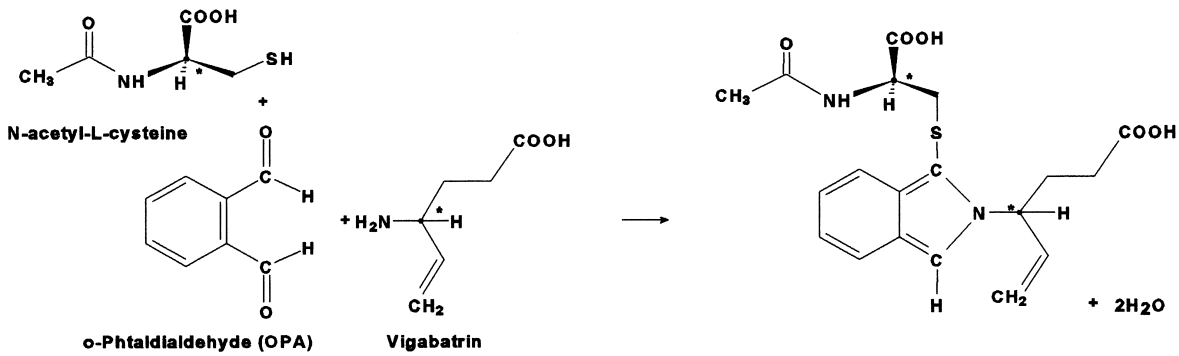

Fig. 1. Reaction of vigabatrin with OPA and *N*-acetyl-L-cysteine.

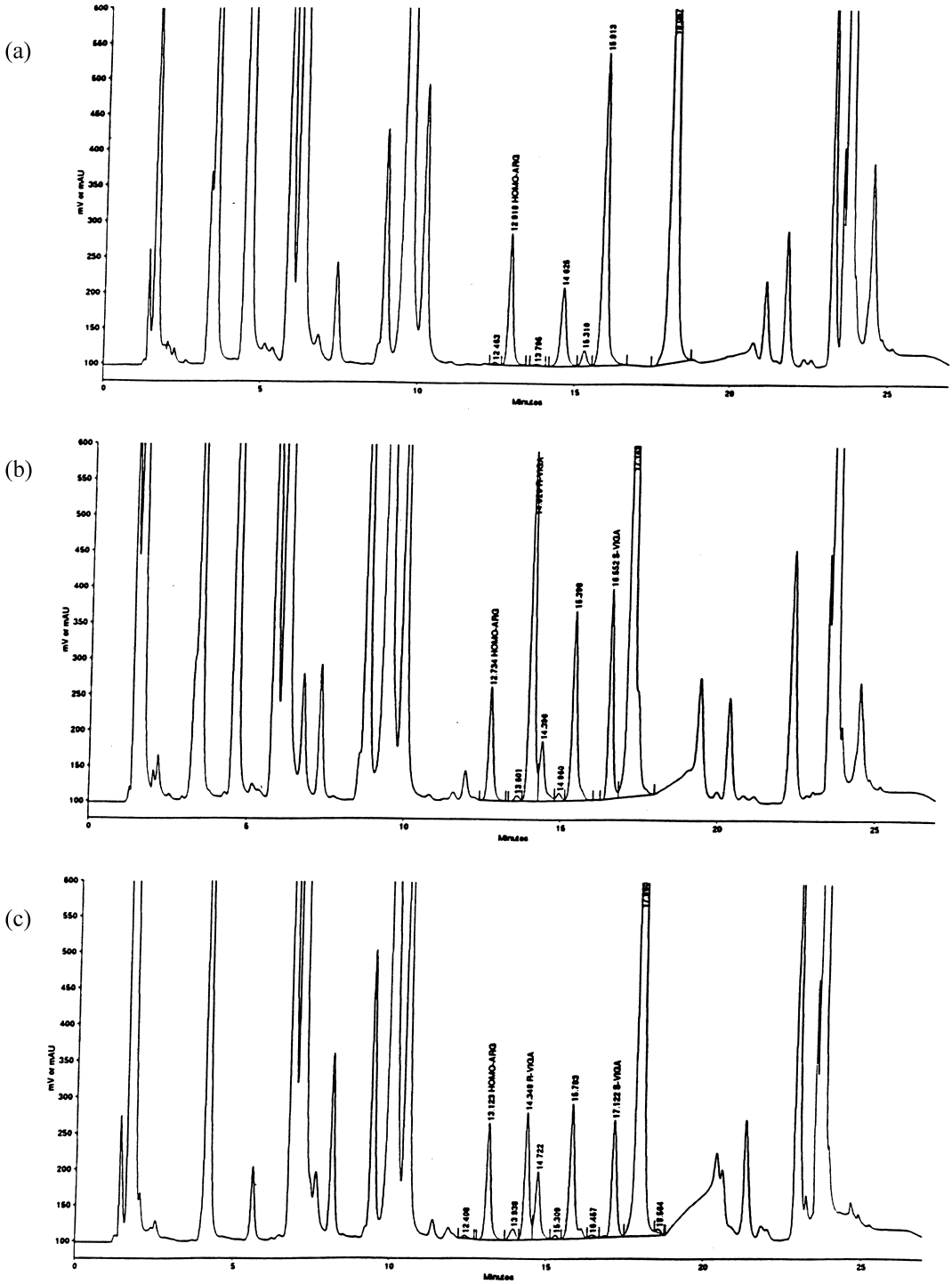

Fig. 2. HPLC chromatograms of (a) a blank patient sample (not on vigabatrin therapy), (b) a patient sample during vigabatrin therapy containing 24.2 mg/l (*R*)-(−)-vigabatrin and 14.5 mg/l (*S*)-(+)-vigabatrin, and (c) a calibration sample containing 7.96 mg/l of both enantiomers. Retention times are 12.7 min for the internal standard, 14.0 min for (*R*)-(−)-vigabatrin and 16.6 min for (*S*)-(+)-vigabatrin.

mined once from the pure (*R*)-(−)- and (*S*)-(+)-compounds.

Column life-time, often a problem using OPA derivatization, is extended by using a small sample injection volume of 5 μl, made possible by a higher sensitivity gain setting of the detector. Replacing the top layer of the column when necessary (broad or split peaks) and reversing the flow direction will prolong the column life-time even more.

3.3. Recovery

Recovery of both enantiomers was estimated by comparison of peak heights in extracted spiked bovine serum with those in standard solutions and appeared to be 88.2±4.5% ($n=3$) and 92.4±3.0% ($n=3$) for the (*R*)-(−)- and (*S*)-(+)-enantiomer, respectively.

3.4. Precision and reproducibility

Subsequent analysis of bovine serum spiked with 15.4 mg/l (*RS*)-vigabatrin resulted in good reproducibility. The coefficient of variation was 2.8% for (*R*)-(−)-vigabatrin (mean 7.85 mg/l; $n=8$) and 1.1% for (*S*)-(+)-vigabatrin (mean 7.72 mg/l; $n=8$). The day-to-day variability was estimated by analysis of bovine serum spiked with (*RS*)-vigabatrin at two different concentrations (7.7 and 23.1 mg/l) on five separate days and resulted in coefficients of variation of 5.7% (mean 3.78 mg/l) and 3.9% (mean 11.72 mg/l) for the (*R*)-(−)-enantiomer and of 4.2% (mean 3.88 mg/l) and 4.7% (mean 11.64 mg/l) for the (*S*)-(+)-enantiomer.

3.5. Linearity and sensitivity

The method showed excellent linearity for both enantiomers from 0 to 45 mg/l. The linear regression line for (*R*)-(−)-vigabatrin was calculated as $y = -0.191 + 1.020x$ ($n=15$) with $R^2=0.9972$ and for (*S*)-(+)-vigabatrin it was $y = -0.077 + 1.017x$ ($n=15$) with $R^2=0.9974$.

The limit of quantitation was defined as the lowest concentration that can be determined with a coefficient of variation of 10%. For the (*R*)-(−)-enantiomer this limit appeared to be 0.20 mg/l ($n=6$) and for the (*S*)-(+)-enantiomer 0.14 mg/l ($n=6$).

3.6. Interferences

Commonly used antiepileptic drugs (AED) and metabolites do not interfere with the method because they do not react with the derivatizing reagent and are not detected under these chromatographic conditions. The following AED/metabolites were tested: ethosuximide, primidone, phenobarbital, *N*-desmethylsuximide (metabolite of methsuximide), phenytoin, carbamazepine and its 10,11-epoxide and 10,11-*trans*-dihydrodiol metabolites, monohydroxycarbazepine (metabolite of oxcarbazepine), valproic acid, lamotrigine, clobazam and its *N*-desmethyl metabolite, clonazepam, nitrazepam and diazepam and its *N*-desmethyl metabolite. The new anticonvulsant gabapentin reacts with OPA and could be detected with this system but its retention time is much longer (approx. 24 min).

3.7. Patients

With our method it was possible to evaluate the vigabatrin enantiomer levels in serum of adult epileptic patients on vigabatrin racemate. With regression analysis the linear relation between the enantiomers was calculated as $\{(R)\text{-}(-)\} = -0.52 + 1.54*\{(S)\text{-}(+)\}$ with $R^2=0.934$ (Fig. 3). The poor correlation was caused by the fact that, in these samples, the blood sampling time was not stan-

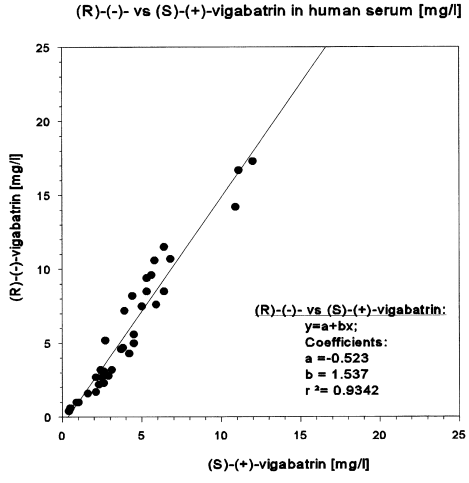

Fig. 3. Relation between (*R*)-(−)- and (*S*)-(+)-vigabatrin concentrations in serum of 34 adult epileptic patients.

dardized in relation to time of drug intake. We expect a higher correlation with standardized sampling time. Data in the literature [6] suggest a linear correlation between the two enantiomers with a mean $(R)/(S)$ ratio of 1.3. A weaker correlation in children is reported in comparison with adults [10]. As illustrated in Fig. 4a,b from a pharmacokinetic curve for one patient after intake of 1000 mg vigabatrin racemate, the $(R)/(S)$ ratio depends on sampling time.

The mechanism behind the difference in (R)-(−)- and (S)-(+)-vigabatrin is still unclear. Possibly the (S)-(+)-enantiomer is less efficiently absorbed from the alimentary tract due to stereospecific amino acid uptake. Another explanation is that only the (S)-(+)-enantiomer is used by GABA-T as a substrate, while the (R)-(−)-enantiomer is inactive and not recognized by GABA-T. Data for the absolute bioavailability of vigabatrin enantiomers have not been reported, due to the absence of an intravenous formulation [11].

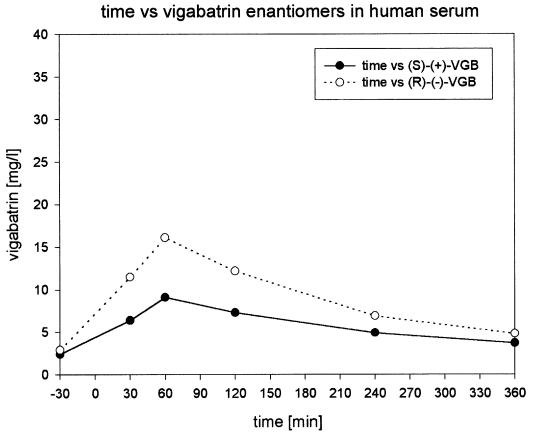

Fig. 4. (a) Concentration vs. time profile of vigabatrin enantiomers in serum; (b) R/S ratio vs. time profile in one adult patient after intake of 1000 mg (RS)-vigabatrin.

In conclusion, our results in patients illustrate the application of our method for the study of the pharmacokinetics of (R)-(−)- and (S)-(+)-vigabatrin. The differences in the pharmacokinetics and pharmacological activity of both enantiomers emphasize the importance of this assay. We will use this reported method for pharmacokinetic studies in patients, especially 24 h curves.

Acknowledgements

We thank the neurologists of the Instituut voor Epilepsiebestrijding, Heemstede, The Netherlands, for their cooperation in relation to patient data and samples.

References

[1] J.P. Leach, G.J. Sills, A. Majid, E. Butler, A. Carswell, G.G. Thompson, M.J. Brodie, Seizure (1996) 229–234.
[2] B.S. Meldrum, K. Murugaiah, Eur. J. Pharmacol. 89 (1983) 149–152.
[3] W. Löscher, C.P. Fassbender, L. Gram, M. Gramer, D. Hoerstermann, B. Zahner, H. Stefan, Epilepsy Res. 14 (1993) 245–255.
[4] L.M. Tsanaclis, J. Wicks, J. Williams, A. Richens, Ther. Drug Monit. 13 (1991) 251–253.
[5] K.D. Haegele, J. Schoun, R.G. Alken, N.D. Huebert, J. Chromatogr. 274 (1983) 103–110.
[6] T.M. Schramm, G.E. McKinnon, M.J. Eadie, J. Chromatogr. 616 (1993) 39–44.
[7] T. Chen, J.J. Contario, J. Chromatogr. 314 (1984) 495–498.
[8] H. Brückner, R. Wittner, J. Chromatogr. 476 (1989) 73–82.
[9] H. Lingeman, W.J.M. Underberg, A. Takadate, A. Hulshoff, J. Liq. Chromatogr. 8(5) (1985) 789–874.
[10] L. Nagarajan, T. Schramm, D.B. Appleton, C.J. Burke, M.J. Eadie, Clin. Exp. Neurol. 30 (1993) 127–136.
[11] E. Rey, G. Pons, G. Olive, Clin. Pharmacokinet. 23(4) (1992) 267–278.