Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography A, 1031 (2004) 237–242
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Determination of vigabatrin in human plasma and urine by high-performance liquid chromatography with UV-Vis detection

Sevil Müge Çetin*, Sedef Atmaca

Department of Analytical Chemistry, Faculty of Pharmacy, Istanbul University, Beyazit, 34116 Istanbul, Turkey

Abstract

A simple and reliable high-performance liquid chromatographic (HPLC) method with UV-Vis detection has been developed and validated for the determination of vigabatrin (VG) in human plasma and urine. The samples were pre-column derivatizated with 1,2-naphthoquinone-4-sulphonic acid sodium salt (NQS). A good chromatographic separation was achieved on a C_{18} column with a mobile phase consisting of acetonitrile and 10 mM orthophosphoric acid (pH 2.5) gradient elution. Tranexamic acid was used as an internal standard (I.S.). The method was linear over the concentration range of 0.8–30.0 μg/ml for both samples. The method is precise (relative standard deviation (R.S.D.) <9.13%) and accurate (relative mean error (RME) <−8.75%); analytical recoveries were 81.07% for plasma and 83.05% for urine. The assay was applied to pharmacokinetic study in a healthy volunteer after a single oral administration of 1 g of vigabatrin.

Keywords: Vigabatrin; Naphthoquinone sulphonic acid

1. Introduction

Vigabatrin (VG) (4-amino-hex-5-enoic acid), is one of the newer generation of antiepileptic drugs. It is a structural analogue of γ-aminobutyric acid (GABA) and is a selective catalytic inhibitory of enzyme GABA transaminase in brain. Vigabatrin is mostly administered as add-on therapy in resistant partial and secondarily generalised seizures which are not satisfactorily controlled by other antiepileptic drugs [1,2].

After oral administration, vigabatrin is well absorbed and maximal concentrations in plasma can be observed within 1–2 h. The elimination half-life is around 5–7 h. It is weakly metabolised and is mainly excreted by the kidney. It shows no significant protein-binding. Its pharmacokinetics is not affected by foods and other drugs [3,4].

Vigabatrin has been determined in human biological fluids, in many different ways with the aim of pharmacokinetic study or therapeutic drug monitoring. For example, by using amino acid analyser with fluorimetric detection following *o*-phthaldialdeyde (OPA) derivatization [5], gas chromatography (GC) with mass spectrometric detection (MS) [6] or thermionic specific detection (TID) [7]. The first method requires the regeneration of the columns after each analysis, the latter assays require complex sample preparation involving double derivatization (esterification of the carboxylic acid moiety and the acetylation of the amino group) procedure and expensive chiral capillary columns. In the developed high-performance liquid chromatographic (HPLC) methods vigabatrin has been analysed either alone [8–11] or with the other drugs such as gabapentin [12–14], haloperidol and amantadine [15]. Most of these methods involve the use of OPA derivatization and fluorimetric detection with isocratic [9,14] or gradient [10,12,13] system. Although the derivatization step is simple and rapid, the instability of the OPA derivatives make them difficult to apply to off-line derivatization. A sensitive HPLC method [11] based on the formation of a fluorescent derivative obtained by the reaction with 4-chloro-7-nitrobenzofurazan (NBD-Cl) has been developed for the assay of vigabatrin in plasma and urine by our laboratory. Among the HPLC analyses, only one method [8] has been reported using UV-Vis detection. In this study, 2,4,6-trinitrobenzenesulphonic acid (TNBS) has been used.

In the present study, an HPLC method with UV-Vis detection is described for the determination of vigabatrin in plasma and urine. The procedure is based on the off-line derivatization of the drug with 1,2-naphthoquinone-4-sulphonic acid sodium salt (NQS) which is the colour labelling reagent for primary and secondary amines. NQS

* Corresponding author. Fax: +90-2124400252.
E-mail address: sevilmuge@yahoo.com (S.M. Çetin).

0021-9673/$ – see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2003.11.020

has been used in HPLC analyses for both off-line [16,17] and on-line derivatization [18–20]. These methods usually employ UV-Vis [16,17,19] and fluorimetric [18,20] detection, however electrometric detection [21] has also been used. In this study, NQS derivatization has provided the sensitivity required for the pharmacokinetic study of vigabatrin.

2. Experimental

2.1. Chemicals and reagents

Vigabatrin and its tablets (Sabril, 500 mg vigabatrin per tablet) were kindly donated by Hoechst Marion Roussel (Istanbul, Turkey). The internal standard (I.S.), tranexamic acid was provided from Daiichi Seiyaku (Tokyo, Japan). NQS was purchased from Sigma (St. Louis, MO, USA). HPLC grade of acetonitrile, methanol, chloroform, ortophosphoric acid, hydrochloric acid, boric acid, potassium chloride, sodium hydroxide were obtained from Merck (Darmstadt, Germany). Water was deionized and purified by a Milli-Q water purification system from Millipore (Bedford, MA, USA).

2.2. Solutions

The stock solution of vigabatrin (1 mg/ml) was prepared in water and diluted with water to give standard solutions of 4.0–150.0 μg/ml. Standard calibration samples were prepared daily by spiking 0.5 ml of drug-free human plasma or urine (diluted with water to a ratio of 1:10) with 100 μl of appropriate vigabatrin standard solutions to achieve final concentrations of 0.8, 1.0, 5.0, 10.0, 15.0, 20.0, 25.0 and 30.0 μg/ml.

The internal standard working solution was prepared by dissolving tranexamic acid in water to obtain a concentration of 0.5 mg/ml.

The reagent solution was freshly prepared in water at 25 mg/ml concentration for the analysis of plasma and urine samples, respectively.

Borate buffer was prepared by dissolving 0.620 g of boric acid and 0.750 g potassium chloride in 100 ml of water. The pH level was adjusted to 9.0 with 0.1 M sodium hydroxide solution and the volume was made up to 200 ml with water.

2.3. Instrumentation

The analyses were performed on a Thermo Separation Products Liquid Chromatograph (TX, USA) which consisted of a P4000 solvent delivery system equipped with a Rheodyne injection valve with 20 μl loop, UV3000 detector set at 448 nm and a SN4000 automation system software. Separations were carried out at room temperature on a Shim-Pack CLS-ODS analytical column, 5 μm particle size, 250 mm × 4.6 mm i.d. (Shimadzu, Tokyo, Japan) with a guard column (4 mm × 3 mm i.d., Phenomenex) packed with the same material. The mobile phase consisting of acetonitrile and 10 mM orthophosphoric acid (pH 2.5) was delivered as a linear gradient at a flow rate of 1 ml/min. The elution profile was as follows: 20% acetonitrile (0–2 min), 25% acetonitrile (2–11 min) and 98% acetonitrile (11–20 min). Before use the mobile phase was degassed by an ultrasonic bath and filtered by a Millipore vacuum filter system equipped with a 0.45 μm HV filter.

2.4. Sample preparation and derivatization

Blood samples were collected into the tubes containing disodium EDTA and centrifuged at 4500 × *g* for 10 min. The resultant plasma and urine samples stored at −20 °C until analysis.

Standard calibration plasma or urine sample was spiked with 20 μl of internal standard solution. Plasma was deproteinized by adding 850 μl of methanol then the mixture was vortex-mixed for 1 min and centrifuged at 4500 × g for 10 min. An aliquot of 1.2 ml of the protein-free supernatant was evaporated to about 0.5 ml under nitrogen at 45 °C. The remaining solution or urine sample was derivatized according to the method previously reported [22] as follows: after adding 500 μl of buffer and 500 μl of NQS solutions the sample was kept at 60 °C for 20 min. Then the mixture was extracted with 2 ml × 2.5 ml of chloroform after cooling and acidifying with 250 μl of 0.1 M HCl. A 4.5 ml aliquot of the organic phase was evaporated to dryness. The residue was dissolved with 100 μl (for plasma)/125 μl (for urine) of acetonitrile–10 mM orthophosphoric acid (pH 2.5) (25: 75, v/v) and the solution was injected into the HPLC system.

2.5. Linearity

Calibration curves were prepared by six replicate analyses of plasma and urine samples spiked with 100 μl each of standard solution of vigabatrin to obtain the concentrations of 0.8, 1.0, 5.0, 10.0, 15.0, 20.0, 25.0 and 30.0 μg/ml, with a fixed concentration (20 μg/ml) of internal standard.

Samples were analysed as described above, and the peak area ratios of vigabatrin to internal standard were plotted against the corresponding drug concentrations.

2.6. Recovery

Recovery study was performed at seven concentration levels of the calibration curve ranging from 1.0 to 30.0 μg/ml. Absolute recoveries were determined ($n = 5$) by comparing the peak area values of the extracted human plasma and urine samples with those of the aqueous standard vigabatrin solutions at the same concentration.

2.7. Assay validation

The intra-day and inter-day accuracy and precision of the method were validated by analyzing the plasma and urine

samples spiked with vigabatrin at low, medium and high concentrations (1.0, 10.0 and 25.0 μg/ml). Determinations were performed with eight replicates on the same day as well as on eight separate days.

2.8. Applicability

The study protocol for the human experiment was approved by the Human Ethics Committee of Istanbul University, Cerrahpasa Faculty of Medicine. The subject (a 47 year-old healthy woman) read and completed the informed consent statement before participating in the study.

Venous blood samples were collected into the tubes containing disodium EDTA prior to dosage and 0.50, 0.75, 1.00, 1.15, 1.30, 1.45, 2.00, 2.15, 3.00, 5.00, 7.50, 12.00, 15.00 and 24.00 h after administration of the drug containing 1 g of vigabatrin. The blood specimens were processed to plasma as described above. Urine samples were also collected at intervals for up to 24 h. The samples were stored at −20 °C until analysis. Urine was diluted between 1:20 and 1:100 with water, depending on its concentration before analysis.

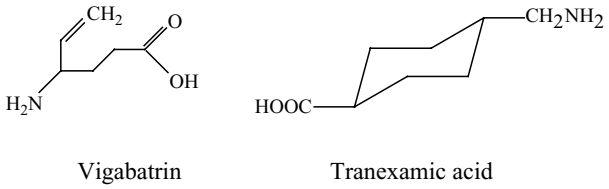
The plasma and urine concentrations were calculated from the regression equations of the calibration curves after analyzing the samples as described above ($n = 6$).

2.9. Stability

Analyte stability was assessed for all phases of the storage and analytical process at three concentration levels (1.0, 15.0 and 30.0 μg/ml) of vigabatrin. The stability in the extraction solvent was determined at 4 °C and room temperature. The stability of the derivative in mobile phase prior to injection into the HPLC was also tested. The freeze-thaw stability was evaluated for these concentrations of vigabatrin in plasma and urine samples for three cycles of thawing at room temperature followed by re-freezing to −20 °C for 24 h. Long-term stability was assessed using samples stored at −20 °C over a period of 8 months.

3. Results

The reliable separation of vigabatrin and internal standard (Fig. 1) was obtained using the gradient system on reversed phase. The retention times of vigabatrin—and internal standard—NQ derivatives were 13.85 and 15.28 min, respectively and the total run time of analysis was 20 min. The representative chromatograms in Fig. 2A–C show, respectively, blank human plasma, plasma spiked with vigabatrin and internal standard, and plasma obtained at 1.5 h after a single dose of 1 g vigabatrin administration from a healthy volunteer.



Vigabatrin Tranexamic acid

Fig. 1. Chemical structures of vigabatrin and internal standard, tranexamic acid.

Table 1
Calibration curve parameters ($n = 6$)

Parameters	Plasma	Urine
Linear range (μg/ml)	0.8–30.0	0.8–30.0
Slope: mean ± S.D.	0.0651 ± 0.001	0.0709 ± 0.002
Intercept: mean ± S.D.	0.0054 ± 0.003	0.0039 ± 0.004
Correlation coefficient: mean ± S.D.	0.9996 ± 0.0001	0.9996 ± 0.001

A linear correlation was obtained between the peak area ratios ($A_{VG/I.S.}$) and over the concentration range 0.8–30.0 μg/ml for each plasma and urine sample. The regression equations were as follows: $A_{VG/I.S.} = 0.0651C + 0.0054$ ($r = 0.9996$) for plasma and $A_{VG/I.S.} = 0.0709C + 0.0039$ ($r = 0.9996$) for urine. Calibration parameters were given on Table 1.

The limit of quantitation (LOQ) values for each sample were accepted as the lowest concentration level on the calibration curves, 0.8 μg/ml, with a relative standard deviation (R.S.D.) of less than 5.20%. Under the experimental conditions, the lower limit of detection (LOD) was found to be 0.5 μg/ml in plasma and urine at a signal-to-noise ratio of 3.

The average absolute recoveries of vigabatrin were of 81.07% from plasma and 83.05% from urine. The mean recovery of the internal standard was found to be 80.36 and 80.60% from plasma and urine, respectively, at the working concentration of 20 μg/ml. The results in Table 2 show no clear relationship between concentration and recovery.

Intra-day and inter-day R.S.D. values were within 1.90 and 5.78% for plasma, 2.70 and 9.13% for urine, respectively, indicating good precision. The accuracy of the method

Table 2
Absolute recovery of vigabatrin from plasma and urine ($n = 5$)

Sample	Concentration (μg/ml)		Recovery (%)	R.S.D. (%)
	Added	Found (mean ± S.D.)		
Plasma	1.00	0.74 ± 0.04	74.00	5.21
	5.00	4.00 ± 0.11	80.00	2.69
	10.00	8.39 ± 0.17	83.90	2.02
	15.00	13.02 ± 0.51	86.80	3.91
	20.00	16.00 ± 0.27	80.00	1.69
	25.00	20.37 ± 0.69	81.48	3.36
	30.00	24.55 ± 0.94	81.83	3.82
Urine	1.00	0.74 ± 0.04	74.00	5.59
	5.00	4.13 ± 0.13	82.60	3.05
	10.00	8.48 ± 0.25	84.80	3.46
	15.00	13.05 ± 0.25	87.00	1.92
	20.00	16.12 ± 0.41	80.60	2.53
	25.00	21.39 ± 0.60	85.56	2.80
	30.00	26.03 ± 0.73	86.77	2.79

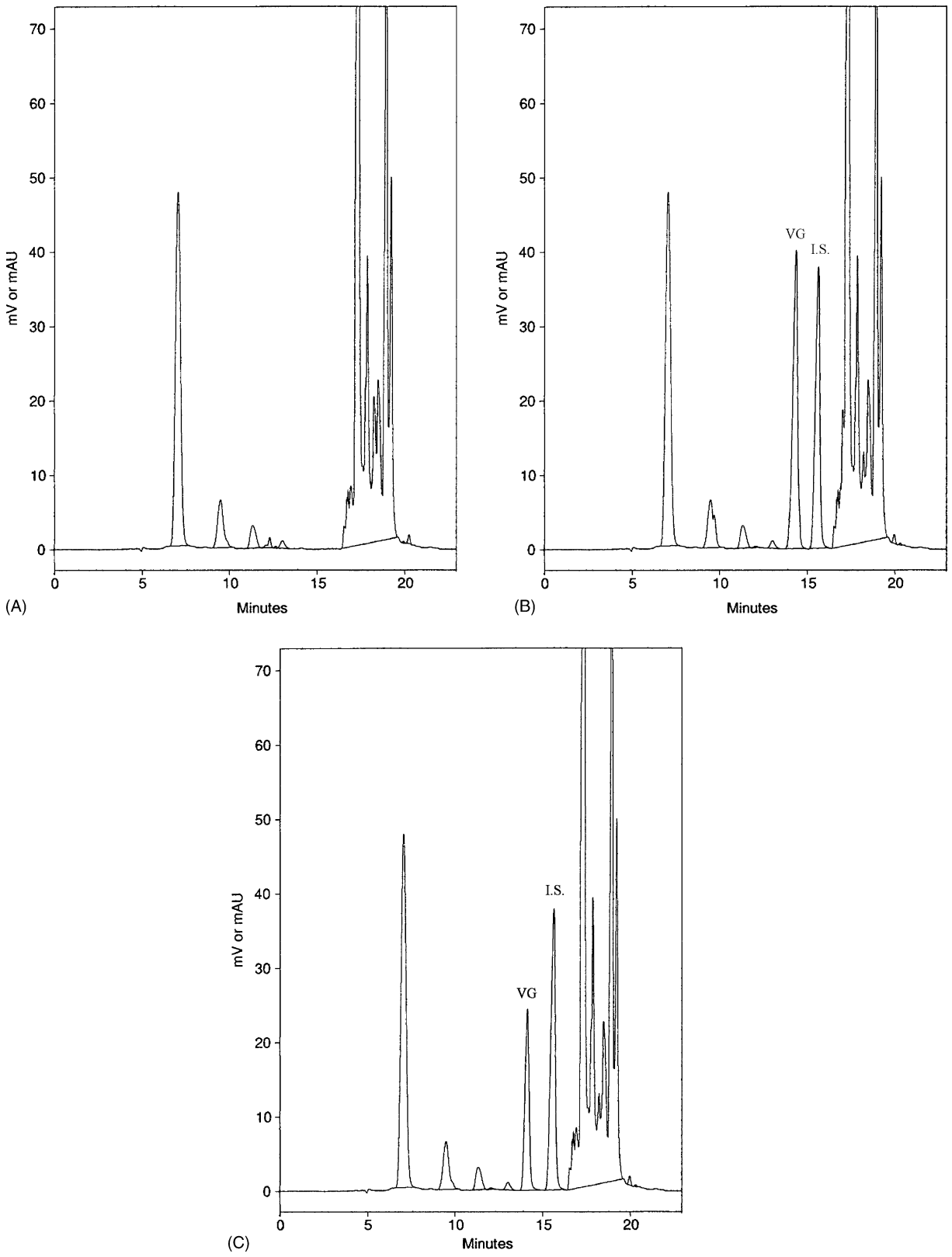


Fig. 2. Representative HPLC chromatograms of (A) a blank plasma; (B) a plasma spiked with 15 μg/ml of vigabatrin (VG) and 20 μg/ml of the internal standard (I.S.); and (C) a plasma obtained at 1.5 h after oral administration of 1 g vigabatrin to a healthy volunteer with 20 μg/ml of the internal standard.

Table 3
Intra-day and inter-day precision and accuracy of vigabatrin in plasma and urine ($n = 8$)

Sample	Concentration (μg/ml)		R.S.D. (%)	RME (%)
	Added	Found (mean ± S.D.)		
Plasma	Intra-day			
	1.0	0.92 ± 0.049	5.32	−7.72
	10.0	9.90 ± 0.188	1.90	−1.05
	25.0	25.10 ± 0.691	2.75	0.38
	Inter-day			
	1.0	0.91 ± 0.053	5.78	−8.59
	10.0	9.78 ± 0.199	2.03	−2.19
	25.0	24.71 ± 0.650	2.63	−1.17
Urine	Intra-day			
	1.0	0.94 ± 0.082	8.75	−5.75
	10.0	10.02 ± 0.271	2.70	0.19
	25.0	25.31 ± 1.059	4.19	1.24
	Inter-day			
	1.0	0.91 ± 0.083	9.13	−8.75
	10.0	9.95 ± 0.481	4.84	−0.51
	25.0	24.98 ± 1.107	4.43	−0.07

expressed as relative mean error (RME) was below −8.75% which was shown to be satisfactory (Table 3).

NQ derivatives of vigabatrin and the internal standard in the extraction solvent were stable for at least 72 h at 4 °C and about 10 h at room temperature, whereas it decomposes quickly in basic reaction medium. The stability in mobile phase was also tested and it was found that the samples were stable for 7 h at 4 °C and 3 h at room temperature. The percentage loss of the samples in mobile phase was about 5%. The plasma and urine samples spiked with vigabatrin were evaluated for the stability following freezing and thawing. The drug was stable for at least three freeze-thaw cycles. The stability of the spiked plasma and urine samples after 8 weeks of storage at −20 °C was evaluated as well, no significant change was observed for either sample.

Commonly prescribed antiepileptic drugs (carbamazapine, valproic acid, pirimidone, phenobarbital, clonazepam, ethosuximide, lamotrigine, gabapentin) and metabolites (10-hydroxycarbamazapine, carbamazapine-10,11-epoxide) were analysed for possible interference. No interference was observed because they did not react with NQS except gabapentin which was derivatized but showed no interference peak under the chromatographic conditions.

To check the clinical applicability of the method, the pharmacokinetics of vigabatrin was investigated in a healthy volunteer after a single oral administration of 1 g of the drug. The concentration–time profile is shown in Fig. 3. The results of analysis revealed a maximum plasma concentration (C_{max}) of 23.51 μg/ml was attained at 2 h (t_{max}) of dosing. Plasma concentration was measurable up to 24 h. The elimination half-life ($t_{1/2}$) of the drug and area under the curve (AUC) were 4.95 h and 119.15 μg h/ml, respectively. Fig. 4

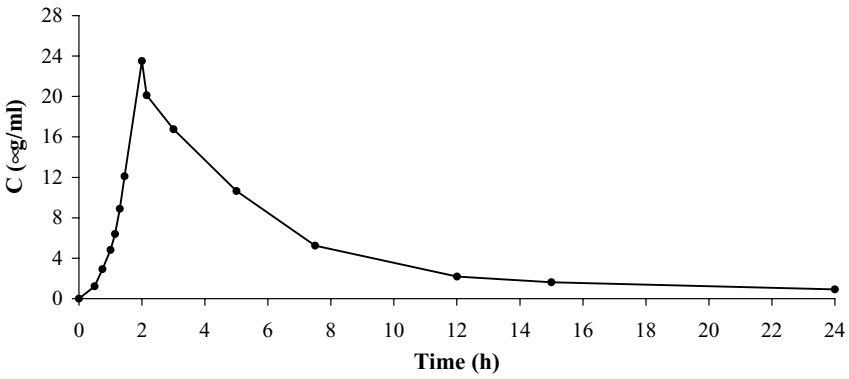


Fig. 3. A representative plasma concentrations versus time profile of vigabatrin in a healthy volunteer after a single 1 g oral dose.

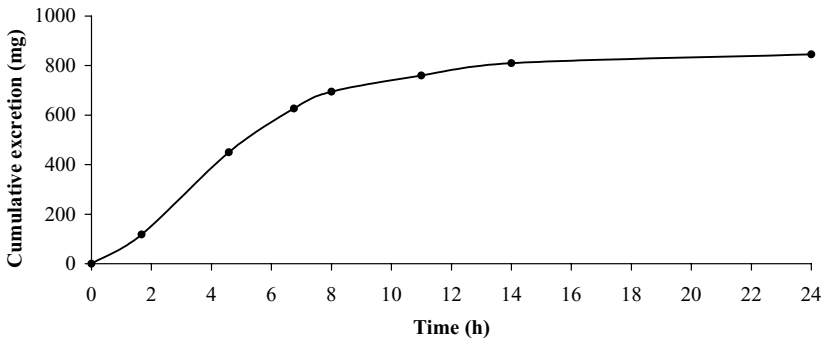


Fig. 4. Cumulative excretion of vigabatrin in urine of a healthy volunteer after a single 1 g oral dose.

shows the cumulative urinary excretion of vigabatrin for the same subject after this dose. It appears that 84.56% of drug was excreted unchanged within 24 h. These parameters are in agreement with those reported previously [3].

4. Discussion

An HPLC method based on the reaction between vigabatrin and NQS has been reported previously for the determination of this drug in tablets and the optimum reaction conditions have been described [22]. Some modifications were made in order to determine vigabatrin in plasma and urine by HPLC using this reaction, e.g., the NQ derivatives were extracted with chloroform instead of chloroform–*n*-butanol (4:1), because the use of chloroform extraction provided higher analyte stability and recovery. Moreover, it markedly minimised the interferences of endogenous amino acids, the excess of NQS, the degradation products of NQS. When the isocratic system, acetonitrile-10 mM orthophosphoric acid (pH 2.5) (25:75), used in the previous study was applied, the resolution was poor and the run time was long (30 min). The gradient elution with these solutions provided the optimum resolution with a short time analysis.

Vigabatrin exhibits a very low UV absorption with $\varepsilon_{207.8nm} = 182.6$ l/mol cm in 0.1 M HCl. Consequently, the sensitivity is very low by conventional UV-spectrophotometric methods. Attachments of chromophoric group to vigabatrin facilitates and increases the sensitivity of its detection. NQS was chosen as the derivatizing reagent because of its reactivity and formation of a chromophoric group that is detectable both by UV and fluorescence. Since the level of vigabatrin in biological fluids is not too low, in this study UV-Vis detector was preferred over fluorescence detector. Moreover, this detector is commonly available in a laboratory and not expensive as the fluorimetric one. When compared to some HPLC studies carried out with fluorescence detection [9–11] (LOQ values of these studies were in the range of 0.14–0.5 μg/ml) the sensitivity of the proposed method is lower (0.8 μg/ml). However, there are HPLC–fluorescence detection methods that are either similar [13] (0.65 μg/ml) or lower [14] (2 μg/ml) in sensitivity. In any case, the level of the sensitivity permits the use of proposed method not only for drug monitoring but also pharmacokinetic study of vigabatrin. When compared to OPA derivatization, the presented method offers an advantage, because NQS derivatization provides highly stable derivatives extractable into an organic phase.

Among the HPLC analyses, there is only one report [8] involving UV-Vis detection after TNBS derivatization and therapeutic monitoring of vigabatrin. This method provides higher detection limit (1 μg/ml) and lower extraction recovery (73%) when compared with the present study. Another drawback of this report is minimal explanation of the method and validation data. However, LOQ value (1 μg/ml) is close to that of the developed method involving NQS derivatization.

5. Conclusion

In this study, a simple, sensitive and reliable method consisting of a single step liquid-phase extraction and RP-HPLC using gradient elution and UV-Vis detection was developed and fully validated for the determination of vigabatrin in human plasma and urine. The method showed high selectivity, precision and accuracy for the use in pharmacokinetic study and therapeutic monitoring of vigabatrin.

Acknowledgements

The authors would like to thank the Research Fund Istanbul University for the sponsorship of this study (Project numbers T-53/23072002 and UDP-131/18042003).

References

[1] S.M. Grant, R.C. Heel, Drugs 41 (1991) 889.
[2] A. Guberman, Can. J. Neurol. Sci. 23 (Suppl. 2) (1996) 13.
[3] E. Rey, G. Pons, G. Olive, Clin. Pharmacokinet. 23 (1992) 267.
[4] P.N. Patsalos, J.S. Duncan, Rev. Contemp. Pharmacother. 6 (1995) 447.
[5] J. Grove, R.G. Alken, P.J. Schechter, J. Chromatogr. 306 (1984) 383.
[6] K.D. Haegele, J. Schoun, R.G. Alken, N.D. Huebert, J. Chromatogr. 274 (1983) 103.
[7] T.M. Schramm, G.E. McKinnon, M.J. Eadie, J. Chromatogr. 616 (1993) 39.
[8] S. George, L. Gill, R.A. Braitwaite, Ann. Clin. Biochem. 37 (2000) 338.
[9] L.M. Tsanaclis, J. Wicks, J. Williams, A. Richens, Ther. Drug Monit. 13 (1991) 251.
[10] T.A.C. Vermeij, P.M. Edelbroek, J. Chromatogr. B 716 (1998) 233.
[11] S. Erturk, E.S. Aktas, S. Atmaca, J. Chromatogr. B 760 (2001) 207.
[12] N. Wad, G. Kramer, J. Chromatogr. B 705 (1998) 154.
[13] N. Ratnaraj, P.N. Patsalos, Ther. Drug Monit. 20 (1998) 430.
[14] D.F. Chollet, L. Goumaz, C. Juliano, G. Anderegg, J. Chromatogr. B 746 (2000) 311.
[15] G. Huesgen, C. Roos, J. Kinkeldei, J. Lab. Med. 24 (2000) 14.
[16] J.R.L. Smith, A.U. Smart, F.E. Hancock, M.V. Twigg, J. Chromatogr. 483 (1989) 341.
[17] P. Campíns-Falcó, A. Sevillano-Cabeza, C. Molíns-Legua, M. Kholmann, J. Chromatogr. B 687 (1996) 239.
[18] Y. Kobayashi, H. Kubo, T. Kinoshita, Anal. Biochem. 160 (1987) 392.
[19] J. Saurina, S. Hernández-Cassou, J. Chromatogr. A 676 (1994) 311.
[20] P. Edder, A. Cominoli, C. Corvi, J. Chromatogr. A 830 (1999) 345.
[21] Y. Nakahara, Y. Takeda, Chromatographia 26 (1988) 363.
[22] S.M. Cetin, S. Atmaca, Acta Pharm. Turc. 4 (2002) 57.