ELSEVIER

Journal of Chromatography B, 779 (2002) 307–312

JOURNAL OF CHROMATOGRAPHY B

www.elsevier.com/locate/chromb

Short communication

High-performance liquid chromatographic method for the determination of gabapentin in human plasma

Z. Zhu*, L. Neirinck

Pharmascience, 6111 Royalmount Avenue, Suite 100, Montreal, Quebec, H4P 2T4, Canada

Received 23 January 2002; received in revised form 28 May 2002; accepted 31 May 2002

Abstract

A sensitive high-performance liquid chromatography (HPLC) method using UV detection for the determination of gabapentin in human plasma has been developed. In this method, gabapentin was extracted from human plasma with a reversed-phase solid-phase extraction (SPE) cartridge followed by derivatization with phenylisothiocyanate. Analysis was achieved by using a HPLC system that was equipped with a UV detector. The quantitation limit of gabapentin in human plasma was 0.03 μg/ml. The method is sensitive with excellent selectivity and reproducibility and it has been applied to a bioequivalence clinical study with great success.

Keywords: Gabapentin

1. Introduction

Gabapentin [1-(aminomethyl) cyclohexaneacetic acid] (Fig. 1) is a novel antiepileptic drug that is effective in a number of seizure models. It is an oral anticonvulsant for the treatment of partial and generalized tonic–clonic seizures [1]. It is well absorbed and excreted completely by humans and there is no significant binding to human protein [2].

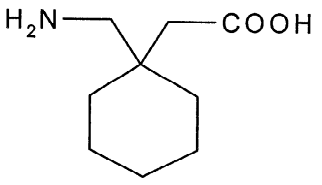
Numerous pharmacokinetic studies have been performed with gabapentin, and several methods for the determination of gabapentin in human plasma have been published. Among those published methods for the analysis of gabapentin in human plasma or serum, there are seven methods using a high-performance liquid chromatography (HPLC) technique [3–9], and one method using liquid chromatography–tandem mass spectrometry (LC–MS–MS) [10]. Others use gas chromatography (GC) [11], gas chromatography–mass spectrometry (GC–MS) [12] or capillary electrophoresis (CE) [13]. Hengy and Kölle's HPLC method [3] involved a multi-step of derivatization with 2,4,6-trinitrobenzenesulfonic acid (TNBS) followed by an extraction procedure. While the use of *o*-phthalaldehyde-3-mercaptopropionic acid (OPA-MPA) derivatives have been described [4], they are only stable for 25 min, making them difficult to apply to off-line derivatization. Other HPLC methods have been described, typically with high detection limits ranging from 0.06 to 0.5 μg/ml.

*Corresponding author. Tel.: +1-514-340-5040; fax: +1-514-342-4118.

E-mail address: zzhu@pharmascience.com (Z. Zhu).

1570-0232/02/$ – see front matter
PII: S1570-0232(02)00399-9

(A):



(B):

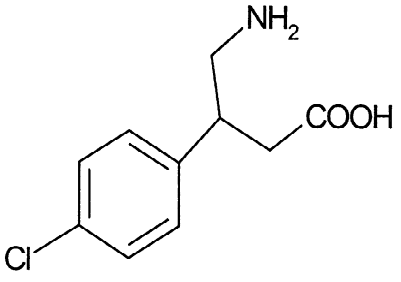


Fig. 1. Chemical structures of (A) gabapentin; (B) internal standard (baclofen).

According to the Compendium of Pharmaceuticals and Specialties [14], after a single dose of 400 mg gabapentin, C_{max} is expected to be about 5.5 μg/ml and $t_{1/2}$ is about 6 h. Hence, 48 h after a single 400 mg dose of gabapentin, the concentration is expected to be around 0.03 μg/ml. Therefore, it was necessary to develop a method that would be able to detect gabapentin at 0.03 μg/ml level.

The method described in this paper is a sensitive and robust method for the routine analysis of gabapentin in human plasma, with detection limit of 0.03 μg/ml, while enabling off-line derivatization. The method is HPLC with UV detection and involves a reversed-phase solid-phase extraction (SPE) followed by a derivatization with phenylisothiocyanate (PITC). It is easy to operate, with an 8 min run time, and allows processing of over 100 samples per day. This method is suitable for the analysis of plasma from pharmacokinetic or clinical studies.

2. Experimental

2.1. Materials

Gabapentin was obtained from Teva Tech (Kfar Saba, Israel) and baclofen was from Fine Chemicals (Cape Town, South Africa). PITC was purchased from Chromatographic Specialties (Brockville, Canada). All other chemicals were purchased from Fisher Scientific (Nepean, Canada). HPLC-grade water was obtained from a laboratory Nano-Pure water purification system. The drug-free human plasma for preparation of standard curve and quality control samples was obtained from Biological Specialty (Colmar, PA, USA).

2.2. Stock solutions and standards

Stock solutions of gabapentin (1.00 mg/ml), and internal standard (1.00 mg/ml baclofen, Fig. 1) were prepared in a methanol–water (50:50) solution. A seven-point calibration standard curve, ranging from 0.03 to 10 μg/ml was prepared by spiking the drug-free human plasma with appropriate amounts of gabapentin. The quality control samples (at three concentration levels, i.e., 0.09, 3.5 and 8 μg/ml) were prepared in a similar manner from the stock solution. Before the spiking, all drug free plasma was tested to make sure there was no endogenous interference at the retention times for gabapentin, and the internal standard. The quality control samples were extracted with the calibration standards to verify the integrity of the method.

2.3. Solid-phase extraction

Plasma samples (0.5 ml) were placed into glass culture tubes. Internal standard (0.1 ml of 50 μg/ml baclofen) and 0.25 ml 1 *M* sodium phosphate monobasic buffer were added. The solid-phase extraction cartridge (Bond-Elut, C_{18}, 100 mg, 1 ml) was activated with 1 ml methanol followed by 0.5 ml of sodium phosphate monobasic buffer. The plasma and buffer mixture was then passed through the conditioned cartridge by gravity. The cartridge was washed with 0.1 *M* sodium phosphate monobasic

buffer, follow by 0.1 *M* HCl. The cartridge was then eluted with 1 ml of methanol.

2.4. Derivatization

0.1 *M* NaOH was added to the eluent and the mixture was evaporated to dryness under a nitrogen evaporator (Tubor Vap). 0.15 ml of derivatizing reagent (0.35% PITC in methanol) was added to dissolve and react with the residue. The reaction took place at room temperature for 20 min. The reaction mixture was then evaporated to dryness under a nitrogen evaporator. The residue was reconstituted with 250 μl of mobile phase and 25 μl was injected into the HPLC system for analysis.

2.5. Chromatography and quantitation

An Agilent 1100 series HPLC system with a UV–Vis detector was used for this study. The separation was achieved by using a 100×4.6 mm, 5 μm Hypersil HyPurity Elite C_{18} column with mobile phase consisting of 0.04 *M* sodium acetate buffer–methanol–acetonitrile (48:40:12, v/v). The mobile phase was delivered at a flow-rate of 1.1 ml/min. A peak height ratio method was used for quantitation. The gabapentin concentration in human plasma samples was determined by a standard curve that was analyzed with weighted least-squares linear regression. The weighting factor was $1/x^2$.

3. Results

3.1. Limit of quantitation, linearity and precision

The representative calibration equation for this method was: $y = 0.0001x + 0.000425$. The calibration curves were linear for gabapentin in the concentration range of 0.03–10 μg/ml with an average regression of 0.9994 ($n = 5$). The limit of quantitation (LOQ) in human plasma for gabapentin was 0.03 μg/ml.

Inter-assay precision was determined by analyzing five calibration curves with quality control samples on 5 different days. The intra-assay precision was determined by analyzing six replicates of quality control samples extracted on the same day. The results of inter-day and intra-day precision for gabapentin in human plasma are presented in Table 1.

3.2. Recovery

The absolute recovery was determined by comparing the peak height of extracted human plasma samples with the peak height of solutions prepared at the same concentrations. The analysis was done for six replicates at concentrations of 0.09, 3.5 and 8 μg/ml. The average recovery of gabapentin from spiked human plasma samples was 81.9%. The average recovery ($n = 18$) of the internal standard was found to be 81.6%. The results of gabapentin recovery at different concentrations are presented in Table 2.

3.3. Specificity

Six different sources of drug-free human plasma were screened and no endogenous interference was observed at the retention time of gabapentin and internal standard. A chromatogram of extracted blank human plasma sample, a representative chromatogram of extracted plasma sample containing 0.03 μg/ml gabapentin as well as a representative chromatogram of an extracted plasma sample containing 7.5 μg/ml of gabapentin are shown in Fig. 2.

Table 1
Assay variability of gabapentin in human plasma

Concentration added (μg/ml)	Concentration found (μg/ml)	R.E.[a] (%)	RSD (%)	*n*
Inter-day				
0.09	0.0866±0.003	−3.7	3.6	30
3.5	3.56±0.08	1.8	2.3	14
8.0	7.88±0.25	−1.5	3.1	30
Intra-day				
0.03	0.0311±0.001	3.7	2.6	6
0.09	0.0847±0.001	−5.9	1.4	6
3.5	3.52±0.05	0.5	1.5	6
8.0	7.75±0.06	−3.1	0.8	6

[a] R.E. = Relative error.

Table 2
Extraction recovery of gabapentin in human plasma

	Gabapentin concentration (μg/ml)					
	Added	Found	Added	Found	Added	Found
	0.09	0.0801	3.5	2.78	8.0	6.21
	0.09	0.0794	3.5	2.95	8.0	6.28
	0.09	0.0761	3.5	2.62	8.0	6.56
	0.09	0.0754	3.5	2.60	8.0	6.17
	0.09	0.0807	3.5	2.85	8.0	6.51
	0.09	0.0804	3.5	2.87	8.0	6.10
Mean	0.09	0.0787	3.5	2.88	8.0	6.30
RSD[a] (%)	–	2.7	–	4.7	–	2.7
Recovery (%)	–	87.4	–	79.4	–	78.8

[a] RSD = Relative standard deviation.

Six commonly used over-the-counter (OTC) drugs were also tested for possible interference. No interference was observed at the retention time of gabapentin and internal standard. The OTC drugs tested were the following: caffeine, acetylsalicylic acid, ibuprofen, acetaminophen, phenylpropanolamine and dextromethorphan.

3.4. Stability

The stability of derivatized gabapentin and internal standard in the mobile phase (processed sample stability) were tested, and it was concluded that the processed samples are stable at room temperature for at least 65 h. The processed sample stability was evaluated by comparing the extracted plasma samples that were injected immediately (time 0), with the samples that were re-injected 65 h after sitting in the auto-sampler at room temperature. Evaluation was based on back-calculated concentrations. The human plasma samples containing gabapentin were also evaluated for the stability following freeze–thaw. The freeze–thaw stability evaluation was conducted by comparing the stability samples that had been frozen and thawed three times, with the plasma samples thawed only once. Gabapentin in human plasma was stable for at least three freeze–thaw cycles.

The stability of spiked human plasma samples after 8 weeks of storage at −20 °C was evaluated as well. The stability evaluation involved an analysis of the low, mid and high quality control samples that were stored at −20 °C for 8 weeks together with freshly spiked standard curve and quality control samples. The analysis was performed on the same day. The peak height ratio of gabapentin/internal standard was determined. No significant change (i.e., less than 4% of difference) was observed under the conditions described, demonstrating that plasma samples containing gabapentin are stable for at least 8 weeks at −20 °C.

3.5. Application

The method has been applied to a study with more than 1600 plasma samples from human volunteers. Nineteen blood samples were drawn from each volunteer over a period of 48 h after an oral administration of 400 mg gabapentin. Samples were stored at −20 °C until analyzed. Fig. 3 shows a characteristic concentration versus sampling time profile of gabapentin in human plasma from a male volunteer.

4. Discussion

One phenomenon we had observed was that the recovery of gabapentin decreases with increasing concentration as shown in Table 2. This is most likely due to hydrophilicity of gabapentin that causes the loss of recovery at higher concentration. Further optimizing the extraction procedure should improve the recovery of gabapentin.

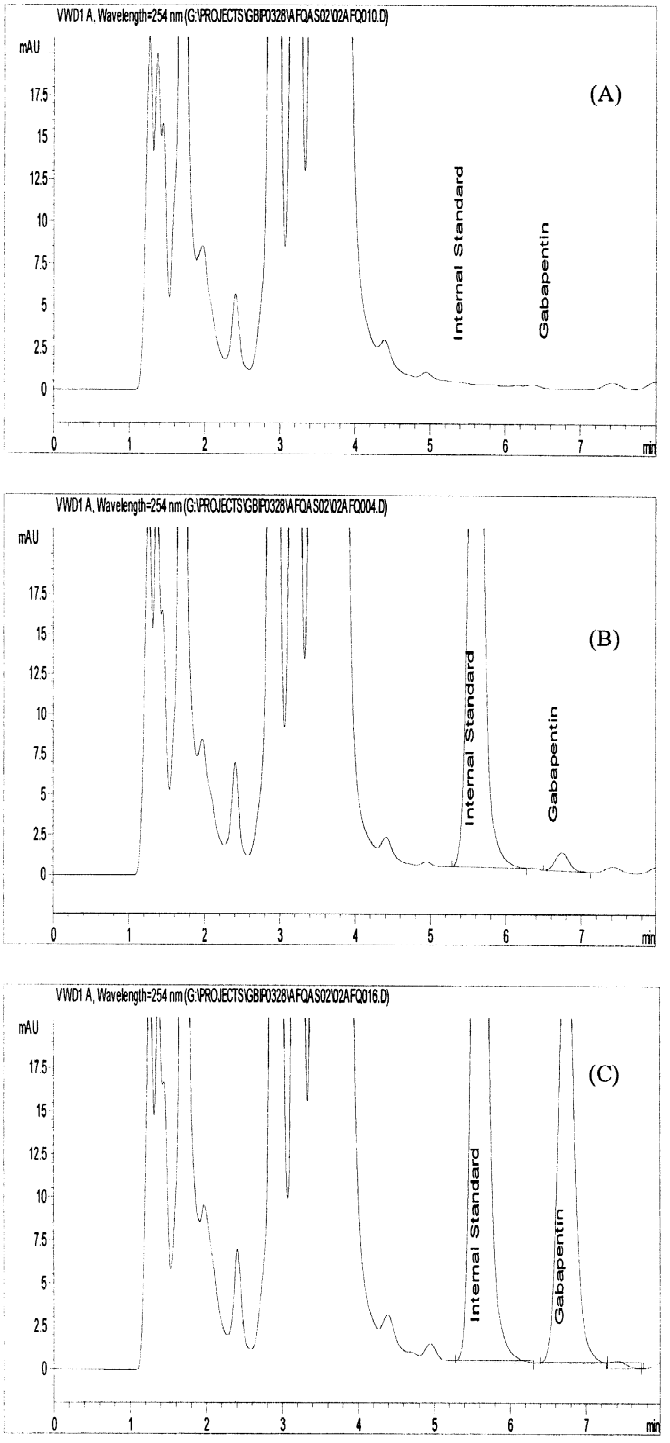


Fig. 2. Chromatograms of extracts of human plasma samples. (A) Plasma containing no gabapentin; (B) plasma spiked with 0.03 μg/ml gabapentin; (C) plasma spiked with 7.5 μg/ml gabapentin.

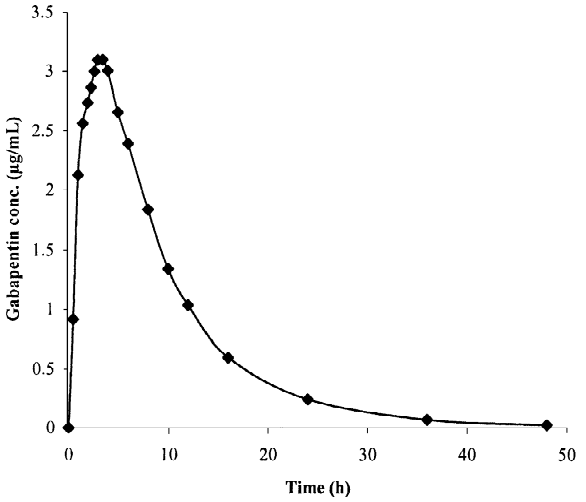


Fig. 3. Plasma levels of gabapentin following an oral dose of 400 mg to a human male volunteer.

PITC is recognized as the most widely used precolumn derivatization reagent for amino acid analysis. Precolumn derivatization of gabapentin with PITC is a very simple process. It can be achieved at room temperature and results in complete derivatization. The gabapentin PITC derivative is very stable. It can be stored at room temperature for at least 65 h without degradation. NaOH was added to the extraction eluent to neutralize the solution and to introduce a basic condition for the derivatization. In addition, since PITC degrades when it is in contact with water, it is important to make sure that the extraction residue is free of water before the addition of PITC. For the same reason, PITC should be stored in an airtight container and exposure to air should be minimized during handling. It is preferable to prepare the PITC methanol solution daily. During the process of internal standard selection, several candidates were investigated which included tryptophan, lysine, phenylalanine and 4-(amino-methyl) cyclohexane carboxylic acid. They either eluted too early or demonstrated a lack of consistency during derivatization. However, the speed of derivatization of baclofen was similar to that of gabapentin which allowed for a method with a consistent analyte/internal standard ratio. A non-polar reversed-phase column separated the residue derivatization reagent from the derivatized analytes. Since the derivatives of PITC had a very strong absorbance at 254 nm, UV detection at this wavelength provided enough sensitivity for the analysis. An isocratic condition was used to simplify the condition and to shorten the analysis time.

5. Conclusion

A precolumn derivatization reversed-phase HPLC method for the determination of gabapentin is presented. This method is a very robust and it is easy to operate with excellent reproducibility. The derivative is very stable and the analysis time is only 8 min which allows one to do off-line derivatization. It is suitable for routine analysis of gabapentin in human plasma samples. This method has been applied with great success to a clinical study that had more than 1600 plasma samples.

Acknowledgements

The authors wish to thank M. Ghamlouche and J.F. Blackburn for providing the validation results.

References

[1] D. Chadwick, Lancet 343 (1994) 89.
[2] J.P. Leach, M.J. Brodie, Seizure 4 (1995) 5.
[3] H. Hengy, E.U. Kölle, J. Chromatogr. 341 (1985) 473.
[4] G. Forrest, G.J. Sills, J.P. Leach, M.J. Brodie, J. Chromatogr. B 681 (1996) 421.
[5] P.H. Tang, M.V. Miles, T.A. Glauser, T. DeGrauw, J. Chromatogr. B 727 (1999) 125.
[6] D.F. Chollet, L. Goumaz, C. Juliano, G. Anderegg, J. Chromatogr. B 746 (2000) 311.
[7] Q. Jiang, S. Li, J. Chromatogr. B 727 (1999) 119.
[8] N. Ratnaraj, P.N. Patsalos, Ther. Drug Monit. Aug. 20 (1998) 430.
[9] N. Wad, G. Krämer, J. Chromatogr. B 705 (1998) 154.
[10] D.R. Ifa, M. Falci, M.E. Moraes, F.A. Bezerra, M.O. Moraes, G. de Nucci, J. Mass Spectrom. 36 (2001) 188.
[11] W.D. Hooper, M.C. Kavanagh, R.G. Dickinson, J. Chromatogr. 529 (1990) 167.
[12] L.L. Garcia, Z.K. Shihabi, K. Oles, J. Chromatogr. B 669 (1995) 157.
[13] M.M. Kushnir, J. Crossett, P.I. Brown, F.M. Urry, J. Anal. Toxicol. 23 (1) (1999) 1.
[14] Canadian Pharmacists Association, Compendium of Pharmaceuticals and Specialties, 36th Edition, 2001, p. 1025.