BIOMEDICAL CHROMATOGRAPHY
Biomed. Chromatogr. **19**: 63–67 (2005)
Published online in Wiley InterScience (www.interscience.wiley.com).
DOI: 10.1002/bmc.417

ORIGINAL RESEARCH

Gas chromatography–tandem mass spectrometry analysis of gabapentin in serum

Cristiana Gambelunghe,[1] Giuseppina Mariucci,[2] Michela Tantucci[2] and Maria Vittoria Ambrosini[2]*

[1]Department of Clinical and Experimental Medicine-Laboratorio delle Attività Motorie e Sportive, University of Perugia, Perugia, Italy
[2]Department of Experimental Medicine and Biochemical Sciences, University of Perugia, Perugia, Italy

Received 2 April 2004; accepted 27 April 2004

ABSTRACT: A new highly sensitive analytical method for determining gabapentin [1-(aminomethyl) cyclohexaneacetic acid; Neurontin®] in serum using gas chromatography/tandem mass spectrometry (GC-MS/MS) was developed. GC-MS/MS was applied to determine the levels of gabapentin in serum samples of mice at 1 and 6 h after oral or intraperitoneal treatment (300 mg/kg). At 1 h, the concentrations of the drug were 4.02 ± 0.42 and 4.32 ± 0.28 μg/mL in mice treated orally and intraperitoneally, respectively. At 6 h, drug levels decreased by about 66% in both groups. The method, coupling two stages of mass analysis, could be very useful in identifying the drug in complex mixtures such as blood and urine. Moreover, it is easy and rapid to perform, and sensitive enough to allow the presence of the drug to be determined at very low detection limits. It is a very reliable method for both clinical and experimental monitoring of gabapentin. Copyright © 2004 John Wiley & Sons, Ltd.

KEYWORDS: gabapentin; GC/MS/MS analysis; serum

INTRODUCTION

Gabapentin, [1-(aminomethyl) cyclohexaneacetic acid; Neurontin®] is a relatively new antiepileptic drug (AED) for both adjunctive therapy and monotherapy in the treatment of patients who are not adequately controlled with standard antiseizure drugs. It is structurally related to the neurotransmitter γ-aminobutyric acid (GABA), but it does not possess high affinity for either $GABA_A$ or $GABA_B$ receptors. It is not converted metabolically into GABA or a GABA agonist and does not inhibit GABA uptake or degradation (Taylor *et al.*, 1998). Gabapentin is water-soluble, rapidly absorbed and able to cross the blood–brain barrier. It binds to a specific receptor associated with the L-amino acid transport system, and its anticonvulsant activity may be related to its effects on brain amino acid concentrations or metabolism (Taylor, 1994). Recently, it has been suggested that gabapentin selectively inhibits Ca^{2+} influx by inhibiting voltage-operated Ca^{2+} channels in a subset of excitatory and inhibitory presynaptic terminals, thereby attenuating synaptic transmission (Van Hoft *et al.*, 2003). In spite of extensive literature on the therapeutic efficacy of gabapentin, there is no consensus on its molecular mechanism of action.

*Corresponding to: M. V. Ambrosini, Department of Experimental Medicine and Biochemical Sciences, Division of Cellular and Molecular Biology, University of Perugia, Via del Giochetto, 06122 Perugia, Italy.
E-mail: neurbisp@unipg.it

Abbreviations used: AED, antiepileptic drug; AMD, automated method development; DTE, dithioerythritol; GABA, γ-aminobutyric acid; MSTFA; *N*-methyl-*N*-trimethylsilyltrifluoroacetamide.

Published online 7 October 2004

Copyright © 2004 John Wiley & Sons, Ltd.

Although gabapentin was originally developed for treating partial seizures, it has been demonstrated to be effective against chronic neuropathic pain. Therefore, the drug is widely used to treat various neuropathic pain conditions (Rosenberg *et al.*, 1997; Rosner *et al.*, 1996) such as chronic post-herpetic pain (Rowbotham *et al.*, 1998; Stacey and Glanzman, 2003), painful diabetic neuropathy, central neuropathic pain following lesions of the CNS and migraine (Finnerup *et al.*, 2002; Pappagallo, 2003).

Gabapentin is particularly useful in special populations like the elderly and children, as well as in patients suffering from liver disease. Indeed, it is not metabolized by the liver, does not induce hepatic enzymes and is not bound to plasma proteins. The drug is entirely excreted unchanged in urine and, therefore, dosage adjustment is necessary in patients with renal impairment (Harden, 1994). Gabapentin displays dose-dependent, saturable absorption due to saturation of active transport across the gut via the L-amino acid transporter system (Stewart *et al.*, 1993; Stevenson *et al.*, 1997). As the drug is absorbed by means of the above-mentioned active transport mechanism, it is reasonable to hypothesize that significant and clinically meaningful inter-individual variability may exist in its absorption. Therefore, measuring gabapentin serum concentrations may be useful in assessing compliance and evaluating risks of toxicity.

Several methods for gabapentin detection in human serum and urine have been published. They describe the measurement of gabapentin using liquid chromatography (Gauthier and Gupta, 2002), gas–liquid

chromatography (Wolf *et al.*, 1996), high-performance liquid chromatography (Gidal *et al.*, 2000; Jiang and Li, 1999; Tang *et al.*, 1999; Windsor and Radulovic, 1995; Zhu and Neirink, 2002) or gas chromatography–mass spectrometry (Kushnir *et al.*, 1999). We present here a highly sensitive and specific analytical method for detecting gabapentin in the serum of mice by means of gas chromatography/tandem mass spectrometry (GC/MS/MS).

EXPERIMENTAL

Reagents and chemicals. Reagents and solvents were high-performance liquid chromatographic grade and purchased from Merck (Darmstadt, Germany). Gabapentin was supplied by Sigma (Sigma Chemical Co., St Louis, MO, USA). Octadecyl (C_{18}) columns for solid-phase extraction were obtained from Varian (Varian Inc., Harbor City, CA, USA). *N*-methyl-*N*-trimethylsilyltrifluoroacetamide (MSTFA), ammonium iodide (NH_4I) and dithioerythritol (DTE) came from Fluka (Buchs, Switzerland).

Animals. The study was carried out on adult male CD1 mice (n = 10, 25–28 g, Harlan, Italy) randomly divided into two groups of five to test the possible influence of oral and intraperitoneal administration on gabapentin pharmacokinetics. Mice tested for oral administration had water *ad libitum* but were deprived of food 15 h before the test. Mice tested for intraperitoneal administration had access to food and water *ad libitum* until the test. To avoid any cyrcadian influence the experiments were performed between 8:00 and 15:00.

Gabapentin pharmacokinetics. Gabapentin was dissolved in saline immediately before being used and was administered orally by gavage or intraperitoneally (300 mg/kg/4 mL). To determine the hematic concentrations, blood samples from each animal were collected twice, at 1 and 6 h from drug administration, by retro-orbital puncture.

The experiment was performed in accordance with Italian and European Community Guidelines, with the approval of the institutional Bioethical Committee.

Serum sample preparation. Serum samples were obtained by centrifuging the blood samples collected from each mouse at 1 and 6 h after the treatment. Then, according to Kushnir *et al.* (1999), 700 µL of acetonitrile were added to 500 µL of serum and then centrifuged for 10 min at 6000 rpm. The supernatant liquid was evaporated under a stream of nitrogen at 50°C, the residue was taken up in 1 mL of 0.1 M HCl and then applied to a C_{18} column, previously conditioned with 1 mL of methanol and 1 mL of 0.1 M HCl. The column was washed with 1 mL of 0.1 M HCl dried under vacuum for 1 min and the analytes were then eluted with 500 µL of 2% NH_4OH in methanol. After evaporation of the solvent, the residue was derivatized with 50 µL of MSTFA-NH_4I-DTE (1000:2:4 v/w/w), then incubated for 30 min at 70°C to obtain the bis-(trimethylsilil) ether of gabapentin (gabapentin-TMS).

GC-MS analysis. GC-MS/MS analyses were carried out using a Varian Saturn 2000 mass detector equipped with a Varian CP3800 gas chromatograph (Varian Inc., Harbor City, CA, USA). Instrument control and data processing were performed with an IBM computer and Saturn 2000 workstation data processing system. GC separation was achieved on a Chrompack (4330 EA Middelburg, The Netherlands) capillary column CP–SIL 8CB-MS (length 30 mm, inside diameter 0.25 mm, film thickness 0.25 µm), operated with helium at a flow rate of 1 mL/min and temperature programming of 120°C for 2 min ramped at 15°C/min to 225°C for 1 min and finally ramped at 20°C/min to 280°C and held for 4 min. Injections of 1 µL were effected at 250°C in the splitless mode (0.8 min) into a split–splitless injector. The transfer line was heated to 280°C and the ion trap temperature was 220°C.

MS/MS method development. Following examination of the electronic impact (EI) mass spectrum of gabapentin–TMS, it was determined that optimal sensitivity would be achieved through MS/MS isolation and collision induced dissociation (CID) of the molecular ion at m/z 226. Because the resonant mode of CID tended to produce more intense product ion, the resonant mode was chosen for quantitative analysis. Some runs with automated method development (AMD) were carried out to determine the optimal CID voltage for dissociation of the isolated parent ion at m/z 226.

Analytical conditions for MS/MS detection of gabapentin are shown in Table 1.

Calibration, quantitation and extraction recovery. An external standard calibration curve was prepared from derivatized standards of gabapentin–TMS in methanol at 1, 3, 5, 7 and 9 µg/mL. Concentrations of gabapentin in serum samples were calculated from the calibration curve as µg/mL. The detection limit of gabapentin–TMS, evaluated with decreasing concentrations of analyte until a response equivalent to three times the background noise, was 0.1 ng/mL. Extraction recovery (90%) was calculated by spiking 100 ng/mL of the analytes to 500 µL blank solution of serum and comparing this representative peak area with the peak area of gabapentin–TMS standard at the same concentration. The analysis was repeated seven times.

Statistical differences between serum concentrations of gabapentin at 1 and 6 h were verified using Student's t test for paired data.

Table 1. Ion trap parameters for MS-MS analysis of GBP

Emission current	80 µA
Count threshold	1 counts
Multiplier offset	200 V
Scan time	0.660 s
Maximum ionization time	25,000 µs
Ionization parameters	Ionization storage level m/z 48.0 Ejection amplitude 20.0 V
Isolation parameters	Parent ion mass m/z 226.0 Isolation window 3.0
Dissociation parameters	Waveform type resonant Excitation storage level m/z 100.0 Excitation amplitude 4.45 V Excitation time 80 ms

Copyright © 2004 John Wiley & Sons, Ltd.

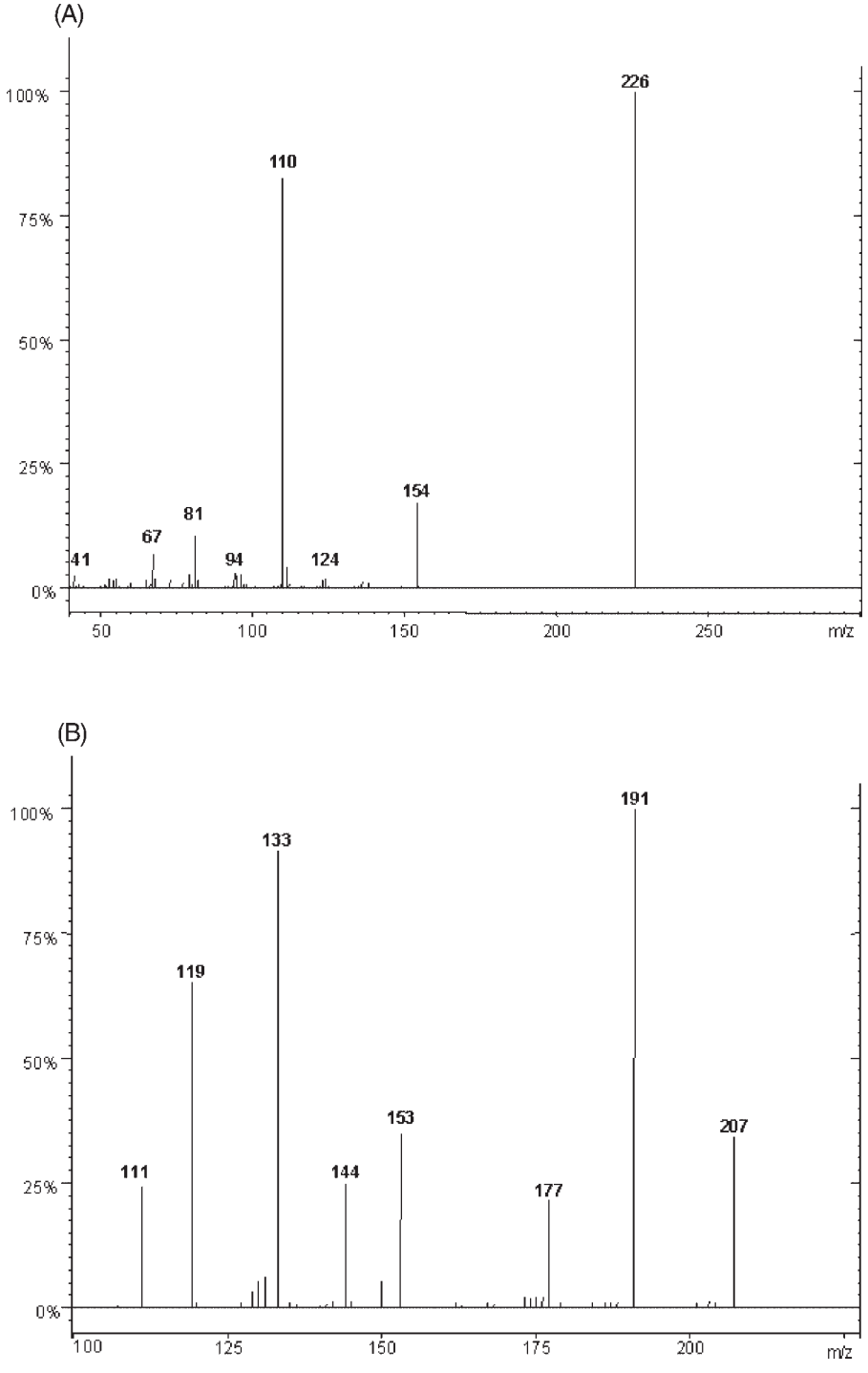

Figure 1. Mass spectrum (A) and tandem mass spectrum (B) of gabapentin–TMS.

RESULTS AND DISCUSSION

The analytical method we developed gave us both mass spectrum [Fig. 1(A)] and MS/MS spectrum [Fig. 1(B)] of gabapentin–TMS. Linear regression analysis of the standard demonstrated excellent linearity ($y = 485x + 14{,}746$, $r = 0.997$) in the range between 1 and 9 μg/mL gabapentin–TMS.

Mean serum concentrations at 1 and 6 h after admistration of 300 mg/kg of gabapentin are shown in Fig. 2. One hour after oral administration the serum gabapentin concentration did not differ from that detected at 1 h in mice treated intraperitoneally. Analogously, serum levels of the drug decreased by 66% at 6 h, independent of the administration route. These results are in agreement with pharmacokinetics studies

Copyright © 2004 John Wiley & Sons, Ltd.

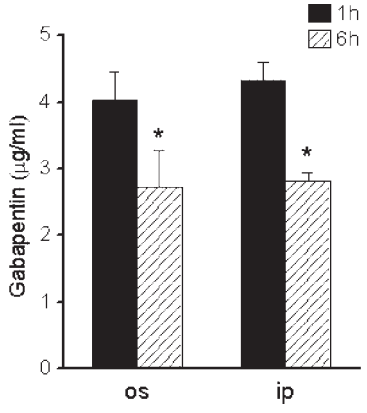

Figure 2. Mean serum concentrations of gabapentin at 1 and 6 h after oral (o.s.) and intraperitoneal (i.p.) admistration. *$p < 0.01$ vs 1 h. (Student's *t*-test for paired data).

(Windsor *et al.*, 1995; Ouellett *et al.*, 2001; Gidal *et al.*, 2000), which reported that gabapentin is rapidly absorbed after oral administration, reaches maximum blood concentration within 2–3 h, is not metabolized by the liver, does not induce hepatic enzymes, is not protein bound, and, has a half-life of 5–7 h. Moreover, the data indicate that its pharmacokinetics is not influenced by the administration route.

The GC-MS/MS method we set up to determine gabapentin concentration in serum samples, coupling two stages of mass analysis, can be very useful in identifying this drug in a complex mixture such as blood and urine. In fact, if soft ionization of the sample produces predominantly $[M + H]^+$ ions, the second stage of MS can be used to obtain mass spectrum which identifies this component in the mixture. Tandem mass spectrometry is also very useful in eliminating interferences because during biological sample processing endogenous components are often extracted, together with drugs and their metabolites, and are therefore introduced into the GC-MS system. As a result of co-eluting interferences, deformed chromatographic peaks are produced and sometimes the ion signal at the m/z of interest is produced by more than one compound. In our experience, this GC-MS/MS method is very effective in resolving these problems and allows a selective elimination of the interferences arising from the matrix and therefore, by eliminating the background, improves the detection limits.

CONCLUSION

Although gabapentin is a drug that is widely used due to its antiepileptic and antinociceptive properties, its bioavailability may vary greatly inter- and intra-subjects because of its particular active absorption by the gut and excretion by the kidney (Ouellet *et al.*, 2001). For this reason, determinations of blood concentrations of the drug should be performed in order to monitor its extent of absorption and toxicity, particularly in patients with impaired renal function, in whom its clearance is reduced and half-life becomes longer.

Application of GC-MS/MS analysis to determine gabapentin in serum gives excellent results. Therefore, this method may be very reliable in both clinical and experimental monitoring of the drug and, when compared with other previously described methods (Gauthier *et al.*, 2002; Kusnhir *et al.*, 1999; Ratnaraj *et al.*, 1998, Wolf *et al.*, 1996), it is easier to perform and sensitive enough to allow the presence of the drug to be determined at very low detection limits.

REFERENCES

Finnerup NB, Gottrup H and Jensen TS. Anticonvulsants in central pain. *Expert Opinion on Pharmacotherapy* 2002; **3**(10): 1411.

Gauthier D, Gupta R. Determination of gabapentin in plasma by liquid chromatography with fluorescence detection after solid-phase extraction with a C_{18} column. *Clinical Chemistry* 2002; **48**: 2259.

Gidal BE, Radulovic LL, Kruger S, Rutecki P, Pitterle M and Bockbrader HN. Inter- and intra-subject variability in gabapentin absorption and absolute bioavailability. *Epilepsy Research* 2000; **40**: 123.

Harden CL. New antiepileptic drugs. *Neurology* 1994; **44**: 787.

Jiang Q and Li S. Rapid high-performance liquid chromatographic determination of serum gabapentin. *Journal of Chromatography B* 1999; **727**: 119.

Kushnir MM, Crossett J, Brown PI and Urry FM. Analysis of gabapentin in serum and plasma by solid-phase extraction and gas chromatography-mass spectrometry for therapeutic drug monitoring. *Journal of Analytical Toxicology* 1999; **23**: 1.

Ouellet D, Bockbrader HN, Wesche DL, Shapiro DY and Garofalo E. Population pharmacokinetics of gabapentin in infants and children. *Epilepsy Research* 2001; **47**: 229.

Pappagallo M. Newer antiepileptic drugs: possible uses in the treatment of neuropathic pain and migraine. *Clinical Therapy* 2003; **25**(10): 2506.

Ratnaraj N, Patsalos PN. A high performance liquid chromatography micromethod for the simultaneous determination of vigabatrin and gabapentin in serum. *Theraputic Drug Monitoring* 1998; **20**: 430.

Rosenberg JM, Harrell C, Ristic H, Werner RA and De Rosayro AM. The effect of gabapentin on neuropathic pain. *Clinical Journal of Pain* 1997; **13**: 2251.

Rosner H, Rubin L and Kestenbaum A. Gabapentin adjunctive therapy in neuropathic pain states. *Clinical Journal of Pain* 1996; **12**: 56.

Rowbotham M, Harden N, Stacey B, Bernstein P and Magnus Miller L. Gabapentin for the tretment of postherpetic neuralgia: a randomized controlled trial. *Journal of the American Medical Association* 1998; **280**: 1837.

Stacey BR and Glanzman RL. Use of gabapentin for postherpetic neuralgia: results of two randomized, placebo-controlled studies. *Clinical Therapy* 2003; **25**(10): 2597.

Stevenson CM, Kim J and Fleisher D. Colonic absorption of antiepileptic agents. *Epilepsia* 1997; **38**: 63.

Stewart BH, Kugler AR, Thompson PR and Bockbrader HN. A saturable transport mechanism in the intestinal absorption of gabapentin is the underlying cause of the lack of proportionality between increasing dose and drug levels in plasma. *Pharmaceutical Research* 1993; **10**: 276.

Tang PH, Miles MV, Glauser TA and DeGrauw T. Automated microanalysis of gabapentin in human serum by high-performance liquid chromatography with fluorometric detection. *Journal of Chromatography B* 1999; **727**: 125.

Taylor CP. Emerging perspectives on the mechanism of action of gabapentin. *Neurology* 1994; **44**(Suppl. 5): S10.

Copyright © 2004 John Wiley & Sons, Ltd.

Taylor CP, Gee NS, Su TZ, Kocsis JD, Welty DF, Brown JP, Dooley DJ, Boden P and Singh L. A summary of mechanistic hypotheses of gabapentin pharmacology. *Epilepsy Research* 1998; **29**: 233.

Van Hoft JA, Dougherty JJ, Endeman D, Nichols RA and Wadman WJ. Gabapentin inhibits presynaptic Ca^{2+} influx and synaptic transmission in rat hippocampus and neocortex. *Euoropean Journal of Pharmacology* 2003; **449**: 221.

Windsor BL and Radulovic LL. Measurement of a new anticonvulsant, (*S*)-3-(aminomethyl)-5-methylhexanoic acid, in plasma and milk by high-performance liquid chromatography. *Journal of Chromatography B* 1995; **674**: 143.

Wolf CE, Saady JJ and Poklis A. Determination of gabapentin in serum using solid-phase extraction and gas-liquid chromatography. *Journal of Analytical Toxicology* 1996; **20**: 498.

Zhu Z and Neirinck L. High-performance liquid chromatographic method for the determination of gabapentin in human plasma. *Journal of Chromatography B* 2002; **779**: 307.

Copyright © 2004 John Wiley & Sons, Ltd.