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Rapid quantification of gabapentin in human plasma by liquid chromatography/tandem mass spectrometry

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Abstract

A simple, sensitive and rapid liquid chromatography/tandem mass spectrometry (LC–MS/MS) method was developed and validated for the quantification of gabapentin, a new antiepileptic drug, in human plasma using its structural analogue, 1,1-cyclohexane diacetic acid monoamide (CAM) as internal standard. The method involved a simple protein precipitation by means of acetonitrile followed by a rapid isocratic elution with 10 mM ammonium formate buffer/acetonitrile (20/80, v/v, pH 3.0) on Waters Symmetry® C_{18} reversed phase chromatographic column and analyzed by mass spectrometry in the multiple reaction monitoring mode. The precursor to product ion transitions of m/z 172 → 154 and m/z 200 → 182 were used to measure the analyte and the IS, respectively. The assay exhibited a linear dynamic range of 40–10 000 ng/mL for gabapentin in human plasma. The limit of detection and lower limit of quantification in human plasma were 10 and 40 ng/mL, respectively. Acceptable precision and accuracy were obtained for concentrations over the standard curve ranges. A run time of 2 min for each sample made it possible to analyze a throughput of more than 400 human plasma samples per day. The validated method has been successfully used to analyze human plasma samples for application in pharmacokinetic, bioavailability or bioequivalence studies.

Keywords: Gabapentin; 1,1-Cyclohexane diacetic acid monoamide (CAM); Liquid chromatography/tandem mass spectrometry; Human plasma; Pharmacokinetic study

1. Introduction

Gabapentin, 1-(aminomethyl-1-cyclohexyl) acetic acid, is a new antiepileptic drug currently being introduced in therapy worldwide [1,2]. Gabapentin is a structural analogue of the inhibitory neurotransmitter γ-aminobutyric acid (GABA). Gabapentin crosses the blood–brain barrier and is employed for the treatment of partial seizures. The mode of action of gabapentin is not completely understood, since it is neither a GABA agonist nor an inhibitor of GABA uptake or degradation. However, it has been demonstrated that gabapentin increases the GABA level in the brain [3,4]. 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 such as chronic post-herpetic pain, painful diabetic neuropathy, central neuropathic pain following lesions of the CNS and migraine [5–10]. 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 [11].

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 [12]. For this reason, determinations of blood concentrations of gabapentin may be useful in assessing compliance and evaluating risks of toxicity.

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Different methods have been reported for the quantification of gabapentin in biological fluids. These methods are based on high-performance liquid chromatography (HPLC) with ultraviolet (UV) [13–16] or fluorescence (F) [17–23] detection; gas chromatography (GC) with flame ionization (FI) [24,25] or mass spectrometric (MS) [26–28] detection and capillary electrophoresis (CE) [29,30]. Recently, Gambelunghe et al. [31] reported a sensitive method for determining gabapentin in serum using gas chromatography/tandem mass spectrometry (GC-MS/MS). The HPLC and CE methods require derivatization of gabapentin to produce a chromophore, detectable by UV/F. The GC methods require derivatization of gabapentin to improve the volatility and avoid column interactions. Generally, for routine analysis of large series, the derivatization step increases the time of sample preparation and the cost of the method. Another inconvenience is the zwitterionic characteristic of gabapentin, which renders it extremely difficult to extract from biological samples. Thus, when using conventional HPLC, GC or CE, long extraction procedures (multi-step liquid–liquid or multi-step solid-phase extractions) are required.

Quantification of drugs in biological matrices by liquid chromatography/tandem mass spectrometry (LC–MS/MS) is becoming more common, owing to the improved sensitivity and selectivity of this technique [32–46]. LC–MS/MS requires less extensive sample preparation since gabapentin can be detected directly without derivatization, and thus sample preparation time is reduced. Ifa et al. [47] reported an LC–MS/MS method for the quantification of gabapentin in human plasma using acetaminophen as an internal standard. Recently, Carlsson and Reubsaet [48] developed an LC–MS/MS method for quantification of gabapentin in blood using (*S*)-(+)-α-amino-cyclohexane-propionic acid hydrate as an internal standard.

The purpose of this investigation was to explore the high selectivity and sensitivity of triple quadrupole MS system with an electrospray interface for the development and validation of a robust reversed phase LC–MS/MS method in multiple reaction monitoring mode for the quantification of gabapentin in human plasma using its structural analogue, 1,1-cyclohexane diacetic acid monoamide (CAM) as the internal standard. It was essential to establish an assay capable of quantifying gabapentin at concentrations down to 40 ng/mL. At the same time, it was expected that this method would be efficient in analyzing large number of plasma samples obtained for pharmacokinetic, bioavailability or bioequivalence studies after therapeutic doses of gabapentin. The procedure requires a single protein precipitation without any derivatization step and unlike to Carlsson and Reubsaet method [48], evaporation-concentration steps are not required. The advantages of the method presented in this paper in comparison to that of Ifa et al. [47] are in the following ways: (1) less plasma was used; 100 μL instead of 200 μL; the volume of samples to be collected per time point from an individual during study is reduced significantly, allowing inclusion of additional points; (2) more sensitive; sensitivity of 40 ng/mL (∼ca. 200 pg on-column) was achieved with 5 μL sample injected in comparison to 50 ng/mL (∼ca. 2000 pg on-column) with 40 μL sample injection; the sensitivity could be further improved by sample concentration; (3) rapidity; sample turnaround time of 2 min compared to 4 min makes it an attractive procedure in high-throughput bioanalysis of gabapentin.

2. Experimental

2.1. Chemicals

Gabapentin and 1,1-cyclohexane diacetic acid monoamide (CAM), used as internal standard (IS), were obtained from our R&D Department (Hyderabad, India). Chemical structures are presented in Scheme 1. HPLC-grade LiChrosolv methanol and LiChrosolv acetonitrile were purchased from Merck (Darmstadt, Germany). Formic acid and ammonium formate were purchased from Merck (Worli, Mumbai, India). HPLC Type I water from Milli-Q system (Millipore, Bedford, MA, USA) was used. All other chemicals were of analytical grade.

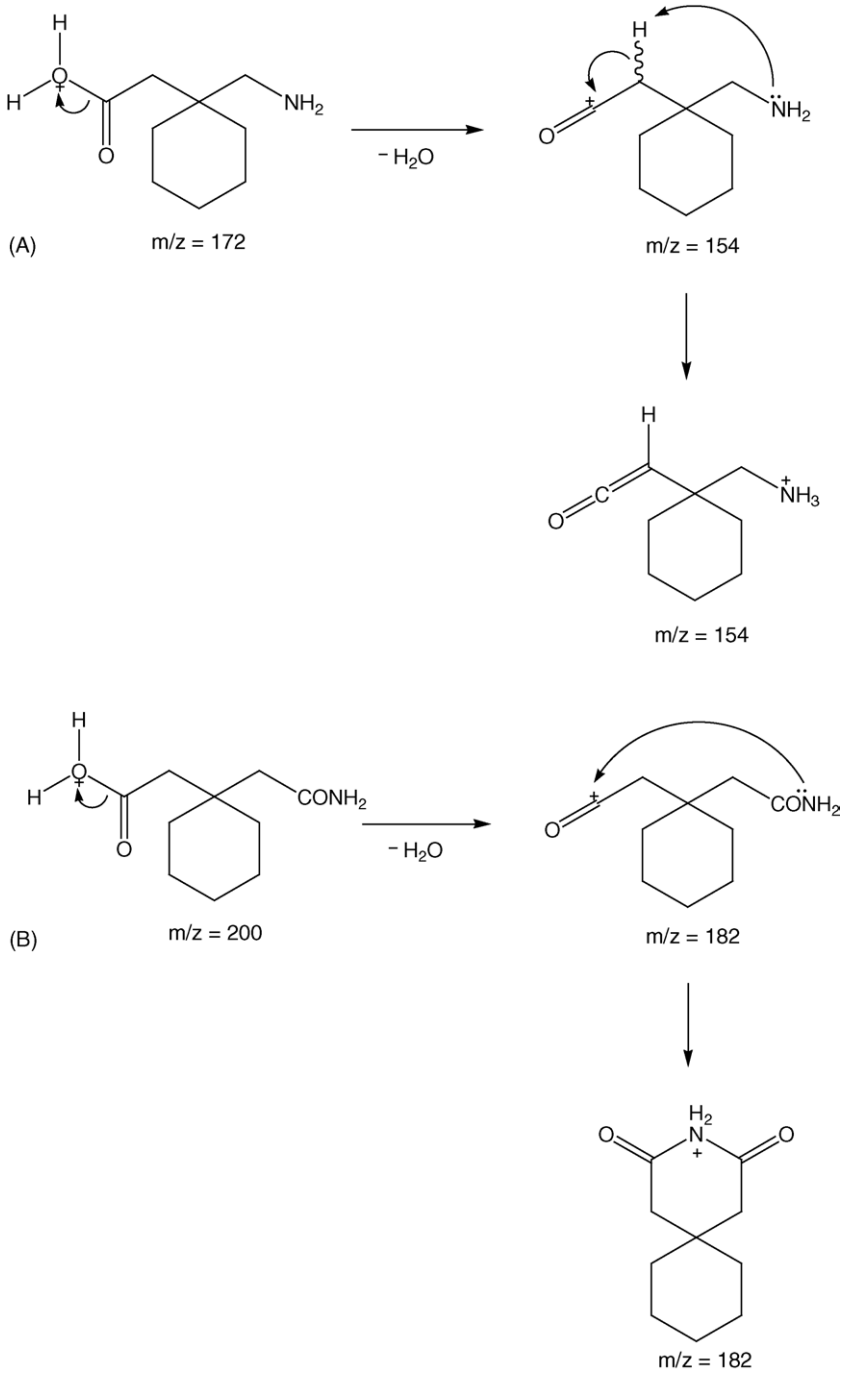
2.2. LC–MS/MS instrument and conditions

The HPLC Agilent 1100 Series (Agilent Technologies, Palo Alto, USA) is equipped with a G1312A binary pump, a G1379A degasser, a G1367A autosampler equipped with a G1330B thermostat, a G1316A thermostatted column compartment and a G1323B control module. The chromatography was on Waters symmetry C_{18} column (5 μm, 150 mm × 4.6 mm i.d.) at 30 °C temperature. The mobile phase composition was a mixture of 10 mM ammonium formate buffer/acetonitrile (20/80, v/v, pH adjusted to 3.0 with formic acid), which was pumped at a flow-rate of 1.0 mL/min.

Mass spectrometric detection was performed on an API 4000 triple quadrupole instrument (MDS-SCIEX, Toronto, Canada) using MRM. A turbo electrospray interface in positive ionization mode was used. The main working parameters of the mass spectrometer are summarized in Table 1. Data processing was performed on Analyst 1.4 software package (SCIEX).

2.3. Sample preparation

Sample preparation involved a simple protein precipitation with acetonitrile. Aliquots (100 μL) of human plasma were subjected for precipitation with addition of 100 μL of IS working solution (5 μg/mL) and 500 μL of acetonitrile. The samples were vortex mixed for 20 s and centrifuged at 8000 rpm for 3 min. The upper layer (400 μL) was transferred into injector vials and a 5 μL aliquot was injected into chromatographic system.



Scheme 1. Dissociation route for (A) gabapentin and (B) 1,1-cyclohexane diacetic acid monoamide, CAM (IS) (see text for CID conditions).

2.4. Bioanalytical method validation

Standard stock solutions of gabapentin (1 mg/mL) and the IS (1 mg/mL) were separately prepared in 10 mL volumetric flasks with methanol. Working solutions for calibration and controls were prepared from the stock solution by adequate dilution using diluent (water/methanol, 50/50, v/v). The IS working solution (5 μg/mL) was prepared by diluting its stock solution with diluent. Working solutions (50 μL) were added to 950 μL drug-free human plasma to obtain gabapentin concentration levels of 40, 80, 200, 400, 800, 2000, 4000 and 10 000 ng/mL. Quality control (QC) samples were prepared as a bulk based on an independent weighing of standard drug, at concentrations of 40 ng/mL (LLOQ), 120 ng/mL (low), 3000 ng/mL (medium) and 8000 ng/mL (high) as a single batch at each concentration. These samples were divided into aliquots in microcentrifuge tubes (Tarson, 1.5 mL) and stored in the freezer at below −50 °C until analysis.

Table 1
Tandem mass spectrometer main working parameters

Parameter	Value
Source temperature (°C)	250
Dwell time per transition (ms)	200
Ion source gas (gas 1) (psi)	9
Ion source gas (gas 2) (psi)	20
Curtain gas (psi)	10
Collision gas (psi)	8
Ion spray voltage (V)	5500
Entrance potential (V)	10
Declustering potential (V)	50 (analyte) and 50 (IS)
Collision energy (V)	17 (analyte) and 15 (IS)
Collision cell exit potential (V)	10 (analyte) and 12 (IS)
Mode of analysis	Positive
Ion transition for gabapentin (*m*/*z*)	172.1/154.1
Ion transition for CAM (*m*/*z*)	200.1/182.4

A calibration curve was constructed from a blank sample (a plasma sample processed without the IS), a zero sample (a plasma processed with the IS) and eight non-zero samples covering the range 40–10 000 ng/mL including LLOQ. The calibration curves were generated using the analyte to IS peak area ratios by weighted ($1/x^2$) least-squares linear regression on five consecutive days. The acceptance criterion for a calibration curve was a correlation coefficient (r) of 0.99 or better, and that each back-calculated standard concentration must be within 15% deviation from the nominal value except at the LLOQ, for which maximum acceptable deviation was set at 20%. At least 67% of non-zero standards were required to meet the above criteria, including acceptable LLOQ and upper limit of quantification.

The within-batch precision and accuracy were determined by analyzing five sets of quality control samples in a batch. The between-batch precision and accuracy was determined by analyzing five sets of quality control samples on three different batches. The quality control samples were randomized daily, processed and analyzed in position either (a) immediately following the standard curve, (b) in the middle of the batch, or (c) at the end of the batch. The acceptance criteria for within- and between-batch precision were 20% or better for LLOQ and 15% or better for the other concentrations, and that the accuracy was 100 ± 20% or better for LLOQ and 100 ± 15% or better for other of concentrations.

Recovery of gabapentin from the extraction procedure was determined by a comparison of the peak area of gabapentin in spiked plasma samples (five low, medium and high quality controls) with the peak area of gabapentin in samples prepared by spiking extracted drug-free plasma samples with the same amounts of gabapentin at the step immediately prior to chromatography. Similarly, recovery of IS was determined by comparing the mean peak areas of extracted quality control samples ($n = 10$) to mean peak areas of IS in samples prepared by spiking extracted drug-free plasma samples with the same amounts of IS at the step immediately prior to chromatography.

3. Results and discussion

In order to develop a method with the desired LLOQ (40 ng/mL), it was necessary to use MS–MS detection, as MS–MS methods provide improved limit of detection (LOD) for trace-mixture analysis [32]. The inherent selectivity of MS–MS detection was also expected to be beneficial in developing a selective and sensitive method. The positive ion TurboIonspray product ion mass spectrum of gabapentin and the IS are shown in Fig. 1A and B, respectively. $[M + H]^+$ was the predominant ion in the Q1 spectrum and was used as the precursor ion to obtain the product ion spectra. The most sensitive mass transition was from *m*/*z* 172 to *m*/*z* 154 for gabapentin and *m*/*z* 200 to *m*/*z* 182 for the IS. The structure of the protonated molecules and neutral losses are shown in Scheme 1, proposing a dissociation route for gabapentin and IS. LC-MRM is a very powerful technique for pharmacokinetic studies since it provides sensitivity and selectivity requirements for analytical methods. Thus, the MRM tech-

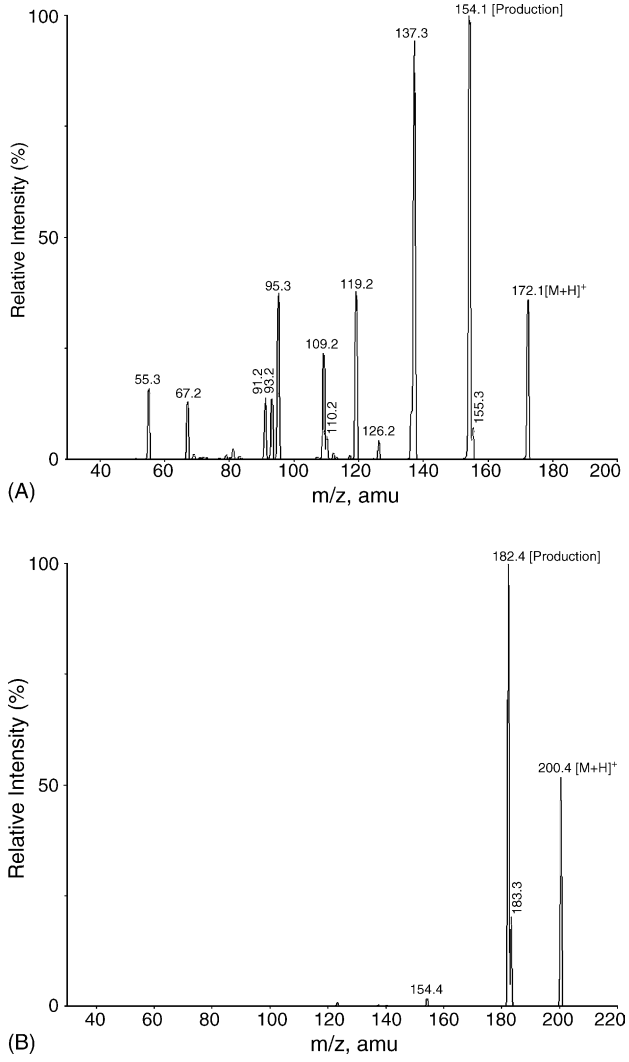


Fig. 1. Full scan positive ion TurboIonspray product ion mass spectra of (A) gabapentin and (B) IS.

nique was chosen for the assay development. The MRM state file parameters were optimized to maximize the response for the analyte. The parameters presented in Table 1 are the result of this optimization.

3.1. Method development

The chromatographic conditions, especially the composition of mobile phase, were optimized through several trials to achieve good resolution and symmetric peak shapes for the analyte and the IS, as well as short run time. Modifiers such as formic acid and ammonium formate alone or in combination in different concentrations were added. It was found that a mixture of 10 mM ammonium formate buffer/acetonitrile (20/80, v/v; pH 3.0) could achieve this purpose and was finally adopted as the mobile phase. The formic acid was found to be necessary in order to lower the pH to protonate the gabapentin and thus deliver good peak shape. The percentage of formic acid was optimized to maintain this peak shape whilst being consistent with good ionization and fragmentation in the mass spectrometer.

The tandem mass spectrometer allows the selective detection of substances with varying masses or fragments without chromatographic separation. The development of the chromatographic system was focused on short retention times in order to assure high throughput, paying attention to matrix effects as well as good peak shapes. The high proportion of organic solvent (10 mM ammonium formate/acetonitrile (20/80, v/v; pH 3.0)) eluted the analyte and the IS at retention times of 1.2 and 1.4 min, respectively. A flow-rate of 1 mL/min produced good peak shapes and permitted a runtime to 2 min.

Internal standard is necessary for determination of analyte in biological samples. For an LC–MS/MS analysis, utilization of stable isotope-labeled drugs as internal standards proves to be helpful when significant matrix effect is possible. However, there are also many problems with the use of stable isotope-labeled internal standards. The major problems involve inadequate isotopic purity and stability, which often impose unfavorable impact on highly sensitive quantitative analyses. In the initial stages of this work, several compounds were investigated to find a suitable internal standard and finally CAM (Scheme 1), structurally related to gabapentin, was found to be best for the present purpose. Clean chromatograms were obtained and no significant direct interferences in the MRM channels at the relevant retention times were observed. However, in ESI, signal suppression or enhancement may occur due to co-eluting endogenous components of the sample matrix. These potential matrix effects were evaluated by spiking blank plasma extracts at the low and high QC levels. The resulting chromatograms were compared with those obtained for clean standard solutions at the same concentrations. Six independent plasma lots were used with six samples from each lot. The results (data not shown) showed that there was no significant difference between peak responses for spiked plasma extracts and clean solutions.

3.2. Assay performance and validation

The calibration curve was linear over the concentration range 40–10 000 ng/mL. The best linear fit and least squares residuals for the calibration curve were achieved with a $1/x^2$ weighing factor, giving a mean linear regression equation for the calibration curve of $y = 0.0003x - 0.0086$ where y is the peak area ratio of the analyte to the IS and x the concentration of the analyte. The correlation coefficient (r) for gabapentin was above 0.999. Table 2 summarizes the calibration curve results.

The specificity of the method was examined by analyzing ($n = 8$) blank human plasma extract (Fig. 2A) and an extract spiked only with the internal standard (Fig. 2B). As shown in Fig. 2A, no significant interference in the blank plasma traces was observed from endogenous substances in drug-free human plasma at the retention time of the analyte. Similarly, Fig. 2B shows the absence of direct interference from the internal standard to the MRM channel of the analyte. Fig. 2C depicts a representative ion-chromatogram for the LLOQ (40 ng/mL). The product ion-chromatogram obtained from an extracted plasma sample of a healthy volunteer who participated in a bioequivalence study conducted on 24 persons, is depicted in Fig. 3. Gabapentin was unambiguously identified and was quantified as 2890 ng/mL.

The extraction recovery of gabapentin was 75.3% on average, and the dependence on concentration is negligible. The recovery of the IS was 82.8% at the concentration used in the assay (5 μg/mL). Recovery of the analyte and IS were high and with the consistency in the recovery of gabapentin

Table 2
Precision and accuracy data of back-calculated concentrations of calibration samples for gabapentin in human plasma

Concentration added (ng/mL)	Concentration found (ng/mL) (mean ± S.D.; $n = 5$)	Precision (%)	Accuracy (%)
40	40.6 ± 2.5	6.2	100.8
80	78.8 ± 1.7	2.2	98.0
200	203.4 ± 8.8	4.3	99.0
400	399.7 ± 11.4	2.8	99.3
800	799.0 ± 11.7	1.4	99.3
2000	2048.4 ± 37.2	1.8	101.7
4000	4038.8 ± 111.1	2.7	100.2
10000	10028.3 ± 101.3	1.0	99.7

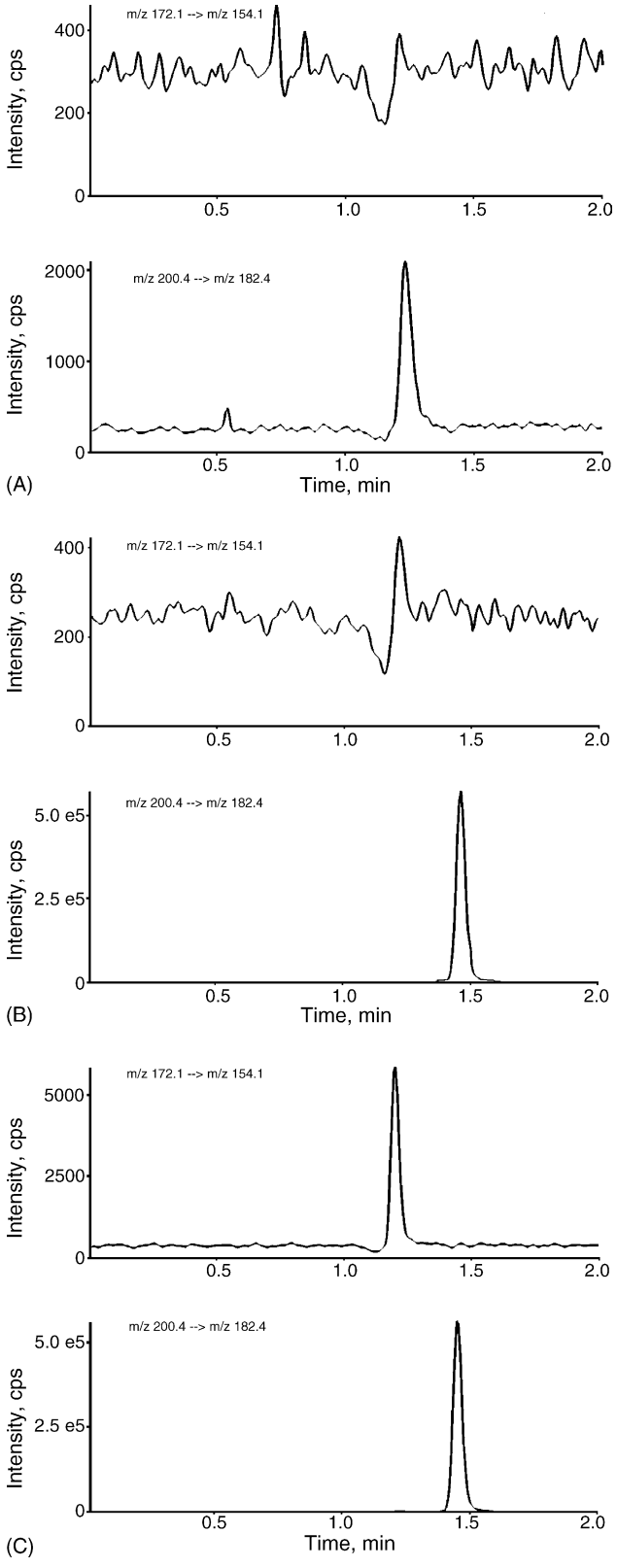


Fig. 2. MRM ion-chromatograms for gabapentin and the IS resulting from the analysis of (A) blank (drug and internal standard free) human plasma, (B) blank (drug-free spiked with IS) human plasma and (C) 40 ng/mL (LLOQ) of gabapentin spiked with IS.

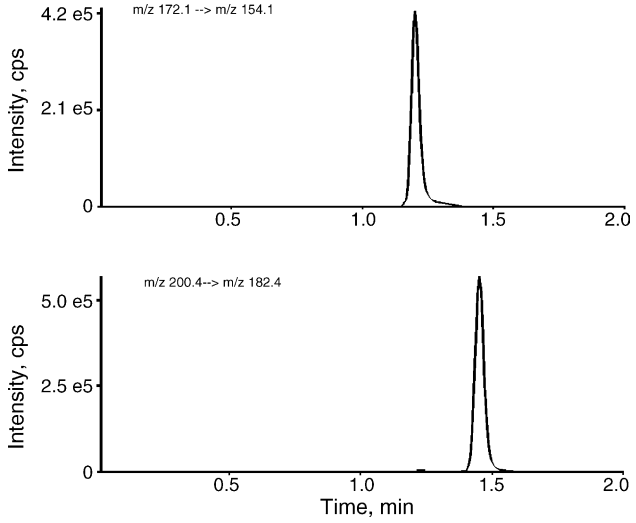


Fig. 3. MRM ion-chromatograms resulting from the analysis of volunteer plasma sample after the administration of a 900 mg oral single dose of gabapentin. The sample concentration was determined to be 2890 ng/mL.

and IS, the assay has proved to be robust in high-throughput bioanalysis.

The LOD demonstrated that the analyte gave a signal-to-noise ratio (S/N) of ≥3 for 10 ng/mL extracted/injected. The LLOQ, the lowest concentration in the standard curve that can be measured with acceptable accuracy and precision was found to be 40 ng/mL in human plasma. The mean response for the analyte peak at the assay sensitivity limit (40 ng/mL) was ≈12.1-fold greater than the mean response for the peak in five blank human plasma samples at the retention time of the analyte. Excellent sensitivity was observed for a 5 μL injection volume; the LLOQ corresponds to ca. 200 pg on-column. The between-batch precision at the LLOQ was 4.1% and the between-batch accuracy was 106.1% (Table 3). The within-batch precision was 6.6% and the accuracy was 106.7% for gabapentin.

The middle and upper quantification levels of gabapentin ranged from 120 to 8000 ng/mL in human plasma. For the between-batch experiments, the precision ranged from 4.9 to 7.7% and the accuracy ranged from 101.2 to 106.0% (Table 3). For the within-batch experiments, the precision and accuracy for the analyte met the acceptance criteria (<±15%).

The upper concentration limits can be extended with acceptable precision and accuracy to 100 μg/mL by a 20-fold dilution with control human plasma. These results suggest that sample with concentrations greater than the upper limit of the calibration curve can in this way be assayed to obtain acceptable data (Table 3).

These results show that the method is accurate and precise over the concentration range 40–10 000 ng/mL.

3.3. Stability studies

The stability of the analyte and IS in human plasma under different temperature and timing conditions, as well as the

Table 3
Precision and accuracy of the method for determining gabapentin concentrations in plasma samples

Concentration added (ng/mL)	Within-batch precision ($n = 5$)			Between-batch precision ($n = 3$)		
	Concentration found (ng/mL) (mean ± S.D.)	Precision (%)	Accuracy (%)	Concentration found (ng/mL) (mean ± S.D.)	Precision (%)	Accuracy (%)
40	42.8 ± 2.8	6.6	106.7	42.5 ± 1.7	4.1	106.1
120	124.4 ± 6.7	5.4	103.4	121.8 ± 9.4	7.7	101.2
3000	2992.2 ± 165.3	5.5	99.5	3051.6 ± 151.9	4.9	101.4
8000	8441.4 ± 281.4	3.3	105.2	8507.8 ± 472.2	5.5	106.0
100[a]	101.3 ± 2.8	2.8	101	104.7 ± 6.3	6.0	104.7

[a] The sample concentration was 100 μg/mL and was processed with 20-fold dilution.

Table 4
Stability of gabapentin in human plasma

Sample concentration (ng/mL) ($n = 5$)	Concentration found (ng/mL)	Precision (%)	Accuracy (%)
Short-term stability for 24 h in plasma			
120	119.1	3.8	99.2
8000	8401.3	2.4	105.0
Three freeze-thaw cycles			
120	127.8	5.9	106.5
8000	7759.6	3.1	96.9
Autosampler stability for 25 h			
120	118.4	4.6	98.6
8000	7806.8	6.2	97.5
Stability for 30 days at <−50 °C			
120	126.4	8.6	105.3
8000	7855.2	7.2	98.2

stability in stock solution, was evaluated as follows. All the stability studies were carried out at two concentration levels (120 and 8000 ng/mL as low and high values) with five determinations for each.

For short-term stability determination, stored plasma aliquots were thawed and kept at room temperature for a period of time exceeding that expected to be encountered during the routine sample preparation (around 24 h). Samples were extracted and analyzed as described above and the results are given in Table 4. These results indicate reliable stability behavior under the experimental conditions of the regular analytical procedure.

The stability of QC samples kept in the autosampler for 25 h was also assessed. The results indicate that solutions of gabapentin and IS can remain in the autosampler for at least 25 h, without showing significant loss in the quantified values, indicating that samples should be processed within this period of time (Table 4).

The data representing the stability of gabapentin in plasma at two QC levels over three freeze and thaw cycles are given in Table 4. These tests indicate that the analyte is stable in human plasma for three freeze and thaw cycles, when stored at below −50 °C and thawed to room temperature.

Table 4 also summarizes the long-term stability data for gabapentin in plasma samples stored for a period of 30 days at below −50 °C. The stability study of gabapentin in human plasma showed reliable stability behavior, as the mean of the results of the tested samples were within the acceptance criteria of ±15% of the initial values of the controls. These findings indicate that storage of gabapentin in plasma samples at below −50 °C is adequate, and no stability-related problems would be expected during routine analysis for pharmacokinetic, bioavailability or bioequivalence studies.

The stability of stock solutions was tested and established at room temperature for 2, 27 h and under refrigeration (∼4 °C) for 30 days. The recoveries for gabapentin and IS were 101.5 (CV 0.8%), 99.4 (CV 1.3%), 96.8 (CV 1.2%) and 100.6 (CV 1.9%), 100.7 (CV 2.4%), 99.2 (CV 2.1%) respectively. The results revealed optimum stability for the prepared stock solutions throughout the period intended for their daily use.

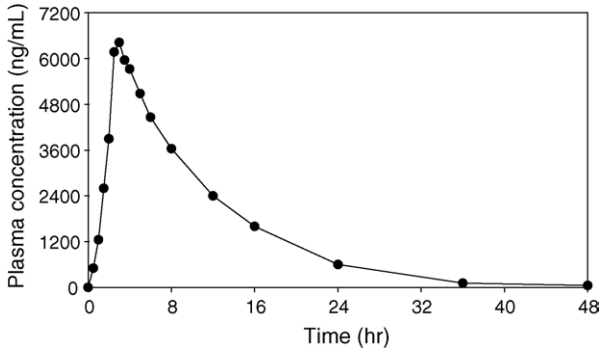


Fig. 4. Representative plasma concentration vs. time curve obtained from a subject after the single dose oral administration of 900 mg of gabapentin.

3.4. Application

The validated method has been successfully used to quantify gabapentin concentrations in the human plasma samples after the administration of a single 900 mg oral dose of gabapentin. The representative concentration versus time profile of a subject receiving a single dose of gabapentin is presented in Fig. 4.

4. Conclusion

In summary, LC–MS/MS method for the quantitation of gabapentin in human plasma was developed and fully validated as per FDA guidelines [49]. This method offers significant advantages over those previously reported, in terms of improved sensitivity and selectivity, faster run time (2 min) and lower sample requirements. Thus the volume of samples to be collected per time point from an individual during trial is reduced significantly, allowing inclusion of additional points. With dilution integrity up to 20-fold, we have established that the upper limit of quantification is extendable up to 100 μg/mL. Hence, this method is useful for single and multiple ascending dose studies in human subjects. The current method has shown acceptable precision and adequate sensitivity for the quantification of gabapentin in human plasma samples obtained for pharmacokinetic, bioavailability or bioequivalence studies. The desired sensitivity of gabapentin was achieved with an LLOQ of 40 ng/mL, which has a within- and between-batch CV of 6.6 and 4.1%, respectively. The sensitivity could be further improved by sample concentration.

Gabapentin was shown to be stable in routine analysis conditions and in human plasma for up to 30 days when stored at below −50 °C. The simplicity, protein precipitation and sample turnover rate of 2 min per sample, make it an attractive procedure in high-throughput bioanalysis of gabapentin. The validated method allows quantification of gabapentin in the 40–10 000 ng/mL range.

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References

[1] M.C. Walker, P.N. Patsalos, Pharm. Ther. 67 (1995) 351–384.
[2] P. Gareri, T. Gravina, G. Ferreri, G.D. Sarro, Prog. Neurobiol. 58 (1999) 389–407.
[3] O.A. Petroff, F. Hyder, D.L. Rothman, R.H. Mattson, Epilepsia 41 (2000) 675–680.
[4] O.A. Petroff, D.L. Rothman, K.L. Behar, D. Lamoureux, R.H. Mattson, Ann. Neurol. 39 (1996) 95–99.
[5] N.B. Finnerup, H. Gottrup, T.S. Jensen, Expert Opin. Pharmacother. 3 (2002) 1411–1420.
[6] M. Pappagallo, Clin. Therapy 25 (2003) 2506–2538.
[7] J.M. Rosenberg, C. Harrell, H. Ristic, R.A. Werner, A.M. de Rosayro, Clin. J. Pain 13 (1997) 251–255.
[8] H. Rosner, L. Rubin, A. Kestenbaum, Clin. J. Pain 12 (1996) 56–58.
[9] M. Rowbotham, N. Harden, B. Stacey, P. Bernstein, L.M. Magnus, J. Am. Med. Assoc. 280 (1998) 1837–1842.
[10] B.R. Stacey, R.L. Glanzman, Clin. Therapy 25 (2003) 2597–2608.
[11] J.A. van Hoft, J.J. Dougherty, D. Endeman, R.A. Nichols, W.J. Wadman, Eur. J. Pharmaco. 449 (2003) 221–228.
[12] D. Ouellet, H.N. Bockbrader, D.L. Wesche, D.Y. Shapiro, E. Garofalo, Epilepsy Res. 47 (2001) 229–241.
[13] H. Hengy, E.U. Kölle, J. Chromatogr. 341 (1985) 473–478.
[14] G.L. Lensmeyer, T. Kempf, B.E. Gidal, D.A. Wiebe, Ther. Drug Monit. 17 (1995) 251–258.
[15] A. Fraser, W. MacNeil, Recent Developments in Therapeutic Drug Monitoring and Clinical Toxicology, Marcel Dekker, New York, 1992, pp. 313–320.
[16] Z. Zhu, L. Neirinck, J. Chromatogr. B 779 (2002) 307–312.
[17] G. Forrest, G.J. Sills, J.P. Leach, M.J. Brodie, J. Chromatogr. B: Biomed. Appl. 681 (1996) 421–425.
[18] N. Wad, G. Krämer, J. Chromatogr. B: Biomed. Appl. 705 (1998) 154–158.
[19] N. Ratnaraj, P.N. Patsalos, Ther. Drug Monit. 20 (1998) 430–434.
[20] Q. Jiang, S. Li, J. Chromatogr. B: Biomed. Appl. 727 (1999) 119–123.
[21] P.H. Tang, M.V. Miles, T.A. Glauser, T. DeGrauw, J. Chromatogr. B: Biomed. Appl. 727 (1999) 125–129.
[22] D. Gauthier, R. Gupta, Clin. Chem. 48 (2002) 2256–2259.
[23] D.F. Chollet, L. Goumaz, C. Juliano, G. Anderegg, J. Chromatogr. B: Biomed. Appl. 746 (2000) 311–314.
[24] W.D. Hooper, M.C. Kavanagh, R.G. Dickinson, J. Chromatogr. 529 (1990) 167–174.
[25] C.E. Wolf, J.J. Saady, A. Poklis, J. Anal. Toxicol. 20 (1996) 498–501.
[26] M.M. Kushnir, J. Crossett, P.I. Brown, F.M. Urry, J. Anal. Toxicol. 23 (1999) 1–6.
[27] L.L. Garcia, Z.K. Shihabi, K. Oles, J. Chromatogr. B 669 (1995) 157–162.
[28] D.C. Borrey, K.O. Godderis, V.I. Engelrelst, D.R. Bernard, M.R. Langlois, Clin. Chim. Acta 354 (2005) 147–151.
[29] S.Y. Chang, F.Y. Wang, J. Chromatogr. B 799 (2004) 265–270.
[30] M.S. Lee, LC/MS Applications in Drug Development, Wiley, New York, 2002.
[31] C. Gambelunghe, G. Mariucci, M. Tantucci, M.V. Ambrosini, Biomed. Charomtogr. 19 (2005) 63–67.
[32] M. Jemal, Biomed. Chromatogr. 14 (2000) 422–429.
[33] N.V.S. Ramakrishna, K.N. Vishwottam, S. Puran, S. Manoj, M. Santosh, S. Wishu, M. Koteshwara, J. Chidambara, B. Gopinadh, B. Sumatha, J. Chromatogr. B 805 (2004) 13–20.
[34] N.V.S. Ramakrishna, K.N. Vishwottam, S. Puran, S. Manoj, M. Santosh, M. Koteshwara, J. Mass Spectrom. 39 (2004) 824–832.
[35] N.V.S. Ramakrishna, K.N. Vishwottam, S. Puran, M. Koteshwara, S. Manoj, M. Santosh, J. Chromatogr. B 809 (2004) 117–124.
[36] N.V.S. Ramakrishna, K.N. Vishwottam, S. Puran, M. Koteshwara, S. Manoj, M. Santosh, J. Chidambara, S. Wishu, B. Sumatha, J. Chromatogr. B 809 (2004) 243–249.
[37] N.V.S. Ramakrishna, M. Koteshwara, K.N. Vishwottam, S. Puran, S. Manoj, M. Santosh, J. Pharm. Biomed. Anal. 36 (2004) 505–515.
[38] N.V.S. Ramakrishna, K.N. Vishwottam, S. Manoj, M. Koteshwara, J. Chidambara, D.P. Varma, Biomed. Chromatogr. 19 (2005), doi:10.1002/bmc.477, January 14 [Epub ahead of print].
[39] N.V.S. Ramakrishna, K.N. Vishwottam, M. Koteshwara, S. Manoj, M. Santosh, D.P. Varma, J. Pharm. Biomed. Anal. (2005), doi:10.1016/j.jpba.2005.05.021.

[40] N.V.S. Ramakrishna, K.N. Vishwottam, S. Manoj, M. Koteshwara, S. Wishu, D.P. Varma, Biomed. Chromatogr. 19 (2005), doi:10.1002/bmc.498, April 12 [Epub ahead of print].
[41] N.V.S. Ramakrishna, K.N. Vishwottam, S. Manoj, M. Koteshwara, S. Wishu, D.P. Varma, Biomed. Chromatogr. 19 (2005), doi:10.1002/bmc.510, April 26 [Epub ahead of print].
[42] N.V.S. Ramakrishna, K.N. Vishwottam, S. Manoj, M. Koteshwara, M. Santosh, J. Chidambara, B.R. Kumar, Rapid Commun. Mass Spectrom. 19 (2005) 1970–1978.
[43] N.V.S. Ramakrishna, K.N. Vishwottam, S. Manoj, M. Koteshwara, M. Santosh, B.R. Kumar, Y. Anjaneyulu, J. Mass Spectrom., in press.
[44] N.V.S. Ramakrishna, K.N. Vishwottam, S. Manoj, M. Koteshwara, M. Santosh, Y. Anjaneyulu, Chromatographia, in press.
[45] R.V.S. Nirogi, V.N. Kandikere, M. Shukla, K. Mudigonda, S. Maurya, R. Boosi, A. Yerramilli, Anal. Chim. Acta, in press.
[46] R.V.S. Nirogi, V.N. Kandikere, M. Shukla, K. Mudigonda, S. Maurya, R. Boosi, A. Yerramilli, Rapid Commun. Mass Spectrom., in press.
[47] D.R. Ifa, M. Falci, M.E. Moraes, F.A.F. Bezerra, M.O. Moraes, G. de Nucci, J. Mass Spectrom. 36 (2001) 188–194.
[48] K.C. Carlsson, J.L.E. Reubsaet, J. Pharm. Biomed. Anal. 34 (2004) 415–423.
[49] Guidance for Industry: Bioanalytical Method Validation, US Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research, Rockville, MD, 2001.