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Sample preparation and determination of gabapentin in venous and capillary blood using liquid chromatography–tandem mass spectrometry

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Abstract

An analytical method for the determination of gabapentin in serum obtained from venous blood samples has been developed using high-performance liquid chromatography (HPLC)–tandem mass spectrometry. In addition, a comparative study between capillary plasma samples and venous serum samples was carried out. This demonstrates the potential for the use of the described analytical system using very small amounts of blood. As internal standard (*S*)-(+)-α-amino-cyclohexane-propionic acid hydrate was used. Gabapentin and the internal standard are structural isomers, but have different *m*/*z* values for the fragments after collision induced dissolution. Gabapentin has 172 → 154 and 172 → 136 transitions and amino-cyclohexane–propionic acid hydrate has a 172 → 126 transition which can be detected in tandem MS. Analysis of gabapentin was carried out on a C8 HPLC column using an isocratic mobile phase consisting of ammonium acetate (pH 3.0; 5 mM)–methanol (96:4, v/v). The analytical method was validated for venous serum samples. Limit of detection was 1.6 ng/ml and lower limit of quantification was 7.5 ng/ml. R.S.D. values and bias values were within the range of acceptance for all concentration levels. The method developed for venous serum samples is being used in a gabapentin monitoring study using population pharmacokinetic modeling.

Keywords: Gabapentin; LC–MS/MS; Capillary blood samples; Clinical drug monitoring

1. Introduction

Gabapentin, 1-(aminomethyl-1-cyclohexyl) acetic acid, is an antiepileptic drug related to γ-aminobutyric acid (GABA) (Fig. 1). 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 shown that gabapentin increases the GABA level in the brain [1]. Gabapentin has a demonstrated analgesic effect in patients with chronic neuropathic pain states [2]. Gabapentin is seldom monitored by blood sampling as is common for many other antiepileptic drugs. However, as population pharmacokinetic (PK) models develop, closer

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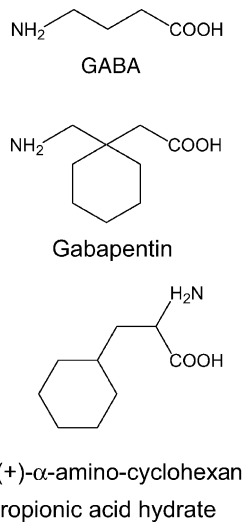

Fig. 1. Chemical structures of GABA, gabapentin and the internal standard (*S*)-(+)-α-amino-cyclohexane–propionic acid hydrate.

monitoring of gabapentin concentrations in serum or plasma becomes more important. The population PK models are able to handle complex data sets with many parameters at the same time, and will probably be of increasing importance in clinical monitoring of drug regimens.

Several analytical methods have been reported for the determination of gabapentin in human plasma or serum. These methods are based on high-performance liquid chromatography (HPLC) with ultraviolet (UV) and fluorescence (F) detection [3–8]; gas chromatography (GC) with flame ionization (FI) and mass spectrometric (MS) detection [9–11] and capillary electrophoresis (CE) [12]. The HPLC and CE methods require derivatisation of gabapentin to produce a chromophore, detectable by UV/F. The GC methods require derivatisation of gabapentin to improve the volatility and avoid column interactions. Generally, for routine analysis of large series, the derivatisation step increases the time of sample preparation and the cost of the method. Another inconvenience is the zwitterionic characteristic of gabapentin, which renders it 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–mass spectrometry (LC–MS) is becoming more common, owing to the improved sensitivity and specificity of this technique [13,14]. LC–MS requires less extensive sample preparation since gabapentin can be detected directly without derivatisation, and thus sample preparation time is reduced. A method for determination of gabapentin by LC tandem MS (LC–MS/MS) using electrospray ionization has been developed with linear calibration curve over the range 50–10,000 ng/ml [15].

This paper describes a method for sample collection and sample preparation for venous blood samples and for small amounts of blood using capillary tubes. A method for LC–MS/MS analysis with high sensitivity for gabapentin was developed and validated for venous blood samples. In addition, the possibility of using capillary blood samples for the quantification of gabapentin was explored by comparing results from venous serum samples and capillary plasma samples from patients using gabapentin. The small amount of blood in capillary blood samples require an analytical method with high sensitivity for gabapentin, as well as a method for sample collection and preparation. Capillary blood sampling can simplify routine blood sampling, and allow the patient to do the sampling at home.

2. Experimental

2.1. Chemicals and standard solutions

Gabapentin (produced by Parke-Davis) was a gift from the National Center for Epilepsy in Norway. (*S*)-(+)-α-Amino-cyclohexane–propionic acid hydrate (internal standard, IS) was purchased from Sigma–Aldrich AS (Oslo, Norway). All other chemicals used were of analytical grade.

Stock solutions of gabapentin (2 mg/ml) and IS (472 μg/ml) were prepared in water. Working standard solutions of IS for venous blood sample preparation (11.8 μg/ml) and for capillary blood sample preparation (2.36 μg/ml) were prepared from stock solution by dilution with water. Working standard solutions for the calibration curves of gabapentin were made from stock solution by dilution with water. All solutions were stored at approximately −20 °C until needed.

2.2. Sample preparation for venous blood samples

Human blood needed for method validation was collected from healthy, drug-free volunteers. Serum was obtained by centrifugation of blood. Aliquots (200 μl) of human serum were mixed with IS solution (20 μl of working standard solution). Blank serum samples were also spiked with gabapentin from the working standard solutions to concentrations of 6.75, 13.5, 67.5, 101.25, 135, 337.5, 675, 1012.5, 1350, 3375, 6750 and 10125 ng/ml. The samples were then precipitated with 800 μl cold (4 °C) acetonitrile. The tubes were vortexed for 30 s and allowed to stand at room temperature for at least 3 min. The tubes were then centrifuged at 17,000 × *g* for 10 min at room temperature. The supernatant (500 μl) was collected and dried under nitrogen gas at 50 °C. The residue was dissolved in 200 μl water and vortexed for 10 s. The tubes were centrifuged at 17,000 × *g* for 10 min at room temperature. The supernatant (100 μl) was collected and frozen at −20 °C until sample analysis. The samples were thawed at room temperature, and then vortexed for 10 s. Aliquots of 40 μl were transferred into microvials, capped and placed in an autosampler.

2.3. Sample preparation for capillary samples

Capillary blood samples were obtained from patients and drug-free, healthy volunteers using lancets to prick the finger. The blood was drawn into heparinized capillary tubes (Baxter, USA). In addition, venous blood withdrawn from a drug-free, healthy volunteer, collected in an unheparinized test tube was also used. This blood sample was spiked with gabapentin from stock solution to a concentration of 5000 ng/ml and the blood was then transferred to the capillary tubes.

The capillary blood was centrifuged in a microhematocrit centrifuge (Lars Ljungberg, Sweden). The capillary tubes were then stored at −20 °C until sample preparation. At sample preparation, the capillary tubes were taken directly from −20 °C and cut into two halves while the sample was still frozen, separating the plasma from the rest. Plasma (20 μl) was drawn out of the capillary tube with a Finn pipette and transferred to a test tube. The aliquots were spiked with IS solution (10 μl of working standard solution for capillary blood samples) and vortexed. The blank plasma from healthy volunteers was spiked with gabapentin and IS. The samples were precipitated with 70 μl acetonitrile at 4 °C. The tubes were vortexed for 20 s and allowed to stand at room temperature for at least 3 min. The tubes were centrifuged at 17,000 × *g* for 10 min at room temperature. The supernatant (50 μl) was collected and dried under nitrogen at 50 °C. The residue was dissolved in 50 μl water and vortexed for 10 s. The tubes were centrifuged at 17,000 × *g* for 10 min at room temperature. The supernatant (40 μl) was collected and frozen at −20 °C until sample analysis. The samples were thawed at room temperature, and vortexed for 10 s. Aliquots of 30 μl were transferred into microvials, capped and placed in an autosampler.

2.4. Chromatographic conditions

The HPLC system consisted of a Tsp SCM1000 vacuum degasser, Tsp SpectraSystem P4000 quaternary gradient pump and a Tsp SpectraSystem AS3000 autosampler. Detection was carried out by a Finnigan LCQ^{duo} ion trap mass spectrometer. Xcalibur^{TM} version 1.0 software was used to control this system and to perform data acquisition (all LC–MS/MS equipment Instrument-Teknikk AS, Østerås, Norway). Separation was performed on a 2.1 mm × 30 mm (3.5 μm) ZORBAX® SB-C8 Rapid Resolution column (Agilent, Oslo, Norway). To protect the analytical column, a guard column was used (2.1 mm × 12.5 mm ZORBAX® 300SB-C3, Agilent, Oslo, Norway). Isocratic elution was carried out with ammonium acetate (pH 3.0; 5 mM)–methanol (96:4, v/v) at a flow rate of 0.4 ml/min. Time for analysis was 4 min.

2.5. MS/MS conditions

The chromatographic system was connected to the MS detector using an API electrospray interface. The detector was operated in the positive ion mode. Sheath gas (N_2) flow was set at 60 units, auxiliary gas (N_2) flow at 20 units. The spray voltage was set at 5 kV, the capillary temperature at 250 °C. Both gabapentin and IS were detected in the MS/MS mode by monitoring the 172 → 154 transition for gabapentin and the 172 → 126 transition for IS. Isolation width was

set at $m/z = 2$ in both cases. Helium gas was used to cause collision-induced dissociation at 24% relative collision energy for both ions.

2.6. Validation procedure

For the inter-run statistics, calibration curves with the concentrations of 6.75, 13.5, 67.5, 101.25, 135, 337.5, 675, 1012.5, 1350, 3375, 6750 and 10125 ng/ml, and sets of three validation samples with the concentrations of 7.5, 500 and 7725 ng/ml were analyzed in four separate runs with two replicates of each sample. For the intra-run statistics, five replicates were analyzed. The first two replicates of validation samples in the intra-run experiment were also used for the fifth day inter-run calculation. From this design the accuracy, precision, linearity, limit of detection (LOD) and limit of quantification (LOQ) could be determined. Quantification of the standards and samples was based on area measurement of the ratio between the MRM signals for gabapentin ($172 \rightarrow 154$) and IS ($172 \rightarrow 126$).

2.7. Calibration standards, samples for validation and quality control

The calibration samples were prepared by spiking serum from healthy volunteers with working standard solutions of gabapentin. Validation samples were prepared in blank serum at concentrations of 7.5, 500 and 7725 ng/ml. Quality control samples (5000 ng/ml) were prepared by spiking of blank serum.

3. Results and discussion

3.1. Choice of internal standard

The IS is a structural isomer of gabapentin (Fig. 1) and was chosen because it behaves similarly to gabapentin in the analytical system. Although both substances have the same exact monoisotopic mass, they can be detected individually since they have different fragmentation patterns. Fig. 2 shows the full scan fragmentation spectra of both gabapentin and IS. There was no interference between gabapentin and IS when measuring the $172 \rightarrow 154$ transition and the $172 \rightarrow 126$ transition, respectively.

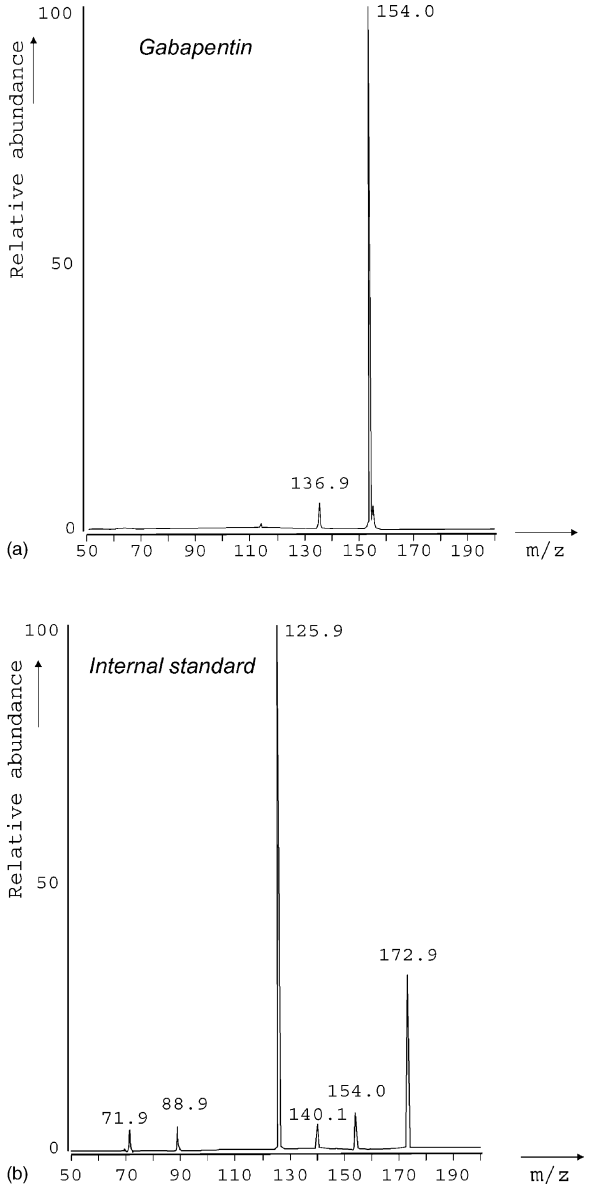

Fig. 2. The full scan fragmentation spectra of the LC–MS/MS assay for gabapentin (a) and IS (b). In both cases, m/z 172 was selected as parent ion for fragmentation at 24%.

3.2. Validation results

Linearity, LOD, LOQ and both intra- and inter-run precision and accuracy were determined to assess the performance of the method. Because of the wide range of concentrations studied, two calibration areas were

Table 1
Regression coefficients for the calibration curve ($y = bx + c$[a]) for gabapentin concentrations below 135 ng/ml

	b	c[a]	r
Day 1	100.2	−4.318	0.9975
Day 2	80.82	−9.870	0.9959
Day 3	123.7	−10.88	0.9960
Day 4	108.5	−0.1312	0.9984
Day 5	119.4	1.091	0.9976
Average	106.5	−4.822	0.9971
Standard deviation	17.06	5.464	

[a] The intercept c was found not to be statistically different from zero.

Table 2
Correlation and regression coefficients for the calibration curve ($y = ax^2 + bx + c$) for gabapentin concentrations above 135 ng/ml

	a	b	c	r
Day 1	0.9741	118.2	58.34	0.9965
Day 2	1.173	104.9	29.77	0.9994
Day 3	0.4896	164.7	108.1	0.9989
Day 4	0.9002	137.2	60.12	0.9991
Day 5	0.6266	155.8	139.8	0.9995
Average	0.8327	136.2	79.22	0.9987
Standard deviation	0.2741	24.98	44.02	

chosen, the lower part ranging from 7.5 to 135 ng/ml, and the upper part ranging from 135 to 10125 ng/ml. Tables 1 and 2 show the statistical data for the standard calibration curves of gabapentin. The ratio between gabapentin and IS is represented by x, the concentration in ng/ml by y. By regression analysis, two separate calibration curves were determined. The calibration curve shows linearity from 7.5 ng/ml to at least 135 ng/ml ($y = bx + c$). The intercept was not statistically different from zero. From 135 to 10125 ng/ml, the calibration curve fits a quadratic polynomial ($y = ax^2 + bx + c$).

Table 3 shows the validation data for the intra- and inter-run precision (R.S.D.) and accuracy (bias) at three concentration levels. R.S.D. values and bias values where within the range of acceptance for all levels. LOD at 3 × (S/N) ratio were calculated to be 1.6 ng/ml and LLOQ was demonstrated to be 7.5 ng/ml.

The freeze–thaw stability of gabapentin was investigated by analyzing two replicates of samples from serum spiked with gabapentin that had been frozen and thawed 0, 1, 2 and three times respectively. The nominal (theoretical) concentration of the samples was 500 ng/ml, and the average measured concentrations were 564, 559, 557 and 538 ng/ml, respectively. The overall standard deviation was 16 ng/ml (2.8%). These results demonstrate that the samples could be thawed and refrozen without compromising the integrity of the samples. Frozen gabapentin serum samples have been reported to be stable for 6 months at −15 °C [10] and gabapentin in serum is stable for at least 1 month at 4 °C [5]. Aqueous solutions of gabapentin has a 1-year stability period [10].

No peaks for gabapentin or IS were observed in human blank serum under the LC–MS/MS conditions described (Fig. 3a). The mass chromatogram of a patient sample spiked with IS is shown in Fig. 3b. The retention times of gabapentin and IS were approximately 2.5 and 2.9 min, respectively. No interfering ion suppression was observed.

Table 3
Accuracy (bias) and precision data for intra- and inter-run validation

	Nominal (theoretical) concentration gabapentin (ng/ml)	Measured concentration gabapentin (ng/ml)	R.S.D. (%)	Bias (%)
Intra-run ($n = 5$)	7.5	8.4 ± 0.8	9.3	12.5
	500	508 ± 29	5.7	1.5
	7725	8486 ± 875	10.3	9.9
Inter-run ($n = 5$)	7.5	7.7 ± 0.7	8.7	2.8
	500	483 ± 62	12.8	−3.4
	7725	8903 ± 668	7.5	15.2

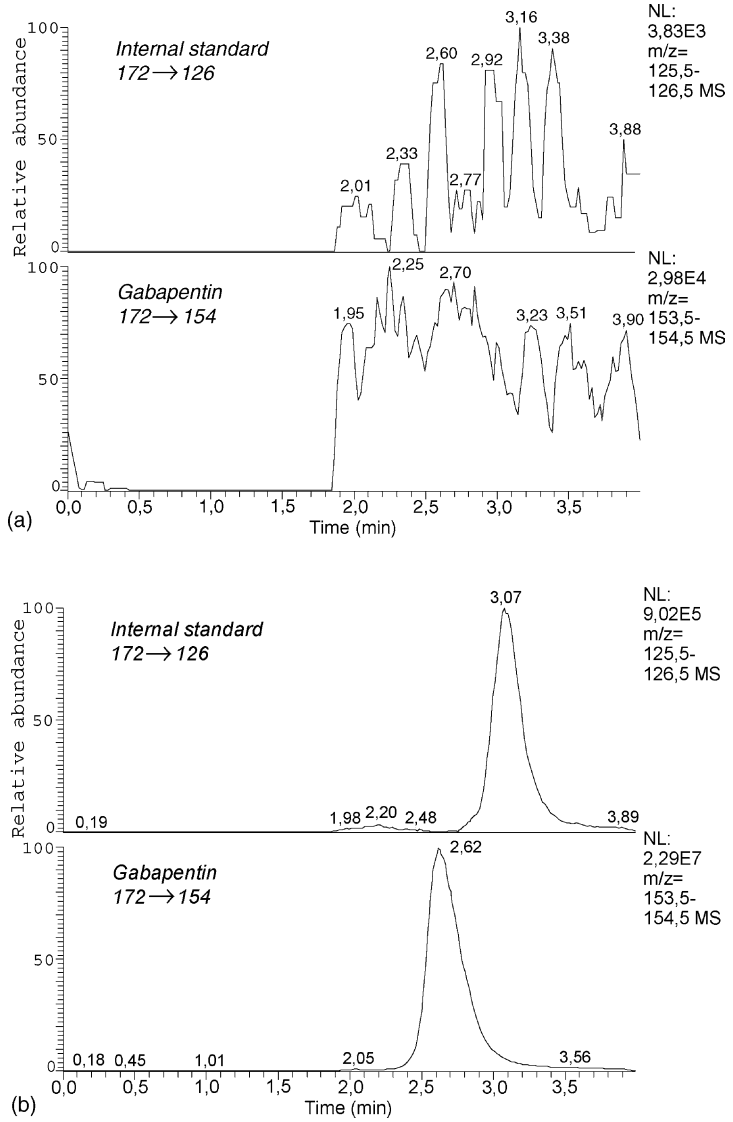

Fig. 3. (a) Ion traces of both gabapentin and IS in blank human serum. No peaks for gabapentin or IS were observed. (b) Ion traces of both gabapentin and IS in a patient sample spiked with IS. The calculated concentration of gabapentin in this sample was 3830 ng/ml.

3.3. The use of the analytical method for the analysis of venous serum samples from patients participating in a pharmacokinetic population study

The method described for venous serum samples is currently being used in a gabapentin monitoring study that will be presented elsewhere. The monitoring is performed by implementation of a population pharmacokinetic (PK) model. The population PK model was developed from the 8 h serum profiles of 13 patients using gabapentin for neuropathic pain. The serum samples were analyzed by the LC–MS/MS method described in this paper. For the monitoring, patients are followed for 2 months with serum sampling every week. The serum samples are analyzed by LC–MS/MS and the serum concentrations are entered into the monitoring tool Win-USCPACK®. The results of the population PK data analysis are used to monitor

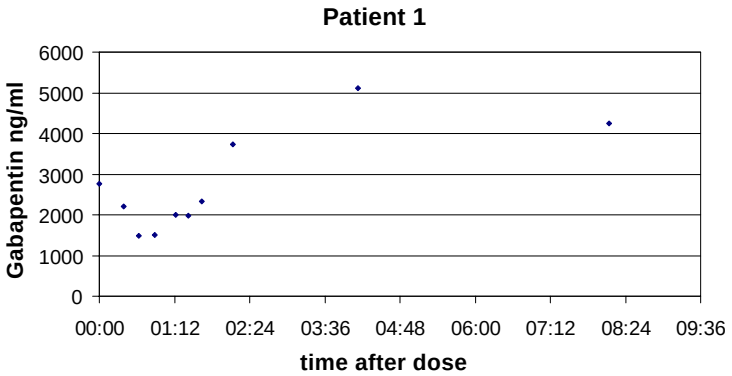

Fig. 4. Serum concentration vs. time curve for a patient using gabapentin for neuropathic pain. The patient was in steady state, and the samples were collected after the patient had taken a dose of 600 mg gabapentin (at time = 0). The curve shows that this patient had a lag time for absorption of the drug of about 50 min and a maximum concentration of approximately 5000 ng/ml at 4 h after dosing.

the patient regimen with 1 week delay after sampling. If necessary, the samples could be analyzed immediately, and the results implemented within hours of sampling.

Fig. 4 shows the serum concentration versus time curve for a patient using gabapentin as an analgesic for neuropathic pain with the patient in steady state. Samples were collected after the patient had taken a dose of 600 mg gabapentin (at time = 0). The curve shows that this patient had a lag time for absorption of the drug of about 50 min and a maximum concentration of approximately 5000 ng/ml at 4 h after dosing. Fig. 3b shows a typical chromatogram of a sample from this patient.

3.4. The possible use of the analytical method for the analysis of capillary plasma samples

The method presented in this paper allows the analysis of small amounts of serum (~50 μl). The feasibility of capillary samples is dependent on the distribution of the drug in the blood and body fluids. Because gabapentin is taken orally, a lag time for absorption is observed. Once a drug is absorbed in the gastrointestinal tract, the distribution in the blood occurs instantly. Capillary blood sampling can be used if the distribution of the drug to capillary blood occurs at the same rate as for venous blood, resulting in comparable concentrations in capillary blood and venous blood. This manner of sampling is used in several types of analysis requiring small amounts of blood, often in combination with analytical strips (for example, for the measuring of blood sugar levels in diabetic patients). These methods can be performed by the patients themselves at home. Capillary blood sampling for gabapentin would be a useful alternative to venous blood sampling. Compared to venous blood sampling, the risk of complications for the patient and the pain involved is reduced. This could be of particular interest when monitoring children, but also for

Table 4
Capillary samples of gabapentin

Nominal (theoretical) concentration gabapentin (ng/ml)	Measured concentration ± S.D. gabapentin (ng/ml)	Number of replicates n	R.S.D. (%)	Bias (%)
500	248.5 ± 23.6	2	9.5	−50.3
2500	2539.0 ± 337.2	2	13.3	1.6
5000	5891.0 ± 824.7	4	14.0	17.8
5000[a]	5572.5 ± 578.6	2	10.4	11.4
7725	7370.7 ± 929.4	4	12.6	−4.6
10000	8755.8 ± 553.4	2	6.3	−12.4

[a] Spiked full blood.

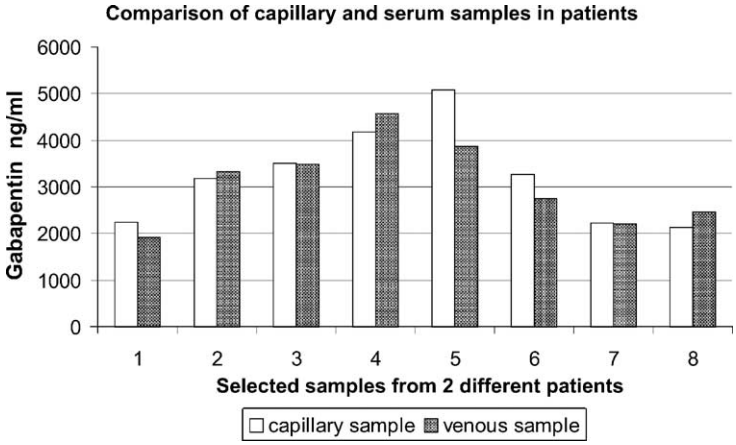

Fig. 5. Comparison of the analytical results of venous and capillary blood samples from patients using gabapentin. The patients were in steady state when participating. Samples 1–4 are obtained from a patient using 400 mg of gabapentin twice a day. Samples 5–8 are obtained from a patient using 300 mg of gabapentin three times a day. These pilot results indicate that the method for capillary blood can be used to monitor gabapentin serum concentration levels in patients.

the development of pharmacokinetic population models. Gabapentin has been demonstrated to be stable in serum at 4 °C for at least 1 month [5] and could be transported at low temperature to the analytical lab for centrifugation and further sample preparation. Results and statistics for the capillary plasma samples are presented in Table 4. Capillary samples from patients using gabapentin as analgesic treatment were taken simultaneously with venous blood sampling. The venous and capillary samples were analyzed, and the results compared (Fig. 5). These pilot test results indicate that capillary plasma samples have an acceptable accuracy in the concentration range usually found in patients (about 3000–10,000 ng/ml). In a well developed population PK model for gabapentin, this method seems to be acceptable for routine monitoring. When higher accuracy is required or with very low doses, regular venous blood sampling should be used.

The limit of detection acquired in this method allows both analysis of samples for academic and research purposes, and for the possible dilution of samples, when only very small volumes of blood can be obtained. Routine analysis of samples from patients with long-term use of gabapentin will usually have concentrations that range from 2000 to 3000 ng/ml and upwards. Gabapentin is a relatively new drug and the possible use of the drug in acute pain conditions is still under investigation. It can therefore be of use to be able to analyze samples with low concentrations of gabapentin.

4. Conclusion

The validated method for analyzing gabapentin presented in this paper is robust and can be used for analyzing large amounts of samples. This signals the possibility for routine analyses of gabapentin, which is necessary for the development of population pharmacokinetic models for the monitoring of gabapentin regimens. This can be of importance, both for patients using gabapentin as antiepileptic treatment and patients using it for neuropathic pain. Since the method presented is sensitive, the use of small amounts of sample was investigated. Comparison between capillary and venous blood samples demonstrates the potential of the method. The use of capillary samples would make sampling easier and less painful for the patient.

References

[1] O.A. Petroff, F. Hyder, D.L. Rothman, R.H. Mattson, Epilepsia 41 (2000) 675–680.
[2] M. Rowbotham, N. Harden, B. Stacey, P. Bernstein, L. Magnus-Miller, JAMA 280 (1998) 1837–1842.

[3] H. Hengy, E.U. Kolle, J. Chromatogr. 341 (1985) 473–478.
[4] N. Wad, G. Kramer, J. Chromatogr. B: Biomed. Sci. Applic. 705 (1998) 154–158.
[5] Q. Jiang, S. Li, J. Chromatogr. B: Biomed. Sci. Appl. 727 (1999) 119–123.
[6] G.L. Lensmeyer, T. Kempf, B.E. Gidal, D.A. Wiebe, Ther. Drug Monit. 17 (1995) 251–258.
[7] G. Forrest, G.J. Sills, J.P. Leach, M.J. Brodie, J. Chromatogr. B: Biomed. Appl. 681 (1996) 421–425.
[8] P.H. Tang, M.V. Miles, T.A. Glauser, T. DeGrauw, J. Chromatogr. B: Biomed. Sci. Applic. 727 (1999) 125–129.
[9] M.M. Kushnir, J. Crossett, P.I. Brown, F.M. Urry, J. Anal. Toxicol. 23 (1999) 1–6.
[10] C.E. Wolf, J.J. Saady, A. Poklis, J. Anal. Toxicol. 20 (1996) 498–501.
[11] W.D. Hooper, M.C. Kavanagh, R.G. Dickinson, J. Chromatogr. 529 (1990) 167–174.
[12] L.L. Garcia, Z.K. Shihabi, K. Oles, J. Chromatogr. B: Biomed. Appl. 669 (1995) 157–162.
[13] A.P. Bruins, TrAC Trends Anal. Chem. 13 (1994) 81–90.
[14] W. Muck, Pharmazie 54 (1999) 639–644.
[15] D.R. Ifa, M. Falci, M.E. Moraes, F.A. Bezerra, M.O. Moraes, G. de Nucci, J. Mass Spectrom. 36 (2001) 188–194.