Journal of Pharmaceutical and Biomedical Analysis 56 (2011) 771–777

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Simple and accurate quantitative analysis of ten antiepileptic drugs in human plasma by liquid chromatography/tandem mass spectrometry

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ARTICLE INFO

Article history:
Received 12 May 2011
Received in revised form 14 July 2011
Accepted 18 July 2011
Available online 23 July 2011

Keywords:
Antiepileptic drugs
Liquid chromatography (LC)–tandem mass spectrometry (MS/MS)
Human plasma

ABSTRACT

A simple, accurate, and sensitive liquid chromatography (LC)–tandem mass spectrometry (MS/MS) method has been developed for the simultaneous quantification of 10 antiepileptic drugs (AEDs; gabapentin (GBP), levetiracetam (LEV), valproic acid (VPA), lamotrigine (LTG), carbamazepine-10,11-epoxide (CBZ-epoxide), zonisamide (ZNS), oxcarbazepine (OXC), topiramate (TPM), carbamazepine (CBZ), phenytoin (PHT)) in human plasma as a tool for drug monitoring. d_{10}-Phenytoin (d_{10}-PHT) and d_6-valproic acid (d_6-VPA) were used as internal standards for the positive- and negative-ionization modes, respectively. Plasma samples were precipitated by the addition of acetonitrile, and supernatants were analyzed on a C18 reverse-phase column using an isocratic elution. Detection was carried out in selected reaction monitoring (SRM) mode. The calibration curves were linear over a 50-fold concentration range, with correlation coefficients (r^2) greater than 0.997 for all AEDs. The intra- and inter-day precision was less than 12%, and the accuracy was between 85.9 and 114.5%. This method was successfully used in the identification and quantitation of AEDs in patients undergoing mono- or polytherapy for epilepsy.

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1. Introduction

Carbamazepine (CBZ), phenytoin (PHT) and valproic acid (VPA) are well-known antiepileptic drugs (AEDs). Since the 1993s, new AEDs, including gabapentin (GBP), lamotrigine (LTG), levetiracetam (LEV), oxcarbazepine (OXC), topiramate (TPM), and zonisamide (ZNS), have been approved and introduced to the market [1]. These AEDs have differing mechanisms of action that indirectly affect voltage-dependent calcium channels, sodium channel blockage, and GABAergic inhibition of drug interactions [2]. Patients use monotherapy or polytherapy to control seizures. However, polytherapy often causes undesirable side effects, due to drug–drug interactions among co-administered AEDs. Administration of GBP with CBZ increased plasma concentrations of carbamazepine 10,11-epoxide (CBZ-epoxide), which also possesses antiepileptic activity [3,4]. Thus, it is necessary to simultaneously monitor AEDs, including their active metabolites, to develop therapies without side effects. Various analytical tools have been developed for therapeutic drug monitoring of AEDs, including high-performance liquid chromatography (HPLC) coupled with ultraviolet detection [5–9], an evaporative light-scattering detector [10], fluorescence polarization immunoassay [11,12], and an enzyme-multiplied immunoassay technique [13,14]. However, these methods require time-consuming and laborious extraction procedures or relatively large sample volumes (~1 mL) as well as lengthy chromatographic run times, limiting their throughput capacity and sensitivity. Recently, Subramanian et al. [15] reported a liquid chromatography atmospheric pressure chemical ionization mass spectrometry (LC–APCI-MS) method with solid-phase extraction (SPE) to simultaneously detect six AEDs and three metabolites in human plasma. However, this method cannot be applied to routinely prescribed drugs, such as GBP, LEV, and VPA.

In the present study, we established a fully validated, rapid, and accurate LC–MS/MS method for the simultaneous quantification of nine frequently prescribed AEDs in human plasma. CBZ-epoxide, an active metabolite of CBZ, was included in this assay because it is pharmacologically active. Felbamate was not included in this

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doi:10.1016/j.jpba.2011.07.019

Table 1
Multiple reaction monitoring parameters for the antiepileptic drugs used in this study.

Antiepileptic drugs	Ion mode	Precursor ion (*m*/*z*)	Product ion (*m*/*z*)	DP[a] (V)	CE[b] (V)
PHT	Positive	253	182	70	47
GBP	Positive	172	154	70	18
LTG	Positive	256	211	70	39
CBZ	Positive	237	194	70	23
CBZ-epoxide	Positive	253	210	70	19
OXC	Positive	253	208	70	28
LEV	Positive	171	126	70	20
VPA	Negative	143	143	−54	−15
TPM	Negative	338	78	−90	−60
ZNS	Negative	211	119	−61	−21
d_{10}-PHT (IS)	Positive	263	192	70	25
d_6-VPA (IS)	Negative	149	149	−66	−15

[a] Declustering potential.
[b] Collision energy.

assay because it is not considered a first-line medication, due to aplastic anemia and hepatic failure [16]. This method includes a simple, one-step sample preparation and reversed-phase HPLC chromatographic separation with multiple reaction monitoring (MRM) detection.

2. Experimental

2.1. Chemicals

CBZ, CBZ-epoxide, OXC, PHT, TPM, VPA, and ZNS were purchased from Sigma–Aldrich (St. Louis, MO, USA). GBP, LTG, LEV, d_{10}-PHT, and d_6-VPA were purchased from Toronto Research Chemicals (North York, ON, Canada). HPLC-grade acetonitrile and methanol were purchased from Merck (Darmstadt, Germany). All other chemicals and solvents were of the highest analytical grade available.

2.2. Preparation of standards and quality control samples

Stock solutions of GBP, LEV, VPA, LTG, CBZ-epoxide, ZNS, OXC, TPM, CBZ, and PHT, were prepared by dissolving the compounds in methanol at 10, 20, 10, 25, 10, 5, 10, 100, 10, and 10 mg/mL, respectively. The stock solutions were further diluted with methanol to obtain working standard solutions at several concentrations. The calibration curves were obtained using 6 calibration standards, i.e., blank plasma samples prepared by the addition of the working solutions to drug-free blank plasma, giving final concentrations of 0.8, 2, 4, 8, 20, and 40 μg/mL for GBP and TPM, 1.2, 3, 6, 12, 30, and 60 μg/mL for LEV and OXC, 10, 25, 50, 100, 250, and 500 for VPA, 0.4, 1, 2, 4, 10, and 20 μg/mL for LTG and CBZ, 0.2, 0.5, 1, 2, 5, and 10 μg/mL for CBZ-epoxide, 1.6, 4, 8, 16, 40, and 80 μg/mL for ZNS, and 4, 10, 20, 40, 100, and 200 μg/mL for PHT. Calibration curves for AEDs in human plasma were derived from their peak area ratios relative to that of the IS using linear regression with $1/x$ as a weighting factor. Quality control (QC) samples were prepared at four concentration levels (LLOQ, low, medium and high) for final concentrations of 0.8, 1, 6, and 30 μg/mL for GBP and TPM, 1.2, 1.5, 9, and 45 μg/mL for LEV and OXC, 10, 12.5, 75, and 375 for VPA, 0.4, 0.5, 3, and 15 μg/mL for LTG and CBZ, 0.2, 0.25, 1.5, and 7.5 μg/mL for CBZ-epoxide, and 1.6, 2, 12, and 60 μg/mL for ZNS, and 4, 5, 30, and 150 μg/mL for PHT. A mixed stock solution of d_{10}-PHT and d_6-VPA was diluted to 1 μg/mL of each compound in methanol for routine use as an internal standard (IS). All prepared plasma samples and stock solutions were stored at −80 °C (Revco ULT 1490 D-N-S; Western Mednics, Asheville, NC, USA).

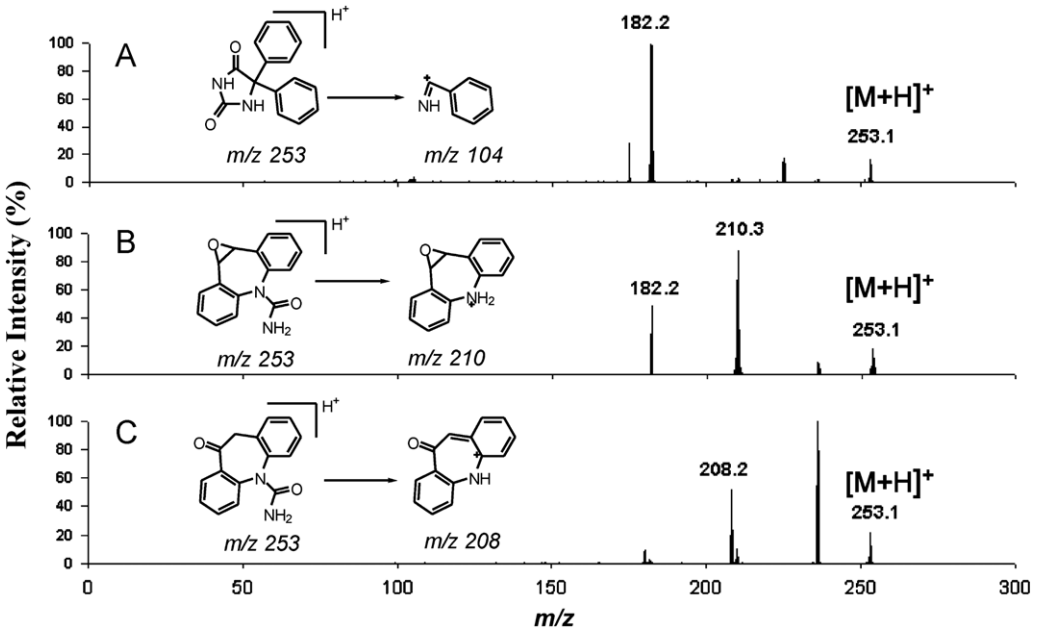

Fig. 1. Product-ion mass spectra of PHT (A), CBZ-epoxide (B), and OXC (C).

2.3. Characterization of product ions using tandem mass spectrometry

To characterize the product ions of GBP, LEV, VPA, LTG, CBZ-epoxide, ZNS, OXC, TPM, CBZ, PHT, d_{10}-PHT, and d_6-VPA, 1 μM solutions of each compound were infused separately into the mass spectrometer at a flow rate of 10 μL/min. The precursor ions and the pattern of fragmentation were monitored in positive- and negative-ion modes. The most abundant fragment ion observed in the MS/MS scan was selected for the quantitation of each drug.

2.4. Sample preparation

Sample preparation was performed by protein precipitation with acetonitrile. A 490-μL aliquot of acetonitrile containing d_{10}-PHT and d_6-VPA (1 μg/mL each) was added to 10-μL aliquots of plasma samples and vortexed. After centrifugation (9000 × *g*, 10 min, 4 °C), a 5-μL aliquot of the supernatant was injected into the LC–MS/MS system. All prepared samples were kept in an autosampler at 4 °C until injection.

2.5. Liquid chromatography/mass spectrometry

Agilent 1100 series (Agilent, Wilmington, DE, USA) LC system consisting of an autosampler, binary pump, and column oven was used for chromatography. The separation was performed on a Luna C18 column (100 × 2.0 mm, 3 μm; Phenomenex, Torrance, USA) with an isocratic elution. The mobile phase consisted of 5 mM ammonium formate buffer (pH 7.8) and 90% acetonitrile containing 5 mM ammonium formate (60:40, v/v). The flow rate was 0.2 mL/min. The HPLC system was coupled to a Qtrap 4000 triple–quadrupole mass spectrometer (AB Sciex, Foster City, CA, USA) equipped with an electrospray ionization (ESI) source. The turbo ion-spray interface was operated in both positive- and negative-ion modes with nitrogen as the nebulizing, turbo spray, and curtain gas, with the optimum values set at 40, 50, and 20 psi, respectively. The turbo-gas temperature was set at 600 °C, and the ESI needle voltages in positive- and negative-ion modes were adjusted to 5500 V and −4500 V, respectively. Quadrupoles Q1 and Q3 were set to unit resolution. The mass spectrometric parameters for SRM and product-ion mode are shown in Table 1. Data processing was performed using the Analyst software (ver. 1.4.1; Applied Biosystems).

2.6. Method validation

Method validation was carried out according to the currently accepted United States Food and Drug Administration's bioanalytical method validation procedures [17].

2.6.1. Selectivity

Selectivity was evaluated by comparing chromatograms of six different batches of drug-free plasma to ensure that no interfering peaks were present at the respective retention times of the analytes at the lower limit of quantification (LLOQ). The visible interferences were tested with blank plasma samples and plasma samples spiked with AEDs at the lower limit of quantification (LLOQ).

2.6.2. Linearity

Linearity was investigated over the designated concentration range for all analytes (Table 2). Calibration curves were obtained using 6 calibration standards for six different days and processed by weighed ($1/x$) least-squares linear regression analysis.

Table 2
HPLC retention time, calibration range, and correlation coefficient for the antiepileptic drugs used in this study.

Antiepileptic drugs	Retention time (min)	Calibration range (μg/mL)	Correlation coefficient (r)
GBP	1.96	0.8–40	0.9982
LEV	2.21	1.2–60	0.9987
VPA	2.70	10–500	0.9994
LTG	4.14	0.4–20	0.9972
CBZ-epoxide	5.02	0.2–10	0.9981
ZNS	5.32	1.6–80	0.9991
OXC	5.88	1.2–60	0.9984
TPM	8.43	0.8–40	0.9992
CBZ	8.63	0.4–20	0.9978
PHT	9.92	4.0–200	0.9967
d_{10}-PHT (IS)	9.94	NA	NA
d_6-VPA (IS)	2.75	NA	NA

2.6.3. Accuracy and precision

The accuracy and precision (presented as the coefficient of variation; CV) of the assay were assessed by analyzing QC samples at four different concentrations. Accuracy (%) was determined from the percentage ratio of the measured nominal concentration (mean of measured/nominal × 100). The intra-day CV and accuracy of the method were evaluated based on the analysis of six samples. The CV and accuracy for inter-day assays were assessed at the same concentration and repeated on six different days.

2.6.4. Recovery and matrix effects

Recovery and matrix effects were assessed at two concentrations for each drug by comparing the peak areas of triplicates at each concentration for analyte standards in methanol and standards spiked before and after protein precipitation in human plasma. Absolute recovery was calculated as the ratio of the mean peak area of an analyte spiked before extraction to the mean peak area of the same analyte spiked in methanol at the same concentration multiplied by 100. Relative recovery was calculated as the ratio of the mean peak area of an analyte spiked before extraction to the mean peak area of the same analyte spiked post-extraction in the same matrix multiplied by 100. The matrix effect was evaluated by comparing the mean peak area of an analyte spiked post-extraction to the mean peak area of an equivalent concentration of the same analyte standard in methanol. To further test whether cellular matrix can cause any ion suppression, each drug solution (10 μg/mL) was infused post-column at a flow of 10 μL/min, resulting in constant product ion intensity. Then, plasma extracts was injected onto the column and the changes in ion intensities were monitored to evaluated ion suppression [18].

2.6.5. Stability

To study the stability of the drugs in human plasma, plasma samples were spiked with two different concentrations of each analyte. Stability was assessed by analyzing three replicate samples after three different manipulations: (1) short-term storage (6 h at room temperature); (2) three freeze–thaw cycles; and (3) post-treatment storage (24 h at room temperature). The concentrations obtained were compared with the nominal values of the QC samples. The stabilities of the stock solutions of 10 analytes after 3 weeks at 4 °C and after 4 months at 80 °C were evaluated by comparison with freshly prepared solutions of the same concentrations. Stability was defined as <10% loss of the initial drug concentration.

2.7. Clinical application

The clinical applicability of the present method was evaluated by analyzing plasma taken from 25 patients undergoing mono- or

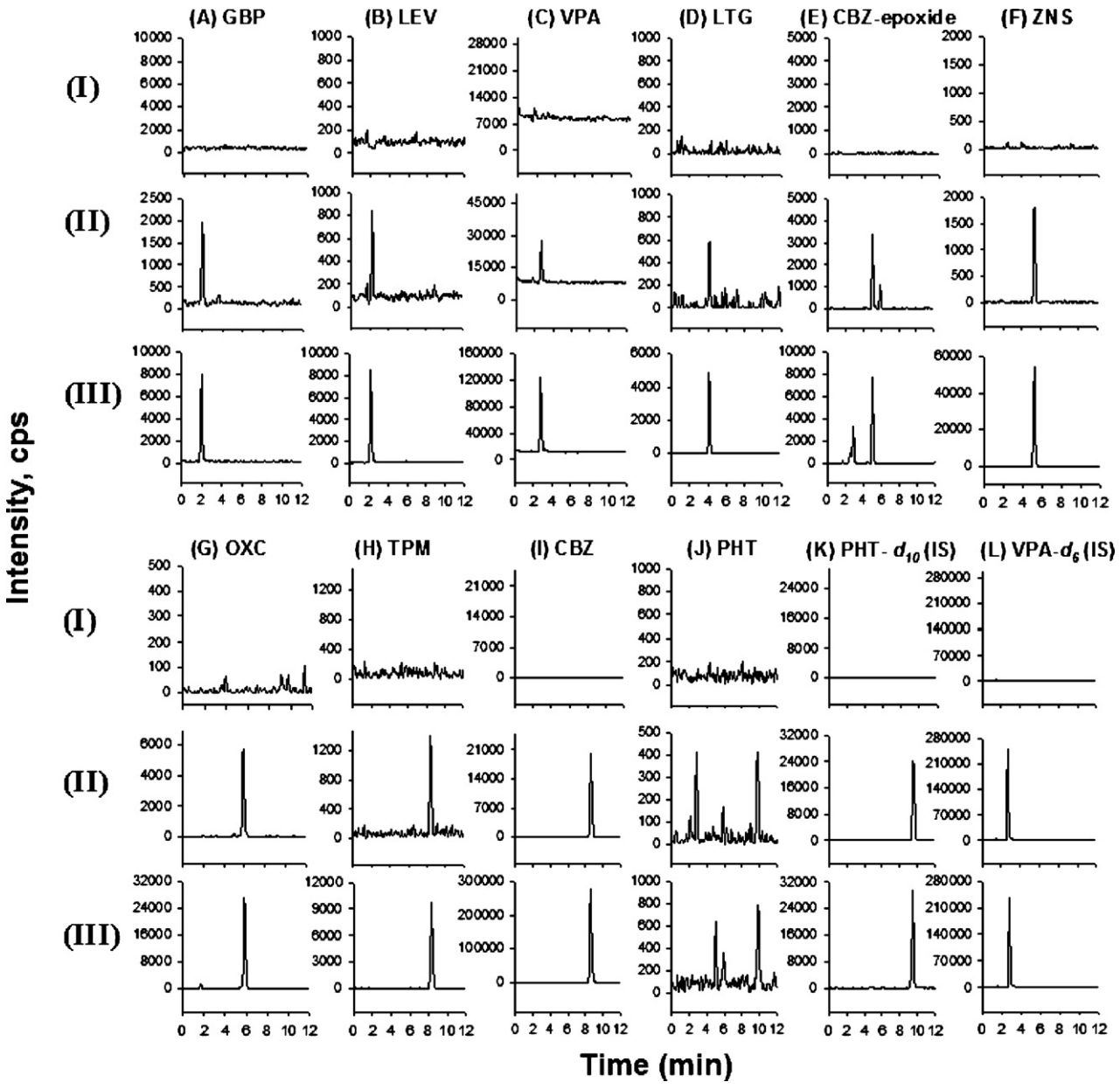

Fig. 2. Representative chromatograms of GBP (A), LEV (B), VPA (C), LTG (D), CBZ-epoxide (E), ZNS (F), OXC (G), TPM (H), CBZ (I), and PHT (A): blank plasma (I), blank plasma spiked with LLOQ levels of antiepileptic drugs and the IS (1 μg/mL) (II), and plasma samples obtained from patients undergoing treatment (III).

poly-therapy for the treatment of epilepsy. Different drug types and their plasma concentrations were evaluated.

3. Results and discussion

3.1. Chromatography optimization

All the analytes investigated in this study generated the prominent protonated molecular ion $[M+H]^+$ in positive-ion mode and the deprotonated molecular ion $[M-H]^-$ in negative-ion mode. Based on signal intensity and fragmentation pattern, the analytes were divided into two ionization groups: positive-ion mode for GBP, LEV, LTG, CBZ-epoxide, OXC, CBZ, and PHT and negative-ion mode for VPA, ZNS, and TPM. Thus, two different ISs were included in the present method: d_{10}-PHT for positive-ion-mode drugs and d_6-VPA for negative-ion-mode drugs. The MRM transition and optimized collision-induced dissociation conditions are given in Table 1.

Sample preparation and chromatographic conditions were optimized for simple, rapid, and practical quantitative analysis. Simple protein precipitation (50× acetonitrile added to 10 μL of plasma sample) was sufficient to detect all AEDs within the target concentration ranges. The large dilution of plasma with acetonitrile minimized matrix effects. When organic modifiers and acid modifiers were investigated, the combination of acetonitrile with 5 mM ammonium formate (pH 7.8) as a modifier resulted in higher ionization efficiency and better chromatographic separation than the combinations of methanol and acetic acid, acetonitrile and acetic acid, and acetonitrile and formic acid. Among the drugs tested in this study, PHT, OXC, and CBZ-epoxide had the same precursor ion at m/z 253 and showed a common fragment ion at m/z 182 in their product-ion spectra, although the relative intensities of this product ion varied depending on the drug. Thus, those three drugs should be chromatographically separated to avoid interference. CBZ-epoxide, OXC and PHT were successfully separated using our analytical system with retention times of 5.02, 5.88, and

Table 3
Accuracy and precision of the LC–MS/MS method for each analyte.

Compounds	Added (μg/mL)	Intra-day (n = 6)			Inter-day (n = 6)		
		Precision		Accuracy (%)	Precision		Accuracy (%)
		Measured (μg/mL)	RSD (%)		Measured (μg/mL)	RSD (%)	
PHT	4	4.44 ± 0.265	6.0	111	4.39 ± 0.247	5.6	109
	5	5.56 ± 0.249	4.5	111	5.49 ± 0.225	4.1	110
	30	26.6 ± 1.25	4.7	86.5	26.6 ± 1.23	4.6	87.7
	150	158 ± 10.8	6.8	98.7	148 ± 14.9	10	99.1
GBP	0.8	0.699 ± 0.0362	5.2	87.3	0.753 ± 0.0857	11	93.1
	1	0.997 ± 0.0804	8.1	99.7	1.03 ± 0.0593	5.8	106
	6	6.43 ± 0.278	4.3	107	5.85 ± 0.564	9.6	96.6
	30	33.2 ± 1.74	5.2	105	29.9 ± 2.72	9.1	103
LTG	0.4	0.389 ± 0.0151	3.9	97.3	0.391 ± 0.0367	9.4	94.0
	0.5	0.530 ± 0.0357	6.7	106	0.512 ± 0.0526	10	104
	3	3.15 ± 0.225	7.2	105	2.88 ± 0.212	7.3	96.1
	15	16.7 ± 0.817	4.9	105	15.7 ± 1.04	6.6	105
CBZ	0.4	0.341 ± 0.0143	4.2	85.2	0.387 ± 0.0458	12	90.6
	0.5	0.496 ± 0.0412	8.3	99.2	0.505 ± 0.0465	9.2	106
	3	3.24 ± 0.129	4.0	108	3.06 ± 0.204	6.7	102
	15	16.6 ± 1.08	6.5	105	15.7 ± 1.18	7.5	107
CBZ-epoxide	0.2	0.178 ± 0.0164	9.2	87.3	0.190 ± 0.0232	12	89.5
	0.2	0.257 ± 0.0193	7.5	103	0.261 ± 0.0186	7.1	108
	1.5	1.60 ± 0.0644	4.0	106	1.56 ± 0.105	6.7	104
	7.5	8.16 ± 0.630	7.7	103	7.77 ± 0.621	8.0	105
OXC	1.2	1.16 ± 0.125	11	96.8	1.15 ± 0.133	12	93.7
	1.5	1.47 ± 0.136	9.3	98.2	1.48 ± 0.120	8.1	104
	9	8.82 ± 0.722	8.2	98.0	8.87 ± 0.815	9.2	98.7
	45	48.9 ± 2.70	5.5	103	47.5 ± 4.46	9.4	108
LEV	1.2	1.17 ± 0.108	9.3	97.1	1.14 ± 0.128	11	92.9
	1.5	1.52 ± 0.0896	5.9	101	1.55 ± 0.129	8.3	103
	9	9.32 ± 0.702	7.5	103	8.68 ± 0.730	8.4	95.0
	45	49.1 ± 2.43	4.9	104	44.3 ± 4.09	9.2	101
VPA	10.	10.9 ± 0.351	3.2	109	10.8 ± 0.807	7.5	108
	12.5	13.5 ± 0.723	5.3	108	13.5 ± 0.584	4.3	111
	75	70.0 ± 2.27	3.2	93.3	69.4 ± 2.61	3.8	90.9
	375	389 ± 31.6	8.1	99.0	377 ± 25.0	6.6	97.4
TPM	0.8	0.90 ± 0.0188	2.1	112	0.843 ± 0.096	11	105
	1	1.09 ± 0.0253	2.3	109	1.08 ± 0.0628	5.8	108
	6	5.66 ± 0.190	3.4	94.3	5.63 ± 0.398	7.1	92.4
	30	30.8 ± 2.97	9.6	97.7	29.4 ± 2.29	7.8	96.4
ZNS	1.6	1.66 ± 0.0896	5.4	103	1.66 ± 0.164	9.9	104
	2	2.14 ± 0.0795	3.7	107	2.19 ± 0.0498	2.3	112
	12	10.6 ± 0.339	3.2	88.0	11.2 ± 0.710	6.3	91.7
	60	59.4 ± 5.78	9.7	94.6	59.9 ± 4.88	8.2	98.0

Table 4
Stability, matrix effects, and recovery for each analyte.

Compounds	Added (μg/mL)	Stability (%)			Matrix effects and recovery (%)		
		Short-term (room temp.) 6 h	Post-preparative stability (15 °C) 24 h	Three freeze–thaw cycles (−80 °C ↔ RT)	Matrix effect (%)	Relative recovery (%)	Absolute recovery (%)
PHT	5	97.4	112	89.9	100	90.9	91.0
	150	95.6	93.0	94.5	102	88.0	89.8
GBP	1	94.8	98.4	88.9	69.7	94.6	66.0
	30	95.6	99.7	101	70.5	92.6	65.3
LTG	0.5	96.3	108	91.9	105	89.3	93.9
	15	102	107	99.5	107	87.9	93.7
CBZ	0.5	102	108	91.9	111	95.1	105
	1.5	98	101	95.0	105	90.1	94.6
CBZ-epoxide	0.25	99.4	107	87.3	113	90.7	103
	7.5	97.4	101	94.4	104	90.2	93.7
OXC	1.5	89.0	106	85.8	112	95.3	107
	45	87.2	99.3	91.4	110	90.0	98.9
LEV	1.5	91.9	106	95.6	98.1	94.8	93.0
	45	97.4	93.3	86.5	89.2	97.8	87.2
VPA	12.5	101	102	98.3	104	90.3	94.1
	375	102	101	101	105	89.4	93.5
TPM	1	107	110	98.2	106	91.5	97.1
	30	99.1	98.3	95.5	105	91.0	95.7
ZNS	2	102	100	94.7	112	95.5	107
	60	98.4	99.9	94.3	107	90.6	96.5

9.92 min, respectively (Fig. 1). We also checked the possible interference due to ion source fragmentation of trans-diol-carbazepine, a metabolite of OXC as reported by Corso et al. [19]. Trans-diol-carbazepine was eluted much earlier (retention time of 2.0 min) and no interference was expected (data not shown). Several commercially available C18 columns were examined to optimize the chromatographic peak shape, peak width, and separation. The Luna C18 column generated better peak shape, narrow peak width, and optimal peak separation under isocratic conditions. Under these optimized chromatographic conditions, all target drugs were separated and analyzed within a 12-min run time (Table 2).

3.2. Method validation

The specificity and selectivity of the method were investigated by preparing and analyzing human plasma blanks from six different batches of human plasma. No interference was observed in drug-free plasma samples at the retention times of the target drugs. Additionally, no carry-over effect was observed in our system. Typical chromatograms for blank plasma (Fig. 2(I)), plasma spiked with LLOQ levels of the AEDs (Fig. 2(II)), and plasma collected from patients being treated for epilepsy (Fig. 2(III)) are presented. The calibration curve was constructed using six different concentrations and processed by weighed ($1/x$) least squares linear regression analysis. The calibration ranges for each drug were from 2–5-fold lower to 2-fold higher than the concentration of serum reference ranges of each drug for therapeutic drug monitoring proposed by Patsalos et al. [20]. The calibration curves for the drugs were linear over the concentration ranges, with correlation coefficients (r) higher than 0.993. Signal-to-noise ratios at LLOQ were higher than 10 for all drugs included in the assay (Table 2).

The intra- and inter-day variations of target drug determinations in human plasma over the entire concentration ranges are summarized in Table 3. The intra-day coefficients of variation were between 2.1 and 11.0%, and accuracies ranged from 85.2 to 112%. The inter-day coefficients of variation were between 2.3 and 12.0%, and the accuracy was between 87.7 and 112%.

To determine whether matrix interference affected the analysis of AEDs, blank plasma sample was injected into the LC–MS/MS system while individual drug was introduced continuously post-column. No significant matrix interference was observed near retention time of AEDs (data not shown). The absolute recovery of AEDs was between 89.2 and 107%. The absolute recovery of GBP was relatively low (~70%) although significant matrix effect was not observed in post-column infusion. Based on the high recovery, relatively low intra- and inter-day CVs, and good linearity of GBP, the present method is suitable for stable detection and quantitation of GBP.

Stock solutions of GBP, LEV, VPA, LTG, CBZ-epoxide, ZNS, OXC, TPM, CBZ, and PHT in methanol were stable for at least 3 weeks at 4 °C and for 4 months at −80 °C. No significant degradation of any of the analytes in human plasma occurred after short-term storage for 6 h at room temperature, three freeze–thaw cycles, or post-treatment storage for 24 h at room temperature, within ±15% deviation from the nominal concentrations (Table 4).

3.3. Clinical application

The validated method was applied to drug monitoring of AEDs in plasma collected from patients, and the detection frequency and estimated concentrations of the drugs are given in Table 5. Among 25 patients, LTG was detected in 20 patients, with concentrations ranging from 0.8 to 13.0 μg/mL. More than two drugs were monitored in 19 of the 25 patients, and a maximum of six drugs (LEV, LTG, ZNS, TPM, CBZ, and PHT) were detected in one patient.

Table 5
Concentration of antiepileptic drugs in plasma from patients treated using combination therapy with ten drugs.

Compounds	Drugs detected	
	Concentration (μg/mL)	Number
PHT	3.0–15.7	4
GBP	5.9	1
LTG	0.8–13.0	20
CBZ	7.5–14.4	6
CBZ-epoxide	1.3–3.0	6
OXC	3.0–33.1	2
LEV	NA	0
VPA	42.1–113.9	11
TPM	0.9–9.7	7
ZNS	7.1–35.2	2

4. Conclusions

An LC–MS/MS method for simultaneous quantification of nine AEDs (GBP, LEV, VPA, LTG, ZNS, OXC, TPM, CBZ, and PHT) and one active metabolite (CBZ-epoxide) in human plasma was developed with acceptable accuracy and precision. To the best of our knowledge, this method is most rapid (12 min per sample) and simple (protein precipitation) compared to previously reported simultaneous quantitation methods for monitoring AEDs. The quantitation can be performed using small volumes of human plasma (10 μL), allowing efficient use of limited blood samples. The developed method was successfully applied to the identification and quantitation of plasma samples from patients who were undergoing treatment. Thus, this method may be useful for therapeutic drug screening of co-administered epileptic drugs in plasma as well as pharmacokinetic studies of these drugs.

Acknowledgements

This work was supported by the Basic Research Program through the National Research Foundation of Korea (2010-0005370), Ministry of Education, Science, and Technology, Korea.

References

[1] S.M. LaRoche, S.L. Helmers, The new antiepileptic drugs, JAMA 291 (2004) 605–614.
[2] M. Mula, F. Monaco, Epilep. Disord. 11 (2009) 1–9.
[3] H. Breton, M. Cociglio, F. Bressolle, H. Peyriere, J.P. Blayac, D. Hillaire-Buys, Liquid chromatography–electrospray mass spectrometry determination of carbamazepine, oxcarbazepine and eight of their metabolites in human plasma, J. Chromatogr. B: Analyt. Technol. Biomed. Life Sci. 828 (2005) 80–90.
[4] R.S. Fisher, Emerging antiepileptic drugs, Neurology 43 (1993) S12–S20.
[5] M. Contin, M. Balboni, E. Callegati, C. Candela, F. Albani, R. Riva, A. Baruzzi, Simultaneous liquid chromatographic determination of lamotrigine, oxcarbazepine monohydroxy derivative and felbamate in plasma of patients with epilepsy, J. Chromatogr. B: Analyt. Technol. Biomed. Life Sci. 828 (2005) 113.
[6] M. Contin, S. Mohamed, C. Candela, F. Albani, R. Riva, A. Baruzzi, Simultaneous, HPLC–UV analysis of rufinamide, zonisamide, lamotrigine, oxcarbazepine monohydroxy derivative and felbamate in deproteinized plasma of patients with epilepsy, J. Chromatogr. B: Analyt. Technol. Biomed. Life Sci. 878 (2010) 461–465.
[7] R. Mandrioli, N. Ghedini, F. Albani, E. Kenndler, M.A. Raggi, Liquid chromatographic determination of oxcarbazepine and its metabolites in plasma of epileptic patients after solid-phase extraction, J. Chromatogr. B: Analyt. Technol. Biomed. Life Sci. 783 (2003) 253–263.
[8] T.A. Vermeij, P.M. Edelbroek, Robust isocratic high performance liquid chromatographic method for simultaneous determination of seven antiepileptic drugs including lamotrigine, oxcarbazepine and zonisamide in serum after solid-phase extraction, J. Chromatogr. B: Analyt. Technol. Biomed. Life Sci. 857 (2007) 40–46.
[9] T. Yoshida, K. Imai, S. Motohashi, S. Hamano, M. Sato, Simultaneous determination of zonisamide, carbamazepine and carbamazepine-10,11-epoxide in infant serum by high-performance liquid chromatography, J. Pharm. Biomed. Anal. 41 (2006) 1386–1390.
[10] M.K. Manoj Babu, Simultaneous separation and quantitation of four antiepileptic drugs—a study with potential for use in patient drug level monitoring, J. Pharm. Biomed. Anal. 34 (2004) 315–324.

[11] D.J. Berry, P.N. Patsalos, Comparison of topiramate concentrations in plasma and serum by fluorescence polarization immunoassay, Ther. Drug Monit. 22 (2000) 460–464.
[12] J. Ma, P.L. Zhu, J.W. Xie, Z.P. Jia, J.S. Yang, Restricted-access media high pressure liquid chromatography vs fluorescence polarization immunoassay for analysis of carbamazepine in human plasma, Acta Pharmacol. Sin. 23 (2002) 87–91.
[13] R. Gorodischer, P. Burtin, Z. Verjee, P. Hwang, G. Koren, Is saliva suitable for therapeutic monitoring of anticonvulsants in children: an evaluation in the routine clinical setting, Ther. Drug Monit. 19 (1997) 637–642.
[14] B. Rambeck, T.W. May, M.U. Jurgens, V. Blankenhorn, U. Jurges, E. Korn-Merker, A. Salke-Kellermann, Comparison of phenytoin and carbamazepine serum concentrations measured by high-performance liquid chromatography, the standard TDx assay, the enzyme multiplied immunoassay technique, and a new patient-side immunoassay cartridge system, Ther. Drug Monit. 16 (1994) 608–612.
[15] M. Subramanian, A.K. Birnbaum, R.P. Remmel, High-speed simultaneous determination of nine antiepileptic drugs using liquid chromatography–mass spectrometry, Ther. Drug Monit. 30 (2008) 347–356.
[16] C.M. Dieckhaus, C.D. Thompson, S.G. Roller, T.L. Macdonald, Mechanisms of idiosyncratic drug reactions: the case of felbamate, Chem. Biol. Interact. 142 (2002) 99–117.
[17] United States Food and Drug Administration, US FDA Guidance for Industry/Bioanalytical Method Validation, US FDA, Rockville, MD, 2001.
[18] B.K. Matuszewski, M.L. Constanzer, C.M. Chavez-Eng, Strategies for the assessment of matrix effect in quantitative bioanalytical methods based on HPLC–MS/MS, Anal. Chem. 75 (2003) 3019–3030.
[19] G. Corso, O. D'Apolito, G. Paglia, The accuracy of oxcarbazepine (OXC) quantification by a liquid chromatography/tandem mass spectrometry method is influenced by the ion source fragmentation of its metabolite trans-diol-carbazepine (DHD), Rapid Commun. Mass Spectrom. 21 (2007) 269–272.
[20] P.N. Patsalos, D.J. Berry, B.F.D. Bourgeois, J.C. Cloyd, T.A. Glauser, S.I. Johannessen, I.E. Leppik, T. Tomson, E. Perucca, Antiepileptic drugs—best practice guidelines for therapeutic drug monitoring: a position paper by the subcommission on therapeutic drug monitoring. ILAE Commission on Therapeutic Strategies, Epilepsia 49 (2008) 1239–1276.