ELSEVIER

Journal of Chromatography B, 770 (2002) 217–225

JOURNAL OF CHROMATOGRAPHY B

www.elsevier.com/locate/chromb

Separation of carbamazepine and five metabolites, and analysis in human plasma by micellar electrokinetic capillary chromatography

Maria Augusta Raggi[a], Vincenzo Pucci[a], Alessandra Maurizio[a], Jan Muzikar[b], Ernst Kenndler[b,*]

[a]*Department of Pharmaceutical Sciences, University of Bologna, Bologna, Italy*
[b]*Institute for Analytical Chemistry, University of Vienna, Währingerstraße 38, A 1090 Vienna, Austria*

Abstract

A rapid and feasible method was developed for the analysis of carbamazepine and its five metabolites (10,11-dihydro-10,11-epoxycarbamazepine, 10,11-dihydro-10,11-dihydroxycarbamazepine, 10,11-dihydro-10-hydroxycarbamazepine, 2-hydroxycarbamazepine and 3-hydroxycarbamazepine) in human plasma. Separation of the analytes is based on micellar electrokinetic chromatography, in untreated fused-silica capillary (48.5/40.0 cm length, 50 μm I.D.) with phosphate buffer (30 m*M*, pH 8.00) as background electrolyte, containing 50 m*M* sodium dodecylsulfate, and methanol (15%, v/v) as organic modifier. Clean up of human plasma samples was carried out by means of a solid-phase extraction procedure, which gave a high extraction yield for all six carbamazepines (>88%). The overall precision of the method gives a mean RSD of about 1.8%. The limit of quantitation for all analytes is ≤0.30 μg ml^{-1}, the limit of detection ≤0.12 μg ml^{-1}.

Keywords: Carbamazepine; Carbamazepine metabolites

1. Introduction

Carbamazepine (5H-dibenzo[b,f]azepine-5-carboxamide, CBZ, Fig. 1) is the drug of choice in the treatment of many forms of epilepsy [1,2]. Recently it has been introduced in clinical psychiatry in the treatment of schizophrenia for its mood-stabilising properties [3,4]. CBZ is usually administered per os at daily doses ranging from 200 to 1200 mg which give rise to CBZ plasma levels in the 4–12 μg ml^{-1} range [5]. These plasma levels are considered safe in order to obtain good clinical response and avoid otherwise severe and potentially lethal toxic effects.

The drug undergoes extensive hepatic metabolism by means of the cytochrome P450 system, mainly by the CYP 3A4 and CYP 2C8 subtypes [6,7]. The most important products of this and subsequent metabolic steps are 10,11-dihydro-10,11-epoxycarbamazepine (CBZ-EP; for the structural formulae and symbols of all metabolites see Fig. 1) and 10,11-dihydro-10,11-dihydroxycarbamazepine (CBZ-DiOH). CBZ-EP has considerable anticonvulsant activity, however, it seems also to cause toxic effects, such as neurotoxic ones [8]. Plasma levels of CBZ-EP are largely induction-dependent. Seemingly CBZ-DiOH has no pharmacological properties [9]. Other minor metabolites of CBZ are 10,11-dihydro-10-hydroxycarbamazepine (CBZ-10OH), 2-hydroxycarbamazepine (CBZ-2OH) and 3-hydroxycarbamazepine (CBZ-3OH) [10].

*Corresponding author. Tel.: +43-1-4277-523-05; fax: +43-1-4277-9523.

E-mail address: ernst.kenndler@univie.ac.at (E. Kenndler).

1570-0232/02/$ – see front matter
PII: S0378-4347(01)00524-2

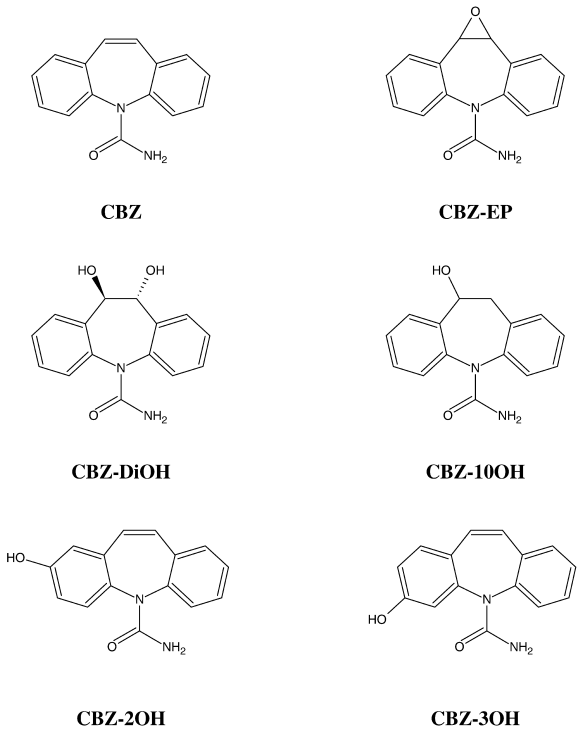

Fig. 1. Structural formulae of carbamazepine and metabolites.

The simultaneous administration of other drugs can often alter the plasma level of CBZ; in particular, phenobarbital, phenytoin [11,12] and fluoxetine [13] usually increase CBZ plasma levels. It is thus of clear importance to constantly monitor CBZ and its main metabolites, particularly in patients undergoing polypharmacy. The analytical results obtained can also enable the gathering of several information on pharmacokinetic interactions between CBZ and other central nervous system (CNS) drugs.

Routine clinical analysis of CBZ is usually based on immunological methods [14,15], which have the disadvantage of scarce selectivity: in fact, cross-reaction can occur with both CBZ-EP and CBZ-DiOH [16]. In past years, gas-chromatography (GC) was the mostly used technique for the analysis of CBZ in plasma, although it required a complex derivatisation procedure [13,17]. More recently high-performance liquid chromatography (HPLC) [18–29], sometimes coupled with mass spectrometry (MS) [30], has become common as well. Most papers report on analysis of CBZ [18–28], only occasionally together with its main metabolites CBZ-EP and CBZ-DiOH in plasma. Others report analysis of CBZ in different matrices such as urine [19,30] or hair [22]. Only one work by Pienimäki et al. [29] deals with the determination of metabolites other than the two main ones in plasma. However, the proposed procedure is laborious and time-consuming; it consists of a liquid–liquid extraction and needs considerably long chromatographic run times. Recently, we have developed a more rapid HPLC method [31] using solid-phase extraction for sample pretreatment for the determination of CBZ and its five metabolites.

Besides GC and HPLC, micellar electrokinetic chromatography (MEKC) has been used to separate and quantify CBZ in serum [32–35]. Very recently Thormann's group [36] compared the use of differently coated capillaries for MEKC analysis of CBZ and CBZ-EP in serum and plasma, and quantified these two analytes after liquid–liquid extraction [37].

The aim of the present investigation is the development of a rapid and feasible method for the separation of CBZ and the two main metabolites together with three minor ones. The method is based on MEKC combined with diode-array detection. In a first step, separation conditions were worked out that enable baseline resolution for all analytes. Then in combination with a solid-phase extraction (SPE) step, the method was proved for its suitability to quantify the analytes in real samples, in spiked blank plasma of healthy persons, and finally in plasma taken from epileptic patients.

2. Experimental

2.1. Chemicals

Carbamazepine (5H-dibenzo[b,f]azepine-5-carboxamide, CBZ) and its metabolites 10,11-dihydro-10,11-epoxycarbamazepine (CBZ-EP); 10,11-dihydro-10,11-dihydroxycarbamazepine (CBZ-DiOH); 10,11-dihydro-10-hydroxycarbamazepine CBZ-10OH); 2-hydroxycarbamazepine (CBZ-2OH) and 3-hydroxycarbamazepine (CBZ-3OH) were kindly provided by Novartis Pharma AG (Basel, Switzerland).

Boric acid, sodium dihydrogen phosphate and sodium hydroxide were analytical grade; acetonitrile,

methanol and water (all from E. Merck, Darmstadt, Germany) were HPLC grade. Sodium dodecylsulfate (SDS) was purchased from Serva (Heidelberg, Germany). Tetrahydrofuran (THF, HPLC grade) and phosphoric acid (analytical grade) were from Carlo Erba (Milan, Italy). Ultrapure water (18.2 MΩ cm) was obtained by means of a Millipore MilliQ apparatus (Milford, MA, USA).

Stock solutions of the analytes in methanol at concentration of 1 mg ml^{-1} were stable for at least 3 months when stored at −20°C. Standard working solutions were prepared by diluting each stock solution with a mixture of phosphate buffer (30 m*M*, pH 8.00) and methanol (85:15, v/v). The standard solutions were prepared every day.

2.2. Instruments

Variation of the conditions to adjust separation for all analytes was carried out by means of P/ACE System 2100 (Beckman, San Ramon, CA, USA), equipped with UV–vis detector, operated at 214 nm. These measurements were done in an uncoated fused-silica capillary (Supelco, PA, USA) of 27.0 cm total length (effective length 21.0 cm) and 50 μm/360 μm I.D./O.D., thermostated at 25.0°C. A constant voltage of +12 kV was applied, with currents typically less than 60 μA. Injection was made from the anodic side of the capillary by 35 mbar pressure for 1 s.

Quantitative analysis of carbamazepine and its metabolites was performed with a ^{3D}CE capillary electrophoresis automated apparatus (Agilent Technologies, Palo Alto, CA, USA), equipped with a diode array detector set at 205 nm. MEKC was run in untreated fused-silica capillaries (Supelco, PA, USA) of 48.5/40.0 cm length and 50 μm/363 μm I.D./O.D. The cartridge was maintained at 25.0°C. The sample solutions were loaded into the capillary at the anodic end by pressure injection at 50 mbar for 10 s. The instrument was operated at +25 kV with typical currents of about 60 μA.

The background electrolyte (BGE) for MEKC was phosphate buffer (30 m*M*, pH 8.00) modified with 15% (v/v) methanol, and containing 50 m*M* SDS. The buffer was filtered (0.20 μm, Albet-Jacs-020-25) prior to use.

Careful washing of the capillary was essential in order to obtain reproducible results and to avoid interference due to the matrix and the analytes interacting with the internal wall of the capillary. Thus, at the beginning of every working day the capillary was rinsed with: water (5 min), 0.1 *M* NaOH (3 min), water (6 min) and BGE (5 min). After each electrophoretic run the capillary was flushed with BGE for 2 min. At the end of the day the capillary was washed with water (10 min), with 1 *M* NaOH (10 min) and with water (20 min). It was then air-dried for 3 min.

2.3. Sample pretreatment

For the SPE procedure Oasis HLB (30 mg, 1 ml) cartridges (Waters, Milford, MA, USA) were used. Conditioning and equilibration were carried out by passing 1 ml of methanol through the cartridge followed twice by 1 ml of water each. Subsequently, 250 μl of blank or patient plasma were diluted with 500 μl of water, and the solution was then loaded onto the already equilibrated cartridge. After washing twice with 1 ml of water and once with 50 μl of methanol, the cartridge was dried under vacuum (40 kPa) for 30 s and the analytes eluted with 1 ml THF.

The eluate was then dried by means of a rotary evaporator, the residue redissolved in 250 μl of phosphate buffer (30 m*M*, pH 8.00) containing 15% (v/v) methanol and filtered through a syringe filter of 0.2 μm pore size (Whatman, Clifton, NJ, USA) prior to injection into the HPCE instrument.

2.4. Method validation

2.4.1. Calibration curves

The calibration curves were set up by adding known amounts of analyte standard solutions to blank plasma, obtained from healthy volunteers. The resulting mixture was subjected to the entire analytical procedure (including SPE). The peak areas of the analytes were plotted against the respective concentrations (expressed as μg ml^{-1}), and the lines were obtained by means of the least-squares method.

2.4.2. Precision and recovery assays

A suitable amount of CBZ and its five metabolites was added to blank plasma or patient plasma samples

and subjected to the described analysis. The concentrations of analytes added were: 0.25, 5.00 and 15.00 μg ml^{-1} for CBZ, CBZ-EP and CBZ-DiOH; 0.25, 1.00 and 5.00 μg ml^{-1} for CBZ-10OH and CBZ-2OH; 0.30, 1.00 and 5.00 μg ml^{-1} for CBZ-3OH. The procedure was repeated six times within the same day to obtain repeatability and six times over different days to obtain intermediate precision. The extraction yield or absolute recovery was calculated as the ratio of peaks areas of the analytes extracted from plasma to those obtained analysing standard solutions of carbamazepines having equivalent concentrations with the combined SPE-MEKC method described.

2.5. Human plasma sampling

Plasma samples were taken from epileptic patients under therapy with CBZ from the Institute of Clinical Neurology, University of Bologna (Italy), and put into vials containing EDTA as the anticoagulant. The blood was immediately centrifuged for 20 min at 1400 *g* and the supernatant plasma frozen and maintained at −20°C until analysis that was carried out usually within 1 month. The same procedure was used to separate plasma from the blood of healthy volunteers (''blank'' plasma).

3. Results and discussion

3.1. Development of the separation conditions

The separation of carbamazepine and its two main metabolites (CBZ-DiOH and CBZ-EP) was demonstrated by MEKC in aqueous solutions by Härtter et al. [34] in a common borate buffer (pH 9.2, 50 m*M* SDS). Their results could be reproduced by us (data not shown). However, we proved that this system is not suitable for the separation of the other metabolites. It was found that under the conditions described CBZ-10OH is not fully separated from CBZ-DiOH, and CBZ-3OH and CBZ-2OH are nearly co-eluting, too. An additional reason for our intention to modify the buffer conditions was the observation that in pure aqueous BGEs peak splitting occurred when the samples were dissolved and injected in mixed aqueous–organic solvents. Dissolution in such solvents containing organic modifiers were found necessary due to the limited solubility of the analytes, especially upon pretreatment of the real serum samples that contained seemingly some lipoid residues.

The addition of organic modifiers in MEKC has nevertheless narrow limits due to the potential dissolution of the micelles, because high concentration of organic solvents might significantly increase the critical micellar concentration. For this reason we used BGEs with relatively low content of methanol, namely up to 20% (v/v). The resulting total electrophoretic mobilities are shown in Fig. 2. It can be seen that they are slightly decreasing with increasing methanol concentration. As the EOF mobility decreases much more pronouncedly (see also Fig. 2), the apparent mobilities (resulting after correction by the EOF mobility) must decrease with increasing organic solvent concentration pronouncedly as well.

The compromise between sufficient resolution of the analytes and short analysis time results in the selection of the BGE consisting of 30 m*M* phosphate buffer, pH 8.0, containing 50 m*M* SDS and 15% (v/v) MeOH: as can be seen from Fig. 3, baseline separation of all analytes is obtainable within 15 min. This system was taken further for the determination of the analytes in human plasma.

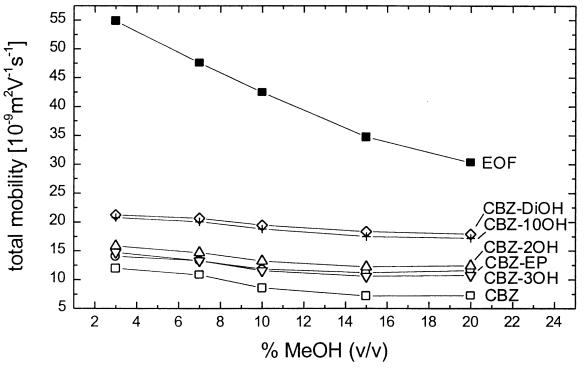

Fig. 2. Dependence of the total mobility of the analytes and the mobility of the EOF on the concentration of methanol in the BGE (phosphate buffer, 30 m*M*, pH 8.00; 50 m*M* SDS).

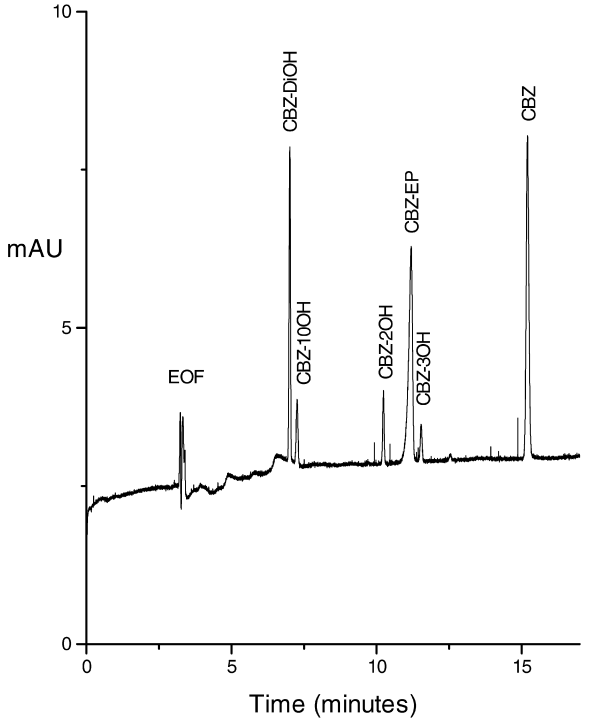

Fig. 3. MEKC of a standard solution containing 5 μg ml^{-1} of CBZ, CBZ-EP and CBZ-DiOH, and 1 μg ml^{-1} of CBZ-10OH, CBZ-2OH and CBZ-3OH. Conditions: BGE phosphate buffer (30 m*M*, pH 8.00) modified with 15% (v/v) methanol, 50 m*M* SDS. Capillary length 48.5/40.0 cm, I.D. 50 μm. Voltage +25 kV. Temperature 25.0°C. Detector wavelength 205 nm.

3.2. Application to real samples

3.2.1. Determination of the analytes in spiked plasma samples

3.2.1.1. Qualitative analysis. Sample pretreatment for quantitative analysis was carried out using a recently developed SPE procedure for the purification of human plasma samples [31] with minor changes. The SPE procedure uses Oasis HLB cartridges (see Experimental section); the analytes are eluted with tetrahydrofuran, the eluate is brought to dryness and the residue redissolved with a mixture of 30 m*M* phosphate buffer, pH 8.00, and 15% (v/v) methanol.

The electropherogram of a blank plasma sample and the same sample spiked with the analytes (5.0

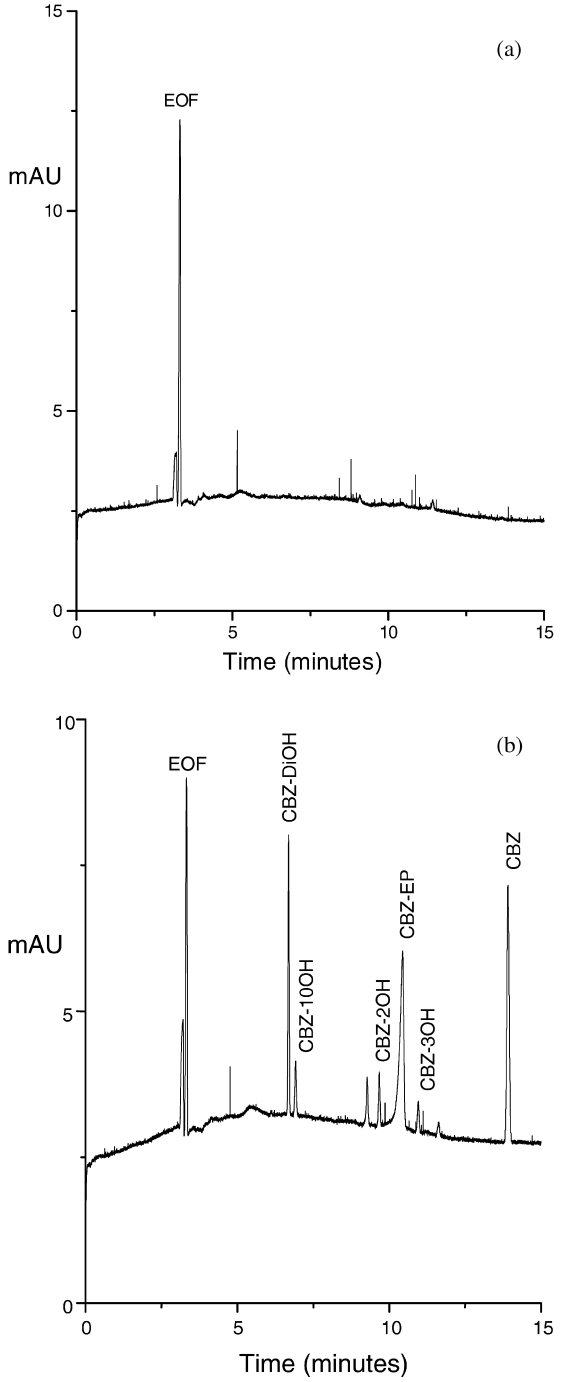

Fig. 4. (a) MEKC of blank plasma after the extraction procedure. (b) MEKC of the blank plasma shown in (a), spiked with a standard solution containing 5 μg ml^{-1} of CBZ, CBZ-EP and CBZ-DiOH, and 1 μg ml^{-1} CBZ-10OH, CBZ-2OH and CBZ-3OH, and subjected to SPE procedure. Conditions as in Fig. 3.

Table 1
Assay characteristics

Compound	Parameters[a]				LOQ (μg ml^{-1})	LOD (μg ml^{-1})
	Range (μg ml^{-1})	*a*	*b*	*r*		
CBZ	0.25–15.00	1.23	5.10	0.9997	0.25	0.10
CBZ-EP	0.25–15.00	1.56	5.59	0.9993	0.25	0.10
CBZ-DiOH	0.25–15.00	0.99	2.65	0.9997	0.25	0.10
CBZ-10OH	0.25–5.00	1.07	3.49	0.9989	0.25	0.10
CBZ-2OH	0.25–5.00	1.30	3.05	0.9988	0.25	0.10
CBZ-3OH	0.30–5.00	0.80	2.05	0.9993	0.30	0.12

[a] Calibration curve: $y = a + bx$, where x is the analyte concentration, expressed as μg ml^{-1}, and y is the peak area, expressed as arbitrary units; r is the linear correlation coefficient.

μg ml^{-1} of CBZ, CBZ-EP and CBZ-DiOH and 1.0 μg ml^{-1} of CBZ-10OH, CBZ-2OH and CBZ-3OH) are shown in Fig. 4a and b. Comparison of the two electropherograms shows that no interfering peak appears at the retention times of the analytes; thus carbamazepine and its five metabolites can be reliable identified.

3.2.1.2. Quantitation. Calibration curves from spiked blank plasma samples were set up for the particular analytes in the respective concentration ranges. The ranges are given in Table 1, together with the parameters of linear regression (obtained from 10 different concentrations). It can be seen that good linearity is achieved. The limits of detection

Table 2
Extraction yield and precision data for spiked blank plasma samples

Compound	Amount added to blank plasma (μg ml^{-1})	Extraction yield (%)[a]	Repeatability (RSD%)	Interday precision (RSD%)
CBZ	0.25	98.8	0.9	2.4
	5.00	97.9	0.2	1.8
	15.00	93.7	0.7	1.3
CBZ-EP	0.25	100.1	1.9	2.9
	5.00	97.3	1.7	2.8
	15.00	92.8	0.9	2.1
CBZ-DiOH	0.25	95.7	1.0	3.3
	5.00	89.4	0.6	2.0
	15.00	88.9	0.5	1.7
CBZ-10OH	0.25	98.0	1.0	2.9
	1.00	95.6	0.8	2.9
	5.00	88.1	1.4	1.6
CBZ-2OH	0.25	97.5	1.5	3.3
	1.00	91.2	1.5	3.2
	5.00	89.9	0.2	1.7
CBZ-3OH	0.25	98.3	1.3	3.2
	1.00	93.4	1.8	3.0
	5.00	89.8	1.5	2.5

[a] Each value is the mean of six independent assays. The extraction yield was calculated from peak areas from spiked samples compared to peak areas of the same analyte concentration in standard solutions.

(LOD) and quantification (LOQ) were calculated according to USP 24 guidelines [38] and are reported in the same Table 1. The LOQ is in the range of a few hundred ng ml^{-1}, a level that is sufficiently low for pharmacological and clinical purposes.

The extraction yield or absolute recovery was evaluated on blank plasma samples spiked with three different concentrations of the carbamazepines. The yield was calculated from the measured analyte concentrations in these samples, compared with those obtained from pure aqueous reference solutions (after application of the same analytical procedure). The results are reported in Table 2. The mean extraction yield was 96.8% for CBZ and 93.7% for the metabolites. Good repeatability and intermediate precision resulted as well (Table 2): the mean RSD of peak areas was less than 2.4% for CBZ and 3.3% for the metabolites for concentrations higher than 250 ng ml^{-1}.

3.3. Application to patient plasma

The validated method was applied to the determination of carbamazepines in the plasma of epileptic patients treated with Tegretol® tablets. The electropherogram of a plasma sample from a patient treated with 600 mg per day of CBZ is shown in Fig. 5. CBZ, CBZ-EP and CBZ-DiOH are detectable. It should be noted that the first identification of the analytes was not carried out from the retention times, as the EOF mobility might change from run to run. This is a well-known phenomenon, especially when real samples are injected, that contain matrix components with the tendency to absorb onto capillary wall. Thus preliminary identification was based on apparent mobilities.

The concentrations of CBZ, obtained by interpolation on the corresponding calibration curve, was 5.2 μg ml^{-1}, that of CBZ-EP and CBZ-DiOH were 1.0 and 3.6 μg ml^{-1}, respectively. In this plasma sample CBZ-10OH, CBZ-2OH and CBZ-3OH were not present above the LOD of the method (0.10, 0.10 and 0.12 μg ml^{-1}, respectively).

In order to evaluate the accuracy of the method, known amounts of the standard analyte solutions were added to known amounts of plasma samples from patients treated with Tegretol, and the resulting mixture analysed. The recovery of the added analytes was then calculated. These recovery assays were repeated at three different levels (the concentrations added are reported in Table 3) and three times for each level. The accuracy data are satisfactory: the recovery values range from 86.5 to 96.8% for CBZ and from 85.4 to 100.3% for the metabolites.

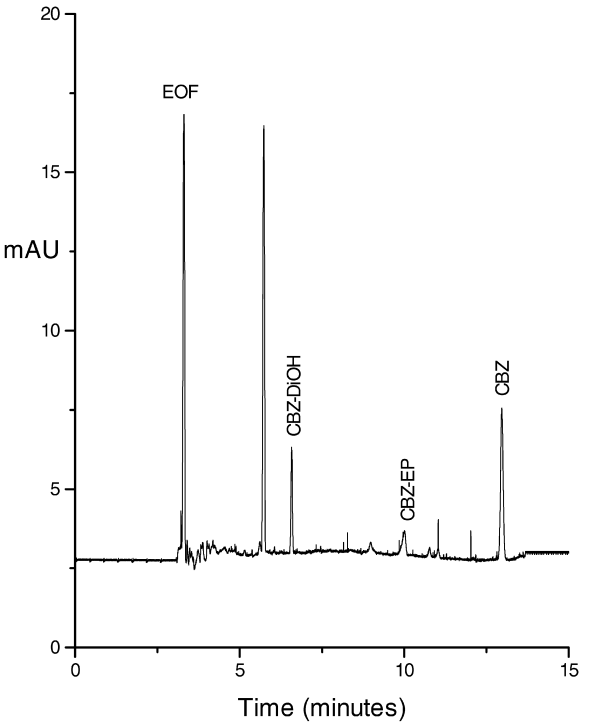

Fig. 5. MEKC of a plasma sample of an epileptic patient treated with Tegretol®. Conditions as in Fig. 3.

In addition to the example already given above, the method has been applied to the analysis of seven plasma samples from different epileptic patients. In all samples, the concentrations of the minor metabolites were always very low, in most cases lower than the LOQ values. Only in one sample we found a CBZ-3OH level of 325 μg ml^{-1} and in another one a CBZ-10OH concentration of 340 μg ml^{-1}. However, the separation selectivity allows for the discrimination of all six analytes in all cases. This can be concluded from Fig. 6, which shows the electropherogram of a patient plasma sample to which standard solutions of the analytes had been added. Note that the clinically important compounds to be

Table 3
Accuracy assays in human plasma of a patient undergoing therapy with Tegretol®: three independent measurements each, after application of the SPE-MEKC procedure

Compound	Amount added to sample ($\mu g\ ml^{-1}$)	Mean recovery (%±SD)
CBZ	2.0	95.5±0.8
	5.0	96.8±1.7
	8.0	86.5±0.3
CBZ-EP	2.0	99.3±1.3
	5.0	90.8±2.2
	8.0	87.2±0.3
CBZ-DiOH	2.0	83.9±0.9
	5.0	88.6±4.3
	8.0	85.4±0.1
CBZ-10OH	1.0	97.9±5.3
	3.0	92.0±0.9
	5.0	87.7±0.1
CBZ-2OH	1.0	97.8±5.2
	3.0	88.6±1.1
	5.0	85.2±0.4
CBZ-3OH	1.0	100.3±0.5
	3.0	95.4±0.9
	5.0	90.9±0.4

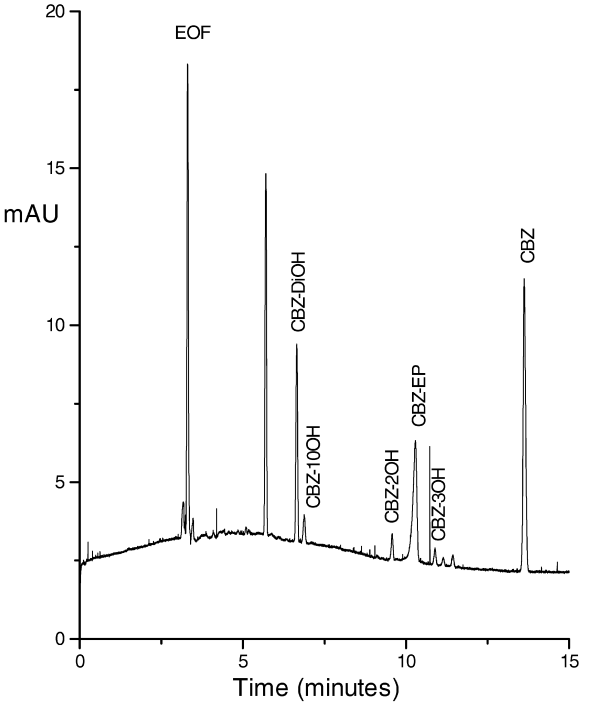

Fig. 6. MEKC of the plasma sample of a patient shown in Fig. 5, spiked with a standard solution containing 5 $\mu g\ ml^{-1}$ of CBZ, CBZ-EP and CBZ-DiOH, and 1 $\mu g\ ml^{-1}$ of CBZ-10OH, CBZ-2OH and CBZ-3OH. Conditions as in Fig. 3.

determined are CBZ and CBZ-EP. Furthermore, other drugs simultaneously administered to the epileptic patients (phenobarbital, lamotrigine, clonazepam, phenytoin, olanzapine) gave no interference in the assay. In all cases peak identity and peak purity was assessed from its spectra recorded by the diode array detector.

4. Conclusions

The MEKC method developed allows for a rapid and accurate separation, identification and quantification of CBZ and five metabolites in human plasma. The combination of SPE pretreatment and MEKC separation and quantitation has the advantage of being more rapid and achieving a better yield than liquid–liquid extraction procedures proposed in literature [34]; moreover only a small amount of plasma sample (250 μl) is necessary for one analysis. The method shows good precision and accuracy, and is thus useful for clinical monitoring of patients under therapy with CBZ.

Acknowledgements

A grant for A.M. from the Faculty of Pharmacy, University of Bologna is acknowledged. The authors would like to thank Dr Fiorenzo Albani (Institute of Clinical Neurology, University of Bologna Italy) for providing plasma samples of patients treated with Tegretol®. Providing pure compounds for the development of this assay by Novartis Pharma AG (Basel, Switzerland) is acknowledged, too.

References

[1] M.A. Rogawski, R.J. Porter, Pharmacol. Rev. 42 (1990) 224.
[2] F. Albani, R. Riva, A. Baruzzi, Pharmacopsychiatry 28 (1995) 235.
[3] T. Okuma, Neuropsychobiology 27 (1993) 138.
[4] H.M. Emrich, M. Dose, R. Wolf, Neuropsychobiology 27 (1993) 158.
[5] R.H. Mattson, Epilepsia 36 (1995) 22.
[6] M. Kerr, K.E. Thummel, C.J. Wurden, S.M. Klein, D.L. Kroetz, F.J. Gonzalez, R.H. Levy, Biochem. Pharmacol. 47 (1994) 1969.
[7] C.R. Valentine, J.L. Valentine, J. Seng, J. Leakey, D. Casciano, Cell Biol. Toxicol. 12 (1996) 155.
[8] F. Semah, F. Gimnez, E. Longer, D. Laplane, A. Thuillier, M. Baulac, Ther. Drug Monit. 16 (1994) 537.
[9] J.W. Faigle, K.F. Feldman, in: R. Levy, R. Mattson, B. Meldrum, J.K. Penry, F.E. Dreyfuss (Eds.), Antiepileptic Drugs, 3rd ed, Raven Press, New York, 1989, p. 491.
[10] P. Myllynen, P. Pienimäki, H. Raunio, K. Vähäkangas, Hum. Exp. Toxicol. 12 (1998) 668.
[11] M. Eichalbaum, M. Thomson, G. Tybring, L. Bertilson, Clin. Pharmacokinet. 10 (1985) 89.
[12] E. Perucca, Br. J. Clin. Pharmacol. 18 (1984) 401.
[13] G.E. von Unruh, W.D. Paar, Biomed. Environ. Mass Spectrom. 13 (1986) 651.
[14] M. Oellerich, in: H. von Schliack, H.C. Hopf (Eds.), Diagnostik in der Neurologie, Thieme Verlag, Stuttgart, 1988, p. 263.
[15] K. Matsumoto, H. Kikuchi, S. Kano, H. Iri, H. Takahashi, M. Umino, Clin. Chem. 34 (1988) 141.
[16] J.F. Wilson, L.M. Tsanaclis, J.E. Perrett, J. Williams, J.F.C. Wicks, A. Richens, Ther. Drug Monit. 14 (1992) 98.
[17] J.T. Burke, J.P. Thenot, J. Chromatogr. 340 (1985) 199.
[18] C.K. Tsaprounis, M. Kajbaf, J.W. Gorrod, J. Clin. Pharmacol. 16 (1991) 257.
[19] R.D. Chelberg, S. Gunawan, D.M. Treiman, Ther. Drug Monit. 10 (1988) 188.
[20] K.M. Matar, P.J. Nicholls, A. Teckle, S.A. Bawazir, M.I. Al-Hassan, Ther. Drug Monit. 21 (1999) 559.
[21] J.M. Potter, A. Donnelly, Ther. Drug Monit. 20 (1998) 652.
[22] L.A. Saris, G.J. Brekelmans, G.J. van der Linden, R.V. Rademaker, P.M. Edelbroek, J. Chromatogr. B 691 (1997) 409.
[23] D. Chollet, E. Castella, P. Combe, V. Arnera, J. Chromatogr. B 683 (1996) 237.
[24] R.P. Remmel, S.A. Miller, N.M. Graves, Ther. Drug Monit. 12 (1990) 90.
[25] W. Kuhnz, H. Nau, Ther. Drug Monit. 6 (1984) 478.
[26] H. Liu, M. Delgado, S.T. Iannaccone, L.J. Forman, C.M. Eggers, Ther. Drug Monit. 15 (1993) 317.
[27] M.C. Rouan, J. Campestrini, V. Le Clanche, J.B. Lacaillon, J. Godbillon, J. Chromatogr. 573 (1992) 65.
[28] E. Tanaka, J. Chromatogr. B 688 (1997) 155.
[29] P. Pienimäki, S. Fuchs, J. Isojärvi, K. Vähäkangas, J. Chromatogr. B 673 (1995) 97.
[30] J.L. Maggs, M. Pirmohamed, N.R. Kitteringham, B.K. Park, Drug Metab. Dispos. 25 (1997) 275.
[31] R. Mandrioli, F. Albani, G. Casamenti, C. Sabbioni, M.A. Raggi, J. Chromatogr. B 762 (2001) 109.
[32] W. Thormann, S. Lienhard, P. Wernly, J. Chromatogr. 636 (1993) 137.
[33] K.J. Lee, G.S. Heo, N.J. Kim, D.C. Moon, J. Chromatogr. 608 (1992) 243.
[34] S. Härtter, B. Jensen, C. Hiemke, M. Leal, H. Weigmann, K. Unger, J. Chromatogr. B 712 (1998) 253.
[35] K. Makino, Y. Goto, M. Sueyasu, K. Futagami, Y. Kataoka, R. Oishi, J. Chromatogr. B 695 (1997) 417.
[36] R. Kuldvee, W. Thormann, Electrophoresis 22 (2001) 1345.
[37] W. Thormann, R. Theurillat, M. Wind, R. Kuldvee, J. Chromatogr. A 924 (2001) 429.
[38] United States Pharmacopoeia, 24th ed., United States Pharmacopoeial Convention, Rockville, MD, 2000, pp. 2150–2151.