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Short communication

Development of a simple column-switching high-performance liquid chromatography (HPLC) method for rapid and simultaneous routine serum monitoring of lamotrigine, oxcarbazepine and 10-monohydroxycarbazepine (MHD)

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Abstract

Using isocratic column-switching high-performance liquid chromatography (HPLC) we established a group method for automated quantitative analysis of the antiepileptic drugs lamotrigine, oxcarbazepine and its metabolite 10-monohydroxycarbazepine (MHD) that are also used in psychiatry as mood stabilizers. Samples were cleaned from interfering proteins and lipids by transfer onto a pre-column, using a PerfectBond® C-8 material, with 8% acetonitrile in water as a pre-column eluent. Separation was performed by elution onto the analytical column (Betasil® C6 5 μm, 250 mm × 4.6 mm) at a flow rate of 1.0 ml/min with potassium dihydrogenphosphate buffer (20 mmol/l, pH3.0)/acetonitrile (70/30; v/v) as analytical eluent. UV-spectrophotometric detection was set to 215 nm for all three compounds. The analytical run was finished within 18 min. Detection limit was 30 ng/ml for lamotrigine, 35 ng/ml for oxcarbazepine and 25 ng/ml for 10-monohydroxycarbazepine. The method was found to be suitable for automated analysis of serum samples of patients treated with lamotrigine and oxcarbazepine.

Keywords: Column-switching; Lamotrigine; Oxcarbazepine; 10-monohydroxycarbazepine; Therapeutic drug monitoring

1. Introduction

Lamotrigine and oxcarbazepine are well known as antiepileptic therapeutics against (focal) epileptic seizures in children and adults. Oxcarbazepine (10,11-dihydro-10-oxo-5H-dibenz[b,f]azepine-5-carboxamide) structurally differs from its tricyclic analogue carbamazepine in a 10-keto group (Fig. 1). Oxcarbazepine has an elimination half-life of 1–5 h and is metabolised to the pharmacological active compound of 10-monohydroxycarbazepine (MHD, monohydroxy derivate), which is known to have the relevant activity [1]. Therefore, serum levels of oxcarbazepine are lower than those of its metabolite. The therapeutic reference range for MHD is proposed to be between 10 and 20 mg/l [2]. Oxcarbazepine turned out to have potency as mood stabilizer in bipolar disorders, in manic episodes as well as in acute depression, and is sometimes added to an antidepressant medication [3]. Lamotrigine is a phenyltriazine derivate (Fig. 1) that structurally differs from all other antiepileptic drugs. It undergoes glucuronidation by uridine-glucuronyl-transferase isoenzyme 1A4 (UGT1A4); the cytochrome – P450 – system is not involved. The 2N-glucuronide is not known to have any therapeutic activity [4]. Since 2003, lamotrigine is approved to the German market to prevent depressive episodes in patients with bipolar disorders. Lamotrigine inhibits voltage-activated sodium channels; the presynaptic release of the excitatory neurotransmitter glutamate is decreased. Therapeutic drug monitoring of lamotrigine is suggested in order to control patients with infrequent seizures [5]. For its use as mood stabilizer no recommendation is given [6]. A therapeutic reference range for lamotrigine has not yet been established, neither for the use as antiepileptic nor for the use in bipolar disorders; therefore,

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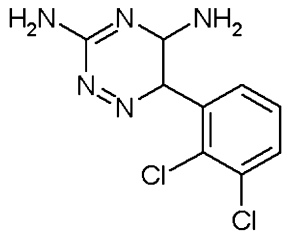
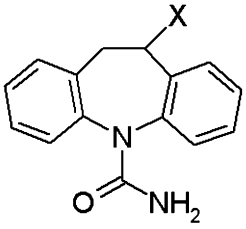
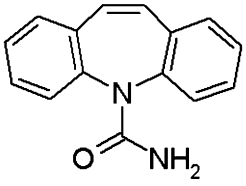


lamotrigine



X : =O, oxcarbazepine
X : -OH, 10 - monohydroxycarbazepine



carbamazepine

Fig. 1. Chemical structures of lamotrigine, oxcarbazepine, 10-monohydroxycarbazepine (MHD) and carbamazepine.

we decided on a tentative therapeutic range mentioned before [7].

High-performance liquid chromatography (HPLC) methods for the three analytes have already been published [8–11]. The benefit of the method described in this paper is a simplification by switching from off-line to on-line drug – extraction using HPLC column-switching, which saves time, manpower and amount of serum that can be reduced to 100 μl.

2. Material and methods

2.1. Chemicals

Lamotrigine (CAS number 84057-84-1) was kindly supplied by Glaxo Smith Kline (Durham, Great Britain). Oxcarbazepine (CAS number 28721-07-5) and its main metabolite 10-monohydroxycarbazepine were offered by Novartis (Basel, Switzerland). Structures of the substances are shown in Fig. 1.

Additional chemicals used for the preparation of the eluents were of HPLC or analytical grade.

Potassium dihydrogenphosphate and ortho-phosphoric acid, Suprapur® 85% forming the buffer solution, were obtained from Merck KGaA (Darmstadt, Germany). Acetonitrile Ultra Gradient HPLC grade was obtained from J.T. Baker (Mallinckrodt Baker B.V., Deventer, Holland).

Water was deionized and filtered by means of a Millipore system by Millipore GmbH (Eschborn, Germany).

Human drug-free serum was prepared from whole blood drawn from healthy volunteers (Bezirksklinikum Regensburg, Germany).

2.2. Sample preparation

For validation experiments, stock solutions of lamotrigine, oxcarbazepine and 10-monohydroxycarbazepine were prepared by solubilising 5 mg of lamotrigine and 10-monohydroxycarbazepine and 1.6 mg oxcarbazepine in 5 ml of ethanol each. Oxcarbazepine was diluted by deionized water to 200 μg/ml. Dilution of the serum matrix with aqueous solution was below 10%. A calibration series of five concentrations were used for lamotrigine and four concentration levels for oxcarbazepine and for 10-monohydroxycarbazepine, respectively. Quality control samples containing the analytes were prepared in the same way as the calibration samples.

Stock solutions were stored at −20 °C.

The pre-column eluent – 8% acetonitrile in water – was set up by adding 160 ml acetonitrile to deionized water to obtain a total volume of 2000 ml. The mixture was degassed in an ultrasonic bath for 5 min. Pre-column eluent was also used to rinse the injection needle of the autosampler between injections in order to avoid carry-over effects by adhering proteins or drug remnants.

The isocratic analytical mobile phase consisted of 20 mmol/l phosphate buffer (5.44 g potassium dihydrogenphosphate in 2000 ml deionized water) and was supplemented with acetonitrile (70/30, %v/v). The pH-value was adjusted to 3.0 using ortho-phosphoric acid.

Serum samples of patients treated with lamotrigine and oxcarbazepine were sent to an external laboratory, while a total of 500 μl of each serum specimen was taken off for method development. Samples were centrifuged directly after delivery for 10 min at 4000 U/min and either rapidly analysed or stored at −20 °C.

2.3. Instrumentation

A Perfect Bond® C-8 material 20 μm, 20 mm × 2.1 mm (MZ-Analysentechnik, Mainz, Germany) served as pre-column, which was protected by an inline filter (RECIPE GmbH, Munich, Germany). The analytical column was a Betasil® C-6 column, 250 mm × 4.6 mm, 5 μm (Thermo Electron Corporation, Dreieich, Germany).

Analysis of the specimens was carried out on a Dionex HPLC system (Dionex GmbH, Idstein, Germany) consisting of a GINA 50 autosampler, a dual ternary low-pressure gradient pump P680 for delivering the pre-column eluent and the analytical eluent at the same time at a flow rate of 1.3 ml/min each. The process of switching from the pre-column to the analytical column was executed by an electric 10-port valve incorporated in a thermostatted column compartment, set to 25 °C. At 0–5 min, serum samples were delivered to the pre-column by pre-column eluent

(8% acetonitrile in water). At the same time the analytical eluent flushed the analytical column for preparing separation of the drug mixture. At 5–10 min, the switching valve was set to the analytical position, the analytical eluent delivered the matrix-free drug mixture to the analytical column in backflush mode. The valve was set back to the starting position from 10 on to 18 min to prepare the pre-column for the next sample injection. The wavelength of the variable photodiode array UV-detector PDA-100 was set to 215 nm. The chromatogram was evaluated and integrated by the Chromeleon software Version 6.3 SP1 Build 587.

2.4. Quantification and validation of the method

Quantification experiments were based on calibration of peak heights for oxcarbazepine and MHD and peak areas for lamotrigine. The method validation included precision, linearity, recovery limit of detection (LOD), as well as specificity of lamotrigine, oxcarbazepine and MHD from human serum. Linearity data were assessed by analysing three standard calibration samples at each concentration. Precision and recovery data were evaluated by independently prepared quality control samples within the same concentration range as the calibration serum samples.

We evaluated *precision data and linearity* of the method by calculating the coefficient of variation (C.V.) in replicates of three of four (oxcarbazepine and MHD) to five (lamotrigine) different concentration levels of day-to-day analysis (intraday precision) and between-day analysis (interday precision, three different days) of the analytes. Nominal concentrations for lamotrigine were 1.250–2.500–5.000–10.000–15.000 ng/ml for determination of precision and 500–2.500–10.000–15.000 ng/ml for set-up linearity data, 250–500–1.000–2.000 ng/ml for oxcarbazepine and 5.000–10.000–20.000–40.000 ng/ml for MHD.

Recoveries were expressed by comparing peak areas/heights of solutions of the analytes in serum and in pre-column eluent after column-switching procedure, the concentration of the pre-column solution was set to be 100% expected. Three different drug concentrations per analyte were used for recovery experiments (lamotrigine 100–1000–5000 ng/ml, MHD 100–1000–10000 ng/ml, oxcarbazepine 100–500–1000 ng/ml).

The *limit of detection* was estimated by comparing peak heights of oxcarbazepine and MHD and peak areas of lamotrigine derived from drug-free serum and from standard solutions of the drug mixture. Referring to the background of the matrix, a signal-to-noise ratio of 3:1 was assessed. Confirmation of the estimated LOD was made by analysing 10 different concentrations evenly distributed starting with the value of the lowest estimated concentration to be detected. The values were verified by setting up a linear regression over the data points. *The limit of quantification* (LOQ) was calculated from the values obtained from standard deviation methods. Experimental verification of LOQ included precision, recovery and linearity data at the respective concentrations.

Data are presented as mean ± standard deviation (S.D.) unless otherwise indicated.

For testifying the *specificity* of the method, potential interferences of concomitantly given drugs were judged by their retention times. For this reason, human serum was spiked by the drugs tested.

3. Results

3.1. Retention times

The presented method yielded retention times of 9.45 ± 0.06 min ($n = 36$) for lamotrigine, 11.10 ± 0.004 min ($n = 36$) for MHD and 16.05 ± 0.02 min ($n = 36$) for oxcarbazepine. All substances were not subject to interferences with endogenous compounds. The analytical run could be finished after 18 min including precleaning of the specimens.

Table 1
Precision data (coefficient of variation [%]) for determination of human blank serum spiked with different concentrations of lamotrigine, 10-monohydroxycarbazepine (MHD) and oxcarbazepine

Analyte	Concentration (ng/ml)	Intraday precision (C.V.%)	Interday precision (C.V.%)
Lamotrigine	30 (LOQ)	0.6071	8.9497
	1250	0.4285	1.1323
	2500	0.1217	0.3426
	5000	0.0571	1.0486
	10000	0.0141	0.8248
	15000	0.0886	2.5511
MHD	60 (LOQ)	3.2501	5.3694
	5000	0.3034	0.9241
	10000	0.0338	0.5606
	20000	0.0139	1.1217
	40000	0.1344	1.1150
Oxcarbazepine	90 (LOQ)	2.0537	7.0911
	250	1.4414	7.2765
	500	0.5804	6.8106
	1000	0.6109	5.9972
	2000	0.4835	4.0783

Table 2
Linearity data ($n = 12$, four concentration levels on three different days) showing the coefficients of correlation (r^2), the slopes and intercepts of the linear regression lines of lamotrigine, 10-monohydroxycarbazepine (MHD) and oxcarbazepine

Analyte	Day	Linearity (correlation coefficient r^2)	Linearity (slope)	Linearity (intercept)
Lamotrigine	1	0.9992	0.0337	2.9227
	2	0.9968	0.0320	6.4393
	3	1.0000	0.0331	−1.1301
MHD	1	0.9999	0.0444	15.506
	2	0.9999	0.0439	19.448
	3	0.9999	0.0432	23.672
Oxcarbazepine	1	0.9999	0.0266	0.519
	2	0.9995	0.0248	−0.2239
	3	0.9996	0.0256	−0.1959

3.2. Precision

Coefficient of variation resulting from intraday experiments within the analysed concentration range was always below 0.5% of mean for lamotrigine, below 0.3% of mean for MHD and below 1.5% of mean for oxcarbazepine.

Interday variation was <2.6% of mean for lamotrigine, <1.1% of mean for MHD and <7.3% of mean for oxcarbazepine (Table 1).

3.3. Linearity

The calibration curves showed high correlation coefficients (r^2) comparing day-to-day peak areas for lamotrigine and peak heights for oxcarbazepine and its metabolite with spiked concentrations of the analytes in serum. Linearity could be demonstrated over the whole concentration range investigated. For lamotrigine r^2 was always above 0.9968, for MHD r^2 was 0.9999 and for oxcarbazepine r^2 was 0.9995 and higher (Table 2).

3.4. Recovery

The expected recoveries were determined to be in a range of 95–107% (concentration range 100–5000 ng/ml) for lamotrigine and 101–103% (concentration range 100–10000 ng/ml) for MHD. For oxcarbazepine, expected recovery varied from 97 to 120% (concentration range 100–1000 ng/ml) (Table 3).

3.5. Limit of detection/limit of quantification

The limit of detection proofed to be 10.0 ng/ml for lamotrigine, 30.0 ng/ml for oxcarbazepine and 20.0 ng/ml for its metabolite 10-monohydroxycarbazepine. The limit of quantification was 30.0 ng/ml for lamotrigine, 90.0 ng/ml for oxcarbazepine and 60.0 ng/ml for its metabolite 10-monohydroxycarbazepine (Fig. 4). Precision results from intraday and interday experiments at limit of quantification were displayed as coefficients of variation (Table 1). Recovery of lamotrigine at LOQ was 96 ± 1.4%. For oxcarbazepine and its metabolite we found recoveries to be 102 ± 3.4% and 109 ± 1.5% (Table 3). Experimental verification of linearity ranging between 10 and 30 ng/ml for lamotrigine, 30 and 90 ng/ml for oxcarbazepine and 20 and 60 ng/ml for MHD showed correlation coefficients (r^2) of 0.9875 and above for lamotrigine, 0.9986 for oxcarbazepine and 0.9995 for MHD (Table 4).

Table 3
Recovery data (% expected ± S.D., $n = 3$) deriving from quality control serum samples in a concentration range of 30–5000 ng/ml for lamotrigine, 60–10000 ng/ml for 10-monohydroxycarbazepine (MHD) and 90–1000 ng/ml for oxcarbazepine compared to dilutions of the analytes in pre-column eluent (8% acetonitrile in water)

Analyte	Concentration (ng/ml)	Recovery ± S.D. (% expected)
Lamotrigine	30	96 ± 1.4
	100	107 ± 15.0
	1000	95 ± 0.4
	5000	96 ± 0.5
MHD	60	109 ± 1.5
	100	101 ± 1.2
	1000	103 ± 0.2
	10000	101 ± 0.6
Oxcarbazepine	90	102 ± 3.4
	100	120 ± 1.3
	500	102 ± 1.1
	1000	97 ± 0.8

Table 4
Linearity data set-up over triplicates of analysis showing the coefficients of correlation (r^2), the slopes and intercepts of the linear regression lines of lamotrigine, 10-monohydroxycarbazepine (MHD) and oxcarbazepine between the limit of detection (LOD) and the limit of quantification (LOQ)

Analyte	Concentration range (ng/ml)	Linearity (correlation coefficient r^2)	Linearity (slope)	Linearity (intercept)
Lamotrigine	10–30	0.9875	0.0172	−0.0069
MHD	20–60	0.9995	0.0386	−0.0132
Oxcarbazepine	30–90	0.9986	0.0263	0.2288

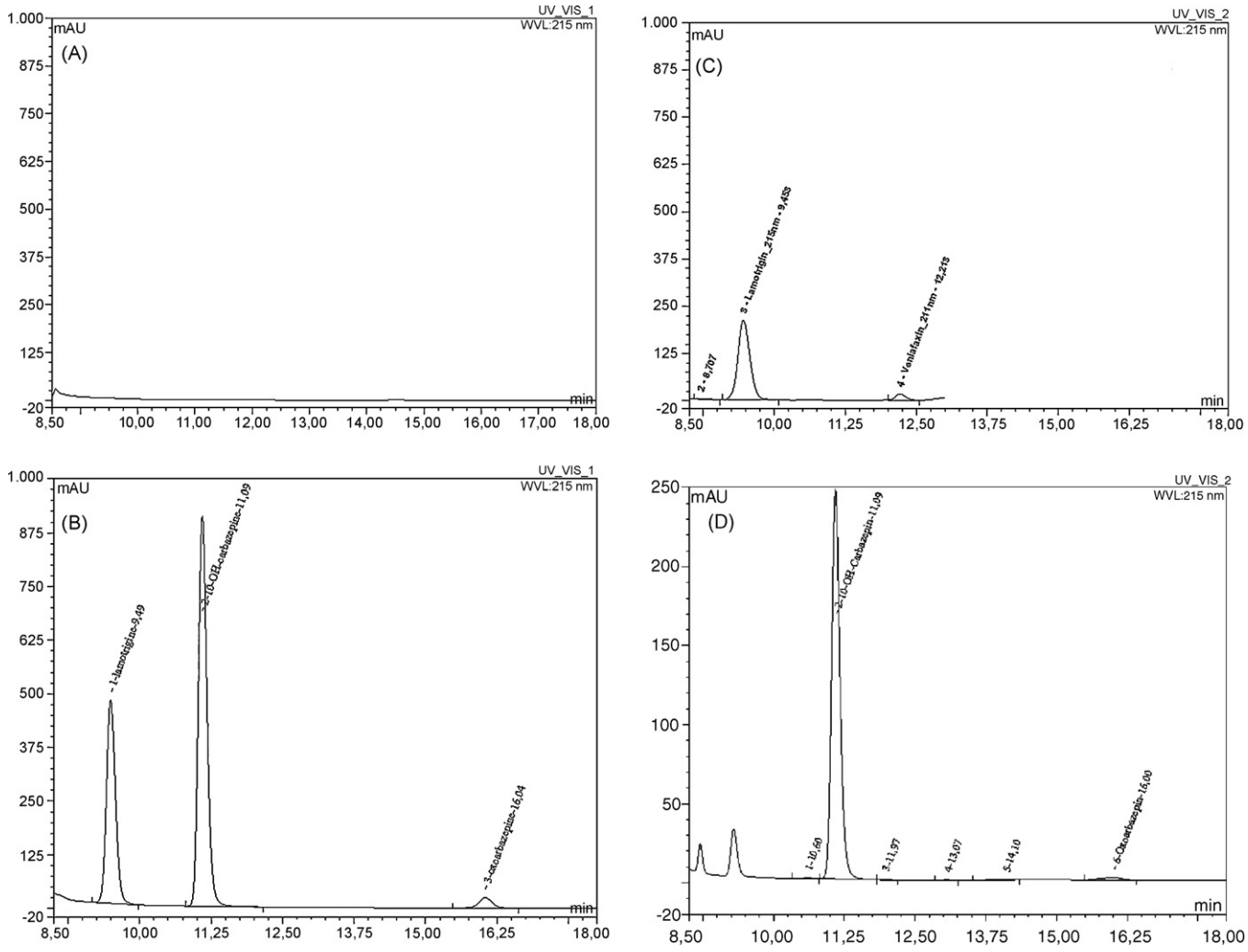


Fig. 2. Representative chromatograms of a blank serum sample (A), a serum calibration sample containing the analytes lamotrigine (5000 ng/ml), oxcarbazepine (1000 ng/ml) and its metabolite 10-monohydroxycarbazepine (MHD) (20000 ng/ml) (B), a serum sample of a patient receiving 100 mg lamotrigine (concentration 3363 ng/ml) (C), a serum sample of a patient receiving 300 mg oxcarbazepine (total concentration of oxcarbazepine and metabolite 5605 ng/ml) (D).

3.6. Specificity

The method was tested for its specificity by analysing internistic and psychiatric drugs, which are commonly coadministered with the analytes. Depending on their chemical structure, some of the drugs are eluted together with the solvent; others are detected at some time within the analytical run. Co-elution with lamotrigine only has to be expected with 9-hydroxyrisperidone (9.8 min), N-desmethylclozapine (9.8 min) and metoprolol (8.8 min). Oxcarbazepine is subject to analytical interference with nortriptyline (16.1 min) and perazine (16.3 min), 10-monohydroxycarbazepine with clozapine (11.3 min) and chlordiazepoxide (11.5 min). Retention times of all substances tested are listed in Table 5.

3.7. Application to patient samples

The method has been successfully applied to routine therapeutic drug monitoring of lamotrigine, oxcarbazepine and its

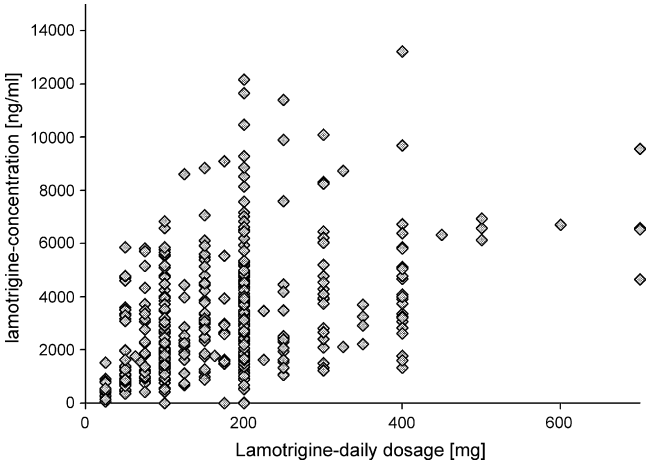


Fig. 3. Method application on therapeutic drug monitoring of lamotrigine specimen ($n = 472$): dose–concentration relationship.

Table 5
Compounds studied for possible interferences with lamotrigine (retention time 9.45 ± 0.06 min), oxcarbazepine (retention time 16.05 ± 0.02 min), and MHD (retention time 11.10 ± 0.004 min)

Drugs tested for possible interferences	Detection (retention time) (min)
Benzodiazepines	
Alprazolam	15.3
Bromazepam	15.3
Chlordiazepoxide	11.5
Clobazam	Not detected
Lorazepam	Not detected
Nitrazepam	15.5
Oxazepam	Not detected
Triazolam	10.8
Antidementive drugs	
Donepezil	13.2
Galantamine	Not detected
Rivastigmine	8.7
Antidepressants (non-tricyclic)	
Citalopram	19.6
Desmethylcitalopram	18.2
Desmethylvenlafaxine	8.2
Fluoxetine	Not detected
Fluvoxamine	Not detected
Paroxetine	Not detected
Reboxetine	Not detected
Sertraline	Not detected
Venlafaxine	11.6
Neuroleptics	
9-OH Risperidone	9.8
Aripiprazole	37.9
Chlorprotixene	Not detected
Clozapine	11.3
Dehydroaripiprazole	32.3
N-Desmethylclozapine	9.8
Duloxetine	Not detected
Fluphenazine	Not detected
Haloperidol	Not detected
Levomepromazine	Not detected
Olanzapine	Not detected
Desmethylolanzapine	Not detected
Perazine	16.3
Promethazine	8.6/12.5/22.8
Quetiapine	14.4
Risperidone	10.2
Ziprasidone	Not detected
Zuclopenthixole	Not detected
Tricyclic antidepressants	
Amitriptyline	Not detected
Clomipramine	8.4
Desipramine	12.4
Doxepine	20.9
Imipramine	Not detected
Maprotiline	20.9
Nortriptyline	16.1
Trimipramine	10.0
Antiepileptics	
Carbamazepine	22.8
Carbamazepine-10,11-epoxid	13.9
Others	
Biperiden	Not detected
Metoprolol	8.8
Propranolol	14.2

The compounds were chosen from the comedication actually observed in the specimens sent to our laboratory.

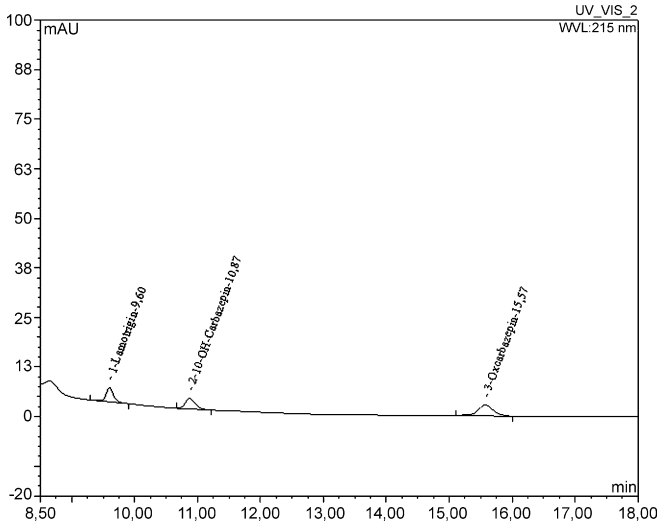


Fig. 4. Chromatogram of a spiked serum sample showing the limit of quantification (LOQ) of lamotrigine (30 ng/ml), oxcarbazepine (90 ng/ml) and 10-hydroxy-10,11-dihydrocarbazepine (MHD) (60 ng/ml).

main metabolite 10-monohydroxycarbazepine within the concentration ranges determined (Fig. 2). Exemplary, a lamotrigine dose–concentration relationship was set up over 472 patient serum samples (Fig. 3).

4. Conclusion and discussion

The HPLC method described for the determination of lamotrigine, oxcarbazepine and its active metabolite 10-monohydroxycarbazepine turned out to be rapid, precise, accurate and specific over the entire therapeutic range, using the advantages of an on-line sample preparation by column-switching procedure. For separation of lamotrigine, oxcarbazepine and MHD in human serum, injection of 100 μl serum turned out to be sufficient. Life of the pre-column as well as the analytical column could therefore be saved to 75 and 250–300 serum injections, respectively. The analytical run could be finished 18 min after injection, including precleaning of the sample.

For calibration of lamotrigine serum samples, peak areas showed higher linearity and precision compared to peak heights. Intraday variations within the concentration levels specified were always below 2% for all analytes, indicating high precision of the method. An imprecision of up to 2% for lamotrigine and MHD and below 8% for oxcarbazepine among inter-day measurement experiments is considered acceptable. Serum stock solutions of the analytes have been tested for autosampler stability. The results were in accordance with previous findings. Lamotrigine areas were comparable within 2 days and peak heights for oxcarbazepine and its metabolite MHD were comparable within 24 h, when kept in the autosampler at room temperature [12,13]. The application of this HPLC method offers the possibility of a simultaneous detection of various antidepressants and neuroleptics within the same run that might be prescribed to patients suffering from bipolar disorders.

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