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Determination of oxcarbazepine and its metabolites in postmortem blood and hair by means of liquid chromatography with mass detection (HPLC/APCI/MS)

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Abstract

A typical use of hair analysis in forensic toxicology is the documentation of previous drug administration. This is illustrated in a suicidal death of a 58-year-old epileptic patient who was treated with oxcarbazepine and probably with levomepromazine. The toxicological analysis carried out by HPLC/APCI/MS included also the hair (6 cm length) besides postmortem blood. The method was validated for levomepromazine, oxcarbazepine (OXCBZ) and its two metabolites, 10-hydroxycarbazepine (CBZ-10OH) and trans-diol-carbazepine (CBZ-diOH) in various biological matrices. The analysis of the postmortem blood indicated oxcarbazepine and its two main metabolites were present at therapeutic concentrations; levomepromazine was detected at a fatal concentration. In three 2-cm segments of hair, oxcarbazepine and its two metabolites were detected; however, levomepromazine was not detected in this specimen. As a result of complex chemical-toxicological investigation it was confirmed the information that the decedent. was an epileptic patient and was treated with oxcarbazepine for at least 6 months before death. In addition, he took a toxic dose of levomepromazine in order to commit suicide. The analysis revealed differences between the concentration levels of oxcarbazepine and its active metabolite CBZ-10OH in postmortem specimens and hair, suggesting different mechanisms of penetration of metabolites and their precursors into this matrix.
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Keywords: Trileptal; Oxcarbazepine; Hair; LC/APCI/MS

1. Introduction

Oxcarbazepine (Trileptal, Novartis; Ch Tolep, I) is an antiepileptic drug currently registered in over 50 countries and recently accepted for registration worldwide [1,2]. It is already considered a first line therapy for a range of epilepsy indication [3].

Oxcarbazepine (OXCBZ) as a 10-keto analoque of carbamazepine (CBZ) has a similar therapeutic profile to CBZ but seems to be better tolerated [4]. The side-effect profile of carbazepine is also similar in nature to CBZ, although the frequency and severity of side-effects has been shown to be lower [5]. A key difference between the two drugs is that OXCBZ unlike CBZ is not metabolized to an epoxide derivative. As the epoxide is responsible for some of the toxic effects of CBZ, the lack of epoxidation of OXCBZ is probably one reason for its better side-effect profile [3].

Oxcarbazepine undergoes reductive metabolism of the keto group to form the active monohydroxy derivative 10-hydroxy-10,11-dihydrocarbamazepine (OXCBZ-10OH). This metabolite predominates in plasma after oral dosing whereas OXCBZ reaches only low levels. OXCBZ-10OH is thus the main compound responsible for the antiepileptic activity of OXCBZ in man. There is a linear relationship between dose and serum levels of the drug and its metabolite in the usual clinical dose ranges. The primary metabolite is conjugated to a glucuronide compound. A minor amount is oxidized to an inactive dihydroxy derivative 10,11-dihydroxy-10,11-dihydrocarbamazepine (OXCBZ-diOH) [1,3,4,6,7].

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Simultaneous determination of oxcarbazepine and its metabolites for therapeutic drug monitoring was reported earlier [1,2,4,8,9], HPLC with UV detection seems to be the most important method for these purposes. Solid phase extraction (SPE) was chosen to for the sample pretreatment because this technique is more feasible and less polluting than the traditional liquid–liquid extraction used in other methods and allows for high extraction yields with good selectivity [2].

The toxicological investigation included also the analysis of levomepromazine in biological matrix of the case examined. Levomepromazine (Tieman®;D; Nozinan® – Specia, F), previously known as methotrimeprazine, is among the oldest neuroleptics belonging to the phenothiazine group. It has a broad range of beneficial effects in the terminal phase of many illnesses resulting from its combined antipsychotic, axiolytic and sedative activity [10].

The aim of this investigation was the implementation of the reliable method based on HPLC with mass detection in positive chemical ionization mode (HPLC/APCI/MS) for simultaneous determination levomepromazine, oxcarbazepine and its two metabolites namely OXCBZ-10OH and OXCBZ-diOH in human (postmortem) blood and hairs. In fatality hair analysis recording chronic drug intake during a period of life of several months for sampling allows for extending retrospectively the time window of medico-legal investigation [11–15].

The present method was employed to investigate the history of oxcarbazepine administration confronted with the toxicological data at the time of death of a subject who had been treated with this drug for epilepsy.

2. Case report

The deceased was found dead at home. According to the report, the victim was a 58-year-old man (M.G.). In the past, he was a psychiatric patient, also treated for of epilepsy. He had attempted suicide on several occasions. He drank alcohol occasionally. For a short time before his death, he started to be treated also with levomepromazine (*Tisercin*).

The postmortem examination did not explain the cause of death; macroscopic examinations of the internal organs of the deceased did not demonstrate any morphological changes.

The toxicological analysis included the blood, urine and hair of the deceased taken during autopsy. The blood and urine of the victim were tested for alcohol and the analysis revealed 1.9 and 3.2‰ of ethanol, respectively.

3. Experimental

3.1. Chemicals and reagents

All chemicals and solvents were of analytical grade.

Oxcarbazepine, 10-hydroxy-10,11-dihydrocarbamazepine (CBZ-10OH) from Novartis Pharma (Basle, Switzerland); 10,11-epoxy-10,11-dihydrocarbamazepine (CBZ-epoxy), 10,11-dihydroxy-10,11-dihydrocarbamazepine (CBZ-diOH), levomepromazine, fluphenazine and diazepam-d_5 from SIGMA (USA). Acetonitrile, *n*-hexane, methanol, acetone and solid phase columns LiChrolut RP-18 – were obtained from Merck (Darmstadt, Germany). Ammonium carbonate and formic acid from Riedel-de Haën (Seelze, Germany).

3.2. Materials

3.2.1. Drug

Free samples of peripheral autopsy blood were taken from normal subjects spiked with drugs of interest; hair taken from voluntaries was used for the development and validation of the method applied.

3.2.2. Postmortem autopsy specimens

Samples of peripheral vein blood and hair (6 cm) were collected at an autopsy which was performed at the Institute of Forensic Medicine Collegium Medicum Jagiellonian University in Kraków during the 24 h following death. The samples were kept frozen (−22 °C) until the analyses were performed.

3.3. Calibrators and controls

For the calibrator blood and hair samples, three methanol working solutions of OXCBZ, CBZ-10OH and CBZ-diOH and levomepromazine were prepared at the following concentrations: 1.00, 10.00 and 100.00 μg/ml; two methanol working solutions of CBZ-epoxy and fluphenazine at concentrations 1.00, 10.00 μg/ml and one of diazepam-d_5 at concentration 1.00 μg/ml. The QC working solutions were prepared separately by a different analyst. Calibrator working solutions were made from different source lots. All working solutions were stored at −20 °C when not in use. Daily calibration samples were prepared by fortifying 1.0 ml of “blank” blood with know amounts of OXCBZ, CBZ-OH, CBZ-diOH and levomepromazine at concentrations ranging from 0, 0.05, 0.10, 0.20, 0.50 and 1.00 μg/ml for low series; concentrations at 1.00, 2.00, 5.00, 10.00 and 20.00 μg/ml for high series and 50 mg “blank” hair with known amounts of OXCBZ, CBZ-10OH, CBZ-diOH at concentrations ranging from 0.50, 1.00, 2.00, 5.00, 10.00, 50.00 and 100.00 ng/mg. Low, medium and high QC blood specimens were also prepared daily at concentrations 0.10, 0.25 and 0.75 μg/ml for low series concentrations OXCBZ, CBZ-10OH, CBZ-diOH, levomepromazine and 2.00, 7.50 and 15.00 μg/ml for high series concentrations OXCBZ, CBZ-10OH, CBZ-diOH, levomepromazine. Low, medium and high QC hair specimens were prepared at concentrations 1.00, 25.00 and 75.00 ng/mg for OXCBZ, CBZ-10OH, CBZ-diOH.

For the internal standards (IS), a methanol working solution of 1.00 μg/ml (for low concentrations); 10.00 μg/ml (for high concentrations) of CBZ-epoxy, fluphenazine for

blood and 1.00 μg/ml of diazepam-d_5 for hair were prepared and stored at −20 °C when not use. Two hundred microliters of these working solutions were added to each blood sample prior to extraction, giving a final IS concentration of 0.20 μg/ml (for low) and 2.00 μg/ml (for high) for CBZ-epoxy and levomepromazine, respectively. Fifty microliters working solution of diazepam-d_5 was added to each hair sample prior to extraction, giving a final IS concentration of 1.00 ng/mg, respectively.

3.4. Sample preparation

3.4.1. Blood

Calibrators, and QC samples were prepared in separate propylene *ToxTubes* (50 ml) by the addition of 1.0 ml of blank blood, appropriate amount of calibrator or QC working solutions and IS while gently vortexing. Double samples 1.0 ml of forensic autopsy blood was pipetted into tubes and IS added while gently vortexing. Tubes were twisted on and allowed to equilibrating for 1 h at room temperature. To each tube were added: 1.0 ml of 0.1 M ammonium-acetate buffer (pH 5.0) and 60 μl ß-glucuronidase (30 U/ml) with arylsulfatase (60 U/ml), then mixed and the mixture was kept at 37 °C overnight for 16 h. Afterwards supernatant was neutralized with 1 M NaOH and 2 ml of 0.1 M ammonium-carbonate buffer (pH 9.3), the tube was twisted on and vortexed gently. Finally tubes were centrifuged for 10 min at 14,000 × *g* and supernatant was decanted into clean *Toxtubes*. Samples were extracted by means of solid phase extraction (SPE). The extraction columns were conditioned with sequential rinses of 1 ml double deionized water and 4 ml ammonium-carbonate buffer (pH 9.3). Between each rinse, the columns were aspirated but not allowed to dry. The 4 ml sample of spiked blood was then loaded onto the column and was pulled through by vacuum at rate of 1 ml/min. After the samples were loaded, the columns were washed with 2 ml ammonium-carbonate buffer and then dried under vacuum for 30 min. The analytes were diluted with 1 ml of 0.1% acetate acid in methanol in 10 ml silanized glass tubes. Eluates were dried under a stream of nitrogen at 37 °C. The residues were diluted with 100 μl of mixture of A and B mobile phases (1:1, v/v) and 10 μl were injected into LC/MS system.

3.4.2. Hair

Calibrators, QC and forensic autopsy hair samples were subjected a decontamination procedure by using *n*-hexane, and afterwards acetone in an ultrasonic bath for 30 s. Hair sample was divided into three segments of approximately 2 cm. The hair samples were pulverized in mill-ball (Retsch MM 200, Germany). Calibrators, and QC samples were prepared in separate silanized glass vials (10 ml) by the addition of 50 mg of blank hair, appropriate amount of calibrator or QC working solutions and IS. Double samples of 50 mg of forensic autopsy hair was put into tubes and IS was added, then to each vial 1 ml of 0.1 M chloride acid was added. The vials were capped and vortexed gently and the specimens incubated at 57 °C for 24 h, then neutralized with 1 M NaOH and treated with 2 ml buffer carbonate–ammoniate to pH 9.3. The vials were centrifuged for 10 min at 14,000 × *g* and supernatant was decanted into clean glass bottle. Sample were then pretreated by solid phase extraction (SPE). These extraction columns were conditioned with sequential rinses of the following: 1 ml of deionized water and 4 ml ammonium-carbonate buffer (pH 9.3). The 3 ml sample of spiked blood was then loaded onto the column and was pulled through by vacuum at rate of 1 ml/min. After the samples were loaded, the columns were washed 2 ml ammonium-carbonate buffer and then dried under vacuum for 30 min. The analytes were eluted 1 ml 0.1% acetic acid in methanol into 10 ml silanized glass tubes. Eluates were dried under a stream of nitrogen at 37 °C. The residues were diluted with 60 μl of mixture A and B mobile phases (1:1, v/v) and 20 μl were injected into LC/MS.

3.5. Chromatographic and detection system conditions

A Finnigan MAT (San Jose, CA, USA) liquid chromatograph equipped with a pump Model TSP 4000 and an autosampler Model TSP AS 3000 with a 20 μl injection loop were used in gradient mode. The chromatographic separation was performed with a LiChroCART column 125 mm × 3 mm i.d., 5 μm particle size, filled with Purospher RP 18 and a LiChroCART precolumn 4 mm × 4 mm i.d., particle size 5 μm filled with LiChrospher 60 RP – select B (Merck, Darmstadt, Germany). The mobile phase consisted of a gradient mixture of the phase [A] which was 0.1% formic acid in demineralised water and [B] which was 95% acetonitrile + 5% of the phase [A]. The flow rate was 0.4 ml/min. The gradient was programmed as follows: 95% [A] and 5% [B] for 2 min, followed by a linear change to 30% [A] and 70% [B] in 30 min; 30% [A] and 70% [B] was held for 2 min, then changed to 95% [A] and 5% [B] for 8 min.

A LCQ Finnigan MAT mass detector, ion trap equipped with an APCI source was used. The APCI inlet conditions were as follows: sheath gas (nitrogen) pressure 70 p.s.i., heated vaporizer temperature 400 °C, heated capillary temperature 150 °C, discharge current 5 μA. Mass spectra of the substances involved were taken between 50 and 650 *m/z* with positive ionization mode at 4.0 V capillary voltage. For quantitation following ions were taken – *m/z*: 271.0 + 208.3 for CBZ-diOH, 255.0 + 237.2 for CBZ-OH, 253.1 + 208.3 for OXCBZ and CBZ-epoxy (IS), 329.2 for levomepromazine, 438.2 for fluphenazine (IS) and 290.3 for diazepam-d_5 (IS).

3.6. Data analysis

Each specified ion was automatically selected, retention times were calculated, and peak abundances determined. All data were checked for interferences, peak shape and baseline determination. Calibration, using internal standardization, was done by liner regression analysis over a maximum concentration range from 0.05 to 20.00 μg/ml for

OXCBZ and its metabolites and levomepromazine (blood) and 0.50–100.00 ng/mg for OXCBZ and its metabolites (hair). For each standard curve, a minimum of five different concentrations was used. Data were fit to liner least-squares regression curves with a weighting factor of $1/x$.

3.7. Selectivity

To evaluate peak – purity and selectivity, "blank" blood and hair samples (no analyte or IS added) were analyzed with each batch to check for peaks that might interfere with detection of analytes or IS.

To assess possible interferences, control samples were spiked individually to contain 10.00 μg/ml four β-blokers (acebutolol, betaksolol, metoprolol, propranolol), carbazepine, benzodiazepines (alprazolam, bromazepam, clonazepam, estazolam, flunitrazepam, oxazepam, midazolam), phenothiazines (thioridazine, promazine, perazine, prometazine), opioids (narceine, noscapine, thebaine, tramadol, papaverine), TCA (amitriptyline, doxepine, imipramine, clomipramine, opipramol) and metoclopramide. The above drugs were chosen because their RT values were close to RT values of drugs of interest in the LC/MS system which was applied.

3.8. Linearity, carry-over and limits of quantitation and detection

Calibration curves were prepared daily by spiking "blank" blood and hair with corresponding analytical working solutions to obtain calibration concentrations at 0.05, 0.10, 0.20, 0.50 and 1.00 μg/ml OXCBZ, CBZ-10OH, CBZ-diOH and levomepromazine (for low series concentrations); 1.00, 2.00, 5.00, 10.00; 20.00 μg/ml OXCBZ, CBZ-10OH, CBZ-diOH and levomepromazine (for high series concentrations) and 0.50, 1.00, 2.00, 5.00, 10.00, 50.00 and 100.00 ng/mg for OXCBZ, CBZ-10OH and CBZ-diOH. Validation samples were prepared in triplicate at following concentrations: 0.01, 0.025, 2.50, 5.00 and 10.00 μg/ml of OXCBZ, CBZ-10OH, CBZ-diOH and levomepromazine (for blood) and 0.01, 0.025, 125.00, 150.00 and 200.00 ng/mg of OXCBZ, CBZ-10OH, CBZ-diOH (for hair) to assess the method's accuracy above and below the calibration curve. Negative quality control samples were analyzed after each linearity sample to evaluate potential carry-over.

The limits of detection (LOD) of the method was determined by analyzing validation samples ($n = 5$) to check whether acceptance criteria were met for each analyte. The LOD was defined as the lowest concentration at which the analyte ion signal-to-noise ratio (determined by peak area) was $\geq 3/1$, and chromatography (peak shape and resolution) and relative retention time (±2% of target RT) were acceptable. The LOQ was defined at the lowest concentration that meat LOD criteria and had analyte quantification within ±20% of target value.

3.9. Accuracy and precision

Inter- and intra-assay accuracy and precision data for OXCBZ and its metabolites and levomepromazine in blood samples and for OXCBZ and its metabolites in hair samples were determined with the low, medium and high QC. Intra-assay data were assessed by comparing data from within one run ($n = 10$) and inter-assay data were determined between five separate runs ($n = 34$). Accuracy, expressed as percentage, was calculated by taking the difference between mean calculated concentrations and target concentrations, dividing by the calculated mean and multiplying by 100. Precision, expressed as percent relative standard deviation (%R.S.D.), was determined by calculating the percent ratio of the standard deviation divided by the calculated mean concentration time 100.

3.10. Extraction efficiency

The recovery or extraction efficiency (%) for each analyte was determined at low, medium and high concentrations ($n = 5$). Relative recovery was assessed by adding IS working solution to one set of spiked blood and hair samples before extraction and to the second set after extraction but prior to evaporation. A third set of samples was prepared for the determination of absolute recovery. Analyte and IS working solutions were added to clean tubes followed by evaporation and analysis. The relative extraction efficiency was calculated by comparing the peak area ratios of analyte to internal standard for each compound in the first set with the appropriate peak area ratios in the second, and the absolute extraction efficiency was determined by comparing peak area ratios between the first and third set.

4. Results

4.1. Selectivity

Blank blood and hair were analyzed with each validation run ($n = 6$). All six samples were free of co-eluting peaks at the retention times of CBZ, CBZ-OH, CBZ-diOH, levomepromazine and their internal standards (CBZ-epoxy and diazepam-d_5).

Of the 27 interference compounds added to low validation sample none yielded analyte concentrations outside the ±20% limits of expected concentration.

4.2. Linearity, carry-over and limits of quantitation and detection

An overview of characteristic calibration data over a range from to 1.00 μg/ml (low series concentrations) and to 20 μg/ml (high series concentrations) for OXCBZ and its metabolites and levomepromazine in blood and up to 100.00 ng/mg for OXCBZ and its metabolites in hair are

Table 1
Characteristics of OXCBZ and its two metabolites CBZ-10OH and CBZ-diOH; and levomepromazine calbratrion curves[a] for blood (low and high series) and OXCBZ and its two metabolites CBZ-10OH and CBZ-diOH for hair

Analyte	Range (μg/ml)	Regression equation of calibrators[b]	Correlation coefficient (r)[b]	LOD[c] and LOQ[d] (μg/ml)
Blood-low series				
Oxcarbazepine	0.05–1.00	$y = 0.93(0.02)x + 0.02(0.01)$	0.994 (0.002)	0.05
CBZ-10OH	0.05–1.00	$y = 1.38\ (0.14)x + 0.01(0.01)$	0.999 (0.002)	0.05
CBZ-diOH	0.05–1.00	$y = 2.13(0.16)x + 0.01(0.01)$	0.991 (0.003)	0.05
Levomepromazine	0.05–1.00	$y = 0.97(0.04)x + 0.01(0.01)$	0.990 (0.003)	0.05
Blood-high series				
Oxcarbazepine	1.00–20.00	$y = 0.012(0.001)x + 0.042(0.002)$	0.994 (0.001)	0.05
CBZ-10OH	1.00–20.00	$y = 0.086(0.007)x + 0.007(0.005)$	0.999 (0.001)	0.05
CBZ-diOH	1.00–20.00	$y = 0.094(0.008)x + 0.005(0.006)$	0.993 (0.001)	0.05
Levomepromazine	1.00–20.00	$y = 0.056(0.002)x - 0.012(0.004)$	0.9975 (0.001)	0.05

Analyte	Range (ng/mg)	Regression equation of calibrators[b]	Correlation coefficient (r)[b]	LOD[c] and LOQ[d] (ng/mg)
Hair				
Oxcarbazepine	0.50–100.00	$y = 0.072(0.003)x + 0.050(0.006)$	0.996 (0.001)	0.50
CBZ-10OH	0.50–100.00	$y = 0.25(0.02)x + 0.45(0.04)$	0.999 (0.002)	0.50
CBZ-diOH	0.50–100.00	$y = 0.37(0.01)x + 0.62(0.05)$	0.997 (0.001)	0.50

[a] $N = 5$.
[b] Mean value and standard error.
[c] Limits of detection.
[d] Limits of quantitation.

presented in Table 1. A linear relationship between concentration and peak area was demonstrated. The calibration range tested in this method refers to the wide range of concentrations, occurring in the examined case.

Additional quality control samples ($n = 3$) were analyzed to evaluate to upper limit of linearity and potential carry-over. The 5.00 μg/ml of OXCBZ and its metabolites and levomepromazine in blood and 125.00 ng/mg CBZ and its metabolites in hair sample quantified within the acceptable criteria of ±20% of target concentration. Negative samples were analyzed between samples of increasing analyte concentration. No detectable carry-over occurred following the 5.00 μg/ml OXCBZ and its metabolites and levomepromazine in blood samples; however quantifiable amounts of OXCBZ, CBZ-OH, CBZ-diOH and levomepromazine (0.15, 0.11, 0.15 and 0.15 μg/ml, respectively) were measured in the negative sample following the 10.00 μg/ml of this analytes in blood sample. A similar procedure was adopted in the case of hair analysis. No detectable carry-over occurred following the 150.00 ng/mg OXCBZ and its metabolites for hair samples. Quantifiable amounts of OXCBZ, CBZ-OH, CBZ-diOH (0.68, 1.00, 1.24 ng/mg, respectively) were measured in the negative sample following the 200.00 ng/mg of this analytes for hair sample.

4.3. Accuracy and precision

Precision and accuracy of the method were evaluated at three concentrations over the linear dynamic range (low, medium and high); for blood samples the analyses were carried out in low and high concentration series. Data for both intra-assay ($n = 10$) and inter-assay ($n = 34$) are presented in Table 2. Intra-assay accuracy (percent difference between mean and target concentrations) and precision (%R.S.D.) ranged from −4.2 to 11.5 and 0.9 to 8.6%, respectively. Inter-assay accuracy and precision ranged from −4.3 to 2.3 and 1.7 to 7.5%, respectively.

4.4. Extraction efficiency

The extraction efficiencies of the method for the four analytes in blood and the three analytes in hair in quality control samples ($n = 5$) are presented in Table 3 as percent recovery. The method provided good relative and absolute recoveries of 62.0–94.4% and 39.0–77.1%, respectively, for all analytes across the linear dynamic range.

4.5. Application to authentic blood and hair samples

In the course of a comprehensive toxicological analysis based on the developed analytical procedure, oxcarbazepine and the metabolites were determined in postmortem blood and hair, while levomepromazine was determined only in blood of the deceased.

As a result of a toxicological screening of blood it was concluded that the only possible cause of death was a levomepromazine overdose, possibly in interaction with ethanol.

The analysis of 6 cm long hair revealed the presence of oxcarbazepine and their metabolites it was confirmed the information that the decedent was an epileptic patient and was treated with oxcarbazepine for at least 6 months before death.

The xenobiotics were identified in the analyzed biological extracts based on chromatographic and mass spectrometric parameters. The analytical documentation for the

Table 2
Accuracy and precision for the simultaneous determination of OXCBZ, CBZ-10OH, CBZ-diOH and levomepromazine (low and high series) in human blood and OXCBZ, CBZ-10OH, CBZ-diOH in hair by LC/APCI/MS

Analyte (μg/ml); (ng/mg)	Intra-assay ($N = 5$)			Inter-assay ($N = 21$)		
	Mean (μg/ml); (ng/mg)	Accuracy[a] (%)	Precision[b] (%)	Mean (μg/ml); (ng/mg)	Accuracy[a] (%)	Precision[b] (%)
Blood-low series						
OXCBZ						
0.10	0.11	5.9	5.2	0.10	3.7	5.6
0.25	0.26	5.1	5.8	0.24	−4.3	4.6
0.75	0.74	−1.3	1.3	0.75	0.6	2.7
CBZ-10OH						
0.10	0.11	8.2	6.2	0.10	5.3	7.3
0.25	0.27	7.1	6.1	0.25	0.5	5.2
0.75	0.76	1.7	3.0	0.75	0.6	2.7
CBZ-diOH						
0.10	0.11	9.9	6.3	0.11	10.7	7.5
0.25	0.27	6.1	5.7	0.26	4.4	6.2
0.75	0.77	2.2	3.8	0.76	0.8	2.8
Levomepromazine						
0.10	0.11	11.5	5.5	0.11	12.3	4.9
0.25	0.26	3.2	4.1	0.27	6.1	6.1
0.75	0.78	4.2	1.9	0.77	3.0	2.1
Blood-high series						
OXCBZ						
2.00	2.15	7.0	5.2	2.16	7.4	2.9
7.50	7.65	2.0	2.1	7.68	1.9	3.4
15.00	14.98	−0.2	1.4	14.88	−0.8	2.9
CBZ-10OH						
2.00	2.12	4.8	4.5	2.11	5.0	2.2
7.50	7.61	1.3	3.9	7.71	2.7	2.0
15.00	15.12	3.2	0.9	15.21	1.3	2.5
CBZ-diOH						
2.00	2.11	4.5	3.8	2.15	7.0	2.0
7.50	7.35	−1.8	1.9	7.42	−1.1	1.9
15.00	14.92	−0.5	1.1	14.82	−1.2	2.4
Levomepromazine						
2.00	2.12	5.5	4.2	2.11	5.2	3.0
7.50	7.30	−2.7	2.1	7.45	−0.6	2.9
15.00	14.91	−0.7	1.7	14.81	−1.4	2.8
Hair						
OXCBZ						
1.00	1.08	7.7	7.9	1.06	5.7	5.2
25.0	26.62	6.1	2.8	26.74	6.5	2.2
75.00	78.28	4.4	2.3	79.00	5.1	2.1
CBZ-10OH						
1.00	0.97	−3.1	8.6	0.96	−3.8	7.2
25.00	27.10	7.7	5.1	27.22	8.1	1.7
75.00	79.38	5.5	2.8	78.44	4.4	1.8
CBZ-10OH						
1.00	0.96	−4.2	7.3	0.96	−4.3	7.4
25.00	26.51	5.7	5.1	27.00	7.4	2.1
75.00	79.21	5.3	2.2	79.12	5.2	2.4

Units (μg/ml) for blood and (ng/mg) for hair.
[a] Percent difference between mean and target concentration.
[b] Percent relative standard deviation.

Table 3
Percent extraction efficiencies of OXCBZ and its two metabolites CBZ-10OH and CBZ-diOH and levomepromazine from human blood samples ($n = 5$) and OXCBZ and its two metabolites CBZ-10OH and CBZ-diOH from hair samples ($n = 5$) of research subjects

Analytes (μg/ml); (ng/mg)	Relative recovery[a] ± S.D. (%)	Absolute recovery[b] ± S.D. (%)
Blood–low series		
OXCBZ		
0.10	79.3 ± 4.3	58.4 ± 3.7
0.25	73.3 ± 3.5	55.6 ± 4.3
0.75	75.1 ± 4.7	53.4 ± 5.5
CBZ-10OH		
0.10	85.0 ± 6.1	73.0 ± 5.1
0.25	88.6 ± 4.4	77.1 ± 3.7
0.75	87.3 ± 2.9	73.1 ± 4.3
CBZ-diOH		
0.10	91.2 ± 3.4	69.7 ± 4.3
0.25	90.1 ± 4.4	70.0 ± 3.5
0.75	93.7 ± 2.9	68.3 ± 4.7
Levomepromazine		
0.10	65.0 ± 3.7	41.9 ± 3.9
0.25	67.4 ± 4.1	40.0 ± 4.1
0.75	59.1 ± 5.0	43.7 ± 4.3
Blood–high series		
OXCBZ		
2.00	75.3 ± 3.3	55.4 ± 3.9
7.50	77.3 ± 4.4	61.0 ± 4.7
15.00	78.1 ± 3.7	55.4 ± 3.9
CBZ-10OH		
2.00	88.7 ± 4.3	69.5 ± 6.9
7.50	85.0 ± 3.7	71.3 ± 2.8
15.00	81.3 ± 4.7	69.9 ± 4.1
CBZ-diOH		
2.00	92.4 ± 2.6	70.1 ± 3.7
7.50	90.9 ± 3.5	71.3 ± 2.8
15.00	94.4 ± 4.4	70.0 ± 3.8
Levomepromazine		
2.00	62.0 ± 2.7	39.5 ± 2.5
7.50	69.1 ± 4.2	40.0 ± 3.8
15.00	62.8 ± 3.1	47.0 ± 2.9
Hair		
OXCBZ		
2.00	75.4 ± 4.3	59.4 ± 4.1
7.50	79.0 ± 3.4	59.5 ± 5.3
15.00	72.1 ± 3.7	59.4 ± 5.1
CBZ-10OH		
2.00	83.7 ± 2.7	70.5 ± 5.3
7.50	87.0 ± 4.1	72.1 ± 3.0
15.00	82.1 ± 4.8	68.4 ± 3.7
CBZ-diOH		
2.00	93.7 ± 3.7	69.1 ± 2.7
7.50	93.4 ± 4.2	75.3 ± 2.9
15.00	91.3 ± 3.1	69.0 ± 4.1

Units (μg/ml) for blood and (ng/mg) for hair.
[a] Relative recovery was assessed by comparing results when IS was added to samples before and after SPE.
[b] Absolute recovery was assessed by comparing results from extracted samples to results from methanolic samples (non-extracted).

Table 4
Toxicological determination of oxcarbazepine and metabolites in blood and hair of the deceased M.G.

MATERIAL	Hair			Blood
	Segment I	Segment II	Segment III	
Xenobiotics	Concentration			
	(ng/mg)			(μg/ml)
OXCBZ	3.9	10.4	13.0	0.79
CBZ-10OH	18.4	53.9	105.9	13.96
CBZ-diOH	0.5	1.2	3.0	0.23
Levomepromazine	n.d.	n.d.	n.d.	1.96

n.d.: Not detected.

selected hair segment (No. 3) of the victim for OXCBZ and the metabolites CBZ-10OH and CBZ-diOH is presented in Fig. 1.

Table 4 illustrates the results of quantitative analysis of blood and hair of the victim achieved employing the above method.

5. Discussion

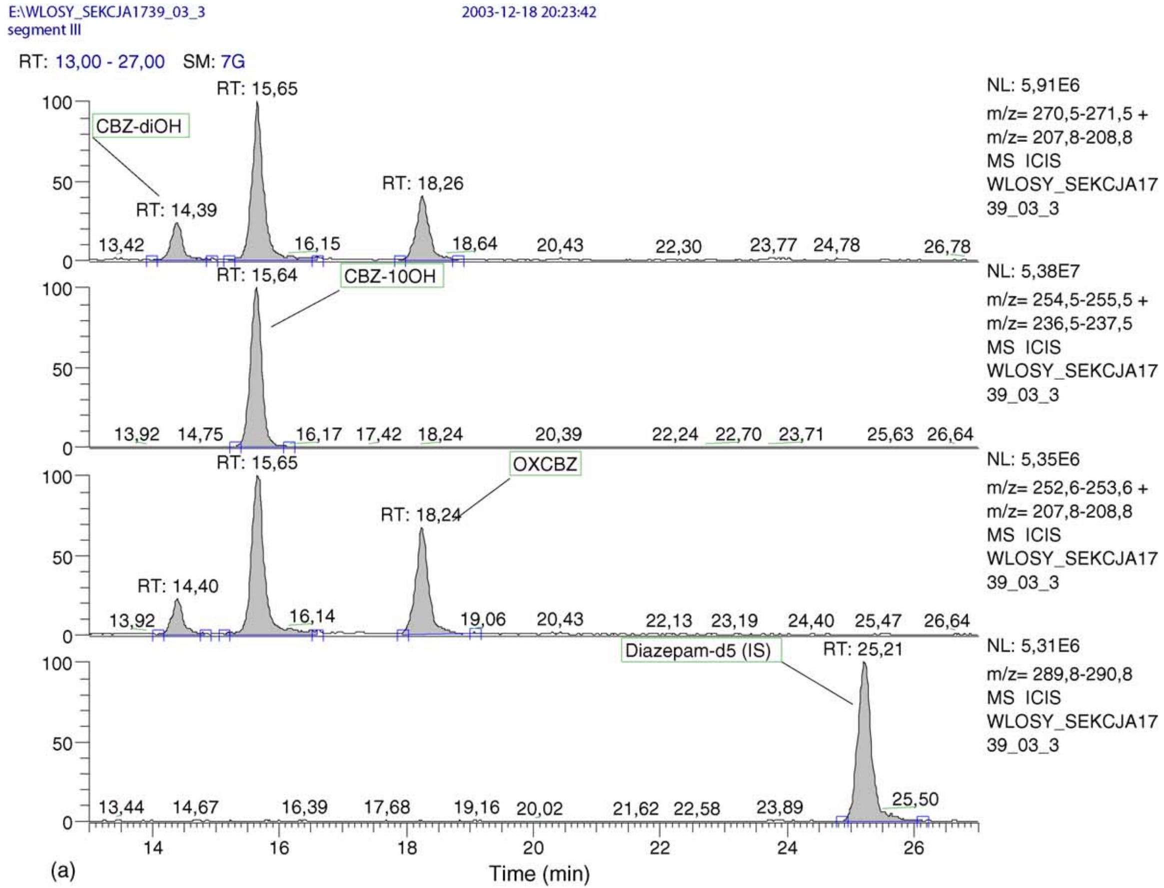
Detection and identification of oxcarbazepine and its metabolite have been developed and documented by toxicological analyses of blood and urine originating from epileptic patients [1–9]. As it follows from the available sources, chromatographic methods have been employed, but, to the best of our knowledge, never using mass spectrometry.

Moreover, the analysis of hair samples has become more feasible thanks to the high sensitivity of the most modern techniques as liquid chromatography with mass detection [13–16], used in the presented case (HPLC/APCI/MS).

The developed analytical method, which includes a broad spectrum of validation procedures for OXCBZ, CBZ-10OH and CBZ-diOH presented in this report, was employed for the investigation of postmortem blood and hair samples of the victim. The procedure may be also successfully employed for hair examinations in monitoring pharmacotherapy of epileptic patients receiving this drug.

In the interpretation of the results of the chemical-toxicological expertise, the decisive opinion on the cause of death was based on the high level of levomepromazine in the postmortem material collected from the deceased, which indicated a case of fatal poisoning. In documented fatal poisonings with levomepromazine, the blood of victims showed the precursor concentration values as ranging between 0.8 and 8 μg/g [17–19]. The case study included additionally distribution and redistribution of levomepromazine and its metabolites in a fatal poisoning was a subject of our previous examination [19].

The subsequent analysis of blood demonstrated the presence of oxcarbazepine and its metabolites. The results unambiguously indicated that M.G. had been on oxcarbazepine therapy, what was confirmed by his medical history. The con-



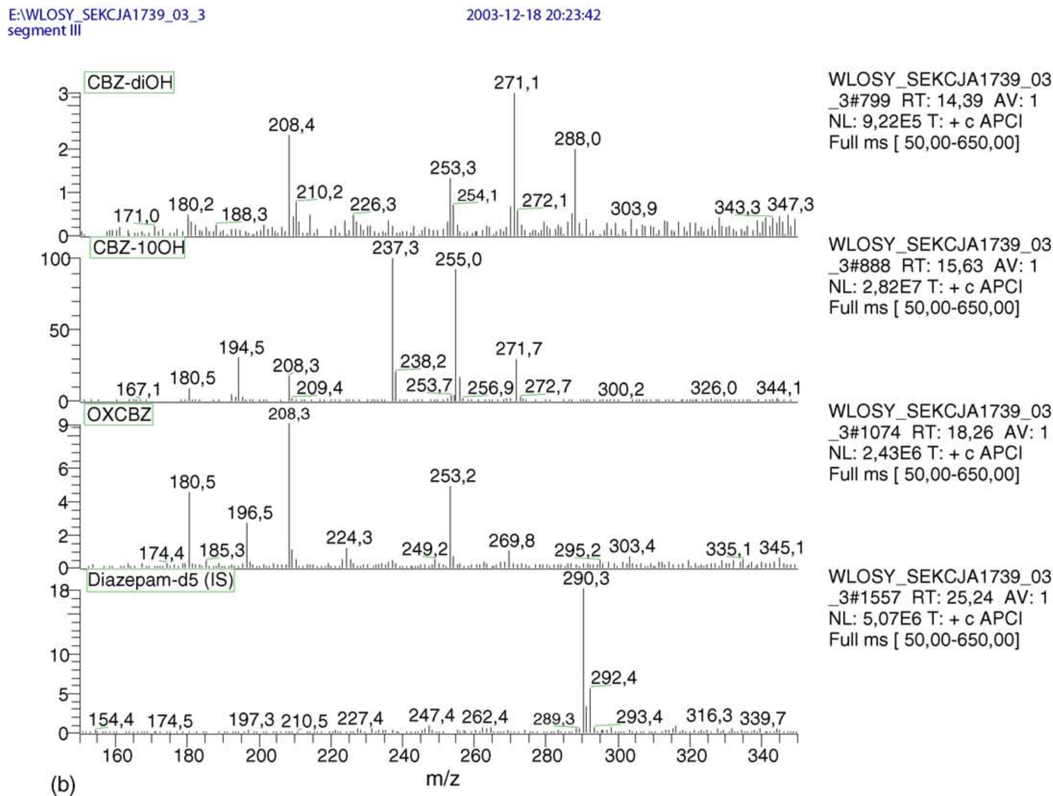


Fig. 1. Chromatograms (a) and mass spectra (b) of OXCBZ, CBZ-10OH, CBZ-diOH and diazepam-d_5 of hair sample (III segment) of the victim.

centration values of OXCBZ and its metabolites in blood obtained in the course of our investigations approximated the levels of these xenobiotics reported by some authors in clinical material originating from patients with epilepsy [2,4].

The analysis of the deceased's hair demonstrated the presence of OXCBZ and its two metabolites, CBZ-10OH and CBZ-diOH, thus confirming the data from his medical history stating that M.G. had been taking the drug for epilepsy at least over a period of 6 months determined by the hair length. The results expressed as the relative metabolite/precursor concentration ratios in blood and hair suggest a difference in the process of incorporation of the precursor and metabolites into hair, what is most likely affected by the physico-chemical properties of the molecules, and especially their polarity. This issue was stressed by Pragst et al. [20] in his report on tricyclic antidepressants; in his opinion, the process of incorporation of metabolites into hair structure was less effective when compared to the precursors.

While analyzing the high relative concentration ratios of CBZ-10OH/OXCBZ one may draw conclusions about process of OXCBZ metabolism in the human body. No data on the levels of this drug and its metabolites in postmortem materials have been published to date. In addition, no fatal poisonings with oxcarbazepine have been documented.

In our extensive toxicological practice we have also not observed fatal poisonings with OXCBZ, in contrast to numerous cases of fatal poisonings with carbamazepine (CBZ), which was a subject of our previous investigations [21–23].

Oxcarbazepine is a new generation anti-seizure agent, which is relatively new on the market (it was introduced during the nineties) [2]; one may expect that the interest in this therapeutic substance will increase. It can be concluded that the proposed method is suitable for reliable therapeutic drug monitoring in hair and will most likely find its use in toxicological analysis of postmortem and clinical material.

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