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Determination of oxcarbazepine and its related substances using UHPLC method with UV detection

Jelena Žirojević, Katica Drljevic-Đurić, Aleksandra Đurđević*

[1] Medicines and Medical Devices Agency of Serbia, Vojvode Stepe 458, Belgrade, Serbia

*Author for correspondence, E-mail: aleksandra.djurdjevic@alims.gov.rs

Summary

A simple and rapid ultra - high performance liquid chromatographic (UHPLC) method for the separation and determination of oxcarbazepine and its related substances in tablets, was developed. Chromatographic separation of oxcarbazepine from its related substances (degradation and by-products) was achieved on a reversed phase C_{18} column (Pinnacle DB C18, RESTEK, 100 x 2.1 mm, 1.9 µm particle size) using gradient program with water (as mobile phase A) and acetonitrile (as mobile phase B), at a flow rate 0.5 mL min^{-1} and UV detection at 254 nm. The column temperature was 30°C.The method has good selectivity towards oxcarbazepine and its related substances. The accuracy of the method for oxcarbazepine assay, expressed as mean recovery was 101.8 %, and of the method for quantification of degradation products was 95.9 % – 103.3 %. Limit of quantification for oxcarbazepine and its degradation products ranged from 0.60 µg mL^{-1} to 1.30 µg mL^{-1}. The linearity range for oxcarbazepine assay was from 2.4 to 3.60 mg mL^{-1} (R^2=0.999). Mean value for oxcarbazepine assay from the tablets was 299.92 mg (label claim 300 mg). The method can be used for routine analysis and the quality control of oxcarbazepine drug substance and its formulated products.

Key words: oxcarbazepine, related substances, UHPLC method, gradient elution

Introduction

10,11-dihydro-10-oxo-5H-dibenz[b,f]azepine-5-carboxamide (Oxcarbazepine) is an anticonvulsant and mood stabilizing drug used in the treatment of epilepsy and reduction in anxiety symptoms accompanying the epilepsy seizures. Oxcarbazepine is structurally a derivative of carbamazepine which is also an effective anticonvulsant [1–3].Oxcarbazepine is not official in any Pharmacopoeia. Considering the biological significance of oxcarbazepine, several quantitative analytical procedures have been reported for its determination in various matrices [4]. In pharmaceutical formulations such methods include spectroscopic [5–9], HPTLC (*High Performance Thin Layer Chromatography*) [10, 11], HPLC (*High Performance Liquid Chromatography*) [12, 13], GC (*Gas Chromatography*) [14], and voltammetry [15]. The process synthesis of oxcarbazepine involves many steps so that many by-products could be carried over to the final product. Also the storage condition and temperature variations may lead to the formation of degradation products in formulations. The fast and reliable method is required for analysis of related substances in oxcarbazepine formulations. The focus of the present study was to develop and accurate, selective and reproducible method for the determination of oxcarbazepine in the presence of low levels of its degradation and by-products. Examined by products were: CGP 52118, CGP 83596, CGP 16554 and CGP 84426 and degradation products were: CGP26202, GP 47705, CGP18671, G 32883, GP477 3 and GP 51045.

The developed method was UHPLC with gradient elution. The mobile phase was composed of water and acetonitrile without addition of any additives.

1. Experimental

2.1 Materials and reagents

All reagents were of analytical reagent grade. Gradient grade acetonitrile was obtained from Merck AG, Darmstadt, Germany. Deionized water (EasyPure) was used throughout the study. Diluent was mixture of acetonitrile and water (80:20 V/V). Standard substances of oxcarbazepine and its related substances as well as Trileptal® film tablets, 300 mg label claim were supplied by Novartis Pharma Stein AG, Switzerland.

2.2 Apparatus

Experiments were done on the Nexera X2 UPLC modular system composed LC-30 AD pump, an SPD-M 20A diode array detector an SIL-30AC auto-injector, a DGU 20 A5 degasser and CBM 20A system controller (all from Shimadzu, Kyoto, Japan). A reversed phase C_{18} (Pinnacle DB C18, 100 x 2.1 mm, 1.9 μm particle size, RESTEK)

column was used for separation. Maximum applied pressure was 1000 bar. Chromatographic data were analysed with Shimadzu Lab Solutions software.

2.3 Chromatographic conditions

The mobile phase was water (mobile phase A) and acetonitrile (mobile phase B). Before delivering into the system it was filtered through 0.45 μm PTFE filter and degassed using vacuum. The analysis was carried out under gradient conditions using a flow rate of 0.5 mL min^{-1} at 30^0C temperature. Gradient conditions are given in Table I.

Table I Gradient conditions
Tabela I Uslovi gradijenta

Step	Time [min]	A [%]	B [%]
1	0	92	8
2	6	92	8
3	11	40	60
4	11.1	92	8
5	13	92	8

Chromatograms were recorded at 254 nm for both oxcarbazepine and related substances.

2.4 Analytical procedure

For testing suitability of chromatographic system test solution (*System Suitability Test* – SST) consisting of oxcarbazepine and 10 related substances (Fig. 1) was prepared. Concentration of each compound in this solution was approximately 3 μg mL^{-1} which corresponds to 0.1 % of working concentration for assay. SST solution was used to determine resolution factor.

For assay of oxcarbazepine in Trileptal® film tablets standard solution was prepared by weighing and dissolving standard substance oxcarbazepine to yield concentration approximately 3 mg mL^{-1}.

For quantification of related substances mix solution consisting of oxcarbazepine and its degradation products was prepared. Concentration of oxcarbazepine, GP 47703, GP 47705 and GP 51045 were approximately 3 μg mL^{-1}which corresponds to 0.1 % of working concentration for assay. Concentration of CGP 26202 and CGP 18671 were approximately 6 μg mL^{-1}which corresponds to 0.2 % of working concentration for

assay. Concentration of G 32883 was approximately 9 μg mL^{-1}which corresponds to 0.3 % of working concentration for assay.

In order to simulate situation in real sample and to prove selectivity of UHPLC method solution of a synthetic mixture of oxcarbazepine and its 10 related substances was made in which oxcarbazepine concentration was 3 mg $mL^{-1}$and all related substances were at the level 0.1 % (approximately 3 μg mL^{-1}).

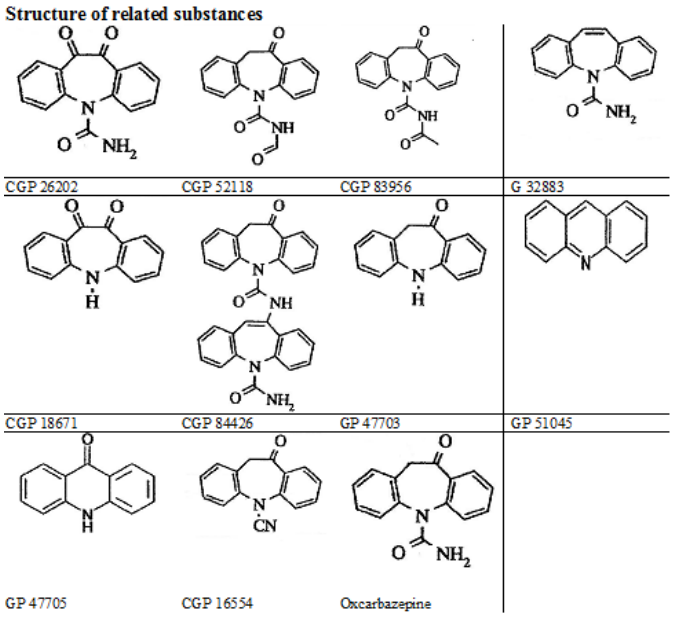

Figure 1. Molecular structures ofoxcarbazepine and its related substances (degradation and synthesis by-products substances)

Slika 1. Molekulske strukture okskarbamazepina i njegovih srodnih supstanci (degradacioni proizvodi i sporedni proizvodi iz procesa sinteze)

2.5 Sample preparation

20 tablets were accurately weighed and finally powdered. An accurately weighed portion of the powder equivalent to 300 mg of oxcarbazepine was transferred to a 100 mL volumetric flask. A 70 mL of diluent was added and sonicated for 15 min., than thoroughly shaken for 15 minutes and brought to volume with diluent and filtered.

2. Results and discussion

2.1 Method development

During the method development top priority was given to complete separation of oxcarbazepine from its related substances. We required resolution ≥ 1.4 between each pair of peaks. Also, repeatability of peak area should give relative standard deviation ≤ 3 % for each peak in solution for quantification of degradation products (n = 6) and ≤ 1 % for oxcarbazepine in solution for assay.

Molecular structures of oxcarbazepine and its related substances studied in this work are presented in Figure 1. All these materials were subjected to separation by reversed phase UHPLC. We found that separation and resolution were pH independent, so the pH of the mobile phase was not adjusted. Acetonitrile was used as solvent modifier to improve separation. Since we could not met the required quality chromatographic separation using isocratic elution we optimized the gradient elution with water as phase A and acetonitrile as phase B. Several gradient conditions were tried, applying *One Factor at Time Approach*, in which gradient slope was varied, bearing in mind that total time of analysis should be less than 15 min. The temperature of column was varied between 25^0C and 40^0C with 30^0C giving the best resolution. The flow rate was subjected to optimization with the aim to shorten the total time of analysis without adversely affecting the resolution. Column characteristics and high applied pressure allowed good flow rate at 0.5 ml/min. At this flow rate the retention of oxcarbazepine and related substances is satisfactory so that subsequent analysis was made with flow rate of the mobile phase 0.5 mL min^{-1}. Injected volume of the analyte was varied from 0.1 µL to 1 µL and obtained peak areas and relative standard deviations were satisfactory with 1 µL of injected volume. A typical chromatogram of a SST solution is given in Figure 2.

The peaks were identified according to their retention times by comparison with co-injected individual standards. Reproducible peak shapes were obtained under the optimum conditions. Therefore the C_{18} column choice was good. The retention time (t_R), the relative retention time (RRT), relative response factors and resolutions were determined and recorded in Table II.

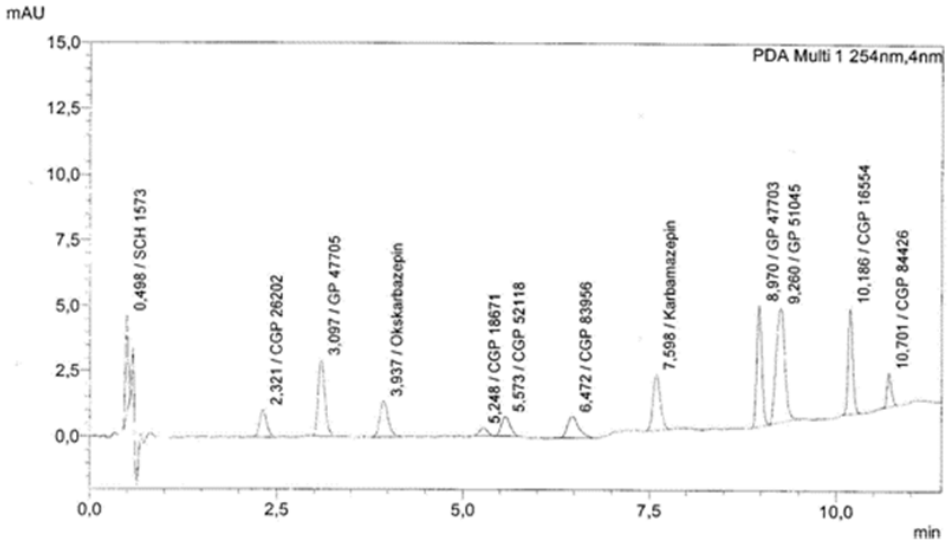

Figure 2. Chromatogram of SST solultion under gradient conditions with water as mobile phase A and acetonitrile as mobile phase B at 30^{0}C and UV detection at 254 nm

Slika 2. Hromatogram rastvora za proveru pogodnosti sistema u uslovima gradijentnog eluiranja sa dodom kao mobilnom fazom A i acetonitrilom kao mobilnom fazom B na 30^{0}C i detekcijom na 254 nm

Table II Chromatographic parameters of oxcarbazepine and its related substances

Tabela II Hromatografski parametri okskarbamazepina i njegovih srodnih supstanci

Compound	Type*	Rt (min)	RRT	RRF	Rs
CGP 26202	DP	2.32	0.59	1.22	-
GP 47705	DP	3.10	0.79	0.09	4.2
Oxcarbazepine	DS	3.94	1	1	4.2
CGP 18671	DP	5.25	1.33	0.86	6.1
CGP 52118	BP	5.57	1.41	-	1.5
CGP 83956	BP	6.47	1.64	-	3.5
G 32883	DP	7.60	1.93	1.1	4.7
GP 47703	DP	8.97	2.28	0.4	7.9
GP 51045	DP	9.26	2.35	0.1	1.5
CGP 16554	BP	10.19	2.59	-	5.2
CGP 84426	BP	10.70	2.72	-	3.8

*DP – Degradation product, BP – By-product, DS – Drug substance

2.2 Accuracy for degradation products

Standard mixture containing known amounts of degradation products were analysed by UHPLC under optimal conditions. The accuracy of the method was checked for 5 different concentration levels spinning the range 20 % – 120 % from specification limits. Defined quantity of each of the degradation product was added to the placebo and chromatographed. Mean recoveries are given in the Table III.

Table III Accuracy of the method expressed as mean *Recovery*
Tabela III Tačnost metode izražena kao srednja *Recovery* vrednost

Compound	20 %	50 %	70 %	100 %	120 %	Mean (%)
CGP 26202	96.3	104.9	96.6	98.0	101.9	99.5
GP 47705	95.9	97.8	95.5	97.9	98.3	97.1
Oxcarbazepine	103.0	104.2	100.6	104.8	103.7	103.3
CGP 18671	95.0	101.9	102.5	98.2	98.3	99.1
G 32883	96.1	104.5	95.7	103.8	99.4	99.9
GP 47703	97.9	95.8	95.1	95.3	95.2	95.9
GP 51045	98.8	98.6	98.3	98.0	98.0	98.3

2.3 Accuracy for oxcarbazepine assay

The accuracy of the method for quantification of drug substance was determined by applying the method to placebo to which known amount of oxcarbazepine corresponding to 80 %, 100 % and 120 % of label claim had been added. At each level five determinations were performed. Mean *Recoveries* at each level are 102.0 %, 101.5 % and 102.0 %, respectively and 101.8 % mean value.

2.4 Selectivity

To demonstrate the selectivity of the method the placebo was prepared and spiked with oxcarbazepine and each of the related substances and it was found that the excipients don't interfere either with oxcarbazepine or any of related substances. This indicated that method is selective for separation and determination of oxcarbazepine and its degradation products in film tablets (Figure 3).

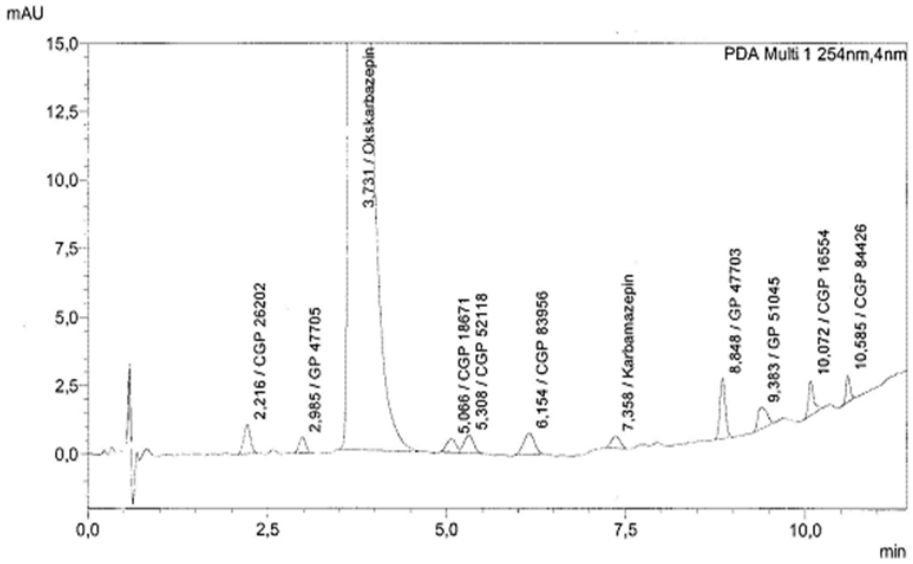

Figure 3. Chromatogram of placebo solution spiked with oxcarbazepine and its related substances

Slika 3. Hromatogram placebo rastvora sa dodatkom okskarbamazepina i njegovih nečistoća

2.5 Linearity

Calibration graphs (concentration vs. peak area) were constructed at 5 concentration levels for oxcarbazepine and its degradation products. At each concentration level 3 determinations were made. Table IV. gives concentration range, linearity equation, correlation coefficient (R^2), precision expressed as RSD (*Relative Standard Deviation*) and LOQ for all compounds.

Table IV Linear regression data for determination of oxcarbazepine and its related substances

Tabela IV Podaci linearne regresije za metodu za određivanje sadržaja okskarbamazepina i njegovih srodnih supstanci

Parameter / Compound	Concentration range (μg mL^{-1})	Regression equation	R^2	RSD (%)	LOQ (μg mL^{-1})
Related substances					
CGP 26202	1.28 – 7.68	1E+06X – 139.72	0.998	1.24	1.28
GP 47705	0.65 – 3.90	2E+07X – 59.61	1.000	1.62	0.65
Oxcarbazepine	0.64 – 3.82	1E+06X + 196.56	0.995	1.40	0.64
CGP 18671	1.22 – 7.32	2E+06X + 247.38	0.995	2.95	1.22
G 32883	1.85 – 11.1	2E+06X – 187.73	0.998	1.87	1.85
GP 47703	0.60 – 3.6	4E+06X – 83.83	1.000	0.32	0.60
GP 51045	0.58 – 3.48	2E+07X – 655.66	0.999	0.17	0.58
Oxcarbazepine assay (mg mL^{-1})					
Oxcarbazepine	2.40 – 3.60	2E+06X + 31695	1.000	0.18	-

2.6 Method application

The validated UHPLC method was applied for determination of oxcarbazepine and its degradation products in Trileptal® film tablets, 300 mg (Novartis, Pharmastein AG, Stein, Swiss). Specification limits for assay of oxcarbazepine were 285.0 – 315.0 mg/tablet. Shelf-life specification included determination of CGP 26202 (≤ 0.2 %), CGP 18671 (≤ 0.2 %), G 32883 (≤ 0.5 %), other individual (≤ 0.1 %), sum of other degradation products (≤ 0.5 %) and total of degradation products (≤ 1.0 %). Samples are prepared in triplicate and analysed. Typical chromatogram is presented in Figure 4. Mean value, expressed as the percentage of the label claim, was 99.76 % indicating that the amount of oxcarbazepine in the tablets met the requirements (95 % – 105 % of the

label claim). Amount of degradation produced in sample solution could be calculated either using peak areas of corresponding peaks in quantification solution or using RRT and RRF values given in Table II. In analysed sample of Trileptal® film tablets amount of all related substances were bellow specification limits.

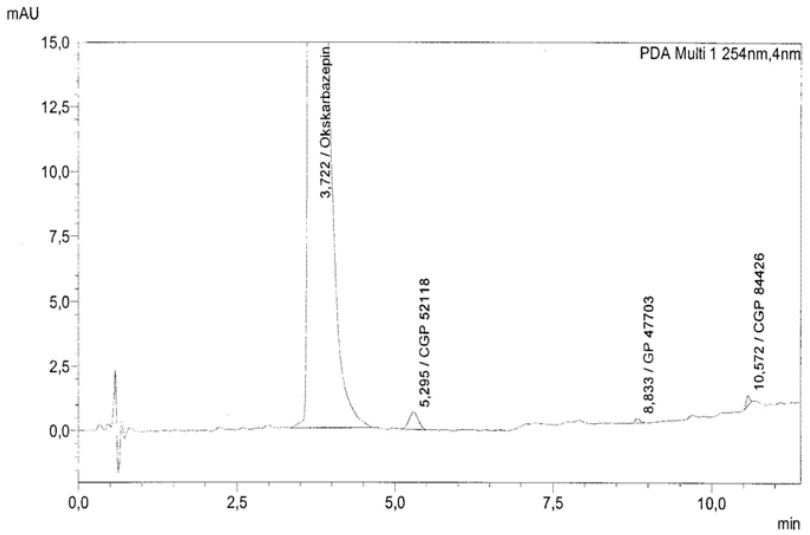

Figure 4. Chromatogram of Trileptal® film tablets obtained under optimal conditions

Slika 4. Hromatogram Trileptal® film tableta dobijen pod optimalnim hromatografskim uslovima

3. Conclusion

The described gradient reversed phase UHPLC method for the determination of oxcarbazepine and its degradation products in dosage form has been evaluated for accuracy, precision, selectivity, linearity and LOQ. The developed method has an advantage over existing literature methods with respect to the total time of analysis and consumption of organic solvents. It is suitable not only for separation and determination of degradation products but also for monitoring of synthesis by-products and it has applicability in routine analysis of oxcarbazepine.

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Određivanje okskarbamazepina i srodnih supstanci orimenom UHPLC metode sa UV detekcijom

Jelena Žirojević, Katica Drljevic-Đurić, Aleksandra Đurđević*

Agencija za lekove i medicinska sredstva Srbije, Vojvode Stepe 458, Beograd, Srbija

*Autor za prepisku, E-mail: aleksandra.djurdjevic@alims.gov.rs

Kratak sadržaj

Razvijena je jednostavna i brza hromatografska metoda visokih performansi (UHPLC) za razdvajanje i određivanje okskarbamazepina i nečistoća u tabletama. Hromatografsko razdvajanje okskarbamazepina od srodnih supstanci (degradacionih proizvoda i sporednih proizvoda sinteze) izvršeno je na reverzno-faznoj C18 koloni (Pinnacle DBC 18, RESTEK, 100 mm x 2,1 mm, 1,9 µm veličine čestica) pri gradijentnom programu eluiranja sa vodom kao mobilnom fazom A i acetonitrilom kao mobilnom fazom B, pri brzini protoka mobilne faze 0,5 mL $min.^{-1}$ i pri UV detekciji na 254 nm. Temperatura kolone bila je 30°C. Limit kvantifikacije za okskarbamezepin i degradacione proizvode bio je u oblasti od 0,60 mL^{-1} do 1,30 µg mL^{-1}.Tačnost metode za određivanje sadržaja okskarbamazepina, izražena kao srednja *Recovery* vrednost bila je 101,8 % a za kvantifikaciju degradacionih proizvoda 95,9 % – 103,3 %. Opseg linearnosti za određivanje karbamazepina je 2,40 mg mL^{-1} do 3,60 mg mL^{-1} (R^2=0,999). Srednja vrednost određivanja sadržaja okskarbamazepina u tabletama iznosila je 299,92 mg (nominalno, 300 mg). Predložena metoda može se koristiti u rutinskoj kontroli kvaliteta aktivne supstance iz doziranih oblika koji sadrže okskarbamazepin.

Ključne reči: okskarbamazepin, degradacioni proizvodi, UHPLC metoda, gradijentno eluiranje