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Short communication

Automated microanalysis of oxcarbazepine and its monohydroxy and transdiol metabolites in plasma by liquid chromatography

M.C. Rouan*, M. Decherf, V. Le Clanche, J.B. Lecaillon, J. Godbillon

Laboratoires Ciba-Geigy, Biopharmaceutical Research Center, 2 & 4, rue Lionel Terray, BP 308, 92506 Rueil-Malmaison Cédex, France

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Abstract

An automated high-performance liquid chromatographic method for the simultaneous determination of oxcarbazepine and its monohydroxy and transdiol metabolites in plasma is described. 5,6-Dihydro-11-oxo-11H-dibenz[b,e]azepine-5-carboxamide was used as internal standard. Liquid–solid extraction from plasma (100 μl) on 50 mg Bond-Elut C_{18} cartridges was automatically performed by the Automatic Sample Preparation with Extraction Columns (ASPEC) system. A reversed-phase column (ODS Hypersil, 3 μm particle size, 4 cm × 4.6 mm I.D.) was used with acetonitrile–methanol–0.01 *M* potassium dihydrogenphosphate as mobile phase. The eluted compounds were detected at 210 nm. The limit of quantitation was 0.2 μmol/l for oxcarbazepine and 0.1 μmol/l for its metabolites. No interference with concomitantly administered anti-epileptic drugs such as phenobarbital, phenytoin, valproic acid or carbamazepine, was found.

1. Introduction

Oxcarbazepine (10,11-dihydro-10-oxo-5H-dibenz[b,f]azepine-5-carboxamide; Fig. 1) (OXC) is a keto-analogue of the well established anti-epileptic drug carbamazepine. In contrast to the oxidative metabolism of CBZ, OXC is rapidly reduced to a monohydroxy derivative, 10,11-dihydro-10-hydroxycarbamazepine (MHD; Fig. 1), in man [1]. This active metabolite predominates in plasma after oral dosing whereas OXC reaches only low levels. MHD is thus the main compound responsible for the anti-epileptic activity of OXC in man. A large part of MHD is eliminated in the urine as glucuronides [2]. A minor amount is oxidized to an inactive dihydroxy derivative, 10,11-dihydro-10,11-trans-dihydroxycarbamazepine (DHD; Fig. 1).

A number of analytical methods involving gas chromatography [3] or high-performance liquid chromatography (HPLC) [4–9] have been described for the simultaneous determination of OXC and its two main metabolites in plasma or serum. These methods require a plasma volume (0.5–1 ml) not adequate for investigations in pediatric patients. They involve time-consuming liquid–liquid extraction and evaporation of the organic solvent before analysis. Recently, Hartley *et al.* [10] have published a HPLC method requiring only 100 μl of plasma and using a rapid

* Corresponding author.

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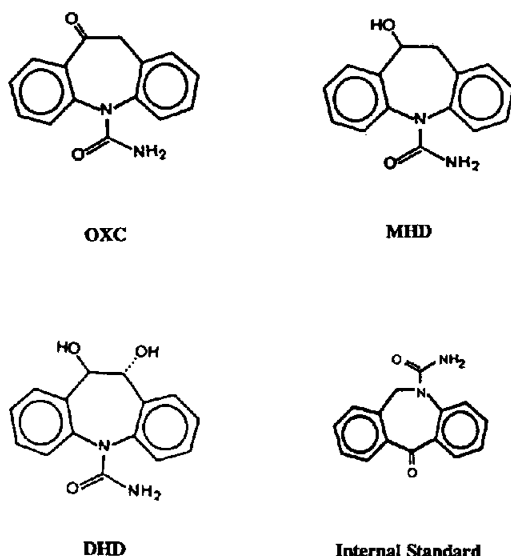

Fig. 1. Chemical structure of oxcarbazepine, its metabolites and the internal standard.

liquid–solid extraction. The limit of detection of the method was estimated to be 50–100 ng/ml for OXC and the metabolites. The present method describes a HPLC procedure for the assay of OXC and its metabolites in plasma. The method involves liquid–solid extraction from 100 μl plasma. It is more sensitive than the method previously described by Hartley *et al.* [10], and it has the advantage to be fully automatable by the Automatic Sample Preparation with Extraction Columns (ASPEC) system.

2. Experimental

2.1. Materials

Oxcarbazepine, MHD, DHD and the internal standard 5,6-dihydro-11-oxo-11H-dibenz[b,e]-azepine-5-carboxamide (Fig. 1) were provided by Ciba-Geigy (Basle, Switzerland).

2.2. Reagents

Potassium dihydrogenphosphate and dipotassium hydrogenphosphate were purchased from Merck (Darmstadt, Germany). Acetonitrile (HPLC quality) and methanol (RPE-ACS quality) were from Carlo Erba France (Rueil-Malmaison, France). Water was purified and deionized using a Milli-Q reagent-grade water system (Millipore, Bedford, MA, USA).

2.3. Apparatus

The chromatographic system consisted of a Model 303 pump (Gilson, Villiers-le-Bel, France), an ASPEC system (Gilson) and a Model Spectroflow 783 variable-wavelength UV detector (Kratos, Ramsey, NJ, USA) set at 210 nm and 0.016 AUFS with a response time of 1 s. A Model C-R4A integrator-recorder (Shimadzu, Kyoto, Japan) was used for data capture.

2.4. Column

A pre-packed column (stainless-steel tube, 4 cm × 4.6 mm I.D.), filled with ODS Hypersil, 3 μm particle size (Société Française Chromato Colonne, Eragny, France) was used. A guard-column (stainless-steel tube, 3.3 cm × 4.6 mm I.D.) was placed between the pump and the injector. It was tap-filled in our laboratory with pellicular ODS material, 37–53 μm particle size (Whatman, Clifton, NJ, USA). A Whatman pre-column (reversed-phase material, 1 cm × 3 mm I.D.) was placed between the injector and the analytical column.

2.5. Extraction columns

Bond-Elut C_{18} (50 mg) extraction columns of 1-ml capacity were used. They were manufactured by Analytichem International and purchased from Prolabo (Paris, France).

2.6. Calibration solutions

An internal standard solution at a concentration of 198 μmol/l was prepared in methanol–water (50:50, v/v). Stock calibration solutions (400 μmol/l) were prepared by dissolving OXC, MHD or DHD in the internal standard solution. In order to obtain the calibration solutions

dilutions were made with the internal standard solution.

2.7. Sample handling

Aliquots of 100 μl plasma, 100 μl water and 10 μl internal standard (or calibration) solution were introduced into a vial and vortex-mixed for a few seconds. The vial was then placed on the ASPEC system. Sample handling was then automatically performed by the ASPEC system. The automated sequences are described in Table 1. Each sample was prepared separately during the chromatography of the previous sample. After each liquid transfer, the needle was rinsed with 1 ml of water, with a flow-rate of 400 μl/s.

2.8. Chromatography

The mobile phase was acetonitrile–methanol–0.01 *M* potassium dihydrogenphosphate (pH 5.0) (9:11:80, v/v/v). Chromatography was carried out at room temperature with a flow-rate of 2 ml/min. The pressure at the top of the pre-column was *ca.* 140 bar.

3. Results and discussion

The described method has been adapted with a few modifications from that previously published for the assay of carbamazepine in plasma [11].

OXC, MHD, DHD and the internal standard were well separated from plasma components within 16 min (Fig. 2). The retention times were 3.7 min for DHD, 4.9 min for MHD, 9.5 min for OXC and 14.0 min for the internal standard. The time required by the ASPEC system for sample handling was 11 min. Diluted plasma samples were found to be stable for at least 6 h on the rack of the ASPEC system at room temperature. The pressure at the top of the pre-column (*ca.* 140 bar) began to increase already at the second working day. Therefore the pre-column was changed every day. The life time of the analytical column lasted for *ca.* 500 injections of plasma extracts.

The retention times of OXC and its metabolites were hardly dependent on the pH of the mobile phase. Nevertheless a salt solution (pH 5.0) was used in the mobile phase to stabilize the retention times of the plasma components and the concomitantly administered drugs that could

Table 1
Automated procedure for the extraction and the injection

Step	Liquid dispensed		Dispensing flow-rate (μl/s)	Pressurizing air volume (μl)
Conditioning	Methanol Water	(1 ml) (1 ml)	100	60
Loading	Diluted plasma	(200 μl)	25	100
Washing	0.02 *M* K_2HPO_4 Water–methanol (95:5, v/v)	(2 ml) (2 ml)	100	200
Elution	Methanol	(250 μl)	50	100
Dilution of the extract	Water	(950 μl)	800	2000[a]
Injection via a 500-μl loop	Diluted extract	(1 ml)	25	–

[a] 2 ml of air were used to mix the extract with water by bubbling.

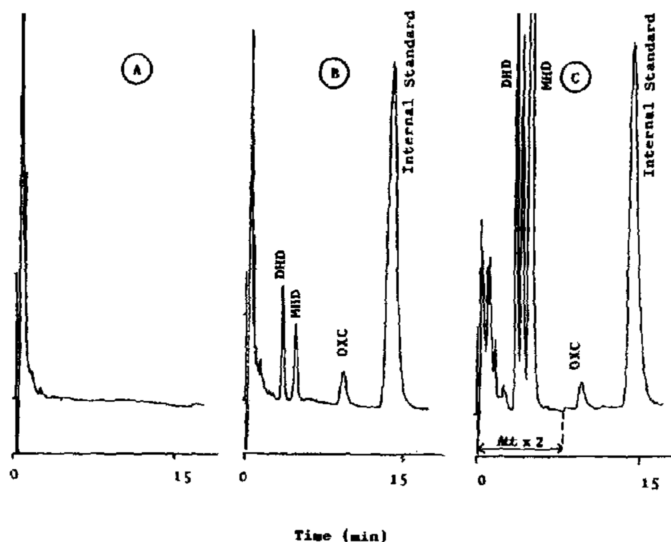

Fig. 2. Chromatograms of extracts of (A) blank plasma, (B) spiked plasma containing 1 μmol/l of OXC and its metabolites and 20 μmol/l of internal standard, and (C) plasma collected from a child (2.5 years) given oral doses of OXC and phenobarbital concurrently (concentrations found, OXC: 0.7 μmol/l, MHD: 32 μmol/l, DHD: 9.1 μmol/l).

possibly interfere. A salt solution (pH 7.8) was used as washing solvent because this resulted in plasma blanks free from significant interferences.

The extraction yield from plasma was 87% for OXC, 84% for MHD, and 82% for DHD (at concentrations of 1 μmol/l for OXC and DHD, and 10 μmol/l for MHD) as compared with direct injection of a standard solution.

Calibration graphs were obtained by plotting the peak-height ratio (OXC or MHD or DHD/ internal standard) *versus* the concentration of either compound in the sample. The equations were calculated by the least-squares method using linear regression with a weighting factor of $1/(\text{concentration})^2$ [12]. Typical equations and correlation coefficients (r) were: $y = 0.205x - 0.003$, $r = 0.9992$, for OXC; $y = 0.501x + 0.002$, $r = 0.9999$, for MHD; $y = 0.649x + 0.004$, $r = 0.9999$, for DHD, where y is the peak-height ratio and x is the concentration. The concentrations in the calibration plasma samples ranged from 0.2 to 10 μmol/l for OXC and from 0.1 to 10 μmol/l for MHD and DHD.

The within-day accuracy and precision of the method were assessed by assaying series of six plasma samples spiked with different concentrations of OXC, MHD and DHD. The results are shown in Table 2. The between-day accuracy and precision were assessed by analysing plasma samples spiked with different concentrations over 1 week (Table 2). The limit of quantitation, corresponding to a mean recovery [(found/given concentration) · 100] between 85 and 115% and a coefficient of variation lower than 15%, was 0.2 μmol/l (50 ng/ml) for the determination of OXC and 0.1 μmol/l (25 ng/ml for MHD and 27 ng/ml for DHD) for the determination of MHD and DHD.

The method was applied to the assay of plasma samples collected from subjects treated with OXC. No interference from anti-epileptic drugs such as phenobarbital, phenytoin, valproic acid and carbamazepine, was found in patients treated concomitantly with OXC and one of these drugs. Valproic acid was not extracted from plasma. The other compounds were eluted with retention times of 4.2 min (phenobarbital), 6.9 min (epoxy-carbamazepine), 18 min (phenytoin) and 20 min (carbamazepine). A chromatogram is shown in Fig. 2 for a child (2.5 years) treated with OXC and phenobarbital. Suitable sensitivity and reproducibility were obtained. An example

Table 2
Within- and between-day precision and accuracy of the assay

Nominal plasma concentration (μmol/l)	Within-day ($n = 6$)		Between-day ($n = 4$)	
	Nominal/found (mean) (%)	C.V. (%)	Nominal/found (mean) (%)	C.V. (%)
OXC				
0.2	109	10	105	13
0.5	98	4	104	3
2	98	2	98	2
10	100	2	99	3
MHD				
0.1	105	3	102	7
0.2	88	3	98	3
2	101	2	95	4
10	102	1	98	5
DHD				
0.1	104	4	114	6
0.2	100	3	100	2
2	101	1	94	9
10	101	2	97	10

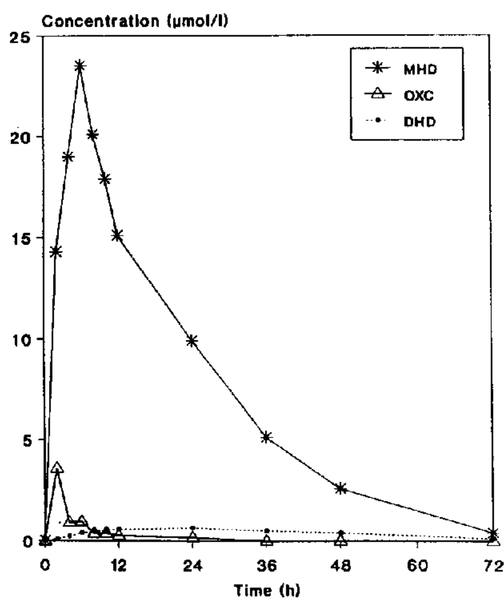

Fig. 3. Plasma profiles of oxcarbazepine and its metabolites in a volunteer given a single oral dose of 600 mg of oxcarbazepine.

of plasma profiles is given in Fig. 3 for a healthy male volunteer who received a 600-mg dose of OXC as 2 tablets of 300 mg.

4. Conclusion

The described procedure permits the automated and rapid analysis of oxcarbazepine and its two main metabolites in plasma. The method is sensitive and can be used for pediatric patients and small animals by using a plasma volume of only 100 μl.

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