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Narrow-bore liquid chromatographic assay for oxcarbazepine and its major metabolite in rat brain, liver and blood microdialysates

K. Van Belle[a], V. De Koster[a], S. Sarre[a], G. Ebinger[b], Y. Michotte[a,*]

[a]*Department of Pharmaceutical Chemistry and Drug Analysis, Pharmaceutical Institute, Vrije Universiteit Brussels, Laarbeeklaan 103, B-1090 Brussels, Belgium*

[b]*Department of Neurology, University Hospital, Vrije Universiteit Brussels, Laarbeeklaan 103, B-1090 Brussels, Belgium*

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Abstract

An isocratic narrow-bore high-performance liquid chromatographic assay with UV detection is described for the detection of oxcarbazepine and its major metabolite, 10,11-dihydro-10-hydroxycarbamazepine, using carbamazepine as the internal standard. This method is sufficiently sensitive to allow quantification of oxcarbazepine and its metabolite in rat brain, liver and blood microdialysates.

1. Introduction

Oxcarbazepine (OX) (10,11-dihydro-10-oxocarbamazepine: GP 47680) is a new antiepileptic drug, recently released (Trileptal), and chemically related to carbamazepine (CBZ) [1].

OX has been found to be as effective and as well tolerated as CBZ in the treatment of generalized tonic–clonic seizures and/or partial seizures and in the treatment of trigeminal neuralgia [2].

The major metabolic pathway for OX is a reduction to the pharmacologically active 10,11-dihydro-10-hydroxycarbamazepine (MOX) (GP 47779) by non-inducible cytosolic reductases. OX is a prodrug immediatly metabolized to MOX and present at low concentrations [3].

Microdialysis is an *in vivo* sampling technique that allows the study of pharmacokinetics, simultaneously in different tissues, in laboratory animals [4,5]. Due to the relatively small recovery obtained with dialysis, only a small fraction of the unbound drug and metabolite under investigation is collected in small volume samples. This results in low dialysate concentrations to be analyzed.

A new trend in drug analysis is the use of miniaturized LC devices, such as narrow-bore (2 mm I.D.) [6], microbore (1 mm I.D.) [7], and capillary (320 μm I.D.) [8] columns. The smaller I.D.s of the columns lead to more concentrated peaks for small volume samples and thus increased mass sensitivity. Other advantages are improved separation efficiency, higher speed of analysis and less solvent consumption. High-performance liquid chromatographic (HPLC) sys-

* Corresponding author.

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tems are reported in the literature for the analysis of OX in human plasma samples. These assays use conventional (>4 mm I.D.) columns and are not sensitive enough for the analysis of microdialysates [9–11].

Here we report a narrow-bore LC system with UV detection, using CBZ as the internal standard, for the determination of OX and MOX in rat brain, blood and liver dialysates.

2. Experimental

2.1. Materials

The LC–UV system consisted of a Gilson 305 piston pump (Villers le Bel, France) with a manometric module 805 as pulse dampener, connected to a Kontron 433 UV detector (path length: 10 mm, cell volume: 8 μl) (Milan, Italy).

Separation was performed on a Spheri-5ODS column, 5 μm particle size, 22 × 0.21 cm I.D. and a Spheri-5ODS guard column (5 μm, 3 × 0.21 cm I.D.) (Bioanalytical Systems, West Lafayette, IN, USA).

Samples (50 μl) were injected with a Gilson-ASPEC automated injector. Integration was carried out on a Merck D-2500 chromato-integrator (Darmstadt, Germany).

2.2. Chemicals and reagents

OX, MOX and CBZ were gifts from Ciba-Geigy (Basle, Switzerland). All other chemicals were analytical reagent grade or better and purchased from Merck (Darmstadt, Germany).

Stock solutions of OX, MOX and CBZ (100 μg/ml) were made in methanol and kept at −20°C. Further dilutions were made in freshly prepared and filtered (0.2-μm membrane filter) artificial cerebrospinal fluid (CSF). The ion concentrations in the artificial CSF are: 1.35 m*M* Ca^{2+}, 131.9 m*M* Cl^-, 3.0 m*M* K^+, 144.5 m*M* Na^+, 1.1 m*M* Mg^{2+}, 0.242 m*M* HPO_4^{2-} and 20 m*M* HCO_3^- (pH 7.6).

2.3. Chromatographic conditions

The mobile phase consisted of filtered (0.2-μm membrane filter) 0.05 *M* KH_2PO_4 (adjusted to pH 6.5 with 5 *M* NaOH)–acetonitrile (78:22, v/v). The flow-rate was 0.4 ml/min and analysis was carried out at room temperature. The sensitivity of the detector was set at 0.01 AUFS and the detection wavelength was 220 nm.

To 40 μl of the standard and sample solutions 25 μl of the internal standard solution (CBZ, 500 ng/ml) were added. A 50-μl aliquot of this mixture was injected onto the column.

2.4. In vivo microdialysis experiments

A male albino Wistar rat (weighing 250 g) was anaesthetized with a ketamine/diazepam mixture (50/5 mg/kg) during the whole experimental period. Rigid microdialysis probes (CMA 10, 3-mm membrane length, 0.52 mm diameter and 20 kDa molecular mass cut-off) were placed, simultaneously, in the jugular vein, liver and cerebellum with the use of guide cannulae. The probes were connected to a microinjection pump (CMA 100) and perfused with artificial CSF (cerebellum) or with physiological saline (0.9% NaCl) (blood, liver) at a flow-rate of 2 μl/min. Collection of dialysates started 1 h after probe insertion and the sampling time was 20 min (sample volume 40 μl).

Dialysates were collected in basal (pre-dose) conditions during 1 h. Then OX was administered (intraperitoneal (i.p.), 10 mg/kg) and dialysates were collected for another 6 h. The dialysates obtained are clean and free of proteins so no sample preparation is necessary.

3. Results and discussion

3.1. Assay characteristics

OX and MOX are neutral molecules so their retention times are not influenced by changes in pH. Therefore, initially a mobile phase consist-

ing of water (78%) and acetonitrile was chosen. Using this mobile phase, interfering peaks occurred in the plasma and liver dialysates. Thus, water was replaced by a buffer solution (pH 6.5) resulting in clean blank dialysates under basal conditions. Fig. 1A shows a blank plasma dialysate.

The mean retention times (min) for MOX, OX and CBZ and the coefficients of variation (C.V.) over 6 days were respectively 4.9 (0.8%), 10.2 (0.4%) and 20.6 (0.2%). A chromatogram of a 100 ng/ml standard of OX and MOX with CBZ as internal standard is shown in Fig. 1B.

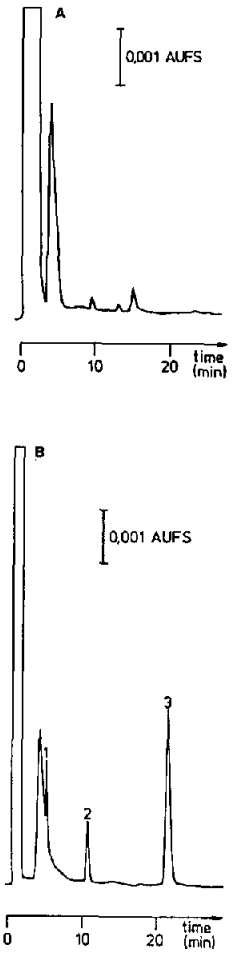

Fig. 1. (A) Chromatogram of a blank plasma dialysate obtained under basal conditions, and (B) a chromatogram of a standard (100 ng/ml). Peaks: 1 = MOX, 2 = OX, and 3 = CBZ.

In the LC assay described by Noirfalise and Collinge [9] a conventional column (I.D. 4 mm) was used to determine OX and MOX. However, the determination limits (OX: 150 ng/ml, MOX: 1000 ng/ml) obtained were insufficient to determine these drugs in the collected dialysates. In the assay described here we used a narrow-bore column to obtain higher and sufficient sensitivity.

The sensitivity criteria were calculated for six standards using the method described by Oppenheimer *et al.* [12]. The detection limit (the actual net response that may *a priori* be expected to lead to detection) was 10 ng/ml for both drugs (C.V. for OX: 10.7%, C.V. for MOX: 9.8%). The determination limit (the level at which measurement precision will be satisfactory for quantitative determination) was 25 ng/ml for both drugs (C.V. for OX: 3.4%, C.V. for MOX: 4.3%).

Good linearity ($n = 6$) was exhibited for OX and MOX over the concentration range 25–1000 ng/ml. The coefficients of correlation and their coefficient of variation (C.V.) were respectively 0.9998 (0.03%) and 0.9997 (0.02%).

3.2. Analytical precision

Within-run precision was determined by comparing the peak-height ratios for 6 standard curves ranging from 25 to 1000 ng/ml on the same day. The run-to-run precision of standard curves in the range from 50 to 500 ng/ml was determined on 6 different days. The results of the within-run and run-to-run precision are summarized in Table 1.

3.3. Method precision and accuracy

Known amounts of OX and MOX were added to blank blood, liver and CSF (brain) dialysates. The results of the obtained recovery are given in Table 2.

Table 1
Analytical precision for MOX and OX

MOX			OX		
Concentration (ng/ml)	Peak-height ratio (mean ± S.D.)	C.V. (%)	Concentration (ng/ml)	Peak-height ratio (mean ± S.D.)	C.V. (%)
Within-run precision (n = 6)					
0	–	–	0	–	–
25	0.108 ± 0.004	3.7	25	0.094 ± 0.003	3.2
50	0.231 ± 0.007	3.0	50	0.168 ± 0.003	1.8
100	0.474 ± 0.013	2.7	100	0.406 ± 0.010	2.5
250	1.018 ± 0.024	2.4	250	0.983 ± 0.016	1.6
500	2.243 ± 0.057	2.5	500	1.909 ± 0.050	2.6
1000	3.938 ± 0.055	1.4	1000	3.391 ± 0.069	2.0
Run-to-run precision (n = 6)					
0	–	–	0	–	–
50	0.227 ± 0.015	6.6	50	0.168 ± 0.012	7.1
100	0.444 ± 0.021	4.7	100	0.369 ± 0.020	5.4
250	1.127 ± 0.077	6.8	250	0.973 ± 0.049	5.0
500	2.063 ± 0.107	5.2	500	1.874 ± 0.116	6.2

3.4. Measurement of OX and its metabolite in rat brain, liver and blood dialysates

The described method was used to measure dialysate concentrations of drug and metabolite in the cerebellum, liver and blood of the rat. Fig. 2 shows chromatograms of cerebellum (A), liver (B), and blood (C) dialysates 1 h after administration of OX (i.p.: 10 mg/kg). The dialysate concentrations for the free drugs in the extracellular fluid and plasma are less than the real values due to the less than 100% recovery of the probes.

The real extracellular tissue and plasma concentrations are necessary to calculate pharmacokinetic parameters or to correlate the free, pharmacological active, concentration with its effect, and can be calculated using the internal standard technique [13] as *in vivo* calibration method for the microdialysis. At present, this *in vivo* calibration method for OX and MOX is being validated in our laboratory.

Fig. 3 shows the dialysate concentration–time curves of OX and its metabolite for cerebellum (A), liver (B), and blood (C).

Table 2
Method precision and accuracy for MOX and OX

Sample	Recovery (mean ± S.D, n = 6) (%)	C.V. (%)
MOX		
Plasma	99.8 ± 2.7	2.7
Liver	99.8 ± 1.2	1.2
Brain	100 ± 2.1	2.1
OX		
Plasma	98.9 ± 2.4	2.4
Liver	99.9 ± 1.3	1.3
Brain	99.9 ± 2.1	2.1

4. Conclusion

A narrow-bore LC assay is described giving adequate separation and sensitivity to determine OX and the active metabolite concentrations in rat cerebellum, liver and blood microdialysates.

In vivo microdialysis has become an important

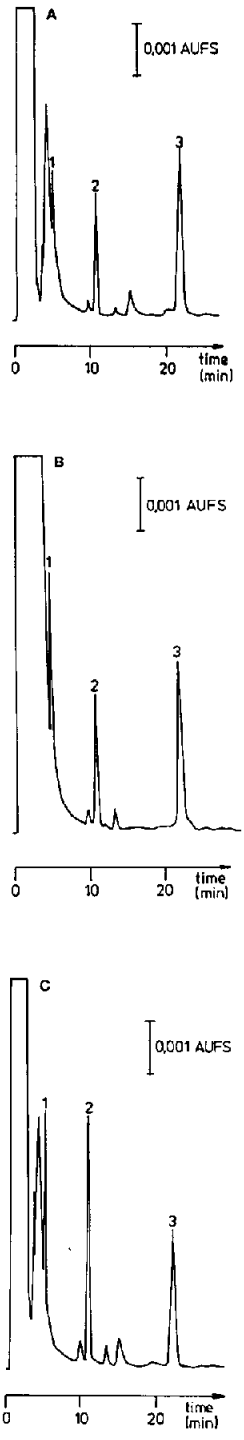

Fig. 2. Chromatograms of cerebellum (A), liver (B), and blood (C) dialysates after administration of OX (10 mg/kg i.p.).

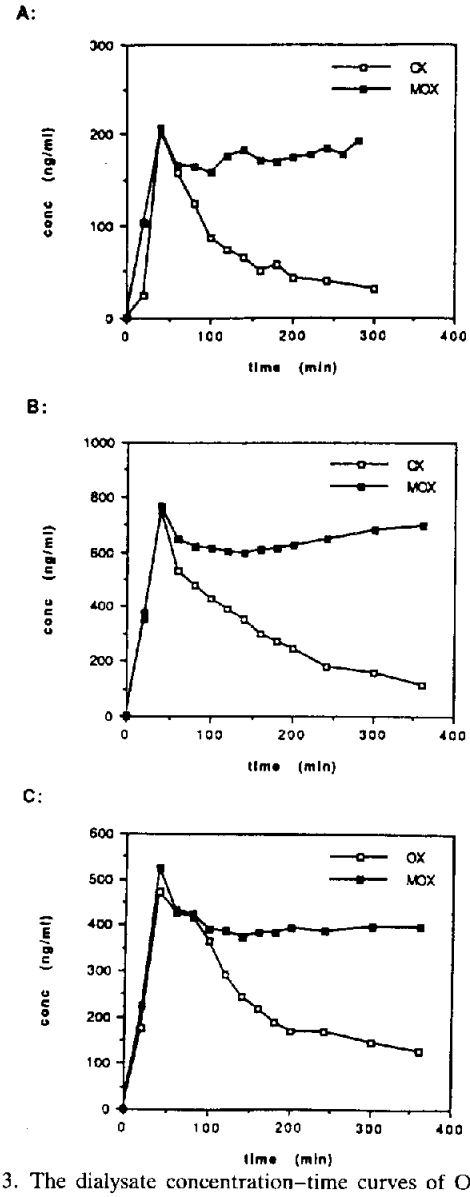

Fig. 3. The dialysate concentration–time curves of OX and MOX for cerebellum (A), liver (B), and blood (C) after administration of OX (10 mg/kg i.p.).

tool in drug development. For highly protein-bound drugs and for potent drugs microdialysis is of limited value due to the lack of sufficiently sensitive analytical techniques. For such determinations miniaturized-column LC may become of great value.

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