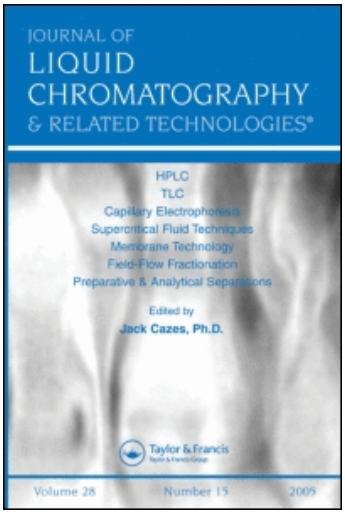

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Simultaneous HPLC Analysis of Carbamazepine and Carbamazepine Epoxide in Human Brain Microdialysate

R. D. Scheyer[ab]; M. J. During[c]; J. A. Cramer[ab]; B. R. Toftness[ab]; J. M. Hochholzer[b]; R. H. Mattson[ab]
[a] Department of Neurology, Yale University School of Medicine, New Haven, Connecticut [b] Section of Neurosuqyy Neurology Service-127 West Haven Veterans Administration Medical Center, West Haven, Connecticut [c] Yale University School of Medicine, New Haven, Connecticut

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SIMULTANEOUS HPLC ANALYSIS OF CARBAMAZEPINE AND CARBAMAZEPINE EPOXIDE IN HUMAN BRAIN MICRODIALYSATE

R. D. SCHEYER[1,3], M. J. DURING[2], J. A. CRAMER[1,3], B. R. TOFTNESS[1,3], J. M. HOCHHOLZER[3], AND R. H. MATTSON[1,3]

[1]*Department of Neurology*
[2]*Section of Neurosurgery*
Yale University
School of Medicine
New Haven, Connecticut 06520
[3]*Neurology Service-127*
West Haven Veterans Administration Medical Center
950 Campbell Avenue
West Haven, Connecticut 06516

ABSTRACT

Intracerebral microdialysis has become a standard method for neurochemistry studies and is becoming recognized as an important new method for pharmacological studies. The technique permits repeated measurement of drug concentrations in the brain extracellular fluid with minimal disturbance of the neuronal environment. The sample volumes are exceedingly small, on the order of tens of microliters. This is countered by the purity of the samples, reducing the need for extraction prior to assay. We present a HPLC assay capable of reliably measuring the concentrations of the antiepileptic drug carbamazepine and its metabolites carbamazepine-10,11-epoxide and carbamazepine-10,11-*trans*-diol.

INTRODUCTION

Microdialysis is a promising technique for studying drug pharmacokinetics. However, the small sample volumes require new assays or modifications of standard assays. We present a technique which has proven satisfactory for the analysis of antiepileptic drug concentrations from microdialysis catheters implanted in epilepsy patients undergoing intracranial electrical recording for seizure localization in preparation for epilepsy surgery.

To measure clinical concentrations of antiepileptic drugs (AEDs) in human cerebral microdialysate, we have modified an assay previously used in our laboratory for the measurement of carbamazepine (CBZ) and the active metabolite carbamazepine-10,11-epoxide (CBZE) concentrations in human serum (1). That method, an adaptation of the methods of Sawchuck (2) and Szabo (3), required the addition of cyheptamide as an internal standard, followed by an alkaline extraction into methylene chloride. Microdialysis provides protein free samples of sufficient purity that extraction is unnecessary.

MATERIALS AND METHODS

Microdialysis is conducted in patients undergoing depth electrode evaluation for the localization of seizure foci prior to epilepsy surgery. The

details of the implantation and microdialysis apparatus itself have been presented elsewhere (4). The microdialysis catheter is made of regenerated cellulose, (300 μm o.d., molecular weight cutoff 5000, Cuprophan, Enka Glanstoff, Germany); inflow and outflow tubing is made of fused silica. The catheter is perfused with an artificial extracellular fluid (ECF) composed of a sterile solution of NaCl 135 mMol, KCl 3 mMol, $CaCl_2$ 1.2 mMol, MgCl2 1 mMol, and ascorbate 200 uMol buffered with sodium mono- and di-phosphate to pH 7.35. The perfusion rate is typically 2.5 μl/min, but may be varied from 0.25 to 5.0 μl/min.

Chemicals

Water, acetonitrile, and methanol, all HPLC grade, were obtained from Fisher Scientific (Springfield, NJ, USA). CBZ was obtained from Supelco Inc. (Bellefonte, PA USA), CBZE was a gift of Ciba-Geigy Corp. (Summit, NJ USA), carbamazepine-10,11-*trans*-diol (CBZD) was a gift of George Szabo (Boston V.A. Medical Center, Boston MA, USA).

Chromatography

The analytical column is an Econosphere C18, 3 micron (10 cm × 4.6 mm I.D.) (Alltech Associates, Deerfield, IL, USA). A Spectra Physics 8880 autosampler is used to inject 20 μl of sample to overfill a 10 μl sample loop (the surplus is necessary to ensure sufficient injected volume and to avoid injection of air onto the column). To minimize volume loss, samples are

not processed prior to injection. A Spectra Physics SP8800 ternary pump (Spectra Physics, San Jose, CA, USA) is operated in isocratic mode with a mobile phase of water, acetonitrile, and methanol 60/23/17 (v/v). The column temperature is maintained at 40°C, and the flow rate is 0.6 ml/ml.

Peaks are detected at 210 nm by a Spectra Physics Spectra 100 variable wavelength detector; peak areas are computed with a Spectra Physics Chromjet 2 channel integrator.

Spiked samples are used to generate external standard curves. A stock solution of 4 μg/ml CBZ, CBZE, and CBZD in mobile phase is further diluted with mobile phase to 2.0, 1.0, 0.5, 0.2, 0.1, and 0.05 μg/ml concentrations.

Standards are injected at the beginning and end of each assay run. The standard curve is generated by a linear regression of the peak areas of the first set of standards. The second set is used to verify assay stability over the course of the run. Standards and samples are injected every 20 minutes, with mobile phase blanks alternating with samples as a precaution against unanticipated late-eluting peaks.

In vitro within run C.V.'s were computed on replicates of 10 samples, between run C.V.'s were computed on replicates of 5 samples performed

on 3 days. *In vivo* C.V.s were calculated on replicates of 4 within run and 4 between run injections of pooled dialysate from a patient receiving CBZ. A portion of the pool was spiked with CBZ and CBZE to assess assay repeatability in perfused dialysate at higher concentrations.

RESULTS

A calibration chromatogram is presented in figure 1.

In vitro:

CBZ and CBZD concentrations as low as 0.02 μg/ml, and CBZE concentrations of 0.01 μg/ml, were readily quantifiable. C.V.s are presented in table 1.

Patient samples:

Patient microdialysate CBZ and CBZE concentrations were 0.26 and 0.08 μg/ml respectively. C.V.s were 8.9% and 1.6% within run, and 6.8% and 7.9% between run for CBZ and CBZE. The pool was then spiked with CBZ and CBZE resulting in concentrations of 3.41 and 2.21 μg/ml. C.V.s were 1.7% and 1.3% within run, and 1.6% and 2.0% between run. The CBZD peak, visible on the chromatogram, was not quantified. A patient chromatogram is shown in figure 2.

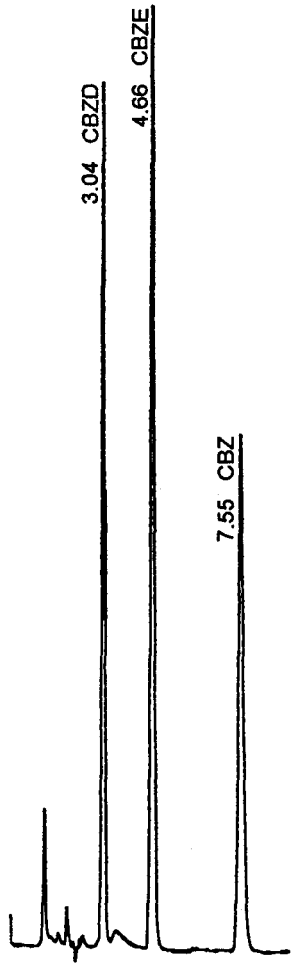

Figure 1 Calibration chromatogram (1.0 μg/ml). (Retention times in minutes.)

TABLE 1

In Vitro C.V.'s

Drug	Concentration (ug/ml)	C.V. within run	C.V. between run
CBZ	2.0	0.7 %	0.9 %
	1.0	0.3	2.0
	0.5	3.8	2.1
	0.2	1.4[a]	
	0.1	0.5[a]	
	0.05	3.9[a]	
	0.02	6.3[a]	
CBZE	0.3	0.9 %	1.8 %
	0.15	0.7	3.0
	0.075	0.5[b]	5.9
	0.03	1.6[a]	
	0.015	7.2[a]	
	0.0075	5.4[a]	
	0.0038	10.5[a]	
CBZD	0.6	2.1 %	3.4 %
	0.3	0.8	4.1
	0.15	0.5	7.7
	0.06	1.8[a]	
	0.03	3.3[a]	
	0.015	0.0[a]	
	0.0075	25.5[a]	

[a] n = 4
[b] n=6

DISCUSSION

Microdialysis of human epileptic tissue offers a unique opportunity to study the concentrations of AEDs in the vicinity of their putative receptors. The technique, although invasive, does not add significantly to the risks of electrode implantation being performed for seizure localization.

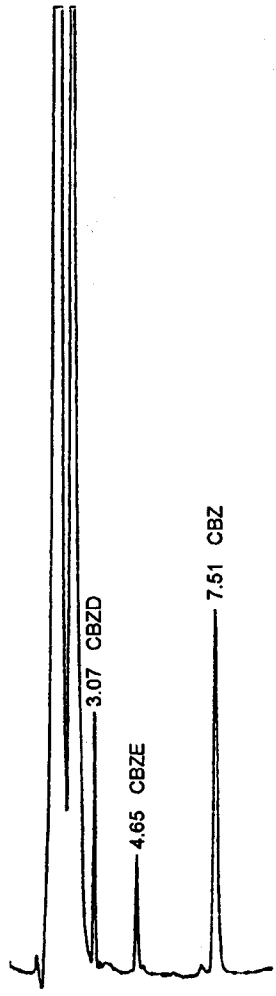

Figure 2 Patient chromatogram.
Direct injection of brain dialysate.

At usual perfusion rates, the sample volumes obtained are quite small, typically about 30 µl (5), but assay sensitivity has been sufficient to quantify CBZ and CBZE concentrations in cerebral microdialysate volumes of <20 µl. A CBZD peak was visible in some patient chromatograms but was not quantified as it is not believed biologically active.

The microdialysis membrane removes large molecules (> approximately 5000 Dalton), and replaces the extraction procedures required by standard assays. The small volumes would make such extractions difficult. To reduce the effect of measurement errors when transferring small volumes, no internal standard is added. This places extra demands on the stability of the analytical column. Repeat controls at the end of each chromatographic run check for possible drift. An internal standard could be added to the ECF prior to perfusion. If this were done, a fraction of the standard (dependent on perfusion rate) might diffuse out of the catheter. This would complicate its use as a chromatographic reference, as well as adding safety concerns and altering the neuronal environment.

Despite the absence of an internal standard, our data have been very consistent. The assay has proven suitable for assessment of brain concentrations of the antiepileptic drug CBZ and its active metabolite CBZE.

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