

**Liquid Chromatography / Tandem Mass Spectrometry for the  
Determination of Carbamazepine and Its Metabolite  
in Rat Blood Collected by an Automated Blood Sampling System**

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## Overview

- LC/MS/MS methods were developed and validated for the determination of carbamazepine and its metabolite carbamazepine-10,11-epoxide in rat plasma.
- Automated blood sampler Culex® was used for the collection of rat blood at preprogrammed intervals after oral administration of carbamazepine
- Automated blood sampling and LC/MS/MS analyzing improve quality, throughput and precision for small volume/lower concentration samples.
- The significant difference in PK results between automated blood sampling and traditional manual withdraw has been observed, with more consistent and meaningful data for the automated blood sampling system and the predicted stress influences on the traditional manual approach.

## Introduction

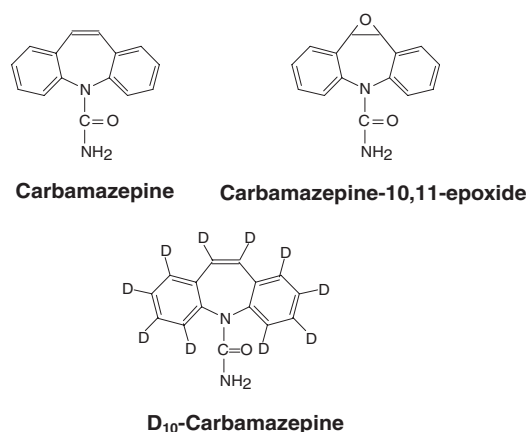
Carbamazepine (CBZ) (5-H-dibenz[b,f]azepine-5-carboxamide) is an anticonvulsant used in clinical practice as first-line treatment for generalised tonic-clonic and partial seizures [1]. Over the last two decades, thirty-three metabolites of CBZ have been isolated and identified in the urine from patients on an oral dose [2]. Of these metabolites, carbamazepine 10,11-epoxide (CBZ-E) is the most important one from a clinical point of view. CBZ-E is pharmacologically as active as the parent compound in experimental animals.

Figure 1 shows the structures of CBZ, CBZ-E and D<sub>10</sub>-carbamazepine. A fast, sensitive and specific LC/MS/MS method for the simultaneous determination of CBZ and its metabolite CBZ-E in rat plasma is described. After

administration of CBZ, blood samples were periodically collected from awake, freely moving animals by a Culex automated blood sampler. The PK parameters of CBZ and CBZ-E were evaluated. This study has demonstrated that Culex automated blood sampling system provided a powerful tool for unattended pharmacokinetic and pharmacodynamic studies involving low stress freely moving rats.

## Methods

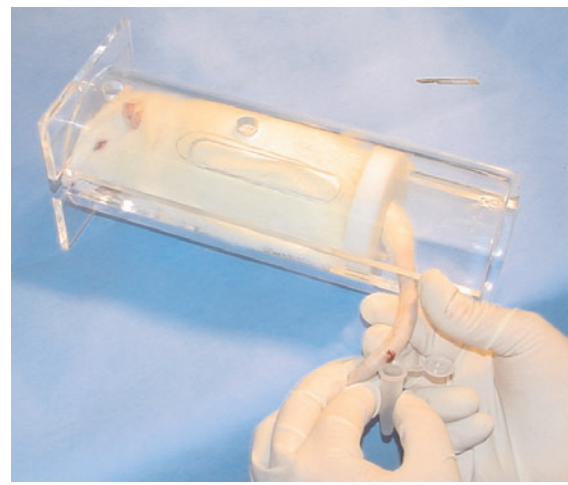
Male Sprague-Dawley rats weighting 300-400 g were used for the study. For automatic sampling, rats were implanted with a jugular vein catheter (CX-2010, BAS, West Lafayette, IN, USA) and/or femoral vein catheter (CX-2020, BAS). After surgery, the rats were installed in the Ratur™ (BAS) and allowed to recover for one day with free access to food and water. The rats were fasted overnight before dosing orally with CBZ at a dose of 5 mg/kg. A volume of 0.25 mL blood sample was withdrawn from the jugular vein into a vial, containing heparine and kept in a refrigerated fraction collector according to a preset schedule in Culex (BAS) automated blood sampling system. For manual sampling, rats were restrained in a restraint box and the blood samples were collected via the tail vein. Figures 2 and 3 illustrate the automated blood sampling and manual sampling system. The analytical method consists of a liquid-liquid extraction procedure and electrospray LC/MS/MS analysis. The samples were then analyzed off-line by reverse phase liquid chromatography electrospray tandem mass spectrometry. The LC/MS/MS system was equipped with a BAS PM-80 pump coupled to a Finnigan LCQ Deca ion trap mass spectrometer (ThermoQuest, San Jose, CA, USA). The mass spectrometer was operated in ESI positive ion mode. The column was a Supelco Discovery C8, 5 mm, 150 x 2.1 mm with. A mobile phase containing 0.5% acetic acid and 30% acetonitrile at a flow-rate of 0.4 mL/min. D<sub>10</sub>-Carbamazepine (D10-CBZ) is used as the internal standard for all compounds.



F1 Structures of carbamazepine and related compounds



F2 Culex Automated Blood Sampling System



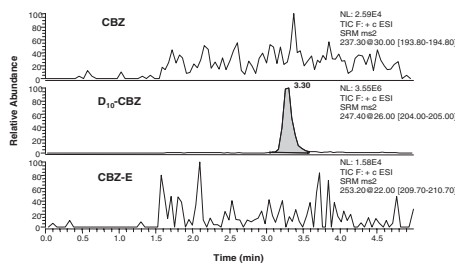
F3 Manual sampling system

## Results

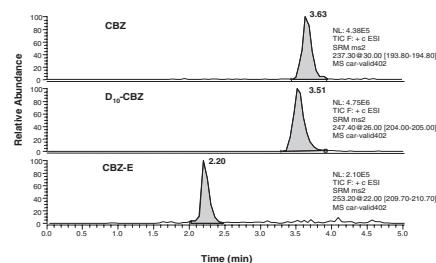
Quantitation was conducted using the selected reaction monitoring (SRM) mode. Table 1 summarizes the product ion spectra of the two analytes and D<sub>10</sub>-carbamazepine. The best sensitivities and minimum interferences were achieved by monitoring the transitions stated in Table 1. The chromatographic separation was achieved within 5 min using a C8 (150 x 2.1 mm) 5 mm column with a mobile phase containing 0.5% acetic acid and 30% acetonitrile at a flow-rate of 0.4 mL/min. Figures 4, 5 and 6 show typical chromatograms of an extracted drug-free rat plasma blank, plasma spiked with all

Compounds	[M+H] <sup>+</sup>	Product ions (m/z)	Ionization width (m/z)	Collision energy (%)	SRM ion combination
CBZ	237	220, 194	1	30	237→194
CBZ-E	253	236, 210	1	22	253→210
D <sub>10</sub> -CBZ	247	230, 204	1	26	247→204

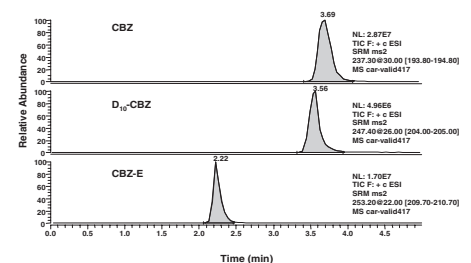
T1 Positive product ion mass parameters of CBZ, CBZ-E and D<sub>10</sub>-CBZ



F4 Representative chromatogram of extracted blank rat plasma



F5 Representative chromatogram of two analytes from an extracted rat plasma LLOQ (5ng/mL) sample



F6 Representative chromatogram of two analytes from an extracted rat plasma (500ng/mL) sample

analytes at 5 ng/mL (LLOQ) and plasma spiked with all analytes at 500 ng/mL, respectively. No endogenous rat plasma components were observed at the retention times corresponding to all two analytes and D<sub>10</sub>-carbamazepine (internal standard). The lower limit of quantitation (LLOQ) is 5 ng/mL for each analyte, based on 0.1 mL aliquots of rat plasma. The extraction recovery of analytes from rat plasma was over 87%. Linearity is observed over the range of 5-2000 ng/mL. The intra- and inter-day accuracy and precision values for QC samples are present in Table 2. The precision values (coefficient of variation) at the three concentrations in the intra-assay study varied between 2.6 and 5.2% for CBZ and 4.9 and 9.5% for CBZ-E while that of inter-assay study varied between 4.0 and 4.7% for CBZ and 8.7 and 9.6% for CBZ-E. The accuracy (relative error) values for all three concentrations deviated less than 4.7% FOR CBZ and 5.2% for CBZ-E from the corresponding nominal concentrations. This method has been used for the pharmacokinetic study of CBZ and CBZ-E.

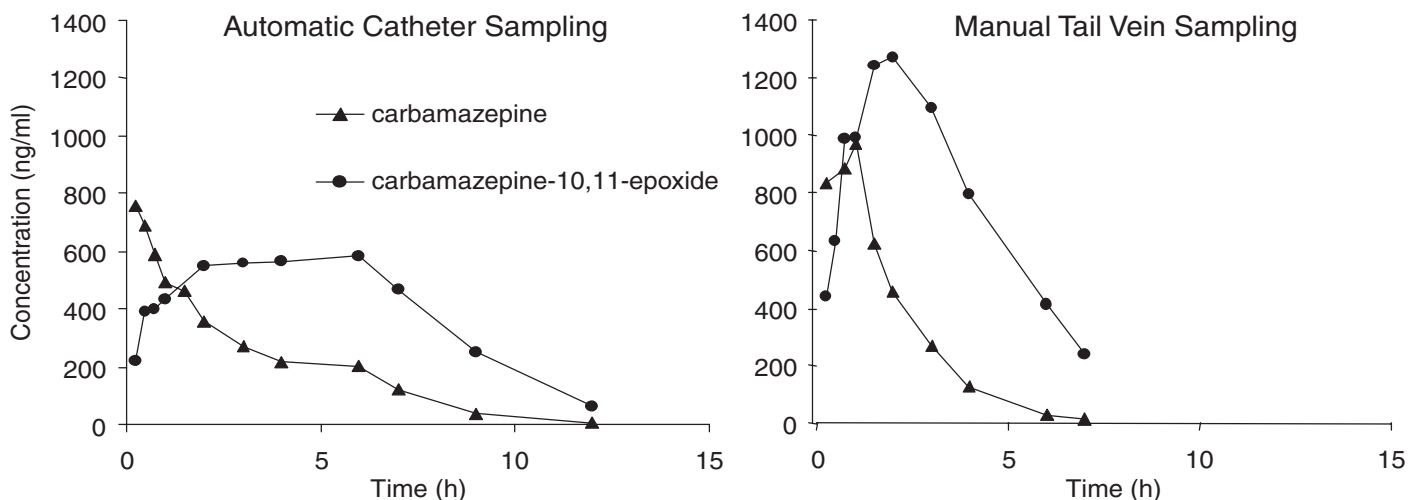
Compounds	Nominal Concentration (ng/mL)	Inter-day variation (n = 12)			Intra-day variation (n = 4)		
		Measured	CV	Relative	Measured	CV	Relative
		Con. (ng/mL)	(%)	error (%)	Con. (ng/mL)	(%)	error (%)
CBZ	5	4.9	4.5	2.0	4.8	5.2	4.5
	50	50.7	4.0	1.4	51.8	3.0	3.6
	1000	953	4.7	4.7	969	2.6	3.1
CBZ-E	5	5.2	9.6	4.0	5.1	6.3	1.5
	50	47.7	9.1	4.6	49.5	9.5	1.1
	1000	1052	8.7	5.2	990	4.9	1.0

T2 Accuracy and precision for CBZ and CBZ-E from rat plasma

The precision values (coefficient of variation) at the three concentrations in the intra-assay study varied between 2.6 and 5.2% for CBZ and 4.9 and 9.5% for CBZ-E while that of inter-assay study varied between 4.0 and 4.7% for CBZ and 8.7 and 9.6% for CBZ-E. The accuracy (relative error) values for all three concentrations deviated less than 4.7% FOR CBZ and 5.2% for CBZ-E from the corresponding nominal concentrations. This method has been used for the pharmacokinetic study of CBZ and CBZ-E.

Culex automated blood sampler is designed to collect whole blood, urine and feces over a long period of time from awake, freely-moving animals like rats, dogs and primates as shown in Fig. 2 for a Culex system. Each unit can house four rats. The Culex system collects blood in sealed, refrigerated vials, which are transferable to a 96-well plate for automated sample preparation. It provides simultaneously automated serial blood sampling, behavior monitoring and microdialysis from a rodent metabolism cage for PK, PD and CNS effect studies [3].

After giving a single oral dose of 5 mg/kg of CBZ, the rat was sampled by an automated blood sampling system or by traditional manual draws. The plasma was collected at specific time points for the determination of the analytes. The plasma concentration-time plot of CBZ and CBZ-E for both automatic and manual sampling is shown in Figure 7.



F7 The plasma concentration-time profiles of CBZ and CBZ-E in rat following a single 5 mg/kg oral administration by Culex and manual sampling

It has been recognized that stress of conventional sampling from awake rats drastically influences rodent physiology (blood pressure, blood flow) and biochemistry (stress hormone release, metabolism, even protein expression) [4]. Automated blood sampling permits awake cannulated animals to be sampled stress free (without restraint and human handling). The latter situation provides the most consistent and meaningful data for PK studies. Table 3 shows PK parameters of CBZ and its epoxide metabolite in plasma from the same rat using two different blood sampling methods. It can be seen clearly that when PK data are obtained from an animal under stress, the normal parameters of C<sub>max</sub>, T<sub>max</sub>, AUC and CL can all vary very substantially.

	Compound	C <sub>max</sub> (ng/mL)	T <sub>max</sub> (hr)	AUC (ng hr/mL)	CL (mL/hr)
Manual	CBZ	972	1.0	2086	2.4
	CBZ-E	1270	2.0	7520	0.7
Automatic	CBZ	759	0.3	2392	2.1
	CBZ-E	584	6.0	4727	1.1

T3 PK parameters of carbamazepine and its epoxide metabolite in plasma from the same rat using two different sampling methods

## Discussion

LC/MS/MS with automated blood sampling system has been proven to be powerful for the pharmacokinetic study. The current studies demonstrate the significant difference in PK results between automated blood sampling and manual withdraw, with more consistent and meaningful data for the automated blood sampling system and the predicted stress influences on the traditional manual approach.

## References

- [1] D. Chadwick, Lancet 354 (1999) 13-19.
- [2] K. Lertratanakoon, M. G. Horing, Drug Metab. Dispos, 10 (1982) 1-10.
- [3] www.culex.net
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