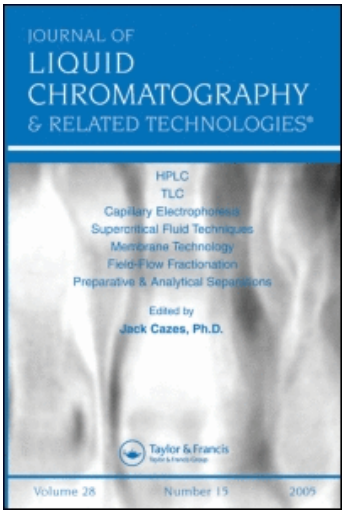

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Determination of Temazepam and Its Active Metabolite, Oxazepam in Plasma, Urine and Dialysate Using Solid-Phase Extraction Followed by High Performance Liquid Chromatography

J. Chopineau[a]; F. Rivault[a]; V. Sautou[a]; M. F. Sommier[a]
[a] Laboratoire de Pharmacie Clinique et Biotechnique, U.F.K Pharmacie, Clermont-Ferrand, Cedex, France

To cite this Article Chopineau, J. , Rivault, F. , Sautou, V. and Sommier, M. F.(1994) 'Determination of Temazepam and Its Active Metabolite, Oxazepam in Plasma, Urine and Dialysate Using Solid-Phase Extraction Followed by High Performance Liquid Chromatography', Journal of Liquid Chromatography & Related Technologies, 17: 2, 373 — 383
To link to this Article: DOI: 10.1080/10826079408013358
URL: http://dx.doi.org/10.1080/10826079408013358

JOURNAL OF LIQUID CHROMATOGRAPHY, 17(2), 373-383 (1994)

DETERMINATION OF TEMAZEPAM AND ITS ACTIVE METABOLITE, OXAZEPAM IN PLASMA, URINE AND DIALYSATE USING SOLID-PHASE EXTRACTION FOLLOWED BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

J. CHOPINEAU, F. RIVAULT,
V. SAUTOU, AND M. F. SOMMIER
Laboratoire de Pharmacie Clinique et Biotechnique
U.F.R. Pharmacie
Place Henri Dunant
63003 Clermont-Ferrand Cedex, France

ABSTRACT

A sensitive and rapid assay of temazepam and its active metabolite, oxazepam in plasma, urine and dialysate was developed. This assay involves solid phase extraction followed by HPLC analysis. Mean recoveries were 97 % for temazepam and 70 % for oxazepam. The limit of detection was 5 ng/ml of biological fluids for each compound. One advantage of this method lies in the solid phase extraction which is more rapid and convenient than liquid liquid extraction.

This assay maybe useful for monitoring concentrations of temazepam and oxazepam in plasma, urine and dialysate of patients under continuous ambulatory peritoneal dialysis (CAPD).

INTRODUCTION

Temazepam (1 chloro 2,3 dihydro 3-hydroxy-1-methyl-5-phenyl-1H-1,4 benzodiazepin-2-one) is a benzodiazepin used in the short term treatment of insomnia. It is prescribed for to uraemic patients being hemodialysed or under CAPD. We are investigating the behaviour of temazepam and its entry through the peritoneum in uraemic patients under CAPD.

Temazepam is metabolised to an inactive glucuroconjugated compound (1) but also to oxazepam, an active metabolite (2). We developed an assay method for both temazepam and oxazepam in various fluids (plasma, urine and dialysate). These compounds were separated by gas chromatography (3,4,5) or high performance liquid chromatography (6,7,8,9,10) after extraction from biological fluids.

We have developed a method of separation by HPLC after solid phase extraction. The two compouds were extracted from plasma, urine and dialysate and then analysed using the same procedure.

MATERIALS AND METHODS

Reagents

Compounds and reagents were of analytical grade (>99% purity).

Temazepam was generously given by Wyeth France (Paris, France), and climazolam by Hoffman Laroche (Basel, Switzerland). Oxazepam was purchased from Sigma Chemicals (St Louis, USA). Other reagents methanol (Carlo Erba, Milan, Italy) and acetonitrile (Prolabo, Paris, France).

Potassium phosphate buffer (0.01M, pH 5.6) was obtained by diluting 10 ml of 1M potassium phosphate buffer (pH 5.6) to 1 l with distilled water. The 1M buffer was prepared by mixing 94.8 ml of 1M potassium dihydrogenphosphate solution (KH_2PO_4, Merck, Darmstadt, Germany) with 5.2 ml of 1M dipotassium hydrogen phosphate solution (K_2HPO_4, Merck, Darmstadt, Germany).

Reagents used for solid-phase extraction were methanol, distilled water, acetonitrile-distilled water (30:70 v/v) and acetonitrile-distilled water (20:80 v/v).

Stock solutions (1 mg/ml) were prepared by dissolving 10 mg of temazepam, oxazepam or climazolam in 10 ml of methanol. Standard solutions containing a mixture of temazepam, oxazepam and climazolam were prepared as detailed in Table I (climazolam as internal standard was at a set concentration of 400 ng/ml).

TABLE I : Preparation of standard solutions using stock solutions (1 mg/ml) of temazepam, oxazepam and climazolam

	1	2	3	4	5
OXAZEPAM	10 µl (20 ng/ml)	25 µl (50 ng/ml)	50 µl (100 ng/ml)	100 µl (200 ng/ml)	250 µl (500 ng/ml)
TEMAZEPAM	10 µl (20 ng/ml)	25 µl (50 ng/ml)	50 µl (100 ng/ml)	100 µl (200 ng/ml)	250 µl (500 ng/ml)
CLIMAZOLAM	200 µl (400 ng/ml)	200 µl (400 ng/ml)	200 µl (400 ng/ml)	200 µl (400 ng/ml)	200 µl (400 ng/ml)

HPLC procedure

The HPLC system consisted of a L5000 LC Controller module equipped with a pump (655 A-11 liquid chromatography), a L4250 UV-Visible detector and a D2000 chromato-integrator (all from Merck-Hitachi, Darmstadt, Germany).

The analysis was performed on a Lichrocart 125-4 Lichrospher 100 RP 18 endcapped 5 micron analytical column (Merck, Darmstadt, Germany). A Lichrocart 4mm X 4 mm ID Lichrosorb RP 18-5 micron precolumn was used (Merck, Darmstadt, Germany)). The mobile phase was acetonitrile-potassium phosphate buffer (0,01 M, pH 5.6) (40-60 v/v). The analysis was performed in isocratic mode. The flow rate was 1.6 ml/min. The UV detector wavelength was 254 nm.

The injection volume was obtained with a 20 microlitre injection loop.

The column was flushed daily with methanol-water (50:50 v/v)

Solid-phase extraction

The solid-phase extraction was performed using the Vac-Elut system (Analytichem International, Harbor City, USA) and C18 100 mg Bond Elut cartridges (Analytichem International, Harbor City, USA).

The extraction procedure comprised four steps:

* Sample preparation ,deproteination: 1 ml of acetonitrile-distilled water (30:70 v/v) was added to 1 ml of plasma or 1 ml of urine or 10 ml of dialysate. After homogenisation (vortex mixing for 10 s), the samples were centrifuged for 5 min at 4000g.

* Conditioning of the cartridges : 2 ml of methanol and then 2ml of distilled water were run through the cartridges.

The sample (first prepared) was then run through the conditioned cartridges.

* Washing : 2 ml of acetonitrile-distileled water (20:80 v/v) was run through the cartridges. This was followed by drying for 3 or 4 min.

* Elution : the cartridges were eluted with four 200 microlitre volumes of methanol.

The eluate was then evaporated under nitrogen and the residue taken up in 100 microlitres of methanol which 20 microlitres were injected.

Quantification

The internal standard was climazolam. Calibration curves were obtained by plotting the peak area ratios (peak area of temazepam or oxazepam over peak area of

climazolam) as a fonction of concentration of temazepam or oxazepam per ml of plasma, urine or dialysate. Concentration of temazepam or oxazepam for a patient was calculated by interpolation from the calibration curve.

RESULTS AND DISCUSSION

Chromatography

With our chromatographic system, we can separate the two compounds (oxazepam and temazepam) and the internal standard as shown in figure 1. Retention times are stated on the chromatogram.

Quantitative analysis : validation of the method

* Linearity : good linearity was observed for temazepam and oxazepam within the concentration ranges studied (20–500 ng/ml) for each biological fluid was (plasma, urine, dialysate). Slopes and correlation coefficient are given in table II.

* Precision : this was evaluated by intra–assay and inter–assay variabilities. Results are given in tables III and IV.

* Limit of quantification : 5 ng/ml for each compound in all three biological fluids.

* Recoveries : these were calculated at two different concentrations : 50 and 200 ng/ml. Values for recoveries are given in table V.

Figure 2 shows a chromatogram of a blank plasma extract and plasma sample containing 100 ng/ml of oxazepam and temazepam.

Figure 3 shows a chromatogram of a blank urine extract and urine sample containing 100 ng/ml of oxazepam and temazepam.

Figure 4 shows a chromatogram of a blank dialysate extract and dialysate sample containing 100 ng/ml of oxazepam and temazepam.

Clinical application

This assay was used to estimate the entry of temazepam and its active metabolite, oxazepam through the peritoneum during CAPD in uraemic patients.

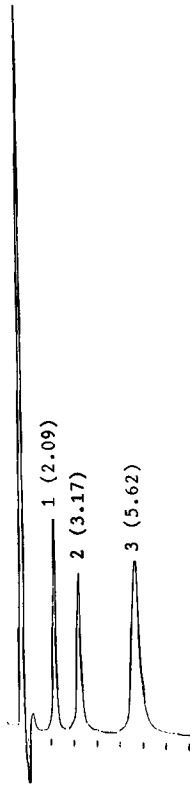

Figure 1 : Chromatographic separation of mixture containing oxazepam 100 ng/ml (peak 1), temazepam 100 ng/ml (2) and climazolam 400 ng/ml (3).

TABLE II : Linearity of the method : values of slopes and correlation coefficients.

		Slope	Correlation coefficient
Temazepam	**Plasma**	0,00486	0,999
	Urine	0,00520	0,999
	Dialysate	0,00501	0,999
Oxazepam	**Plasma**	0,00322	0,999
	Urine	0,00370	0,999
	Dialysate	0,00441	0,999

TABLE III: Precision of the method; intra-day assay variability

		TEMAZEPAM		OXAZEPAM	
BIOLOGICAL FLUID	CONCENTRATION ADDED (ng/ml)	CONCENTRATION FOUND (ng/ml)*	COEFFICIENT OF VARIATION (%)	CONCENTRATION FOUND (ng/ml)*	COEFFICIENT OF VARIATION (%)
Plasma	50	49.1+/- 3.7	7.5	52.6 +/- 5.1	9.7
n = 10	200	202.7 +/- 12.2	6.0	211.4 +/- 12.8	6.1
Urine	50	48.9 +/- 2.4	4.9	52.2 +/- 3.8	7.3
n = 10	200	203.3 +/- 5.8	2.9	207.0 +/- 5.8	2.8
Dialysate	50	48.0 +/- 4.4	9.2	48.3 +/- 6.1	12.6
n = 10	200	202.0 +/- 10.6	5.2	200.1 +/- 11.6	5.8

*** mean +/- standard deviation**

TABLE IV: Precision of the method; inter-day assay variability

		TEMAZEPAM		OXAZEPAM	
BIOLOGICAL FLUID	**CONCENTRATION ADDED (ng/ml)**	**CONCENTRATION FOUND (ng/ml)***	**COEFFICIENT OF VARIATION (%)**	**CONCENTRATION FOUND (ng/ml)***	**COEFFICIENT OF VARIATION (%)**
Plasma	50	48.7 +/- 4.1	8.4	49.0 +/- 5.1	10.2
n = 10	200	206.3 +/- 9.6	4.7	210.0 +/- 11.6	5.5
Urine	50	47.1 +/- 2.4	5.1	48.0 +/- 4.6	9.6
n = 10	200	199.1 +/- 5.8	2.9	203.4 +/- 5.7	2.8
Dialysate	50	44.9 +/- 5.4	12.0	52.7 +/- 5.7	10.8
n = 10	200	211.9 +/- 13.3	6.3	209.7 +/- 8.9	4.2

*** mean +/- standard deviation**

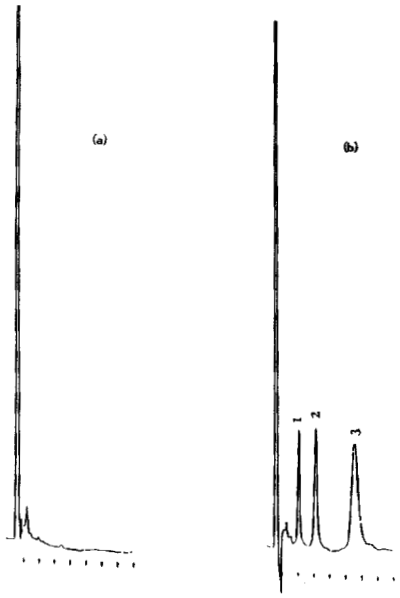

Figure 2: Chromatograms of blank plasma extract (a) and plasma sample (b) containing 100 ng/ml of oxazepam (1) and temazepam (2) and 400 ng/ml of climazolam (3).

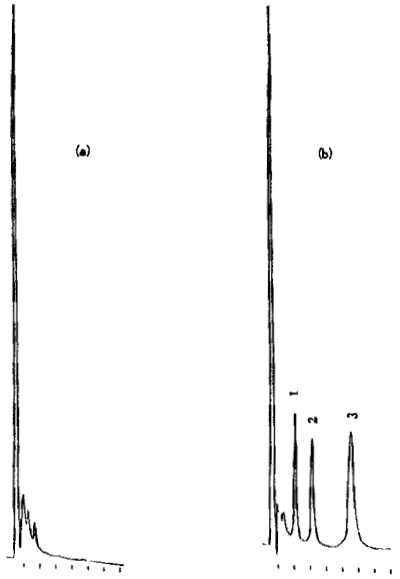

Figure 3 : Chromatograms of blank urine extract (a) and urine sample (b) containing 100 ng/ml of oxazepam (1) and temazepam (2) and 400 ng/ml of climazolam (3)

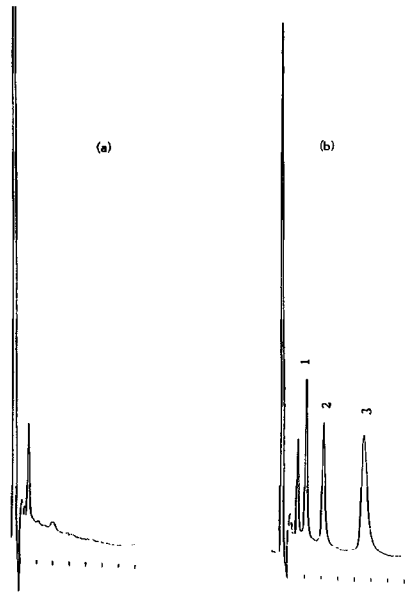

Figure 4 : Chromatograms of blank dialysate extract (a) and dialysate sample containing 100 ng/ml of oxazepam (1) and temazepam (2) and 400 ng/ml of climazolam (3).

TABLE V: Recoveries of temazepam and oxazepam in plasma, urine and dialysate

COMPOUND	BIOLOGICAL FLUID	CONCENTRATION (ng/ml)	RECOVERY (%)	COEFFICIENT OF VARIATION (%)
Temazepam	Plasma	50	96.3	13.8
		200	97.1	9.1
	Urine	50	99.0	9.6
		200	99.8	8.6
	Dialysate	50	96.2	12.5
		200	94.4	9.9
Oxazepam	Plasma	50	60.1	9.5
		200	74.0	9.4
	Urine	50	60.2	13.5
		200	74.5	13.8
	Dialysate	50	69.6	11.8
		200	85.3	14.2

In conclusion, this rapid and convenient assay method involving solid phase extraction followed by HPLC analysis is suitable for routine clinical monitoring of temazepam and oxazepam in plasma, urine and dialysate.

ACKNOWLEDGMENTS

The authors thank H. CONFOLENT and F. PICQ for the assay of temazepam and oxazepam.

REFERENCES

-1-H.J. SCHWARTZ, Br. J. Clin. Pharmacol., 8 : 23S-29S (1979).

-2-A.LOCKNISKAR, D.J. GREENBLATT, Biopharm. Drug. Dispos., 11 : 499-506 (1990).

-3-M. DIVOLL, D.J. GREENBLATT, J. Chromatogr., 222 : 125-128 (1981).

-4-P.D. KROBOTH, R.B. SMITH, R. RAULT, M.R. SILVER, M.I. SORKIN, J.B. PUSCHETT, R.P. JUHL, Clin. Pharmacol. Ther., 37 : 453-459 (1985).

-5-P. LILLSUNDE, T. SEPPÄLÄ, J. Chromatogr., 533 : 97-110(1990).

-6-G.W. KUNSMAN, S.E. MANNO, M.A. PRZEKOP, B.R. MANNO, K.A. LLORENS, C.M. KUNSMAN, J. Chromatogr., 568 : 427-436 (1991)

-7-T. STEBLER, T.N. GUENTERT, J. Chromatogr., 564 : 330-337 (1991).

-8-B.K. LOGAN, D.T. STAFFORD, I.R. TEBBETT, C.M. MOORE, J. Anal. Toxicol., 24 : 154-159 (1990).

-9-S.E. PATTERSON, J. Pharm. Biomed. Anal., 4 : 271-274 (1986).

-10-C.E. LAU, S. DOLAN, M. TANG, J. Chromatogr., 416 : 212-218 (1987).

Received: June 5, 1993
Accepted: June 20, 1993