



Chlordiazepoxide and Metabolites in Serum

Waters Corporation



This is an Application Brief and does not contain a detailed Experimental section.

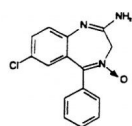
Abstract

This application brief demonstrates analysis of chlordiazepoxide and metabolites in serum.

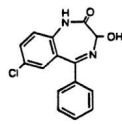
Introduction

The compounds used in this study are –

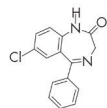
1. Norchlordiazepoxide
2. Oxazepam
3. Nordazepam
4. Chlordiazepoxide
5. Demoxepam (I.S.)



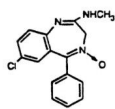
1. Norchlordiazepoxide



2. Oxazepam

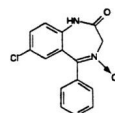


3. Nordazepam



4. Chlordiazepoxide

· HCl



5. Demoxepam

Experimental

HPLC Method

Column:	Symmetry C ₁₈ , 3.9 x 150 mm, 5 μm
Guard column:	Sentry Guard Column, 3.9 x 20 mm, 5 μm
Part number:	Column - WAT046980, Guard - WAT054225
Mobile phase:	20 mM potassium phosphate, pH 7.0/acetonitrile/methanol 56:21:23
Flow rate:	1.0 mL/min

Injection volume:

20 μ L of reconstituted porcine serum extract

Detection:

UV @ 240 nm

Oasis[®] HLB Extraction Method

Oasis[®] HLB 1 cc/30 mg Extraction Cartridge
Part Number WAT094225

Condition:

1 mL methanol/1 mL water

Load:

1 mL spiked porcine serum
with 1.5 μ g/mL demoxepam (I.S.)

Wash:

1 mL 5% methanol in water

Elute:

1 mL methanol

Evaporate and Reconstitute:

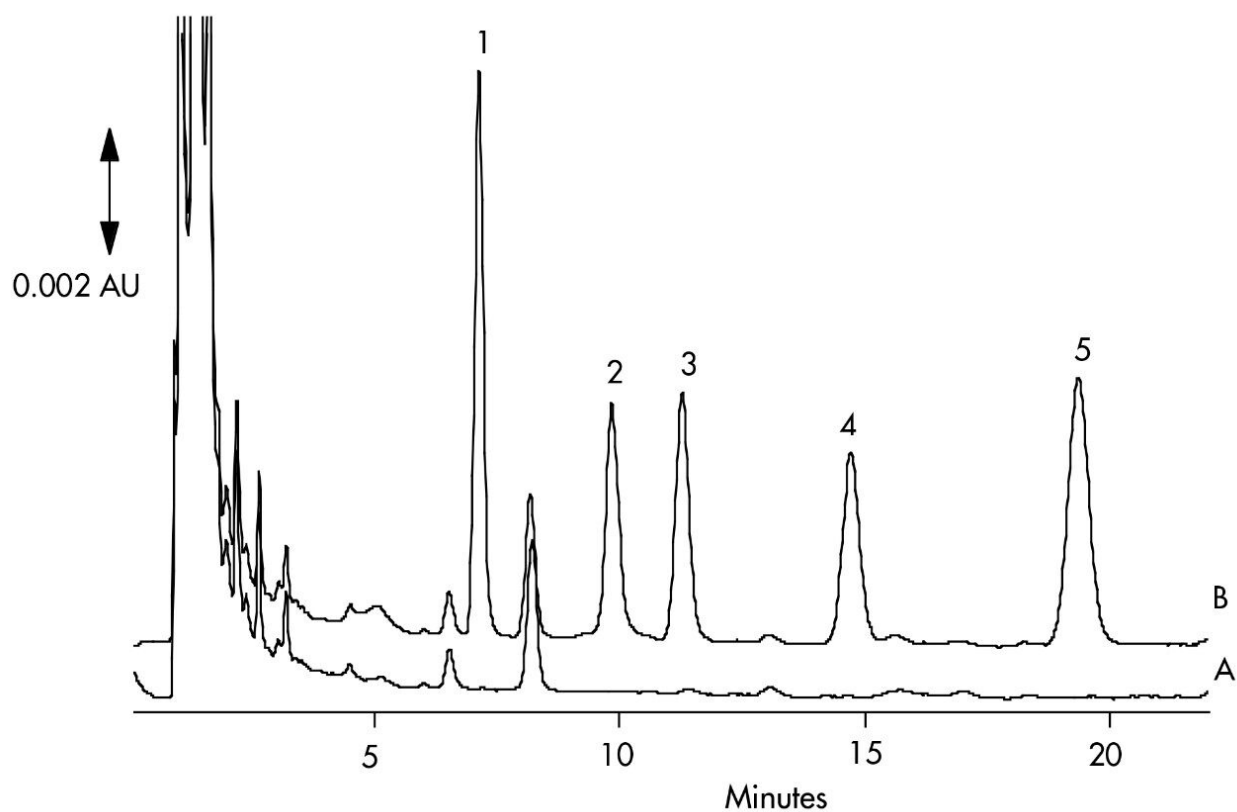
40 °C under nitrogen stream
200 μ L of 20 mM potassium phosphate pH
7/methanol 80:20 (v/v)

Results and Discussion

Compound	Concentration $\mu\text{g/mL}$	% Recovery	%RSD (n-6)
1. Norchloridiazepoxide	0.200	97.8%	4.3%
	0.040	96.5%	5.5%
2. Oxazepam	0.200	104.0%	2.6%
	0.040	90.0%	2.5%
3. Nordazepam	0.200	101.0%	3.0%
	0.040	98.9%	1.2%
4. Chlordiazepoxide	0.200	90.0%	2.4%
	0.040	100.0%	4.1%

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Chromatogram of Serum Extracts: A) Blank B) Spiked Sample



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