

Extraction of Diazepam on Strata™ X

Column: Synergi™ 4 µm Polar-RP 80 Å, LC Column 150 x 4.6 mm, Ea

Dimensions: 150 x 4.6 mm ID

Order No: 00F-4336-E0

Elution Type: Gradient

Eluent A: 20 mM KH₂PO₄ (pH=2.5)

Eluent B: MeOH

Gradient Profile:	Step No.	Time (min)	Pct A	Pct B
	1	0	75	25
	2	3	75	25
	3	8	30	70
	4	11	30	70
	5	14	75	25

Flow Rate: 1.5 mL/min

Col. Temp.: ambient

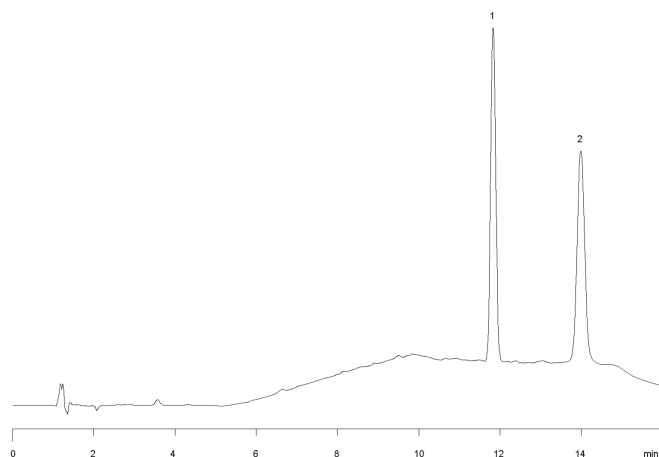
Detection: UV-Vis Abs.-Variable Wave.(UV) @ 254 nm (ambient)

Analyst Note: SecurityGuard™ Guard Cartridge System extends column lifetime.

- SecurityGuard Cartridges, POLAR-RP 4 x 3.0mm, 10/Pk Part No.: AJ0-6076

- Holder Part No.: KJ0-4282

14618



ANALYTES:

- 1 Desmethyldiazepam
- 2 Diazepam (Valium)



Sample Preparation Details

for HPLC Application ID No.: 14618

Extraction of Diazepam on Strata™ X

PRODUCT DESCRIPTION:

Strata™-X 33 µm Polymeric Reversed Phase, 30 mg / 1 mL, Tubes , 100/Pk

Order No.: 8B-S100-TAK

SOLID PHASE EXTRACTION (SPE) PROCEDURE:

Note: The solvent volumes shown below are for a 30 mg bed mass.

The solvent volumes will need to be adjusted for a smaller or larger bed mass.

Condition:

Load:

Load: 1 ml analyte
and IS spiked
Porcine serum (conc.=0.8 µg/mL; diluted 1:1 with 100 mM KH₂PO₄, pH 6.0)

Wash:

Dry:

1 min @ 10 in. Hg

Elute:

Final Prep and Analysis:

Reconstitute with 200 µL mobile phase

Inject: 50 µL on HPLC UV-Vis Abs.-Variable Wave.(UV) @ 254 nm (ambient)

ANALYTES:	Spiked Conc. (ng/mL)	Log P	pKa	% Rec	%RSC (n=0)
1 Desmethyldiazepam	0.8			98	
2 Diazepam	0.8			98	

Note: This method is designed as a convenient starting point for further investigation and can be tailored to meet your extraction goals.
Call your local Phenomenex Representative for assistance in method development and optimization techniques.

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