

## ***Extraction of Benzodiazepines from plasma using Strata ä X-C***

### **SPE method:\***

*Condition:* 2mL methanol

*Equilibrate:* 2mL deionized water

*Load:* 2mL of analyte & IS [conc. = 0.8 µg/mL] spiked Porcine plasma (1:1 dilution with 100mM KH<sub>2</sub>PO<sub>4</sub>, pH 6.0); sample acidified with 2% H<sub>3</sub>PO<sub>4</sub>/mL

*Wash 1:* 2mL 0.1 N HCl

*Wash 2:* 2mL 100% methanol

*Elute:* 2mL 5% NH<sub>4</sub>OH/methanol

Blow down under slow stream of N<sub>2</sub> and reconstitute with mobile phase buffer

Inject: 50µL on HPLC

**\*Note:** the solvent volumes shown above are for a 60mg bed mass. The solvent volumes will need to be adjusted for a smaller or larger bed mass.

### **HPLC conditions**

Synergi Max RP, 4µ, 80Å, 150x4.6mm; UV=254nm

A=0.1% TFA/H<sub>2</sub>O (pH=2.1), C = 0.1% TFA/ACN

<i>Time (min)</i>	<i>%A</i>	<i>%C</i>	<i>Flow (mL/min)</i>
0	95	5	1.5
10	5	95	1.5

### **% Recovery**

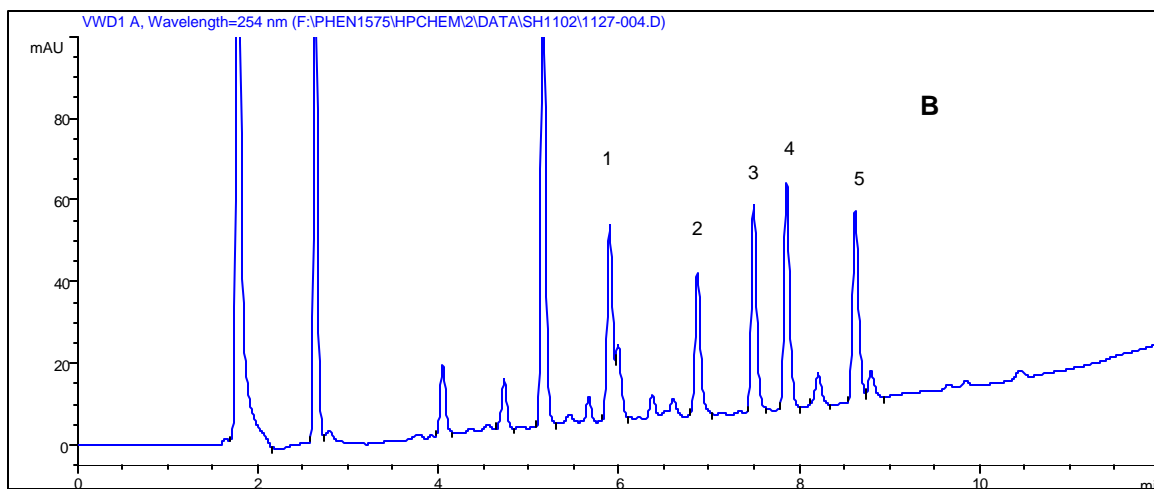
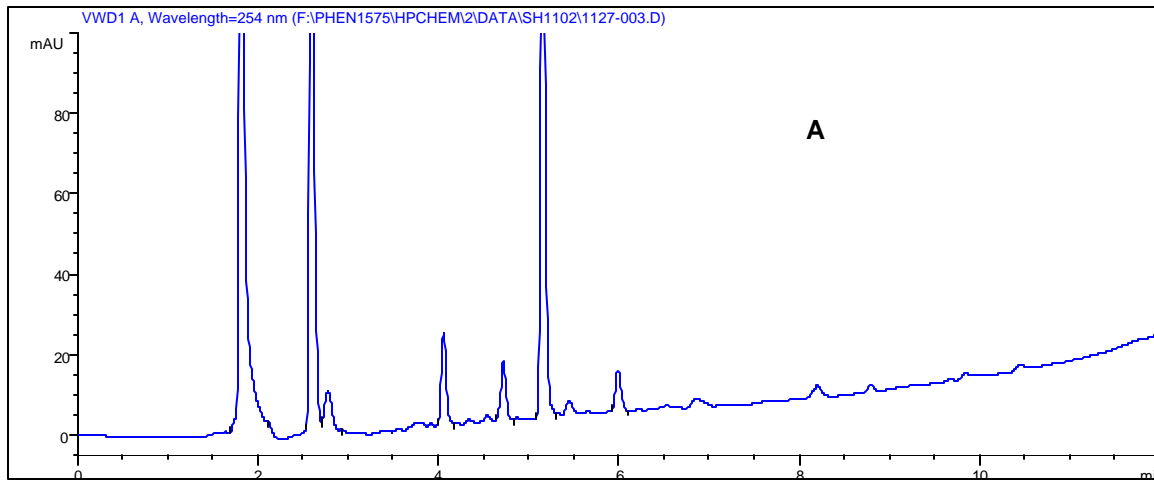
<i>Analyte</i>	<i>strata-X-C</i>	<i>% RSD</i>
Bromazepam	102	0.55
Flurazepam	105	2.71
Diazepam	93	2.86
Flunitrazepam	96	3.86

### **Ordering Information**

Order Number	Description
8B-S029-TAK	strata-X-C 30mg/1mL Tubes (100/Box)
8B-S029-UBJ	strata-X-C 60mg/3m L Tubes (50/Box)
8B-S029-ECH	strata-X-C 100mg/6mL Tubes (30/Box)
8B-S029-FCH	strata-X-C 200mg/6mL Tubes (30/Box)
8B-S029-HCH	strata-X-C 500mg/6mL Tubes (30/Box)
8E-S029-AGB	strata-X-C 96-Well Plate 10mg/well (2/Box)
8E-S029-TGB	strata-X-C 96-Well Plate 30mg/well (2/Box)

\* This method is designed as a convenient starting point for further investigation. This method can be tailored to meet your extraction goals. Phenomenex makes no guarantee regarding the accuracy or completeness of the method.

**Chromatogram of plasma extracts by strata-X-C: A) Blank B) Spiked sample**



1. Bromazepam
2. Flurazepam
3. Diazepam
4. Oxazepam (IS)
5. Flunitrazepam