

SPE Method for Benzodiazepines in Serum

Application Brief

Condition: Add 5 mL methanol to the cartridge. Apply vacuum and discard the eluant. Repeat with 5 mL water. Do not allow the sorbent to go dry at any point during this step.

Load: Add 1 mL 0.1 M sodium acetate to 4 mL serum. Mix, then add to the cartridge. Apply vacuum and discard the eluant.

Rinse: Add 3 mL water to the cartridge. Apply vacuum and discard the eluant. Leave vacuum on for 30 seconds after all of the water has passed through the cartridge. Centrifuge the cartridge at 1000–1500 rpm for 5 minutes.¹

Elution: Place a collection tube beneath the cartridge. Add 3 mL acetone² to cartridge. Apply vacuum and collect the eluant. Concentrate to dryness using gentle heat (<45°C) and under a stream of nitrogen (transfer to a small vial may be necessary). Dissolve the residue in 200 µL methanol. Inject 20 µL into the HPLC.

- ¹ Centrifuging removes additional water which aids in sample concentration.
- ² Methanol will provide adequate recoveries. Acetone was used to decrease the amount of time required to concentrate the sample to dryness.

Equipment

- AccuBOND[®] ODS (C18) 6 mL/500 mg cartridge (P/N 188-1356)
- vacuum manifold (P/N 5185-5754, 10-port) (P/N 5185-5765, 20-port)

Reagents

- water (HPLC grade)
- methanol (HPLC grade)
- sodium acetate (prepare at 0.10M in water)
- acetone (pesticide grade)



Compound	k	\bar{x}	Std. Dev.
Bromazepam	4.58	62	7
Nitrazepam	5.58	99	8
Clonazepam	6.04	76	5
Oxazepam (Serax)	7.36	78	3
Flunitrazepam (Rohypnol)	8.26	65	3
Lorazepam (Ativan)	8.86	64	1
Chlordiazepoxide (Librium)	9.52	88	2
Clobazam	10.78	77	4
Temazepam (Restoril)	11.32	61	3
Desmethyldiazepam	13.04	80	3
Diazepam (Valium)	17.82	78	2

Serum spiked at 1 µg/mL

$n = 6$

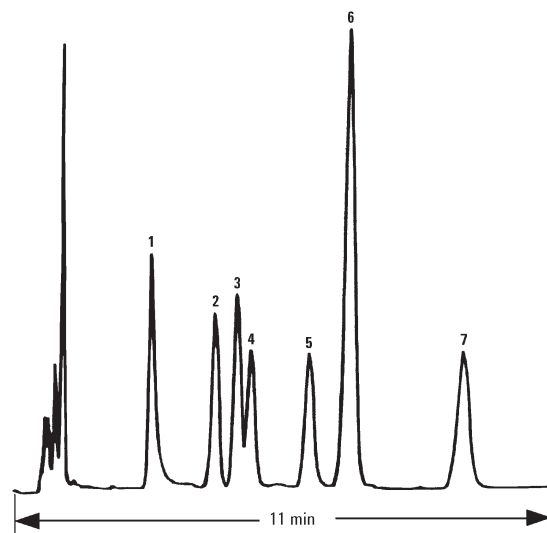
k = partition ratio (a measure of retention)

\bar{x} = % recovery

Serum Extract Containing Benzodiazepines

Column: C8, 15 cm x 4.6 mm I.D.
5 microns
Mobile Phase: Acetonitrile/Methanol/
0.005M KH_2PO_4 (15/30/55)
Flow Rate: 2 mL/min
Temperature: 40°C
Detector: Diode array at 245 nm

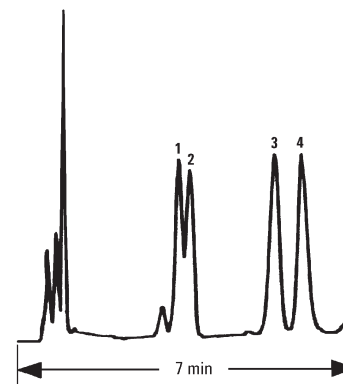
1. Bromazepam
2. Oxazepam
3. Flunitrazepam
4. Lorazepam
5. Temazepam
6. Desmethyldiazepam
7. Diazepam



Steroid Extract Containing Benzodiazepines

Column: C8, 15 cm x 4.6 mm I.D.
5 microns
Mobile Phase: Acetonitrile/Methanol/
0.005M KH_2PO_4 (15/30/55)
Flow Rate: 2 mL/min
Temperature: 40°C
Detector: Diode array at 245 nm

1. Nitrazepam
2. Clonazepam
3. Chlordiazepoxide
4. Clobazam



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