

SPESolid Phase
Extraction

Application Note

World Leaders in Sample Preparation

SPE197**ISOLATION OF BENZODIAZAPINES
FROM SERUM FOR HPLC ANALYSIS**

Spe-ed™ Cartridge	Cat. No. 12001- Octadecyl C18/18%, 100mg/1mL. Process with <i>Spe-ed Mate</i> .
Sample Preparation	Adjust pH to 9.6 with 0.1 M sodium borate buffer (pH 9.6)
Cartridge Conditioning	2 x 1mL of methanol, followed immediately by 1mL of water. DO NOT ALLOW CARTRIDGE TO RUN DRY!
Sample Addition	Add internal standards*. Add up to 2mL of prepared sample. Aspirate sample completely through cartridge.
Cartridge Wash	2 sequential 0.50mL volumes of water. Air-dry cartridge under vacuum for 3 minutes.
Analyte Elution	2 sequential 0.2mL volumes of methanol. Dry eluate under N ₂ and minimal heat. Reconstitute with 100uL of mobile phase. Inject 15-20uL of mobile phase inject 15-20uL onto HPLC.
HPLC conditions	Ultrasphere ODS, 4.6mmx 150mm,5 micron.
Column	35% acetonitrile, and 65% 7 mM K ₂ HPO ₄
Mobile Phase	buffer adjusted to pH 3.7 with 0.1M phosphoric acid
Flow Rate	2.0mL / minute.
Detector	272NM
Temperature	55°C

*Internal standard is nitrazepam. Dilute in 0.1M sodium borate buffer from stock solution (nitrazepam in methanol) daily.

The logo for Applied Separations, featuring the company name in a bold, italicized sans-serif font with horizontal lines above the word 'Applied'.

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