

**SPE**Solid Phase  
Extraction

# Application Note

World Leaders in Sample Preparation

**SPE154****ISOLATION OF PHENOBARBITAL  
FROM SERUM FOR HPLC**

<b>Spe-ed™ Cartridge</b>	Cat. No. 12001-Octadecyl C18/18%, 100mg/1mL. Process with <i>Spe-ed Mate</i> .
<b>Cartridge Conditioning</b>	2 x 1mL methanol 2 x 1mL of distilled water <b>DO NOT ALLOW CARTRIDGE TO RUN DRY!</b>
<b>Sample Addition</b>	Add 500uL serum, and 100uL 0.1 M phosphate buffer (pH 3.5) and 100uL internal standard, 200ug/mL 5-p-methylphenyl-5-phenyl hydantoin (MPPH)
<b>Cartridge Wash</b>	3 x 1mL distilled water.
<b>Analyte Elution</b>	2 x 200uL methanol. Evaporation to dryness under nitrogen, and reconstitute in 200uL mobile phase, or inject directly.
<b>CG Conditions</b>	
<b>Column</b>	C18, 10 micron, 2.1mm x 250mm
<b>Mobile Phase</b>	25%acetonitrile/75% 3.5 mM H <sub>3</sub> PO <sub>4</sub>
<b>Flow</b>	1.5mL/minute
<b>Detector</b>	50°C.
<b>Injection</b>	200nm 0.1 a.u.f.s. 50uL

**Note:** *Since sample matrix interferences and concentrations may vary from sample to sample, it may be necessary to adjust the wash and elution solvent/solution strength and/or volume to optimize isolation.*

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

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