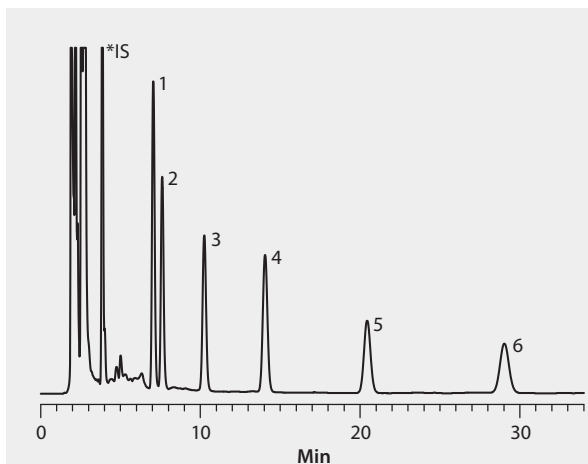


HPLC Analysis of Barbiturates in Serum on Discovery® C18 after SPE using Discovery® DSC-18Lt

Barbiturates are commonly abused and among the most widely tested compounds in clinical, forensic, or therapeutic drug monitoring applications. Shown here is the baseline separation of a set of barbiturates on a Discovery C18 HPLC column after extraction from spiked serum with Discovery DSC-18Lt SPE. Highest grade HPLC solvents were used to supply low background interference and low particulate contamination for robust, trouble-free operation. Cerilliant and Sigma-Aldrich reference standards provided reliable identification and quantification. A Zymark® RapidTrace® SPE Workstation was used in this study.

market focus	Forensics and Toxicology
sample preparation	SPE (Solid Phase Extraction)
sample/matrix	0.5 mL porcine serum spiked with 0.5 µg/mL or 1.0 µg/mL each analyte then diluted with 0.5 mL water
SPE tube/cartridge	Discovery DSC-18Lt, 500 mg/3 mL (52613-U)
condition	2 mL methanol; 2 mL DI water
sample addition	1 mL at 0.75 mL/min
washing	2 mL 5% methanol, then vacuum or air dry for 5-10 min
elution	1-2 mL methanol
eluate post-treatment	dry eluate with nitrogen purge (40 °C; 15-20 min), reconstitute in 20 µL mobile phase
column	Discovery C18, 15 cm x 4.6 mm, 5 µm preceded by a 2 cm C18 guard column and 0.5 µm frit filter (504955)
mobile phase	[A] methanol; [B] water (40:60, A:B)
flow rate	1 mL/min
column temp.	30 °C
detector	UV, 214 nm
injection	30 µL, diluted porcine serum extract
Application No.	G001056



Barbiturates from serum using 500 mg/3 mL Discovery DSC-18Lt SPE tubes and Zymark's RapidTrace SPE Workstation.
 *IS = Barbitol (internal standard).
 Sample Info: 0.5 mL porcine serum spike with 0.5 µg/mL or 1.0 µg/mL each analyte then diluted with 0.5 mL water.

Compound	Efficiency of Recovery		
	Concentration (µg/mL)	%Recovery	%RSD (n=6)
1. Phenobarbital	0.5	96.2	±1.6
	1.0	94.9	±1.7
2. Aprobarbital	0.5	98.5	±2.1
	1.0	100.8	±0.8
3. Butabarbital	0.5	97.2	±1.9
	1.0	98.7	±1.8
4. Mephobarbital	0.5	99.7	±2.4
	1.0	101.0	±2.0
5. Pentobarbital	0.5	96.4	±1.7
	1.0	96.4	±1.9
6. Secobarbital	0.5	98.2	±1.7
	1.0	97.7	±1.8

SPE Method For RapidTrace SPE Workstation Application

- Condition & equilibrate each tube/well with 2 mL MeOH & 2 mL DI Water
- Load sample
- Wash each tube/well with 2 mL 5% MeOH
- Vacuum or air dry for 5-10 min
*This removes any excess water from the sorbent.
 The presence of water in the final eluent may prolong eluent evaporation.*
- Elute with 1-2 mL MeOH
- Dry eluate with nitrogen purge (40 °C; 15-20 min)
- Reconstitute with 200 µL mobile phase
- Quantify against internal or external standards via HPLC analyses

Related Products

- analytical column
 Discovery® C18 HPLC Column ([Supelco 504955](#))
- SPE tube or plate
 Discovery® DSC-18Lt SPE Tube ([Supelco 52613-U](#))
- standard
 Aprobarbital ([Sigma A7023](#))
 Barbiturate Mix-5 solution ([Cerilliant B-041](#))
 Butabarbital solution ([Cerilliant B-024](#))
 Mephobarbital ([Fluka 1386000](#))
 Pentobarbital solution ([Cerilliant P-010](#))
 Phenobarbital solution ([Cerilliant P-008](#))
 Secobarbital solution ([Cerilliant S-002](#))