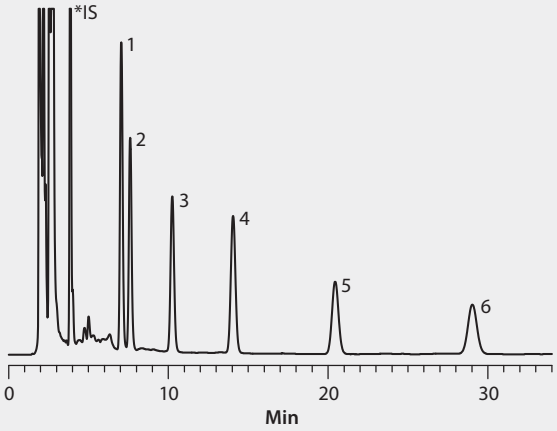
Email: techserv@sial.com

# 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 spikd 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 × 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 = Barbital (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.

**Efficiency of Recovery**

| Compound | 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**

1. Condition & equilibrate each tube/well with 2 mL MeOH & 2 mL DI Water
2. Load sample
3. Wash each tube/well with 2 mL 5% MeOH
4. 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.*
5. Elute with 1–2 mL MeOH
6. Dry eluate with nitrogen purge (40 °C; 15–20 min)
7. Reconstitute with 200 µL mobile phase
8. 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)