



## SPE Application Note for Barbiturates from Urine (HCX method)

The following method was developed for the extraction of barbiturate drugs from urine, including: butobarbital, amobarbital, cyclobarbital and phenobarbital. Typical recoveries for these drugs are > 85%.

### EXTRACTION PROCEDURE

**ISOLUTE® SPE Column:** Confirm HCX 130 mg/3 mL Part # 902-0013-B

**Pre-treatment:** Add 1 mL of 0.05 M phosphate buffer, pH 6.0 to 2 mL sample. Spike with 100 uL of internal std.

**Solvation:** Condition the column with 1 mL of methanol at 2 to 4 mL/min.

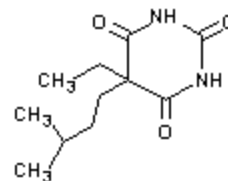
**Equilibration:** Rinse column with 1 mL of 0.01M HCl at 2 to 4 mL/min.

**Sample application:** Load the sample at 1 to 2 mL/min.

**Interference elution:** Rinse the column with 2 mL of ACN/acetone/0.01 M HCl, 15:15:70, at 2 to 4 mL/min. Dry on vacuum box for 1 minute at -20 psig.

**Analyte elution:** Elute analytes with 1.5 mL hexane/ethyl acetate/acetone, 50:40:10, at 1.5 mL/min. Evaporate to dryness. Reconstitute in 50uL BSTFA. Heat for 15 min at 60 C.

**Structure** These compounds are barbituric acids. Amobarbital is shown as an example.



**Structural considerations** Advantage is taken of both the hydrophobic character and other characteristics of this group of analytes for the extraction.

**Matrix considerations** The analyte is being extracted from an aqueous matrix of high ionic strength. There are potential interferences from this matrix. A mixed mode retention mechanism allows a rigorous clean-up procedure.

**Analytical method** GC-MS.

Column: DB-5 capillary, 15 m x 0.25 mm i.d. x 0.25 um film.  
Initial Temp: 100 C  
Temp Ramp: 15 C / min

IST 1032 A

Last Revised: 23-Mar-99

Page 1 of 2



Final Temp: 250 C for 3 mins  
Detection: MS

- Reagents**
- a) 0.05 M phosphate buffer, pH 6.0. Weigh potassium dihydrogen orthophosphate (6.8 g) into a 1L volumetric flask containing 100 mL of deionized water. Adjust the pH to 6.0 (+/- 0.1) using 1.0 M potassium hydroxide. Dilute to mark with deionized water.
  - b) 0.01 M Hydrochloric acid. Add 2 mL of concentrated HCl to 200 mL deionized water in a 250 mL volumetric flask. Mix and dilute to mark with deionized water.
  - c) ACN/acetone/0.01 M HCl 15:15:70. Add 15 mL of acetonitrile and 15 mL of acetone to a 100 mL volumetric flask. Dilute to mark with 0.01 M hydrochloric acid.
  - D) Hexane/ethyl acetate/acetone 50:40:10. Add 40 mL of ethyl acetate and 10 mL of acetone to a 100 mL volumetric flask. Dilute to mark with hexane.

**General comments** Derivatization is recommended but not required prior to GC analysis.

ISOLUTE column part numbers represent the product configuration of choice for use with a vacuum sample processing station. For 96-well and alternative column configurations compatible with any SPE automation system, please contact Biotage.

© 2006 Argonaut Technologies, now Biotage company. All rights reserved. ISOLUTE is a registered trademark of Argonaut Technologies, now a Biotage company.

**United States and Canada**

T: + 1 434 9792319  
Toll-Free: +1 800 446 4752  
ordermailbox@biotage.com

**Sweden**

Biotage  
T: + 46 18 56 59 00  
order@eu.biotage.com

**United Kingdom, EIRE**

Biotage  
T: + 44 1443 811811  
eurosales@eu.biotage.com

**Japan**

Biotage  
T: + 81 422 281233  
order@biotage.co.jp

