



BARBITURATES IN EQUINE URINE FOR GC/MS CONFIRMATIONS

UCT Part Numbers:

XRDAH206 - 200 mg XtrackT[®] DAU Extraction Column in 6 mL cartridge, with CLEAN-THRU[®] Tips

or

XCDAH206 - 200 mg XtrackT[®] DAU Extraction Column in 6 mL cartridge without Tips

CLTTP050 - CLEAN-THRU[®] Tips

SBSTFA-0-1 – SELECTRA-SIL[®] BSTFA, 10 x 1gm vial pack

OPTIONAL:

STMPAH-0-1 – SELECTRA-SIL[®] TMPAH, 10 x 1gm vial pack

1. PREPARE SAMPLE:

To 2 mL of urine add internal standard(s) and 1 mL of 100 mM phosphate buffer (pH= 5.0)

Mix/vortex.

Sample pH should be 5.0 ± 0.5

Adjust pH accordingly with 100 mM monobasic or dibasic sodium phosphate

2. CONDITION XTRACKT[®] DAU EXTRACTION COLUMN:

1 x 3 mL CH₃OH

1 x 3 mL D.I. H₂O

1 x 1 mL 100 mM phosphate buffer (pH= 5.0)

NOTE: Aspirate at full vacuum or pressure

3. APPLY SAMPLE:

Load at 1 to 2 mL/ minute

4. WASH COLUMN:

1 x 3 mL D.I. H₂O

1 x 1 mL 100 mM acetic acid

Dry column (5 minutes at full vacuum or pressure)

1 x 2 mL Hexane

5. ELUTE BARBITUATES:

1 x 3 mL Hexane/ Ethyl Acetate (50: 50)

Collect eluate at 1 to 2 mL / minute

6. DRY ELUATE:

Evaporate to dryness at < 40 °C

Reconstitute with 100 µL Ethyl Acetate

7. QUANTITATIVE

Add 50 µL of both Ethyl Acetate and BSTFA

(OPTIONAL DERIVATIZATION

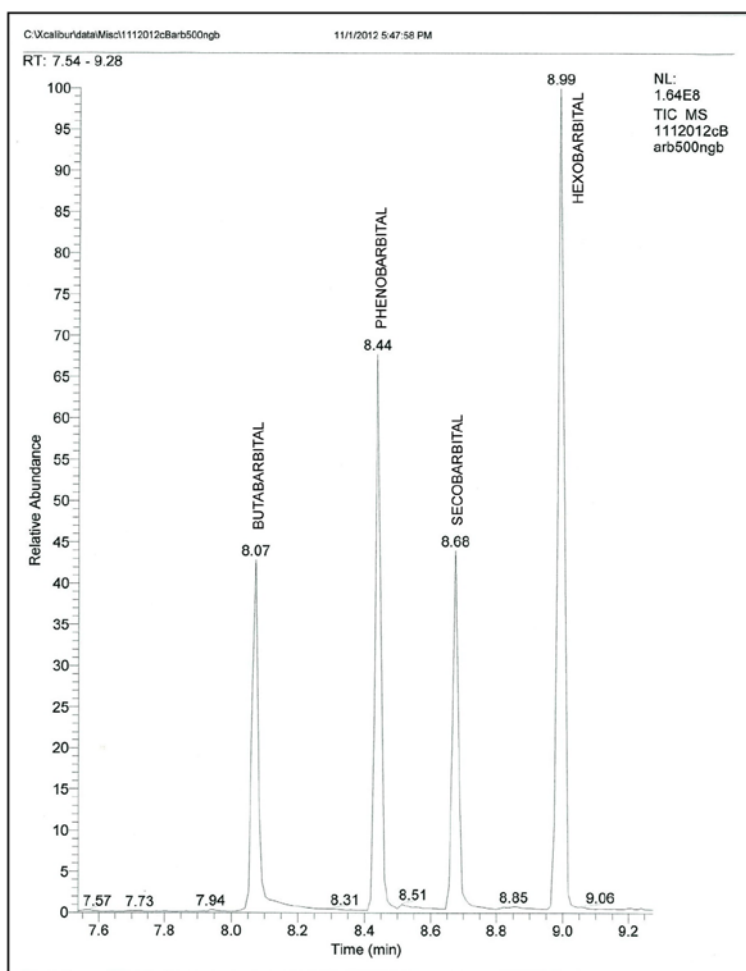
Add 25-50 µL of 0.2 M TMPAH

Reaction occurs in injection port)

Inject 1 to 2 µL onto gas chromatograph

CHROMATOGRAM

Underivatized Barbituates



Mass Spec Table

Compound	Primary Ion*	Secondary Ion	Tertiary Ion
Butabarbital	156	141	157
Phenobarbital	204	232	117
Secobarbital	168	167	195
Hexobarbital [†]	221	157	236

*Quantitation Ion

[†]Suggested internal standard for GC/MS

Other Barbituates that can be extracted using this protocol

Compound	Primary Ion*	Secondary Ion	Tertiary Ion
Amobarbital	156	141	157
Butalbital	168	167	181
Pentobarbital	156	141	195
Thiopental	172	157	173

*Quantitation Ion

Mass Spec Ion Table for derivatized barbituates

Compound	Primary Ion*	Secondary Ion	Tertiary Ion
Butalbital	196	195	209
Butalbital-D ₅ [†]	201	214	
Amobarbital	169	184	185
Pentobarbital	169	184	112
Secobarbital	196	195	181
¹³ C ₄ Secobarbital [†]	200	185	
Phenobarbital	232	146	175
Phenobarbital-D ₅ [†]	237	151	

*Quantitation Ion

[†]Suggested internal standard for GC/MS