AMPHETAMINES IN BLOOD, PLASMA/SERUM, URINE, OR TISSUE USING CLEAN SCREEN® DAU SPE AND LC-MS/MS ANALYSIS

UCT Part Numbers

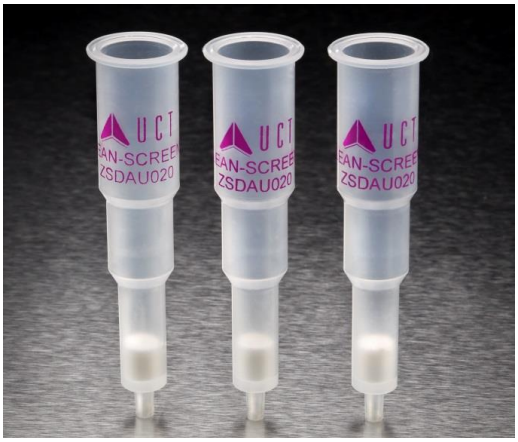
CSDAU206
Clean Screen® DAU
200 mg, 6 mL Column

SLPFPP50ID2.1-1.8UM
Selectra® PFPP UHPLC Column
50 X 2.1 mm, 1.8 µm

SLPFPPGDC20-1.8UM
Selectra® PFPP Guard Column
10 X 2.0 mm, 1.8 µm

SLGRDHLDR-HP
Guard Column Holder

CLINICAL FORENSICS



Summary:

Amphetamines are a group of drugs that stimulate the central nervous system (CNS). These drugs can be administered in the body through many ways. While oral consumption is the most common route, they also can also be snorted, smoked and injected intravenously. Increasing abuse potential and dependence liability of amphetamine & methamphetamine have caused the DEA/FDA to classify these drugs as Schedule II controlled substances. The ease of manufacturing has made methamphetamine one of the most frequently encountered substance in drug related cases. Designer drugs metylenedioxymethamphetamine (MDMA) and methylenedioxyamphetamine (MDA) are methylenedioxy derivatives of methamphetamine and amphetamine respectively. Phentermine is a schedule IV drug that is not heavily abused but is known to exert effects that are similar to Amphetamine.

This application note describes a simple and robust solid-phase extraction (SPE) procedure for amphetamines in blood, plasma/serum, urine and tissue samples. The mixed-mode functionality of the Clean Screen® DAU SPE cartridge ensures efficient extraction of the amphetamines while removing undesired matrix components and yielding clean extracts. UHPLC separation was carried out using a Selectra® PFPP column prior to detection by tandem mass spectrometry (MS/MS). The PFPP (pentafluorophenylpropyl) stationary phase can undergo dipole–dipole and pi–pi interactions, imparting unique selectivity and retention mechanisms to the column that distinguish it from other stationary phases. In this application excellent retention of the polar amphetamines, including baseline separation of the isobaric methamphetamine and phentermine, was obtained in less than 4.5 minutes.

SPE Procedure:

1) **Sample Preparation:**

- Add appropriate volumes of internal standard to 1 -2 mL of blood, plasma/ serum, urine, or 1 g (1:4) tissue homogenate
- Mix/vortex briefly and let stand for 5 minutes
- Add 3 mL of 100 mM phosphate buffer (pH 6.0)
- Mix/vortex briefly
- For blood, plasma/ serum tissue homogenate samples, centrifuge for 10 minutes at 2000 rpm (discard pellet after loading sample onto SPE cartridge)

2) **Condition SPE Sorbent:**

- 1 x 3 mL methanol
- 1 x 3 mL 100 mM phosphate buffer (pH 6.0)

3) **Apply sample:**

- Load sample at 1-2 mL/minute

4) **Wash Sorbent:**

- 1 x 3 mL 0.1 M HCl
- 1 x 3 mL methanol

5) **Dry Sorbent:**

- Dry SPE cartridge for 2 mins at 80-100 psi

6) **Elute:**

- 1 x 3 mL ethyl acetate/ IPA/ NH_4OH (78:20:2)
- Collect eluate at 1-2 mL/minute

7) **Evaporate Eluent:**

- Evaporate the eluent for 5 minutes to remove NH_4OH (40°C, gentle stream of N_2)
- Add 100 µL of 1% HCl in methanol to prevent volatization of the drugs and loss during evaporation

Note: it is important to remove the NH_4OH prior to adding 1% HCl in methanol, otherwise a white precipitate (NH_4Cl) will form.

8) **Reconstitute:**

- Reconstitute samples in 100 µL of mobile phase (alternative volumes may also be used)

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LC-MS/MS Parameters:

System: Shimadzu LCMS-8050	
UHPLC Column: Selectra® PFPP (50 X 2.1 mm, 1.8 µm)	
Guard Column: Selectra® PFPP (10 X 2.0 mm, 1.8 µm)	
Column Temperature: 40°C	
Flow Rate: 0.5 mL/min	
Injection Volume: 2 µL	
Autosampler temperature: 10°C	
Gradient Program:	
Time (min)	% Mobile Phase A (0.1% Formic Acid in Water)	% Mobile Phase B (0.1% Formic Acid in Methanol)
0.0	100	0
0.5	70	30
3.0	60	40
3.5	0	100
4.5	0	100
4.6	100	0
6.0	100	0

Results:

Recovery - Blood				
Analyte	**10 ng/mL (n=3)**	**Rel. Std Dev (%)**	**100 ng/mL (n=3)**	**Rel. Std Dev (%)**
Amphetamine	96%	2.55	92%	0.78
Methamphetamine	95%	2.59	92%	1.72
Phentermine	104%	3.37	95%	8.16
MDA	100%	4.80	94%	1.16
MDMA	97%	3.36	94%	0.64
MDEA	93%	1.87	91%	0.36
Recovery - Urine				
Analyte	**10 ng/mL (n=3)**	**Rel. Std Dev (%)**	**100 ng/mL (n=3)**	**Rel. Std Dev (%)**
Amphetamine	104%	2.85	95%	2.02
Methamphetamine	103%	3.38	93%	3.03
Phentermine	117%	4.08	107%	5.25
MDA	106%	2.27	96%	3.04
MDMA	105%	2.82	96%	2.62
MDEA	102%	2.16	94%	2.41

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Chromatograms:

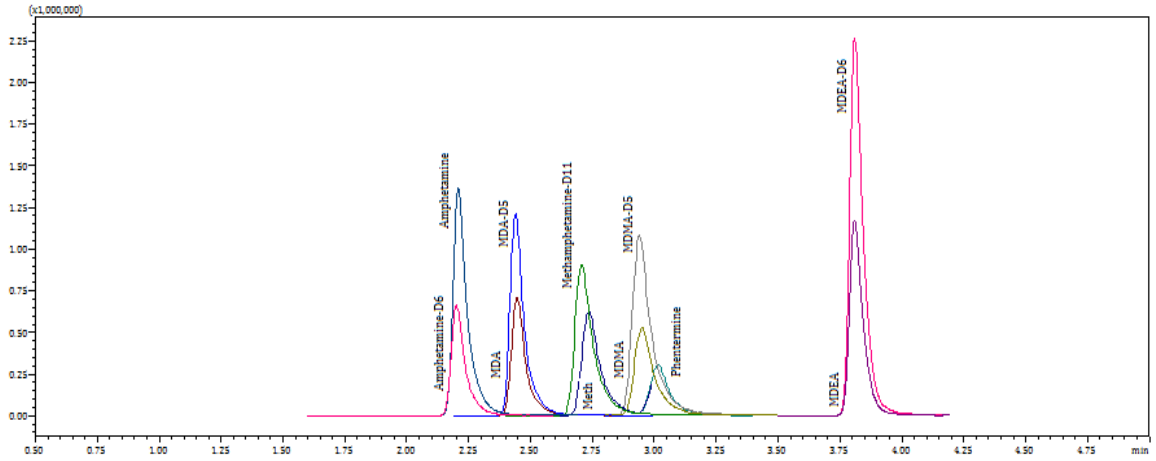

Figure 1. Chromatogram of a 10 ng/mL extracted sample.

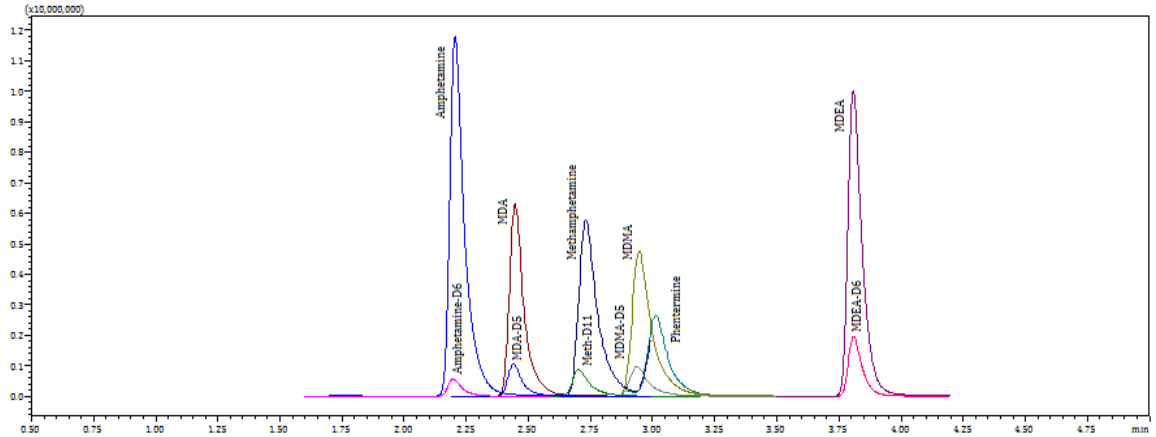

Figure 2. Chromatogram of a 100 ng/mL extracted sample.

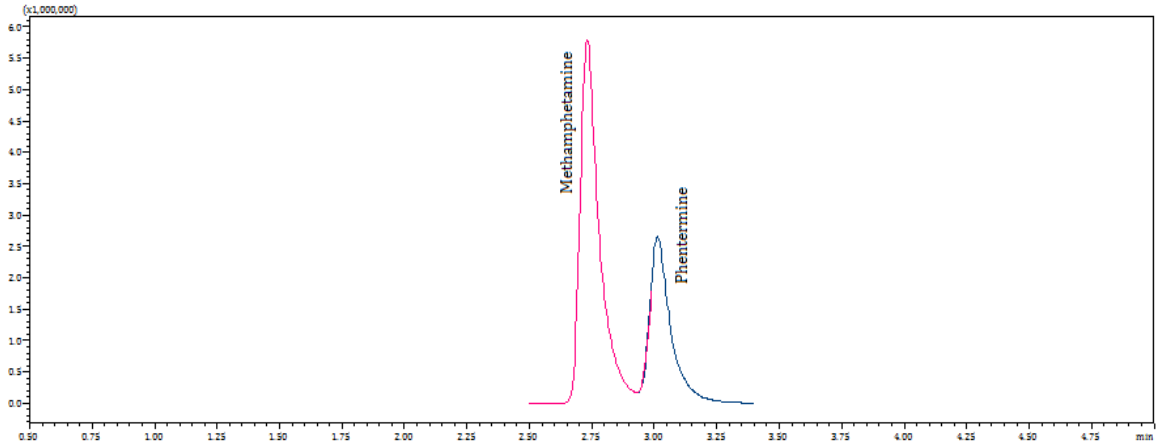

Figure 3. Chromatogram showing excellent baseline separation of methamphetamine & phentermine

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Calibration Curves:

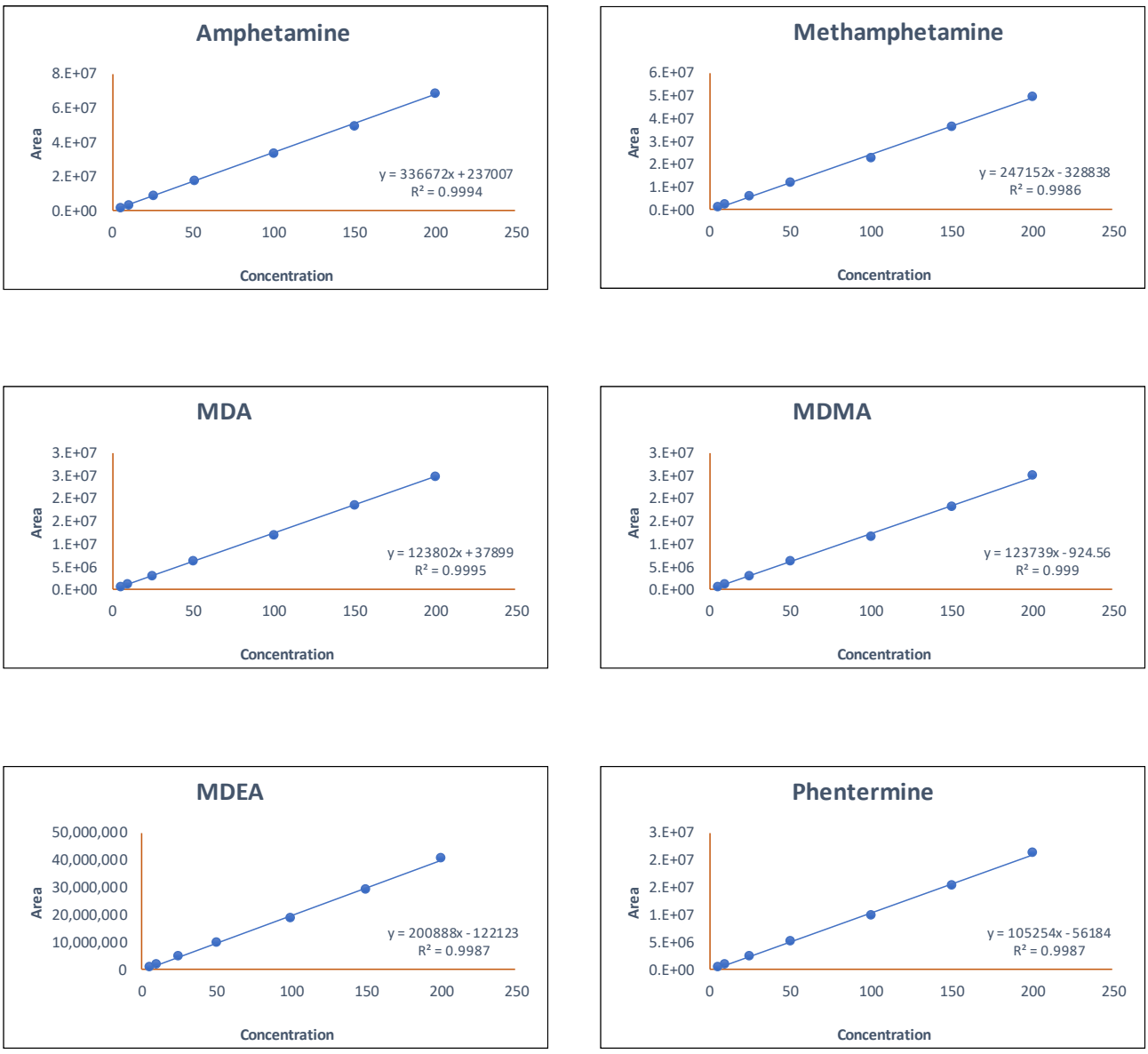

Figure 4. Calibration curve for the six amphetamines (5, 10, 25, 50, 100, 150 & 200 ng/mL).

Note: For accurate quantitation of recoveries and to prevent saturation of the MS detector, a calibration curve ranging from 5-200 ng/ml was utilized for this study. Depending upon the requirements of an individual testing lab, a calibration curve with a wider concentration range may be required for routine analysis.

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