



SYMPATHOMIMETIC AMINES IN BLOOD, PLASMA/SERUM, AND URINE BY LC-MS/MS OR GC-MS CLEAN SCREEN[®] DAU EXTRACTION COLUMN

Part #

CSDAU – CLEAN SCREEN[®] DAU

PFAA-0-1 – SELECTRA-SIL[®] PFAA

SPFPOH-1 – SELECTRA-SIL[®] PFPOH

SHFAA-0-1 – SELECTRA-SIL[®] HFAA

SBSTFA-1-1 – SELECTRA-SIL[®] BSTFA w/ 1% TMCS

SLDA50ID21-5UM – SELECTRA[®] DA HPLC Column, 50 x 2.1 mm, 5 μ m

1. PREPARE SAMPLE:

To 1 mL of 100 mM phosphate buffer (pH 6.0) add internal standards
Add 1 -2 mL of blood, plasma/ serum, urine, or 1 g (1:4) tissue homogenate
Mix/vortex and let stand for 5 minutes
Add 2 mL of 100 mM phosphate buffer (pH 6.0). Mix/vortex
Sample pH should be 6.0 ± 0.5 .
Adjust pH accordingly with 100 mM monobasic or dibasic sodium phosphate.
Centrifuge for 10 minutes at 2000 rpm and discard pellet

2. CONDITION CLEAN SCREEN[®] EXTRACTION COLUMN:

1 x 3 mL CH₃OH
1 x 3 mL D.I. H₂O
1 x 3 mL 100 mM phosphate buffer (pH 6.0)
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 3 mL 100 mM Acetic Acid
1 x 3 mL CH₃OH
Dry column (5 minutes at full vacuum or pressure)

5. ELUTE SMA'S:

1 x 3 mL CH₂Cl₂/ IPA/ NH₄OH (78:20:2)
Collect eluate at 1 to 2 mL/minute

NOTE: Prepare elution solvent daily
Add IPA/ NH₄OH, mix, then add CH₂Cl₂ (pH 11-12)

6. DRY ELUATE:

Add 100 μ L of 1% HCl in Methanol to each test tube
Evaporate to dryness at < 40 °C

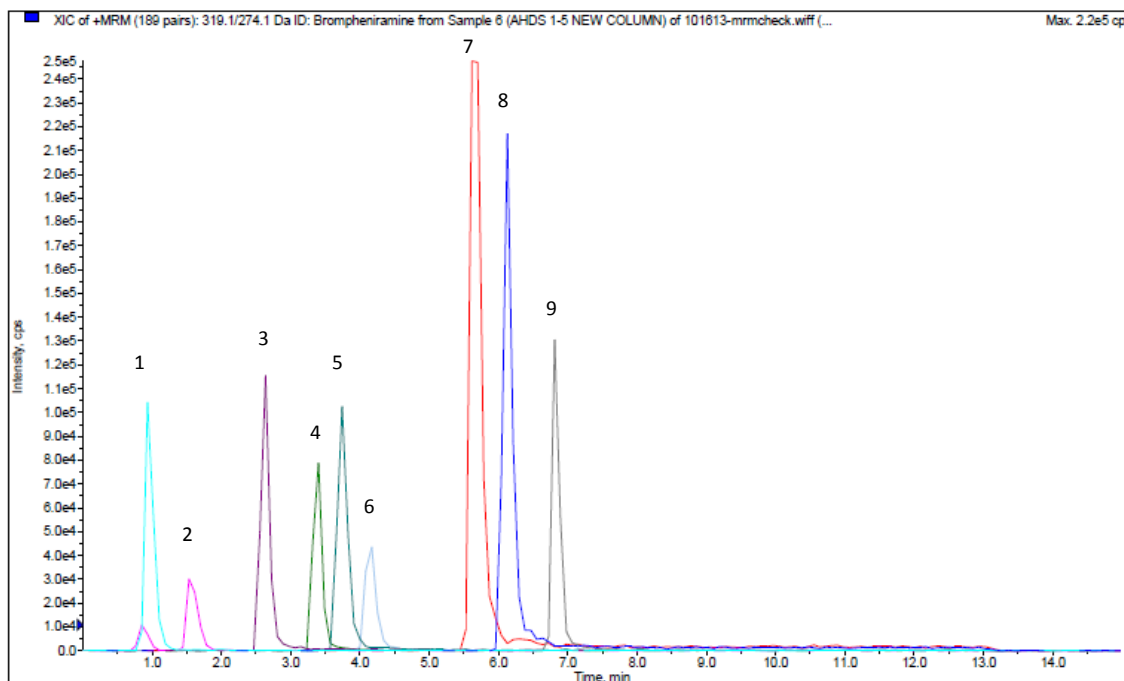
7. RECONSTITUTE / DERIVATIZE:

- **LC-MS/MS:** Reconstitute sample in 100 μ L of mobile phase
Inject 20 μ L.
- **GC-MS:** Fluoroacylate with PFPA (PFAA)
Add 50 μ L PFPA. Over lay with N₂ and cap
*Improved derivatization by addition of PFPOH
React 20 minutes at 70 °C. Evaporate to dryness <40 °C
Reconstitute with 100 μ L Ethyl Acetate

Alternate Derivatization

1. Fluoroacylate with HFPA (HFAA)
Add 50 μ L HFPA. Over lay with N₂ and cap
*Improved derivatization by addition of PFPOH
React 20 minutes at 70 °C. Evaporate to dryness <40 °C
Reconstitute with 100 μ L Ethyl Acetate
2. Form TMS Derivatives by adding 50 μ L BSTFA w/ 1% TMCS and 50 μ L of Ethyl Acetate;
React 45 minutes at 70 °C

INSTRUMENT CONDITIONS (LC-MS/MS):



Analyte	MRM Transitions		Relative Retention Time (min)
	Q1	Q3	
1. Phenylpropanolamine	152.2	134.2	0.95
2. Ephedrine	166.2	148.3	1.55
3. Phentermine	150.2	91.20	2.60
4. Diethylpropion	206.2	100.2	3.40
5. Pheniramine	241.2	167.2	3.75
6. Doxylamine	271.2	167.2	4.15
7. Chlorpheniramine	275.1	230.1	5.65
8. Brompheniramine	319.1	274.1	6.13
9. Diphenhydramine	256.1	152.1	6.82

PARAMETERS

Mobile Phase A: 0.1% Formic Acid in D.I. H₂O

Flow Rate: 0.5 mL/minute

Reconstitute: 100 μ L

LC Column: Selectra[®] DA HPLC Column 50 x 2.1 mm 5 μ m

Instrument: API 3200 Qtrap MS/MS with Shimadzu Prominence UFLC

Mobile Phase B: 0.1% Formic Acid in Methanol

Polarity: Positive

Injection Volume: 20 μ L

Gradient:

Time	%A	%B
0.0	80	20
0.5	80	20
12.00	10	90
12.01	80	20
15.00	STOP	

INSTRUMENT CONDITIONS (GC-MS):**PFPA DERIVATIZATION**

Analyte	Quantify Ion	Qualifier Ion 1	Qualifier Ion 2
Amphetamine	190	91	118
Amphetamine-D ₅	194	92	123
Methamphetamine	204	91	160
Methamphetamine-D ₅	208	92	163
MDA	135	162	325
MDMA	204	162	339
Pseudoephedrine	204	160	119
Phenylephrine	190	119	267
Ephedrine	204	160	119

HFPA DERIVATIZATION

Analyte	Quantify Ion	Qualifier Ion 1	Qualifier Ion 2
Amphetamine	240	118	91
Amphetamine-D ₅	244	123	122
Methamphetamine	254	118	91
Methamphetamine-D ₅	258	213	120
MDA	375	162	135
MDMA	254	210	162
MDEA	268	240	162
Pseudoephedrine	344	254	210
Ephedrine	344	254	169

BSTFA DERIVATIZATION

Analyte (TMS)	Quantify Ion	Qualifier Ion 1	Qualifier Ion 2
Amphetamine	116	192	91
Amphetamine-D ₅	120	197	92
Amphetamine-D ₆	120	198	93
Amphetamine-D ₁₀	120	202	97
Amphetamine-D ₁₂	120	203	98
Methamphetamine	130	206	91
Methamphetamine-D ₅	134	211	92
Methamphetamine-D ₈	137	214	92
Methamphetamine-D ₉	137	215	93
MDA	116	236	135
MDMA	130	250	131
Pseudoephedrine	130	147	294
Ephedrine	130	147	294