



**SYMPATHOMIMETIC AMINES IN BLOOD, PLASMA/SERUM
URINE AND TISSUE FOR GC OR GC/MS
CONFIRMATIONS USING: 200 mg CLEAN SCREEN[®]
EXTRACTION COLUMN**

Part #: ZSDAU020 without Tips or ZCDAU020 with CLEAN-THRU[®] Tips

1. PREPARE SAMPLE

To 1 mL of 100 mM phosphate buffer (pH 6.0) add internal standard(s). * Add 1 mL of blood, plasma/serum, urine or 1g of (1:4) tissue homogenate. Mix/vortex
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 as appropriate.

2. CONDITION CLEAN SCREEN[®] EXTRACTION COLUMN

1 x 3 mL CH₃OH.
1 x 3 mL D.I. H₂O.
1 x 1 mL 100 mM phosphate buffer (pH= 6.0).
NOTE: Aspirate at < 3 inches Hg to prevent sorbent drying.

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.
1 x 3 mL CH₃OH.
Dry column (5 minutes at > 10 inches Hg).

5. ELUTE SMA

1 x 3 mL CH₂Cl₂/IPA/NH₄OH (78:20:2); Collect eluate at
1 to 2 mL/minutes.
NOTE: Prepare elution solvent daily. Add IPA/NH₄OH, mix, then add CH₂Cl₂.

6. CONCENTRATE ELUATE

Add 30 μ L silylation grade DMF to eluate.
Evaporate to 30 μ L at < 40°C.

ALTERNATE DRYING PROCEDURE

Evaporate for 4 min.
Add 100 μ L of 1% HCl in methanol.
Evaporate to dryness.

7. FLUOROACYLATE WITH PFPA (PFAA)***

Add 50 μ L PFPA (PFAA). Overlay with N₂ and cap.
Improved derivatization by addition of 50 μ L PFPOH.****
React 20 minutes at 70°C. Evaporate to dryness at < 40°C.
Reconstitute with 100 μ L ethyl acetate.

8. QUANTITATE

Inject 1 to 2 μ L onto gas chromatograph.
For MSD monitor the following ions:

ANALYTE (PFPA)	Primary Ion**	Secondary	Tertiary	Cerilliant#
Amphetamine-D5*	194	92	123	A-005
Amphetamine	190	91	118	A-007
Methamphetamine-D5*	208	92	163	M-004
Methamphetamine	204	91	160	M-009
Pseudoephedrine	204	160	119	P-035
Ephedrine	204	160	119	E-011
Phenylephrine	190	119	267	
Methylenedioxyamphetamine	135	162	325	M-012
Methylenedioxymethamphetamine	204	162	339	M-013

* Suggested internal standards for GC/MS: D₅-Amphetamine and D₅-Methamphetamine

** Quantification Ion

*** Part # SPFAA-0-1,10, 25,100

**** Part # SPFPOH-1,10,25,100

ALTERNATE DERIVATIZATION

7. Form TMS derivatives:

Add 50 µL BSTFA with 1% TMCS and 50 µL of ethyl acetate.
React 45 minutes at 70°C.

8. QUANTITATE

Inject 1 to 2 µL onto gas chromatograph.

For MSD monitor the following ions:

ANALYTE (TMS)	Primary Ion**	Secondary	Tertiary	Cerilliant#
Amphetamine-D5*	120	197	92	A-005
Amphetamine-D6*	120	198	93	A-044
Amphetamine-D10*	120	202	97	A-038
Amphetamine-D12*	120	203	98	A-019
Amphetamine	116	192	91	A-007
Methamphetamine-D5*	134	211	92	M-023
Methamphetamine-D8*	137	214	92	
Methamphetamine-D9*	137	215	93	M-091
Methamphetamine	130	206	91	M-004
Pseudoephedrine	130	147	294	P-035
Ephedrine	130	147	294	E-011
Methylenedioxyamphetamine	116	236	135	M-012
Methylenedioxymethamphetamine	130	250	131	M-013
Para-Methoxyamphetamine	116	222	121	NMID1908

ALTERNATE DERIVATIZATION

7. Form 4-CB (4-Carboxyhexafluorobutyl chloride)*

derivatives: Add 20 µL 4-CB* and 100 µL of ethyl acetate.
React 45 minutes at 70°C.

8. QUANTITATE

Inject 1 to 2 µL onto gas chromatograph.

For MSD monitor the following ions:

<u>ANALYTE (TMS)</u>	<u>Primary Ion**</u>	<u>Secondary</u>	<u>Tertiary</u>	<u>Cerillinat#</u>
Amphetamine-D5****	298	270	399	A-005
Amphetamine	294	266	248	A-007
Methamphetamine-D5****	312	284	266	M-023
Methamphetamine	308	280	262	M-004
Methylenedioxyamphetamine-D5****	136	434	270	M-010
Methylenedioxyamphetamine	162	429	266	M-012
Methylenedioxymethamphetamine-D5****	312	284	266	M-011
Methylenedioxymethamphetamine	308	280	262	M-013
Methylenedioxyethylamphetamine-D6****	328	165	300	
Methylenedioxyethylamphetamine	322	162	294	M-065

* Part # S4CB-0-10

*** Quantification Ion

**** Suggested internal standards for GC/MS: D₅-Amphetamine and D₅-Methamphetamine