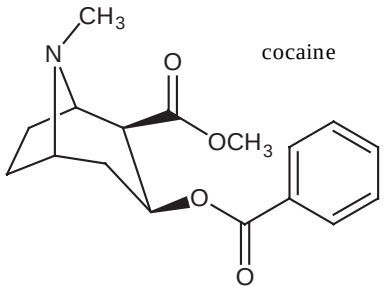
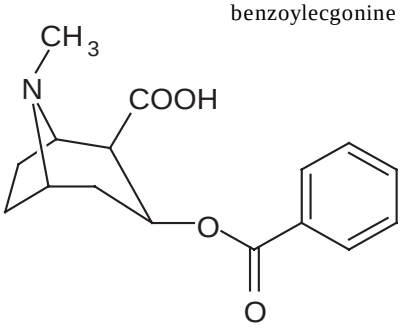
Extraction of Cocaine and Benzoylecgonine from Urine using Strata™ Screen-C

This method is optimized for the extraction and clean-up of cocaine and benzoylecgonine, a metabolite of cocaine, from urine using Strata Screen-C, a mixed-mode sorbent (C8 + SCX). While the metabolite is extracted for SAMSHA drug confirmation, any parent compound present will also be extracted with this method. Benzoylecgonine is an amphoteric compound capable of carrying a positive or negative charge depending on the pH of the solution. The urine sample is loaded at pH 6. The metabolite is negatively charged and thus initially retained by hydrophobic interactions with the C8 portion of the sorbent. Urinary salts and water-soluble contaminants are effectively removed by an aqueous wash. The acid wash step neutralizes the carboxylic acid and protonates the amine group. Benzoylecgonine is now retained by both hydrophobic and ionic interactions. An aggressive organic wash removes any remaining organic contaminants (without a nitrogen group), finishing the clean-up of the sample. A basic organic solution elutes the target analyte. The result is a very clean, concentrated extract.



cocaine



benzoylecgonine

Specimen preparation:
To 5mL of urine add internal standard(s) + 2mL 100mM phosphate buffer (pH 6). Mix/Vortex. Add 1M potassium hydroxide to adjust pH to 6.

Suggested internal standard for GC/MS: d_3-cocaine; d_3-benzoylecgonine.

SPE Method:

Condition
1. 2mL methanol
2. 1mL 100mM phosphate buffer (pH 6)
Load
1. Apply the sample at a rate ≤2mL/min.
Wash/Dry
1. 6mL DI water
2. 3mL 100mM hydrochloric acid
3. 6mL methanol
4. Dry column 5 min at full vacuum (>10" Hg).
Elute Cocaine and Benzoylecgonine
1. With the vacuum turned off, apply 3mL dichloromethane/isopropanol/ammonium hydroxide (78:20:2). Allow solvent to slowly soak into sorbent for 15-30 sec before applying vacuum. Optimal flow rate of elution solvent is ≤2mL/min.

(Important: The volumes shown are for 150mg sorbent mass. The method can be optimized for smaller or larger bed masses, by adjusting the solvent volumes.)

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Derivatize:
Evaporate to dryness at ≤40°C. Add 50µL ethyl acetate + 50µL BSTFA (with 1% TMCS). Cap, mix/vortex and heat for 20 min at 70°C. Allow the solution to cool. Important: Do not evaporate the BSTFA solution.

BSTFA = N ,O-bis(trimethylsilyl)trifluoroacetamide
TMCS = trimethylchlorosilane

Analysis:
Inject 1 to 2µL onto GC column. (recommended: Zebron ZB-5, 15m x 0.25mm x 0.25µm)
Monitor the following ions (MSD):

cocaine	d_3-cocaine	benzoylecgonine-TMS	d_3-benzoylecgonine-TMS	
182	185	240	243	◀ quantification ions
198	201	256	259	
303	306	361	364	

Ordering Information:

Order No.	Description	Unit
8B-S016-EAK	Screen-C Tubes (100mg/1mL)	100/Box
8B-S016-EBJ	Screen-C Tubes (100mg/3mL)	50/Box
8B-S016-SBJ	Screen-C Tubes (150mg/3mL)	50/Box
8B-S016-RBJ	Screen-C Tubes (300mg/3mL)	50/Box
8B-S016-SCH	Screen-C Tubes (150mg/6mL)	30/Box
8B-S016-HCH	Screen-C Tubes (500mg/6mL)	30/Box
8E-S016-CGB	Screen-C 96-Well Plate (25mg/well)	2/Box
8E-S016-DGB	Screen-C 96-Well Plate (50mg/well)	2/Box
7EG-G002-11	Zebron ZB-5 (15m x 0.25m x 0.25µm)	1/Box

Extraction Tips!

1. Preparing solutions

100mM phosphate buffer (pH 6)
Add 13.6g of monobasic potassium phosphate to an empty 1L volumetric flask. Add 900mL DI water to dissolve the solid. Adjust the pH to 6 with 1M potassium hydroxide while stirring. Bring the volume up to the mark with DI water.

1M potassium hydroxide
Add 5.6g of potassium hydroxide to an empty 100mL volumetric flask. Add 90mL DI water to dissolve the solid. Bring the volume up to the mark with DI water.

100mM hydrochloric acid
Add 400mL DI water to an empty 500mL volumetric flask. Add 4.2mL concentrated hydrochloric acid. Bring the volume up to the mark with DI water. Mix.

Dichloromethane/isopropanol/ammonium hydroxide (78:20:2)
Combine 20mL isopropanol with 2mL concentrated ammonium hydroxide. Mix. Add 78mL dichloromethane. Mix.

2. Do not allow the sorbent to dry between the conditioning steps or prior to loading the sample. Excessive drying of the sorbent causes “deconditioning” which may lead to significantly lower and erratic recoveries. To ensure a properly solvated sorbent, apply each solvent immediately after the previous solvent.

3. Always condition the sorbent with the strongest solvent used in the method to ensure the cleanest extraction of the target analytes. In this method, 100mM phosphate buffer (pH 6. is used after methanol.

4. During the wash step, drying the sorbent removes any residual water and will ensure optimal analyte recovery.

5. Prepare the elution solvent daily, as the ammonium hydroxide rapidly dissipates in air.

Questions? Please contact your Phenomenex Technical Representative.

This method is designed as a convenient starting point for further investigation. Phenomenex makes no guarantee regarding the accuracy or completeness of the method.