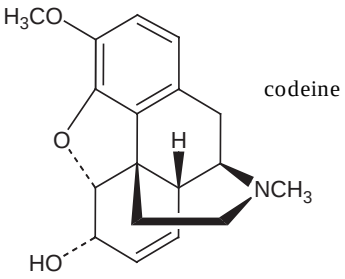
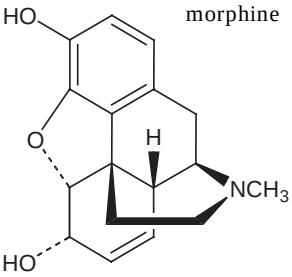
Extraction of Opiates from Urine using Strata™ Screen-C

This method is optimized for the extraction and clean-up of opiates (codeine and morphine) from urine using Strata Screen-C, a mixed-mode sorbent (C8 + SCX). The opiates are present in the urine as glycoside conjugates. Acid hydrolysis is used during specimen preparation to release the conjugated opiates. These opiates are amphoteric compounds capable of carrying a positive or negative charge depending on the pH of the solution. At the loading pH of 8, the compounds are neutral 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 protonates the tertiary amine group on each compound and the codeine and morphine are 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.





Specimen preparation:
Acid hydrolysis: To 5mL of urine add internal standards + 1mL concentrated hydrochloric acid. Mix/Vortex. Heat for 30 min at 100°C. Allow solution to cool to room temperature. Add 2mL 100mM potassium phosphate (pH 6). Mix/Vortex. Adjust sample pH to 8 with 10M potassium hydroxide. Important: the pH should never exceed 8.5, as it will significantly decrease the recovery of morphine.

Suggested internal standard for GC/MS: d_3-codeine and d_3-morphine.

SPE Method:

Condition
1. 2mL methanol
2. 2mL 100mM phosphate buffer (pH 6)
Load
1. Apply the sample at a rate ≤2mL/min
Wash/Dry
1. 2mL DI water
2. 2mL 100mM hydrochloric acid
3. 3mL methanol
4. Dry column 2-5 min at full vacuum (>10" Hg)
Elute Codeine and Morphine
1. With the vacuum turned off, apply 2mL 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):

codeine TMS	d_3-codeine TMS	morphine TMS	d_3-morphine TMS
371	374	429	432 ◀ quantification ions
234	237	287	290
343	346	324	327

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.

10M potassium hydroxide
Add 140g of potassium hydroxide to an empty 250mL volumetric flask. Add 150mL 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.