



SPE Application Note for Codeine and Morphine from Urine

The following method addresses the extraction of the basic drugs morphine and codeine from urine using non-polar and strong cation exchange retention mechanisms.

EXTRACTION PROCEDURE

ISOLUTE® SPE Column: Confirm HCX, 130 mg, 1 or 3 mL
Part numbers 902-0013-A or 902-0013-B

Pre-treatment:

1. Add 0.5 mL of 9 M HCl to 1 mL of urine.
2. Hydrolyze at 120 C for 20 minutes.
3. Add 1 mL of 1 M ammonium acetate buffer, pH~8
4. Add 0.5 mL of conc. ammonium hydroxide.
5. Mix and check that pH is between 7.5 and 8.5.

Solvation: Condition column with 1 mL of methanol at 2 to 4 mL/min.

Equilibration: Rinse column with 1 mL of 0.1M ammonium acetate buffer, pH~8, at 2 to 4 mL/min.

Sample application: Apply sample at 1 to 2 mL/min.

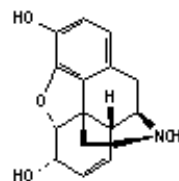
Interference elution: Rinse column with each of the following:

1. 2 mL distilled water at 2-4 mL/min
2. 1 mL 0.01 M HCl at 2-4 mL/min
3. 1 mL methanol at 2-4 mL/min

Dry column under -20 psig vacuum or 20 psig nitrogen for 1 minute.

Analyte elution: Elute analyte with 1 mL of methanol/ammonia (98:2) at 1-2 mL/min.

Structure The structure of morphine is shown here. Codeine has a similar structure, with a methoxy group replacing the hydroxyl group in the 3 position.



Structural considerations The hydrophobic nature of these analytes is exploited for the extraction from urine. The analytes can subsequently be ionized and retained by cation exchange to perform a rigorous clean-up.



Matrix considerations

The analyte is being extracted from an aqueous matrix of high ionic strength.

Analytical method GC-MS

Reagents

1. HCl, 9 M
 2. Ammonium acetate buffer, 1 M, pH~8
 3. Ammonium acetate buffer, 0.1 M, pH~8
 4. Ammonium hydroxide, concentrated
 5. Methanol
 6. Acetic acid, 0.01 M
 7. Ethyl acetate/methanol/ammonia 70:25:5
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General comments

ISOLUTE column part numbers represent the product configuration of choice for use with a vacuum sample processing station. For 96-well and alternative column configurations compatible with any SPE automation system, please contact Biotage.

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