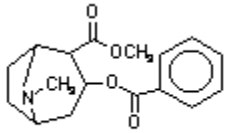
SPE Application Note for
Cocaine and Benzoylecgonine from Urine

The following method has been optimized for the extraction of cocaine and benzoylecgonine from urine for drugs of abuse applications. Typical recoveries of these drugs are > 85%. The procedure may be applied to plasma, serum or whole blood with suitable modifications.

EXTRACTION PROCEDURE

ISOLUTE® SPE Column: Confirm HCX 130 mg/3 mL Part # 902-0013-B

Pre-treatment:	Add 1 mL of 0.05 M phosphate buffer, pH 6.0 to 2 mL urine.
Solvation:	Rinse column with 1 mL of methanol at 2 to 4 mL/min.
Equilibration:	Rinse column with 1 mL of deionized water at 2 to 4 mL/min, followed by 1 mL of 0.05M phosphate buffer (pH=6) at 2 to 4 mL/min
Sample application:	Apply sample at 1-2 mL/min.
Interference elution:	Elute interferences with 2 mL of deionized water at 2 to 4 mL/min, followed by 1 mL of 0.01 M HCl at 2 to 4 mL/min, and then 2 mL of methanol at 2 to 4 mL/min Dry the column for 0.5 min at -20 psig.
Analyte elution:	Elute the analytes with 1.2 mL of methanol/ammonia, 98: 2 at 1 to 2 mL/min Evaporate to dryness. Add 0.5 mL of BSTFA and heat for 15 min at 60 C. Inject into GC. Prepare fresh elutio solvent daily.
Structure	Cocaine is shown. 
Structural considerations	The analytes are relatively non-polar, with a basic group which can be utilized in the extraction.
Matrix considerations	This analyte is present in an aqeous matrix of high ionic strength. A mixed mode extraction mechanism enables a rigorous interference elution step to mininize interferences.
Analytical method	GC-MS Column: DB-5 capillary 15 m x 0.25 mm i.d. x 25 ul film

Initial Temp: 140 C for 1 min
Temp ramp: 15 C / min
Final Temp: 260 C for 3 mins
Detection: MS

Reagents

a) 0.05 M phosphate buffer, pH 6.0. Add 6.8 g of potassium hydrogen orthophosphate to a 1 L volumetric flask containing 900 mL of deionized water. Dissolve. Adjust the pH to 6.0 (+/-0.1) with 1.0 M potassium hydroxide, and make up to the mark with deionized water.
b) 0.01M hydrochloric acid. Add 2 mL of concentrated HCl to a 200 mL of deionized water in a 250 mL volumetric flask. Mix thoroughly. Dilute to mark.
c) Methanol/ammonia 98:2. Add 2 mL of concentrated ammonia to 98 mL of methanol. THIS REAGENT SHOULD BE MADE UP FRESH DAILY.
d) BSTFA containing 1% TMCS. Sigma Part # T6381.

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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