



SPE Application Note for THC-COOH from Urine (HAX)

This method was developed for the extraction of the tetrahydrocannabinol carboxylic acid metabolite from urine using a mixed non-polar and anion exchange retention mechanism. Typical recoveries of the analyte are > 75%.

EXTRACTION PROCEDURE

ISOLUTE® SPE Column: Confirm HAX 200 mg/10 mL Part # 903-0020-H

Pre-treatment: Add 100 µL of internal standard standard to 3 mL of urine. Hydrolyze with 0.1 mL of NaOH, 10 M. Heat for 15 minutes at 60 C. Cool. Add 0.5 mL of glacial acetic acid.

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

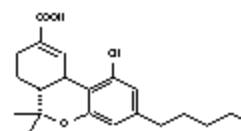
Equilibration: Rinse the column with 1 mL of deionized water at 2 mL/min.

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

Interference elution: Rinse column with 1 mL 0.3 M ammonium acetate buffer, pH 8, at 2 mL/min. Anion exchange counter ion is now acetate. Rinse column with 1 mL of 20 mM ammonium acetate buffer at 2 mL/min, followed by 1 mL of methanol at 2 mL/min.

Analyte elution: Elute analyte with 1 mL of methanol/ethyl acetate/acetic acid 48:50:2, at 1 mL/min.

Structure The analyte has a mainly non-polar structure with a carboxylic acid group.



Structural considerations Due to the presence of a carboxylic acid group, this compound will require derivatization prior to the analytical determination.

Matrix considerations The matrix is of high ionic strength and contains a number of potential interference compounds. The mixed retention mechanism used in this extraction allows a rigorous interference elution procedure.

Analytical method GC, GC-MS

Column: DB-5 capillary, 15 m x 0.25 mm i.d. x 0.25 µm
Initial temp: 180 C for 1 min

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Temp. ramp: 20 C/min
Final temp:- 280 C for 1 min
Detection: MS

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- Reagents**
1. 10 M NaOH
 2. Glacial acetic acid
 3. Methanol
 4. 0.3 M ammonium acetate buffer, pH between 7.5 and 8.5
 5. 20 mM ammonium acetate buffer, pH between 7.5 and 8.5
 6. Methanol/ethyl acetate/acetic acid 48:50:2
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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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