



SPE Application Note for Extraction of NIDA-5 (SAMHSA-5) Analytes Using RapidTrace

This method details the extraction of the NIDA-5 (SAMHSA-5) drug of abuse classes using the same SPE column (ISOLUTE HCX 130 mg/1 mL) and single set of reagents (8 in total) on the RapidTrace.

EXTRACTION PROCEDURE

ISOLUTE® SPE Column: ISOLUTE HCX 130 mg/1 mL, part # 902-0013-A

Pre-treatment: Amphetamine & Metamphetamine:
Add phosphate buffer (pH 6.0, 0.05 M, 0.5 mL) and internal standard (methamphetamine-D5 for GC-MS, 100 uL) to urine (1 mL).

Cocaine & Benzoyllecognine:
Add phosphate buffer (pH 6.0, 0.05 M, 0.5 mL) to urine (2 mL).

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

Phencyclidine:
1. Add 100 uL of internal standard (phencyclidine -D5 for GC-MS)
2. Add phosphate buffer (0.05 M, pH 6.0, 1 mL) to 2 mL urine.
3. Check sample pH is in the range of 4-6.

THC-COOH:
1. Add internal standard (100 uL) to 3 mL of urine.
2. Hydrolyze with NaOH (10 M, 0.1 mL).
3. Heat for 15 minutes at 60 C. Cool.
4. Add glacial acetic acid (0.5 mL).

Solvation: Condition column with methanol (1 mL) at a flow rate of 10 mL/min. This is the same for all drug classes.

Equilibration: Amphetamine & Methamphetamine:
Rinse the column with deionized water (1 mL) at a flow rate of 10 mL/min.
Rinse column with phosphate buffer (pH 6.0, 0.05 M, 1 mL) at a flow rate of 10 mL/min.

Cocaine & Benzoyllecognine:
As above, using flow rates of 6 mL/min.

Codeine & Morphine:



1. Rinse column with deionized water (1 mL) at 10 mL/min.
2. Rinse with ammonium acetate buffer (0.1 M, pH~8, 1 mL) at 10 mL/min.

Phencyclidine:

Condition column with phosphate buffer (0.05 M, pH 6, 1 mL) at 10 mL/min.

THC-COOH:

Rinse column with water (1 mL) at 10 mL/min

Rinse column with HCl (0.01 M, 1 mL) at 10 mL/min

Sample application: Amphetamine & Methamphetamine:
Load sample (1.7 mL) at a flow rate of 2 mL/min.

Cocaine & Benzoyllecognine:
Load sample at a flow rate of 1.5 mL/min.

Codeine & Morphine:
Apply sample volume (3.2 mL) at 2 mL/min.
Rinse with deionized water (2 mL) at 6 mL/min.
Purge cannula with deionized water (4 mL) at 30 mL/min.

Phencyclidine:
Load sample (3.3 mL) at 2 mL/min.

THC-COOH:
Load sample (3.8 mL) at 2 mL/min.

Interference elution: Amphetamine & Methamphetamine:
1. Rinse column with deionized water (1 mL) at a flow rate of 10 mL/min.
2. Purge cannula with deionized water (4.0 mL) at 30 mL/min.
3. Rinse column with HCl (0.01M, 1 mL) at 10 mL/min.
4. Rinse column with methanol (2 mL) at 10 mL/min.
5. Dry column using vacuum for 1 minute at -20 psig.

Cocaine & Benzoyllecognine:
As above, using a flow rate of 6 mL/min instead of 10 mL/min.
Dry the column for 0.5 min at -20 psig

Codeine & Morphine:
1. Rinse column with each of the following:
2. Rinse with HCl (0.01 M, 1 mL) at 3 mL/min.
3. Rinse with methanol (1 mL) at 3 mL/min.
4. Dry column under -20 psig vacuum or 20 psig nitrogen for 1 minute.

Phencyclidine:
Rinse with distilled water (2 mL) at 6 mL/min.
Purge cannula with distilled water (4 mL) at 30 mL/min.
Rinse with HCl (0.01 M, 1 mL) at 10 mL/min
Rinse with methanol (2 mL) at 6 mL/min



Dry column for 0.2 min at -20 psig.

THC-COOH:

Rinse column with water (1 mL) at 6 mL/min.

Purge cannula with water (4 mL) at 30 mL/min.

Rinse column with of acetonitrile/acetone/0.01 M HCl (15:15:70 v/v, 1 mL) at 6 mL/min

Dry under -20 psig nitrogen for 1 minute.

Analyte elution: To elute analytes, apply first volume of elution solvent to extraction cartridge. Soak for two minutes. Add second volume of elution solvent to extraction cartridge and collect.

Elute analytes as described for each drug class below, followed by flushing the cannula as follows:

Purge cannula with methanol (5 mL) at 30 mL/min.

Purge cannula with deionized water (5 mL) at 30 mL/min.

Amphetamine & Methamphetamine:

Before elution, add 0.1 mL of tartaric acid solution to the collection vial, so that the column eluate mixes with the tartaric acid solution on elution. This forms a less volatile 'semi-tartrate' complex with the eluted amphetamine, reducing loss on subsequent evaporation steps.

Collect amphetamines with methanol/ammonium hydroxide (98:2 v/v, 1.2 mL) at 1.5 mL/min.

Cocaine & Benzoyllecognine:

Elute sample with methanol/ammonia, (98:2 v/v, 1.2 mL) at 1.5 mL/min

Evaporate eluate to dryness.

Add 0.5 mL of BSTFA and heat for 15 min at 60 C.

Codeine & Morphine:

Collect analyte with methanol/ammonia (98:2 v/v, 1 mL) at 1.5 mL/min.

Phencyclidine:

Collect sample with methanol/ammonia (98:2 v/v, 1.2 mL) at 1.5 mL/min

THC-COOH:

Collect sample with hexane/ethyl acetate/acetone (50:40:10 v/v, 1.5 mL) at 1.5 mL/min.

Structure Various



Structural considerations Basic and acidic ionizable drugs with varying polarity and pKa values.

Matrix considerations The matrix is complex and contains a variety of potential interference compounds (salts, small organics, etc.). A mixed-mode extraction mechanism enables a rigorous interference elution step to minimize interferences.

Analytical method GC-MS

Reagents

1. Deionized water
2. Methanol
3. 0.05M Potassium Phosphate Buffer, pH 6
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.
4. 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.
5. 1M Ammonium Acetate Buffer, pH 8
6. Acetonitrile/Acetone/0.01M Hydrochloric Acid (15:15:70, v/v)
Add 15 mL of acetonitrile and 15 mL of acetone to a 100 mL volumetric flask. Dilute to mark with 0.01 M HCl.
7. Hexane/Ethyl Acetate/Acetone (50:40:10, v/v)
Add 40 mL of ethyl acetate and 10 mL of acetone to a 100 mL volumetric flask. Dilute to mark with hexane.
8. Methanol/ammonia 98:2, v/v.
Add 2 mL of concentrated ammonia to 98 mL of methanol. THIS REAGENT SHOULD BE MADE UP FRESH DAILY.

General comments Recoveries from in-house work:
Benzoyllecgonine 91%
Amphetamine 90%
Opiates 84–95%
Phencyclidine (PCP) 88%
THC 97%

For individual classes, see the corresponding application note:
Amphetamine and methamphetamine, IST1053A
Cocaine and Benzoyllecgonine, IST1062A
Opiates (codeine, morphine etc), IST1056A
Phencyclidine (PCP), IST1058A
THC, IST1059A

If the addition of tartaric acid solution is not compatible with your analytical methodology, an alternative approach is to use a keeper solvent during evaporation.

DERIVATIZATION
Amphetamine & Methamphetamine:



1. Evaporate the column eluate to dryness at 40 Celcius.
2. Add 50 uL of pentafluoropropionic anhydride and 50 uL of ethyl acetate to the collection vial.
3. React at room temperature for 5 min.
4. Evaporate to dryness with nitrogen at room temperature.
5. Reconstitute in 50 uL ethyl acetate and inject 1-2 uL into GC.

Cocaine & Benzoylecognine:

1. Evaporate eluate to dryness.
2. Add 0.5 mL of BSTFA and heat for 15 min at 60 Celcius.
3. Inject into GC.

Phencyclidine:

Evaporate sample to dryness with nitrogen at room temperature.
Reconstitute in 50 uL of BSTFA and heat at 70 Celcius for 15 min.
Inject 2 uL into GC.

THC-COOH:

Evaporate eluate to dryness.
Derivatize with 50 uL BSTFA at 60 Celcius for 15 min.
Inject into GC.

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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