



Agilent SampliQ EVIDEX Methods for the Extraction of Drugs of Abuse from Urine

Technical Note

Abstract

Agilent SampliQ EVIDEX – Rapid Solid Phase Extraction of Drugs of Abuse from Urine

- Designed for NIDA* drug classes
- Step-by-step instructions
- Robust analytical methods
- One cartridge for all Agilent methods
- Accurate, reproducible results

The benchmarks of any test are accuracy and repeatability. Agilent SampliQ EVIDEX incorporates a specially designed phase and carefully developed methods to provide the confidence that true, accurate values are obtained. When using EVIDEX methods, typical results will show relative standard deviations (RSDs) of less than 5% for all drugs tested.

Agilent has designed each EVIDEX method to be tolerant of inevitable measurement errors. The volumes and concentrations of the reagents have been designed to allow for slight deviations from the stated method values. Consequently, minor errors do not adversely affect the results.

*National Institute on Drug Abuse



Agilent Technologies

Introduction

Thank you for choosing Agilent SampliQ EVIDEX SPE cartridges. The following instructions are designed to take full advantage of the EVIDEX cartridge chemistry and provide the highest possible extraction efficiencies. Please take time to read the following instructions over carefully before beginning your extractions.

Amphetamines

Reagents

0.1 M K_2HPO_4 (pH 6.0 \pm 0.1) – 1.74 g potassium phosphate, dibasic (anhydrous) in 100 mL DI water. Adjust to pH 6.0 \pm 0.1 with phosphoric acid.

1.0 M acetic acid – 5.7 mL glacial acetic acid diluted with DI water to 100 mL.

Methylene chloride/isopropyl alcohol/HCl (60/40/1) – 60 mL methylene chloride, 40 mL isopropyl alcohol, 1 mL concentrated hydrochloric acid. Make fresh daily.

Toluene/acetonitrile (95/5) – 95 mL toluene, 5 mL acetonitrile

PFPA – Pentafluoropropionic acid anhydride

5% sodium bicarbonate – 5 g of sodium bicarbonate in 100 mL DI water. Make fresh weekly.

Other Reagents

Methanol
DI water

Sample Preparation

1. Add 3 mL 0.1 M K_2HPO_4 (pH 6.0) to 5 mL urine.
2. Mix.

Condition

1. Add 6 mL methanol to the cartridge.
2. Apply vacuum.
3. Add 6 mL 0.1 M K_2HPO_4 (pH 6.0) to the cartridge when the methanol reaches the upper frit.
4. Stop the vacuum when the 0.1 M K_2HPO_4 reaches the upper frit.
5. Do not let the phase go dry during any of the conditioning steps.

Load

1. Add the urine sample to the cartridge.
2. Apply vacuum.
3. The flow rate should not exceed 5 mL/min.
4. Stop the vacuum after the urine sample has just passed below the upper frit.

Rinse

1. Add 3 mL of water to the cartridge.
2. Apply vacuum.
3. Add 3 mL 1 M acetic acid to the cartridge when the water reaches the upper frit.
4. Add 3 mL methanol to the cartridge when the 1 M acetic acid reaches the upper frit.
5. Leave the vacuum on for 2 to 3 minutes after the methanol leaves the cartridge.
6. All flow rates should not exceed 5 mL/min.

Elute

1. Place a small collection tube* beneath each cartridge.
2. Add 3 mL methylene chloride/isopropyl alcohol/HCl (60/40/1) to the cartridge.
3. Immediately apply vacuum and collect the eluant.
4. The flow rate should not exceed 5 mL/min.

Analysis Preparation

1. Concentrate the eluant to dryness under a very gentle stream of nitrogen. Do not heat the sample. Evaporation under a very mild vacuum is satisfactory.
2. Remove the sample from the evaporator as soon as the solvent has completely evaporated. **Do not overdry.**
3. Add 0.5 to 1.0 mL toluene/acetonitrile (95/5) then 25 μ L PFPA to the dry sample. Close with a PTFE (Teflon) lined cap.
4. Mix to make sure that all of the dry sample residue comes in contact with the solvent.
5. Heat at 45 °C for 10 minutes.
6. Cool to room temperature.
7. Add 1 mL 5% sodium bicarbonate.
8. Vortex until the top layer is clear (usually 30 to 60 seconds).
9. Transfer 100 to 200 μ L of the top layer to a small vial.** Close with a PTFE (Teflon*) lined cap. Do not transfer any of the bottom (aqueous) layer.
10. Inject 1 to 2 μ L of the top layer.

* 13 x 100 mm screw cap test tube is recommended.

** 2 mL autosampler vial is recommended.

Cocaine (Benzoylecgonine)

Reagents

10 N NaOH – 40 g sodium hydroxide in 100 mL water.

0.1 N HCl – 0.83 mL hydrochloric acid diluted with DI water to 100 mL.

Acetonitrile/0.1 N HCl (40/60) – 40 mL acetonitrile, 60 mL 0.1 N HCl

Ethylacetate/hexane (50/50) – 50 mL ethyl acetate, 50 mL hexane

BSTFA/1% TMCS – 9 mL N,O-bis(trimethylsilyl)tri-fluoroacetamide, 0.1 mL trimethyl-chlorosilane, or can be purchased premixed.

Other Reagents

Methanol

Hexane

DI water

Ethyl acetate

Acetic acid

Sample Preparation

1. Add 3 mL 0.1 M K_2HPO_4 (pH 6.0) to 5 mL urine.
2. Mix.

Condition

1. Add 6 mL methanol to the cartridge.
2. Apply vacuum.
3. Add 6 mL 0.1 M K_2HPO_4 (pH 6.0) to the cartridge when the methanol reaches the upper frit.
4. Stop the vacuum when the 0.1 M K_2HPO_4 reaches the upper frit.
5. Do not let the phase go dry during any of the conditioning steps.

Load

1. Add the urine sample to the cartridge.
2. Apply vacuum.
3. The flow rate should not exceed 5 mL/min.
4. Stop the vacuum after the urine sample has passed below the upper frit.

Rinse

1. Add 3 mL of water to the cartridge.
2. Apply vacuum.
3. Add 3 mL 0.1 N HCl to the cartridge when the water reaches the upper frit.
4. Add 3 mL methanol to the cartridge when the 0.1 N HCl reaches the upper frit.
5. Leave the vacuum on for 2 to 3 minutes after the methanol leaves the cartridge.
6. All flow rates should not exceed 5 mL/min.

Elute

1. Place a small collection tube* beneath each cartridge.
2. Add 3 mL methylene chloride/isopropyl alcohol/ NH_4OH (78/20/2) to the cartridge.
3. Apply vacuum and collect the eluant.
4. The flow rate should not exceed 5 mL/min.

Analysis Preparation

1. Concentrate the eluant to dryness under a stream of nitrogen and at a temperature not exceeding 45 °C.
2. Remove the sample from the evaporator as soon as the solvent has completely evaporated. **Do not overdry.**
3. Apply vacuum and collect the eluant.
4. The flow rate should not exceed 5 mL/min.
5. Transfer the concentrated sample to a small screw cap vial** using ethyl acetate/hexane (50/50).
6. Concentrate the eluant to dryness under a stream of nitrogen and at a temperature not exceeding 45 °C.
7. Remove the sample from the evaporator as soon as the solvent has completely evaporated. **Do not overdry.**
8. Add 75 μ L ethyl acetate then 25 μ L BSTFA/1% TMCS to the dry sample. Close with a PTFE (Teflon*) lined cap.
9. Mix to make sure that all of the dry sample residue comes in contact with the solvent.
10. Heat at 60 °C for 10 minutes. Mix.
11. Cool to room temperature.
12. Inject 1 to 2 μ L.

*13 x 100 mm test tube is recommended.

**2 mL autosampler vial is recommended.

Opiates

Reagents

0.1 M K_2HPO_4 (pH 6.0) – 1.74 g potassium phosphate, dibasic (anhydrous) in 100 mL DI water. Adjust to pH 6.0 ± 0.1 with phosphoric acid.

0.1 N HCl – 0.83 mL hydrochloric acid diluted with DI water to 100 mL.

Methylene chloride/isopropyl alcohol/ NH_4OH (78/20/2) – 78 mL methylene chloride, 20 mL isopropyl alcohol, 2 mL ammonium hydroxide. Make fresh daily.

Methylene chloride/isopropyl alcohol (78/20) – 78 mL methylene chloride, 20 mL isopropyl alcohol.

BSTFA/1% TMCS – 9 mL N,O-bis(trimethylsilyl)tri-fluoroacetamide, 0.1 mL trimethyl-chlorosilane, or can be purchased premixed.

Other Reagents

Methanol
DI water
Ethyl
Ethyl acetate

Sample Preparation

1. Add 0.5 mL HCl and 5 mL urine to a screw top test tube (16 x 125 mm). Close with a PTFE (Teflon) lined cap and mix.
2. Heat at 120 °C for 20 minutes.
3. Cool to room temperature.
4. Add 0.75 mL 10 N NaOH. Mix
5. Adjust to pH 6.5 to 7.5 with ~2.5 mL 0.5 M phosphoric acid.

Condition

1. Add 6 mL methanol to the cartridge.
2. Apply vacuum.
3. Add 6 mL 0.1 M K_2HPO_4 (pH 6.0) to the cartridge when the methanol reaches the upper frit.
4. Stop the vacuum when the 0.1 M K_2HPO_4 reaches the upper frit.
5. Do not let the phase go dry during any of the conditioning steps.

Load

1. Add 3 mL 0.1 M K_2HPO_4 (pH 6.0) to the cartridge.
2. Attach an 8 mL reservoir to the top of the cartridge using a coupling fitting.
3. Immediately add the urine sample to the reservoir.
4. Apply vacuum.
5. The flow rate should not exceed 5 mL/min.
6. Stop the vacuum after the urine sample has just passed below the upper frit.

Rinse

1. Remove the reservoir.
2. Add 3 mL of water to the cartridge.
3. Apply vacuum.
4. Add 3 mL 0.1 M sodium acetate (pH 4.5) to the cartridge when the water reaches the upper frit.
5. Add 3 mL methanol to the cartridge when the 0.1 M sodium acetate reaches the upper frit.
6. Leave the vacuum on for 2 to 3 minutes after the methanol leaves the cartridge.
7. All flow rates should not exceed 5 mL/min.

Elute

1. Place a small collection tube* beneath each cartridge.
2. Add 3 mL methylene chloride/isopropyl alcohol/ NH_4OH (78/20/2) to the cartridge.
3. Transfer the concentrated sample to a small screw cap vial** using methylene chloride/isopropyl alcohol (78/20).
4. Concentrate the eluant to dryness under a stream of nitrogen and at a temperature not exceeding 45 °C.
5. Remove the sample from the evaporator as soon as the solvent has completely evaporated. **Do not overdry.**
6. Add 75 μ L ethyl acetate then 25 μ L BSTDFA/1% TMCS to the dry sample. Close with a PTFE (Teflon) lined cap.
7. Mix to make sure that all of the dry sample residue comes in contact with the solvent.
8. Heat at 60 °C for 10 minutes. Mix.
9. Cool to room temperature.
10. Inject 1 to 2 μ L.

*13 x 100 mm test tube is recommended.

**2 mL autosampler vial is recommended.

Marijuana (THC-COOH)

Reagents

10 N NaOH – 40 g sodium hydroxide in 100 mL DI water.

0.1 M K_2HPO_4 (pH 6.0) – 1.74 g potassium phosphate, dibasic (anhydrous) in 100 mL DI water. Adjust to pH 6.0 ± 0.1 with phosphoric acid.

0.1 M sodium acetate (pH 4.5) – 0.82 g in 100 mL DI water. Adjust to pH 4.5 ± 0.1 with glacial acetic acid.

Methylene chloride/isopropyl alcohol/ NH_4OH (78/20/2) – 78 mL methylene chloride, 20 mL isopropyl alcohol, 2 mL ammonium hydroxide. Make fresh daily.

Methylene chloride/isopropyl alcohol (78/20) – 78 mL methylene chloride, 20 mL isopropyl alcohol.

BSTFA/1% TMCS – 9 mL N,O-bis(trimethylsilyl)trifluoroacetamide, 0.1 mL trimethylchlorosilane, or can be purchased premixed.

Other Reagents

Methanol

DI water

Ethyl acetate

Hydrochloric acid

Phosphoric acid

Sample Preparation

1. Add 0.5 mL 10 N NaOH and 5 mL urine to a screw top test tube (16 x 125 mm). Close with a PTFE (Teflon) lined cap and mix.
2. Heat at 60 °C for 20 minutes.
3. Cool to room temperature.
4. Adjust to pH 3.5 to 4.0 with ~2 mL acetic acid.

Condition

1. Add 6 mL methanol to the cartridge.
2. Apply vacuum.
3. Add 6 mL 0.1 N HCl to the cartridge when the methanol reaches the upper frit.
4. Stop the vacuum when the 0.1 N HCl reaches the upper frit.
5. Do not let the phase go dry during any of the conditioning steps.

Load

1. Add 3 mL 0.1 N HCl to the cartridge.
2. Attach an 8 mL reservoir to the top of the cartridge using a coupling fitting.

3. Immediately add the urine sample to the reservoir.
4. Apply vacuum.
5. The flow rate should not exceed 5 mL/min.
6. Stop the vacuum after the urine sample has just passed below the upper frit.

Rinse

1. Remove the reservoir.
2. Add 3 mL of water to the cartridge.
3. Apply vacuum.
4. Add 3 mL acetonitrile/0.1 N HCl (40/60) to the cartridge when the water reaches the upper frit.
5. Add 3 mL hexane to the cartridge when the acetonitrile/0.1 N HCl solution reaches the upper frit.
6. Leave the vacuum on for 2 to 3 minutes after the hexane leaves the cartridge.
7. All flow rates should not exceed 5 mL/min.

Elute

1. Place a small collection tube* beneath each cartridge.
2. Add 3 mL ethyl acetate/hexane (50/50) to the cartridge.
3. Apply vacuum and collect the eluant.
4. The flow rate should not exceed 5 mL/min.

Analysis Preparation

1. Concentrate the eluant to dryness under a stream of nitrogen and at a temperature not exceeding 45°C.
2. Remove the sample from the evaporator as soon as the solvent has completely evaporated. **Do not overdry.**
3. Transfer the concentrated sample to a small screw cap vial** using methylene chloride/isopropyl alcohol (78/20).
4. Concentrate the eluant to dryness under a stream of nitrogen and at a temperature not exceeding 45 °C.
5. Remove the sample from the evaporator as soon as the solvent has completely evaporated. **Do not overdry.**
6. Add 75 μ L ethyl acetate then 25 μ L BSTFA/1% TMCS to the dry sample. Close with a PTFE (Teflon*) lined cap.
7. Mix to make sure that all of the dry sample residue comes in contact with the solvent.
8. Heat at 60 °C for 10 minutes. Mix.
9. Cool to room temperature.
10. Inject 1 to 2 μ L.

*13 x 100 mm test tube is recommended.

**2 mL autosampler vial is recommended.

Phencyclidine (PCP)

Reagents

0.1 M K_2HPO_4 (pH 6.0) – 1.74 g potassium phosphate, dibasic (anhydrous) in 100 mL DI water. Adjust to pH 6.0 ± 0.1 with phosphoric acid.

0.1 M sodium acetate – 0.82 g in 100 mL DI water. Adjust to pH 4.5 ± 0.1 with glacial acetic acid.

Methylene chloride/isopropyl alcohol/ NH_4OH (78/20/2) – 78 mL methylene chloride, 20 mL isopropyl alcohol, 2 mL ammonium hydroxide. Make fresh daily.

Methylene chloride/isopropyl alcohol (78/20) – 78 mL methylene chloride, 20 mL isopropyl alcohol.

Other Reagents

Methanol
DI water
Ethyl acetate

Sample Preparation

1. Add 3 mL 0.1 M K_2HPO_4 (pH 6.0) to 5 mL urine.
2. Mix.

Condition

1. Add 6 mL methanol to the cartridge.
2. Apply vacuum.
3. Add 6 mL 0.1 M K_2HPO_4 (pH 6.0) to the cartridge when the methanol reaches the upper frit.
4. Stop the vacuum when the 0.1 M K_2HPO_4 reaches the upper frit.
5. Do not let the phase go dry during any of the conditioning steps.

Load

1. Add the urine sample to the cartridge.
2. Apply vacuum.
3. The flow rate should not exceed 5 mL/min.
4. Stop the vacuum after the urine sample has just passed below the upper frit.

Rinse

1. Add 3 mL of water to the cartridge.
2. Apply vacuum.
3. Add 3 mL 0.1 M sodium acetate (pH 4.5) to the cartridge when the water reaches the upper frit.
4. Add 3 mL methanol to the cartridge when the 0.1 M sodium acetate reaches the upper frit.
5. Leave the vacuum on for 2 to 3 minutes after the methanol leaves the cartridge.
6. All flow rates should not exceed 5 mL/min.

Elute

1. Place a small collection tube* beneath each cartridge.
2. Add 3 mL methylene chloride/isopropyl alcohol/ NH_4OH (78/20/2) to the cartridge.
3. Apply vacuum and collect the eluant.
4. The flow rate should not exceed 5 mL/min.

Analysis Preparation

1. Concentrate the eluant to dryness under a stream of nitrogen and at a temperature not exceeding 45 °C.
2. Remove the sample from the evaporator as soon as the solvent has completely evaporated. **Do not overdry.**
3. Transfer the concentrated sample to a small screw cap vial** using methylene chloride/isopropyl alcohol (78/20) for the transfer.
4. Concentrate the eluant to dryness under a stream of nitrogen and at a temperature not exceeding 45 °C.
5. Remove the sample from the evaporator as soon as the solvent has completely evaporated. **Do not overdry.**
6. Add 100 μ L ethyl acetate to the dry sample. Close with a PTFE (Teflon*) lined cap.
7. Mix to make sure that all of the dry sample residue comes in contact with the solvent.
8. Inject 1 to 2 μ L.

*13 x 100 mm test tube is recommended.

**2 mL autosampler vial is recommended.

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