

Extraction of Acidic, Neutral and Basic Compounds from Post Mortem samples using ISOLUTE® 101 Columns

Introduction

This method is suggested for the simultaneous extraction of acidic, neutral and basic compounds from postmortem tissues, blood and urine. The retention mechanism is hydrophobic.

Extraction Procedure

ISOLUTE® SPE Column: ISOLUTE 101 500 mg/6 mL Part number 101-0050-C

Matrix: Complex matrices with various potential interferences (e.g. proteins, lipids, salts etc).

Pre-treatment:

Blood:
Homogenize 5 mL blood in phosphate buffer (pH7.4, 50 mL) at 20,000rpm.

Liver or brain:
Homogenize 1 g in phosphate buffer (pH7.4, 50 mL) at 20,000rpm.

All matrices:
1. Centrifuge at 3500rpm for 2min. at 20 C.
2. Transfer the supernatant to a glass tube (spike with standard solution for reproducibility etc. as necessary)

Standard Preparation: Non-aqueous samples: Spike a solvent similar to sample matrix. Aqueous samples: Spike reagent water or 10 to 20 mM buffer. An appropriate buffer is usually the same as that used in the equilibration step.

Solvation: Condition the column with methanol (8 mL) at a flow rate of 10 mL/min.

Equilibration: Equilibrate with phosphate buffer (pH7.4, 8 mL) at a flow rate of 10 mL/min.

Sample application: Load sample (50 mL) onto the column at a flow rate of 1 mL/min.

Interference elution: Elute interferences with de-ionized water (12 mL) at a flow rate of 10 mL/min. Remove excess water by passing a gentle stream of nitrogen through the column for 60 seconds.

Analyte elution: To elute analytes, apply first volume of elution solvent to extraction column. Soak for two minutes. Add second volume of elution solvent to extraction column and collect. Elute acidic, neutral and basic drugs with ethyl acetate/isopropanol (3:1, v/v. 25 mL) at a flow rate of 1 mL/min. Carry out liquid-liquid extraction to fractionate the acidic, neutral and basic drugs.

Analytical method: GC

Reagents:

1. Phosphate buffer (pH7.4)
2. De-ionized water
3. Methanol (distill before use)
4. Ethyl acetate (distill before use)
5. Isopropanol (distill before use)

Results:

Recoveries of 96-100% are obtainable using this method.

Reference:

General unknown screening in postmortem tissue and blood samples: A semi-automatic solid-phase extraction using polystyrene resins followed by liquid-liquid extraction
Stimpfl,-T; Jurenitsch,-J; Vycudilik,-WJ-Anal-Toxicol. 25, March 2001

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