SPE Method Development Recommendations for Toxicological Screening of Postmortem Samples

This represents recommendations for SPE method development. The proposed steps are based on experience with similar analytes and matrices, but have not been verified in Argonaut Technologies' laboratories. Please refer to section below for the analyte and matrix considerations that were made in developing this method.
As for all method development, this procedure should first be developed using pure solvent spiked with analyte. Only after the chemistry is established should spiked matrix samples be tested.
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.

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 # 101-0050-C)

There may be more than one phase that could be effective in the extraction of this compound. The method development should include testing phases in parallel in order to optimize the procedure.

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

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

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

*See general comments

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 (see General Comments).

Structure

Various.

Structural considerations

Various acidic, neutral and basic drugs with varying aromatic character.

Matrix considerations

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

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)

General comments

Recoveries of 96-100% are obtainable using this method.
Full details about the method can be obtained from the following 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,-W
J-Anal-Toxicol. 25, March 2001

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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