SPE Method Development Recommendations for Extraction of Serum Corticosteroids

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 recommended for the extraction of the corticosteroid hormones cortisone, cortisol, and dexamethasone from serum using a non-polar retention mechanism.

EXTRACTION PROCEDURE

ISOLUTE® SPE Column: ISOLUTE C18, 100 mg / 1ml (Part # 220-0010-A)

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:
1. Dilute 1.5ml of serum with 1.5ml of water.
2. Prepare spiked samples by adding corticosteroids.
3. Add enzyme and incubate at 40 celcius for 5-10 min.
4. Add 1.0ml of metaphosphoric acid.
5. Ultracentrifuge for 5min at 3000r/s.

Solvation: Solvate the column with methanol (3x1m).

Equilibration: Rinse the column with water (1 ml) followed by 30mM Sodium dihydrogen phosphate/hydrogen phosphate, pH7.0 (1 ml).

Sample application: Apply 2.7 ml of the supernatant.
Start with a flow rate of 1-2ml/min, then optimise as required.

Interference elution: Wash the column with 1ml water.

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.

1. Elute analytes, with methanol (2.5 ml).
2. Evaporate under nitrogen (at 38 degrees celcius) to dryness.
3. Dissolve residue in 0.01ml methanol and 0.90ml of water.
4. Analyse the recnstituted extract by MMEKC.

Structure	Not shown
Structural considerations	The analytes are non-polar, with varying size and polarity.
Matrix considerations	The matrix is aqueous.
Analytical method	Mixed Micellular electrokinetic capillary chromatography.
Reagents	1. Distilled water 2. Methanol 3. 30mM Sodium dihydrogen phosphate/hydrogen phosphate buffer 4. Metaphosphoric acid.
General comments	The enzymatic hydrolysis using Helix potima and the protein precipitation using metaphosphoric acid ensures maximal corticosteroid recovery. Reference: Determination of serum corticosteroids by mixed-mode micellar electrokinetic capillary chromatography with sodium dodecylsulphate and sodium cholate. Susanne K. Wiedmer et al. Electrophoresis 1997, Vol 18, pp1861-1864.

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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