SPE Method Development Recommendations for Extraction of Steroids from Biological Fluids

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 Biotage 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 proposed for the extraction of steroids from urine and whole blood using a non-polar retention mechanism.

EXTRACTION PROCEDURE

ISOLUTE® SPE Column: Evaluate C8 200 mg / 3 mL (Part # 290-0020-B) and C18 200 mg / 3 mL (Part # 220-0020-B). The end-capped versions of these columns may also be suitable.

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: Urine. To the urine sample add a suitable internal standard (in a water miscible carrier solvent), and centrifuge for 5 mins at 150 g.

Whole blood. At room temperature, sonicate the whole blood sample (1 mL) for 15 mins. Add 0.1M phosphate buffer, pH 6.0 (6 mL), and vortex the mixture for 30s. Centrifuge the sample for 15 mins at 2000rpm. Remove the supernatant, and treat as for urine.

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

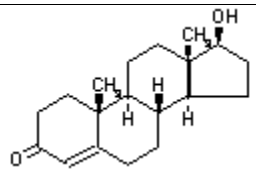
Equilibration: Rinse the column with distilled deionized water (3 mL).

Sample application: Apply the sample (5 mL) at a flow rate of 1-2 mL / min.

Interference elution: Elute interferences with distilled deionized water (3 mL).

Analyte elution: Elute analytes with methanol (2 x 1.5 mL).

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.

Structure	Various, testosterone is shown. 
Structural considerations	The analytes are relatively non-polar compounds, but require derivatisation prior to GC analysis.
Matrix considerations	The analytes are being extracted from a polar aqueous matrix.
Analytical method	GC, GC/MS
Reagents	1. 0.1 M phosphate buffer, pH 6.0 2. Methanol 3. Distilled deionized water
General comments	1. This method is based on the following reference: 'Proposed Confirmatory Procedure for Detecting 5-alpha-Dihydrotestosterone Doping in Male Athletes' by AT Kicman, SB Coutts, CJ Walker and DA Cowan, Clin. Chem, 41/11, 1617-1627 (1995). 2. It may be possible to remove more interferences by rinsing the column with various mixtures (e.g. 50:50 methanol/water, 3 mL) prior to analyte elution. 3. For analysis of conjugated steroids, the extract should be hydrolysed as follows:- Evaporate the methanol extract to dryness. Re-dissolve in phosphate buffer (0.1M, pH 6.8, 0.5 mL) and hydrolyse (2 hrs, 50 C, 2500 Fishman units of beta-glucuronidase added in 0.5 mL phosphate buffer, as before. After hydrolysis, add 100 mg potassium carbonate, extract into diethyl ether (5 mL) and dry with anhydrous sodium sulfate. Derivatise to TMS derivatives and analyse.

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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