SPE Method Development Recommendations for Extraction of Anabolic steroids from Head Hair

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 recommended for the extraction of anabolic steroids from head hair. The mechanism of retention is non-polar.

EXTRACTION PROCEDURE

ISOLUTE® SPE Column: ISOLUTE C18 200mg/3 mL (Part # 220-0020-B)
Optimise for 1 mL columns in addition to the 3 mL column.

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. Wash full length hair with phosphate buffer (pH7.4, 5 mL x 3) 2. Cut hair into pieces, mix and weigh accurately 3. Digest with 1M Sodium hydroxide (2 mL), then heat at 60celcius for 30min. 4. Adjust pH to 5.6 with 6M HCl, mix and centrifuge at 25000rpm for 5min. 5. Add IS
Solvation:	Apply methanol (2 mL)
Equilibration:	Rinse with distilled water (2 mL)
Sample application:	Apply the sample, optimising flow rate (start at 1-2 mL/min).
Interference elution:	Rinse with 10% methanol (2 mL), optimising flow rate (start at 1-2 mL/min).
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 with methanol (2 mL), optimising flow rate (start at 1-2 mL/min). 2. Dry the eluate under nitrogen at 50celcius 3. Analyse by GC-MS

Structure	Various, not shown.
Structural considerations	Heterocyclic derivatives with nitrogen, oxygen and sulfur-containing functional groups.
Matrix considerations	The matrix is aqueous.
Analytical method	GC-MS
Reagents	1. Phosphate buffer (pH7.4) 2. DCM 3. 1M NaOH 4. 6m HCl 5. Deuterated testosterone - Internal standard
General comments	Reference: Detection of analbolic steroids in head hair Deng,-X,-S; Kurosu,-A; Pounder,DJ. J Forensic Sci 1999; 44(2): 343-346 Two chromatographic peaks have been detected for the cis and trans isomers of androstanolone, mestanolone (methylandrostanolone) and oxymethalone. Recoveries are as follows: 35-45% for androstanolone, oxymethalone, chlorotestosterone-acetate, dehydromethyltestosterone, dehydrotestosterone, fluoxymesterone, mestanolone, methyltestosterone, nandrolone 52% for mesterolone, trenbolone 65% bolasterone 24% methenolone 17% stanozolol LODs - 0.002-0.05ng/mg LOQs - 0.02-0.1ng/mg

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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