Purification of Four Anabolic Steroids Using Agilent SampliQ C8 and Amino SPE Tubes

Technical Overview

Introduction

Urine analysis is the matrix of choice for detecting the illegal use of anabolic steroids in food-producing livestock.

Free steroids get conjugated in biological systems. Prior to sample extraction, these conjugated steroids in urine must be enzymatically hydrolyzed to the free steroids. The free steroids are then isolated, purified, and concentrated using an Agilent SampliQ C8 solid phase extraction (SPE) tube. Further sample cleanup is achieved by passing the eluent from the C8 SPE through an Agilent SampliQ Amino SPE tube to remove acids, like uric acid. The amino functional group absorbs the acids, while allowing the steroids to pass through. After evaporation of the eluent from the amino SPE tube, the residue is taken up in the appropriate solvent for analysis.

The four representative anabolic steroids used were prednisolone, dexamethasone, 1,4-androstadiene-3,17-dione, and norgestrel, with five replicates each. After initially spiking urine with the steroids, following the above procedure and using HPLC analysis, recoveries for three of the steroids were 95% to 99%, with norgestrel's recovery at 86% and RSDs 1.0% or lower. The combined use of SampliQ C8 and Amino SPE tubes provided high recoveries with excellent reproducibility.

Study Purpose and Methodology

The purpose of this study was to identify SPE cleanup conditions for anabolic steroids. Prednisolone, dexamethasone, 1, 4-androstadiene-3,17-dione, and norgestrel were used as representative compounds. Figure 1 shows the chemical structures of the compounds.

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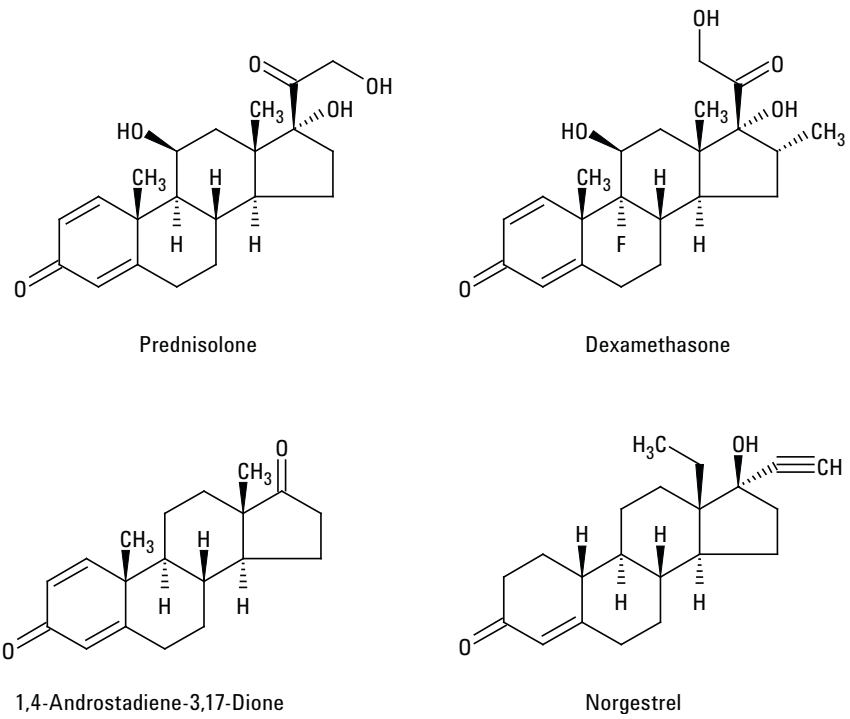

Figure 1. Steroid structures.

An aliquot (5 mL) of human urine was adjusted to pH 5.2 by the addition of 2 M sodium acetate buffer (2 mL, pH 5.2). The steroids were spiked into the sample, corresponding to an individual 4 µg/mL steroid level in urine. The steroids were subjected to enzymatic hydrolysis, conducted by adding 50 µL of β-glucuronidase type HP-2 from helix pomatia juice (Sigma-Aldrich, Cat No: G7017) and incubating the samples at 55 °C for 3 hrs. Then the samples were centrifuged at 2,000 rpm for 10 minutes at 5 °C [1].

A SampliQ C8 SPE tube (3 mL tube, 500 mg, p/n 5982-1035) was used for cleanup and concentration of the enzymatic hydrolyzed urine sample. The hydrolyzed sample was applied to the C8 SPE tube as illustrated in Figure 2.

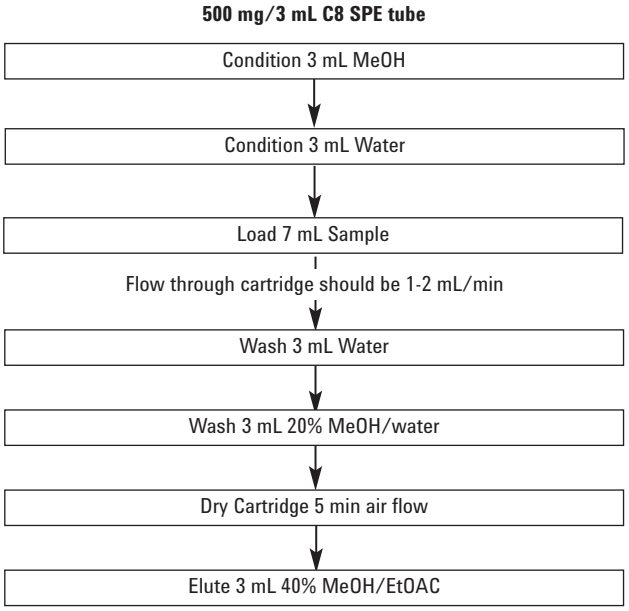

Figure 2. Sample cleanup scheme using Agilent SampliQ C8 SPE.

A SampliQ Amino tube (3 mL tube, 500 mg, p/n 5982-1835) was used to further purify the sample by removing uric and other acids typically found in urine. The eluted sample from the C8 SPE tube was applied to the amino SPE tube as illustrated in Figure 3. The combined effluent was evaporated to dryness at ambient temperature under a stream of nitrogen. The residue was dissolved in methanol-water (50:50 (v/v), 400 µL), and analyzed at 254 nm using external standards.

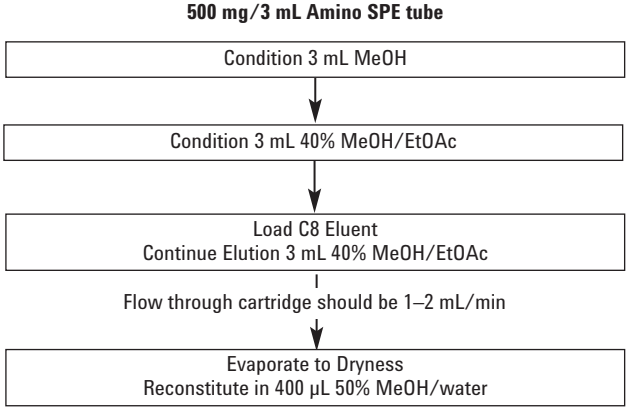

Figure 3. Sample cleanup scheme using Agilent SampliQ Amino SPE.

Results and Discussion

The average of five recoveries for three of the steroids ranged from 95% to 99%, with the recovery of the fourth steroid at 86% (Table 1). Percent RSD for the five replicate recoveries was 1.0% or less. A representative chromatogram for the four steroids after SPE is shown in Figure 4.

Table 1. Recovery data.

	Prednisolone % Recovery	Dexamethasone % Recovery	Andro* % Recovery	Norgestrel % Recovery
Recovery #1	98	98	94	86
Recovery #2	98	97	94	86
Recovery #3	100	98	95	86
Recovery #4	100	98	95	87
Recovery #5	99	99	96	88
Average	99	98	95	86
% RSD	0.9	0.8	1.0	1.0

* 1,4-Androstadiene-3,17-Dione

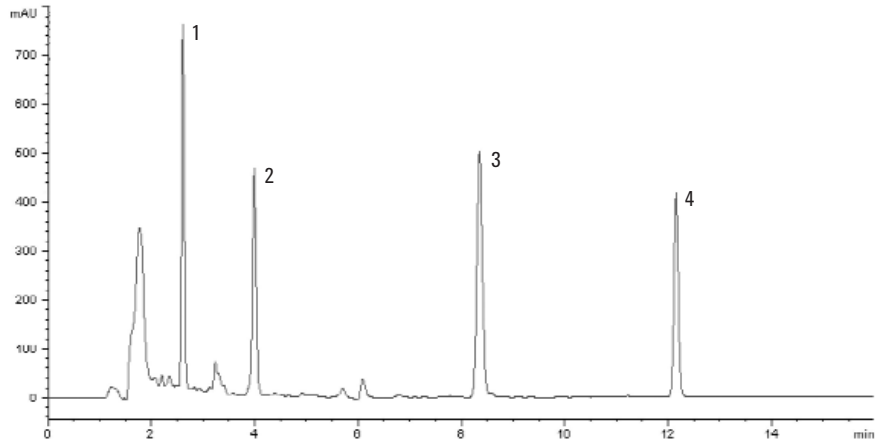

Figure 4. Representative sample chromatogram, 5 µg/mL 1. Prednisolone, 2. Dexamethasone, 3. 1,4-Androstadiene-3,17-Dione, and 4. Norgestrel.

HPLC Analysis

Column:	Agilent ZORBAX Rapid Resolution Eclipse Plus C18, 4.6 mm × 150 mm, 3.5 µm, (p/n 959963-902)
Mobile phase:	A: H_2O (0.1% Formic acid)
	B: ACN (0.1% Formic acid)
Gradient profile:	Time (min.) %B 0.0 40 5.0 40 16.0 75 17.0 75 17.1 40
Flow rate:	1 mL/min
Column temperature:	25 °C
Detection DAD:	254 nm
Injection Volume:	30 µL

Conclusion

The combined use of Agilent SampliQ C8 and Amino SPE tubes provided high recoveries with excellent reproducibility for the four tested anabolic steroids. This convenient procedure using Agilent's SPE tubes provided a significantly more concentrated and cleaner sample for analysis. One would expect this procedure to be applicable to additional steroids.

Reference

1. S.A. Hewitt, M. Kearney, J.W. Currie, P.B. Young, D.G. Kennedy, *Analytica Chimica Acta* 473 (2002) 99-109

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