



Generic Procedure for the Extraction of Diuretics from Urine Using EVOLUTE ABN SPE Columns

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Diuretics are prescribed to treat various conditions such as heart, liver, kidney or lung diseases. However, their mode of action has been misused in sport and has led to the International Olympic Committee (IOC) and various horse racing authorities banning their use in competition.

This Application Note describes the use of EVOLUTE™ ABN 50 µm (new larger particle size), a water wettable resin-based non-polar SPE sorbent, for the extraction of diuretics from urine. It describes a generic approach to the extraction of diuretics to which additional compounds can be included with minimal method development time. The analyte suite includes thiazides, carbonic anhydrase inhibitors, loop and potassium sparing diuretics with wide ranging pK_a and logP values.

Sample Preparation Procedure

Analytes:

Amiloride, Acetazolamide, Hydrochlorothiazide, Methazolamide, Hydroflumethiazide, Furosemide, Bendoflumethiazide, Bumetanide, Spironolactone and Ethacrynic acid.

EVOLUTE ABN Column Configuration:

EVOLUTE ABN 50 µm 100 mg/3 mL (part number 610-0010-H).

EVOLUTE ABN Procedure:

Column Configuration:	100 mg/10 mL
Sample Pre-treatment:	Dilute urine sample (1-2 mL, 1:1 v/v) with 1% (v/v) formic acid
Column Conditioning:	Methanol (3 mL)
Column Equilibration:	0.1% (v/v) formic acid (3 mL)
Sample Application:	Apply diluted sample
Interference Elution:	Rinse with 95:5 (v/v) water/methanol (3 mL)
Analyte Elution:	Methanol (3 mL)
Post Extraction:	Evaporate to dryness and reconsti-

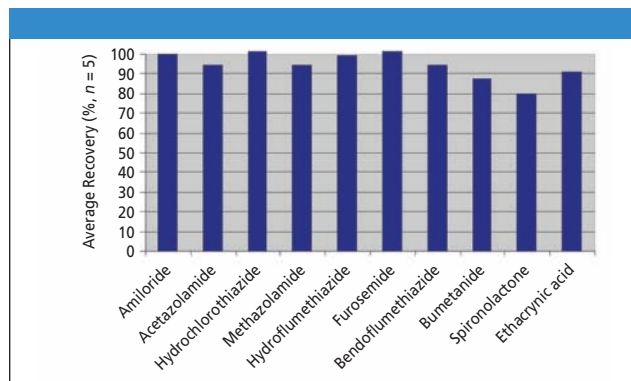


Figure 1: Recoveries for diuretics from urine using EVOLUTE ABN

tute in 50:50 (v/v) H₂O/MeOH (1 mL) for subsequent LC-MS/MS analysis

HPLC Conditions:

Instrument:

Waters 2795 Liquid Handling System & Ultima Pt triple quadrupole mass spectrometer (Waters Assoc., Manchester, UK)

Column:

Zorbax Eclipse XDB C18 3.5 µm analytical column (100 × 2.1 mmID) (Agilent Technologies, Berkshire, UK)
C8 guard column (Agilent Technologies, Berkshire, UK)

Mobile Phase:

0.1% (v/v) aqueous formic acid and MeCN at a flow rate of 0.25 mL/min. 10 / 90 (v/v) MeCN / 0.1% (v/v) aqueous formic acid increasing to 90% (v/v) MeCN over 7 minutes and held for 1 minute.

Gradient:

Injection Volume:

10 µL

Temperature:

Ambient temperature

Conclusions

EVOLUTE ABN has been successfully used to extract a range of diuretics from urine using a generic method (Table I). Recoveries for all analytes were > 80% with excellent RSD's (n=5) as shown in Figure 1.

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Table I: Recovery and % RSD of diuretics from urine

Analyte	% Recovery	% RSD
Amiloride	100	1
Acetazolamide	94	6
Hydrochlorothiazide	104	1
Methazolamide	94	5
Hydroflumethiazide	100	2
Furosemide	102	5
Bendoflumethiazide	94	7
Bumetanide	87	7
Spironolactone	80	10
Ethacrynic acid	91	8