Extraction of Diuretics from Urine using EVOLUTE ABN Columns

Application Note

Introduction

This procedure is recommended for the extraction of a variety of diuretics from urine using non-polar polymeric SPE. The analyte suite includes thiazides, carbonic anhydrase inhibitors, loop and potassium sparring diuretics, with wide ranging pK_a and logP values.

The sample preparation method and suggested analytical procedures are detailed in page 1. Achieved recoveries and % RSD using this methodology are shown on pages 2 and 3.

Sample Preparation Procedure

Analytes

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

EVOLUTE ABN Column Configuration

EVOLUTE ABN 50 µm 100 mg/10 mL, part number 610-0010-H.

EVOLUTE ABN Procedure

Sample:	Spike urine sample with at 50 ng/L concentration of the compounds listed above
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 water/methanol (95:5 v/v, 3 mL)
Analyte Elution:	Methanol (3 mL)
Post Extraction:	Evaporate to dryness and reconstitute in 50:50 (v/v) H_2O/MeOH (1 mL) for subsequent LC-MS/MS analysis

For general guidelines on the use of EVOLUTE ABN SPE columns, request Chemistry Data Sheet TN137 EVOLUTE ABN Columns for Non-polar Solid Phase Extraction in Forensic/Clinical Analysis.

HPLC Conditons

Instrument:	Waters 2795 Liquid Handling System (Waters Assoc., Milford, MA, USA)
Column:	Zorbax Eclipse XDB C18 3.5 µm analytical column (100 x 2.1 mm ID) (Agilent Technologies, Berkshire, UK)
Guard Column:	C8 guard column (Agilent Technologies, Berkshire, UK)
Mobile Phase:	0.1% aqueous formic acid and MeCN at a flow rate of 0.25 mL/min.
Gradient: 90%,	0.1% aqueous formic acid and 10% (v/v) MeCN at a flow rate of 0.25 mL/min increasing to 90% (v/v) MeCN over 7 minutes. The high concentration organic mobile phase was held for 1 minute then returned to the initial starting conditions.
Injection Volume:	10 µL
Temperature:	Ambient temperature

Biotage

Mass Spectrometry

Instrument:	Ultima Pt triple quadrupole mass spectrometer (Waters Assoc., Manchester, UK) equipped with an electrospray interface for mass analysis. Positive ions were acquired in the multiple reaction monitoring mode (MRM)
Desolvation Temperature:	350 °C
Ion Source Temperature:	100 °C
Collision Gas Pressure:	2.9 x 10^{-3}mbar

The base peak in each compound spectrum was attributed to the protonated, [M+H]+ or deprotonated molecular ions [M-H]- and were subsequently used as the precursor ions in the resulting MRM transitions. Positive/negative ion switching was utilized to analyze all the diuretics in this suite. Full MRM transitions and ionization conditions are shown in **Table 1**.

Table 1. Quattro Ultima Pt mass spectrometer parameters

Scan Function	Analyte	MRM Transition	Ionization Polarity	Cone Voltage (V)	Collision Energy (eV)
1	Amiloride	223.1 > 181.1	+	35	12
	Acetazolamide	230.1 > 189.1	+	35	15
2	Hydrochlorothiazide	296.1 > 269.0	-	100	19
3	Methazolamide	237.1 > 195.1	+	35	12
4	Hydroflumethiazide	330.1 > 303.0	-	100	19
5	Furosemide	329.1 > 285.0	-	35	15
6	Bendoflumethiazide	420.1 > 289.1	-	100	22
7	Bumetanide	365.2 > 240.2	+	35	15
8	Spironolactone	417.2 > 341.2	+	35	14
9	Ethacrynic acid	301.1 > 243.1	-	35	12

Results

Table 2. 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

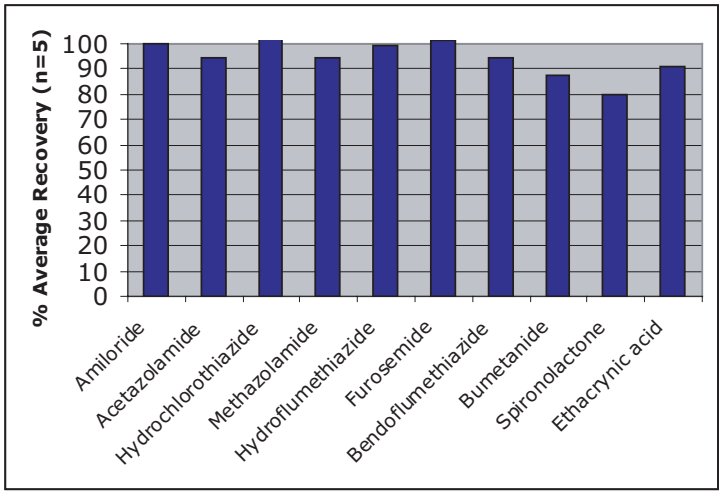

Figure 1. Recoveries (>80%, <10% RSD, n=5) for diuretics from urine using EVOLUTE ABN

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