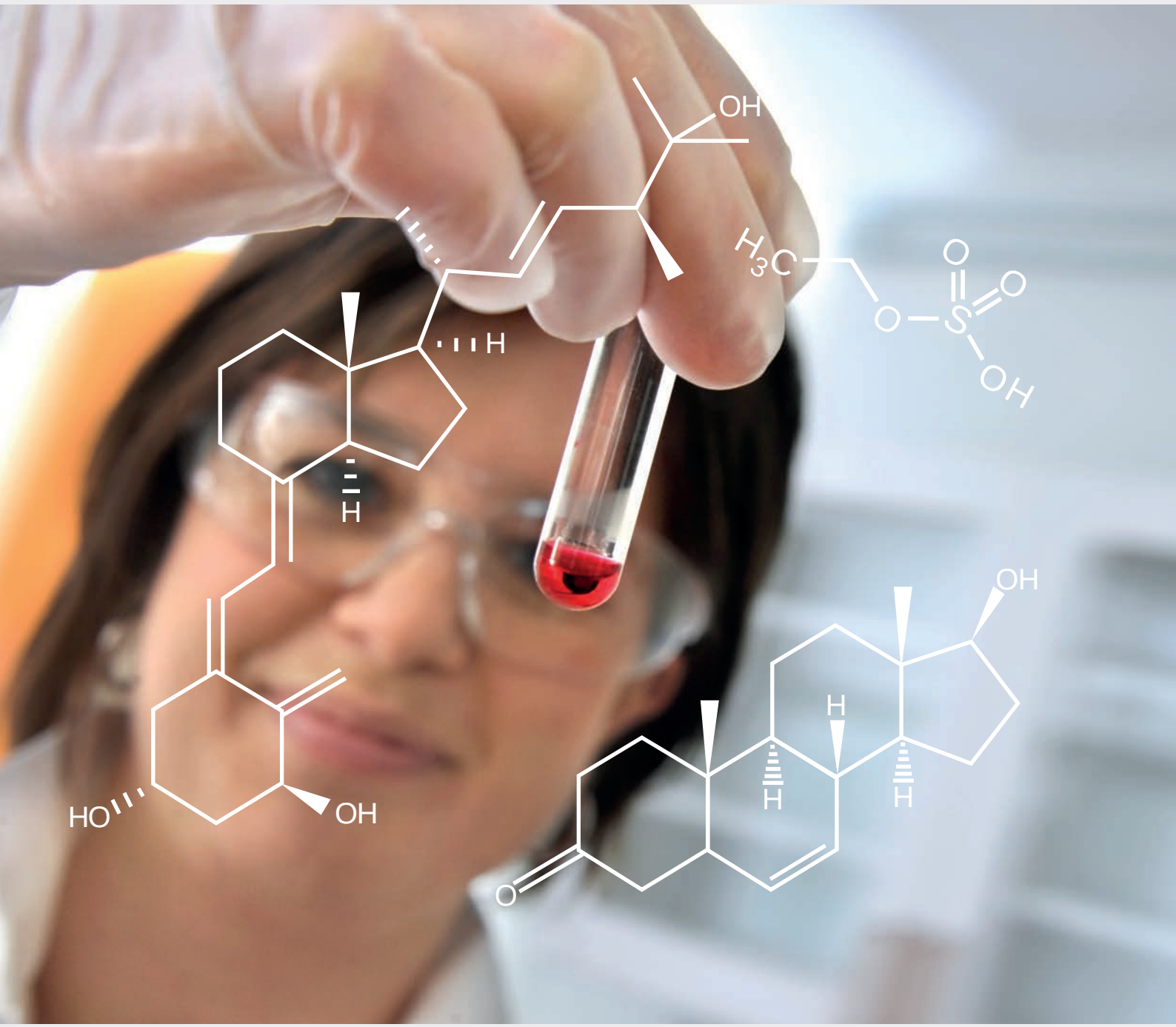
Clinical Applications

Compendium



Biotage®

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Introduction

Welcome to the Biotage Clinical Applications Compendium

This compendium highlights a selection of clinical sample preparation applications from Biotage. Whether you need targeted methods for analytes such as Vitamin D metabolites in serum, or methods suitable for extraction of a wide panel of drugs and metabolites from urine, sample preparation before analysis is essential.

Sample preparation to remove matrix components such as proteins, phospholipids and salts from your biological fluid samples can improve analyte sensitivity, method robustness and improve the quality of analytical data, as well as reducing instrument downtime.

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Choosing the 'right' sample preparation approach for every assay depends on a multitude of factors, including analyte(s), matrix type, analytical methodology, speed, cost per sample, extract cleanliness and sensitivity requirements and throughput needs. The methods outlined in this compendium use the range of Biotage sample preparation products:

- EVOLUTE® EXPRESS Solid Phase Extraction products
- ISOLUTE® SLE+ Supported Liquid Extraction products
- ISOLUTE® PLD+ Protein and Phospholipid Removal products
- ISOLUTE® PPT+ Protein Precipitation products

Detailed extraction and analytical methodology can be found in the full application notes, downloadable from www.biotage.com.

Alcohol Biomarkers

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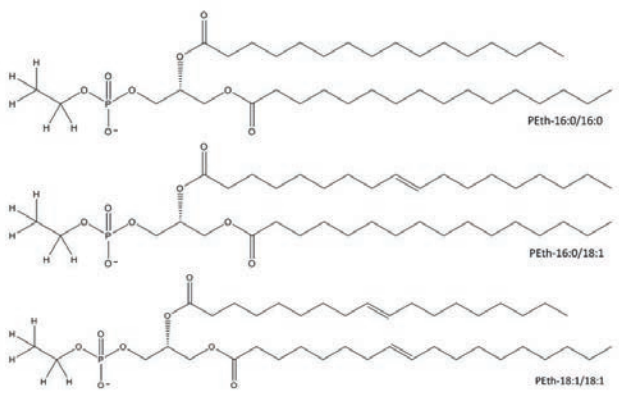
AN876

Extraction of Phosphatidylethanol (PEth) Species from Whole Blood Using ISOLUTE® SLE+ Prior to HPLC-MS/MS Analysis



Chemical structures of three common PEth species.

Analytes
1,2-dipalmitoyl-sn-glycero-3-phosphoethanol (PEth-16:0/16:0), 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoethanol (PEth-16:0/18:1), 1,2-dioleoyl-sn-glycero-3-phosphoethanol (PEth-18:1/18:1).

Format
ISOLUTE® SLE+ 200 µL Supported Liquid Extraction plate, part number 820-0200-P01.

Matrices
Whole blood.

Sample Preparation Method
To whole blood (20 µL), add 6.25% (v/v) aqueous ammonium hydroxide in 30% aqueous methanol (300 µL). Add internal standard. Mix thoroughly and allow to equilibrate. Load 140 µL of the pre-treated sample (equivalent to 8.75 µL whole blood). Apply a pulse of vacuum or positive pressure (3–5 seconds) to initiate flow. Allow the sample to absorb for 5 minutes.

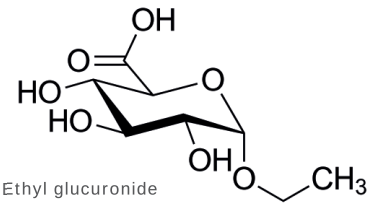
Elute analytes with ethyl acetate (750 uL).

Post Extraction
Dry the extract in a stream of air or nitrogen at 40 °C. Reconstitute the extracts with 150 µL of mobile phase and mix thoroughly before analysis.

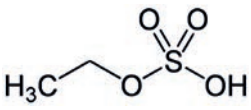
Summary of Results
Recoveries range from 85–89%, with RSDs ~5%. LOQ <30 nmol/L.

AN818

A No-Drydown SPE Method for Biomarkers of Alcohol Consumption in Human Urine Using ISOLUTE® NH2 SPE Columns Prior to LC-MS/MS



Ethyl glucuronide



Ethyl sulfate

Structures of ethyl glucuronide and ethyl sulfate.

Analytes
EtG (Ethyl glucuronide) and EtS (Ethyl sulfate).

Format
ISOLUTE® NH2 100 mg/1 mL columns, part number 470-0010-A.

Matrices
Urine

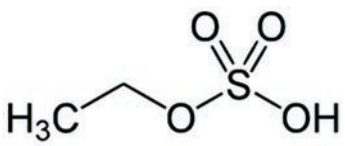
Sample Preparation Method
Dilute urine sample (100 µL) with acetonitrile (1 mL) and add 6M HCl (50 µL). Add internal standard as required and mix. Condition columns with methanol (2 mL), then equilibrate with water (2 mL) followed by acetonitrile containing 0.2% (v/v) acetic acid (2 mL). Load entire sample at a flow rate of 1 mL/min. Dry the column and elute interferences with hexane (1 mL). Dry columns for 10 mins under positive pressure. Elute analytes with 10 mM ammonium formate/formic acid (pH 3, 2 x 750 µL).

Post Extraction
The extract can be injected directly into the analytical system without an additional evaporation step.

Summary of Results
This ISOLUTE NH2 method was able to differentiate between patient positive and patient negative samples over a range of clinical interest.

AN755

Simultaneous Extraction of Ethyl Glucuronide and Ethyl Sulfate from Urine with EVOLUTE® EXPRESS AX Prior to LC-MS/MS Analysis



Structure of Ethyl Sulfate.

Analytes
EtG (Ethyl glucuronide) and EtS (Ethyl sulfate).

Format
EVOLUTE® EXPRESS AX 100 mg/3 mL columns, part number 613-0010-BXG.

Matrices
Urine

Sample Preparation Method
Dilute urine samples with acetonitrile (1:9, v/v) and add internal standard as required. Mix. Condition columns with methanol (3 mL) and equilibrate with water (3 mL) followed by acetonitrile (3 mL). Load the pre-treated sample (2 mL). Elute interferences with acetonitrile (3 mL) followed by methanol (3 mL). Elute analytes with 2% HCl in acetonitrile (3 mL).

Post Extraction
Evaporate to dryness at 40 °C and reconstitute with HPLC grade water (250 µL). Vortex mix and add acetonitrile (250 µL), vortex mix again before analysis.

Summary of Results
Analyte recoveries of >90% with LOQ of 10 ng/mL for EtG and 2 ng/mL for EtS.

Biomarkers

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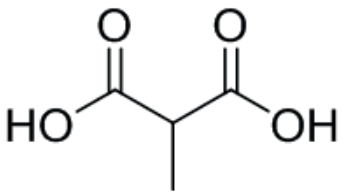
AN861

Extraction of Methylmalonic Acid from Serum Using ISOLUTE® PPT+ Protein Precipitation Plates Prior to LC-MS/MS Analysis



Structure of methylmalonic acid (MMA).

Analytes
Methylmalonic acid (MMA).

Format
ISOLUTE® PPT+ Protein Precipitation plate, part number 120-2040-P01.

Matrices
Serum.

Sample Preparation Method
To serum (100 µL), add 10 µL of ISTD (10 ng/µL). Mix and allow to equilibrate. Place extraction plate in manifold, with an appropriate collection plate in position. Add 800 µL of acetonitrile to each well followed by 100 µL of serum. Mix thoroughly by repeated aspirate/dispense steps. Apply vacuum or pressure to elute the analytes.

Post Extraction
Dry the extract in a stream of air or nitrogen at 40 °C . Reconstitute with 100 µL of 0.4% formic acid (aq) and vortex for 30 seconds before analysis.

Summary of Results
Reproducible recovery (~85%) with low RSD (<2%). Extracts are protein free.

AN851

Extraction of Methylmalonic Acid from Serum Using ISOLUTE® SLE+ Prior to LC-MS/MS Analysis

Analytes
Methylmalonic acid (MMA).

Format
ISOLUTE® SLE+ 200 µL Supported Liquid Extraction plate, part number 820-0200-P01.

Matrices
Serum.

Sample Preparation Method
To serum (100 µL), add 10 µL of ISTD (10 ng/µL). Allow to equilibrate and add 4.6M formic acid (aq) (100 µL). Mix. Load the pre-treated serum (200 µL) onto the plate and apply a pulse of vacuum or positive pressure (3–5 seconds) to initiate flow. Allow the sample to absorb for 5 minutes.

Elute analytes with MTBE (750 µL).

Post Extraction
Evaporate the extract in a stream of air or nitrogen at 40 °C. Addition of 2 µL of ethylene glycol prior to evaporation can help reduce analyte losses due to volatility. Reconstitute extracts with 100 µL of 0.4% formic acid (aq) before analysis.

Summary of Results
High reproducible recoveries >80% and corresponding RSDs of <10%.

AN850

Extraction of Methylmalonic Acid from Serum Using ISOLUTE® PLD+ Prior to LC-MS/MS Analysis

Analytes
Methylmalonic acid (MMA).

Format
ISOLUTE® PLD+ Protein and Phospholipid Removal plate, part number 918-0050-P01.

Matrices
Serum.

Sample Preparation Method
To serum (100 µL), add 10 µL of ISTD (10 ng/µL). Mix and allow to equilibrate. Place extraction plate in manifold, with an appropriate collection plate in position. Add 800 µL of 1% (v/v) formic acid in acetonitrile to each well followed by 100 µL of serum. Mix thoroughly by repeated aspirate/dispense steps. Apply vacuum or pressure to elute the analytes.

Post Extraction
Dry the extract in a stream of air or nitrogen at 40 °C. Reconstitute with 100 µL of 0.4% formic acid (aq) and vortex for 30 seconds before analysis.

Summary of Results
High reproducible recoveries >90% with corresponding RSDs <10%. Extracts are clean with no interference from phospholipids or proteins.

AN849

Extraction of Methylmalonic Acid from Serum Using ISOLUTE® SAX Prior to LC-MS/MS Analysis

Analytes
Methylmalonic acid (MMA).

Format
ISOLUTE® SAX 25 mg Fixed Well plate, part number 500-0025-P01.

Matrices
Serum.

Sample Preparation Method
To serum (100 µL), add 10 µL of internal standard (10 ng/µL). Mix and allow to equilibrate. Add HPLC grade water (190 µL) and vortex. Condition each well with methanol (500 µL) followed by HPLC grade water (500 µL). Load 300 µL of pre-treated sample. Elute interferences with HPLC grade water (500 µL) followed by methanol (500 µL). Elute analytes with 2% formic acid in acetonitrile (600 µL).

Post Extraction
Dry the extract in a stream of air or nitrogen at 40 °C. Reconstitute with 100 µL of 0.4% formic acid (aq) and vortex for 30 seconds before analysis.

Summary of Results
High reproducible recoveries >90% with corresponding RSDs <10%. Extracts are clean with no interference from phospholipids or proteins.

AN847

Extraction of Methylmalonic Acid from Serum Using EVOLUTE® EXPRESS AX Prior to LC-MS/MS Analysis

Analytes
Methylmalonic acid (MMA).

Format
EVOLUTE® EXPRESS AX 30 mg Fixed Well plate, part number 603-0030-PX01.

Matrices
Serum.

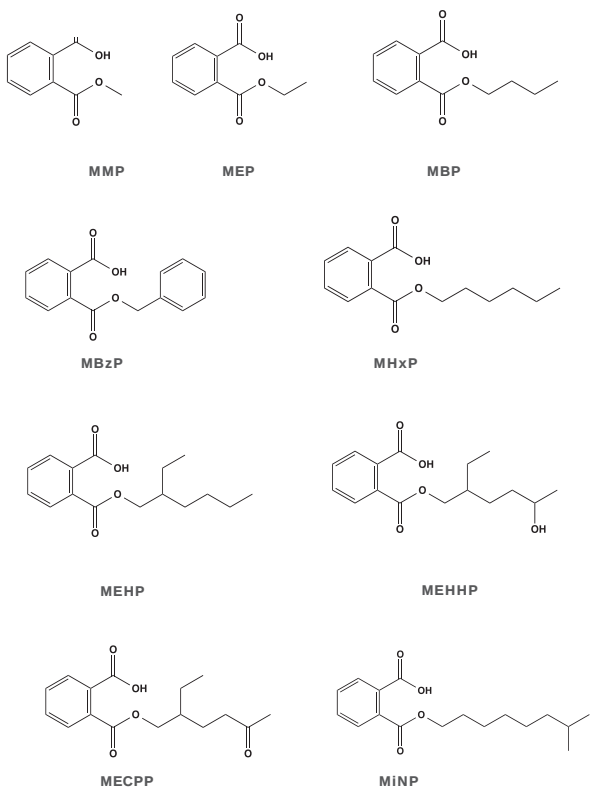
Sample Preparation Method
To serum (100 µL), add 10 µL of ISTD (10 ng/µL). Mix and allow to equilibrate. Add HPLC grade water (290 µL) and vortex. Load pre-treated sample (400 µL) direct to the 96-well plate. Elute interferences with HPLC grade water (1 mL) followed by methanol (1 mL). Elute analytes into a collection plate using 2% formic acid in acetonitrile (1 mL).

Post Extraction
Dry the extract in a stream of air or nitrogen at 40 °C. Reconstitute with 100 µL of 0.4% formic acid (aq) and vortex for 30 seconds before analysis.

Summary of Results
High reproducible recoveries >90% and corresponding RSDs of <10%.

AN826

A High-Throughput SPE Method to Support the Biomonitoring of Phthalate Metabolites in Human Urine Using ISOLUTE® ENV+ Columns Prior to LC-MS/MS



Structures of the target analytes in the phthalate metabolites panel.

Analytes
Monomethyl phthalate (MMP); monoethyl phthalate (MEP); monobutyl phthalate (MBP); monobenzyl phthalate (MBzP); monohexyl phthalate (MHxP); mono (2-ethylhexyl) phthalate (MEHP); mono(2-ethyl-5 hydroxyhexyl) phthalate (MEHHP); mono (2-ethyl-5-carboxypentyl) phthalate (MECPP) and monoisononyl phthalate (MiNP) .

Format
ISOLUTE® ENV+ 50 mg/3 mL columns, part number 915-0005-B.

Matrices
Urine.

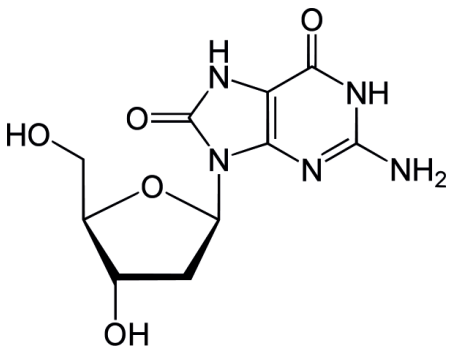
Sample Preparation Method
Hydrolyze urine and add internal standard. Mix and allow to equilibrate. Condition columns with methanol (1 mL) and equilibrate with water (1 mL). Load 500 µL of pre-treated sample. Elute interferences with water/methanol (90/10, v/v, 1 mL), and elute analytes with methanol (2 x 1 mL).

Post Extraction
Evaporate to dryness and reconstitute before analysis.

Summary of Results
ISOLUTE® ENV+ SPE cartridges were successful in providing quantitative analyte recovery, repeatable method precision and minimal matrix suppression for nine phthalate metabolites in urine.

AN759

Extraction of 8-oxoDG from Biological Fluids Using ISOLUTE® ENV+



Structures of (8-oxo-DG).

Analytes
8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxo-DG).

Format
ISOLUTE® ENV+ 50 mg/1 mL columns, part number 915-0005-A.

Matrices
Urine, plasma, saliva, breast milk, seminal plasma, peritoneal fluid.

Sample Preparation Method
Urine: Centrifuge 0.15 mL of urine at 16,100 x g for 15 mins. Add internal standard and dilute 1:1 (v/v) with deionized water. [For all other matrices, see full application note]. Condition column with methanol (1 mL) and equilibrate with water (1 mL). Load sample (1 mL) and elute interferences with 2% (v/v) methanol (aq) (1 mL). Elute analyte with 20% (v/v) acetonitrile in methanol (2 x 300 µL).

Post Extraction
Evaporate to dryness and reconstitute in mobile phase (50 µL). Mix and centrifuge before analysis.

Summary of Results
Recovery >85% with low intra and inter day variability. LOQ in urine <1 pmol/mL.

AN737

Extraction of Organophosphate Pesticide Metabolites From Urine Using EVOLUTE® EXPRESS WAX

Analytes
Dimethylphosphate (DMP), dimethylthiophosphate (DMTP), diethylphosphate (DEP), diethylthiophosphate (DETP), dimethyldithiophosphate (DMDTP) and diethyldithiophosphate (DEDTP).

Format
EVOLUTE® EXPRESS WAX 30 mg Fixed Well plate, part number 604-0030-PX01.

Matrices
Urine

Sample Preparation Method
Dilute urine sample (100 µL) with 2 % formic acid (300 µL) (1:3, v/v). Condition column with methanol (1 mL) and equilibrate with HPLC grade water (1 mL). Load pre-treated sample (400 µL). Elute polar and ionic interferences with HPLC grade water (1 mL). Elute non-polar interferences with acetonitrile (1 mL). Elute analytes with methanol containing ammonium hydroxide (95:5, v/v, 1 mL).

Post Extraction
Evaporate to dryness and reconstitute in mobile phase before analysis.

Summary of Results
Typical recoveries for all analytes are above 85% with relative standard deviations below 10%.

Catecholamine Metabolites

AN874 Extraction of Plasma Catecholamines and Metanephrines Using EVOLUTE® EXPRESS WCX SPE Prior to LC-MS/MS Analysis page 9

AN871 Extraction of Urinary Catecholamines and Metanephrines Using EVOLUTE® EXPRESS WCX SPE Prior to LC-MS/MS Analysis page 10

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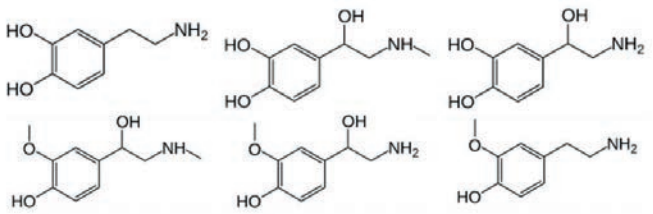
AN874

Extraction of Plasma Catecholamines and Metanephrines Using EVOLUTE® EXPRESS WCX SPE Prior to LC-MS/MS Analysis



Structures of dopamine, epinephrine and norepinephrine (top), metanephrine, normetanephrine and 3-methoxytyramine (bottom).

Analytes
Epinephrine, norepinephrine, dopamine, metanephrine, normetanephrine and 3-methoxytyramine.

Format
EVOLUTE® EXPRESS WCX 10 mg Fixed Well plate, part number 602-0010-PX01.

Matrices
Plasma.

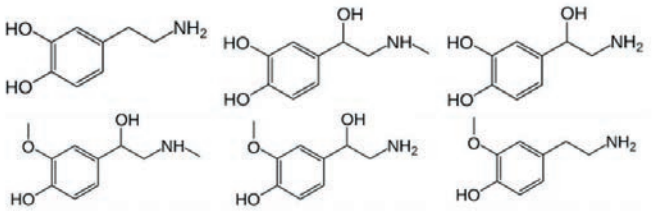
Sample Preparation Method
Mix plasma (300 µL) with 10 µL of internal standard solution and 0.05% formic acid (300 µL). Mix and allow to equilibrate. Load pre-treated plasma (500 µL). Elute interferences with 10 mM ammonium acetate (500 µL) followed by propan-2-ol (500 µL) and finally dichloromethane (500 µL). Elute analytes with 125 µL of water: propan-2-ol (85:15, v/v) containing formic acid (0.1% v/v).

Post Extraction
The extract can be injected directly into the analytical system without an additional evaporation step.

Summary of Results
High, reproducible analyte recoveries. Linearity was determined between 0.04 and 1.28 ng/mL for norepinephrine and dopamine and between 0.02 and 1.28 ng/mL for epinephrine, normetanephrine, metanephrine and 3-methoxytyramine.

AN871

Extraction of Urinary Catecholamines and Metanephrines Using EVOLUTE® EXPRESS WCX SPE Prior to LC-MS/MS Analysis



Structures of dopamine, epinephrine and norepinephrine (top), metanephrine, normetanephrine and 3-methoxytyramine (bottom).

Analytes
Epinephrine, norepinephrine, dopamine, metanephrine, normetanephrine and 3-methoxytyramine.

Format
EVOLUTE® EXPRESS WCX 10 mg Fixed Well plate, part number 602-0010-PX01.

Matrices
Urine

Sample Preparation Method
Mix urine (75 µL) with 10 µL of internal standard solution and 250 mM ammonium acetate solution (150 µL). Mix and allow to equilibrate. ammonium acetate (500 µL). Load pre-treated urine (150 µL). Elute interferences with 10mM ammonium acetate (500 µL) followed by propan-2-ol (500 µL), and dry thoroughly. Elute analytes with 125 µL of water: propan-2-ol (85:15, v/v) containing formic acid (0.1% v/v). Dry. Note that conditioning and equilibration steps are not required when using the EVOLUTE EXPRESS Load-Wash-Elute protocol.

Post Extraction
The extract can be injected directly into the analytical system without an additional evaporation step.

Summary of Results
High reproducible recoveries with LOQs ranging from 0.1 ng/mL (epinephrine) to 2.5 ng/mL (dopamine).

Pain Management Drug Panel

PPS443	Extraction of a Urine Drug Panel Using ISOLUTE® SLE+	page 11
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PPS443

Extraction of a Urine Drug Panel Using ISOLUTE® SLE+

Analytes
56 drug panel—see list in Biotage White Paper PPS443.

Format
ISOLUTE® SLE+ 400 µL Supported Liquid Extraction plate, part number 820-0400-P01.

Matrices
Urine.

Sample Preparation Method
Hydrolyze urine (200 µL) using β glucuronidase enzyme (see PPS443 for hydrolysis conditions). Add 0.1% NH_4OH (200 µL) and mix. Load hydrolyzed sample (400 µL) and apply a pulse of pressure to initiate flow. Allow to absorb for 5 minutes.

Elute analytes with 90:10 (v/v) dichloromethane:2-propanol (DCM:IPA) (2 x 0.75 mL).

Post Extraction
Dry under nitrogen (N_2) at 40 °C. Reconstitute in 90:10 (v/v) 0.1% formic acid in water/0.1% formic acid in methanol before analysis.

Summary of Results
ISOLUTE® SLE+ is recommended if opiates, opioids, benzodiazepines, stimulants (except ritalinic acid), PCP, barbiturates, 9-carboxy-THC, TCAs, meprobamate, carisoprodol, ketamine and norketamine are included in the urine panel.

PPs443

Extraction of a Urine Drug Panel Using EVOLUTE® EXPRESS ABN

Analytes
56 drug panel—see list in Biotage White Paper PPS443.

Format
EVOLUTE® EXPRESS ABN 30 mg Fixed Well plate (600-0030-PX01).

Matrices
Urine.

Sample Preparation Method
Hydrolyze urine (200 µL) using β-glucuronidase enzyme (see PPS443 for hydrolysis conditions). Add 0.1% NH_4OH (200 µL) and mix. Condition wells with methanol (1 mL) and equilibrate with 0.1% NH_4OH (1 mL). Load hydrolyzed sample (400 µL). Elute interferences with 0.1% NH_4OH (1 mL) followed by 10 methanol in water (v/v, 1 mL). Elute analytes with DCM:IPA (90:10, v/v, 2 x 0.75 mL).

Post Extraction
Dry under nitrogen (N_2) at 40 °C. Reconstitute in 90:10 (v/v) 0.1% formic acid in water/0.1% formic acid in methanol before analysis.

Summary of Results
EVOLUTE® EXPRESS ABN works well for some opioid drugs and metabolites, most benzodiazepines, ketamine, norketamine, PCP, 9-carboxy-THC, amitriptyline, nortriptyline, carisoprodol, meprobamate, cocaine and BZE.

PPs443

Extraction of a Urine Drug Panel Using EVOLUTE® EXPRESS CX

Analytes
56 drug panel—see list in Biotage White Paper PPS443.

Format
EVOLUTE® EXPRESS CX 30 mg Fixed Well plate, part number 601-0030-PX01.

Matrices
Urine.

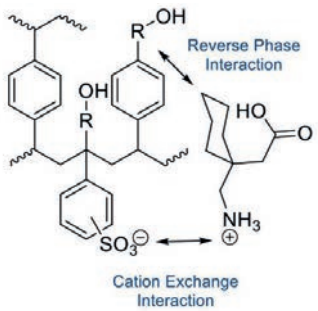
Sample Preparation Method
Hydrolyze urine (200 µL) using β-glucuronidase enzyme (see PPS443 for hydrolysis conditions). Add 4% phosphoric acid (H_3PO_4) (200 µL) and mix. Condition wells with methanol (1 mL) and equilibrate with 4% H_3PO_4 (1 mL). Load hydrolyzed sample (400 µL). Elute interferences with 4% H_3PO_4 (1 mL) followed by 50% methanol in water (v/v, 1 mL). Elute analytes with either:

a. 78:20:2 (v/v) DCM:IPA:NH_4OH(2 x 0.75 mL), or

b. 78:20:2 (v/v) DCM:MeOH:NH_4OH (2 x 0.75 mL).

Post Extraction
Dry under nitrogen (N_2) at 40 °C. Reconstitute in 90:10 (v/v) 0.1% formic acid in water/0.1% formic acid in methanol before analysis.

Summary of Results
If the drugs and metabolites in the urine panel are mostly basic: opiates, opioids, benzodiazepines, PCP, stimulants (except ritalinic acid), TCAs, ketamine, norketamine, and 9-carboxy-THC; then the EVOLUTE® EXPRESS CX method with sample pretreatment using 4% H_3PO_4, a 50% MeOH wash, and elution with 78:20:2 (v/v) DCM:IPA:NH_4OH is recommended. If ritalinic acid, gabapentin and pregabalin are required, the same protocol, except elution with 78:20:2 (v/v) DCM:MeOH:NH_4OH should be used.



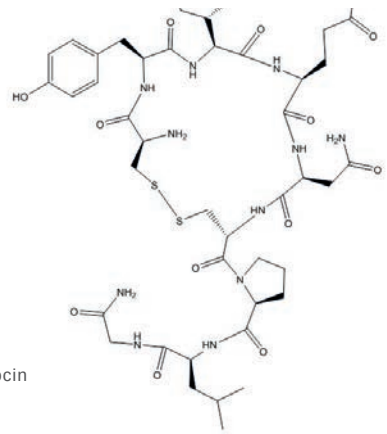
EVOLUTE® EXPRESS CX sorbent's proposed columbic complexation with pregabalin.

Peptides

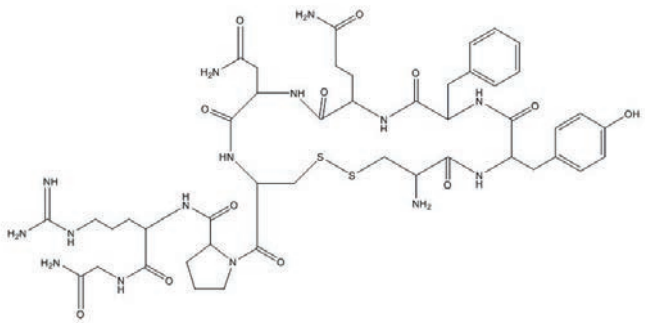
AN882	Extraction of Oxytocin and Vasopressin from Serum Using EVOLUTE® EXPRESS ABN Prior to LC-MS/MS Analysis	page 13
AN869	Extraction of Teicoplanin from Plasma Using EVOLUTE EXPRESS ABN Prior to HPLC-DAD Analysis	page 14

AN882

Extraction of Oxytocin and Vasopressin from Serum Using EVOLUTE® EXPRESS ABN Prior to LC-MS/MS Analysis



Oxytocin



Vasopressin

Analytes
Oxytocin and vasopressin.

Format
EVOLUTE® EXPRESS ABN 30 mg Fixed Well plate, part number 600-0030-PX01.

Matrices
Serum.

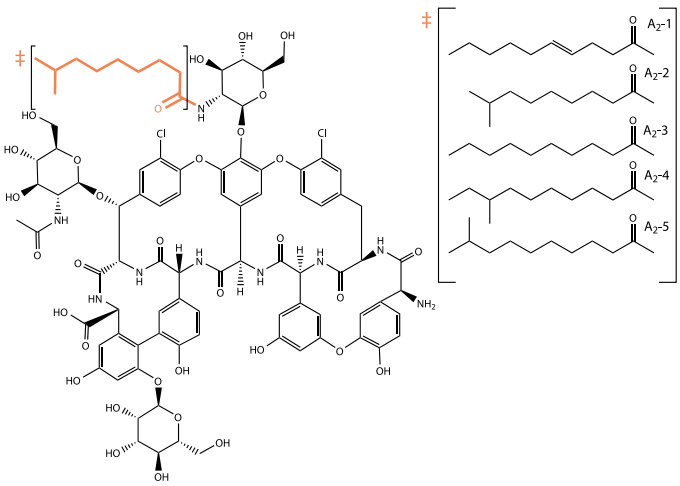
Sample Preparation Method
Dilute serum samples with 1% formic acid (1:1, v/v). Condition wells with methanol (1 mL) and equilibrate with 0.1% formic acid (aq) (1 mL). Load 400 µL of pre-treated serum sample. Elute interferences with 0.1% formic acid (aq) (1 mL). Elute analytes with 5% formic acid in acetonitrile /H_2O (20/80, v/v, 200 µL). *This highly aqueous elution solvent delivers high recoveries of oxytocin and vasopressin, and if desired, the extract can be injected directly into the analytical system without additional processing.* Note that conditioning and equilibration steps are not required when using the EVOLUTE® EXPRESS Load-Wash-Elute protocol.

Post Extraction
Alternatively, to minimize ion suppression, evaporate to dryness at 40 °C and reconstitute the extract with 0.1% formic acid in acetonitrile/H_2O (10/90, v/v, 200 µL) before analysis.

Summary of Results
High reproducible recoveries >70 % with corresponding RSDs <10%. Good sensitivity at concentrations down to 0.2 ng/mL.

AN869

Extraction of Teicoplanin from Plasma Using EVOLUTE® EXPRESS ABN Prior to HPLC-DAD Analysis



Teicoplanin A_2-2 (Major side-chain variants shown in parenthesis).

Matrices
Plasma.

Sample Preparation Method
Dilute plasma (200 µL) in a 1:3 ratio using 2% formic acid (aq). Condition each well with methanol (1 mL), and equilibrate with 0.1% formic acid (aq) (1 mL). Load 800 µL of pre-treated sample. Elute interferences with water (1 mL). Elute analytes with methanol/water (70/30, v/v, 500 µL).

Post Extraction
Evaporate to dryness at 40 ˚C and reconstitute with acetonitrile: 10 mM ammonium acetate pH 4.4 (10/90, v/v, 250 µL) before analysis.

Summary of Results
High recovery (88–100%) with low RSD (<7%). Linear range 2–100 µg/mL.

Analytes
Teicoplanin (as a mixture of A2 variants).

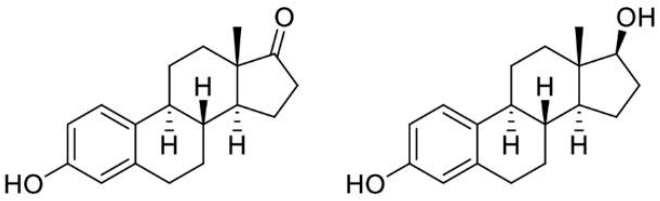
Format
EVOLUTE® EXPRESS ABN 30 mg Fixed Well plate, part number 600-0030-PX01.

Steroids

AN860	Extraction of Estrone and Estradiol from Human Serum Using ISOLUTE® SLE+ Prior to HPLC-MS/MS	page 16
AN778	Extraction of Cortisol from Human Saliva Using ISOLUTE® SLE+ Plates Prior to LC-MS/MS Analysis	page 16
AN777	Extraction Cortisol from Human Urine Using ISOLUTE® SLE+ Plates Prior to LC-MS/MS Analysis	page 17
AN762	Extraction of Low Level Testosterone and Androstenedione from Human Serum Samples Using ISOLUTE® SLE+	page 17
AN740	Extraction of Testosterone and Other Steroid Hormones from Human Plasma Using ISOLUTE® SLE+ 96-Well Plates	page 18
AN602	Extraction of Corticosteroids from Plasma Using ISOLUTE® SLE+ Supported Liquid Extraction Plates	page 18

AN860

Extraction of Estrone and Estradiol from Human Serum Using ISOLUTE® SLE+ Prior to HPLC-MS/MS



Estrone (E1)
Molecular Weight: 270.37

Estradiol (E2)
Molecular Weight: 272.39

Structures of Estrone (E1) and Estradiol (E2).

Analytes
Estrone (E1) and estradiol (E2).

Format
ISOLUTE® SLE+ 400 µL Supported Liquid Extraction plate, part number 820-0400-P01.

Matrices
Serum

Sample Preparation Method
Dilute serum sample (250 µL) with 25% IPA (aq) (100 µL) and add IS. Mix and allow to equilibrate. Load 350 µL of sample onto the plate and apply a pulse of vacuum or positive pressure (3–5 seconds) to initiate flow. Allow the sample to absorb for 5 minutes.

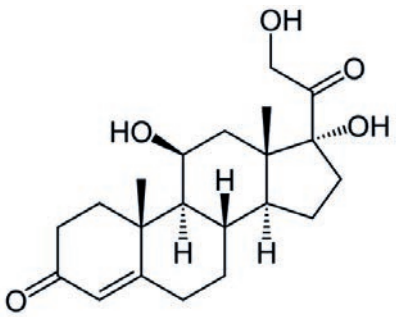
Elute analytes with dichloromethane (DCM) (3 x 600 µL).

Post Extraction
Evaporate to dryness at 40 °C and reconstitute with 250 µL of H_2O/ACN/MeOH (2:1:1, v/v) before analysis.

Summary of Results
High recovery and excellent sensitivity (LOQ of 0.001 ng/mL (estradiol) and 0.002 ng/mL (estrone) was achieved.

AN778

Extraction of Cortisol from Human Saliva Using ISOLUTE® SLE+ Plates Prior to LC-MS/MS Analysis



Structure of cortisol.

Analytes
Cortisol.

Format
ISOLUTE® SLE+ 200 µL Supported Liquid Extraction plate, part number 820-0200-P01.

Matrices
Oral fluid.

Sample Preparation Method
Dilute sample 1:1 (v/v) with water. Load the pre-treated sample (200 µL total volume) onto the plate and apply a pulse of vacuum or positive pressure to initiate flow. Allow the sample to adsorb for 5 minutes.

Elute analytes with MTBE (1 mL).

Post Extraction
Evaporate the extract to dryness. Reconstitute in water:methanol (50:50, v/v) (100 µL) before analysis.

Summary of Results
Recoveries greater than 95% with RSDs below 3%.

AN777

Extraction Cortisol from Human Urine Using ISOLUTE® SLE+ Plates Prior to LC-MS/MS Analysis

Analytes
Cortisol

Format
ISOLUTE® SLE+ 200 µL Supported Liquid Extraction plate, part number 820-0200-P01.

Matrices
Urine

Sample Preparation Method
Dilute sample 1:1 (v/v) with water. Load the pre-treated sample (200 µL total volume) onto the plate and apply a pulse of vacuum or positive pressure to initiate flow. Allow the sample to adsorb for 5 minutes.

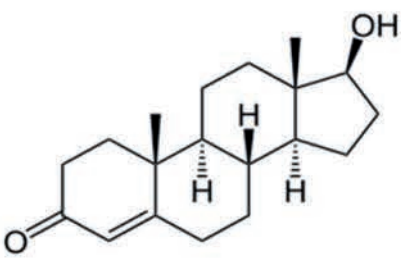
Elute analytes with MTBE (1 mL).

Post Extraction
Evaporate the extract to dryness (40 ºC). Reconstitute in water:methanol (50:50, v/v) (100 µL) before analysis.

Summary of Results
Recoveries greater than 99% with RSDs below 5%.

AN762

Extraction of Low Level Testosterone and Androstenedione from Human Serum Samples Using ISOLUTE® SLE+



Structure of testosterone.

Analytes
Testosterone, androstenedione.

Format
ISOLUTE® SLE+ 400 µL Supported Liquid Extraction plate, part number 820-0400-P01 .

Matrices
Serum

Sample Preparation Method
Dilute human serum (200 µL) with 0.5 mol/L ammonium hydroxide (200 µL), add internal standard and mix.

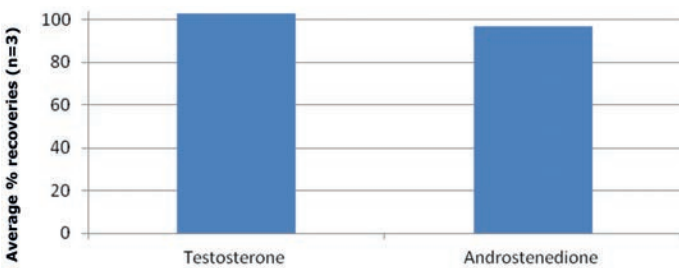
Load the pre-treated sample (400 µL) on to the plate and apply a pulse of vacuum or positive pressure (3–5 seconds) to initiate flow. Allow the sample to absorb for 5 minutes.

Elute analytes with diethyl ether (3 x 500 µL).

Post Extraction
Evaporate the eluate to dryness and reconstitute with 400 µL of methanol:water (1:1, v/v) before analysis.

Additional information: testosterone has an affinity to bind to plastic so the extracts were collected in glass tubes held in a 96 well collection plate.

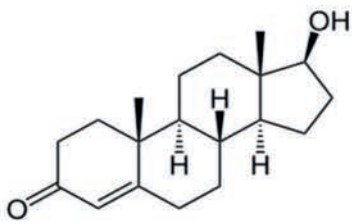
Summary of Results



Average analyte recoveries up to 100 nmol/L.

AN740

Extraction of Testosterone and Other Steroid Hormones from Human Plasma Using ISOLUTE® SLE+ 96-Well Plates



Structure of testosterone.

Analytes
Testosterone, aldosterone, 21-deoxycortisol, 11-deoxycortisol, androstendione, 17-α-hydroxyprogesterone, dehydroepiandrosterone (DHEA), progesterone, androsterone.

Format
ISOLUTE® SLE+ 200 µL Supported Liquid Extraction plate, part number 820-0200-P01.

Matrices
Human plasma

Sample Preparation Method
Dilute human plasma (100 µL) 1:1 with HPLC grade water (100 µL). Load the pre-treated sample (200 µL) on to the plate and apply a pulse of vacuum or positive pressure (3–5 seconds) to initiate flow. Allow the sample to absorb for 5 minutes.

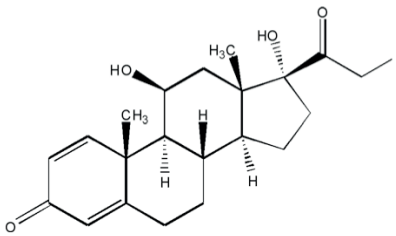
Elute analytes with dichloromethane (1 mL).

Post Extraction
Evaporate to dryness at ambient temperature and reconstitute in 50% methanol (aq) (100 µL). Vortex samples to ensure full reconstitution of all analytes.

Summary of Results
Recoveries ranging from 90–107% (n=7). The LOD for each analyte ranging from 0.5 ng/mL for androstendione to 100 ng/mL for DHEA.

AN602

Extraction of Corticosteroids from Plasma Using ISOLUTE® SLE+ Supported Liquid Extraction Plates



Structure of prednisone.

Analytes
Triamcinolone, prednisolone, hydrocortisone, prednisone, cortisone, betamethasone, dexamethasone, flumethasone, corticosterone, beclomethasone, triamcinolone acetonide, fluocinolone acetonide, budesonide structural isomer 1, budesonide structural isomer 2, 5-pregnen-3β-ol-20-one.

Format
ISOLUTE® SLE+ 200 µL Supported Liquid Extraction plate, part number 820-0200-P01.

Matrices
Plasma.

Sample Preparation Method
Dilute the sample with water (1:1, v/v) and mix. Load the pre-treated plasma (200 uL) onto the plate, and apply a pulse of vacuum or positive pressure (3–5 seconds) to initiate flow. Allow the sample to absorb for 5 minutes.

Post Extraction
Evaporate the extracts to dryness and reconstitute in H_2O/MeOH (80:20, v/v, 500 uL) before analysis.

Summary of Results

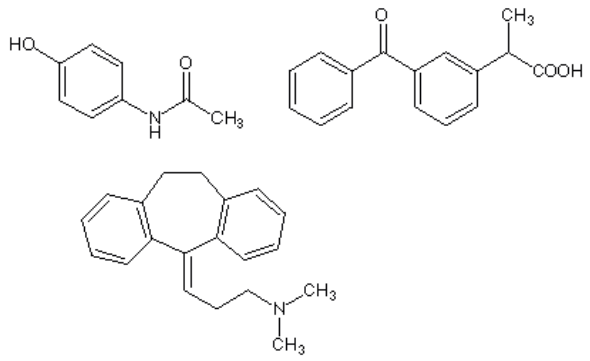
Analyte	% Recovery	RSD (%)
Triamcinolone	93	4
Prednisolone	93	1
Hydrocortisone	98	2
Prednisone	95	1
Cortisone	96	2
Betamethasone	92	2
Dexamethasone	92	2
Flumethasone	91	2
Corticosterone	94	2
Beclomethasone	91	2
Triamcinolone Acetonide	91	3
Fluocinolone Acetonide	90	3
Budesonide Structural Isomer 1	87	3
Budesonide Structural Isomer 2	89	2
5-pregnen-3β-ol-20-one	95	4

Therapeutic Drugs

AN830	Extraction of a Range of Acidic, Basic and Neutral Drugs from Plasma Using ISOLUTE® PLD+ Plates Prior to LC-MS/MS Analysis	page 20
AN811	Extraction of Antiepileptic Drugs from Oral Fluid Using ISOLUTE® SLE+ Prior to LC-MS/MS Analysis	page 21
AN810	Extraction of Mycophenolic Acid (MPA) and Mycophenolic Acid Glucuronide (MPAG) from Serum Using ISOLUTE® SLE+ Prior to LC-MS/MS	page 21
AN805	Extraction of Antiepileptic Drugs from Serum and Urine Using ISOLUTE® SLE+ Prior to LC-MS/MS Analysis	page 22
AN760	Fast Extraction of 10 Tricyclic Anti-depressant Drugs from Urine Using ISOLUTE® SLE+ Columns Prior to LC-MS-MS Analysis	page 23
AN758	Extraction of a Range of Immunosuppressants from Whole Blood Using ISOLUTE® SLE+ for LC-MS/MS Analysis	page 23
AN734	Method for the Extraction of Warfarin from Human Plasma Using ISOLUTE® SLE+	page 24
AN721	Extraction of Tamoxifen and Metabolites from Urine Using ISOLUTE® SLE+	page 25
AN700	Extraction of Diuretics from Urine using EVOLUTE® EXPRESS ABN Columns	page 25
AN603	Extraction of Non-steroidal Anti-inflammatory Drugs (NSAIDs) from Plasma Using ISOLUTE® SLE+ Supported Liquid Extraction Plates	page 26
AN601	Extraction of Tricyclic Anti-depressants from Plasma Using ISOLUTE® SLE+ Supported Liquid Extraction Plates	page 26

AN830

Extraction of a Range of Acidic, Basic and Neutral Drugs from Plasma Using ISOLUTE® PLD+ Plates Prior to LC-MS/MS Analysis



Structures of acetaminophen (neutral), ketoprofen (acidic) and amitryptiline (basic): examples of the broad range of analytes extracted in this application.

Analytes

Acetaminophen, amitriptyline, atenolol, bretylium tosylate, brompheniramine, fluoxetine, metoprolol, mianserin, naltrexone, procainamide, quinidine, ranitidine, salbutamol, sulindac, p-toluamide and ketoprofen.

Format

ISOLUTE® PLD+ Protein and Phospholipid Removal Plate, part number 918-0050-P01.

Matrices

Plasma

Sample Preparation Method

Add internal standard to the plasma sample, mix, and allow to equilibrate. Place the extraction plate in manifold, with an appropriate collection plate in position. Add 400 µL of acetonitrile to each well followed by 100 µL of plasma. Mix thoroughly by repeated aspirate/dispense steps. Apply vacuum or pressure to elute the analytes.

Post Extraction

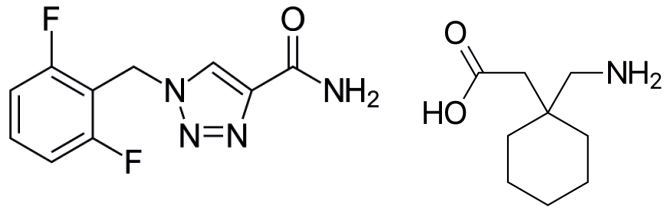
Evaporate to dryness at 40 °C. Reconstitute in 0.1% formic acid aq/methanol (80/20, v/v, 200 µL) before analysis.

Summary of Results

A simple, generic approach to extraction of a broad range of analytes, giving high recoveries with low RSDs.

AN811

Extraction of Antiepileptic Drugs from Oral Fluid Using ISOLUTE® SLE+ Prior to LC-MS/MS Analysis



Structures of rufinamide and gabapentin.

Analytes
Tiagabine, carbamazepine-10,11-epoxide, oxcarbazepine, gabapentin, vigabatrin, rufinamide, felbamate.

Format
ISOLUTE® SLE+ 400 µL Supported Liquid Extraction plate, part number 820-0400-P01.

Matrices
Oral fluid.

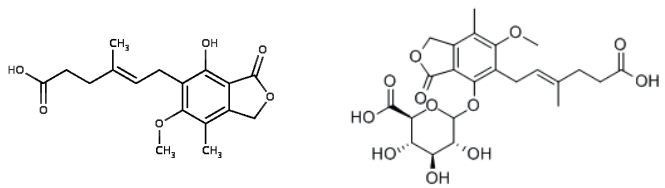
Sample Preparation Method
Add ammonium acetate (5mM, pH 2.9, 250µL) to the sample (100 µL) then add up to 50 µL of internal standard. Mix. Load up to 400 µL of pre-treated oral fluid sample onto the plate and apply a pulse of vacuum or positive pressure (3–5 seconds) to initiate flow. Allow the sample to absorb for 5 minutes. Elute analytes with methyl tert-butyl ether containing 1% (v/v) trifluoroacetic acid (conc) solution (2 x 700 µL).

Post Extraction
Evaporate to dryness and reconstitute sample with mobile phase before analysis.

Summary of Results
Recoveries for the AEDs using this pre-treatment strategy were good for all of the neutral AEDs in either neat or buffered oral fluid and substantially lower for the zwitterionic AEDs. Recovery >80% for felamate, rufinamide, oxcarbazepine, tiagabine and carbamazepine epoxide. Recoveries for gabapentin and vigabatrin were lower and attributed to their zwitterionic characteristics.

AN810

Extraction of Mycophenolic Acid (MPA) and Mycophenolic Acid Glucuronide (MPAG) from Serum Using ISOLUTE® SLE+ Prior to LC-MS/MS



Structure of mycophenolic acid (MPA) and mycophenolic acid glucuronide (MPAG).

Analytes
Mycophenolic acid (MPA) and mycophenolic acid glucuronide (MPAG).

Format
ISOLUTE® SLE+ 200 µL Supported Liquid Extraction plate, part number 820-0200-P01.

Matrices
Serum.

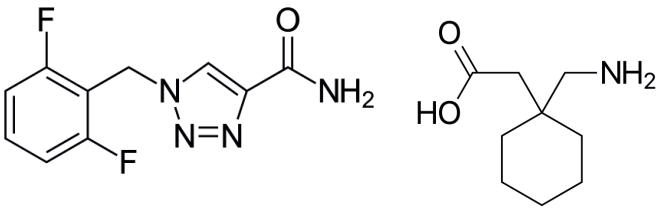
Sample Preparation Method
Add 90 µL of 20% aqueous formic acid to the sample (100 µL) then gently mix. Load the pre-treated sample onto the plate and apply a pulse of vacuum or positive pressure (3–5 seconds) to initiate flow. Allow the sample to absorb for 5 minutes. Elute analytes with ethyl acetate (2 x 500 µL).

Post Extraction
Evaporate to dryness and reconstitute in water:acetonitrile (50:50, v/v, 500 µL) before analysis.

Summary of Results
Recoveries >70% with RSDs <10%.

AN805

Extraction of Antiepileptic Drugs from Serum and Urine Using ISOLUTE® SLE+ Prior to LC-MS/MS Analysis



Structures of rufinamide and gabapentin.

Analytes
Tiagabine, carbamazepine-10,11-epoxide, oxcarbazepine, gabapentin, vigabatrin, rufinamide, felbamate.

Format
ISOLUTE® SLE+ 400 µL Supported Liquid Extraction plate, part number 820-0400-P01.

Matrices
Serum, urine.

Sample Preparation Method

Neutral Antiepileptic Drugs in Serum and Urine:
Add ammonium acetate (5 mM, pH 2.9, 250 µL) to the sample (100 µL) then add up to 50 µL of internal standard and mix.

Load up to 400 µL of pre-treated serum/urine sample onto the plate. Apply a pulse of vacuum or positive pressure (3–5 seconds) to initiate flow. Allow the sample to absorb for 5 minutes.

Elute analytes with methyl tert-butyl ether containing 1% (v/v) trifluoroacetic acid (conc) solution (2 x 700 µL).

Neutral Antiepileptic and Zwitterionic Drugs in Serum and Urine:

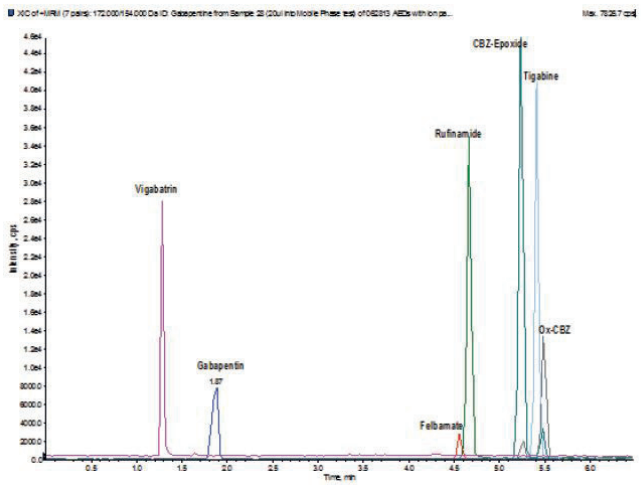
Add 50% aqueous formic acid (100 µL) to the sample (100 µL) then add up to 100 µL of internal standard and mix.

Load up to 300 µL of the pre-treated serum sample onto the plate. Apply a pulse of vacuum or positive pressure (3–5 seconds) to initiate flow. Allow the sample to absorb for 5 minutes.

Elute analytes with methyl tert-butyl ether containing 1% (v/v) trifluoroacetic acid (conc) solution (2 x 700 µL).

Post Extraction
Evaporate to dryness at 40 °C and reconstitute sample in mobile phase before analysis.

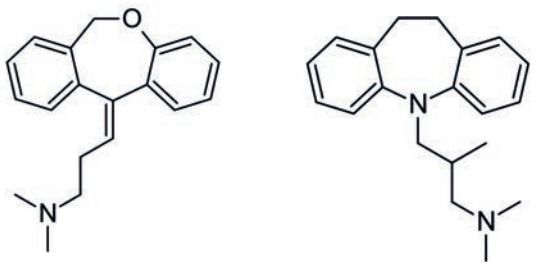
Summary of Results
Recoveries >70% with RSD <10%.



Extracted ion chromatogram of antiepileptic drugs.

AN760

Fast Extraction of 10 Tricyclic Anti-depressant Drugs from Urine Using ISOLUTE® SLE+ Columns Prior to LC-MS-MS Analysis



Structures of doxepin and trimipramine.

Analytes
Trimipramine, imipramine, desipramine, clomipramine, amitriptyline, doxepin, desmethyldoxepin, nortriptyline, paroxetine, sertraline.

Format
ISOLUTE® SLE+ 200 µL Supported Liquid Extraction columns, part number 820-0140-C.

Matrices
Urine.

Sample Preparation Method
Mix urine with concentrated ammonium hydroxide (99:1, v/v). Add internal standard and mix. Load the pre-treated sample (1 mL) onto cartridge. Apply a short pulse of vacuum or positive pressure to initiate flow and allow sample to adsorb for 5 minutes.

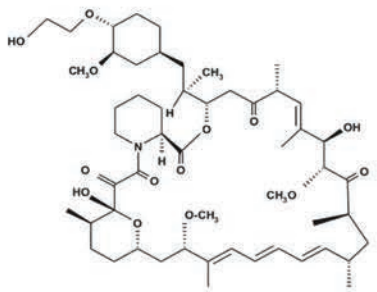
Elute analytes with hexane:isopropanol (98:2, v/v, 2 x 4 mL).

Post Extraction
Evaporate sample to dryness and reconstitute in mobile phase (500 µL) before analysis.

Summary of Results
Recoveries for all of the TCAs were found to be >85% except for paroxetine which was observed at a recovery of 75%.

AN758

Extraction of a Range of Immunosuppressants from Whole Blood Using ISOLUTE® SLE+ for LC-MS/MS Analysis



Structure of everolimus.

Analytes
Sirolimus, tacrolimus, everolimus, cyclosporin A.

Format
ISOLUTE® SLE+ 400 µL Supported Liquid Extraction plate, part number 820-0400-P01.

Matrices
Whole blood.

Sample Preparation Method
In a 2 mL Eppendorf centrifuge tube, pipette whole blood (50 µL). Add HPLC water (250 µL) and vortex for 30 seconds. Centrifuge at 12,000 RPM for 10 minutes.

Load the supernatant (275 µL) onto the plate and apply a pulse of vacuum or positive pressure for 10 seconds. Allow the sample to absorb for 5 minutes.

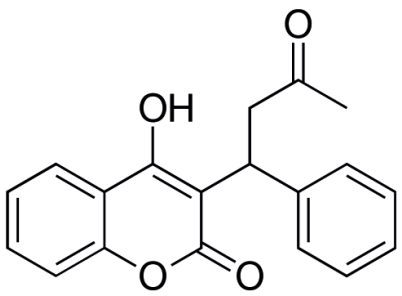
Elute analytes with ethyl acetate (2 x 600 µL).

Post Extraction
Evaporate the extract to dryness (30 °C). Reconstitute in water:acetonitrile (100 µL, 25:75, v/v).

Summary of Results
Recoveries ranged from 60–97%. RSDs were all below 10% for all analytes.

AN734

Method for the Extraction of Warfarin From Human Plasma Using ISOLUTE® SLE+



Structure of warfarin.

Analytes
Warfarin.

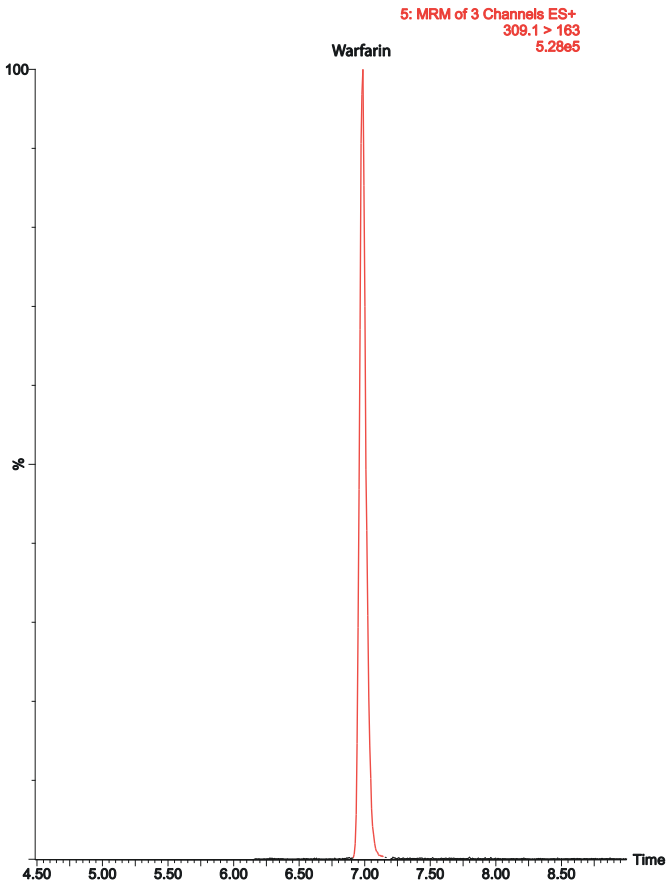
Format
ISOLUTE® SLE+ 200 µL Supported Liquid Extraction plate, part number 820-0200-P01.

Matrices
Human plasma.

Sample Preparation Method
To plasma (100 µL) add 1% formic acid (100 µL), and mix. Load the pre-treated plasma (200 µL) onto the plate and allow to absorb for 5 mins.

Elute analytes with dichloromethane (DCM) (1 mL).

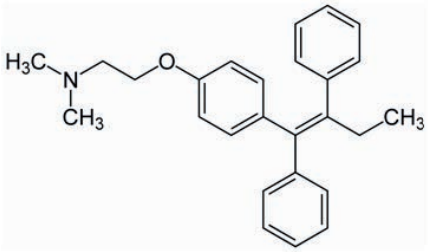
Summary of Results
All results show recoveries above 90% with RSDs below 10%.



Example chromatogram showing warfarin.

AN721

Extraction of Tamoxifen and Metabolites from Urine Using ISOLUTE® SLE+



Structure of tamoxifen.

Analytes
Tamoxifen, endoxifen, 4-OH-tamoxifen, des-methyl-tamoxifen.

Format
ISOLUTE® SLE+ 200 µL Supported Liquid Extraction plate, part number 820-0200-P01.

Matrices
Urine.

Sample Preparation Method
Dilute 100 µL of urine 1:1 (v/v) with 0.5 M NH_4OH. Load the pre-treated urine (200 µL) onto the plate and apply a pulse of vacuum or positive pressure (3–5 seconds) to initiate flow. Allow the sample to absorb for 5 minutes.

Elute analytes with ethyl acetate (2 x 500 µL).

Post Extraction
Evaporate to dryness and reconstitute in 500 µL of 0.1% formic acid in H_2O/MeOH (50:50, v/v) before analysis.

Summary of Results
All results show recoveries above 80% with %RSDs below 10%.

AN700

Extraction of Diuretics from Urine Using EVOLUTE® EXPRESS ABN Columns

Analytes
Amiloride, acetazolamide, hydrochlorothiazide, methazolamide, hydroflumethiazide, furosemide, bendoflumethiazide, bumetanide, spironolactone, ethacrynic acid.

Format
EVOLUTE® EXPRESS ABN 100 mg/3 mL columns (tabless), part number 610-0010-BXG.

Matrices
Urine.

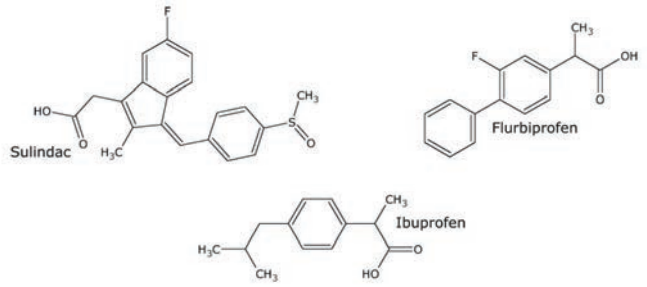
Sample Preparation Method
Dilute urine samples with acetonitrile (1:9, v/v) and add internal standard as required. Mix. Condition columns with methanol (3 mL) and equilibrate with water (3 mL) followed by acetonitrile (3 mL). Load the pre-treated sample (2 mL). Elute interferences with acetonitrile (3 mL) followed by methanol (3 mL). Elute analytes with 2% HCl in acetonitrile (3 mL).

Post Extraction
Evaporate to dryness and reconstitute in H_2O/MeOH (50:50, v/v, 1 mL) for before analysis.

Summary of Results
High analyte recoveries (80–100%) with RSDs of <10% were achieved.

AN603

Extraction of Non-steroidal Anti-inflammatory Drugs (NSAIDs) from Plasma Using ISOLUTE® SLE+ Supported Liquid Extraction Plates



Structures of sulindac, flurbiprofen and ibuprofen.

Analytes
Sulindac, flurbiprofen, ibuprofen.

Format
ISOLUTE® SLE+ 400 µL Supported Liquid Extraction plate, part number 820-0400-P01.

Matrices
Plasma.

Sample Preparation Method
Dilute plasma (1:1, v/v) with 1% formic acid and mix. Load the pre-treated sample (200 µL) onto the plate and apply a pulse of vacuum or positive pressure (3–5 seconds) to initiate flow. Allow the sample to absorb for 5 minutes.

Elute analytes with MTBE (2 x 900 uL).

Post Extraction
Evaporate to dryness and reconstitute in H_2O/MeOH (60:40, v/v, 500 µL).

Summary of Results

Analyte	Recovery	RSDs
Sulindac	92	3
Flurbiprofen	94	3
Ibuprofen	91	10

AN601

Extraction of Tricyclic Anti-depressants from Plasma Using ISOLUTE® SLE+ Supported Liquid Extraction Plates

Analytes
Imipramine, trimipramine, nortriptyline.

Format
ISOLUTE® SLE+ 200 µL Supported Liquid Extraction plate, part number 820-0200-P01.

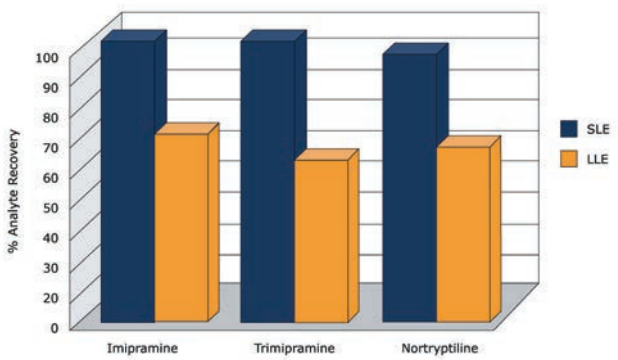
Matrices
Plasma.

Sample Preparation Method
Dilute the sample (100 µL) with 0.5 M NH_4OH (100 µL). Mix. Load the pre-treated sample onto the plate and apply a pulse of vacuum or positive pressure to initiate flow. Allow to absorb for 5 minutes.

Elute analytes with hexane:3-methyl-1-butanol (98:2, v/v, 1 mL).

Post Extraction
Evaporate to dryness and reconstitute in mobile phase (H_2O:ACN:NH_4OH 10:90:0.1, v/v) before analysis.

Summary of Results
Analyte recoveries were >91% with RDS %<4 for all analytes.



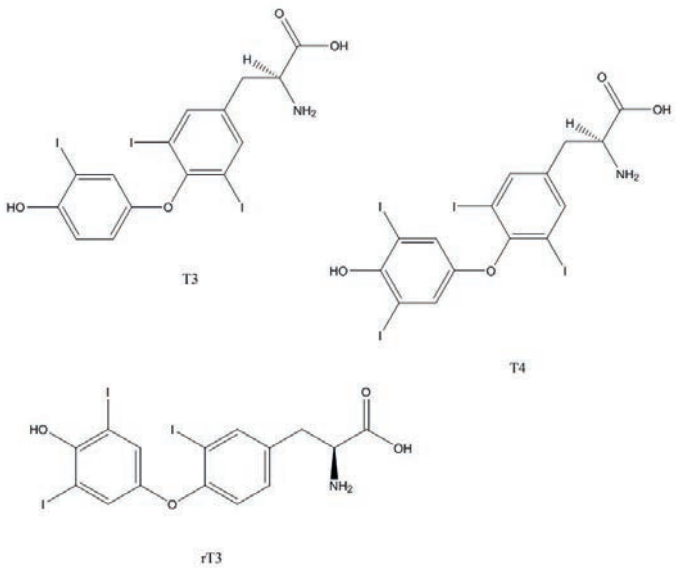
Comparison of analyte recovery using ISOLUTE® SLE+ and LLE.

Thyroid Hormones

AN881 Extraction of Thyroid Hormones: T3, rT3 and T4 from Serum Using EVOLUTE® EXPRESS AX Plates Prior to LC-MS/MS page 27

AN881

Extraction of Thyroid Hormones: T3, rT3 and T4 from Serum Using EVOLUTE® EXPRESS AX Plates Prior to LC-MS/MS



Structures of T3, rT3 and T4.

Analytes
Tri-iodothyronine (T3), reverse tri-iodothyronine (rT3) andthyroxine (T4).

Format
EVOLUTE® EXPRESS AX 30 mg Fixed Well Plate, part number 603-0030-PX01.

Matrices
Serum.

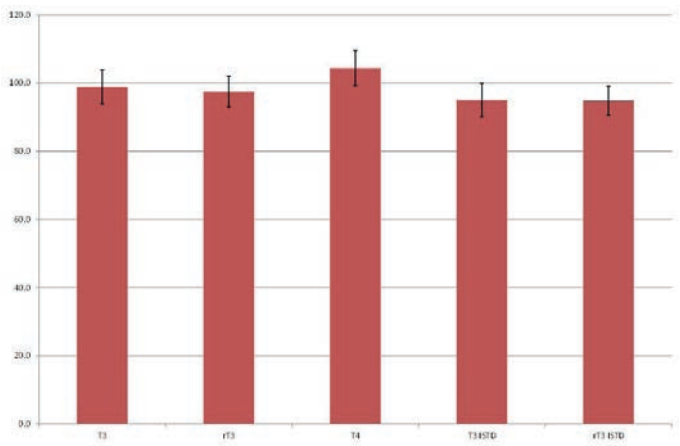
Sample Preparation Method
To serum (200 µL) add internal standard solution (10 µL), then add a further 100 µL of a mixture of citric acid, ascorbic acid and DL-dithiothreitol (25 mg/mL), and vortex mix thoroughly.

Note: *the use of these stabilizers prevents conversion of T4 to T3 and rT3 during extraction.*

Condition each well with methanol (1 mL) and equilibrate with water (1 mL). Load the entire sample volume. Elute interferences with 50 mM NH_4OAc buffer, pH 9 aq (1 mL) followed by MeOH (1 mL), and finally 2% formic acid in DCM (v/v) (1 mL). Elute analytes with MeOH (500 µL).

Post Extraction
Evaporate to dryness at 40 °C and reconstitute with H_2O/MeOH (50/50 (v/v), 150 µL) before analysis.

Summary of Results
High reproducible recoveries >85% with corresponding RSDs <10%. Extremely clean extracts.



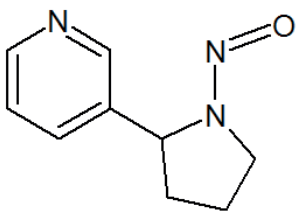
Recovery profile for thyroid hormones extracted at 2 ng/mL.

Tobacco Exposure

AN884	Extraction of Tobacco-Specific Nitrosamines (TSNAs) from Urine Using ISOLUTE® SLE+ Prior to UPLC/MS/MS Analysis	page 28
AN787	Extraction of Nicotine and Metabolites from Urine, Serum, Plasma and Whole Blood Using ISOLUTE® SLE+ Prior to LC-MS/MS Analysis	page 29

AN884

Extraction of Tobacco-Specific Nitrosamines (TSNAs) from Urine Using ISOLUTE® SLE+ Prior to UPLC/MS/MS Analysis



Structure of N-nitrosonornicotine (NNN).

Analytes
NNN (n-nitrosonornicotine), also suitable for NNK (4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone) and NNAL (4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol).

Format
ISOLUTE® SLE+ 1 mL sample volume columns (tabless), part number 820-00140-CG.

Matrices
Urine

Sample Preparation Method
Load 1 mL of the IS spiked urine sample, and apply a pulse of vacuum or positive pressure (3–5 seconds) to initiate flow. Allow the sample to absorb for 5 minutes.

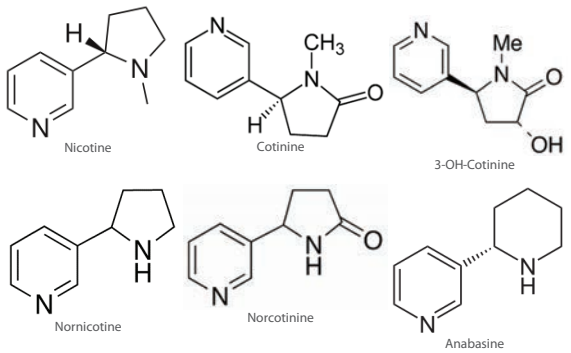
Elute analytes with dichloromethane (DCM) (2 x 1.5 mL).

Post Extraction
Dry under nitrogen (N_2) at 40 °C. Reconstitute in 0.1% formic acid in water/0.1% formic acid in methanol (90:10, v/v) before analysis.

Summary of Results
Limits of quantitation of 10 pg/mL can be achieved.

AN787

Extraction of Nicotine and Metabolites from Urine, Serum, Plasma and Whole Blood Using ISOLUTE® SLE+ Prior to LC-MS/MS Analysis



Structures of nicotine and metabolites.

Analytes

Nicotine, cotinine, 3-OH-cotinine, nornicotine, norcotinine, anabasine.

Format

ISOLUTE® SLE+ 200 µL Supported Liquid Extraction plate, part number 820-0200-P01.

Matrices

Urine, serum, plasma and whole blood.

Sample Preparation Method

To sample matrix (120 µL) add 10 µL internal standard and ammonia solution (25%, 230 µL). Mix well. Load pre-treated sample (150 µL) onto each well. Apply a pulse of vacuum or positive pressure to 3–5 secs.) to initiate flow. Allow the sample to absorb for 5 minutes.

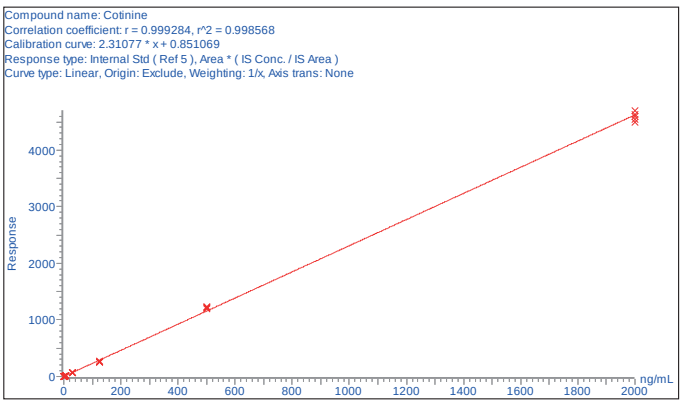
Elute analytes with dichloromethane:isopropanol (95: 5, v/v, 1 mL) into a 2 mL deep well collection plate containing 100 µL methanolic 200 mM HCl in each well.

Post Extraction

Dry the eluate in a stream of air or nitrogen. Reconstitute in methanol:water (10.90, v/v, 200 µL).

Summary of Results

Matrix	Recovery (%) of nicotine	RSD (%)
Urine	97.1	<7
Plasma	95.7	<5
Serum	95.5	<3
Whole Blood	97.9	<4



Shows typical linearity data achieved using this method.

Vitamins

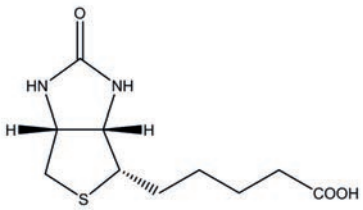
AN880	Extraction of Vitamin B7 (Biotin) from Serum Using EVOLUTE® EXPRESS ABN Prior to LC-MS/MS Analysis		page 31
AN857	Ultra-Sensitive Method for the Determination of 1,25 di-OH Vitamin D2 and 1,25 di-OH Vitamin D3 in Serum Using Supported Liquid Extraction Prior to LC-MS/MS		page 31
AN842	Extraction of 25-hydroxy Vitamin D from Serum Using ISOLUTE® PLD+ Prior to LC-MS/MS Analysis		page 32
AN814	A High-Throughput SPE Method for Extraction of Vitamin B3 (Niacin) and Related Metabolites from Serum Using ISOLUTE® SCX-3 Prior to LC-MS/MS		page 32
AN757	Extraction of Vitamin D Metabolites from Human Serum Using ISOLUTE® SLE+ in 96-Fixed Well Plate Format Prior to LC-MS-MS Analysis		page 33
AN753	Extraction of Retinol, β-Carotene (Vitamin A) and α-Tocopherol (Vitamin E) from Serum Using ISOLUTE® SLE+ 96-Well Plates with APCI-LC-MS-MS Analysis		page 34

Automated Using Biotage® Extrahera™

Methods marked with this icon have been automated using Biotage® Extrahera™, and detailed settings are available.

AN880

Extraction of Vitamin B7 (Biotin) from Serum Using EVOLUTE® EXPRESS ABN Prior to LC-MS/MS Analysis



Structure of Vitamin B7.

Analytes
25-OH vitamin D2 and 25-OH vitamin D3.

Format
EVOLUTE® EXPRESS ABN 10 mg Fixed Well plate, part number 600-0010-PX01.

Matrices
Serum.

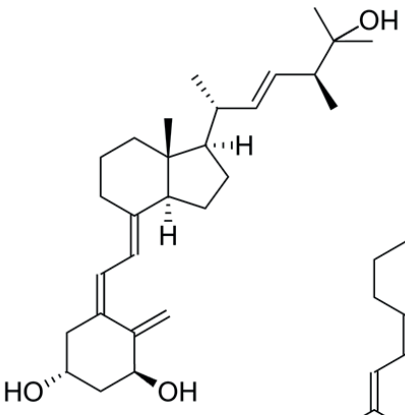
Sample Preparation Method
Dilute serum (200 µL) with 1% formic acid (aq) (200 µL), add internal standard and mix. Condition wells with methanol (500 µL) and equilibrate with 1% formic acid (aq) (500 µL). Load 400 µL of diluted sample. Elute interferences with H_2O (500 µL) followed by H_2O/MeOH (95/5, v/v, 500 µL). Elute biotin with 0.1% NH_4OH in H_2O/MeOH (90/10, v/v, 200 µL). Note that conditioning and equilibration steps are not required when using the EVOLUTE® EXPRESS Load-Wash-Elute protocol.

Post Extraction
Evaporate extract to dryness at 40 °C in a stream of air or nitrogen. Reconstitute the extract with H_2O/ACN (90/10, v/v, 200 µL) before analysis.

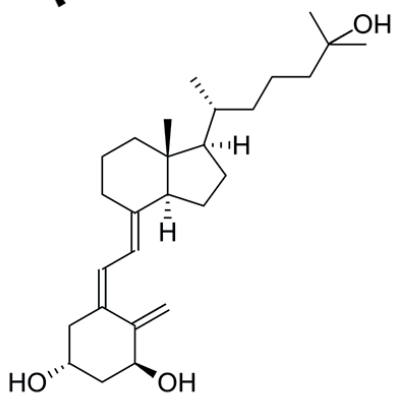
Summary of Results
High reproducible recoveries >80% with corresponding RSDs <10%. Extracts are clean with minimal matrix effects.

AN857

Ultra-Sensitive Method for the Determination of 1,25 di-OH Vitamin D2 and 1,25 di-OH Vitamin D3 in Serum Using Supported Liquid Extraction Prior to LC-MS/MS



1,25 diOH Vitamin D2



1,25 diOH Vitamin D3

Analyte structures.

Analytes
1,25 diOH Vitamin D2 and 1,25 diOH Vitamin D3.

Format
ISOLUTE® SLE+ 400 µL Supported Liquid Extraction plate, part number 820-0400-P01.

Matrices
Serum.

Sample Preparation Method
To serum sample add internal standard, mix and leave to stand for at least 30 mins. Dilute the serum sample with an equal volume of volume of propan-2-ol : water (50:50, v/v) solution, mix. Load pre-treated serum (300 µL) and apply a pulse of vacuum or positive pressure (3–5 sec) to initiate flow. Allow the sample to absorb for 5 minutes.

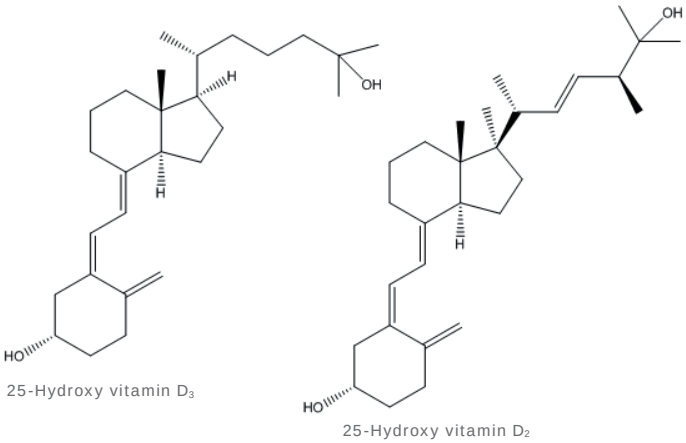
Ensure a collection plate containing 200 µL of derivatization solution (0.25 mg/mL PTAD in ethyl acetate : heptane (8:92, v/v)) in each well is in position. Elute analytes with heptane (2 x 700 uL).

Post Extraction
Evaporate the extracts to dryness at 40 °C and reconstitute in 70% methanol (aq) containing 50 µL/L methylamine (150 µL) before analysis.

Summary of Results
High reproducible recoveries are achieved. Linearity demonstrated from 5 to 500 pg/mL.

AN842

Extraction of 25-hydroxy Vitamin D from Serum Using ISOLUTE® PLD+ Prior to LC-MS/MS Analysis



25-Hydroxy vitamin D_3 25-Hydroxy vitamin D_2

Structures of 25-hydroxy Vitamin D.

Analytes
25-OH vitamin D_2 and 25-OH vitamin D_3.

Format
ISOLUTE® PLD+ Protein and Phospholipid Removal plate, part number 918-0050-P01.

Matrices
Serum.

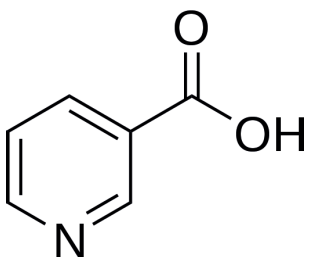
Sample Preparation Method
To serum add internal standard, mix and leave to stand for at least 30 mins. Ensure a suitable collection plate is in position. Apply 400 µL of acetonitrile (MeCN) to each well of the ISOLUTE® PLD+ plate. Add 100 µL of serum with ISTD and mix thoroughly via repeat aspirate/dispense steps. Apply vacuum (-0.2 bar) or 3 psi positive pressure for approximately 5 minutes. For highly particulate laden samples increased pressure or vacuum conditions may be required.

Post Extraction
Dry the extract at 40 °C. Reconstitute using 100 µL of (30/70, v/v) 2 mM ammonium formate, 0.1% formic acid aq/MeOH.

Summary of Results
High analyte recovery with low RSDs was achieved. Linearity was demonstrated from 1–100 ng/mL. Five DEQAS samples were tested using this method and all determined values met acceptability criteria.

AN814

A High-Throughput SPE Method for Extraction of Vitamin B3 (Niacin) and Related Metabolites from Serum Using ISOLUTE® SCX-3 Prior to LC-MS/MS



Structure of Niacin (vitamin B_3).

Analytes
Niacin (nicotinic acid), nicotinuric acid, niacinamide.

Format
ISOLUTE® SCX-3 25 mg Fixed Well plate, part number 533-0025-P01.

Matrices
Serum.

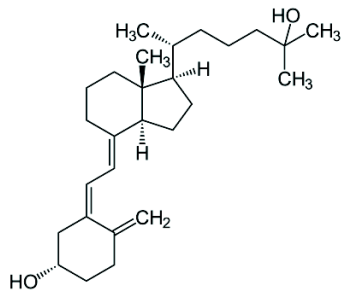
Sample Preparation Method
Dilute serum (50 µL) with aqueous acetic acid (2%, 150 µL). Mix thoroughly. Condition each well with methanol (1 mL) and equilibrate with aqueous acetic acid (2%, 1 mL). Load pre-treated sample (200 µL). Wash each well with water:methanol:acetic acid (68:30:2, v/v/v, 2 x 1 mL) followed by methanol:acetic acid (98: 2, v/v, 2 x 1 mL). Elute analytes with methanol: ammonium hydroxide (95:5, v/v, 2 x 400 µL).

Post Extraction
Evaporate extracts to dryness and reconstitute in 0.1% formic acid (100 µL) prior to analysis.

Summary of Results
This method is a viable option for serum measurements over a relevant concentration range in clinical diagnostics.

AN757

Extraction of Vitamin D Metabolites from Human Serum Using ISOLUTE® SLE+ in 96-Well Plate Format Prior to LC-MS-MS Analysis



Structure of 25-OH vitamin D_3.

Analytes
25-OH vitamin D2 and 25-OH vitamin D3.

Format
ISOLUTE® SLE+ 400 µL Supported Liquid Extraction plate, part number 820-0400-P01.

Matrices
Human Serum.

Sample Preparation Method
Dilute human serum (150 µL) with HPLC grade water:isopropanol (v/v, 50:50), (150 µL), add internal standard. Mix, cap and shake for 60 seconds.

Load the pre-treated serum (300 µL in total) onto the plate and apply a pulse of vacuum or positive pressure to initiate flow (3–5 sec). Allow the sample to absorb for 5 minutes.

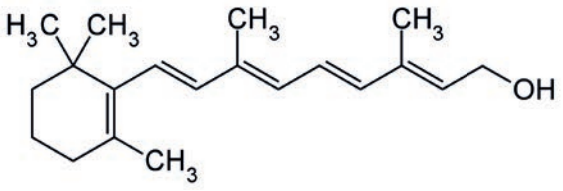
Elute analytes with heptane (2 x 750 µL).

Post Extraction
Evaporate the eluate to dryness without heat and reconstitute in 100 µL of (v/v, 30:70) 2 mM ammonium formate (aq) with 0.1% formic acid: 2 mM ammonium formate (99% MeOH, 1% aq) with 0.1% formic acid before analysis.

Summary of Results
Recoveries were consistently greater than 90% with RSDs <10% and well within the standard deviations provided by DEQAS.

AN753

Extraction of Retinol, β-Carotene (Vitamin A) and α-Tocopherol (Vitamin E) from Serum Using ISOLUTE® SLE+ 96-Well Plates with APCI-LC-MS-MS Analysis



Structure of retinol.

Analytes
Retinol, β-carotene, α-tocopherol.

Format
ISOLUTE® SLE+ 400 µL Supported Liquid Extraction plate, part number 820-0400-P01.

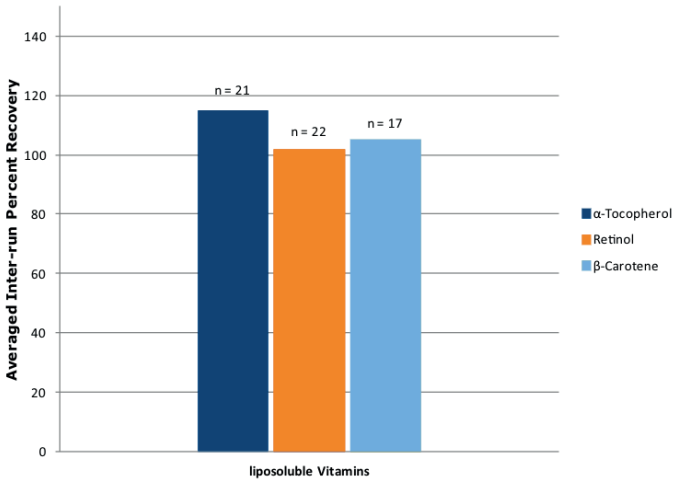
Matrices
Serum.

Sample Preparation Method
Dilute human serum (200 µL) with isopropanol (100 µL). Add internal standard and mix. Load pre-treated samples (~300 µL) onto the plate and apply a short pulse of vacuum or positive pressure (3–5 sec.) to initiate flow. Allow to flow under gravity for 5 minutes.

Elute analytes with hexane:isopropanol (90:10, v/v, 900 µL).

Post Extraction
Evaporate sample to dryness and reconstitute in mobile phase (300 µL) before analysis.

Summary of Results
The average recovery for each target analyte was >95% with the overall RSDs <10%.



Average recoveries for Retinol (102%), α-tocopherol (115%), and β-carotene (105%) from human pooled serum spiked at 100ng/mL. High recoveries are attributed to presence of endogenous vitamins in serum.

Notes

Notes

Notes

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