ISOLUTE® SLE+ Applications

Abstracts and Links

Biotage®

Contents

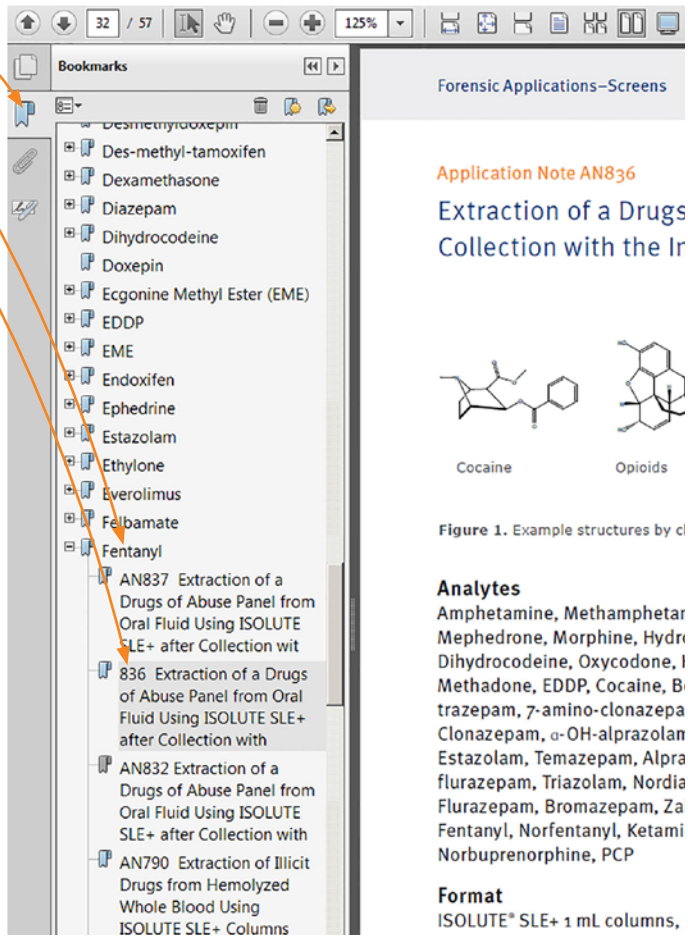
Introduction

The ISOLUTE® SLE+ Applications E-book is designed to give an easy to use overview of the wide range of ISOLUTE SLE+ applications now available. This E-book contains abstracted versions of the wide range of applications produced by Biotage, giving abbreviated forms of the ISOLUTE SLE+ extraction procedures as starting points then clickable links to the website so you can access the full versions.

ISOLUTE® SLE+ plates and columns contain a modified form of diatomaceous earth and are used for the extraction of a diverse range of analytes from aqueous samples such as biological fluids, using a simple load-wait-elute procedure. Various formats, both 96-well plates and columns are available for the extraction of aqueous sample volumes ranging from 10 µL to 10 mL.

Using This E-book

- Firstly **Click here** to log on to the Biotage website.
- Now return to this E-book and use the **bookmark bar** to search for the analyte of interest.
- **Select the analyte** and expand the bookmark to see the associated application notes available.
- **Click on the relevant application** note to take you to the abstracted version from where you will be able to download the complete application note.
- You may also use the contents page to navigate through this E-book.



Sample Preparation Using Supported Liquid Extraction

The supported Liquid Extraction (SLE) process is analogous to traditional liquid-liquid extraction (LLE) and utilizes the same water immiscible solvent systems for analyte extraction. However, instead of shaking the two immiscible phases together, the aqueous phase is immobilized on an inert diatomaceous earth based support material and the water immiscible organic phase flows through the support, alleviating many of the liquid handling issues associated with traditional LLE such as emulsion formation. As a result recoveries are often higher and demonstrate better reproducibility from sample to sample (Figure 1).

In sample preparation, the principles of traditional LLE (partitioning of analytes between aqueous and water immiscible organic solvents) are well known and understood. Traditionally, analytes are extracted from aqueous samples through the addition of an appropriate water immiscible organic solvent. The two immiscible phases are shaken or mixed thoroughly in a separating funnel, and based on relative solubility of the analytes in the two phases, analytes will partition into the organic solvent. The efficiency of the extraction is enhanced by the shaking, which creates a high surface area for the extraction interface allowing partitioning to occur.

Liquid-liquid extraction can give particularly clean extracts of biological fluids, since matrix components such as proteins and phospholipids are not soluble in typical LLE solvents, and are therefore excluded from the final extract. The same benefits are true for supported liquid extraction (SLE) procedures.

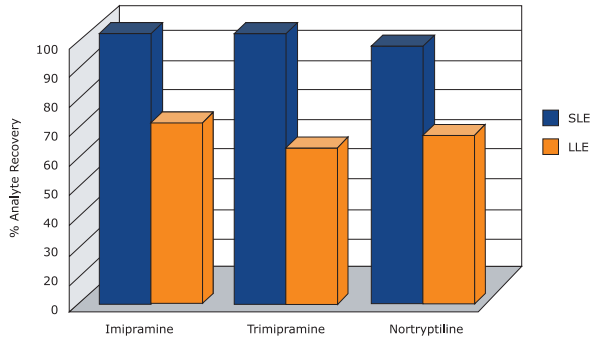


Figure 1. Recovery of tricyclic anti-depressants from plasma, typical ISOLUTE® SLE+ procedure compared with the equivalent liquid-liquid extraction procedure.

Because the same water immiscible solvents are used in SLE, proteins and phospholipids are efficiently removed from the final extract and no additional steps such as protein crash (precipitation) are required.

Using a fast, simple **load-wait-elute** procedure, supported liquid extraction using ISOLUTE® SLE+ products provides inherently cleaner extracts than other simple sample preparation techniques.

The efficient extraction process combining high analyte recoveries, elimination of emulsion formation, and complete removal of matrix interferences such as proteins, phospholipids, and salts results in lower limits of quantitation compared to traditional LLE.

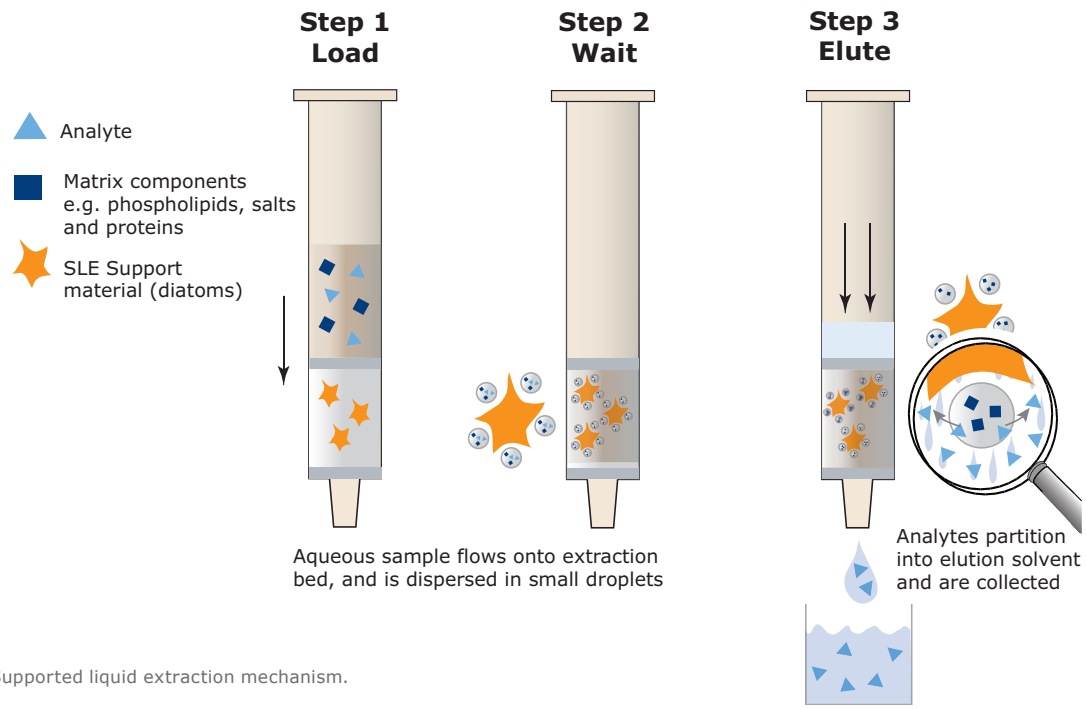
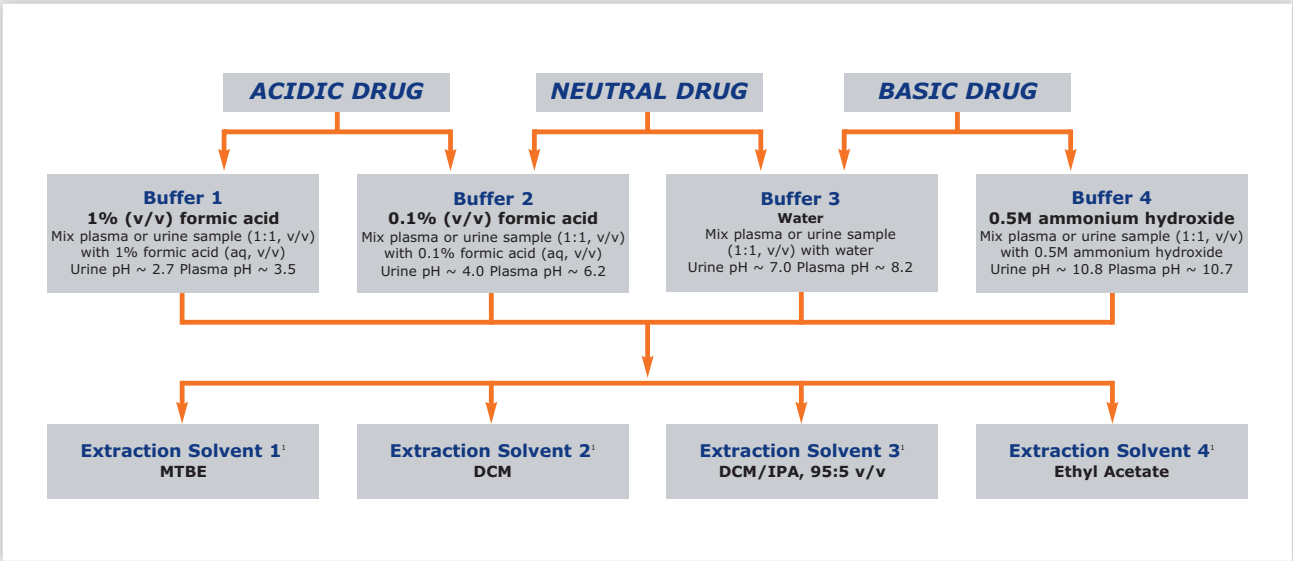


Figure 2. Supported liquid extraction mechanism.

Streamlined Method Development Recommendations

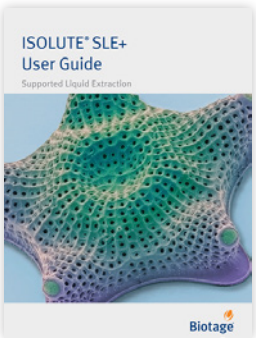
Biotage have developed a simple screening approach based on the principles described in this section, according to analyte(s) functionality. By simply screening 2 pHs, combined with 4 extraction solvents, you can develop a method in minutes.

ISOLUTE®SLE+ Supported Liquid Extraction Method Selection



Using the Chart

- Recommendations can be applied to any format of ISOLUTE® SLE+ product. Appropriate load and elution volumes are detailed in the ISOLUTE SLE+ User Guide.
- Analyte functionality: according to whether your analyte is acidic, neutral or basic, various pH conditions are recommended. For best results, analytes should be in their non-ionized form if possible.
- For acidic drugs, low and intermediate sample pH conditions should be evaluated.
- For basic drugs, intermediate and high sample pH conditions should be evaluated.
- Due to the rapid partition and equilibration of analytes into fresh solvent as the elution solvent passes through the ISOLUTE SLE+ column, extraction efficiency compared to LLE is increased.
- Precise pH control/adjustment may not always be required for low polarity analytes. Optimized pH control is more important for more polar analytes.
- Neutral analytes with no pKa value can be extracted across the pH range. If protein binding is apparent then the use of acid or base in the sample pre-treatment can help to disrupt protein binding, or minimize extraction of unwanted sample components.
- The four extraction solvents selected give a wide range of solvent characteristics and polarity.



ISOLUTE® SLE+ User Guide

For further information download the ISOLUTE® SLE+ User Guide from **www.biotage.com**. Literature part number UI304.V.2

Download ISOLUTE® SLE+ User Guide

Application Notes

Forensic Application Notes

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Clinical Application Notes

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AN805 Extraction of Antiepileptic Drugs from Serum and Urine Using ISOLUTE® SLE+ Prior to LC-MS/MS Analysis

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AN827

Extraction of Amphetamines and Metabolites from Urine (Including Elimination of Sympathomimetic Amine Interferences) Using ISOLUTE® SLE+ Prior to GC/MS Analysis

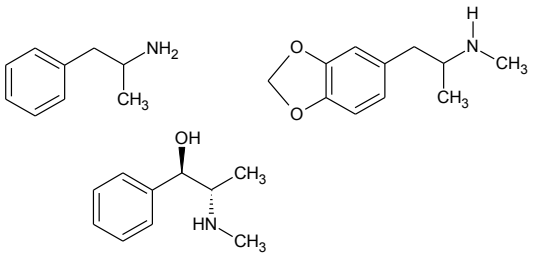

Figure 1. Structures of amphetamine, MDMA and ephedrine.

Analytes

Amphetamine-d_5, amphetamine, methamphetamine, MDMA, MDA, MDEA, ephedrine, pseudoephedrine

Format

ISOLUTE® SLE+ 1 mL columns, part no. 820-0140-C

Matrices

Urine

ISOLUTE® SLE+ Method

To urine (2 mL), add phosphate buffer (pH 6, 0.8M, 1 mL) and vortex. Add sodium periodate (0.3M, 1 mL). Heat for 15 minutes at 60 °C. Allow to cool, add concentrated ammonium hydroxide (85 µL) and vortex. Load 1 mL of the pre-treated urine mixture onto the column and apply a pulse of vacuum or positive pressure (3–5 seconds) to initiate flow. Allow the sample to absorb for 5 minutes.

Apply dichloromethane/isopropanol, (95/5, v/v, 2.5 mL) and allow to flow under gravity for 5 minutes into tubes. Apply a further aliquot of DCM/IPA, (95/5, v/v, 2.5 mL) and allow to flow for another 5 minutes under gravity. Apply vacuum or positive pressure (5–10 seconds) to pull through any remaining extraction solvent.

Remove tubes from the elution rack and add 1% HCl in methanol (100 µL) to each tube.

This devolatilizes the analytes and helps to prevent losses on evaporation. Dry the extract in a stream of air or nitrogen at ambient temperature (20 to 40 L/min) (1.0 bar) for 30 mins.

Add HFBA (100 µL) and ethyl acetate (100 µL) and vortex for 10 seconds. Transfer to a high recovery vial, cap and incubate at 75 °C for 15 minutes. Cool and then evaporate the HFBA at room temperature. On dryness, reconstitute each vial with ethyl acetate (100 µL). Cap and vortex for 10 seconds.

Recovery/Results

Ephedrine and pseudoephedrine are completely removed from the sample. Lower limits of quantitation was 5 ng/mL for amphetamines and metabolites.

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AN776

Extraction of Bath Salts (substituted Cathinones) from Human Urine Using ISOLUTE® SLE+ Columns Prior to GC-MS Analysis

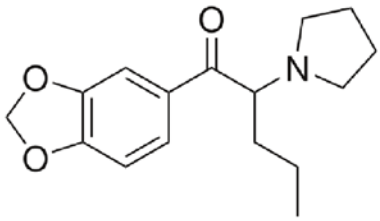

Figure 1. Structures of MDPV.

Analytes
Methcathinone, mephedrone, methedrone, methylone, butylone, ethylone, MDPV, naphyrone

Format
ISOLUTE® SLE+ 1 mL columns, part no. 820-0140-C

Matrices
Urine

ISOLUTE® SLE+ Method
Dilute urine 1:1 (v/v) with 150 mM ammonium hydroxide.

Load the pre-treated sample (1 mL total volume) onto the column and apply a pulse of vacuum or positive pressure to initiate flow. Allow the sample to adsorb for 5 minutes.

Apply MTBE (2 mL) and allow to flow under gravity for 5 minutes. Apply a further aliquot of MTBE (2 mL) and allow to flow under gravity for another 5 minutes. Apply vacuum or positive pressure to pull through any remaining extraction solvent, collecting into a glass culture tube containing 0.2 M hydrochloric acid (100 µL) to add stability during evaporation.

Evaporate the extract to dryness (ambient temperature). Add pentafluoropropionic acid anhydride (PFPA) (50 µL) and ethyl acetate (50 µL) for derivatization. Vortex for 20 seconds, transfer to a high recovery glass vial and cap with a non-split cap. Heat vial in a heating block (70 °C) for 20 minutes. Remove vial and allow to cool. Evaporate the mixture to dryness (ambient temperature). Reconstitute in dichloromethane:isopropanol (95:5, v/v) (100 µL). Cap with a non-split cap and vortex for 30 seconds.

Results
Analyte recoveries ranged from 87–99% a with RSDs below 10% for all analytes.

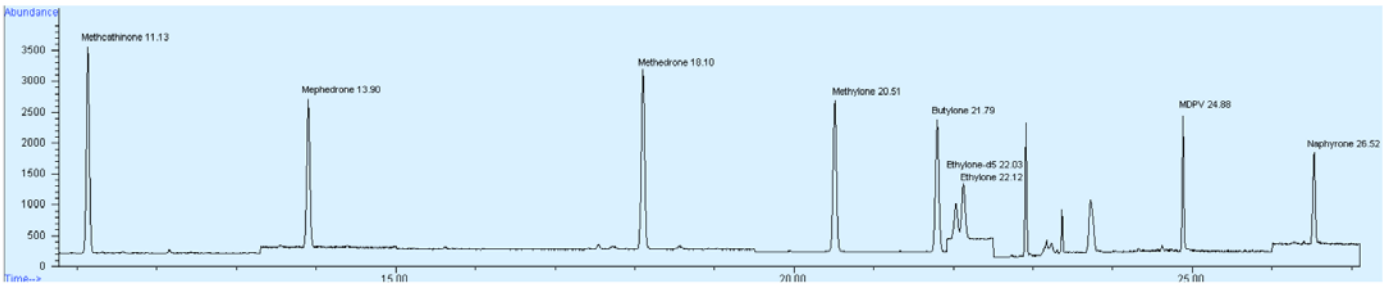

Figure 2. Zoomed chromatogram showing extracted bath salts analytes at 100 ng/mL.

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AN775

Extraction of a Range of Amphetamines and Metabolites from Human Urine Using ISOLUTE® SLE+ Columns Prior to GC-MS Analysis

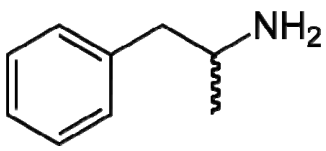

Figure 1. Structure of amphetamine.

Analytes
Amphetamine, amphetamine-d_5, methamphetamine, MDA, MDMA, MDEA

Format
ISOLUTE® SLE+ 1 mL columns, part no. 820-0140-C

Matrices
Urine

ISOLUTE® SLE+ Method
Dilute urine (1 mL) with 15 mM ammonium hydroxide (1 mL).

Load the pre-treated sample (1 mL) onto the column and apply a pulse of vacuum or positive pressure to initiate flow. Allow the sample to adsorb for 5 minutes.

Apply ethyl acetate (4 mL) and allow to flow under gravity for 5 minutes. Apply vacuum or positive pressure to pull through any remaining extraction solvent, collecting into a glass culture tube containing 0.2 M hydrochloric acid (100 µL) to add stability during evaporation.

Evaporate the extract to dryness (ambient temperature). Add pentafluoropropionic acid anhydride (PFPA) (50 µL) and ethyl acetate (50 µL) for derivatization. Vortex for 30 seconds, transfer to a high recovery glass vial and cap with a non-split cap. Heat vial in a heating block (70 °C) for 20 minutes. Remove vial and allow to cool. Evaporate the mixture to dryness (ambient temperature). Reconstitute in dichloromethane:isopropanol (95:5, v/v) (100 µL). Cap with a non-split cap and vortex for 30 seconds.

Recovery/Results
Recoveries ranged from 99–104% with RSDs below 10% for all analytes.

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AN746

Extraction of Amphetamines from Urine Using ISOLUTE® SLE+ Columns

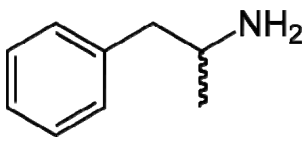

Figure 1. Structure of amphetamine.

Analytes
Ephedrine, amphetamine, methamphetamine, MDMA, MDA, MDEA

Format
ISOLUTE® SLE+ 1 mL columns, part no. 820-0140-C

Matrices
Urine

ISOLUTE® SLE+ Method
Dilute urine (500 µL) 1:1 with 0.5 M ammonium hydroxide (500 µL).

Load pre-treated sample (1 mL) to column followed by a pulse of vacuum to initiate flow and leave for five minutes. Add 0.05 M HCl/Methanol (100 µL) to each collection plate well.

Elute with dichloromethane (2.5 mL). Leave to flow under gravity for 5 minutes then apply a second aliquot of dichloromethane (2.5 mL) followed by a short pulse of vacuum.

Evaporate to dryness and reconstitute in 500 µL 0.1% formic acid (aq) in water/methanol (90/10, v/v).

Recovery/Results
Recoveries of all analytes were >95% at 2 ng/mL

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AN742

Extraction of Amphetamines from Urine Using ISOLUTE® SLE+ 96-Well Plates

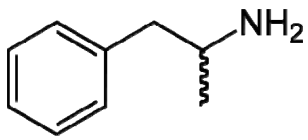

Figure 1. Structure of amphetamine.

Analytes
Ephedrine, amphetamine, methamphetamine, MDMA, MDA, MDEA

Format
ISOLUTE® SLE+ supported liquid extraction Plate, part no. 820-0200-P01

Matrices
Urine

ISOLUTE® SLE+ Method
Dilute urine (100 µL) 1:1 with 0.5 M ammonium hydroxide (100 µL). Load pre-treated sample (200 µL) to plate followed by a pulse of vacuum to initiate flow and leave for five minutes. Add 0.05 M HCl/methanol (100 µL) to each collection plate well.

Elute with dichloromethane (1 mL). Leave to flow under gravity for 5 minutes then apply a second aliquot of dichloromethane (1 mL) followed by a short pulse of vacuum.

Evaporate to dryness and reconstitute in 200 µL 0.1% formic acid (aq) in water/methanol (90/10, v/v).

Recovery/Results
Recoveries of all analytes were >90% at 500 pg/mL .

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AN824

Extraction of Barbiturates from Oral Fluid Using ISOLUTE® SLE+ after Collection with the Oral-Eze® Collection Device Prior to GC/MS Analysis

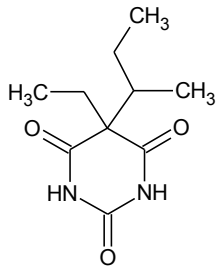

Figure 1. Structure of butabarbital.

Analytes

Butalbarbital, butabarbital, amobarbital, pentobarbital, secobarbital, hexobarbital, phenobarbital

Format

ISOLUTE® SLE+ 400 µL supported liquid extraction columns, part no. 820-0055-B

Matrices

Oral fluid

ISOLUTE® SLE+ Method

Following collection, add 4% ammonium hydroxide (aq) (10 µL) to each collection device.

Load 300 µL of the pre-treated oral fluid onto the column and apply a pulse of vacuum or positive pressure (3–5 seconds) to initiate flow. Allow the sample to absorb for 5 minutes.

Apply dichloromethane/isopropanol (95/5, v/v, 1 mL) and allow to flow under gravity for 5 minutes. Apply a further aliquot of DCM/IPA (95/5, v/v, 1 mL) and allow to flow for another 5 minutes under gravity. Apply vacuum or positive pressure (5–10 seconds) to complete elution.

Dry the extract in a stream of air or nitrogen. Upon dryness, reconstitute with 80 µL ethyl acetate and 20 µL TMAH (trimethylanilinium hydroxide 0.2 M) and vortex for 20 seconds. Transfer to a high recovery glass vial.

Recovery/Results

Recoveries ranged from 95–101%. RSDs were below 9% for all analytes.

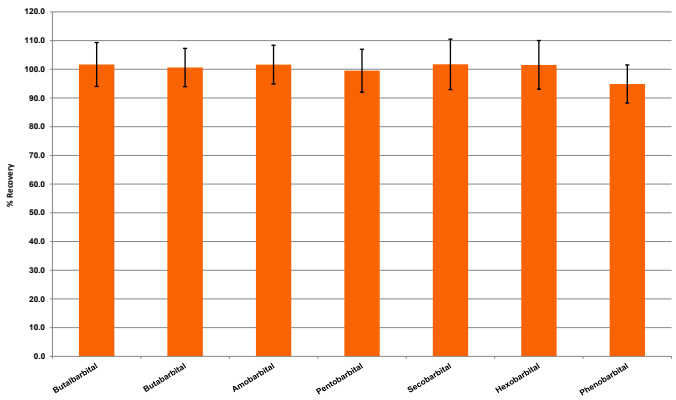

Figure 2. Typical extraction % recoveries (n=7) using the ISOLUTE® SLE+ protocol, at a concentration of 500 ng/mL of oral fluid.

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AN821

Extraction of Barbiturates from Oral Fluid Using ISOLUTE® SLE+ after Collection with the Quantisal™ Collection Device Prior to GC/MS Analysis

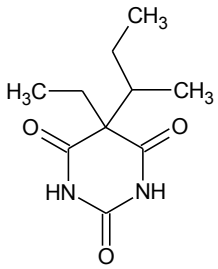

Figure 1. Structure of butabarbital.

Analytes

Butalbarbital, butabarbital, amobarbital, pentobarbital, secobarbital, hexobarbital, phenobarbital

Format

ISOLUTE® SLE+ 400 µL supported liquid extraction columns, part no. 820-0055-B

ISOLUTE® SLE+ 1 mL supported liquid extraction columns, part no. 820-0140-C

Matrices

Oral fluid

ISOLUTE® SLE+ Method

Add concentrated ammonium hydroxide (15 µL) to each collection device.

Load the pre-treated oral fluid (volume as per format) onto the column and apply a pulse of vacuum or positive pressure (3–5 seconds) to initiate flow. Allow the sample to absorb for 5 minutes.

Apply methyl-*tert*-butyl-ether (MTBE) (1 mL for 400 µL format, 2.5 mL for 1 mL format) and allow to flow under gravity for 5 minutes. Apply a further aliquot of MTBE (and allow to flow for another 5 minutes under gravity. Apply vacuum or positive pressure (5–10 seconds) to complete elution.

Dry the extract in a stream of air or nitrogen.

Upon dryness, reconstitute with 80 µL ethyl acetate and 20 µL TMAH (trimethylaniliniumydroxide 0.2M) and vortex for 20 seconds. Transfer to a high recovery glass vial.

Recovery/Results

Recoveries ranged from 88–106%. RSDs were below 6% for all analytes.

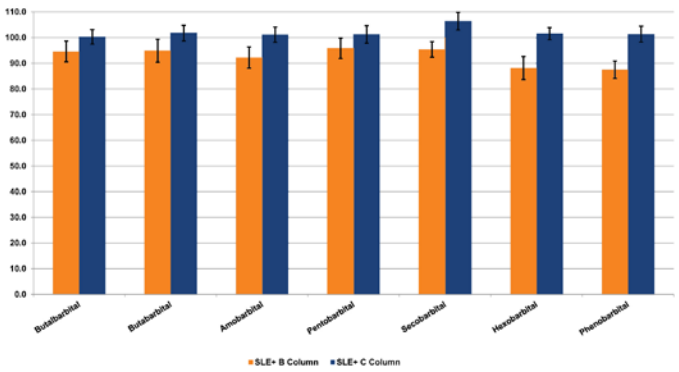

Figure 2. Typical analyte % extraction recoveries (n=7) using the ISOLUTE® SLE+ protocol.

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AN820

Extraction of Barbiturates from Oral Fluid Using ISOLUTE® SLE+ after Collection with the Intercept® Oral Fluid Drug Test Kit Prior to GC/MS Analysis

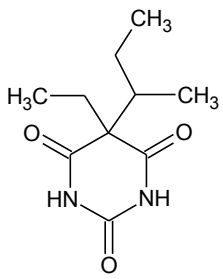

Figure 1. Structure of butabarbital.

Analytes
Butalbarbital, butabarbital, amobarbital, pentobarbital, secobarbital, hexobarbital, phenobarbital

Format
ISOLUTE® SLE+ 400 µL supported liquid extraction columns, part no. 820-0055-B

ISOLUTE® SLE+ 1 mL supported liquid extraction columns, part no. 820-0140-C

Matrices
Oral fluid

ISOLUTE® SLE+ Method
Following collection, add 0.5% ammonium hydroxide (aq) (10 µL) to each collection device. Load the pre-treated oral fluid (300 µL or 1 mL as appropriate) onto the column and apply a pulse of vacuum or positive pressure (3–5 seconds) to initiate flow.

Allow the sample to absorb for 5 minutes. Apply methyl-*tert*-butyl-ether (MTBE) (1 mL for 400 µL format, 2.5 mL for 1 mL format) and allow to flow under gravity for 5 minutes. Apply a further aliquot of MTBE and allow to flow for another 5 minutes under gravity. Apply vacuum or positive pressure (5–10 seconds) to complete elution. Dry the extract in a stream of air or nitrogen. Upon dryness, reconstitute with 80 µL ethyl acetate and 20 µL TMAH (trimethylanilinium hydroxide, 0.2 M) and vortex for 20 seconds. Transfer to a high recovery glass vial.

Recovery/Results
Recoveries ranged 91–104%. RSDs were below 7% for all analytes.

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AN752

Extraction of Barbiturates from Human Urine Using ISOLUTE® SLE+ Columns with GC-MS Analysis

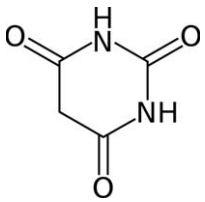

Figure 1. Structure of barbituric acid, the basic structure of all barbiturates.

Analytes
Butalbarbital, butabarbital, amobarbital, pentobarbital, secobarbital, hexobarbital, phenobarbital

Format
ISOLUTE® SLE+ 1 mL columns, part no. 820-0140-C

Matrices
Urine

ISOLUTE® SLE+ Method
To 500 µL of urine add 100mM ammonium acetate pH 5 (500 µL, 1:1, v/v).

Load pre-treated sample (1 mL) to column followed by a pulse of vacuum to initiate flow and allow to absorb for five minutes.

Elute with dichloromethane (2.5 mL). Leave to flow under gravity for 5 minutes, then follow with a further aliquot of dichloromethane (2.5 mL) and allow to flow under gravity for a further five minutes, to complete extraction apply a short pulse of vacuum.

Evaporate to dryness at room temperature (80 L/min) and reconstitute in ethyl acetate (200 µL).

Recovery/Results
Recoveries ranged 103–108% for barbiturates spiked at 10 ng/mL, with RSDs <10% (n=3).

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AN756

Extraction of Benzodiazepines from Human Urine Using ISOLUTE® SLE+ in Column Format Prior to GC-MS Analysis

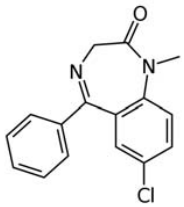

Figure 1. Structure of diazepam.

Analytes

Diazepam, nordiazepam, midazolam, flunitrazepam, 7-aminoflunitrazepam, bromazepam, oxazepam, nitrazepam, flurazepam, temazepam, 7-aminoclonazepam, lorazepam, hydroxyethylflurazepam, estazolam, alprazolam, triazolam, α-hydroxyalprazolam, α-hydroxytriazolam

Format

ISOLUTE® SLE+ 1 mL columns, part no. 820-0140-C

Matrices

Urine

ISOLUTE® SLE+ Method

Dilute human urine (500 µL) with sodium acetate (100 mM, pH 5) (500 µL). Perform enzymatic hydrolysis with β-glucuronidase (H3) ex. H.pomatia (Sigma-Aldrich G8885) 50 µL/mL urine (approx. 4500 U/mL urine) at 60 °C ± 2 °C for 2 hours. Adjustment to pH 7 using 25 % ammonium hydroxide (conc.), 10 µL/mL urine.

Load pre-treated urine (1 mL in total) onto the ISOLUTE SLE+ column followed by a pulse of vacuum to initiate flow, allow the sample to absorb for 5 minutes.

Apply dichloromethane (2.5 mL), wait five minutes to allow the solvent to adsorb, apply a short pulse of vacuum if solvent not fully adsorbed. Apply a second aliquot of dichloromethane (2.5 mL), allow to adsorb for a further 5 minutes and then apply a final pulse of vacuum.

Evaporate the eluate to dryness and reconstitute in 50 µL of ethyl acetate. Sample then derivatized 1:1 with TBDMSTFA, N-tert-butyldimethylsilyl-N-methyltrifluoroacetamide with 1% tert-butyldimethylchlorosilane (Sigma-Aldrich 375934) at 70 °C ± 2 °C for 20 minutes.

Recovery/Results

An LOQ of 40 ng/mL was reached (500 µL matrix) for all analytes with RSDs <10% (n=3).

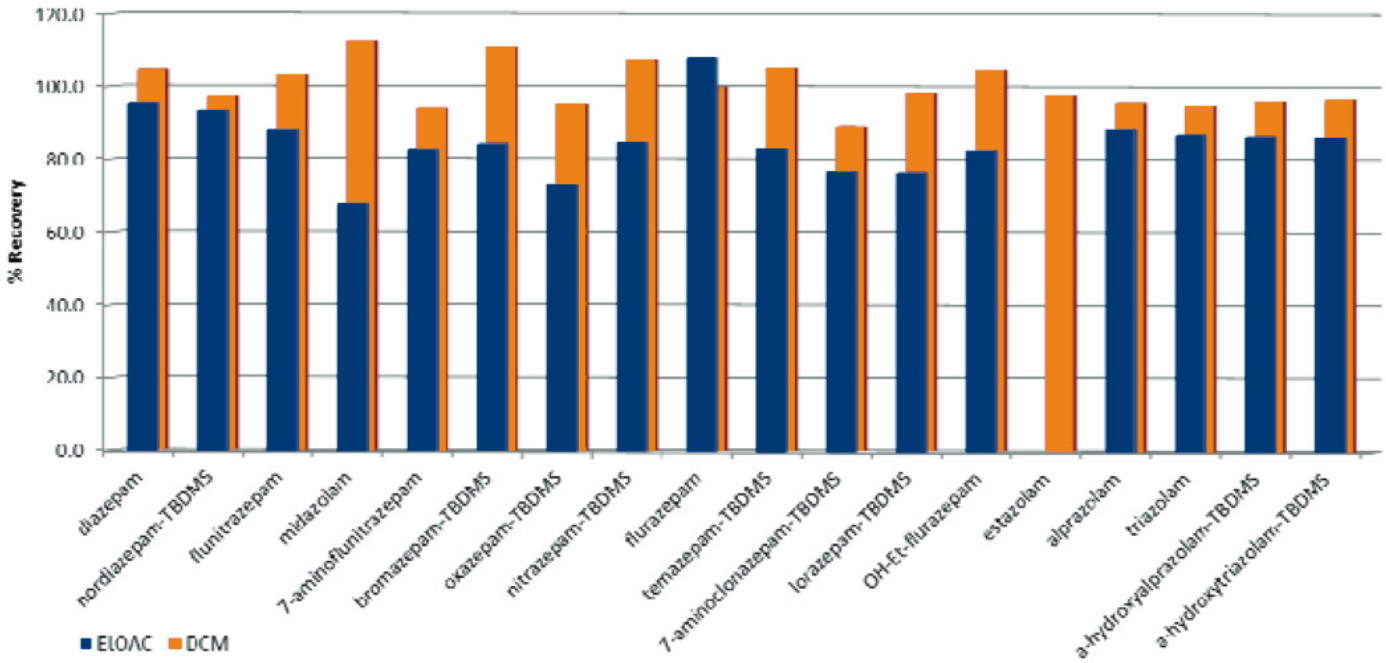

Figure 2. Analyte % recoveries for the full range of extracted benzodiazepines, comparing two different extraction solvents, halogenated and non-halogenated.

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AN751

Extraction of Benzodiazepines from Human Urine Using ISOLUTE® SLE+ 96-Well Plates and Columns Prior to LC-MS/MS Analysis

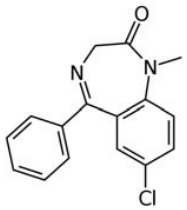

Figure 1. Structure of diazepam.

Analytes

Midazolam, flurazepam, bromazepam, α-hydroxyalprazolam, nordiazepam, nitrazepam, oxazepam, estazolam, alprazolam, lorazepam, triazolam, diazepam, temazepam and flunitrazepam

Format

ISOLUTE® SLE+ 200 µL supported liquid extraction plate, part no. 820-0200-P01

ISOLUTE® SLE+ 1 mL supported liquid extraction columns, part no. 820-0140-C

Matrices

Urine

ISOLUTE® SLE+ Method

To 1 mL of urine add 100mM ammonium acetate pH5 (950 µL) and β-glucuronidase (50 µL) enzyme (~4500 U/mL). Hydrolyze in a water bath at 600 ˚C for 2 hours. 50 µL of enzyme is required per mL of urine used.

Load pre-treated sample (volumes as appropriate for format) followed by a pulse of vacuum to initiate flow and leave for five minutes to completely adsorb. Elute with dichloromethane (one aliquot of 1 mL for SLE+ 200 µL plate, two aliquots if 2.5 mL for SLE+ 1 mL columns). Leave to flow under gravity for 5 minutes, then apply short pulse of vacuum.

Evaporate to dryness at room temperature and reconstitute in HPLC grade water/acetonitrile (70/30, v/v) (200 µL).

Recovery/Results

>85% analyte recoveries with RSDs <5%

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AN825

Extraction of THC, THCA and Carboxy-THC from Oral Fluid Using ISOLUTE® SLE+ after Collection with the Oral-Eze® Collection Device Prior to GC/MS Analysis

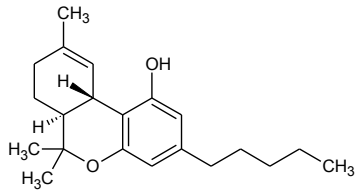

Figure 1. Structure of Δ^9-THC (tetrahydrocannabinol).

Analytes
Δ^9-tetrahydrocannabinol (THC), Δ^9-tetrahydrocannabinolic acid (THCA), 11-nor-Δ^9-carboxy tetrahydrocannabinol (THC-COOH), Δ^9-tetrahydrocannabinol-d_3 (THC-d_3), 11-nor-Δ^9-carboxy tetrahydrocannabinol-d_3 (THC-COOH-d_3) as internal standard

Format
ISOLUTE® SLE+ 400 µL supported liquid extraction columns, part no. 820-0055-B

Matrices
Oral fluid

ISOLUTE® SLE+ Method
Following collection, add 4% ammonium hydroxide (aq) (10 µL) to each collection device. Load 300 µL of the pre-treated oral fluid onto the column and apply a pulse of vacuum or positive pressure (3–5 seconds) to initiate flow. Allow the sample to absorb for 5 minutes.

Apply dichloromethane/isopropanol, (95/5, v/v, 1 mL) and allow to flow under gravity for 5 minutes. Apply a further aliquot of DCM/IPA, (95/5, v/v, 1 mL) and allow to flow for another 5 minutes under gravity. Apply vacuum or positive pressure (5–10 seconds) to complete elution. Dry the extract, then reconstitute with 50 µL ethyl acetate and 25 µL MTBSTFA:TBDMCS 99:1 and vortex for 20 seconds. Transfer to a high recovery glass vial. Place in a heating block set to 70 °C, for 25 minutes. Remove vial from the block and allow cooling.

Recovery/Results
Recoveries ranged from 103–109%. RSDs were below 10% for all analytes.

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AN822

Extraction of THC, THCA and Carboxy-THC from Oral Fluid by ISOLUTE® SLE+ after Collection with the Quantisal™ Collection Device Prior to GC/MS Analysis

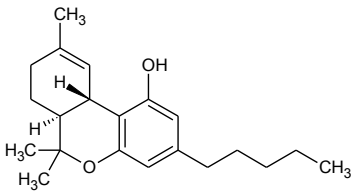

Figure 1. Structure of Δ^9-THC (tetrahydrocannabinol).

Analytes
Δ^9-tetrahydrocannabinol (THC), Δ^9-tetrahydrocannabinolic acid (THCA), 11-nor-Δ^9-carboxy tetrahydrocannabinol (THC-COOH), Δ^9-tetrahydrocannabinol-d_3 (THC-d_3), 11-nor-Δ^9-carboxy tetrahydrocannabinol-d_3 (THC-COOH-d_3) as internal standard

Format
ISOLUTE® SLE+ 400 µL supported liquid extraction columns, part no. 820-0055-B

ISOLUTE® SLE+ 1 mL supported liquid extraction columns, part no. 820-0140-C

Matrices
Oral fluid

ISOLUTE® SLE+ Method
Following collection, add concentrated ammonium hydroxide (15 µL) to each collection device. Load pre-treated oral fluid (volume as dictated by SLE+ format) onto the column and apply a pulse of vacuum or positive pressure (3–5 seconds) to initiate flow. Allow the sample to absorb for 5 minutes. Apply dichloromethane/isopropanol, (95/5, v/v, 1 mL aliquot for ISOLUTE SLE+ 400 µL columns, 2.5 mL aliquot for ISOLUTE SLE+ 1 mL columns) and allow to flow under gravity for 5 minutes. Apply a further aliquot of DCM/IPA and allow to flow for another 5 minutes under gravity.

Apply vacuum or positive pressure (5–10 seconds) to complete elution. Dry the extract then reconstitute with 50 µL ethyl acetate and 25 µL MTBSTFA:TBDMCS (99:1, v/v) and vortex for 20 seconds. Transfer to a high recovery glass vial. Place in a heating block set to 70 °C, for 25 minutes. Remove vial from the block and allow cooling.

Recovery/Results
Recoveries ranged from 75–90% .RSDs were below 10% for all analytes.

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AN819

Extraction of THC, THCA and Carboxy-THC from Oral Fluid by ISOLUTE® SLE+ after Collection with the Intercept® Oral Fluid Drug Test Kit Prior to GC/MS Analysis

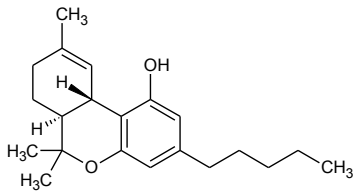

Figure 1. Structure of Δ^9-THC (tetrahydrocannabinol).

Analytes

Δ^9-tetrahydrocannabinol (THC), Δ^9-tetrahydrocannabinolic acid (THCA), 11-nor-Δ^9-carboxy tetrahydrocannabinol (THC-COOH), Δ^9-tetrahydrocannabinol-d_3 (THC-d_3), 11-nor-Δ^9-carboxy tetrahydrocannabinol-d_3 (THC-COOH-d_3) as internal standard

Format

ISOLUTE® SLE+ 400 µL supported liquid extraction columns, part no. 820-0055-B

ISOLUTE® SLE+ 1 mL supported liquid extraction columns, part no. 820-0140-C

Matrices

Oral fluid

ISOLUTE® SLE+ Method

Following collection, add 0.5% ammonium hydroxide (aq) (10 µL) to each collection device. Load contents of the pre-treated oral fluid device onto the column (as appropriate to ISOLUTE SLE+ format) and apply a pulse of vacuum or positive pressure (3–5 seconds) to initiate flow. Allow the sample to absorb for 5 minutes. Apply dichloromethane/isopropanol, (95/5, v/v, 1 mL aliquot for SLE+ 400 µL format, 2.5 mL aliquot for SLE+ 1 mL format) and allow to flow under gravity for 5 minutes. Apply a further aliquot of DCM/IPA, (95/5, v/v, 1 mL) and allow to flow for another 5 minutes under gravity.

Apply vacuum or positive pressure (5–10 seconds) to complete elution. Dry the extract then reconstitute with 50 µL ethyl acetate and 25 µL MTBSTFA:TBDMCS (99:1, v/v) and vortex for 20 seconds. Transfer to a high recovery glass vial. Place in a heating block set to 70 °C, for 25 minutes. Remove vial from the block and allow cooling.

Recovery/Results

Recoveries ranged from 76–94%. RSDs were below 10% for all analytes.

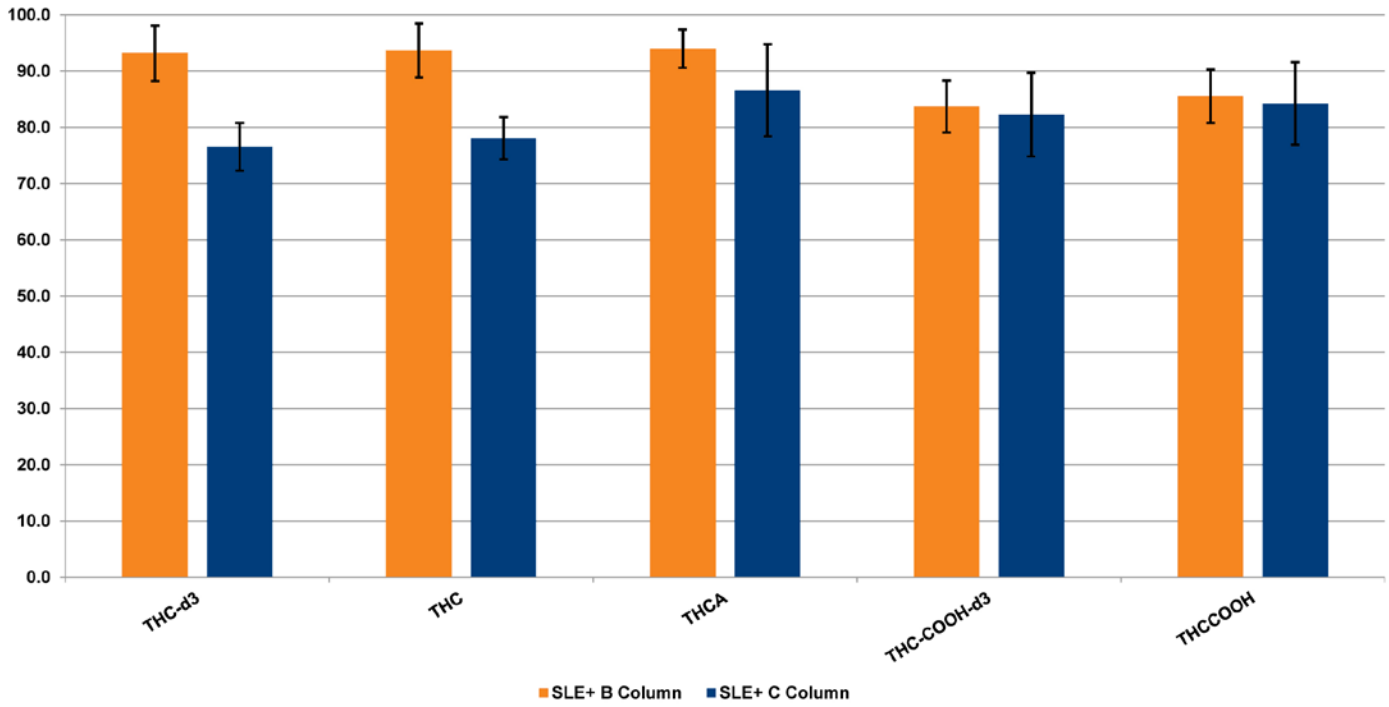

Figure 2. Typical extraction % recoveries (n=7) using the ISOLUTE® SLE+ protocol.

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AN815

Analysis of THC and an Extended Metabolite Suite from Oral Fluid Using ISOLUTE® SLE+ Supported Liquid Extraction Columns Prior to LC-MS/MS

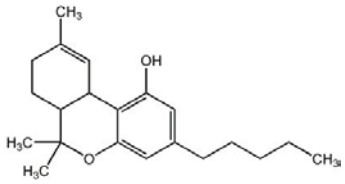

Figure 1. Structure of Δ^9-tetrahydrocannabinol (THC).

Analytes

Δ^9-tetrahydrocannabinol (THC), cannabigerol, cannabidiol, Δ^9-tetrahydrocannabivarin (THCV), 11-hydroxy-Δ^9-tetrahydrocannabinol (11-OH-THC), 11-nor-9-carboxy-Δ^9-tetrahydrocannabivarin (THC-V-COOH), 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol (THC-COOH), 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol glucuronide (THC-COOH-glucuronide), Δ^9-tetrahydrocannabinol glucuronide (THC glucuronide) and Δ^9-tetrahydrocannabinolic acid A (THCA-A)

Format

ISOLUTE® SLE+ 400 µL supported liquid extraction columns, part no. 820-0055-B

ISOLUTE® SLE+ 1 mL supported liquid extraction columns, part no. 820-0140-C

Matrices

Oral fluid

ISOLUTE® SLE+ Method

Remove the paddle from the Quantisal oral fluid collection device and add 10 µL of concentrated formic acid to adjust pH of the sample. Vortex mix thoroughly.

Load pre-treated sample (300 µL for ISOLUTE SLE+ 400 µL columns, 800 µL for ISOLUTE SLE+ 1 mL columns) onto the ISOLUTE SLE+ bed, and apply a pulse of vacuum. Leave for 5 minutes to absorb.

ISOLUTE SLE+ 400 µL format

Elution 1: Apply an aliquot of MTBE (750 µL), wait for 5 minutes

Elution 2: Apply a second aliquot of MTBE (750 µL), wait for 5 minutes then apply a pulse of vacuum or positive pressure to complete elution (10 seconds).

ISOLUTE SLE+ 1 mL format

Elution 1: Apply an aliquot of MTBE (2 mL), wait for 5 min

Elution 2: Apply a second aliquot of MTBE (2 mL), wait for 5 minutes then apply a pulse of vacuum or positive pressure to complete elution (10 seconds).

Dry the combined eluent then reconstitute in 0.1% formic acid in H_2O/ACN (60/40, v/v), 200 µL) and vortex mix thoroughly.

Recovery/Results

The method outlined in this application note achieves high reproducible recoveries for both extraction formats.

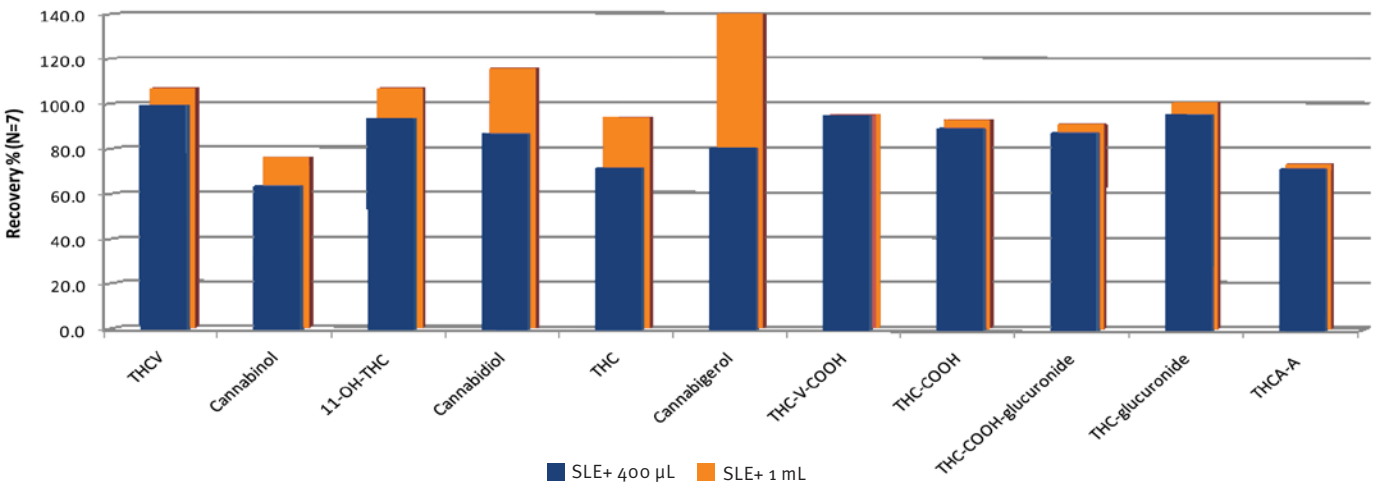

Figure 2. Typical chart of recoveries for both formats using the methods described in this application note.

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AN813

Extraction of Tetrahydrocannabinol (THC) and Metabolites from Whole Blood Using ISOLUTE® SLE+ Prior to LC-MS/MS

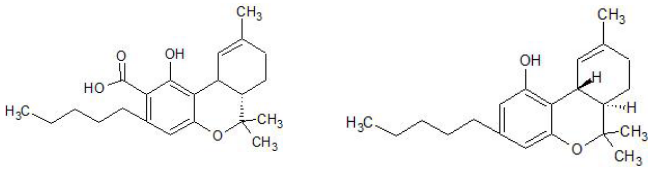

Figure 1. Structure of tetrahydrocannabinol (THC) and tetrahydrocannabinolic acid (THCA).

Analytes
Δ^9-Tetrahydrocannabinol (THC), Δ^9-tetrahydrocannabinol-d_3 (THC-d_3), 11-nor-Δ^9-carboxy tetrahydrocannabinol (THC-COOH), 11-nor-Δ^9-carboxy tetrahydrocannabinol-d_3 (THC-COOH-d_3), 11-hydroxy-Δ^9-tetrahydrocannabinol (11-OH-THC), 11-hydroxy-Δ^9-tetrahydrocannabinol-d_3 (THC-OH-d_3)

Format
ISOLUTE® SLE+ 400 µL supported liquid extraction plate, part no. 820-0400-P01

Matrices
Whole blood

ISOLUTE® SLE+ Method
Add 200 µL of sample, 100 µL of 0.1% Formic Acid and internal standard to each well (up to 30 µL)

Elute with 3 x 600 µL of methyl tert-butyl ether (MTBE) to each well and allow solvent to flow under gravity. Then apply vacuum or positive pressure to pull through any remaining extraction solvent.

Evaporate sample and reconstitute in water: methanol (50:50, v/v, 500 µL).

Recovery/Results
Recoveries for the analytes ranged from 60–107% across a dynamic concentration range from 50 ng/mL to 12.5 ng/mL RSDs <10%.

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AN809

Extraction of THC and Metabolites Including 11-nor-9-carboxy-Δ^9-THC Glucuronide from Urine Using ISOLUTE® SLE+ Prior to LC-MS/MS Analysis

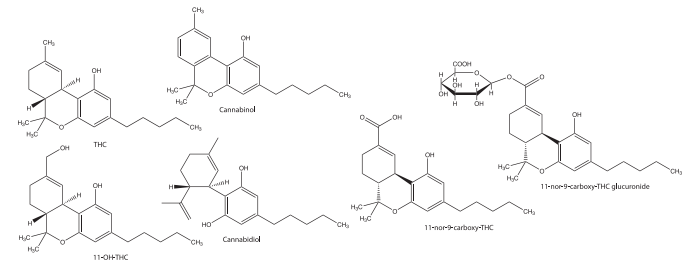

Figure 1. Structure of THC and major metabolites.

Analytes
11-nor-Δ^9-carboxy tetrahydrocannabinol (THC-COOH), 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol glucuronide (THC-COOH-glucuronide), cannabinol, cannabidiol, Δ^9-tetrahydrocannabinol (THC) and 11-hydroxy-Δ^9-tetrahydrocannabinol (11-OH-THC)

Format
ISOLUTE® SLE+ 200 µL supported liquid extraction plate, part no. 820-0200-P01

ISOLUTE® SLE+ 1 mL supported liquid extraction columns, part no. 820-0140-C

Matrices
Urine

ISOLUTE® SLE+ Method
Dilute urine with 25 mM dibutylammonium acetate (1:1, v/v). Vortex mix thoroughly.

Load diluted urine onto each well/column (volume as appropriate to format) and apply a pulse of vacuum or positive pressure to initiate flow. Allow the sample to adsorb for 5 minutes.

Apply ethyl acetate (A single 1 mL aliquot for SLE+ 200 µL, 2 aliquots of 2.5 mL for SLE+ 1 mL) and allow to flow under gravity for 5 minutes. Apply vacuum or positive pressure to pull through any remaining extraction solvent.

Evaporate to dryness. Reconstitute with 0.1% formic acid in water/acetonitrile (70/30, v/v, 200 µL).

Recovery/Results
Analyte recovery >85%.

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AN793

Automated Extraction of Synthetic Cannabinoids (SPICE) from Urine Using ISOLUTE® SLE+ Prior to LC-MS/MS

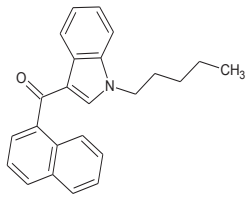

Figure 1. Structure of JWH-018.

Analytes
JWH-018, JWH-073, JWH-200, JWH-250, JWH-250-N-(5-hydroxypentyl), JWH-018-N-5-(pentanoic acid), JWH-073-N-(3-hydroxybutyl), JWH-018-N-(4-hydroxypentyl), XLR-11, UR-144, UR-144 (5-chloropentyl), UR-144-(pentanoic acid), UR-144-(5-hydroxypentyl)

Format
ISOLUTE® SLE+ 400 µL supported liquid extraction plate, part no. 820-0400-P01

Matrices
Urine

ISOLUTE® SLE+ Method
Load 400 µL of hydrolyzed urine samples onto the ISOLUTE® SLE+ 96-well plate. Apply a short pulse of positive pressure and allow samples to absorb for 5 minutes.

Apply ethyl acetate (2 x 700 µL). Apply short pulses of pressure and collect eluent.

Evaporate sample to dryness and reconstitute in mobile phase. Note: Automated workflow in full application note.

Recovery/Results
Averaged recoveries >60% across the synthetic cannabinoids.

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AN791

Extraction of Synthetic Cannabinoids (SPICE) from Oral Fluid Using ISOLUTE® SLE+ 96-well Plates and Columns Prior to LC-MS/MS

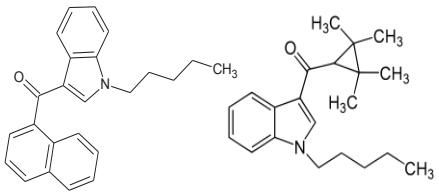

Figure 1. Structure of JWH-018 and UR-144.

Analytes
JWH-018, JWH-073, JWH-200, JWH-250, JWH-250-N-(5-hydroxypentyl), JWH-018-N-5-(pentanoic acid), JWH-073-N-(3-hydroxybutyl), JWH-018-N-(4-hydroxypentyl), XLR-11, UR-144, UR-144 (5-chloropentyl), UR-144-(pentanoic acid), UR-144-(5-hydroxypentyl)

Format
ISOLUTE® SLE+ 400 µL supported liquid extraction plate, part number 820-0400-PO1

ISOLUTE® SLE+ 400 µL columns (Tabless), part number 820-0055-BG

Matrices
Oral fluid

ISOLUTE® SLE+ Method
Mix oral fluid sample (200 µL) with ammonium acetate (100 mM, pH 5, 200 µL).

Load pre-treated oral fluid sample (400 µL) onto the ISOLUTE SLE+ 96-well plate or column. Apply a short pulse of positive pressure and allow samples to sit for 5 minutes.

Apply ethyl acetate (2 x 700 µL). Apply short pulses of pressure and collect eluent.

Evaporate to dryness and reconstitute sample in mobile phase (500 µL).

Recovery/Results
Recoveries for neat oral fluid 65–87% with RSDs <10%.
Recoveries from Orasure Intercept kit 65–110% with %RSDs <10.

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AN780

Extraction of Synthetic Cannabinoids from Hydrolyzed Urine Using ISOLUTE® SLE+ Prior to GC-MS Analysis

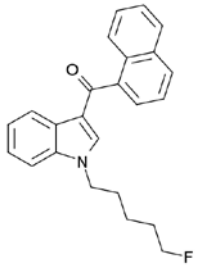

Figure 1. An example of a synthetic cannabinoid, AM2201.

Analytes

UR-144, JWH-073, JWH-018, JWH-250-N-(5-hydroxypentyl), JWH-073 N-(3-hydroxybutyl), AM2201, JWH-018-N-(4-hydroxypentyl), JWH-018-N-5-(pentanoic acid), JWH-200

Format

ISOLUTE® SLE+ 400 µL supported liquid extraction columns, part number 820-0055-B

ISOLUTE® SLE+ 1 mL supported liquid extraction columns, part number 820-0140-C

Matrices

Hydrolyzed urine

ISOLUTE® SLE+ Method

Dilute urine (1 mL) with 100 mM ammonium acetate buffer at pH 5 (950 µL) and β-Glucuronidase enzyme (50 µL, equivalent to approximately 4500 U/mL of urine). Hydrolyze with heat in a water bath at 60 °C for 2 hours.

Load the pre-treated hydrolyzed urine (volumes appropriate to format) onto the column and apply a pulse of vacuum or positive pressure to initiate flow. Allow the sample to adsorb for 5 minutes.

Apply hexane : ethyl acetate (90 : 10, v/v, 1 mL aliquot for SLE+ 400 µL format, 2.5 mL aliquot for 1 mL format) and allow to flow under gravity for 5 minutes. Apply a further aliquot of hexane : ethyl acetate (90 : 10, v/v) and allow to flow for another 5 minutes. Apply vacuum or positive pressure to pull through any remaining extraction solvent.

Dry the extract in a stream of air or nitrogen. Upon dryness, add 50 µL ethyl acetate and 50 µL BSTFA:TMCS 99:01, heat at 70 °C for 30 mins.

Recovery/Results

The average analyte recovery, averaged in 4 urine sets of n=7, was 96%. RSDs were below 10% for all analytes for all donors.

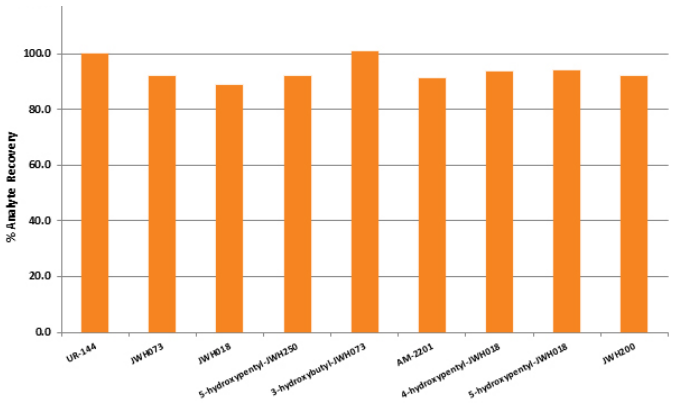

Figure 2. Typical analyte % recoveries for the application analytes (n=7) using the ISOLUTE® SLE+ protocol.

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AN774

Extraction of Synthetic Cannabinoids (SPICE) and Metabolites from Urine, Plasma and Whole Blood Using ISOLUTE® SLE+ Prior to LC-MS/MS Analysis

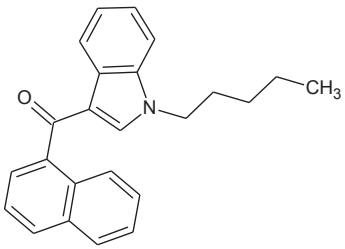

Figure 1. Structure of JWH-018.

Analytes

JWH-018, JWH-073, JWH-200, JWH-250, JWH-250-N-(5-hydroxypentyl), JWH-018-N-5-(pentanoic acid), JWH-073 N-(3-hydroxybutyl), JWH-018-N-(4-hydroxypentyl)

Format

Urine: ISOLUTE® SLE+ 1 mL Cartridge (820-0140-C) or 400 µL 96-well plate (820-0400-P01)

Plasma or Whole Blood: ISOLUTE® SLE+ 1 mL Cartridge (820-0140-C) or 2 mL Cartridge (820-0290-D)

Matrices

Urine, plasma and whole blood

ISOLUTE® SLE+ Method

Urine:

Add β-glucuronidase at a concentration of 5000 units/mL to urine and dilute sample 1:1 with 100 mM ammonium acetate (pH 5). Spike the matrix solution with internal standard. Incubate sample as per instructions with enzyme.

Load pre-treated sample (1 mL onto 820-0140-C or 400 µL onto 820-0400-P01). Apply a short pulse of vacuum or positive pressure and allow sample to flow under gravity for 5 minutes.

Choose ONE of the following elution strategies:

Urine (cartridge): Apply Ethyl Acetate (2 x 3 mL) to 1 mL column. Apply short pulse of vacuum and collect eluent.

Urine (plate): Apply Ethyl Acetate (3 x 500 µL) to 400 µL plate. Apply short pulse of vacuum and collect eluent.

Evaporate to dryness and reconstitute sample in mobile phase.

Plasma or Whole Blood

Dilute plasma (1 mL), whole blood (2 mL) 1:1 (v/v) with HPLC grade water.

Load pre-treated sample (1 mL onto 820-0140-C or 2 mL onto 820-0290-D). Apply a short pulse of vacuum or positive pressure allow sample to flow under gravity for 5 minutes.

Apply Hexane (2 x 4 mL) to 2 mL column. Apply short pulse of vacuum and collect eluent.

Evaporate to dryness and reconstitute sample in mobile phase.

Recovery/Results

The recoveries obtained for the synthetic cannabinoids parent and metabolites ranged from 70–98%.

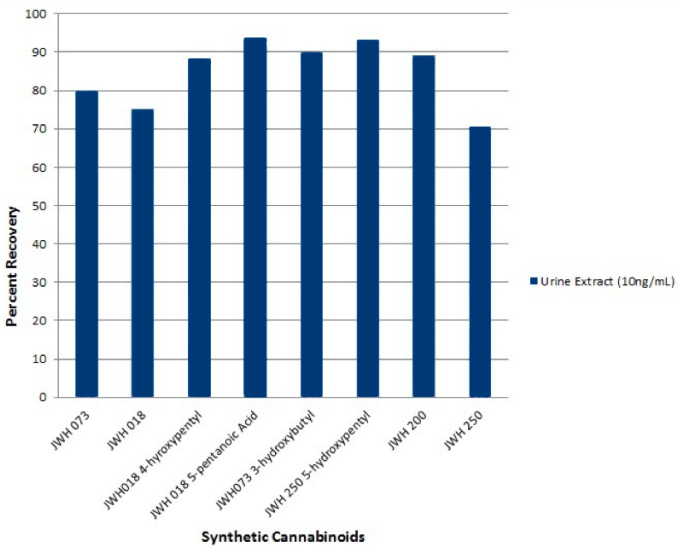

Figure 2. Plot of recoveries for synthetic cannabinoids in urine at 10 ng/mL using SLE+ 1 mL cartridge.

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AN840

Extraction of THC, Hydroxy-THC and Carboxy-THC from Whole Blood Using ISOLUTE® SLE+ Prior to GC/MS Analysis

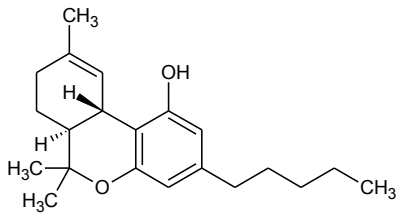

Figure 1. Structure of Δ^9-THC (tetrahydrocannabinol).

Analytes

Δ^9-tetrahydrocannabinol (THC), 11-hydroxy-Δ^9-tetrahydrocannabinol (11-OH-THC), 11-nor-Δ^9-carboxy tetrahydrocannabinol (THC-COOH) with THC-d_3, 11-hydroxy-Δ^9-tetrahydrocannabinol-d_3 (THC-OH-d_3) and 11-nor-Δ^9-carboxy tetrahydrocannabinol-d_3 (THC-COOH-d_3) as internal standard

Format

ISOLUTE® SLE+ 1 mL supported liquid extraction columns, part no. 820-0140-C

Matrices

Whole blood

ISOLUTE® SLE+ Method

To 1.2 mL of whole blood, add 0.4 mL of 0.1% formic acid (aq), and mix thoroughly.

Load 800 µL of the pre-treated whole blood onto the column and apply a pulse of vacuum or positive pressure (3–5 seconds) to initiate flow. Allow the sample to absorb for 5 minutes.

Apply MTBE (3 mL) and allow to flow under gravity for 5 minutes. Apply Hexane (3 mL) and allow to flow for another 5 minutes under gravity. Apply vacuum or positive pressure (5–10 seconds) to pull through any remaining extraction solvent.

Dry the extract in a stream of air or nitrogen.

Upon dryness, reconstitute with 40 µL ethyl acetate and 20 µL BSTFA:TMCS 99:1 and vortex for 20 seconds. Transfer to a high recovery glass vial. Place in a heating block set to 70 °C, for 25 minutes. Remove vial from the block and allow cooling.

Recovery/Results

The optimized SLE+ protocol demonstrated analyte recoveries ranging from 72–83% as shown in Figure 2. RSD's <10% for all analytes.

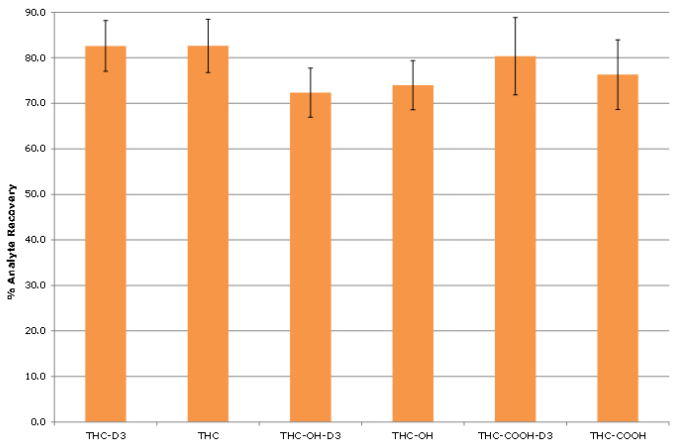

Figure 2. Typical extraction % recoveries (n=7) using the ISOLUTE® SLE+ protocol.

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AN841

Extraction of 11-nor-9-carboxy-tetrahydrocannabinol from Hydrolyzed Urine by ISOLUTE® SLE+ Prior to GC/MS Analysis

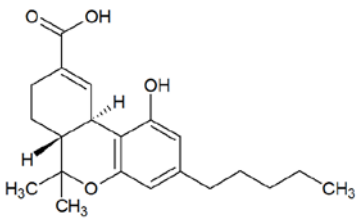

Figure 1. Structure of 11-nor-9-carboxy-tetrahydrocannabinol (carboxy-THC; THC-COOH).

Analytes

11-nor-Δ^9-carboxy tetrahydrocannabinol (THC-COOH) and 11-nor-Δ^9-carboxy tetrahydrocannabinol-d_3 (THC-COOH-d_3) as internal standard

Format

ISOLUTE® SLE+ 1 mL supported liquid extraction columns, part no. 820-0140-C

Matrices

Urine

ISOLUTE® SLE+ Method

To 1 mL of urine, add 950 µL ammonium acetate 50 mM (pH5). Add 50 µL beta-glucuronidase enzyme. Mix. Heat sample for 2 hours at 37 °C.

Load 1 mL of the pre-treated urine onto the column and apply a pulse of vacuum or positive pressure (3–5 seconds) to initiate flow. Allow the sample to absorb for 5 minutes.

Apply MTBE (2.5 mL) and allow to flow under gravity for 5 minutes. Apply a second aliquot of MTBE (2.5 mL) and allow to flow for another 5 minutes under gravity. Apply vacuum or positive pressure (5–10 seconds) to pull through any remaining extraction solvent. See full application note for additional methodology for non-hydrolyzed and base hydrolyzed samples.

Recovery/Results

The optimized ISOLUTE® SLE+ protocols for the three approaches are demonstrated in Figure 2. THC-COOH-d_3 and THC-COOH recoveries from enzyme-hydrolyzed urine were 101% and 100% respectively; from base-hydrolyzed, they were 87% for each. From non-hydrolyzed urine, recoveries were 95% and 93% respectively. For all procedures, RSD values were below 4%.

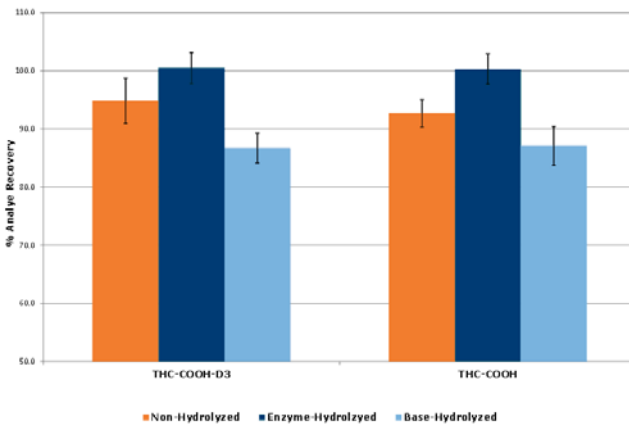

Figure 2. Typical extraction % recoveries (n=7) using the ISOLUTE® SLE+ protocols.

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AN843

Extraction of Anabolic Steroids from Horse Urine Using ISOLUTE® SLE+ Prior to LC-MS/MS Analysis

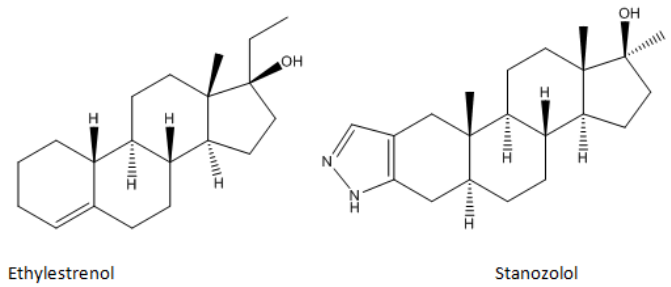

Figure 1. Structures of ethylestrenol and stanozolol.

Analytes
4-ethylestrenol and stanozolol

Format
ISOLUTE® SLE+ 1 mL supported Liquid Extraction plate, 48-well, part number 820-1000-Q01

Matrices
Horse urine

ISOLUTE® SLE+ Method
Take appropriate volume of urine and add same volume of H_2O. Mix. Load the pre-treated sample (800 µL) to each well of the 48-well plate followed by a pulse of vacuum or positive pressure to initiate flow. Leave to absorb for 5 minutes.

Ensure a suitable collection vessel is in place.

Apply 1 mL of MTBE and allow to flow under gravity.

Apply a second 1 mL of MTBE and allow to flow under gravity.

Apply a third 1 mL of MTBE and allow to flow under gravity until the solvent reaches the top frit. Pull through the remaining solvent with vacuum or positive pressure for 10–20 seconds.

Note: DCM is a suitable alternative elution solvent

Evaporate to dryness at 40 °C in a stream of air or nitrogen.

Reconstitute using 500 µL of 20/80 H_2O/ACN with 0.1% Formic acid. Mix gently.

Recovery/Results
Horse urine (gelding and filly) was spiked (n=7 for each matrix) with both analytes at a sample concentration of 40 ng/mL. Both dichloromethane and MTBE were evaluated as elution solvents.

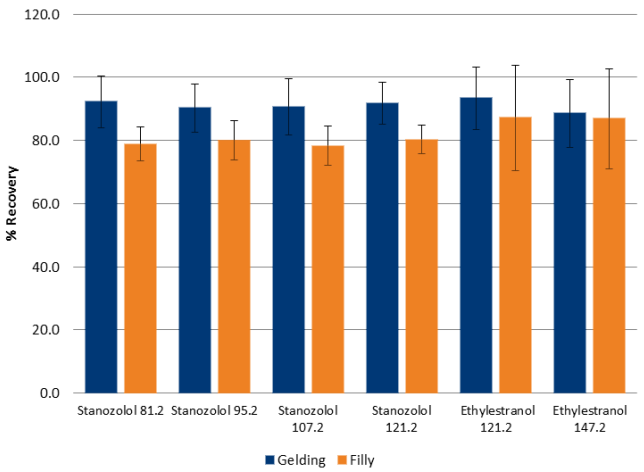

Figure 2. Recovery profile for anabolic steroids with MTBE elution, 20:80 H_2O:ACN 0.1% formic acid reconstitution solution using 48-well plate format.

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AN803

Extraction of Cocaine and Metabolites from Oral Fluid Using ISOLUTE® SLE+ Prior to GC-MS Analysis

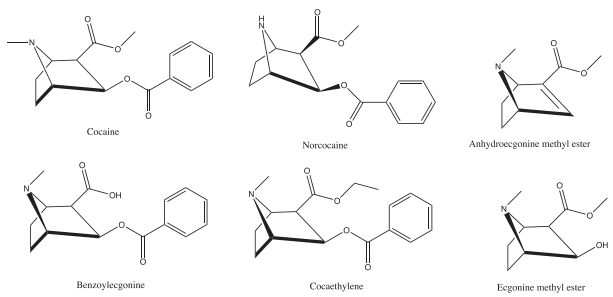

Figure 1. Cocaine structures.

Analytes
Cocaine, AEME, EME, benzoylecgonine, cocaethylene and norcocaine

Format
ISOLUTE® SLE+ 400 µL supported liquid extraction columns, part number 820-0055-B

ISOLUTE® SLE+ 1 mL supported liquid extraction columns, part number 820-0140-C

Matrices
Oral fluid

ISOLUTE® SLE+ Method
The Quantisal™ oral fluid collection device was used to collect samples. One tube collects 1 mL of oral fluid +/- 10% in approximately 3 mL of buffer.

Add internal standard and vortex mix thoroughly. Add 15 µL concentrated ammonium hydroxide (28–30% stock (aq)) to the contents of the tube.

Load the oral fluid/buffer mix (volumes as appropriate to the ISOLUTE SLE+ format) onto the column and apply a pulse of vacuum or positive pressure to initiate flow. Allow the sample to adsorb for 5 minutes.

Apply dichloromethane/isopropanol (95:5, v/v, 1 mL aliquot for ISOLUTE SLE+ 400 µL, 2.5 mL aliquots for ISOLUTE SLE+ 1 mL format) and allow to flow under gravity for 5 minutes. Apply a further aliquot of dichloromethane/isopropanol and allow to flow for another 5 minutes. Apply vacuum or positive pressure to pull through any remaining extraction solvent.

Dry the extract in a stream of air or nitrogen. Upon dryness, add 50 µL ethyl acetate and 50 µL BSTFA:TMCS 99:1. Vortex for 20 seconds and transfer to a high recovery glass vial and cap with a non-split cap. Heat vial in a heating block set to 70 °C, for 20 minutes. Remove vial from the block and allow to cool.

Recovery/Results
Recoveries ranging from 85–99%. RSDs were below 10% for all analytes.

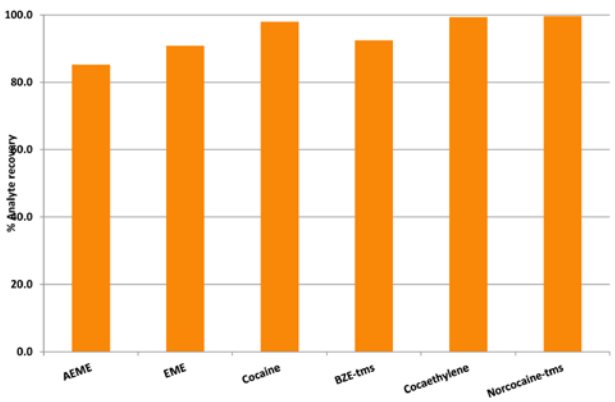

Figure 2. Typical analyte % recoveries for extracted cocaine and metabolites from urine (n=7) using the ISOLUTE® SLE+ protocol.

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AN802

Extraction of Cocaine and Metabolites from Urine Using ISOLUTE® SLE+ Prior to GC-MS Analysis

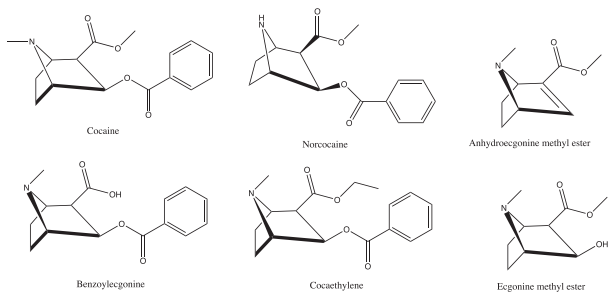

Figure 1. Cocaine structures.

Analytes
Cocaine, AEME, EME, benzoylecgonine, cocaethylene and norcocaine

Format
ISOLUTE® SLE+ 400 µL supported liquid extraction columns, part number 820-0055-B

ISOLUTE® SLE+ 1 mL supported liquid extraction columns, part number 820-0140-C

Matrices
Urine

ISOLUTE® SLE+ Method
Dilute pre-treated urine (1 mL) with 0.1% ammonium hydroxide (1 mL). Add internal standard and vortex mix thoroughly.

Load the pre-treated urine (volumes as appropriate to the format) onto the column and apply a pulse of vacuum or positive pressure to initiate flow. Allow the sample to adsorb for 5 minutes.

Apply dichloromethane/isopropanol (95:5, v/v, 1 mL aliquots for ISOLUTE SLE+ 400 µL format, 2.5 mL aliquots for ISOLUTE SLE+ 1 mL format 2.5 mL) and allow to flow under gravity for 5 minutes. Apply a further aliquot of dichloromethane/isopropanol and allow to flow for another 5 minutes. Apply vacuum or positive pressure to pull through any remaining extraction solvent.

Dry the extract in a stream of air or nitrogen. Upon dryness, add 50 µL ethyl acetate and 50 µL BSTFA:TMCS 99:1. Vortex mix and heat vial in a heating block set to 70 °C, for 20 minutes.

Recovery/Results
Recoveries ranging from 82–105%. RSDs were below 8% for all analytes.

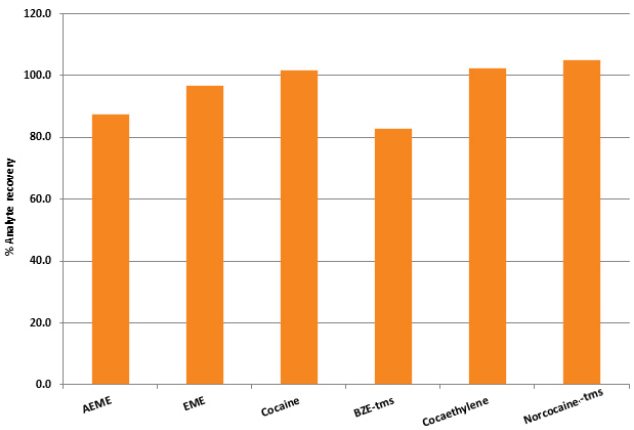

Figure 2. Typical analyte % recoveries for extracted cocaine and metabolites from urine (n=7) using the ISOLUTE® SLE+ protocol.

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AN772

Extraction of Cocaine and Metabolites from Urine Using ISOLUTE® SLE+ Prior to LC-MS/MS Analysis

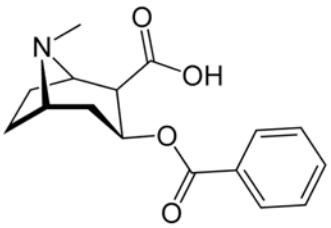

Figure 1. Structure of benzoylecgonine.

Analytes
Cocaine, norcocaine, cocaethylene, benzoylecgonine, ecgonine methyl ester (EME) and anhydroecgonine methyl ester (AEME)

Format
ISOLUTE® SLE+ 200 µL supported liquid extraction plate, part number 820-0200-P01

ISOLUTE® SLE+ 1 mL supported liquid extraction columns, part number 820-0140-C

Matrices
Urine

ISOLUTE® SLE+ Method
Dilute urine (1 mL) with 0.1% ammonium hydroxide (aq) (1 mL). Add internal standard. Vortex mix thoroughly.

Load the pre-treated urine (as appropriate to the SLE+ format) onto the plate and apply a pulse of vacuum or positive pressure to initiate flow. Allow the sample to adsorb for 5 minutes.

Apply DCM/IPA (95:5, v/v) (a single aliquot of 1 mL for the ISOLUTE SLE+ 200 µL plate, two aliquots of 2.5 mL for the ISOLUTE SLE+ 1 mL columns) and allow to flow under gravity for 5 minutes. Apply vacuum or positive pressure to pull through any remaining extraction solvent.

Add 50mM HCl in MeOH to stabilize AEME and EME during evaporation. Evaporate to dryness at 40 °C.

Recovery/Results
Recoveries ranged from 67–100%. RSDs were below 5% for all analytes.

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AN771

Extraction of Cocaine and Metabolites from Hydrolyzed Urine Using ISOLUTE® SLE+ Prior to LC-MS/MS Analysis

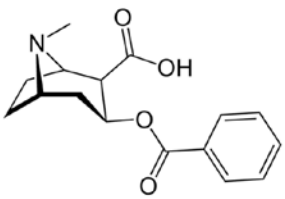

Figure 1. Structure of benzoylecgonine.

Analytes
Cocaine, norcocaine, cocaethylene, benzoylecgonine, ecgonine methyl ester (EME) and anhydroecgonine methyl ester (AEME)

Format
ISOLUTE® SLE+ 200 µL supported liquid extraction plate, part number 820-0200-P01

ISOLUTE® SLE+ 1 mL Supported liquid extraction columns, part number 820-0140-C

Matrices
Hydrolyzed urine

ISOLUTE® SLE+ Method
Dilute urine (1 mL) with 100 mM ammonium acetate buffer at pH5 (950 µL) and β-Glucuronidase enzyme (50 µL, equivalent to approximately 4500U/mL of urine). Add internal standard. Hydrolyze at 60 °C for 2 hours. Cool and add concentrated ammonium hydroxide (28–30% stock) aq (10 µL). Vortex mix thoroughly. Load the hydrolyzed urine (200 µL onto the plate, 1 mL for columns) and apply a pulse of vacuum or positive pressure to initiate flow. Allow the sample to adsorb for 5 minutes.

Apply DCM/IPA (95:5, v/v) (A single aliquot of 1 mL for the plate, two aliquots of 2.5 mL for the columns) and allow to flow under gravity for 5 minutes. Apply vacuum or positive pressure to pull through any remaining extraction solvent. Add 50 mM HCl in MeOH (volumes change with format) to stabilize AEME and EME during evaporation. Evaporate to dryness at 40 °C.

Recovery/Results
Recoveries ranged from 82–100%. RSDs were below 5% for all analytes.

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AN770

Extraction of a Range of Opiates and Metabolites from Human Urine Using ISOLUTE® SLE+ Columns Prior to GC-MS Analysis

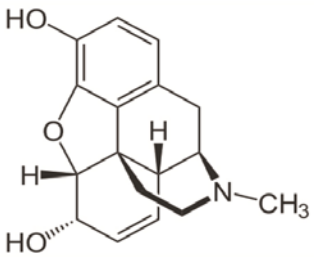

Figure 1. Structure of morphine.

Analytes

Dihydrocodeine, oxycodone, oxymorphone, codeine, morphine, hydrocodone, hydromorphone and 6-monoacetylmorphine (6-MAM)

Format

ISOLUTE® SLE+ 1 mL supported liquid extraction columns, part number 820-0140-C

Matrices

Hydrolyzed urine

ISOLUTE® SLE+ Method

Dilute urine (1 mL) with 100 mM ammonium acetate pH 5 (950 µL) and β-Glucuronidase enzyme (~4500 AU) (50 µL). Hydrolyze with heat in a water bath at 60 °C for 2 hours. Cool and add 25% ammonium hydroxide in water (10 µL) and vortex.

Load the pre-treated sample (1 mL) onto the column and apply a pulse of vacuum or positive pressure to initiate flow. Allow the sample to adsorb for 5 minutes.

Apply dichloromethane:isopropanol (95:5, v/v) (2.5 mL) and allow to flow under gravity for 5 minutes. Apply a second aliquot of dichloromethane:isopropanol (95:5, v/v) (2.5 mL) and allow to flow for another 5 minutes. Apply vacuum or positive pressure to pull through any remaining extraction solvent.

Evaporate the extract to dryness (40 °C). Reconstitute in ethyl acetate (500 µL). Evaporate the extract to dryness (40 °C). Derivatize with BSTFA:TMCS (99:1, v/v) (20 µL) and dichloromethane/Isopropanol (95:5, v/v) (20 µL). Seal with non-split caps and heat in a heating block at 70 °C for 30 minutes.

Recovery/Results

Recoveries ranged from 70–99%. RSDs were all below 5% for all analytes.

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AN741

Extraction of Opiates from Human Urine Using ISOLUTE® SLE+ 96-Well Plates and Columns With LC-MS/MS Analysis

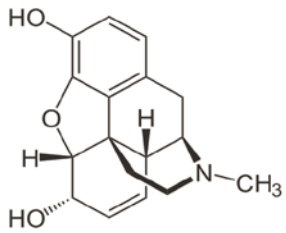

Figure 1. Structure of morphine.

Analytes
Morphine, codeine, oxycodone, 6-acetylmorphine, dihydrocodeine, hydromorphone, hydrocodone, oxymorphone, methadone and its metabolite EDDP

Format
ISOLUTE® SLE+ 200 µL supported liquid extraction plate, part number 820-0200-P01

ISOLUTE® SLE+ 1 mL supported liquid extraction columns, part number 820-0140-C

Matrices
Hydrolyzed urine

ISOLUTE® SLE+ Method
To 1 mL of urine add 100 mM ammonium acetate pH5 (950 µL) and β-glucuronidase (50 µL) enzyme (5000 µ/mL). Hydrolyze 600 °C for 2 hours. Cool and add 25% aqueous ammonium hydroxide (10 µL) and mix.

Load pre-treated sample (volumes as appropriate to the format) followed by a pulse of vacuum to initiate flow and leave for five minutes.

Elute with dichloromethane:isopropanol (95:5, v/v) (for the plate use one aliquot of 1 mL, for the columns use two aliquots of 2.5 mL). Leave to flow under gravity for 5 minutes, then apply short pulse of vacuum. Evaporate to dryness at room temperature (80 L/min) and reconstitute in 0.1% formic acid (aq) (100 µL).

Recovery/Results
Analytes recoveries from 70–102%

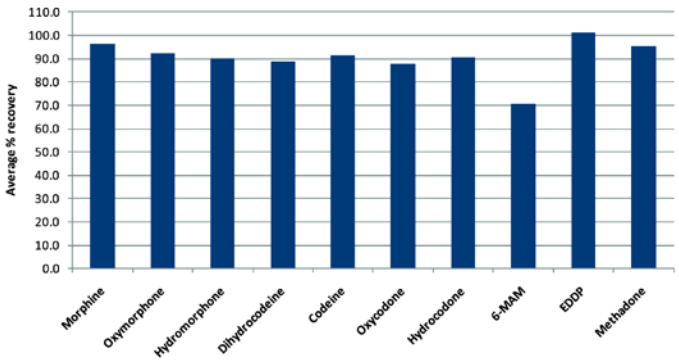

Figure 2. Average analyte recoveries of a range of opiate analytes on the 96 fixed-well plate at 4 ng/mL (n=7).

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AN837

Extraction of a Drugs of Abuse Panel from Oral Fluid Using ISOLUTE® SLE+ after Collection with the Oral-Eze® Collection Device Prior to UPLC-MS/MS Analysis

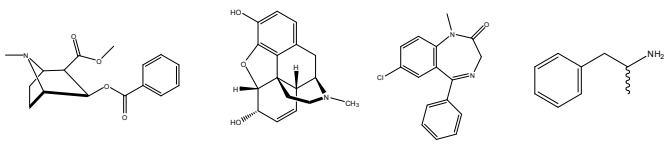

Figure 1. Example structures by class.

Analytes

Amphetamine, methamphetamine, MDA, MDMA, MDEA, mephedrone, morphine, hydromorphone, oxymorphone, dihydrocodeine, oxycodone, hydrocodone, codeine, 6-MAM, methadone, eddp, cocaine, benzoylecgonine, 7-amino-flunitrazepam, 7-amino-clonazepam, nitrazepam, flunitrazepam, clonazepam, α-OH-alprazolam, α-OH-triazolam, oxazepam, estazolam, temazepam, alprazolam, lorazepam, 2-OH-ethyl-flurazepam, triazolam, nordiazepam, diazepam, midazolam, flurazepam, bromazepam, zaleplone, zopiclone, zolpidem, fentanyl, norfentanyl, ketamine, norketamine, buprenorphine, norbuprenorphine, PCP

Format

ISOLUTE® SLE+ 400 µL columns, part no. 820-0055-B
ISOLUTE® SLE+ 1 mL columns, part no. 820-0140-C

Matrices

Oral fluid

ISOLUTE® SLE+ Method

Following oral fluid collection (as per manufacturer instructions), remove paddle, add internal standard as required, and 4% aqueous ammonium hydroxide (10 µL) to each collection device. Vortex mix.

For 400 µL column – Load 300 µL of the pre-treated oral fluid onto the column and apply a pulse of vacuum or positive pressure (3–5 seconds) to initiate flow. Allow the sample to absorb for 5 minutes.

For 1 mL column – Load 600 µL of the pre-treated oral fluid onto the column and apply a pulse of vacuum or positive pressure (3–5 seconds) to initiate flow. Allow the sample to absorb for 5 minutes.

Apply dichloromethane (1 mL for 400 µL columns, 2.5 mL for 1 mL columns) and allow to flow under gravity for 5 minutes. Apply a further aliquot of DCM (1 mL/2.5 mL) and allow to flow for another 5 minutes under gravity. Apply vacuum or positive pressure (5–10 seconds) to pull through any remaining extraction solvent.

Before evaporation, add 50 mM HCl in methanol (100 µL) to each collection tube. This will stabilize amphetamines, bath salts and ketamine, and minimize analyte losses during evaporation. Reconstitute with 200 µL of 5 mM ammonium acetate in 20% methanol.

Recovery/Results

RSDs ranged from 1.2%–9.1%

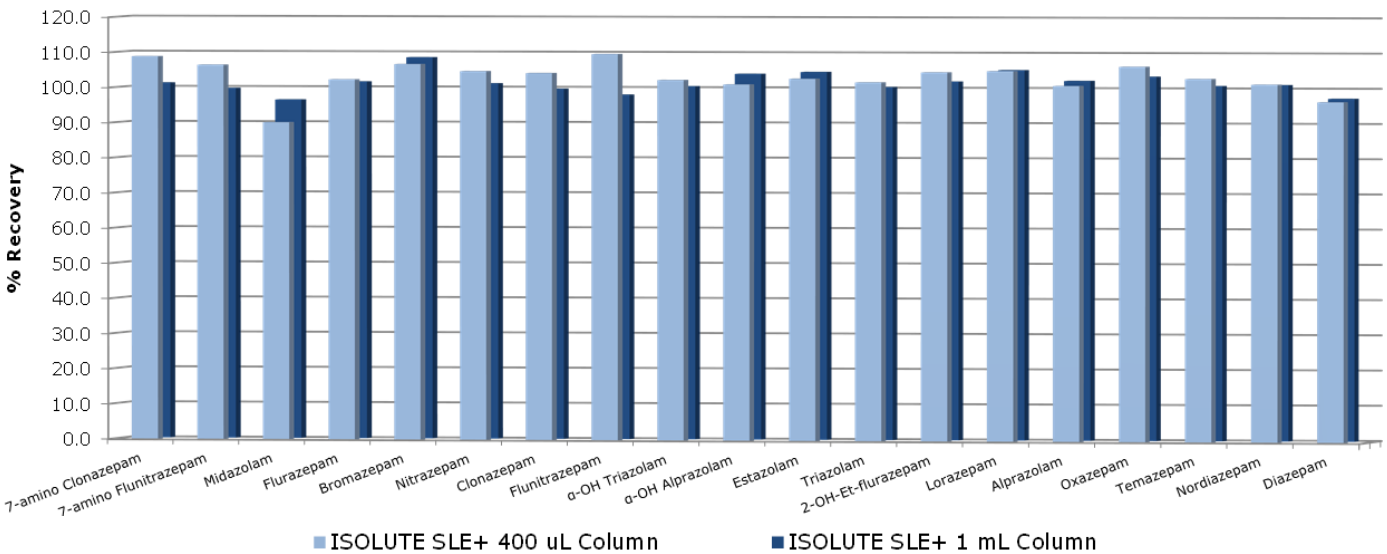

Figure 2. Oral-Eze method scale up for benzodiazepines. Recovery profile for benzodiazepines from Oral-Eze® collected oral fluid using ISOLUTE® SLE+ 400 µL and 1 mL columns.

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AN836

Extraction of a Drugs of Abuse Panel from Oral Fluid Using ISOLUTE® SLE+ after Collection with the Intercept® Oral Fluid Drug Test Kit Prior to UPLC-MS/MS Analysis

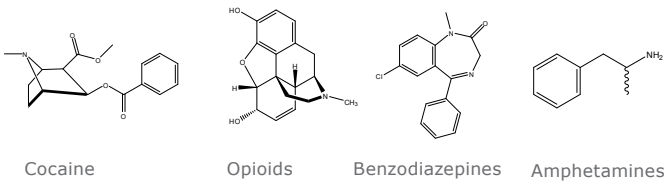

Figure 1. Example structures by class.

Analytes

Amphetamine, methamphetamine, MDA, MDMA, MDEA, mephedrone, morphine, hydromorphone, oxymorphone, dihydrocodeine, oxycodone, hydrocodone, codeine, 6-MAM, methadone, eddp, cocaine, benzoylecgonine, 7-amino-flunitrazepam, 7-amino-clonazepam, nitrazepam, flunitrazepam, clonazepam, α-OH-alprazolam, α-OH-triazolam, oxazepam, estazolam, temazepam, alprazolam, lorazepam, 2-OH-ethyl-flurazepam, triazolam, nordiazepam, diazepam, midazolam, flurazepam, bromazepam, zaleplone, zopiclone, zolpidem, fentanyl, norfentanyl, ketamine, norketamine, buprenorphine, norbuprenorphine, PCP

Format

ISOLUTE® SLE+ 1 mL columns, part no. 820-0140-C

Matrices

Oral fluid

ISOLUTE® SLE+ Method

Following oral fluid collection (as per manufacturer instructions), remove paddle, add internal standard as required, add 0.5% aqueous ammonium hydroxide (10 µL) to each collection device. Vortex mix. Dilute 250 µL of the contents of the device with 250 µL HPLC grade water Vortex mix.

Load 480 µL of the pre-treated oral fluid onto the column and apply a pulse of vacuum or positive pressure (3–5 seconds) to initiate flow. Allow the sample to absorb for 5 minutes. Apply ethyl acetate (3 mL) and allow to flow under gravity for 5 minutes. Apply a further aliquot of ethyl acetate (3 mL) and allow to flow for another 5 minutes under gravity. Apply vacuum or positive pressure (5–10 seconds) to pull through any remaining extraction solvent.

Before evaporation, add 50 mM HCl in methanol (100 µL) to each collection tube. This will stabilize amphetamines, bath salts and ketamine, and minimize analyte losses during evaporation. Reconstitute with 200 µL of 5 mM ammonium acetate in 20% methanol.

Recovery/Results

RSDs ranged from 1.8–9.8%

Amphetamines, Bath Salts and Opiates Recoveries

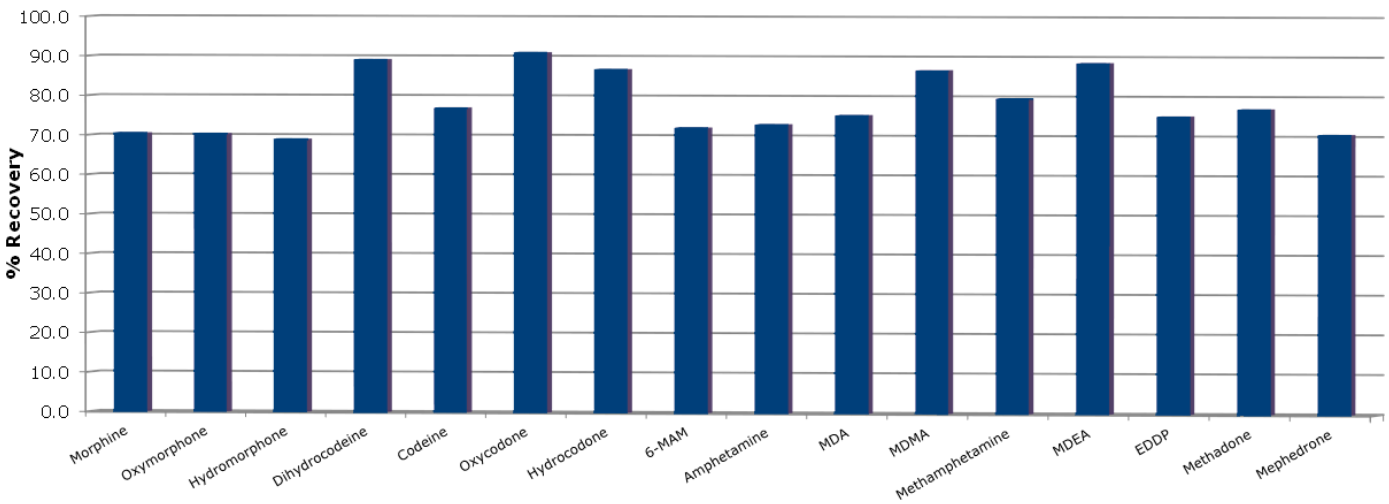

Figure 2. Recovery profile for amphetamines, bath salt and opiates from Intercept® collected oral fluid using ISOLUTE® SLE+ 1 mL columns.

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AN832

Extraction of a Drugs of Abuse Panel from Oral Fluid Using ISOLUTE® SLE+ after Collection with the Quantisal™ Collection Device Prior to UPLC-MS/MS Analysis

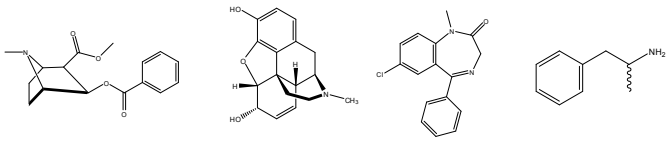

Figure 1. Example structures by class.

Analytes

Amphetamine, methamphetamine, MDA, MDMA, MDEA, mephedrone, morphine, hydromorphone, oxymorphone, dihydrocodeine, oxycodone, hydrocodone, codeine, 6-MAM, methadone, EDDP, cocaine, benzoylecgonine, 7-amino-flunitrazepam, 7-amino-clonazepam, nitrazepam, flunitrazepam, clonazepam, α-OH-alprazolam, α-OH-triazolam, oxazepam, estazolam, temazepam, alprazolam, lorazepam, 2-OH-ethyl-flurazepam, triazolam, nordiazepam, diazepam, midazolam, flurazepam, bromazepam, zaleplone, zopiclone, zolpidem, fentanyl, norfentanyl, ketamine, norketamine, buprenorphine, norbuprenorphine, PCP

Format

ISOLUTE® SLE+ 400 µL columns, part no. 820-0055-B
(also available in tabless format, part number 820-0055-BG)
ISOLUTE® SLE+ 1 mL columns, part no. 820-0140-C
(also available in tabless format, part number 820-0140-CG)

Matrices

Oral fluid

ISOLUTE® SLE+ Method

Following oral fluid collection as per manufacturer instructions, add 15 µL of concentrated ammonium hydroxide to each collection device and vortex mix.

For 400 µL column – Load 200 µL of the pre-treated oral fluid device buffer (equivalent to 50 µL of neat oral fluid) onto the column and apply a pulse of vacuum or positive pressure (3–5 seconds) to initiate flow. Allow the sample to absorb for 5 minutes.

For 1 mL column – Load 500 µL of the pre-treated oral fluid device buffer (equivalent to 125 µL of neat oral fluid) onto the column and apply a pulse of vacuum or positive pressure (3–5 seconds) to initiate flow. Allow the sample to absorb for 5 minutes.

Apply dichloromethane (1 mL for 400 µL columns, 3 mL for 1 mL columns) and allow to flow under gravity for 5 minutes. Apply a further aliquot of dichloromethane and allow to flow for another 5 minutes under gravity. Apply vacuum or positive pressure (5–10 seconds) to pull through any remaining extraction solvent. Before evaporation, add 100 µL of 50 mM HCl in methanol to each collection tube. This will stabilize amphetamines, bath salts and ketamine, and minimize analyte losses during evaporation. Reconstitute with 200 µL of 5 mM ammonium acetate in 20% methanol.

Recovery/Results

The % analyte recoveries for the various drug classes can be seen in Figure 2 RSDs ranged from 1.3–9.3%.

Quantisal™ Method Scale up for Amphetamines, Bath Salts and Opiates

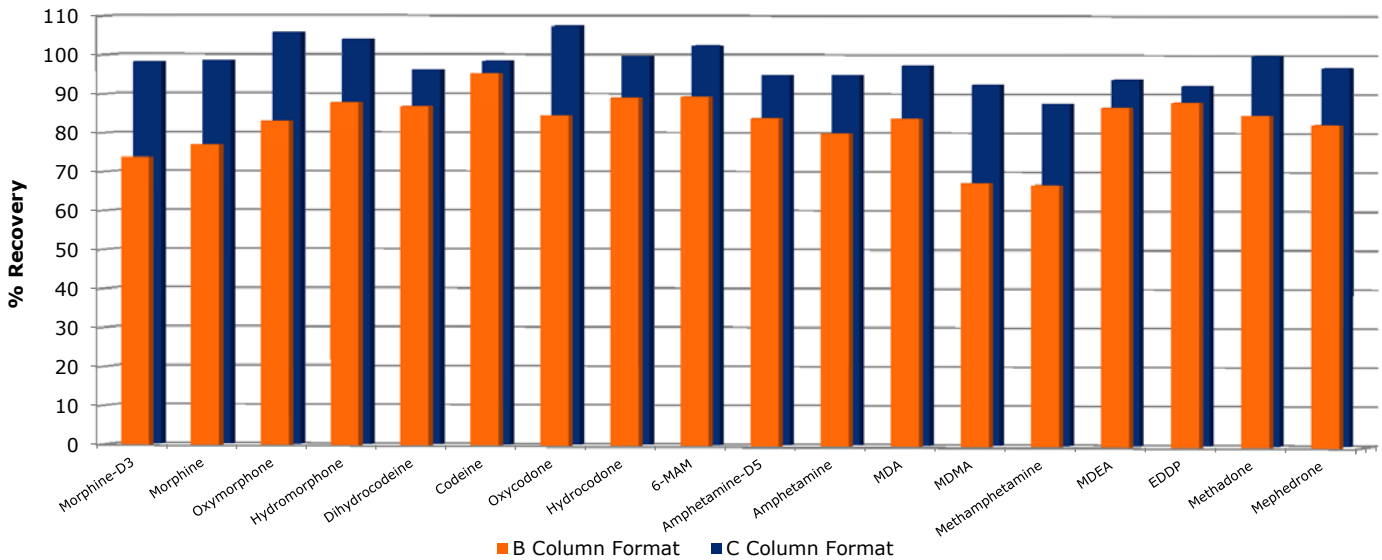

Figure 2. Recovery profile for amphetamines, bath salt and opiates from Quantisal™ collected oral fluid using ISOLUTE® SLE+ 400 µL (B) columns and 1 mL (C) columns.

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AN808

Extraction of Designer Stimulants from Urine Using ISOLUTE® SLE+ Prior to GC/MS Analysis

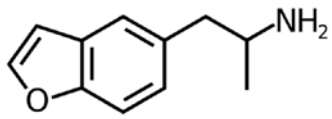

Figure 1. Structure of 5-APB, a chemical found in '*Benzo Fury*'.

Analytes

5-APB, 6-APB, pMMA, BZP, TFMPP, 2C-B, mCPP, amphetamine-d_5 as internal standard

Format

ISOLUTE® SLE+ 1 mL columns, part no. 820-0140-C

Matrices

Urine

ISOLUTE® SLE+ Method

Spike urine sample with ISTD (10 µL in methanol) and dilute spiked sample with ammonium hydroxide (1%, 1:1, v/v). Load the pre-treated urine (1 mL total volume) onto the column and apply a pulse of vacuum or positive pressure to initiate flow. Allow the sample to adsorb for 5 minutes.

Collect extracts into glass tubes containing methanolic HCl (100 µL, 0.2M). Apply methyl tert-butyl ether (MTBE) (2 mL) and allow to flow under gravity for 5 minutes. Apply a further aliquot of MTBE (2 mL) and allow to flow for another 5 minutes. Apply vacuum or positive pressure for 10–20 seconds to elute any remaining extraction solvent.

Evaporate to dryness in a stream of air or nitrogen at ambient temperature for 40 mins and reconstitute. Reconstitute with 0.2M HCl in ethyl acetate (500 µL) and vortex for 20 seconds. Transfer to a high recovery glass vial and evaporate to dryness. Add ethyl acetate (25 µL) and pentafluoropropionic acid anhydride (PFPA) (25 µL) and cap with a non-split cap. Vortex for 20 seconds and heat vial in a heating block set to 70 °C for 30 minutes. Remove vial from the block and allow to cool.

Evaporate at ambient temperature and reconstitute with ethyl acetate (50 µL).

Recovery/Results

Analyte recoveries 105–109% RSDs were below 10% for all analytes for all donors.

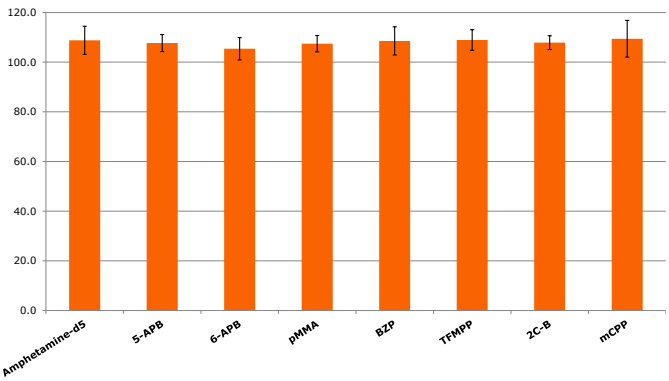

Figure 2. Typical analyte % recoveries for extracted synthetic stimulants (n=7) using this ISOLUTE® SLE+ protocol.

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AN790

Extraction of Illicit Drugs from Hemolyzed Whole Blood Using ISOLUTE® SLE+ Columns Prior to LC-MS/MS Analysis

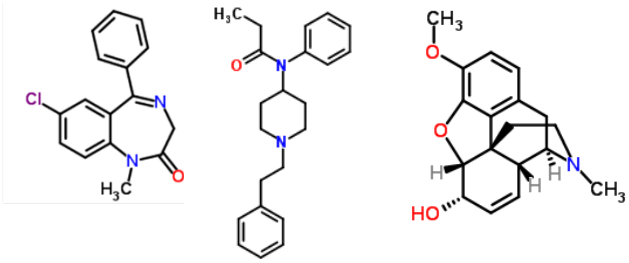

Figure 1. Structures of diazepam, fentanyl, and codeine.

Analytes

Alprazolam, clonazepam, diazepam, flunitrazepam, oxazepam, temazepam, nitrazepam, normeperidine, naltrexone, morphine, codeine, oxymorphone, hydromorphone, oxycodone, hydrocodone, 6-acetyl codeine, 6-acetyl morphine, fentanyl, buprenorphine, EDDP

Format

ISOLUTE® SLE+ 1 mL columns, part no. 820-0140-C
ISOLUTE® SLE+ 2 mL columns, part no. 820-0290-D

Matrices

Whole blood

ISOLUTE® SLE+ Method

Add 400 µL of ammonium hydroxide (2%, v/v) to samples (500 µL) then gently vortex. Add up to 100 µL of internal standard solution to each sample. This can be scaled up for the 2 mL SLE+.

Load pre-treated samples onto columns. Apply a short pulse of vacuum or positive pressure to initiate flow and then allow sample to absorb on column for 5 minutes.

Apply 2 x 3 mL of ethyl acetate (1 mL ISOLUTE SLE+ cartridge) or 2 x 4 mL (2 mL ISOLUTE SLE+ cartridge) to each cartridge and allow solvent to flow under gravity. Apply positive pressure or vacuum for 10–20 seconds to elute any remaining extraction solvent. Evaporate sample to dryness and reconstitute in water:methanol

Recovery/Results

The averaged recoveries (n=7) for drugs spiked into whole blood ranged from 45–92% and 63–104% for the 1 mL and 2 mL column formats, respectively.

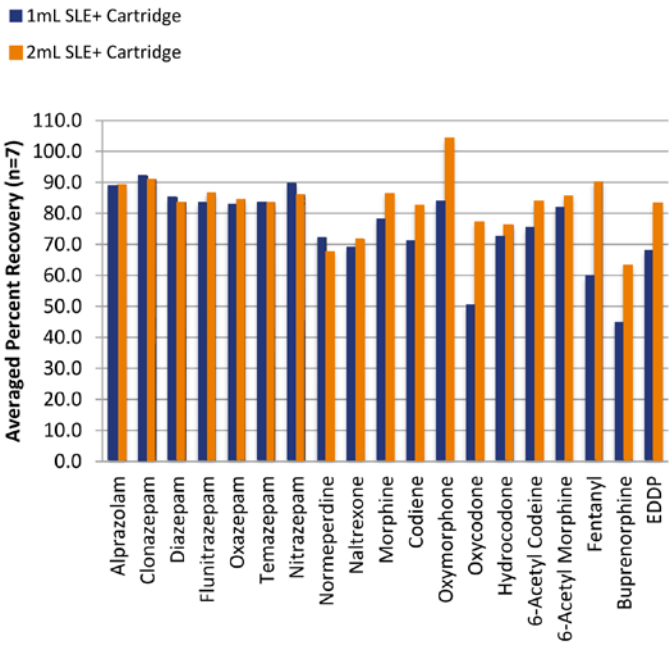

Figure 2. Drugs extracted from hemolysed whole blood using ISOLUTE® SLE+ cartridges. Plot of averaged (n=7) recovery of spiked drugs of abuse (10 ng/mL) from hemolyzed whole blood using ISOLUTE® SLE+ columns.

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AN788

Extraction of Illicit Drugs from Oral Fluid Using ISOLUTE® SLE+ in Column Format Prior to LC-MS/MS Analysis

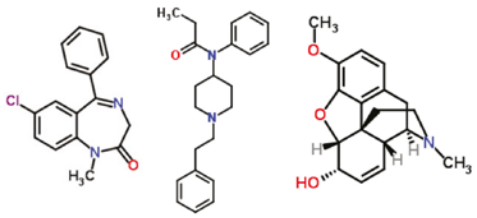

Figure 1. Structures of diazepam, fentanyl and codeine.

Analytes

Alprazolam, clonazepam, diazepam, flunitrazepam, oxazepam, temazepam, nitrazepam, normeperidine, naltrexone, morphine, codeine, oxymorphone, hydromorphone, oxycodone, hydrocodone, 6-acetyl, 6-acetyl morphine, fentanyl, buprenorphine, EDDP

Format

ISOLUTE® SLE+ 400 µL columns (tabless), part no. 820-0055-BG

Matrices

Oral fluid

ISOLUTE® SLE+ Method

Mix oral fluid solution ammonium hydroxide (2%, v/v, 200 µL). Gently vortex the solutions. Load pre-treated samples onto columns. Apply a short pulse of vacuum or positive pressure to initiate flow, and then allow sample to adsorb on column for 5 minutes. Apply ethyl acetate (2 x 600 µL) to each cartridge and allow solvent to flow under gravity. Apply positive pressure or pull slight vacuum as needed during collection process to facilitate a flow rate of 1 mL per minute.

Evaporate sample to dryness and reconstitute in mobile phase (500 µL).

Recovery/Results

The averaged recoveries (n=7) observed for drugs spiked into neat oral solution ranged from 78 to 117% with typical percent relative standard deviations (%RSD) less than 10. The averaged recoveries (n=7) for drugs of interest spiked into oral fluid suspended in the collection kit buffer ranged from 63%–110% with typical %RSDs <10.

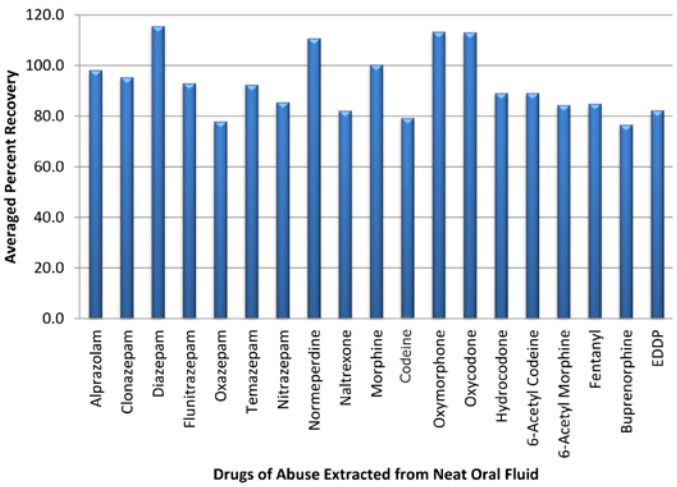

Figure 2. Plot of averaged (n=7) recovery of spiked drugs of abuse (10 ng/mL) from neat solution of oral fluid using ISOLUTE SLE+ 400 µL sample volume columns.

Download Full Application Note »

AN769

Extraction of 45 Multi Class Drugs of Abuse from Urine (Non-Hydrolyzed) Using ISOLUTE® SLE+ Prior to LC-MS/MS Analysis

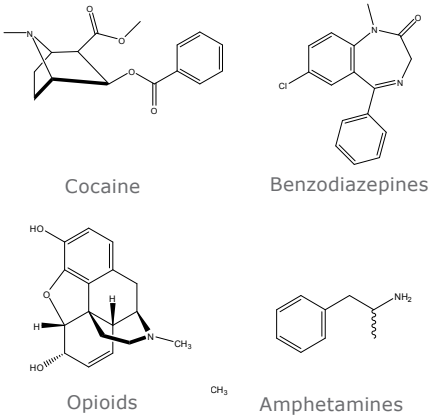

Figure 1. Example structures by class.

Analytes

Amphetamine, methamphetamine, MDA, MDMA, MDEA, methadone, EDDP, mephedrone, morphine, hydromorphone, oxymorphone, dihydrocodeine, oxycodone, hydrocodone, codeine, 6-MAM, cocaine, benzoylecgonine, fentanyl, norfentanyl, ketamine, norketamine, buprenorphine, norbuprenorphine, 7-amino-flunitrazepam, 7-amino-clonazepam, nitrazepam, flunitrazepam, clonazepam, α-OH-alprazolam, α-OH-triazolam, oxazepam, estazolam, temazepam, alprazolam, lorazepam, 2-OH-ethyl-flurazepam, triazolam, nordiazepam, diazepam, midazolam, flurazepam, zaleplone, zopiclone, zolpidem

Format

ISOLUTE® SLE+ 200 SLE plate part no. 820-0200-P01
ISOLUTE® SLE+ 1 mL columns, part no. 820-0140-C

Matrices

Urine (non-hydrolyzed)

ISOLUTE® SLE+ Method

Take 1 mL urine and spike internal standard (10 µL). Add 1 mL of 1% ammonium hydroxide (aq, v/v) and vortex mix thoroughly.

ISOLUTE SLE+ 200 µL plate: Load pre-treated sample (200 µL) to the 96 well-plate followed by a pulse of vacuum or positive pressure to initiate flow. Leave to absorb for 5 minutes.

ISOLUTE SLE+ 1 mL columns: Load pre-treated sample (1 mL) to the columns followed by a pulse of vacuum or positive pressure to initiate flow. Leave to absorb for 5 minutes.

ISOLUTE SLE+ 200 µL plate: Apply 1 mL of dichloromethane/isopropanol (95:5, v/v) and allow to flow under gravity for 5 minutes. Pull through the remaining solvent with vacuum or positive pressure for 10–20 seconds.

ISOLUTE SLE+ 1 mL columns: Apply 2.5 mL of dichloromethane/isopropanol (95/5, v/v) and allow to flow under gravity for 5 minutes.

Apply a second 2.5 mL aliquot of dichloromethane/isopropanol (95/5, v/v) and allow to flow under gravity for 5 minutes. Pull through the remaining solvent with vacuum or positive pressure for 10–20 seconds.

Note: The addition of 100 µL of 50 mM HCl in methanol into the collection plate or culture tube is required prior to or post elution to stabilize multiple analytes (amphetamines, bath salts and ketamine) due to volatility issues during evaporation.

Evaporate to dryness. Reconstitute in 2 mM ammonium acetate in 20% methanol (200 µL for ISOLUTE SLE+ 200 µL plate, 500 µL for ISOLUTE SLE+ 1 mL columns).

Recovery/Results

RSDs (N=7) ranged from 0.8–6.5%. Recoveries greater than >90% were achieved for the majority of the analytes.

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AN768

Extraction of 45 Multi Class Drugs of Abuse from Hydrolyzed Urine Using ISOLUTE® SLE+ Prior to LC-MS/MS Analysis

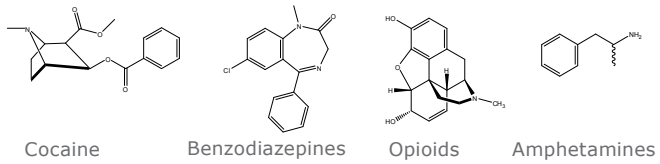

Figure 1. Example structures by class.

Analytes

Amphetamine, methamphetamine, MDA, MDMA, MDEA, methadone, EDDP, mephedrone, morphine, hydromorphone, oxymorphone, dihydrocodeine, oxycodone, hydrocodone, codeine, 6-MAM, cocaine, benzoylecgonine, fentanyl, norfentanyl, ketamine, norketamine, buprenorphine, norbuprenorphine, 7-amino-flunitrazepam, 7-amino-clonazepam, nitrazepam, flunitrazepam, clonazepam, α-OH-alprazolam, α-OH-triazolam, oxazepam, estazolam, temazepam, alprazolam, lorazepam, 2-OH-ethyl-flurazepam, Triazolam, nordiazepam, diazepam, midazolam, flurazepam, zaleplone, zopiclone, zolpidem

Format

ISOLUTE® SLE+ 200 SLE+ plate part no. 820-0200-P01
ISOLUTE® SLE+ 1 mL columns, part no. 820-0140-C

Matrices

Hydrolyzed urine

ISOLUTE® SLE+ Method

Take 1 mL urine and spike with internal standard (10 µL). Add 1 mL of 100 mM ammonium acetate buffer (pH 5) and 50 µL β-Glucuronidase enzyme (equivalent to approximately 4500 U/mL of urine). Hydrolyze at 60 °C for 2 hours. Cool the hydrolyzed urine and add 10 µL of concentrated ammonium hydroxide (28–30% stock, aq) per mL of urine. Vortex mix thoroughly.

ISOLUTE SLE+ 200 µL plate: Load pre-treated sample (200 µL) to the 96 well-plate followed by a pulse of vacuum or positive pressure to initiate flow. Leave to absorb for 5 minutes.

ISOLUTE SLE+ 1 mL columns: Load pre-treated sample (1 mL) to the columns followed by a pulse of vacuum or positive pressure to initiate flow. Leave to absorb for 5 minutes.

ISOLUTE SLE+ 200 µL plate: Apply 1 mL of dichloromethane/isopropanol (95/5, v/v) and allow to flow under gravity for 5 minutes. Pull through the remaining solvent with vacuum or positive pressure for 10–20 seconds.

ISOLUTE SLE+ 1 mL columns: Apply 2.5 mL of dichloromethane/isopropanol (95/5, v/v) and allow to flow under gravity for 5 minutes.

Apply a second 2.5 mL aliquot of dichloromethane/isopropanol (95/5, v/v) and allow to flow under gravity for 5 minutes. Pull through the remaining solvent with vacuum or positive pressure for 10–20 seconds.

Note: The addition of 100 µL of 50 mM HCl in methanol into the collection plate or culture tube is required prior to or post elution to stabilize multiple analytes (amphetamines, bath salts and ketamine) due to volatility issues during evaporation. Evaporate to dryness. Reconstitute in 2 mM ammonium acetate in 20% methanol (200 µL for ISOLUTE SLE+ 200 µL plate, 500 µL for ISOLUTE SLE+ 1 mL columns)

Recovery/Results

RSDs (n=7) ranged from 0.1–9.6%. Recoveries greater than >90% were achieved for the majority of the analytes.

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AN764

Extraction of 22 Pain Management Drugs from Urine Using ISOLUTE® SLE+ in 96-Fixed Well Plate Format Prior to LC-MS-MS

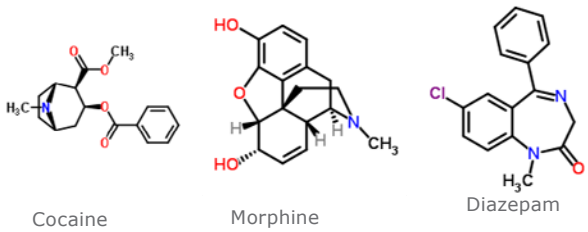

Figure 1. Structures of cocaine, morphine and diazepam.

Analytes

Cocaine, heroin, morphine, codeine, oxymorphone, hydromorphone, oxycodone, hydrocodone, methadone, clonazepam, diazepam, flunitrazepam, oxazepam, temazepam, nitrazepam, alprazolam, methamphetamine, fentanyl, buprenorphine, meperidine, naloxone, naltrexone

Format

ISOLUTE® SLE+ 200 SLE plate part no. 820-0200-P01

Matrices

Urine

ISOLUTE® SLE+ Method

Mix 200 µL of hydrolyzed urine with 200 µL of 2% Ammonium Hydroxide (NH_4OH). Gently vortex solution. Load the pre-treated samples. Apply a short pulse of vacuum or positive pressure to initiate flow and then allow sample to flow under gravity for 5 minutes. Apply 2 x 600 µL of Ethyl Acetate to each well. Apply positive pressure or pull slight vacuum as needed during collection process at a flow rate of 1 mL per minute. Evaporate sample to dryness and reconstitute in mobile phase (500 µL). Dry down degradation of methamphetamine can be decreased by the addition of 50 uL of 50 mM HCl in methanol to elution collection wells prior to sample elution.

Results

The average inter-run recoveries for the target analytes were above 80% with the overall intra-run RSDs <10%.

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AN834

Extraction of NBOMe Designer Drugs from Oral Fluid Using ISOLUTE® SLE+ Prior to Analysis by LDTD-MS/MS

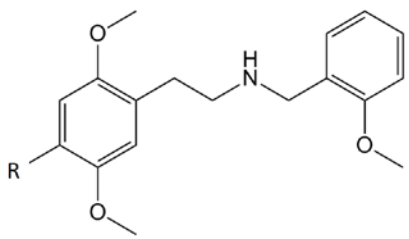

Figure 1. Structures of the NBOMEs studied.

Analytes

25B-NBOMe, 25C-NBOMe, 25D-NBOMe, 25E-NBOMe, 25H-NBOMe, 25I-NBOMe, 25N-NBOMe, 25T2-NBOMe

Format

ISOLUTE® SLE+ 400 µL supported liquid extraction columns, part number 820-0055-B

Matrices

Oral fluid

ISOLUTE® SLE+ Method

Concentration Range 1: 25–1000 ng/mL

To 150 µL of sample, add 25µl-NBOMe-d_3 internal standard (15 µL, (1000 ng /mL in MeOH), and ammonium hydroxide (NH_4OH, 0.1%, 10 µL). Mix.

Load pre-treated oral fluid (175 µL, as above) onto the column and apply a pulse of vacuum or positive pressure (3–5 seconds) to initiate flow. Allow the sample to absorb for 5 minutes.

Apply methyl-tert-butyl-ether (MTBE, 4 x 500 µL) and allow to flow under gravity for 5 minutes. Apply vacuum or positive pressure (5–10 seconds) to complete elution.

Deposit 3 µL of elution phase onto the LazWell plate and let dry. Methodology for a lower calibration range can be found in the full application note on line.

Recovery/Results

LLOQ for 25B-NBOMe and 25I-NBOMe was 0.5 ng/mL with RSDs <10%.

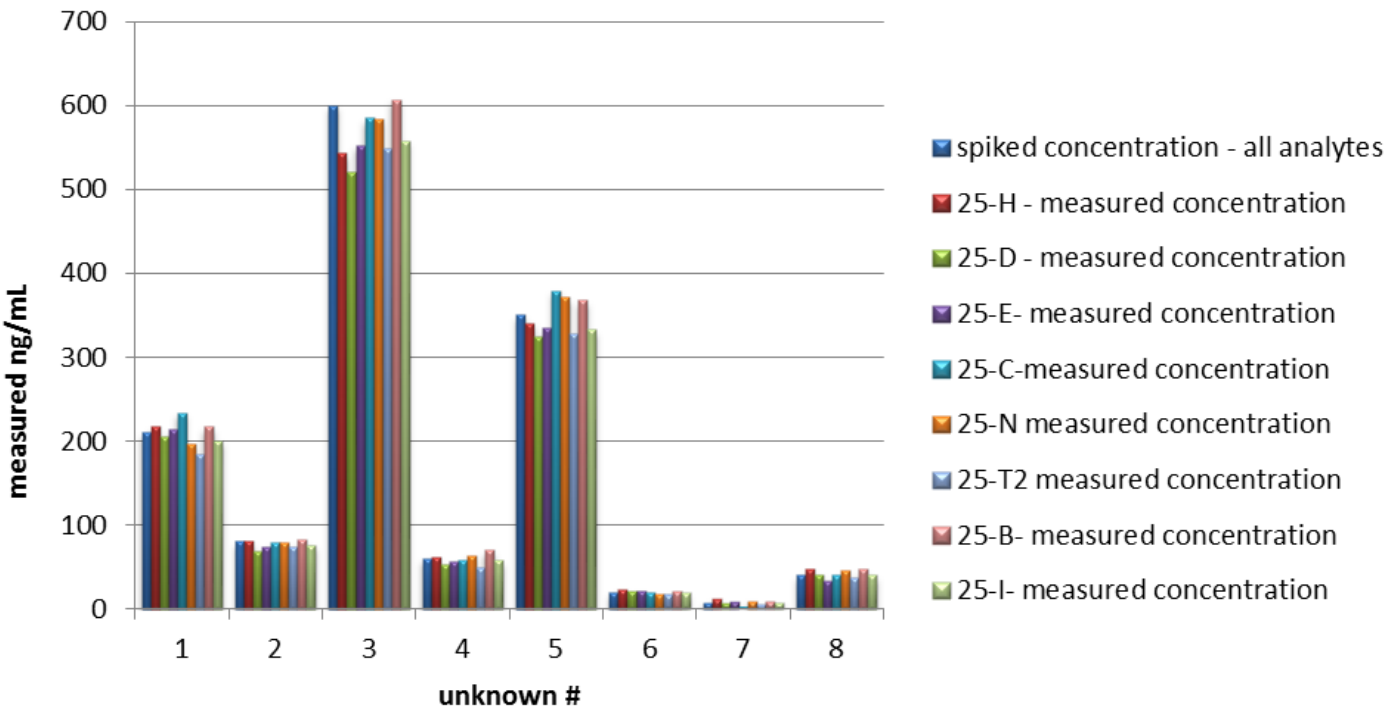

Figure 2. Unknown sample set fortified to eight concentration levels for a panel of NBOMe designer drugs, and extracted using the method detailed in this application note.

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AN829

Extraction of Phencyclidine (PCP) from Urine Using ISOLUTE® SLE+ Prior to GC/MS Analysis

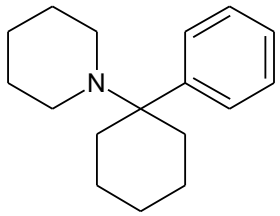

Figure 1. PCP structure.

Analytes
PCP & PCP-d_5

Format
ISOLUTE® SLE+ 1 mL supported liquid extraction columns, part number 820-0140-C (also available in tabless format, part number 820-0140-CG)

Matrices
Urine

ISOLUTE® SLE+ Method
Dilute pre-treated urine (1 mL) with 0.5% ammonium hydroxide (aq) (1 mL). Add internal standard and vortex mix thoroughly.

Load pre-treated urine (1 mL) onto the column and apply a pulse of vacuum or positive pressure (3–5 seconds) to initiate flow. Allow the sample to absorb for 5 minutes.

Apply 1-chlorobutane (2.5 mL) and allow to flow under gravity for 5 minutes. Apply a further aliquot of 1-chlorobutane (2.5 mL) and allow to flow for another 5 minutes under gravity. Apply vacuum or positive pressure to pull through any remaining extraction solvent (5–10 seconds).

To each sample, add 0.2 M methanolic HCl (100 µL). Dry the extract in a stream of air or nitrogen at ambient temperature. Reconstitute with ethyl acetate (200 µL).

Recovery/Results
Recoveries >88%, RSDs <8%.

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AN828

Extraction of *gamma*-Hydroxybutyric Acid (GHB) from Urine Using ISOLUTE® SLE+ Prior to GC/MS Analysis

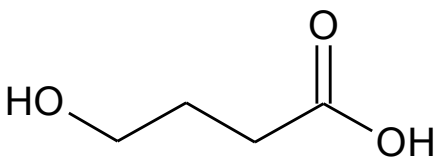

Figure 1. Structure of *gamma*-hydroxybutyric acid (GHB).

Analytes
Gamma-hydroxybutyric acid (GHB) and GHB-d6

Format
ISOLUTE® SLE+ 1 mL 200 µL Fixed Well Plate, part number 820-0200-P01

ISOLUTE® SLE+ 1 mL 400 µL Sample Volume Column part number 820-0055-B

Matrices
Urine

ISOLUTE® SLE+ Method
Dilute pre-treated urine (0.2 mL) with 0.2% formic acid (aq) (0.2 mL). Add internal standard and vortex mix thoroughly.

Load the pre-treated urine (200 µL for both plates and columns) onto each well and apply a pulse of vacuum or positive pressure (3–5 seconds) to initiate flow. Allow the sample to absorb for 5 minutes.

Apply ethyl acetate (1 mL, two aliquots are used for the 400 µL columns) and allow to flow under gravity for 5 minutes. Apply vacuum or positive pressure to pull through any remaining extraction solvent (5–10 seconds).

Dry the extract in a stream of air or nitrogen at ambient temperature. Upon dryness, reconstitute with ethyl acetate (50 µL) and BSTFA/1% TMCS (50 µL) and vortex for 20 seconds.

Recovery/Results
Recoveries ranged 52–69%. RSDs <9%.

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AN816

Extraction of Propofol from Whole Blood Using ISOLUTE® SLE+ Prior to GC/MS Analysis

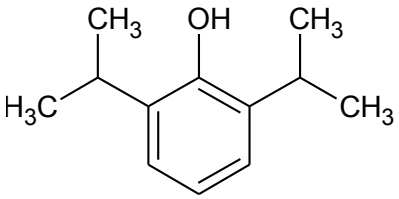

Figure 1. Structure of propofol.

Analytes
Propofol and propofol-d_{17} as internal standard

Format
ISOLUTE® SLE+ 400 µL supported liquid extraction columns, part number 820-0055-B

ISOLUTE® SLE+ 1 mL supported liquid extraction columns, part number 820-0140-C

Matrices
Whole blood

ISOLUTE® SLE+ Method
Add internal standard and dilute whole blood 1:1 (v/v) with HPLC-grade water. Mix.

Load (0.3 mL for ISOLUTE SLE+ 400 µL columns, 0.8 mL for SLE+ 1 mL columns) of the pre-treated whole blood onto the column and apply a pulse of vacuum or positive pressure to initiate flow. Allow the sample to absorb for 5 minutes.

Apply methyl *tert*-butyl ether (MTBE) and allow to flow under gravity for 5 minutes into a glass tube containing 0.5% tetrabutylammonium hydroxide (TBAH) in methanol (10 µL).

Apply vacuum or positive pressure for 10–20 seconds to remove any remaining extraction solvent.

Dry the extract in a stream of air or nitrogen at ambient temperature. Upon dryness, reconstitute with 100 µL heptane and vortex for 20 seconds.

Recovery/Results
Recoveries ranged from 95–104%. RSDs <6%.

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AN812

Extraction of 1,25 di-OH Vitamin D2, 1,25 di-OH Vitamin D3, 25 OH Vitamin D2 and 25 OH Vitamin D3 from Serum Using ISOLUTE® SLE+ Prior to LC-MS/MS Analysis

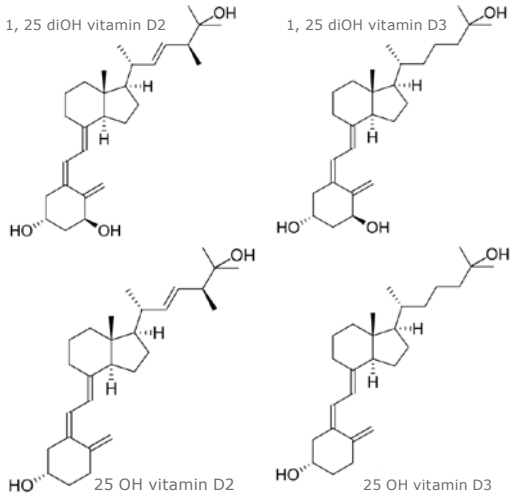

Figure 1. Structure of vitamin D metabolites.

Analytes

1,25-diOH vitamin D3, 25-OH vitamin D3, 1,25-diOH vitamin D2, 25-OH vitamin D2

Format

ISOLUTE® SLE+ 400 µL supported liquid extraction plate, part number 820-0400-P01

Matrices

Serum

ISOLUTE® SLE+ Method

Samples (300 µL) were combined with the required level of internal standard and left to stand for 0.5 hour to reach binding equilibrium.

The samples and standards were then combined with no more than 100 µL of water: propan-2-ol (50:50) and left to stand for an additional 15 minutes for consistent protein disruption.

Load pre-treated sample (0.4 mL) onto each well. Apply a pulse of vacuum or positive pressure to initiate flow. Allow the sample to absorb for 5 minutes. Elute with heptane, (2 x 0.7 mL) and allow solvent to flow under gravity into a 2 mL collection plate already containing 100 µL of 0.5 mg/mL PTAD solution in ethyl acetate: heptane (8:92, v/v). Apply vacuum or positive pressure to elute any remaining extraction solvent.

Note: heptane may be replaced with hexane as an alternative elution solvent, if preferred.

Let collection plate sit for 5 minutes at room temperature. Dry the wells in a stream of air or nitrogen. Reconstitute samples in 100 µL of acetonitrile: water (50:50, v/v).

Recovery/Results

Recoveries for all four derivatized analytes ranged from 75.5% to 92% with RSDs <10%.

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AN811

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

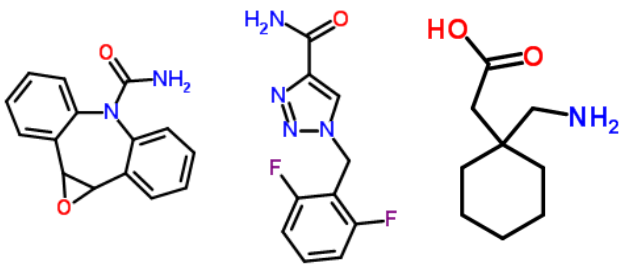

Figure 1. Structures of carbamazepine epoxide, rufinamide and gabapentine.

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

ISOLUTE® SLE+ Method
Add Ammonium Acetate (5mM, pH 2.9, 250µL) to sample (100 µL) then add up to 50 µL of internal standard.

Load up to 400 µL of pre-treated oral fluid sample onto the ISOLUTE SLE+ 96-well plate. Apply a short pulse of positive pressure or vacuum and allow samples to absorb for 5 minutes.

Elute analytes with methyl tert-butyl ether containing 1% (v/v) trifluoroacetic acid (conc) solution (2 x 700 µL). Allow sample to flow through by gravity and collect eluent. Evaporate to dryness and reconstitute sample into mobile phase.

Recovery/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 CBZ Epcx. Recoveries for Gabapentin and Vigabatrin were lower and attributed to their Zwitterionic characteristics.

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AN810

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

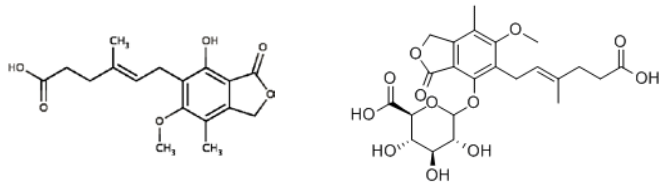

Figure 1. Structure of mycophenolic acid (MPA) and mycophenolic acid glucuronide (MPAG).

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

ISOLUTE® SLE+ Method
Add 90 µL of 20% aqueous formic acid to the samples (100 µL) then gently vortex the solutions.

Load pre-treated samples onto wells. Apply a short pulse of vacuum or positive pressure to initiate flow and then allow sample to absorb on column for 5 minutes.

Apply ethyl acetate (2 x 500 µL) to each well and allow solvent to gravity flow. Apply positive pressure or pull slight vacuum as needed. Evaporate sample and reconstitute in water:acetonitrile (50:50, v/v, 500 µL).

Recovery/Results
Recoveries >70% wih RSDs <10%.

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AN805

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

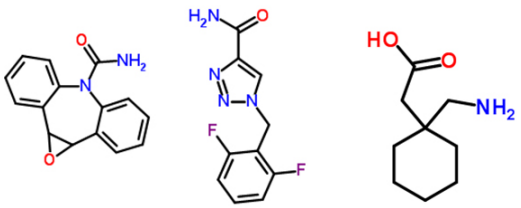

Figure 1. Structures of carbamazepine epoxide, rufinamide and gabapentine.

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

ISOLUTE® SLE+ Method

Sample Preparation for 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 ISOLUTE SLE+ 96-well plate. Apply a short pulse of positive pressure or vacuum and allow samples to sit for 5 minutes.

Elute analytes with methyl tert-butyl ether containing 1% (v/v) trifluoroacetic acid (conc) solution (2 x 700 µL). Allow sample to flow through by gravity and collect eluent. Apply vacuum or positive pressure for 10–20 seconds to remove any remaining extraction solvent.

Sample Preparation for Neutral Antiepileptic and Zwitterionic Drugs in Serum and Urine:
Add 50% aqueous formic acid (100 µL) to the sample (100 µL) Add up to 100 µL of internal standard.

Load up to 300 µL of pre-treated serum sample onto the ISOLUTE SLE+ 96-well plate. Apply a short pulse of positive pressure or vacuum and allow samples to sit for 5 minutes.

Elute analytes with methyl tert-butyl ether containing 1% (v/v) trifluoroacetic acid (conc) solution (2 x 700 µL). Allow sample to flow through by gravity and collect eluent. Apply vacuum or positive pressure for 10–20 seconds to remove any remaining extraction solvent.

Post Extraction
Evaporate to dryness (45 °C for 15 mins) and reconstitute sample in mobile phase.

Recovery/Results
Recoveries >70% with RSD <10%.

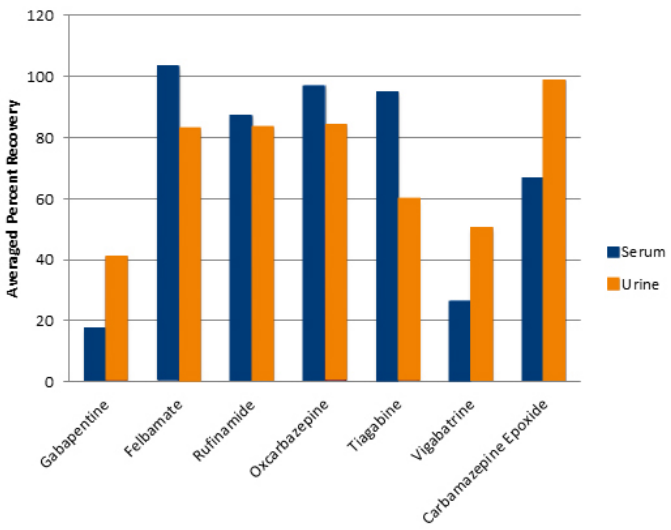

Figure 2. Plot of average recoveries for neutral antiepileptic drugs (n=7) and zwitterionic antiepileptic drugs (n=4) fortified into serum and urine at 20 ng/mL and pretreated with 5 mM ammonium acetate.

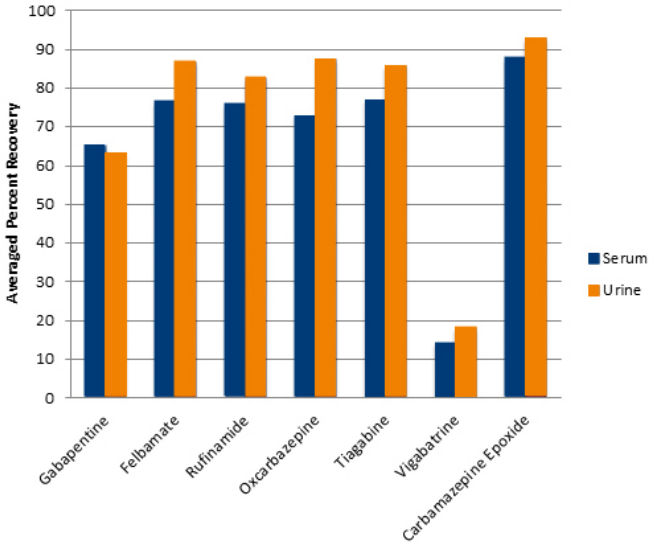

Figure 3. Plot of average recoveries for neutral and zwitterionic antiepileptic drugs (n=7) fortified into serum and urine at 20 ng/mL and pre-treated with 50% aqueous formic acid.

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AN787

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

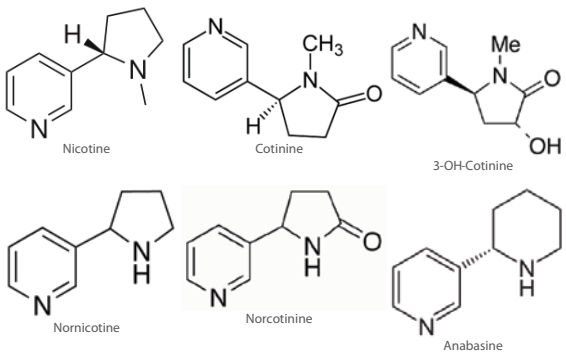

Figure 1. Structures of nicotine and metabolites.

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

ISOLUTE® SLE+ Method
To 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 initiate flow. Allow the sample to absorb for 5 minutes.

Elute with dichloromethane : isopropanol (95: 5, v/v, 1 mL) and allow to flow under gravity into a 2 mL deep well collection plate containing 100 µL methanolic 200 mM HCl in each well. Apply vacuum or positive pressure to elute any remaining extraction solvent.

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

Recovery/Results

Matrix	Recovery (%)	RSD (%)
Urine	787	<8
Plasma	780	<5
Serum	783	<3
Whole Blood	777	<9

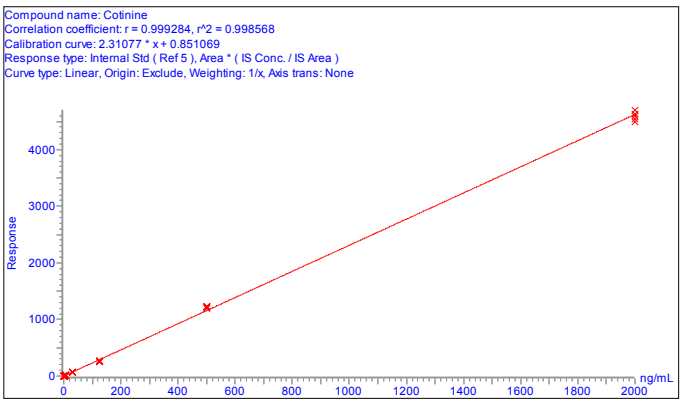

Figure 2. Shows typical linearity data achieved using this method.

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AN778

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

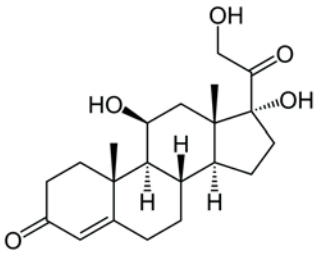

Figure 1. Structure of cortisol.

Analytes
Cortisol, cortisol-d_4

Format
ISOLUTE® SLE+ 200 µL supported liquid extraction plate, part number 820-0200-P01

Matrices
Oral fluid

ISOLUTE® SLE+ 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.

Apply MTBE (1 mL) and allow to flow under gravity for 5 minutes. Apply vacuum or positive pressure to pull through any remaining extraction solvent.

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

Recovery/Results
Recoveries ranged from 96–99% with RSDs below 3% for both cortisol and cortisol D4.

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AN777

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

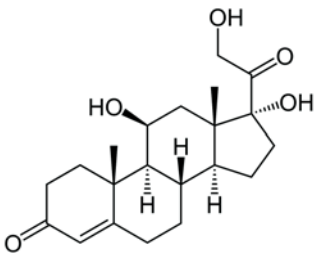

Figure 1. Structure of cortisol.

Analytes
Cortisol, cortisol-d_4

Format
ISOLUTE® SLE+ 200 µL supported liquid extraction plate, part number 820-0200-P01

Matrices
Urine

ISOLUTE® SLE+ 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.

Apply MTBE (1 mL) and allow to flow under gravity for 5 minutes. Apply vacuum or positive pressure to pull through any remaining extraction solvent.

Evaporate the extract to dryness (40 °C). Reconstitute in water:methanol (50:50, v/v) (100 µL).

Recovery/Results
Recoveries ranged from 99–101% with RSDs below 5% for both cortisol and cortisol D4.

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AN762

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

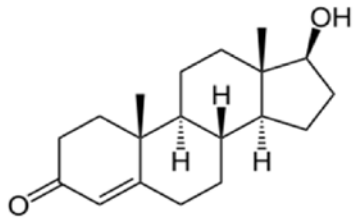

Figure 1. Structure of testosterone.

Analytes
Testosterone, androstenedione

Format
ISOLUTE® SLE+ 400 µL supported liquid extraction plate, part number 820-0400-P01

Matrices
Serum

ISOLUTE® SLE+ Method
Dilute human serum (200 µL) with 0.5 mol/L ammonium hydroxide (200 µL) then mix.

Load pre treated sample (400 µL) to plate followed by a pulse of vacuum to initiate flow and leave for 5 minutes.

Apply 500 µL of diethyl ether and allow to flow under gravity, apply a short pulse of vacuum if solvent not fully absorbed. Apply a second 500 µL of diethyl ether and allow to flow under gravity. Apply a third 500 µL of diethyl ether, allow to flow under gravity and then apply another short pulse of vacuum.

Evaporate the eluate to dryness and reconstitute with 400 µL of methanol:water (1:1, vol:vol).

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.

Recovery/Results

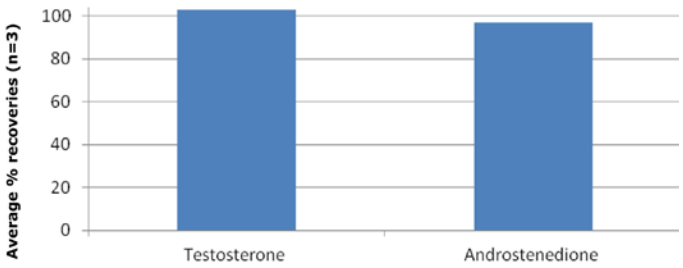

Figure 2. Average analyte recoveries up to 100 nmol/L.

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AN761

Extraction of 1, 25 Dihydroxyvitamin D from Human Serum Using ISOLUTE® SLE+ Prior to LC-MS/MS Analysis

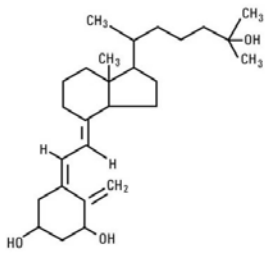

Figure 1. Structure of 1, 25 dihydroxyvitamin D.

Analytes
1α,25-Dihydroxyvitamin D3 (calcitriol), 1α,25-dihydroxyvitamin D2

Format
ISOLUTE® SLE+ 400 µL supported liquid extraction plate, part number 820-0400-P01

Matrices
Serum

ISOLUTE® SLE+ Method
To serum (350 µL), add internal standard solution (10 µL) and vortex mix. Load the pre-treated sample (350 µL) onto the plate and apply a pulse of vacuum or positive pressure) to initiate flow. Allow the sample to adsorb for 5 minutes. Apply ethyl acetate:hexane (50:50, v/v) (900 µL) and allow to flow under gravity for 5 minutes.

Apply a second aliquot of ethyl acetate:hexane (50:50, v/v) (900 µL) and allow to flow under gravity for a further 5 minutes. Apply vacuum or positive pressure to pull through any remaining extraction solvent.

Evaporate the extract to dryness (ambient temperature) . Reconstitute in water:acetonitrile (60:40, v/v) (150 µL) and vortex mix for 60 seconds.

Recovery/Results
Recoveries ranging from 75–78% from extracted serum with RSDs below 10% for both 1,25-dihydroxyvitamin D2 and D3.

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AN760

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

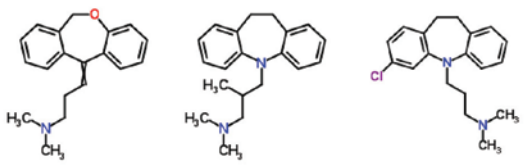

Figure 1. Structures of tricyclic anti-depressant analogues.

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

Format
ISOLUTE® SLE+ 200 µL supported liquid extraction Column, part number 820-0140-C

Matrices
Urine

ISOLUTE® SLE+ Method
Mix urine with concentrated ammonium hydroxide (99:1, v/v) relative to total urine load volume. Add internal standard (optional). Gently vortex solution. Load pre-treated sample (1 mL) onto cartridge. Apply a short pulse of vacuum or positive pressure to initiate flow and then allow sample to sit and adsorb onto column for 5 minutes.

Apply hexane:isopropanol (98:2, v/v) (4mL) to the column. Allow solvent to gravity flow through the column. Apply another aliquot of hexane:isopropanol (98:2, v/v) (4mL) to the column and allow to adsorb. Apply several pulses of positive pressure or vacuum at the end of the collection process to collect final elution solvent.

Evaporate sample to dryness and reconstitute in mobile phase (500 µL).

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

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AN758

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

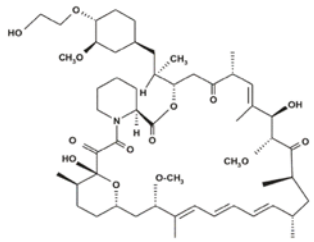

Figure 1. Structure of everolimus.

Analytes
Sirolimus, tacrolimus, everolimus, cyclosporin A

Format
ISOLUTE® SLE+ 400 µL supported liquid extraction plate, part number 820-0400-P01

Matrices
Whole blood

ISOLUTE® SLE+ 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 adsorb for 5 minutes.

Apply ethyl acetate (600 µL) and allow to flow under gravity for 5 minutes. Apply a further aliquot of ethyl acetate (600 µL) and allow to flow for another 5 minutes. Apply vacuum or positive pressure to pull through any remaining extraction solvent.

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

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

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AN757

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

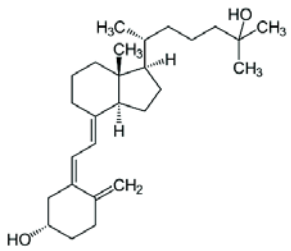

Figure 1. Structure of 25-OH vitamin D3.

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

ISOLUTE® SLE+ Method
Dilute human serum (150 µL) with HPLC grade water:isopropanol (v/v, 50:50) (150 µL) mix, cap and shake for 60 seconds.

Load pre treated serum (300 µL in total) onto the ISOLUTE SLE+ plate followed by a pulse of vacuum to initiate flow, leave the sample to absorb for 5 minutes. Apply heptane (750 µL), wait five minutes to allow the solvent to soak, apply a short pulse of vacuum if solvent not fully adsorbed. Apply a second aliquot of heptane (750 µL), allow to soak for a further 5 minutes and then apply a final pulse of vacuum.

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.

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

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AN754

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

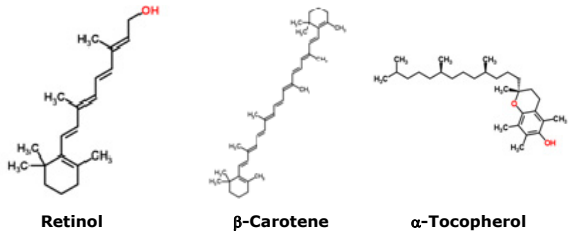

Figure 1. Structures of retinol, β-carotene, α-tocopherol.

Analytes
Retinol, β-carotene, α-tocopherol

Format
ISOLUTE® SLE+ 400 µL supported liquid extraction plate, part number 820-0400-P01

Matrices
Whole blood

ISOLUTE® SLE+ Method
Dilute whole blood (50 µL) with isopropanol (10 µL) and HPLC grade water (70 µL). Spike the mixture with internal standard. Gently vortex solution.

Load pre-treated samples (~130 µL) onto plate. Apply positive pressure or pull slight vacuum to initiate flow and then allow the sample to flow under gravity for 5 minutes.

Apply hexane:isopropanol (v/v, 90:10) (900 µL) to each well, followed by a second application of hexane:isopropanol (v/v, 90:10) (900 µL). Apply positive pressure or pull slight vacuum as needed during collection process. Evaporate sample to dryness and reconstitute in mobile phase (200 µL).

Recovery/Results
The average recovery for each analyte at 200 ng/mL was determined to be >95% RSDs <10%.

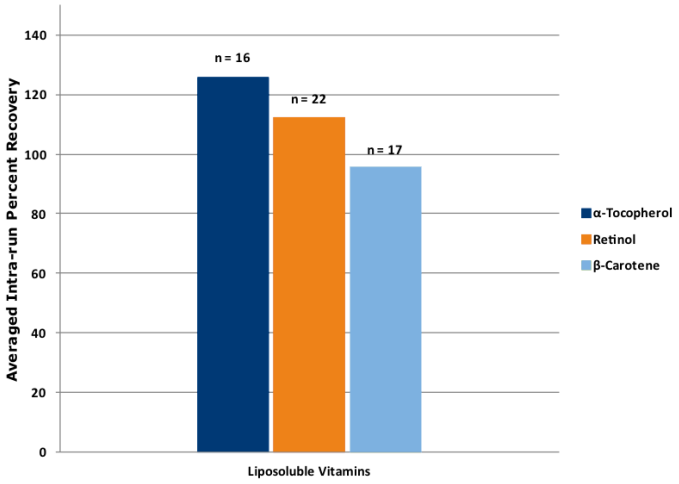

Figure 2. Average percent recoveries for Retinol (112%), α-tocopherol (125%), and β-carotene (96%) from human pooled whole blood spiked at 200 ng/mL. High recoveries are attributed to presence of endogenous vitamins in whole blood.

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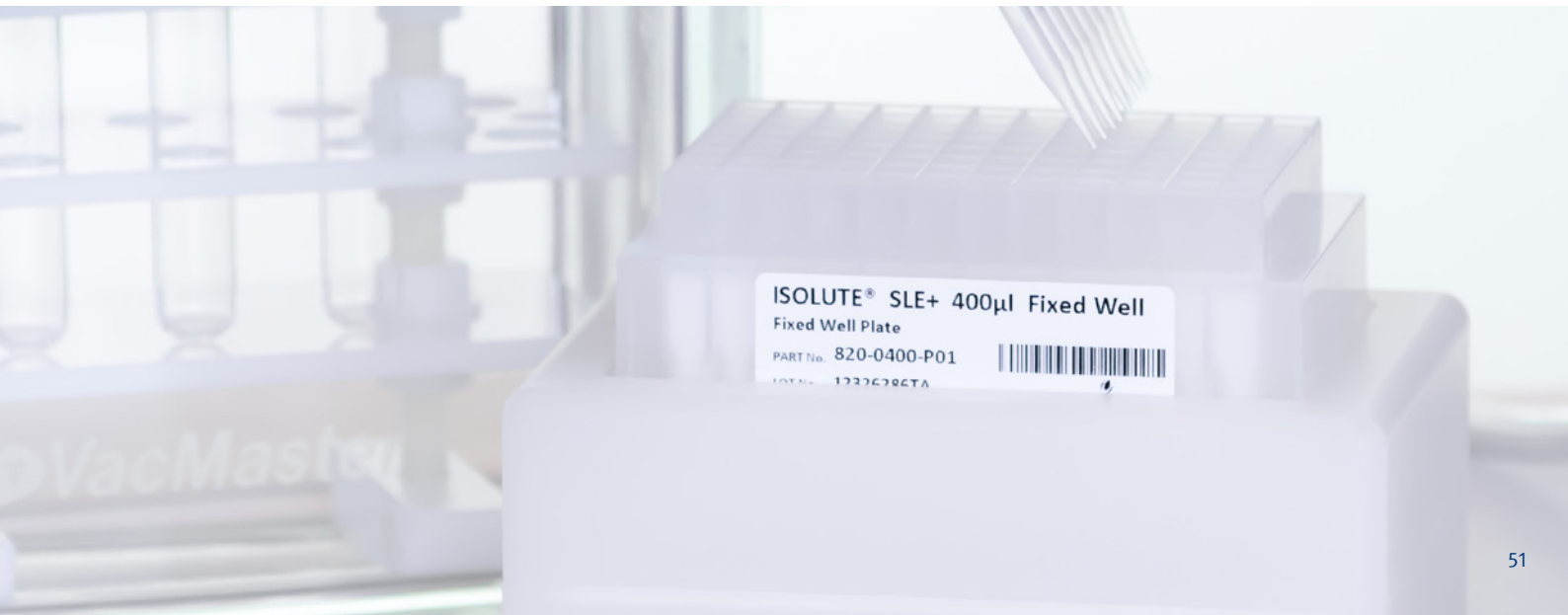

AN753

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

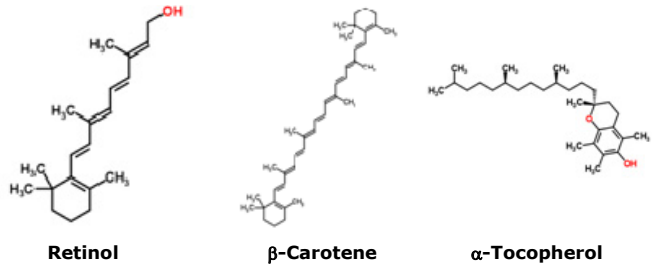

Figure 1. Structure of retinol, β-carotene, α-tocopherol.

Analytes
Retinol, β-carotene, α-tocopherol

Format
ISOLUTE® SLE+ 400 µL supported liquid extraction plate, part number 820-0400-P01

Matrices
Serum

ISOLUTE® SLE+ Method
Dilute human serum (200 µL) with isopropanol (100 µL). Spike the mixture with internal standard. Gently vortex solution.

Load pre-treated samples (~300 µL) onto plate followed by a short pulse of vacuum or positive pressure to initiate flow and allow to flow under gravity for 5 minutes.

Apply hexane:isopropanol (90:10, v/v, 900 µL) to each well, followed by a second application of hexane:isopropanol (90:10 v/v, 900 µL). Apply positive pressure or pull slight vacuum as needed during collection process. Evaporate sample to dryness and reconstitute in mobile phase (300 µL).

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

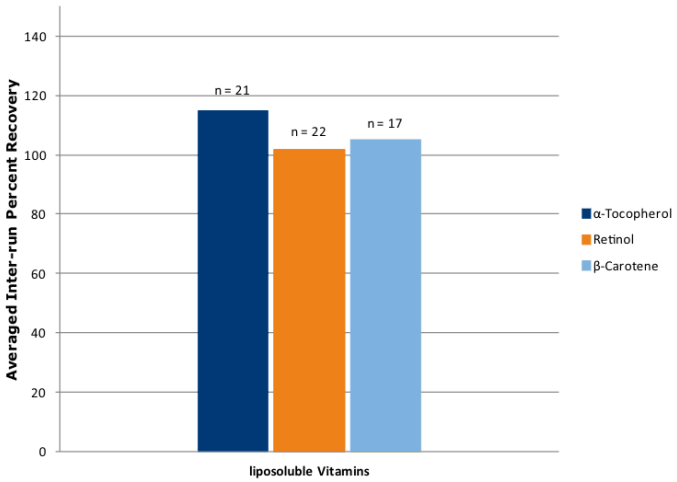

Figure 2. Average recoveries for Retinol (102%), α-tocopherol (115%), and β-carotene (105%) from human pooled serum spiked at 100ng/mL. High recoveries are attributed to presence of endogenous vitamins in serum.

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AN740

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

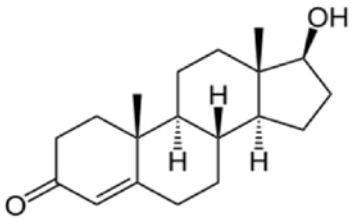

Figure 1. Structure of testosterone.

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

ISOLUTE® SLE+ Method
Dilute human plasma (100 µL) 1:1 with HPLC grade water (100 µL). Load pre-treated sample (200 µL) to plate followed by a pulse of vacuum to initiate flow and leave for five minutes.

Elute with dichloromethane (1 mL) directly in to a collection plate. Leave to flow under gravity for 5 minutes then apply short pulse of vacuum.

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

Recovery/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.

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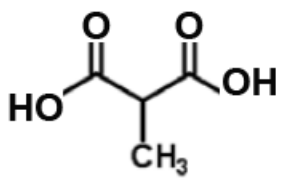
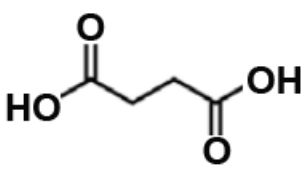
AN738

Extraction of Methylmalonic Acid (MMA) and Succinic Acid (SA) from Human Serum Using ISOLUTE® SLE+ in 96-Well Plates and Columns



Methylmalonic acid (MMA)



Succinic acid (SA)

Analytes
Methylmalonic acid, succinic acid

Format
ISOLUTE® SLE+ 400 µL supported liquid extraction plate, part number 820-0400-P01

Matrices
Serum

ISOLUTE® SLE+ Method
Dilute serum (200 µL) with 4.6 M formic acid (1:1) and allow to stand for 5 minutes.

Load pre-treated sample (400 µL) to plate followed by a short pulse of vacuum to initiate flow and allow sample to absorb for 5 minutes.

Elute with MTBE (1500 µL) allow to flow for 5 minutes then pulse of vacuum).

Evaporate to dryness and reconstitute in 0.4 M formic acid/ methanol (90:10)

Recovery/Results
MMA recoveries >90% with RSD<10%.

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AN734

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

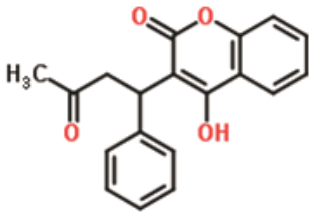

Figure 1. Structure of warfarin.

Analytes
Warfarin

Format
ISOLUTE® SLE+ 200 µL supported liquid extraction plate, part number 820-0200-P01

Matrices
Human plasma

ISOLUTE® SLE+ Method
Dilute 100 µL of plasma 1:1 (v/v) with 1% Formic acid, approximate loading pH ~3.0. Load pre-treated plasma (200 µL) onto the ISOLUTE SLE+ plate, leave the samples to absorb for 5 minutes under gravity and then apply a pulse of vacuum.

Apply 1 mL of dichloromethane, apply a short pulse of vacuum and wait for 5 minutes. minutes.

Recovery/Results
All results show recoveries above 90% with RSDs below 10%, Figure 2. shows an example chromatogram. It should be noted that LOQ values can be improved by concentrating the sample in smaller reconstitution volumes.

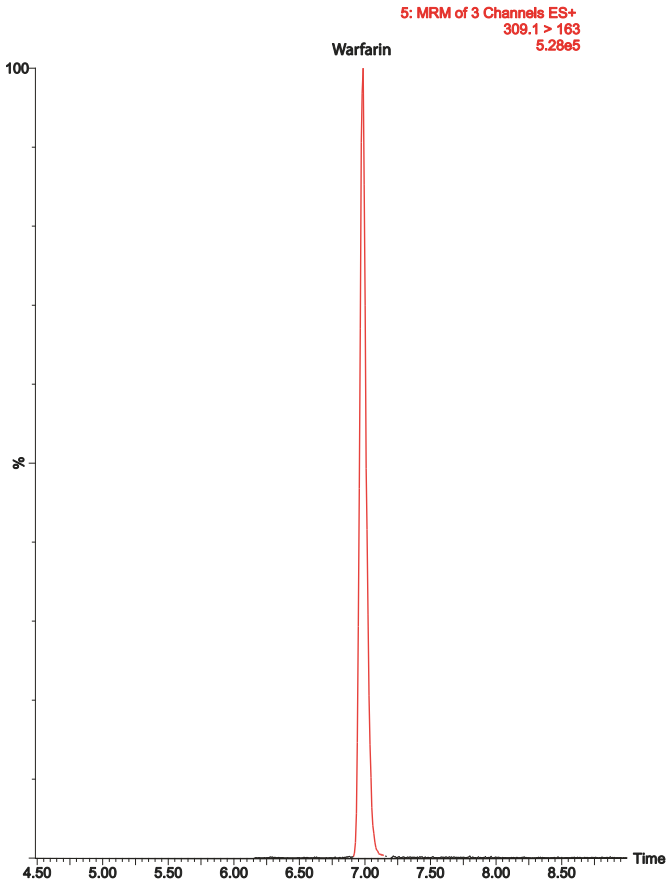

Figure 2. Example chromatogram showing warfarin.

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AN721

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

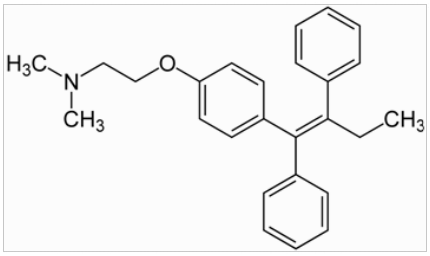

Figure 1. Structure of tamoxifen.

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

ISOLUTE® SLE+ Method
Dilute 100 µL of urine 1:1 (v/v) with 0.5 M NH_4OH. Load pre-treated urine (200 µL) onto the plate and apply a pulse of vacuum to initiate flow. Leave the samples to absorb for 5 minutes.

Apply 500 µL ethyl acetate, apply a short pulse of vacuum and wait for 5 minutes.

Apply a second aliquot 500 µL ethyl acetate, apply a short pulse of vacuum and wait for 5:00 min.

The eluate was evaporated to dryness and reconstituted in 500 µL of 0.1% formic acid 50:50 (v/v) H_2O/MeOH prior to analysis.

Recovery/Results
All results show recoveries above 80% with %RSDs below 10%.

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AN603

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

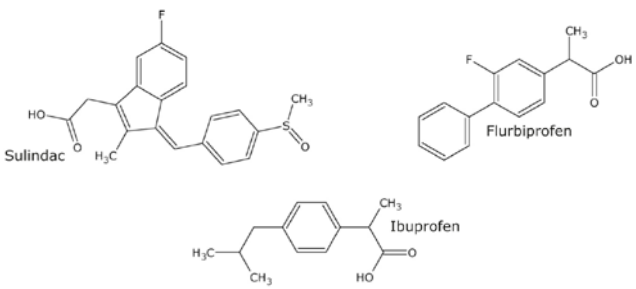

Figure 1. Structures of sulindac, flurbiprofen and ibuprofen.

Analytes
Sulindac, flurbiprofen, ibuprofen

Format
ISOLUTE® SLE+ 400 µL supported liquid extraction plate, part number 820-0400-P01

Matrices
Plasma

ISOLUTE® SLE+ Method
The plasma was diluted 1:1 v/v with 1% formic acid and mixed. The pre-treated plasma was loaded onto the plate, a pulse of vacuum (5–15 seconds) was applied to initiate flow and then the samples were left to absorb for 5 minutes.

Apply two aliquots of MTBE (900 µL). Allow the solvent to flow for 5 minutes under gravity. Apply vacuum for 2 minutes after the second aliquot to complete elution.

The extracts were evaporated to dryness and the analytes reconstituted in 500 µL 60:40 (v/v) H_2O/MeOH prior to analysis.

Recovery/Results

Table 1. NSAID recoveries.

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

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AN602

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

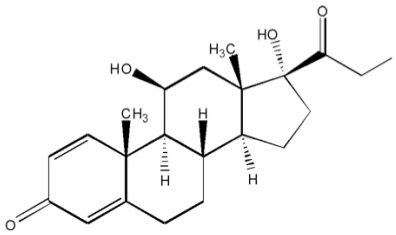

Figure 1. Structure of prednisone.

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

ISOLUTE® SLE+ Method

The sample was diluted 1:1 v/v with water and mixed. The pre-treated plasma was loaded onto the plate, a pulse of vacuum applied to initiate flow and the samples left to absorb for 5 minutes.

Elution: Ethyl acetate (1 x 1 mL) with solvent to flow for 5 minutes under gravity. Apply vacuum (-15 “Hg / -0.5 bar) for 2 minutes to complete elution.

The extracts were evaporated to dryness and the analytes reconstituted in 500 µL of 80:20 (v/v) H_2O/MeOH prior to analysis.

Recovery/Results

Analyte	Recovery	RSDs
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

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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

ISOLUTE® SLE+ Method
The sample (100 µL) was diluted 1:1 with 0.5 M NH_4OH. Load the pre-treated sample onto the plate and apply a pulse of vacuum or positive pressure to initiate flow. Leave the samples to absorb for 5 minutes.

Apply the extraction solvent (1 mL hexane: 3-methyl-1-butanol 98:2 v/v). Allow the solvent to flow for 5 minutes under gravity. Apply vacuum or positive pressure to pull through any remaining elution solvent.

Evaporate to dryness and reconstitute in mobile phase (H_2O:ACN:NH_4OH 10:2:90:0:1, v/v).

Recovery/Results
Analyte recoveries were >91% with RDS %<4 for all analytes using ISOLUTE SLE+.

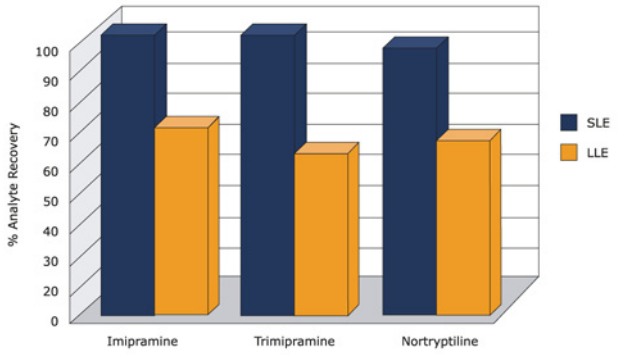

Figure 1. Comparison of analyte recovery using ISOLUTE® SLE+ and LLE.

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