MilliporeSigma

DRUGS OF ABUSE

Application compilation with focus on the LC-MS analysis of drugs of abuse in biological samples

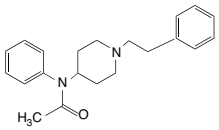
Supelco®
Analytical Products

Table of Contents

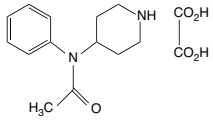
Compound Index

This application compilation highlights different analytical solutions and test methods for several compounds and compound groups, listed and described here:

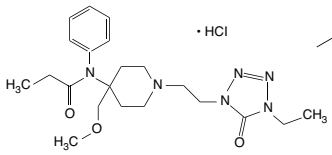
Opioids



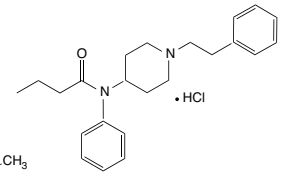
Acetyl fentanyl



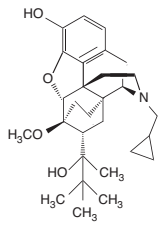
Acetyln orfentanyl oxalate



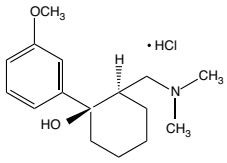
Alfentanil hydrochloride



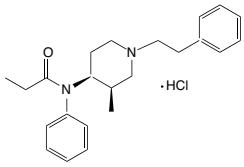
Butyryl fentanyl hydrochloride



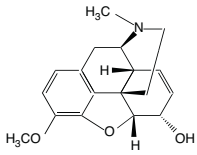
Buprenorphine



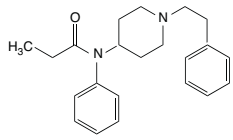
cis-Tramadol hydrochloride



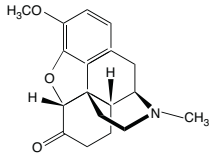
cis-3-methyl Fentanyl hydrochloride



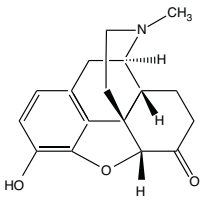
Codeine



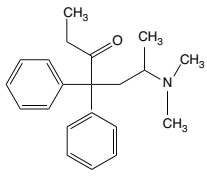
Fentanyl



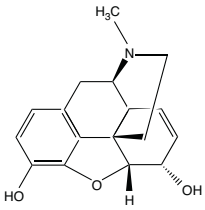
Hydrocodone



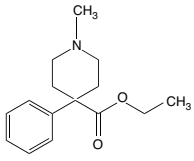
Hydromorphone



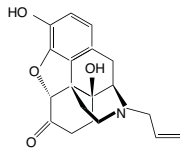
(±)-Methadone



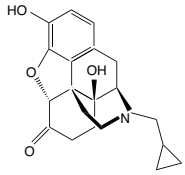
Morphine



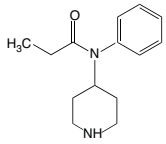
Meperidine



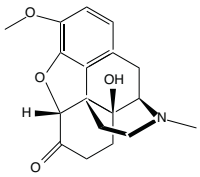
Naloxone



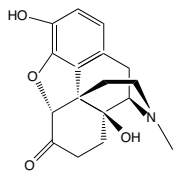
Naltrexone



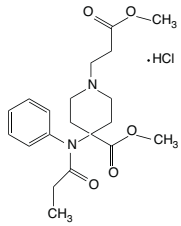
Norfentanyl



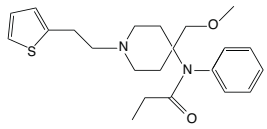
Oxycodone



Oxymorphone

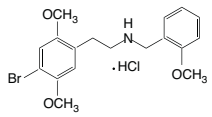


Remifentanil hydrochloride

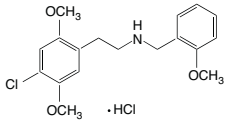


Sufentanil

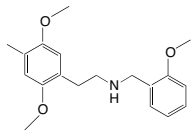
Designer Drugs and Synthetic Hallucinogens



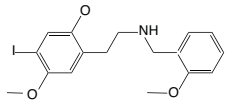
25B-NBOMe hydrochloride



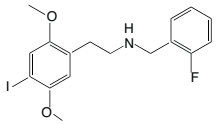
25C-NBOMe hydrochloride



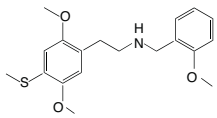
25D-NBOMe



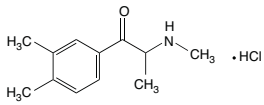
25I-NBOMe



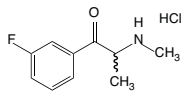
25I-NBF



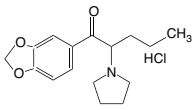
25T-NBOMe



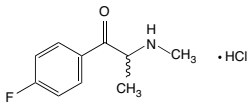
3,4-Dimethylmethcathinone hydrochloride



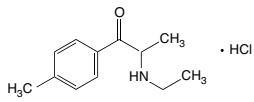
3-Fluoromethcathinone hydrochloride



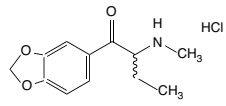
3,4-Methylenedioxypyrovalerone HCl (MDPV)



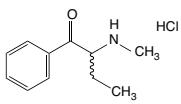
4-Fluoromethcathinone hydrochloride



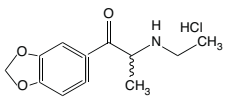
4-Methylmethcathinone hydrochloride



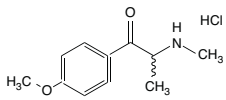
Butylone hydrochloride



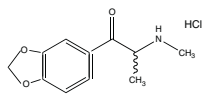
Buphedrone hydrochloride



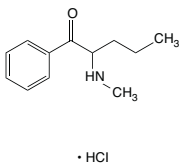
Ethylone hydrochloride



Methedrone hydrochloride

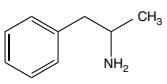


Methylone hydrochloride

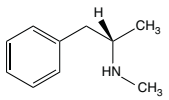


Pentedrone hydrochloride

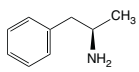
Amphetamine



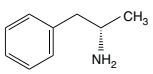
Amphetamine



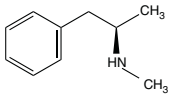
Methylamphetamine



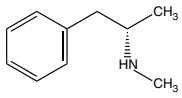
R(-)-Amphetamine



S(+)-Amphetamine

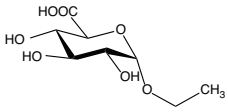


R(-)-Methamphetamine

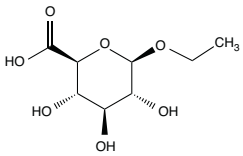


S(+)-Methamphetamine

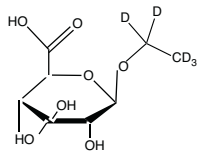
Alcohol



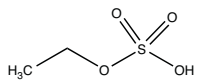
Ethyl glucuronide (EtG)



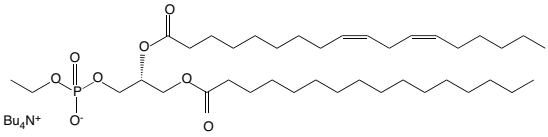
Ethyl-β-D-glucuronide



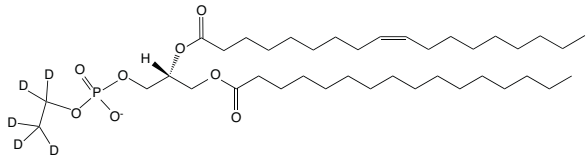
Ethyl-β-D-glucuronide-(ethyl-d5)



Ethyl sulfate (EtS)

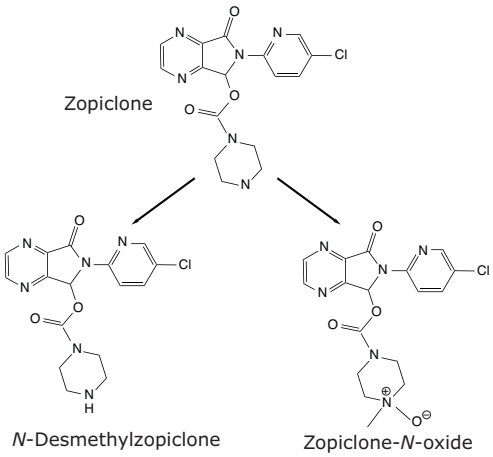


PLPEth



POPEth

Z-drugs



introduction

Alcohols, amphetamines, barbiturates, benzodiazepines, boosters, cannabis or cannabinoids, cathinones (bath salts), cocaine, hallucinogens, kratom, methaqualone, opioids, steroids, Z-compounds, etc. are typically referred to as drugs of abuse.

In most countries, these compound groups are viewed as controlled substances being harmful intoxicants, and for that purpose they require monitoring.

Europe

The European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) is the reference point on drugs in Europe. The purpose of EMCDDA is to provide the EU and its member states, with an accurate status of European drug problems. It provides the data required for drug laws and strategies. EMCDDA collaborates with the EU's different institutions, the Reitox network, candidate and potential candidates to the EU, European Neighbourhood Policy (ENP) area countries, and regional and international organisations.

Through the Reitox network, results from national monitoring programs are collated into different reports such as the **European Drug Report**, and other outputs.

Through the new Security Union Strategy 2020-2024, the Commission aims to increase the EU efforts on tackling illicit drugs, and the new **EU Agenda and Action Plan on Drugs 2021-2025** can provide insight to both political framework and priorities for the next five years. This framework is structured in two main areas: drug demand reduction and drug supply reduction. This policy has three themes: (a) coordination, (b) international cooperation, and (c) information, research, monitoring, and evaluation.

https://www.emcdda.europa.eu/

United States

The Food and Drug Administration (FDA):

- regulates drugs of abuse tests sold to consumers or healthcare professionals
- reviews many of the tests before they are sold for use.

FDA assesses the design and performance of tests and sample collection systems and reviews the test instructions and package inserts. The FDA does not review drugs of abuse tests intended for employment and insurance testing.

https://www.fda.gov/device-advice-comprehensive-regulatory-assistance

https://www.fda.gov/medical-devices/ivd-regulatory-assistance/overview-ivd-regulation

International

The United Nations Office on Drugs and Crime (**UNODC**);

International Narcotics Control Board (**INCB**);

The World Health Organisation (**WHO**);

The Council of Europe and World Customs Organisation (**WCO**).

Monitoring – Drugs of Abuse

Analytical test methods fit for defined purposes are required for accurate quantification of drugs and/or endogenous substances in different biological samples to provide qualitative and quantitative measures of the active drug and/or its metabolite(s).

Bioanalytical chemistry and/or bioanalysis is a discipline aiming at quantitative measurement of drugs and their metabolites, biological and biotic compounds (macromolecules, proteins, DNA, large molecule drugs, metabolites) in biological samples. The term bioanalysis traditionally refers to the measurement of small molecules in biological fluids but over the past two decades this discipline has expanded substantially because of the increased interest in biopharmaceuticals (e.g., proteins and peptides).

Bioanalytical Organisations

There are several national and international bioanalytical organizations active in pharmaceuticals sciences in general and/or bioanalytical chemistry, for example:

AAPS - American Association of Pharmaceutical Scientists

BSAT/APA - Applied Pharmaceutical Analysis

CVG - Canadian LC-MS Group

EIP - European Immunogenicity Platform

EUFEPS - European Federation for Pharmaceutical Scientists

FABIAN (Dutch Bioanalytical Society)

GBC - Global Bioanalysis Consortium

JBF - Japan Bioanalysis Forum

Trends

LC-MS/MS has become the gold standard for confirmatory drug testing, and LC-TOF-MS is used for comprehensive drug screening.

Matrices such as saliva, sweat, hair, and meconium are becoming increasingly more interesting as specimen for drug testing, but urine is still by far the most common and what will be the focus on the following pages.

Characterization and identification of isomeric drug metabolites have become increasingly more important. This technique demands more efficient and relevant separation modes, including chiral recognition for enantiomeric separation purposes.

Miniaturization in both separation and detection for rapid and sensitive drugs of abuse testing gets increasing attention.

Analytical needs

Choosing the best analytical technique for the purpose, with the following method development or method modification, is less difficult when a literature reference can be found for the same or similar needs.

Official test methods are published through different organizations, via databases, and as individual peer-reviewed scientific studies. This knowledge base may provide a good reference point.

Method development typically starts at the desk and not in the laboratory. Method development means to define needs, set goals, make experimental plans, and then to carrying out the practical work. Finally, the new method needs to be verified, validated, and put into routine work.

A few useful questions:

- Is the primary goal quantitative or qualitative analysis?
- If quantitative analysis is required, what levels of accuracy and precision are needed?
- Are analytical standards and certified reference materials available?
- Do you need to perform detection of one or many analytes?
- Is it necessary to identify or resolve all sample components?
- How many different sample matrices would be of interest? Only urine?
- How many samples will be analysed per day
- What will be the total throughput/year?

Define the method goal and the requirements of the new method. Do you really need high resolution, short analysis time, maximum sensitivity? True optimization of a method is a balance between selectivity, speed, and efficiency. Ideally, the development should result in a robust method that provides an acceptable, overall price-per-analysis and ultimately a cost-efficient assay.

Common mistakes in method development are inadequate formulation of method goals, insufficient knowledge of chemistry, trial and error, and use of wrong instrument set-up. These mistakes often result in laborious, time-consuming projects that lead to methods that fail to meet the needs of the laboratory. After defining the goal of the method development, specific information on the sample and the analytes should be sought.

Listed are some of the most common parameters

- nature of the sample (urine/plasma/serum/whole blood, etc.)
- number of compounds/analytes present
- chemical structure (functionality)
- analyte molecular weight, pKa values
- Log P and/or Log D values (hydrophilicity/ hydrophobicity)
- expected concentration ranges
- sample matrix
- sample solubility

Think about the sample as being the central part during all steps. When selecting the most suitable approach, consider sample solubility, how the analytes of interest differ from other compounds, or the sample matrix in the sample.

The analyte perspective

Substances being classified as endogenous are originating from an organism, tissue, or cell. Many organisms also produce small molecule secondary metabolites, biologically active compounds.

That said, most drugs however are synthetic, exogenous small molecules, although some drugs can be proteins, e.g. insulin. Active Pharmaceutical Ingredients (APIs) have traditionally followed the Lipinski rule of five and been relatively hydrophobic molecules. The Lipinski rule states that a compound is more likely to be membrane-permeable and easily absorbed by the body if:

- Its molecular weight is less than 500.
- The compound's lipophilicity, expressed as a quantity known as logP (the logarithm of the partition coefficient between water and 1-octanol), is < 5.
- The number of groups in the molecule able to donate hydrogen atoms to hydrogen bonds (usually the sum of hydroxyl and amine groups in a drug molecule) is < 5.
- The number of groups that can accept hydrogen atoms to form hydrogen bonds (estimated by the sum of oxygen and nitrogen atoms) is < 10.

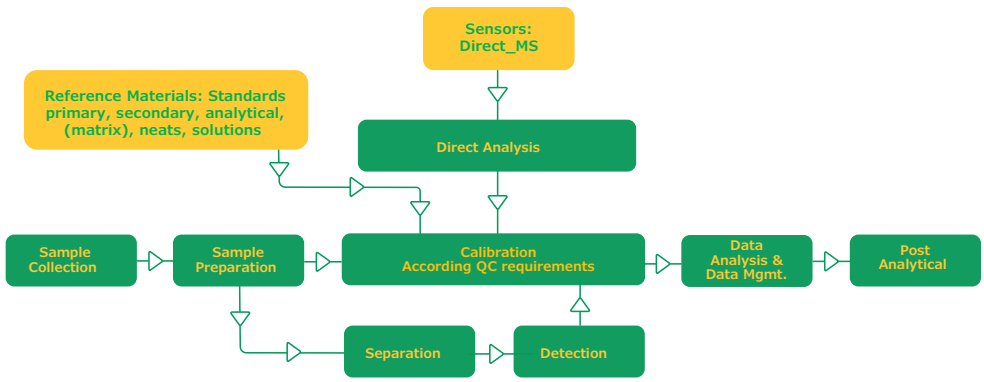
The rules apply only to absorption by passive diffusion of compounds through cell membranes; compounds that are actively transported through cell membranes by transporter proteins are exceptions to the rule. Small molecules are more likely to be absorbed, although some of them are only absorbed after oral administration if given as prodrugs. One advantage small molecule drugs (SMDs) have over "large molecule" biologics is that many SMDs can be taken orally whereas biological drugs generally require injection or another type of administration.

Reversed phase HPLC has been the backbone of pharmaceutical analysis for over 25 years, and the technique has been particularly successful for APIs.

Roughly 10% of marketed drugs do not however follow Lipinski's rule, i.e. many chemotherapeutic agents (anti-cancer drugs) since they are highly hydrophilic.

The analytical Workflow

- Sampling and preservation
- Fortification (if applicable)
- Sample preparation; enrichment (if applicable) or sample purification through protein crash (if applicable)
- Sample adjustments (if applicable)
- Addition of internal standards (calibration protocols, dilution procedures; SOP)
- Chemical analysis
- Data Analysis
- Post Analytical (validation protocol; method transfer protocol)



Biological Samples (Matrix)

The liquid part of blood, devoid of cells and platelets, is termed either plasma or serum depending on how the sample has been prepared.

Since some confusion seems to exist in the literature, a definition could be appropriate. Blood plasma is the liquid portion remaining after the cellular components have been removed from the blood by centrifugation.

An anticoagulant must be added to prevent the blood from clotting before the separation takes place, and the kind of anticoagulant used may be important to know for the analyst. Anticoagulants usually interfere with the clotting process by binding calcium ions, and examples of anticoagulants are sodium citrate, EDTA, potassium oxalate, and heparin. If no anticoagulant is added, clotting will start within minutes and the fibrin clot formed will contain within it the cellular components of the blood. The liquid remaining when the clot is removed is blood serum, and is equivalent to blood plasma, except that it lacks the plasma components that have taken part in the clotting process, mainly the protein fibrinogen.

Tissue is defined as a group or layer of similar cells united to perform specific functions.

Urine is the excrementitious fluid secreted from the blood by the kidneys, passed through the ureters, stored in the bladder, and discharged through the urethra, wherefore a wide variety of metabolic products in both conjugated and unconjugated form are present. Drug of abuse testing is typically carried out with urine samples.

Sample Storage

Plasma, serum, and tissue samples are comparatively stable wherefore no special precautions must be taken, and samples are normally stored in plastic containers at temperatures down to –20 °C. Urine is perhaps the most complicated biological matrix available and will normally need the addition of preservatives to prevent bacterial degradation.

Sample Preparation

Different approaches can be used for sample pre-treatment and purification. A common feature is that loss of analyte during the work-up procedure is inevitable and the actual analyte recoveries must be determined while developing a new method, or otherwise the data produced by the method will be questionable.

The most common practice for determining the analyte recovery is internal standard (I.S.) addition, where the I.S. should be added to the matrix at the start of the analysis. When working with biological matrices one important criterion for the I.S. is that the binding strength of the analytes and the I.S. to proteins and other components of the matrix should be similar.

The I.S. should also have physiochemical properties that are comparable to the analyte and thus behave like the analyte during sample pretreatment (i.e., extraction and purification), yet the properties must be sufficiently unique to allow the I.S. to be clearly discerned from the analyte in the quantitation step. When using LC-MS or LC-MS/MS, isotope dilution is the preferred route for determining analyte recoveries. Deuterated analytes are the ideal internal standards, whose identical chemical properties make them behave exactly as the analytes, but the mass difference makes them easily identifiable in the quantitation procedure.

Liquid-Liquid Extraction (LLE)

LLE was the predominant technique for extraction of molecules of interest from biological matrices in the early ages of small molecule bioanalysis, but its popularity has declined as other, more efficient, techniques have emerged. If LLE is contemplated as a sample pre-treatment alternative, the polarity of the analytes and their ability to bind to proteins are important aspects when selecting solvents for the extraction procedure.

Thus, to quantitatively extract the analyte(s), the chosen solvent should not only dissolve the compounds of interest completely but also be capable of breaking associations to proteins. These requirements are seldom satisfactorily met in practice and low extraction efficiencies are therefore often seen. Another obstacle is that LLE is an inherently non-selective procedure whereby lipids are likely to be co-extracted and may well cause interferences in the ensuing operations. Co-extracted lipids can be removed to some extent, but when LLE is used, the user should expect a significant presence of lipids, even in a purified sample.

The limited compatibility of solvents with plastic vessels is another problem, which makes glass one of the few extraction reservoir materials possible. But since certain groups of molecules tend to bind to glass surfaces it is recommended that all glassware should be silanized prior to use or to work with plastic material. Another problem often associated with LLE is the necessity of using large amounts of toxic and/or flammable solvents, leading to complications with handling and waste disposal. Despite all drawbacks, LLE will continue to be chosen for sample purification in the future.

Protein Precipitation/Protein Crash

The addition of solvent, acids, bases, salts or mixtures thereof to a biological sample will stimulate the precipitation of proteins. This method is an efficient approach in cleaning up samples and is commonly referred to as protein crash. An optimized protocol can remove up to 95% of all proteins in a sample. This method is a “quick-and-dirty” approach wherefore other matrix components, lipids, and minerals, will remain and could be potential causes of column problems (reducing column lifetime, selectivity shifts), ion-suppression/ion-enhancement and thereby lead to inconsistencies or inaccuracies in the detection and quantitation of analytes of interest. When using protein precipitation, the underlying mechanism is to alter the solvation potential of the solvent by lowering the solubility of the solute by the addition of another reagent. The solubility of proteins depends on the distribution of hydrophilic and hydrophobic amino acid

residues on the protein's surface. Proteins that have high hydrophobic amino acid content on the surface have low solubility in an aqueous solvent. Charged and polar surface residues interact with ionic groups in the solvent and increase the solubility of a protein. The addition of miscible solvents such as methanol to a solution may cause proteins in the solution to precipitate. Miscible organic solvents decrease the dielectric constant of water, which in effect allows two proteins to come close together.

Solid Phase Extraction

The use of SPE is today considered as one of the more common techniques for sample work-up. SPE offers several benefits over to LLE, such as improved recoveries, less solvent consumption, smaller sample volumes, and increased sample throughput.

SPE is relatively easy to use, and the technique can be incorporated in dedicated, fully automated sample preparation systems of various formats. Another important advantage with SPE is the wide variety of sorbents commercially available with a variety of functionalities that allow users to choose from different modes of interaction when performing sample purification.

The development of new sorbents in various formats (i.e., discs, syringes, membranes, etc.) is still of interest as the number of analyses demanding highly selective and efficient sample work-up increases. When implementing SPE into an analytical method some simple parameters should be considered:

Sample characteristics: Analyte pK_a, molecular weight, polarity, matrix, interferences, etc.

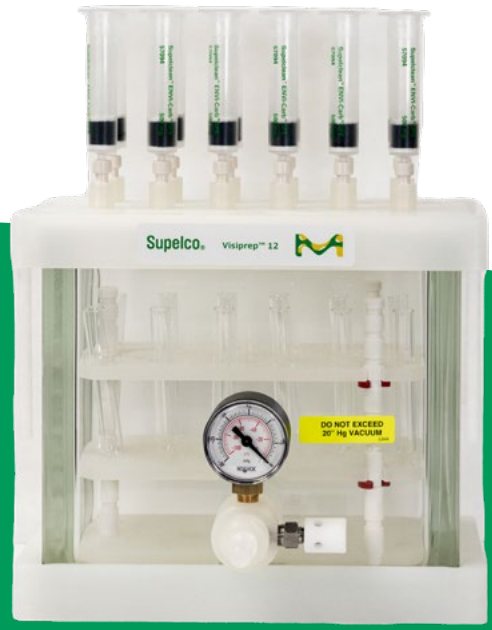
Sorbent selection: Reversed phase, normal phase, ion exchange, adsorption, etc.

Solvent selection: Prepare elution profiles for different solvents.

Method development can thereafter be carried out according to these four steps:

- Conditioning of the solid phase bed.
- Application of the sample.
- Sample pretreatment (multiple steps) to remove loosely bound matrix constituents.
- Elution of the analyte, preferentially with a minimal amount of matrix compounds co-eluting

When a suitable procedure has been established for the compounds of interest, the breakthrough volume of the sorbent should be examined. Knowing the maximum extractable sample amount, both volumetrically and gravimetrically, linearity, repeatability, reproducibility, and recoveries may be determined in order to validate the work-up procedure.



Opioids

Opioids refer to drugs derived from opium, including morphine. Other opioids are semi-synthetic and synthetic drugs such as hydrocodone, oxycodone, and fentanyl, antagonist drugs such as naloxone and endogenous peptides such as the endorphins.

Opiates, and their derivatives are very potent analgesics. Commonly used as therapeutic agents, some of these compounds are also frequently abused as illicit drugs.

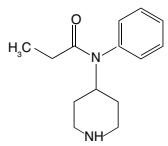
To quantitate opiates and their derivatives, high-performance liquid chromatography (HPLC) has become the preferred technique in most applications. Lately, laboratories are confronted with the continuously increasing demand for higher sample throughput, thus shorter analysis time. A solution was given with the development of Fused-Core® and UHPLC columns. In comparison with the more traditional HPLC columns, the unique feature of the proposed column type is a combination of practical characteristics such as reduced column length, large internal diameter, smaller silica particle size, and higher separation efficiency. On the other hand, for these columns, higher mobile phase flow rates are not sacrificing the improved column efficiency.

- LC-MS Analysis of Fentanyl and Related Compounds in Urine on Ascentis® Express Biphenyl
- LC-MS/MS Analysis of Fentanyl and Related Analogs Using Biocompatible Solid Phase Microextraction
- UHPLC-MS/MS Analysis of Fentanyl and Fentanyl Analogs
- LC-MS Analysis of Pain Management Opioids on Ascentis® Express Phenyl-Hexyl
- UHPLC-MS/MS Determination of Tramadol in Urine
- UHPLC-MS/MS Determination of buprenorphine and norbuprenorphine in Urine
- Analysis of Drugs of Abuse in Urine After Cleanup with Supel™ Swift HLB Solid Phase Extraction 96-well Plates
- DART-MS Analysis of Drugs of Abuse in Human Urine Using C18 SPME LC (SPE-it) Tips

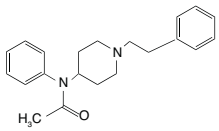
LC-MS Analysis of Fentanyl and Related Compounds in Urine on Ascentis® Express Biphenyl

Fentanyl is a controlled substance and has been categorized as a Schedule II drug under the "Controlled Substance Act" in the United States. Fentanyl and related compounds are synthetic opioids that are at least 100 times more potent than morphine. These main therapeutic applications are intravenous or intramuscular analgesia and sedation and have been widely used for neuroleptic analgesia and surgical anesthesia at doses ranging from 2 – 50 g/mL.

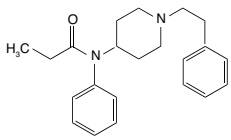
However, the past five years have seen a significant increase in the trafficking and usage of synthetic opioids with a preference for fentanyl. Due to the highly addictive nature of fentanyl and its analogues, several communities worldwide are experiencing an epidemic of opioid-induced overdoses, criminal activity, and lost productivity. In addition to abuse of prescribed fentanyl and other opioids, many "underground" drug laboratories are synthesizing illicit analogues of fentanyl, such as acetyl fentanyl and butyryl fentanyl, which have been designed to evade screening and prosecution by drug enforcement agencies. As the number of opioid drugs and deaths increases, there is a growing need for analytical methods to quickly and accurately determine the concentrations of these drugs in biological samples.



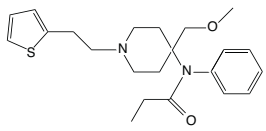
Norfentanyl



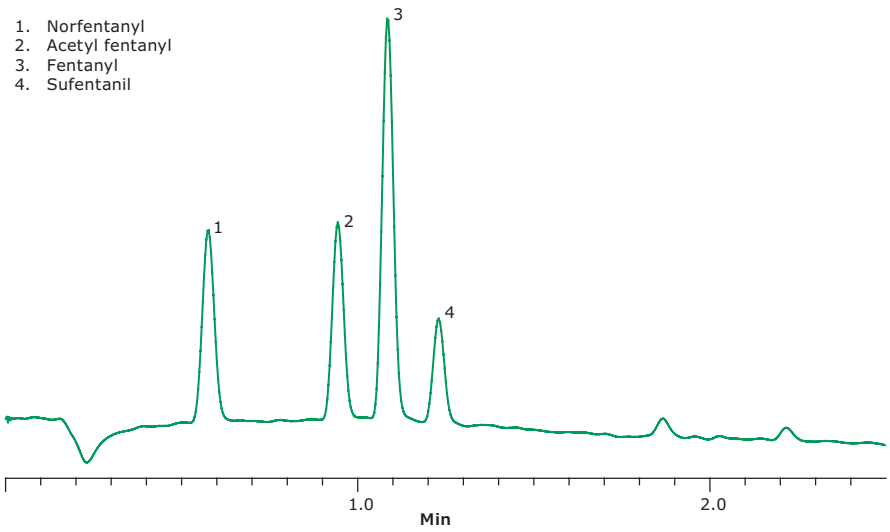
Acetyl fentanyl



Fentanyl



Sufentanil



Chromatographic conditions

Column:	Ascentis® Express Biphenyl 5 cm x 2.1mm I.D., 2.7 µm particles (64057-U)
Mobile phase:	[A] 0.1% formic acid in water; [B] 0.1% formic acid in methanol
Gradient:	40 to 90% B in 1.5 min; held at 90% B for 1 min
Flow rate:	0.6 mL/min
Column temp.:	30 °C
Pressure:	4206 psi (290 bar)
Injection:	0.5 µL
Detector:	MS, ESI(+), SIM, m/z 233, 323, 337, 387
Sample prep:	synthetic urine spiked with norfentanyl (800 ng/mL), acetyl fentanyl (400 ng/mL), fentanyl (800 ng/mL), sufentanil(800 ng/mL), then diluted 1 to 9 in water

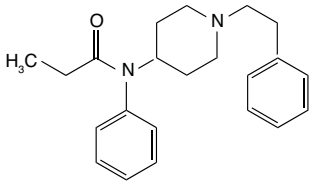
Ascentis® Express Biphenyl

Material list	Cat. No.
Ascentis® Express Biphenyl Column 2.7 µm particle size, L × I.D. 5 cm × 2.1 mm	64057-U
Acetonitrile hypergrade for LC-MS LiChrosolv®	100029
Ethanol gradient grade for liquid chromatography LiChrosolv®	111727
Methanol hypergrade for LC-MS LiChrosolv®	106035
Methanol gradient grade for liquid chromatography LiChrosolv® Reag. Ph Eur	106007
Water for chromatography (LC-MS Grade) LiChrosolv®	115333
Ultrapure water from Milli-Q® IQ 7 series water purification system	ZIQ7005T0C
Ammonium acetate for mass spectrometry, LiChropur™, eluent additive for LC-MS	73594
Ammonium formate eluent additive for LC-MS, LiChropur™, ≥99.0%	70221
Formic acid 98% - 100% for LC-MS LiChropur™	533002
Potassium dihydrogen phosphate anhydrous for HPLC LiChropur™	543841
Sufentanil citrate ≥98% (HPLC)	SML0535
Triethylammonium acetate buffer for HPLC, 0.98-1.02 M	69372
Acetic acid 100% for LC-MS LiChropur™	533001
Acetyl fentanyl solution 1.0 mg/mL in methanol, ampule of 1 mL, certified reference material, Cerilliant®	A-109
Acetyl fentanyl solution 50 µg/mL in methanol, certified reference material, ampule of 1 mL, Cerilliant®	A-129
Fentanyl United States Pharmacopeia (USP) Reference Standard	1269902
Fentanyl solution 1.0 mg/mL in methanol, ampule of 1 mL, certified reference material, Cerilliant®	F-013
Fentanyl solution 100 µg/mL in methanol, ampule of 1 mL, certified reference material, Cerilliant®	F-002
Fentanyl Related Compound G United States Pharmacopeia (USP) Reference Standard	1269979
Norfentanyl oxalate solution 1.0 mg/mL in methanol (as free base), ampule of 1 mL, certified reference material, Cerilliant®	N-031
Sufentanil citrate United States Pharmacopeia (USP) Reference Standard	1623648
Sufentanil Citrate solution 100 µg/mL in methanol (as free base), ampule of 1 mL, certified reference material, Cerilliant®	S-008

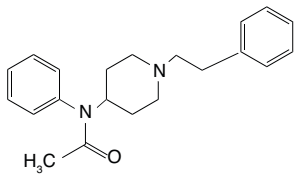
LC-MS/MS Analysis of Fentanyl and Related Analogs Using Biocompatible Solid Phase Microextraction

In this study, fentanyl and related analogues were extracted from urine using a mixed mode SPME LC fiber (research device) and subsequently analyzed using an Ascentis® Express Biphenyl column.

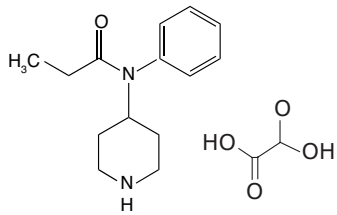
Examination of the structures of these compounds reveals that all these compounds have several sets of delocalized pi electrons either through the benzene ring or centered around the amide functional group. The Ascentis® Express Biphenyl column incorporates ligands with biphenyl moieties which are also rich in pi electrons. Therefore, pi-pi stacking can occur between the analytes and the stationary phase, enabling unique interaction between the compounds and the stationary phase. In addition, the planar structure of the biphenyl ligand enables the column to discriminate structurally similar analytes, allowing for increased resolution of structurally similar compounds.



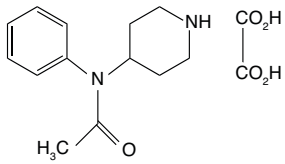
Fentanyl



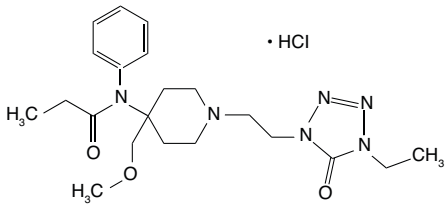
Acetyl fentanyl



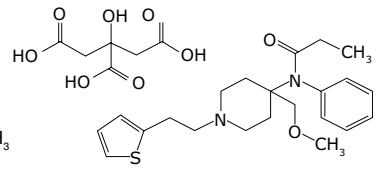
Norfentanyl Oxalate



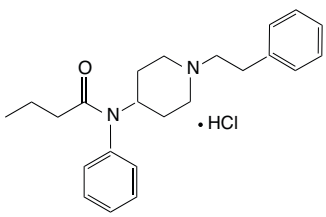
Acetyln norfentanyl oxalate



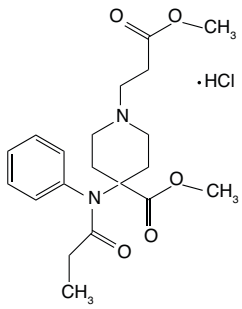
Alfentanil hydrochloride



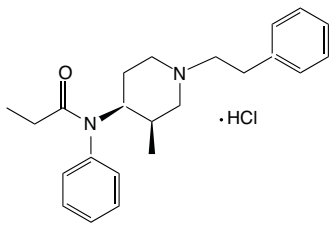
Sufentanil citrate



Butyryl fentanyl hydrochloride



Remifentanil hydrochloride



cis-3-methyl Fentanyl hydrochloride

Extraction

A spiked urine sample was subjected to extraction with a SPME LC fiber. The fiber was conditioned in 50:50 methanol:water (1 mL, 30 min, 800 rpm agitation). The fiber was rinsed off with water (1 mL, 10 s, 800 rpm) prior to extraction. The fiber was immersed into the urine sample and extraction could proceed (1 mL, 30 min, 800 rpm) followed by a water rinse (1 mL, 10 s, 800 rpm). The analytes were desorbed from the fiber using 90:10 methanol:water containing 0.1% (v/v) ammonium hydroxide (200 µL, 30 min, 800 rpm agitation).

The SPME LC technique only extracts the free portion of a drug within a biological sample; therefore, before sample quantitation can occur, a series of extracted standard curves were prepared for each analyte. These calibration samples, which were spiked in synthetic urine, were used to determine the average recovery of each analyte within the spiked samples.

Mixed mode (C8/SCX) fibers (research device) were conditioned in 1 mL of 50:50 (methanol/water) for 30 min with an agitation rate of 800 rpm. Followed by a water rinse for ~ 10 s at 800 rpm.

↓

Fibers were placed into 1 mL spiked urine samples and extracted for 30 min at 800 rpm, followed by a water rinse for ~ 10 s at 800 rpm.

↓

Fibers were desorbed in 200 µL 0.1% ammonium hydroxide in 90:10 methanol: water for 30 min at 800 rpm

↓

Samples were then analyzed by LC-MS/MS

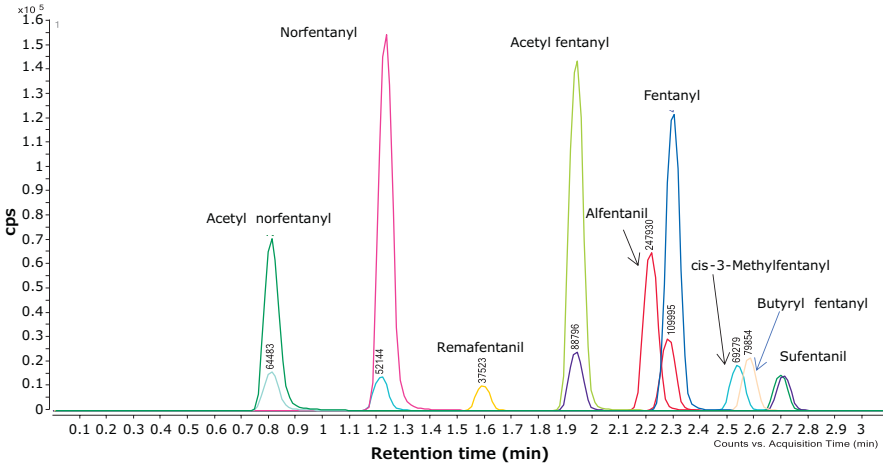
BioSPME

Biocompatible solid phase microextraction (BioSPME) is a variant of solid phase microextraction (SPME) in which the SPME fibers are coated with a non-swelling, biocompatible polymer. The benefit of this design enables minimized binding of biomacromolecules such as proteins and phospholipids but allows extraction of smaller analytes of interest. This coating enables the end-user to directly extract analytes out of complex matrices without the risk of proteins interfering with downstream quantitation of the analytes of interest. Using BioSPME eliminates many steps found in SPE methods and eliminates matrix effects often seen with dilute and shoot approaches.

SPME

LC-MS/MS Analysis of Fentanyl and Related Compounds

Due to the aromatic nature of the analytes of interest, an Ascentis® Express Biphenyl column was employed for the separation of the nine fentanyl analogs. The chromatogram below shows the LC-MS/MS results of the analysis. The Ascentis® Express Biphenyl column provided good resolution of the nine fentanyl analogs which allowed for accurate quantitation of the analytes.



Chromatographic conditions

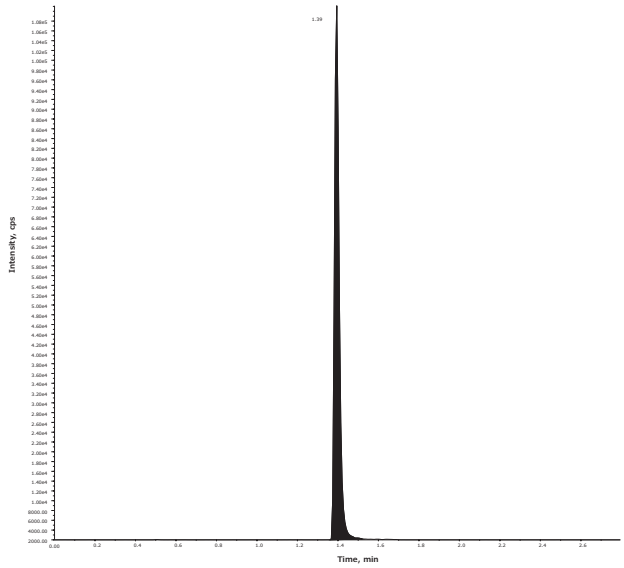
Column :	Ascentis® Express Biphenyl, 5 cm x 2.1 mm, 2.7 µm (**64057-U**)
Mobile phase	A: 0.1% formic acid in water B: 0.1% formic acid in methanol
Gradient:	40% B to 50% B in 2 min, to 80% B in 1 min, hold 80% B for 1 min, to 40% B in 0.1 min and hold at 40% B for 1.4 min
Flow rate:	0.6 mL/min
Column temp:	50 ºC
Injection:	4 µL
Det.:	MS/MS, ESI (+), MRM
Instrument:	Agilent 1290 Infinity II with 6460 QQQ

Material list	Cat. No.
Ascentis® Express Biphenyl Column 2.7 µm particle size, L × I.D. 5 cm × 2.1 mm	**64057-U**
Methanol hypergrade for LC-MS LiChrosolv®	**106035**
Water for chromatography (LC-MS Grade) LiChrosolv®	**115333**
Ultrapure water from Milli-Q® IQ 7 series water purification system	**ZIQ7005T0C**
Formic acid 98% - 100% for LC-MS LiChropur™	**533002**
Acetyl fentanyl solution 1.0 mg/mL in methanol, ampule of 1 mL, certified reference material, Cerilliant®	**A-109**
Acetyl fentanyl solution 50 µg/mL in methanol, certified reference material, ampule of 1 mL, Cerilliant®	**A-129**
Fentanyl United States Pharmacopeia (USP) Reference Standard	**1269902**
Fentanyl solution 1.0 mg/mL in methanol, ampule of 1 mL, certified reference material, Cerilliant®	**F-013**
Fentanyl solution 100 µg/mL in methanol, ampule of 1 mL, certified reference material, Cerilliant®	**F-002**
Fentanyl Related Compound G United States Pharmacopeia (USP) Reference Standard	**1269979**
Norfentanyl oxalate solution 1.0 mg/mL in methanol (as free base), ampule of 1 mL, certified reference material, Cerilliant®	**N-031**
Sufentanil citrate United States Pharmacopeia (USP) Reference Standard	**1623648**
Sufentanil Citrate solution 100 µg/mL in methanol (as free base), ampule of 1 mL, certified reference material, Cerilliant®	**S-008**

Ascentis® Express Biphenyl

UHPLC-MS/MS Determination of Buprenorphine and Norbuprenorphine in Urine on Purospher™ STAR RP-18 endcapped, 2 µm

Buprenorphine is a synthetic opioid used for pain treatment and maintenance medication for opioid addiction. Buprenorphine is metabolized to norbuprenorphine involving enzyme CYP3A4 in the liver. Both the parent compound and its metabolite conjugate to glucuronides. Maximum plasma concentrations are achieved within 30 minutes to three hours after administration. This application presents a LC-MS/MS method for the analysis of buprenorphine and norbuprenorphine in urine using reversed phase LC-MS/MS aided by isotopically labelled internal standards. Human patient samples were analyzed along with standards and control samples. Quantitation of buprenorphine and norbuprenorphine in urine is possible with this method, where the linear range was found to be 1-100 ng/mL and 5 -1000 ng/mL for buprenorphine and norbuprenorphine, respectively.



Norbuprenorphine: 642 ng/mL (standard)

Chromatographic conditions

Column:	Purospher™ STAR RP-18 endcapped, 2 µm Hibar® HR 5 cm x 2.1 mm I.D. (**150646**)
Mobile Phase	A: 0.1% formic acid in Milli-Q® ultrapure water B: 0.1% formic acid in acetonitrile
Gradient:	**Time (min) A (%) B (%)** 0 90 10 0.25 90 10 2 10 90 2.1 90 10 3 90 10
Flow rate:	0.7 mL/min
Temperature:	Column: 40 °C
Pressure:	230 bar (3312 psi) at start of gradient
Injection volume:	10 µL
Diluent:	Mobile Phase A:B (90:10)
Detection:	LC-MS/MS ESI; MRM transitions: 468.6/55.2 (Buprenorphine) and m/z 414.6/83.2 (Norbuprenorphine)
Sample:	Urine samples treated according to in-house sample preparation protocol

Chromatographic Data

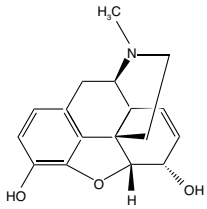
	Compound	Retention Time (min)	Precursor ion (m/z)	Product ions (m/z)
	Void volume	0.2	-	
1	Norbuprenorphine	1.2	414.6	83.2
2	Buprenorphine	1.4	468.6	55.2

Purospher™ STAR

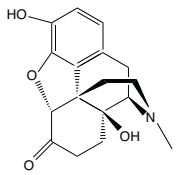
Material list	Cat. No.
Purospher™ STAR RP-18 endcapped (2 µm), Hibar® HR 50 x 2.1 mm ID	**150646**
Acetonitrile hypergrade for LC-MS LiChrosolv®	**100029**
Water for chromatography (LC-MS grade) LiChrosolv®	**115333**
Ultrapure water from Milli-Q® IQ 7 series water purification system	**ZIQ7005T0C**
Formic Acid 98%-100% for LC-MS LiChropur™	**533002**
Buprenorphine NMI Australia	**NMID932**
Norbuprenorphine solution 100 µg/mL in methanol, ampule of 1 mL, certified reference material, Cerilliant®	**N-912**
Norbuprenorphine-D3 solution 1.0 mg/mL in methanol, ampule of 1 mL, certified reference material, Cerilliant®	**N-921**

LC-MS Analysis of Pain Management Opioids on Ascentis® Express Phenyl-Hexyl

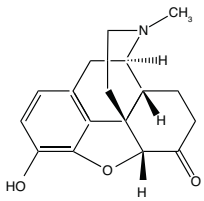
Balancing the management of chronic pain against the real possibility of opioid dependence and subsequent abuse is an ongoing conversation in the healthcare industry. Analysts need reliable tools to identify and quantify the drugs and metabolites in patients to tip the balance toward pain management and away from abuse. LC-MS is becoming the tool of choice for this purpose. Shown here is the rapid, efficient LC-MS analysis of 13 pain manangement opioids on an Ascentis® Express Phenyl-Hexyl U/HPLC column. Supelco® CRMs provided reliable identification and quantification.



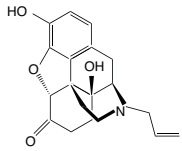
Morphine



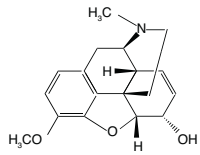
Oxymorphone



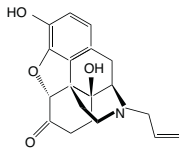
Hydromorphone



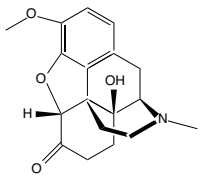
Naloxone



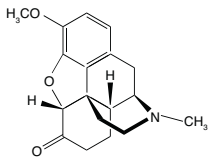
Codeine



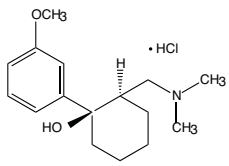
Naltrexone



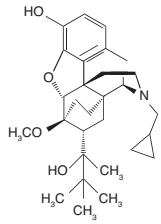
Oxycodone



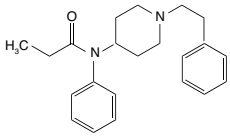
Hydrocodone



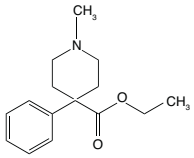
cis-Tramadol hydrochloride



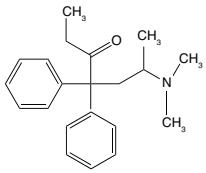
Buprenorphine



Fentanyl

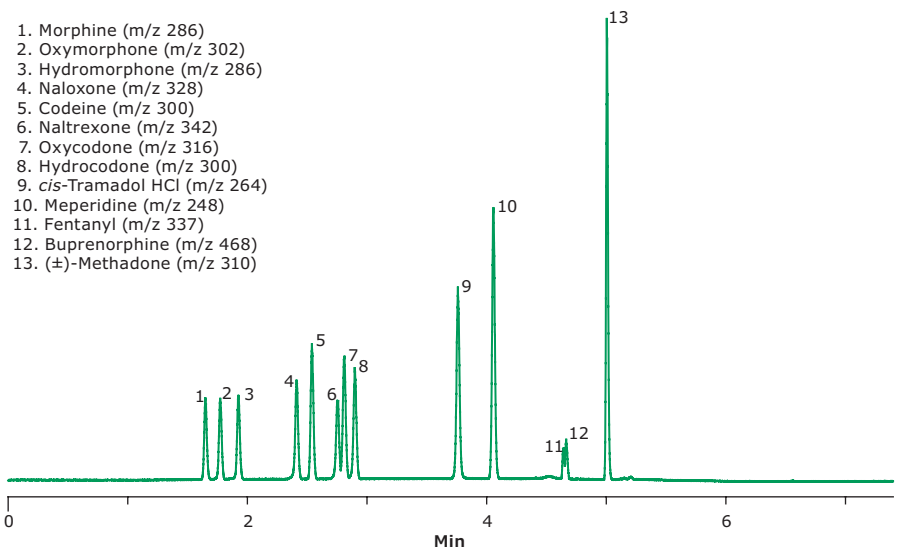


Meperidine



(±)-Methadone

Ascentis® Express Phenyl-Hexyl



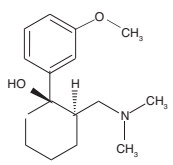
Chromatographic conditions

Column:	Ascentis® Express Phenyl-Hexyl 10 cm x 2.1 mm, 2.7 µm particles (**53336-U**)
Mobile phase:	[A] water with 0.1% formic acid; [B] methanol with 0.1% formic acid
Gradient:	10 to 45% B in 3 min; to 100% B in 2 min; held for 2.4 min
Flow rate:	0.3 mL/min
Column temp.:	30 °C
Pressure:	6940 psi (478 bar)
Injection:	2 µL
Detector:	MS, ESI(+), SIR
Sample:	Pain Management Multi-Component Opiate Mixture-13 (**P-071**) dilute to 30-300 ng/mL in 99:1, water:methanol

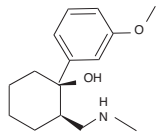
Material list	Cat. No.
Ascentis® Express Phenyl-Hexyl, 2.7 µm HPLC Column 2.7 µm particle size, L × I.D. 10 cm × 2.1 mm	**53336-U**
Acetonitrile hypergrade for LC-MS LiChrosolv®	**100029**
Methanol hypergrade for LC-MS LiChrosolv®	**106035**
Methanol gradient grade for liquid chromatography LiChrosolv® Reag. Ph Eur	**106007**
Water for chromatography (LC-MS Grade) LiChrosolv®	**115333**
Ultrapure water from Milli-Q(R) IQ 7 series water purification system	**ZIQ7005T0C**
Ethanol gradient grade for liquid chromatography LiChrosolv®	**111727**
Acetic acid 100% for LC-MS LiChropur™	**533001**
Ammonium acetate for mass spectrometry, LiChropur™, eluent additive for LC-MS	**73594**
Ammonium formate eluent additive for LC-MS, LiChropur™, ≥99.0%	**70221**
Formic acid 98% - 100% for LC-MS LiChropur™	**533002**
Potassium dihydrogen phosphate anhydrous for HPLC LiChropur™	**543841**
Triethylammonium acetate buffer for HPLC, 0.98-1.02 M	**69372**
Buprenorphine solution 100 µg/mL in methanol, ampule of 1 mL, certified reference material, Cerilliant®	**B-902**
Codeine solution 100 µg/mL in methanol, ampule of 1 mL, certified reference material, Cerilliant®	**C-015**
Fentanyl United States Pharmacopeia (USP) Reference Standard	**1269902**
Hydrocodone United States Pharmacopeia (USP) Reference Standard	**1314960**
Hydromorphone solution 1 mg/mL in methanol, ampule of 1 mL, certified reference material, Cerilliant®	**H-004**
Meperidine solution 1.0 mg/mL in methanol, ampule of 1 mL, certified reference material, Cerilliant®	**M-035**
(±)-Methadone solution 100 µg/mL in methanol, ampule of 1 mL, certified reference material, Cerilliant®	**M-019**
Morphine solution 1.0 mg/mL in methanol, ampule of 1 mL, certified reference material, Cerilliant®	**M-005**
Naloxone United States Pharmacopeia (USP) Reference Standard	**1453005**
Naltrexone United States Pharmacopeia (USP) Reference Standard	**1453504**
Oxycodone United States Pharmacopeia (USP) Reference Standard	**1485191**
Oxymorphone United States Pharmacopeia (USP) Reference Standard	**1488000**
Pain Management Multi-component Opiate Mixture-13 solution 100 µg/mL each component (10 µg/mL Fentanyl), ampule of 1.0 mL, certified reference material, Cerilliant®	**P-071**
cis-Tramadol hydrochloride solution 1.0 mg/mL in methanol (as free base), ampule of 1 mL, certified reference material, Cerilliant®	**T-027**

UHPLC-MS/MS Determination of Tramadol in Urine on Purospher™ STAR RP-18 endcapped, 2 µm

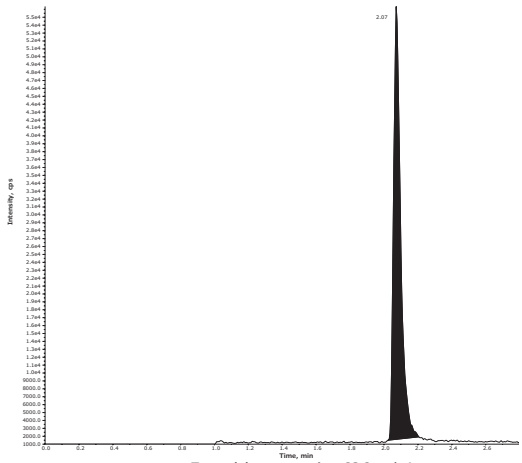
Tramadol is a weak µ-opioid receptor agonist and inhibitor of serotonin/noradrenalin re-uptake used for pain treatment and other indications such as treatment for restless leg syndrome and fibromyalgia. Tramadol is structurally similar to the natural opiate codeine. Tramadol is reported to have a lower risk of developing dependence than codeine. In addition, tramadol is extensively metabolized, where a total of 24 metabolites have been identified. This application presents an LC-MS/MS method for the analysis of tramadol in urine using reversed phase LC-MS/MS aided by isotope-labelled internal standards. Human patient samples were analyzed along with standards and control samples. The linear range was found to be 25-1500 ng/mL.



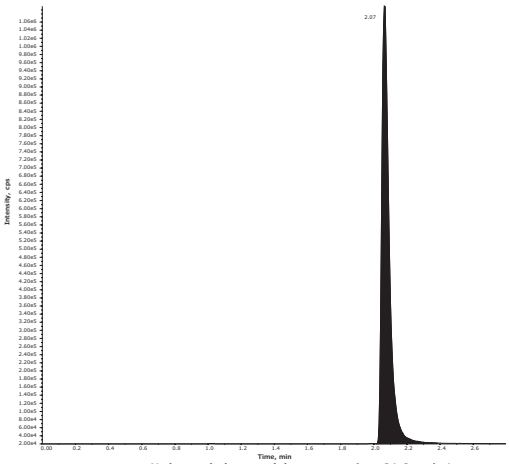
Tramadol



N-desmethyl-tramadol



Tramadol concentration: 23.2 ng/mL



N-desmethyl-tramadol concentration: 21.2 ng/mL

Chromatographic conditions

Column:	Purospher™ STAR RP-18 endcapped, 2 µm Hibar® HR 50-2.1 mm ID (**150646**)
Mobile Phase:	A: 0.1% formic acid in Milli-Q® ultrapure water B: 0.1% formic acid in acetonitrile
Gradient:	

Time (min)	A (%)	B (%)	Flow rate (mL/min)
0	95	5	0.4
0.2	95	5	0.4
2	50	50	0.4
2.5	10	90	0.4
2.8	10	90	0.4
3	95	5	0.5
4.5	95	5	0.5

Flow rate:	See table
Temperature:	Column: 40 °C
Pressure:	170 bar (2448 psi) at start of gradient
Injection volume:	5 µL
Diluent:	Mobile Phase A:B (50:50)
Detection:	LC-MS/MS ESI; MRM transitions: m/z 264/58 (Tramadol) and m/z 250/44 (N-desmethyl-tramadol)
Sample:	Urine samples treated according to in-house sample preparation protocol

Chromatographic Data

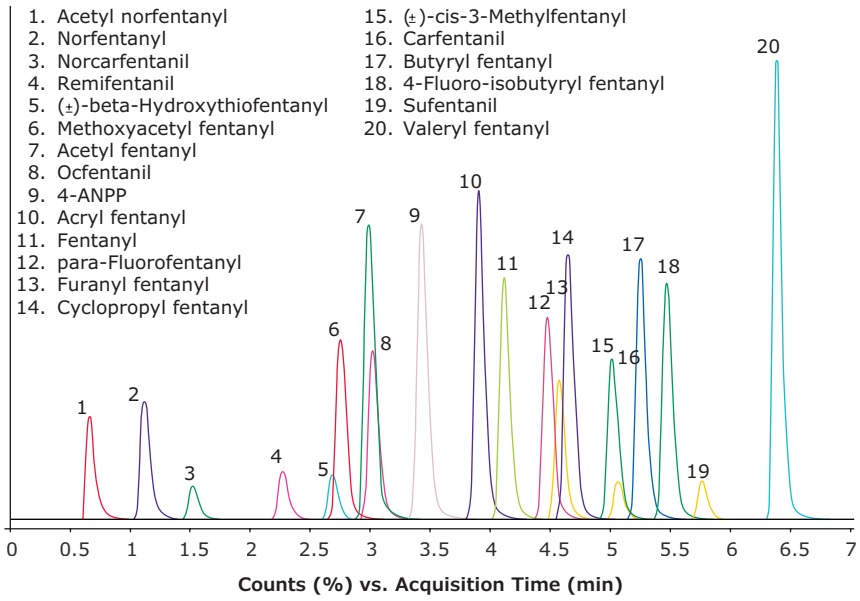
	Compound	Retention Time (min)	Precursor ion (m/z)	Product ions (m/z)
	Void volume	0.2	-	
1	Tramadol	2.07	264	58
2	N-desmethyl-tramadol	2.09	254	44.0.2

Purospher™ STAR

Material list	Cat. No.
Purospher™ STAR RP-18 endcapped, 2 µm Hibar® HR 50 x 2.1 mm ID	**150646**
Acetonitrile hypergrade for LC-MS LiChrosolv®	**100029**
Water for chromatography (LC-MS grade) LiChrosolv®	**115333**
Ultrapure water from Milli-Q® IQ 7 series water purification system	**ZIQ7005T0C**
Formic Acid 98%-100% for LC-MS LiChropur™	**533002**
cis-Tramadol hydrochloride solution 1.0 mg/mL in methanol (as free base), ampule of 1 mL, certified reference material, Cerilliant®	**T-027**
cis-Tramadol-13C, D3 hydrochloride solution 1.0 mg/mL in methanol (as free base), ampule of 1 mL, certified reference material, Cerilliant®	**T-020**
cis-Tramadol-13C, D3 hydrochloride solution 100 µg/mL in methanol (as free base), ampule of 1 mL, certified reference material, Cerilliant®	**T-029**
N-Desmethyl-cis-tramadol hydrochloride solution 1.0 mg/mL in methanol (as free base), ampule of 1 mL, certified reference material, Cerilliant®	**D-023**
N-Desmethyl-cis-tramadol-D3 hydrochloride solution 100 µg/mL in methanol (as free base), ampule of 1 mL, certified reference material, Cerilliant®	**D-110**
O-Desmethyl-cis-tramadol hydrochloride solution 1.0 mg/mL in methanol (as free base), ampule of 1 mL, certified reference material, Cerilliant®	**T-035**

UHPLC-MS/MS Analysis of 20 Fentanyl and Fentanyl Analogs

The ultra-high performance of the Titan™ 1.9 µm particles enable the high-resolution separation of 20 Fentanyl and Fentanyl Analogs in 7 minutes. Titan™ C18 1.9 µm columns are fully compatible to UHPLC instruments and tolerate a maximum column back-pressure of 1000 bar.



Chromatographic conditions

Column:	Titan™ C18, 5 cm × 2.1 mm I.D., 1.9 µm (**577122-U**)
Column temp.:	40 °C
Sample:	1.0 ng/mL in 90:10 methanol:water
Injection volume:	1.0 µL
Mobile phase:	A 0.1% formic acid in water Mobile phase B 0.1% formic acid in acetonitrile
Flow rate:	0.4 mL/min
Gradient:	**Time (min) / %B** 0 / 15 0.3 / 15 5.5 / 30 7.0 / 95 9.0 / 95
Instrument:	Agilent 1290 Infinity II UHPLC Agilent 6495 Triple Quad LC-MS
MS/MS mode:	MRM
Ion mode:	Positive

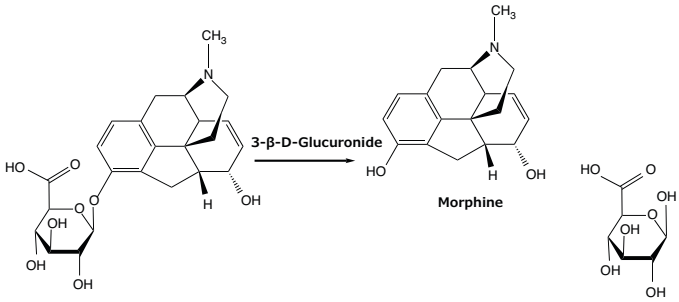
Material list	Cat. No.
Titan™ C18, 5 cm × 2.1 mm I.D., 1.9 µm	**577122-U**
Acetonitrile hypergrade for LC-MS LiChrosolv®	**100029**
Methanol hypergrade for LC-MS LiChrosolv®	**106035**
Water for chromatography (LC-MS Grade) LiChrosolv®	**115333**
Formic acid 98% - 100% for LC-MS LiChropur™	**533002**
Acetyl fentanyl solution 1.0 mg/mL in methanol, ampule of 1 mL, certified reference material, Cerilliant®	**A-109**
Acetyl fentanyl solution 50 µg/mL in methanol, certified reference material, ampule of 1 mL, Cerilliant®	**A-129**
Fentanyl United States Pharmacopeia (USP) Reference Standard	**1269902**
Fentanyl solution 1.0 mg/mL in methanol, ampule of 1 mL, certified reference material, Cerilliant®	**F-013**
Fentanyl solution 100 µg/mL in methanol, ampule of 1 mL, certified reference material, Cerilliant®	**F-002**
Fentanyl Related Compound G United States Pharmacopeia (USP) Reference Standard	**1269979**
Norfentanyl oxalate solution 1.0 mg/mL in methanol (as free base), ampule of 1 mL, certified reference material, Cerilliant®	**N-031**
Sufentanil citrate United States Pharmacopeia (USP) Reference Standard	**1623648**
Sufentanil Citrate solution 100 µg/mL in methanol (as free base), ampule of 1 mL, certified reference material, Cerilliant®	**S-008**

Description	Cat. No.
(±)-cis-3-Methylfentanyl HCl, 100 µg/mL (as free base) in methanol	M-194-0.5ML
(±)-β-Hydroxythiofentanyl HCl, 100 µg/mL (as free base) in methanol	H-130-0.5ML
4-ANPP, 100 µg/mL in methanol	A-139-0.5ML
4-ANPP-D5, 100 µg/mL in methanol	A-157-0.5ML
4-Fluoroisobutyrylfentanyl, 100 µg/mL in methanol	F-050-0.5ML
Acetyl fentanyl-13C6, 100 µg/mL in methanol	A-110-1ML
Acetyl fentanyl-13C6, 50 µg/mL in methanol	A-130-1ML
Acetyl norfentanyl oxalate 1.0 mg/mL (as free base) in methanol	A-115-1ML
Acetyl norfentanyl-13C6 oxalate, 100 µg/mL (as free base) in methanol	A-116-1ML
Acryl fentanyl HCl, 100 µg/mL (as free base) in methanol	A-140-0.5ML
Alfentanil HCl, 1.0 mg/mL (as free base) in methanol	A-071-1ML
Butyryl fentanyl, 100 µg/mL in methanol	B-066-0.5ML
Carfentanil Oxalate, 100 µg/mL in methanol	C-162-1EA
Carfentanil-D5 Oxalate, 100 µg/mL (as free base) in methanol	C-163-1EA
Cyclopropyl fentanyl HCl, 100 µg/mL (as free base) in methanol	C-177-0.5ML
Fentanyl-D5, 100 µg/mL in methanol	F-001-1ML
Furanyl fentanyl HCl, 100 µg/mL (as free base) in methanol	F-046-0.5ML
Furanyl fentanyl-D5 HCl, 100 µg/mL (as free base) in methanol	F-053-0.5ML
Isobutyryl fentanyl HCl, 100 µg/mL (as free base) in methanol	I-038-0.5ML
Methoxyacetyl fentanyl HCl, 100 µg/mL (as free base) in methanol	M-200-0.5ML
Norcarfentanil Oxalate, 100 µg/mL (as free base) in methanol	N-114-1EA
Norfentanyl-D5 oxalate, 1.0 mg/mL (as free base) in methanol	N-055-1ML
Norfentanyl-D5 oxalate, 100 µg/mL (as free base) in methanol	N-030-1ML
Ocfentanil, 100 µg/mL in methanol	O-047-0.5ML
ortho-Fluorofentanyl HCl, 100 µg/mL (as free base) in methanol	F-054-0.5ML
para-Fluorobutyryl fentanyl (PFBF), 100 µg/mL in methanol	F-048-0.5ML
para-Fluorofentanyl, 100 µg/mL in methanol	F-049-0.5ML
Remifentanil acid, 100 µg/mL in acetonitrile	R-026-1ML
Remifentanil HCl, 100 µg/mL (as free base) in methanol	R-024-1ML
Sufentanil-D5, 100 µg/mL in methanol	S-018-1ML
Valeryl fentanyl HCl, 100 µg/mL (as free base) in methanol	V-048-0.5ML
Valeryl fentanyl-D5 HCl, 100 µg/mL (as free base) in methanol	V-068-0.5ML

Analysis of Drugs of Abuse in Urine After Cleanup with Supel™ Swift HLB Solid Phase Extraction 96-well Plates

In this study, we demonstrate the ability to perform cleanup of urine samples using HLB solid phase extraction for the analysis of opioids via LC-MS/MS. The 96-well SPE format (**Figure 1**) utilized is optimal for clinical and other laboratories working in a high-throughput environment.

During analysis of drugs of abuse in urine, the drug metabolites (e.g. morphine) can be present in the glucuronate form (**Figure 2**). In these cases, hydrolysis using a β-glucuronidase enzyme is performed prior to LC-MS analysis of the samples to ensure that the free form of the drug can be analyzed in the samples under investigation. Subsequently, the sample requires a cleanup prior to injection into the LC-MS instrument. Solid Phase Extraction remains the most convenient method for use in such sample cleanups.



Morphine-3-β-D-Glucuronide

Figure 2. β-Glucuronidase Hydrolysis of Morphine-3-β-D-glucuronide to the free Analyte, Morphine

Recovery of Analytes

Synthetic urine, Sigmatrix Urine Diluent, was spiked using the "Pain Management Multi-Component Opiate Mixture-13 solution" diluted to 100 ng/mL for 12 compounds and at 10 ng/mL for fentanyl. A list of the components and the transitions monitored is available in **Table 1**. The following internal standards were used: oxycodone D-3, (±)-Methadone-D9, oxymorphone D-3, hydrocodone D-3, cis-Tramadol-^{13}C, D3, meperidine D-4 were added at 10 ng/mL. The MS transitions monitored with these internal standards are shown in **Table 2**.

β-glucuronidase solution at a concentration of 10 kU/g was prepared in 0.1 M phosphate buffer (pH 6). The bulk sample solution comprised of 3:1:1 Sigmatrix urine diluent: β-glucuronidase (10 KU, pH 6): Phosphate buffer (pH 6.0). The samples underwent digestion for 2 hours at 60 °C with mixing at 200 rpm. The hydrolysis conditions used were previously found to be optimum for using β-glucuronidase enzyme from limpets. The samples were cooled, and the sample solutions adjusted to pH 9 with ammonium hydroxide. The samples were then processed on a Supel™ Swift HLB 96-well plate containing 30 mg/well of HLB sorbent as outlined in **Figure 3**. After sample processing, 75 µL of cleaned sample was diluted with 175 µL of LC-MS grade water to bring the final organic component to 30%. Samples were analyzed on a Sciex 3200 QTrap MS instrument with an Agilent 1290 LC (separation parameters are shown in **Table 3**). Analytes were quantified by a 5-point external calibration curve using standards prepared daily from methanol stock solutions stored in glass vials, final solutions comprised 70:30 methanol: water containing 10 ng/mL of internal standards.

Matrix Effects on Ionization

Samples were processed as described earlier except for no spiked analytes. The cleaned matrix was spiked after processing with both analytes and internal standards. These samples were quantified by a 5-point external calibration curve as described above.

Supel™ Swift HLB

Table 1. Analytes in the "Pain Management Multi-Component Opiate Mixture-13 solution" and MS/MS Detection Parameters

	Compound	log P	pKa	Retention Time (min)	Q1	Q3	DP (V)	CE (V)	EP (V)	CXP (V)	Internal Standard
1	Morphine	0.9	8.2	1.59	286.1	128.1	63	71	8	4	Oxymorphone-D3
2	Oxymorphone	0.8	8.2	1.73	302.1	284.2	46	23	5.5	4	Oxymorphone-D3
3	Hydromorphone	1.1	8.2	1.89	286.1	185.3	61	37	5.5	6	Oxymorphone-D3
4	Naloxone	1.9	7.9	2.38	328.2	310.2	41	23	9	6	Oxycodone-D3
5	Codeine	1.4	8.2	2.7	300.1	114.9	61	61	8	8	Oxycodone-D3
6	Naltrexone	1.9	8.4, 9.9	2.75	328.2	310.2	41	23	9	6	Oxycodone-D3
7	Oxycodone	0.7	8.5	2.83	316.3	241.1	61	38	8	3	Oxycodone-D3
8	Hydrocodone	1.2	8.2	2.84	300.2	199.2	56	35	6.5	6	Hydrocodone-D3
9	Tramadol	1.3	9.4	3.66	264.2	57.9	31	33	6.5	6	Tramadol-D3
10	Meperidine	2.7	8.6	3.98	248.2	220.3	51	29	9	4	Meperidine-D4
11	Fentanyl	4.1	9	4.6	337.2	188.3	46	29	9	4	Meperidine-D4
12	Buprenorphine	5	8.3	4.67	468.3	55.1	86	85	8	4	Meperidine-D4
13	Methadone	3.9	9.2	4.95	310.2	265.2	31	19	4	4	Methadone-D9

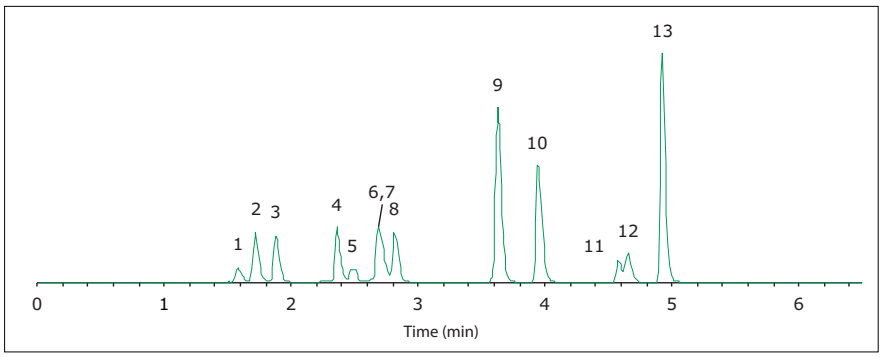
Table 2. Internal Standards used with the 13 Pain Management Compounds and MS-MS Detection Parameters

Internal Standard	Retention Time (min)	Q1	Q3	DP (V)	CE (V)	EP (V)	CXP (V)
Hydrocodone-D3	2.84	303.2	199.2	56	35	6.5	6
Meperidine-D4	3.98	525.2	224.3	51	29	9	4
(±)-Methadone-D9	4.95	319.2	268.2	31	19	4	4
Oxycodone-D3	2.83	319.3	244.1	61	38	8	3
Oxymorphone-D3	1.73	305.1	287.2	46	23	5.5	4
cis-Tramadol-^{13}C, D3	3.66	268.2	57.9	31	33	6.5	6

Pre-extraction	1:1:3 Synthetic urine matrix:β-glucuronidase & mixture of analytes:phosphate buffer (pH 6), incubate 2 hours at 60 °C, cooled, pH adjusted to 9
Conditioning	1000 µL 5% methanol in water
Loading	500 µL of the hydrolyzed urine sample, pH 9
Wash	1000 µL of 5% methanol in water
Elution	500 µL of 100% methanol with 5% formic acid
Post-extraction	Dilute 75 µL of the cleaned sample with 175 µL of LC-MS grade water

Figure 3. Sample Preparation and SPE Method

Table 3. Analytical Conditions for Sciex 3200 QTrap and Agilent 1290 LC Instruments



Representative Chromatogram of the Spiked Urine-Mimic Samples after Cleanup with SPE

Chromatographic conditions

Column:	Ascentis® Express Phenyl-Hexyl 10 cm x 2.1 mm ID, 2.7 µm (**53336-U**)
Mobile Phase:	[A] LC-MS grade water with 0.1% formic acid [B] LC-MS grade acetonitrile with 0.1% formic acid
Gradient:	10% to 45% [B] in 3 minutes, 100% [B] at 5 min and hold 2.4 min
Flow Rate:	0.300 mL/min
Detector:	MS, ESI(+), Scheduled MRM

Results and Discussion

Percent Recovery

A representative chromatogram of a SPE cleaned-up sample spiked at 100 ng/mL (except for fentanyl at 10 ng/mL) is shown in **Figure 4**. Overall, 12 of the 13 analytes showed average recoveries of 73 to 105% (n=96) with an overall recovery of 88% as shown in **Table 4** and **Figure 5**. The lower recovery for buprenorphine is attributed to a log P ~5, which would promote non-specific binding.

For the thirteen analytes, the RSDs associated with the recoveries were <7.2% (n=96) showing consistency across the plate. Absolute recoveries are shown in **Figure 5**.

Without using the assigned internal standard, the absolute recovery across the plate for 12 of the 13 analytes is 70.5 (omitting buprenorphine). Nine of the 13 analytes show recovery at ≥70% as shown in **Figure 6** across the 96 wells.

Table 4. Percent Recovery Across the Supel™ Swift HLB 96 well plate, Analytes were Spiked at 100 ng/mL (except for Fentanyl 10 ng/mL)

Compound	1 Morphine	2 Oxymorphone	3 Hydromorphone	4 Naloxone	5 Codeine	6 Naltrexone	7 Oxycodone
Recovery (%)	88%	94%	94%	74%	105%	75%	92%
RSD (%)	5.40%	4.10%	5.50%	6.90%	7.20%	6.20%	6.70%
Compound	**8 Hydrocodone**	**9 Tramadol**	**10 Meperidine**	**11 Fentanyl**	**12 Buprenorphine**	**13 Methadone**	**Overall***
Recovery (%)	90%	89%	93%	73%	44%	90%	88%
RSD (%)	4.60%	2.00%	3.30%	6.10%	5.80%	2.70%	10.8

*Buprenorphine is omitted

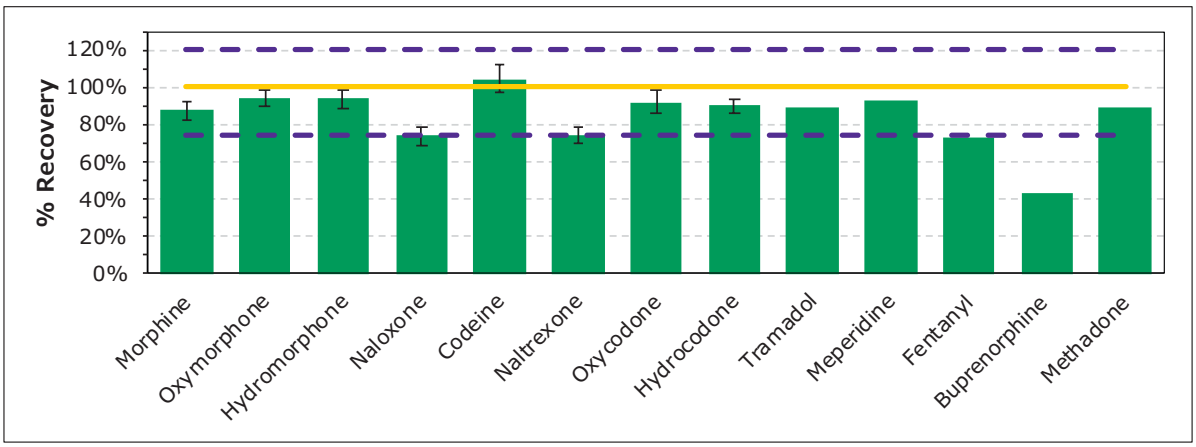


Figure 5. Relative Percent Recovery. Analytes were Spiked at 100 ng/mL Except for Fentanyl at 10 ng/mL. Purple dash lines Represent 75 and 120% Recovery, with the yellow solid line Representing 100% recovery. Analytes are Listed in Elution Order

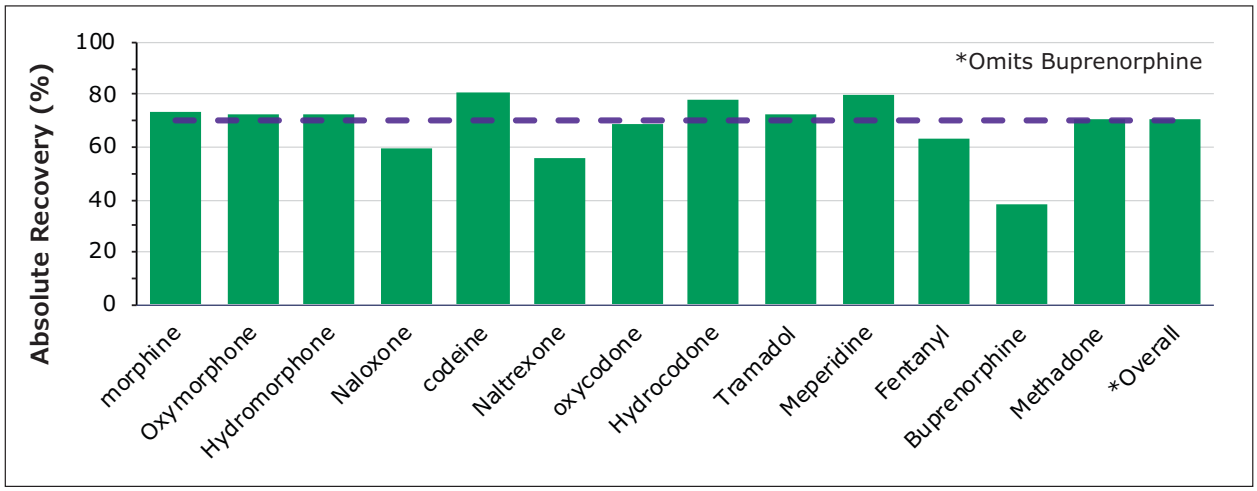


Figure 6. Absolute Percent Recovery Analytes were Spiked at 100 ng/mL Except for Fentanyl at 10 ng/mL. Purple dash lines Represent 70% Absolute Recovery. Analytes are Listed in Elution Order

Matrix Effects

The impact of matrix components was calculated by comparing the signal response of the analyte in 70:30 methanol: water (representing 100%) to a sample that was processed using the SPE procedure outlined and was post-spiked (final extracted samples had 30% methanol present). Across the 13 analytes minimal to no matrix effects (suppression or enhancement) ±10% was observed for most of the analytes as shown in **Figure 7**. Two analytes that were suppressed the most were naloxone (-30%) and naltrexone (-20%). These suppression values would lead to the lower absolute recovery reported in **Figure 6** but are corrected for in relative recovery by use of an internal standard (**Figure 5**).

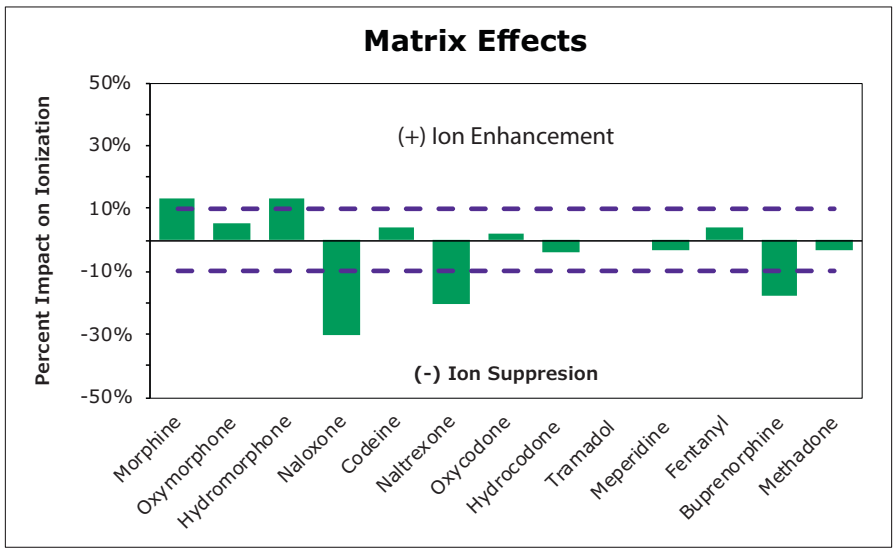


Figure 7. Matrix effects (ion suppression and ion enhancement) Across the 13 analytes. Purple dash lines Represent ±10% Impact on Ionization

Summary

Supel™ Swift HLB SPE is a hydrophilic and lipophilic polymer SPE phase designed for the extraction of a highly broad range of compounds from complex aqueous sample matrices. In this study, we demonstrated the utility of this SPE phase for the preparation of urine samples for the analysis of a series of pain management drugs readily available as a premade mixture. No post-extraction concentration step was required. The relative recoveries of the analytes were in the range of 73-105% with one exception, buprenorphine. The reproducibility across the entire plate was excellent, ≤7.2% RSD. Minimum matrix effects (±10%) were observed after Supel™ Swift HLB SPE cleanup. The developed SPE method can be applied to a wider range of analytes in urine.

Material	Cat No.
Supel™ Swift HLB 96-well plate, 30 mg/well	**57494-U**
Ascentis® Express Phenyl-Hexyl HPLC Column, 2.7 µm particle size, 100 × 2.1 mm ID	**53336-U**
Acetonitrile gradient grade for liquid chromatography LiChrosolv® Reag. Ph Eur	**100029**
Methanol gradient grade for liquid chromatography LiChrosolv® Reag. Ph Eur	**106035**
Water for chromatography (LC-MS Grade) LiChrosolv®	**115333**
Eppendorf® Deep Well Plate 96/1000µL PCR Clean, volume 1000 µL, white border with clear wells, pkg of 20 plates (5 bags x 4 plates)	**EP951032603-20EA**
Seal Plate Film	**Z369659-100EA**
PlatePrep 96-well Vacuum Manifold, starter kit	**575650-U**
Sigmatrix Urine Diluent	**SAE0074**
Pain Management Multi-Component Opiate Mixture-13 solution 100 µg/mL each component (10 µg/mL Fentanyl), ampule of 1.0 mL, certified reference material, Cerilliant®	**P-071-1ML**
Hydrocodone-D3 solution, 100 µg/mL in methanol, ampule of 1 mL, certified reference material, Ceriliant®	**H-005**
Meperidine-D4 solution, 100 µg/mL in methanol, ampule of 1 mL, certified reference material, Ceriliant®	**M-036**
(±)-Methadone-D9 solution, 100 µg/mL in methanol, ampule of 1 mL, certified reference material, Ceriliant®	**M-088**
Oxycodone-D3 solution, 100 µg/mL in methanol, ampule of 1 mL, certified reference material, Ceriliant®	**O-005**
Oxymorphone-D3 solution, 100 µg/mL in methanol, ampule of 1 mL, certified reference material, Ceriliant®	**O-019**
cis-Tramadol-13C, D3 hydrochloride solution, 100 µg/mL in methanol, ampule of 1 mL, certified reference material, Ceriliant®	**T-029**
Formic acid for LC-MS LiChropur™, 97.5-98.5% (T)	**940**
β-Glucuronidase from limpets (Patella vulgata) Type L-II, lyophilized powder, 1,000,000-3,000,000 units/g solid	**G8132**
di-Sodium hydrogen phosphate heptahydrate for analysis EMSURE® ACS	**106575**
Sodium dihydrogen phosphate monohydrate for analysis EMSURE® ACS,Reag. Ph Eur	**106346**
Ammonium hydroxide solution puriss. p.a., reag. ISO, reag. Ph. Eur., ~25% NH3 basis	**30501**
Vials, screw top, clear glass (vial only) volume 40 mL, clear glass vial, thread for 24-400, pkg of 100 ea	**27379**

Designer Drugs and Synthetic Hallucinogens

Designer drugs are structural or functional analogs of a controlled substance that has been designed to mimic the pharmacological effects of the original drug, while avoiding classification as illegal and/or detection in standard drug tests.

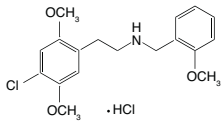
Designer drugs include psychoactive substances that have been designated as new psychoactive substances (NPS) as well as analogs of performance-enhancing drugs such as designer steroids. Because the efficacy and safety of these substances have not been thoroughly evaluated in animal and human trials, the use of some of these drugs may result in unexpected side effects.

NBOMe designer drugs are highly potent synthetic hallucinogens and psychoactive bath salts are a group of recreational designer drugs. The name derives from instances in which the drugs were disguised as bath salts. The white powders, granules, or crystals often resemble Epsom salts, but differ chemically. Bath salts usually contain a cathinone, typically methylenedioxypyrovalerone (MDPV), methylone, or mephedrone. However, the chemical composition varies widely.

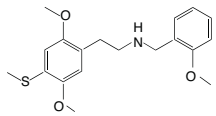
- UHPLC-MS Analysis of NBOMe Designer Drugs in Urine on Ascentis® Express C18 after Solid Phase Extraction (SPE) using HLB plates
- LC-MS Analysis of Illicit Bath Salts on Ascentis® Express HILIC Column
- LC-MS (TOF) Analysis of Illicit Bath Salts in Urine on Ascentis® Express HILIC after Solid Phase Extraction (SPE)

UHPLC-MS Analysis of NBOMe Designer Drugs in Urine on Ascentis® Express C18 after Solid Phase Extraction (SPE) using HLB 96-well plate

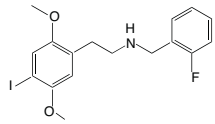
The NBOMe designer drugs comprise various derivatives of the 2C family of illicit psychoactive phenethylamines. Shown here is a rapid sample preparation method for extraction of NBOMe drugs from human urine for LC-MS analysis. NBOMe certified reference materials were used. Optimized reversed phase chromatographic conditions were developed on an Ascentis® Express C18, 2.0 µm column. Sample preparation of spiked human urine was carried out on an HLB 96-well plate. Absolute recovery data was determined. CRMs provided reliable quantification and the highest quality. Dedicated LC-MS solvents gave clean and robust operation.



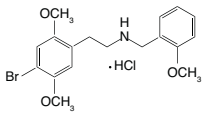
25C-NBOMe hydrochloride



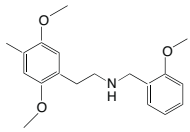
25T-NBOMe



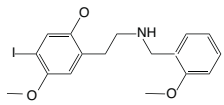
25I-NBF



25B-NBOMe hydrochloride

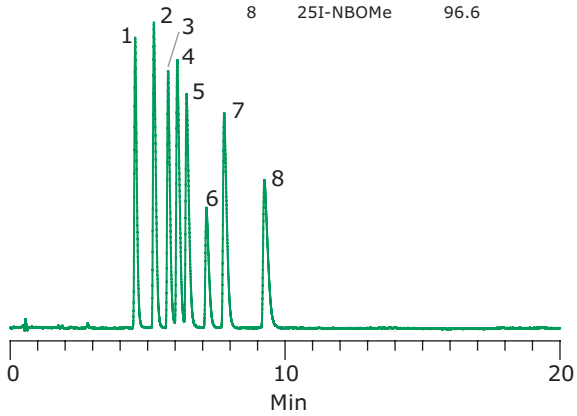


25D-NBOMe



25I-NBOMe

Peak No.	Analyte	Recovery from urine (%)	CV
1	25T-NBOMe	80.3	4.2
2	25C-NBOMe	86.0	4.0
3	25I-NBOH	84.6	4.1
4	25D-NBOMe	84.1	2.8
5	25B-NBOMe	88.2	2.2
6	25I-NBF	88.6	2.7
7	25I-NBMD	90.2	2.2
8	25I-NBOMe	96.6	2.1



Chromatographic conditions

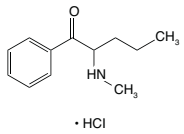
Column:	Ascentis® Express C18, 10 cm x 3 mm I.D., 2 µm particles (**50819-U**)
Column temp.:	50 °C
mobile phase	[A] 20 mM ammonium acetate, pH 4 with acetic acid; [B] acetonitrile; (60:40, A:B)
Flow rate:	0.8 mL/min
Pressure:	7700 psi (531 bar)
Injection:	1 µL
Detector:	UV, 280 nm
sample preparation	SPE (Solid Phase Extraction)
sample/ matrix:	human urine spiked at 100 ng/mL each compound
SPE well plate:	Recommended: Supel™ Swift HLB 96-well pate, 30 mg/well (**57494-U**)
Condition:	1 mL methanol followed by 1 mL water
Sample addition:	1 mL
Washing:	1 mL water followed by 1 mL water:methanol (75:25)
Eluate post-treatment:	evaporate sample to dryness and reconstitute in 1 mL water:acetonitrile (50:50)

SPE

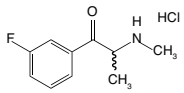
Material list	Cat. No.
Ascentis® Express C18, 10 cm x 3 mm I.D., 2 µm particles	50819-U
Supel™ Swift HLB SPE 96-well plate, 30 mg/well (57494-U)	57494-U
Acetonitrile hypergrade for LC-MS LiChrosolv®	100029
Methanol hypergrade for LC-MS LiChrosolv®	106035
Methanol gradient grade for liquid chromatography LiChrosolv® Reag. Ph Eur	106007
Methanol for HPLC, gradient grade, ≥99.9%	34885
Water for chromatography (LC-MS Grade) LiChrosolv®	115333
Water HPLC Plus	34877
Ultrapure water from Milli-Q® IQ 7 series water purification system	ZIQ7005T0C
Ethanol gradient grade for liquid chromatography LiChrosolv®	111727
Acetic acid 100% for LC-MS LiChropur™	533001
Ammonium acetate for mass spectrometry, LiChropur™, eluent additive for LC-MS	73594
Ammonium formate eluent additive for LC-MS, LiChropur™, ≥99.0%	70221
Formic acid 98% - 100% for LC-MS LiChropur™	533002
Potassium dihydrogen phosphate anhydrous for HPLC LiChropur™	543841
Triethylammonium acetate buffer for HPLC, 0.98-1.02 M	69372
25I-NBOH hydrochloride solution 1.0 mg/mL in methanol (as free base), ampule of 1 mL, certified reference material, Cerilliant®	I-019
25B-NBOMe hydrochloride solution 1.0 mg/mL in methanol (as free base), ampule of 1 mL, certified reference material, Cerilliant®	B-061
25B-NBOMe-D3 hydrochloride solution 100 µg/mL in methanol (as free base), ampule of 1 mL, certified reference material, Cerilliant®	B-062
25C-NBOMe hydrochloride solution 1.0 mg/mL in methanol (as free base), ampule of 1 mL, certified reference material, Cerilliant®	C-131
25C-NBOMe-D3 hydrochloride solution 100 µg/mL in methanol (as free base), ampule of 1 mL, certified reference material, Cerilliant®	C-132
25I-NBOMe hydrochloride solution 1.0 mg/mL in methanol (as free base), ampule of 1 mL, certified reference material, Cerilliant®	I-016
25I-NBOMe-D3 hydrochloride solution 100 µg/mL in methanol (as free base), ampule of 1 mL, certified reference material, Cerilliant®	I-017

LC-MS Analysis of Illicit Bath Salts on Ascentis® Express HILIC Column

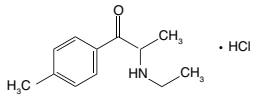
HILIC mode operation on an Ascentis® Express HILIC Column provided baseline resolution of these bath salts, including isobaric compounds. The highest quality LC-MS solvents were used to supply low background interference and low particulate contaminants for robust; trouble-free operation. CRMs provided reliable identification and quantification.



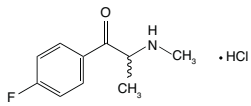
Pentedrone hydrochloride



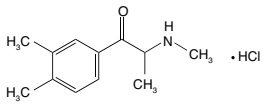
3-Fluoromethcathinone hydrochloride



4-Methylmethcathinone hydrochloride

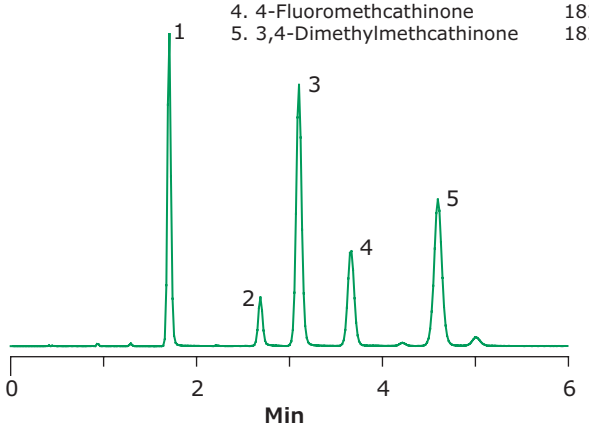


4-Fluoromethcathinone hydrochloride



3,4-Dimethylmethcathinone hydrochloride

Compound	m/z
1. Pentedrone	192
2. 3-Fluoromethcathinone	182
3. 4-Methylmethcathinone	192
4. 4-Fluoromethcathinone	182
5. 3,4-Dimethylmethcathinone	182



Chromatographic conditions

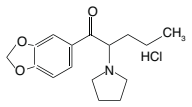
Column:	Ascentis® Express HILIC, 10 cm x 2.1 mm I.D., 2.7 µm particles (**53939-U**)
Mobile phase:	[A] 5 mM ammonium formate in acetonitrile; [B] water; (98:2, A:B)
Flow rate:	0.6 mL/min
Column temp.:	35 °C
Pressure:	267 psi (18.4 bar)
Injection:	2 µL
Detector:	MS, ESI(+) TOF, Summed Ion Chromatogram
Sample:	500 ng/mL in acetonitrile

Ascentis® Express HILIC

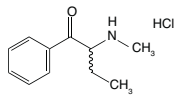
Material list	Cat. No.
Ascentis® Express HILIC, 10 cm x 2.1 mm I.D., 2.7 µm particles	**53939-U**
Acetonitrile hypergrade for LC-MS LiChrosolv®	**100029**
Methanol hypergrade for LC-MS LiChrosolv®	**106035**
Methanol gradient grade for liquid chromatography LiChrosolv® Reag. Ph Eur	**106007**
Ethanol gradient grade for liquid chromatography LiChrosolv®	**111727**
Water for chromatography (LC-MS Grade) LiChrosolv®	**115333**
Ultrapure water from Milli-Q® IQ 7 series water purification system	**ZIQ7005T0C**
Acetic acid 100% for LC-MS LiChropur™	**533001**
Ammonium acetate for mass spectrometry, LiChropur™, eluent additive for LC-MS	**73594**
Ammonium formate eluent additive for LC-MS, LiChropur™, ≥99.0%	**70221**
Formic acid 98% - 100% for LC-MS LiChropur™	**533002**
Potassium dihydrogen phosphate anhydrous for HPLC LiChropur™	**543841**
Triethylammonium acetate buffer for HPLC, 0.98-1.02 M	**69372**
3,4-Dimethylmethcathinone hydrochloride solution 1.0 mg/mL in methanol (as free base), ampule of 1 mL, certified reference material, Cerilliant®	**D-093**
3-Fluoromethcathinone hydrochloride solution 1.0 mg/mL in methanol (as free base), ampule of 1 mL, certified reference material, Cerilliant®	**F-016**
4-Fluoromethcathinone hydrochloride solution 1.0 mg/mL in methanol (as free base), ampule of 1 mL, certified reference material, Cerilliant®	**F-015**
4-Methylmetcathinone hydrochloride solution 1.0 mg/mL in methanol (as free base), ampule of 1 mL, certified reference material, Cerilliant®	**M-155**
Pentedrone hydrochloride 1.0 mg/mL in methanol (as free base), ampule of 1 mL, certified reference material, Cerilliant®	**P-087**

LC-MS (TOF) Analysis of Illicit Bath Salts in Urine on Ascentis® Express HILIC column after Solid Phase Extraction (SPE)

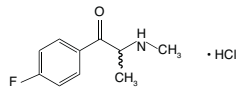
Bath salts are psychoactive designer drugs of the phenethylamine and cathinone families. Shown here is the rapid, sensitive LC-TOF-MS analysis of nine bath salts extracted from human urine using SPE and separated on an Ascentis Express HILIC column. Notice the lack of interfering peaks in the chromatogram demonstrating the effectiveness of the sample cleanup. CRMs were used to ensure reliable MS identification and quantification.



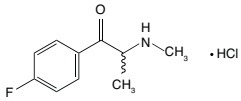
3,4-Methylenedioxypyrovalerone HCl (MDPV)



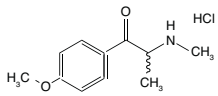
Buphedrone hydrochloride



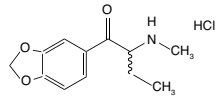
4-Fluoromethcathinone hydrochloride



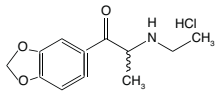
4-Fluoromethcathinone hydrochloride



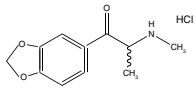
Methedrone hydrochloride



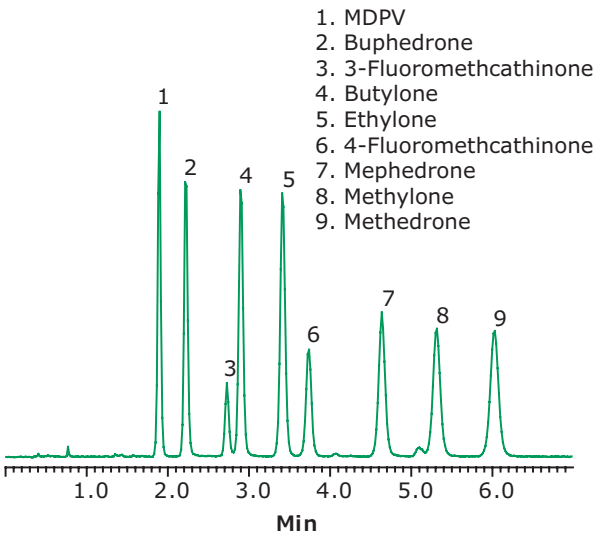
Butylone hydrochloride



Ethylone hydrochloride



Methylone hydrochloride



Chromatographic conditions

Column:	Ascentis® Express HILIC, 10 cm x 2.1 mm I.D., 2.7 µm particles (**53939-U**)
Mobile phase:	(A) 5 mM ammonium formate acetonitrile; (B) 5 mM ammonium formate water; (98:2, A:B); premixed
Flow rate:	0.6 mL/min
Column temp.:	35 °C
Pressure:	1842 psi (127 bar)
Injection:	1 µL
Detector:	MS, ESI(+), TIC, m/z 100-1000
Sample:	200 µg/L ea. in acetonitrile; urine samples spiked at 100 ng/mL with each target analyte (To ensure full ionization of the analytes, spiked samples were treated with formic acid to a final concentration of 0.1% formic acid.)
SPE:	Discovery® DSC-MCAX, 100 mg/1mL (**52782-U**)
Condition:	1 mL 1% formic acid acetonitrile then 1 mL water
Sample addition:	1 mL spiked water blank or urine
Washing:	1 mL water, 1 mL 1% formic acid in acetonitrile, 1 mL water
Elution:	2 mL 10% ammonium hydroxide in acetonitrile
Eluate post-treatment:	thoroughly mix via vortex agitation, evaporate 1 mL aliquot to dryness, reconstitute in 100 µL water:methanol

Ascentis® Express HILIC

Material list	Cat. No.
Ascentis® Express HILIC, 2.7 µm HPLC Column 2.7 µm particle size, L × I.D. 10 cm × 2.1 mm	53939-U
Discovery® DSC-MCAX, 100 mg/1mL, SPE tube	52782-U
Acetonitrile hypergrade for LC-MS LiChrosolv®	100029
Methanol hypergrade for LC-MS LiChrosolv®	106035
Methanol gradient grade for liquid chromatography LiChrosolv® Reag. Ph Eur	106007
Ethanol gradient grade for liquid chromatography LiChrosolv®	111727
Water for chromatography (LC-MS Grade) LiChrosolv®	115333
Ultrapure water from Milli-Q® IQ 7 series water purification system	ZIQ7005T0C
Acetic acid 100% for LC-MS LiChropur™	533001
Ammonium acetate for mass spectrometry, LiChropur™, eluent additive for LC-MS	73594
Ammonium formate eluent additive for LC-MS, LiChropur™, ≥99.0%	70221
Formic acid 98% - 100% for LC-MS LiChropur™	533002
Potassium dihydrogen phosphate anhydrous for HPLC LiChropur™	543841
Triethylammonium acetate buffer for HPLC, 0.98-1.02 M	69372
Mephedrone hydrochloride	M3449
Mephedrone hydrochloride solution 1.0 mg/mL in methanol (as free base), ampule of 1 mL, certified reference material, Cerilliant®	M-138
Methedrone hydrochloride ≥98% (HPLC)	M3699
Methedrone hydrochloride solution 1.0 mg/mL in methanol (as free base), ampule of 1 mL, certified reference material, Cerilliant®	M-147
Buphedrone hydrochloride solution 1.0 mg/mL in methanol (as free base), ampule of 1 mL, certified reference material, Cerilliant®	B-047
Butylone hydrochloride solution 1.0 mg/mL in methanol (as free base), ampule of 1 mL, certified reference material, Cerilliant®	B-045
Ethylone hydrochloride 1.0 mg/mL in methanol (as free base), ampule of 1 mL, certified reference material, Cerilliant®	E-071
3-Fluoromethcathinone hydrochloride solution 1.0 mg/mL in methanol (as free base), ampule of 1 mL, certified reference material, Cerilliant®	F-016
4-Fluoromethcathinone hydrochloride solution 1.0 mg/mL in methanol (as free base), ampule of 1 mL, certified reference material, Cerilliant®	F-015
3,4-Methylenedioxypyrovalerone hydrochloride ≥98% (HPLC)	SML0194
3,4-Methylenedioxypyrovalerone HCl (MDPV) solution 1.0 mg/mL in methanol (as free base), ampule of 1 mL, certified reference material, Cerilliant®	M-146
Methylone hydrochloride 1.0 mg/mL in methanol (as free base), ampule of 1 mL, certified reference material, Cerilliant®	M-140

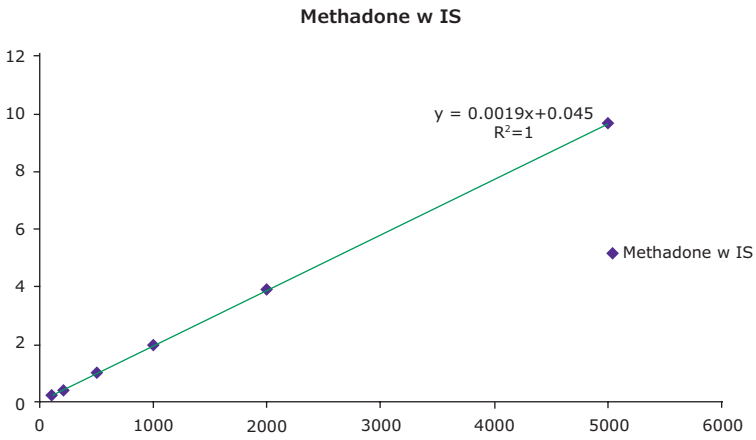
SPE

DART-MS Analysis of Drugs of Abuse in Human Urine Using C18 SPME LC (SPE-it) Tips

Economical and user-friendly sample prep method for DART-MS detection and identification of drugs of abuse in urine.

Urine Summary Full Scan

	R^2 (100–5,000 ng/mL) Linear 1/×	% Recovery 200 ng/mL Spike	% RSD 200 ng/mL Spike	% Recovery 2,000 ng/mL Spike	% RSD 2,000 ng/mL Spike
EDDP	0.998	104	5.1	96	10.4
Cocaine	0.9935	102	7.1	87.6	5.3
Methadone	0.9998	97.5	3.4	103	3.7
Cocaethylene	0.9999	97.6	2.5	102	8.7



Analyte	Sim
Methadone	310.2
Methadone-d_3	313.2
EDDP	278.2
EDDP-d_3	281.2
Cocaine	304.2
Cocaine-d_3	307.2
Cocaethylene	318.2
Cocaethylene-d_3	321.2

Chromatographic conditions

Sample/matrix:	human urine
Desorption process:	Direct MS
Extraction:	60 min with 1 mL sample
SPME fiber:	C18 SPE-it Tips (**57264-U**)
Detector:	MS, Full scan 100-500 amu

Material list	Cat. No.
SPME C18 SPE-it Tips, tray of 96 ea	**57234-U**
Cocaethylene solution 1.0 mg/mL in acetonitrile, ampule of 1 mL, certified reference material, Cerilliant®	**C-010**
Cocaethylene-D3 solution 100 µg/mL in acetonitrile, ampule of 1 mL, certified reference material, Cerilliant®	**C-009**
Cocaine solution 1.0 mg/mL in acetonitrile, ampule of 1 mL, certified reference material, Cerilliant®	**C-008**
Cocaine-D3 solution 100 µg/mL in acetonitrile, ampule of 1 mL, certified reference material, Cerilliant®	**C-004**
EDDP-D3 perchlorate solution 100 µg/mL in methanol (as pyrrolinium), ampule of 1 mL, certified reference material, Cerilliant®	**E-021**
EDDP perchlorate solution 1.0 mg/mL in methanol (as pyrolinium), ampule of 1 mL, certified reference material, Cerilliant®	**E-022**
(±)-Methadone solution 1 mg/mL in methanol, ampule of 1 mL, certified reference material, Cerilliant®	**M-007**
(±)-Methadone-D3 solution 100 µg/mL in methanol, ampule of 1 mL, certified reference material, Cerilliant®	**M-008**

Amphetamine

Amphetamine is a central nervous system stimulant that is used in the treatment of attention deficit hyperactivity disorder (ADHD), narcolepsy and obesity.

Amphetamine increases monoamine and excitatory neurotransmission in the brain, with its most pronounced effects targeting the norepinephrine and dopamine neurotransmitter systems.

At therapeutic doses, amphetamine causes emotional and cognitive effects such as euphoria, change in desire for sex, increased wakefulness, and improved cognitive control. It induces physical effects such as improved reaction time, fatigue resistance, and increased muscle strength. Larger doses of amphetamine may impair cognitive function and induce rapid muscle breakdown.

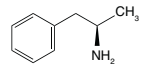
Addiction is a serious risk with heavy recreational amphetamine use but is unlikely to occur from long-term medical use at therapeutic doses.

Amphetamine is a methyl homolog of the mammalian neurotransmitter phenethylamine. The carbon atom adjacent to the primary amine is a stereogenic center, and amphetamine is composed of a racemic 1:1 mixture of two enantiomers.

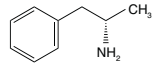
- LC-MS Analysis of Amphetamine Enantiomers on Astec CHIROBIOTIC® V2 in Urine after Solid Phase Extraction (SPE)
- LC-MS Analysis of Methamphetamine Enantiomers on Astec® CHIROBIOTIC® V2 in Urine after SPE
- LC-MS Analysis of Methamphetamine Enantiomers on Astec® CHIROBIOTIC® V2 in Urine following Liquid/Liquid Extraction
- HPLC Analysis of Amphetamines in Urine on Discovery® HS F5 after SPE using Discovery® DSC-MCAX and Standard C18
- GC Analysis of Amphetamines in Urine after SPME

LC-MS Analysis of Amphetamine Enantiomers on Astec® CHIROBIOTIC® V2 in Urine after Solid Phase Extraction (SPE)

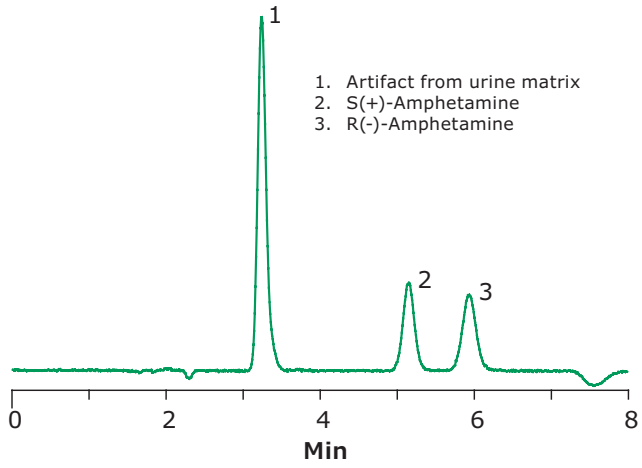
Shown here is the chiral separation of amphetamine enantiomers under MS-compatible conditions on Astec® CHIROBIOTIC® V2 after extraction from urine using SPE. This chiral separation is important because it enables discrimination between legal and illicit sources of drugs. The highest grade mobile phase solvents and additives were used to supply low background interference and low particulate contaminants for robust, trouble-free operation. CRMs provided reliable identification and quantification.



R(-)-Amphetamine



S(+)-Amphetamine



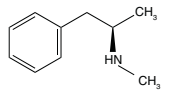
Chromatographic conditions

Column:	Astec® CHIROBIOTIC® V2, 15 cm x 4.6 mm I.D., 5 µm particles (**15023AST**)
Column temp.:	20 °C
Mobile phase:	[A] methanol; [B] water; [C] acetic acid; [D] ammonium hydroxide; (95:5:0.1:0.02, A:B:C:D)
Flow rate:	1 mL/min
Pressure:	1220 psi (84 bar)
Injection:	2 µL
Detector:	MS, ESI(+),SIR m/z 150.1
Sample preparation:	SPE (Solid Phase Extraction)
Sample/matrix:	1 mL of synthetic urine spiked with 50 ng/mL of each enantiomer
SPE tube/ cartridge:	Recommended: Discovery® DSC-MCAX, 100 mg/1mL (**52782-U**)
Condition:	1 mL 1% formic acid in acetonitrile followed by 1 mL water
Sample addition:	1 mL spiked urine sample
Washing:	2 mL water followed by 1 mL 5 mM ammonium phosphate dibasic in 50% methanol followed by 1 mL 25% v/v methanol in water
Elution:	1 mL 10% w/v ammonium hydroxide in acetonitrile
Eluate post-treatment:	evaporate to dryness under nitrogen at 40 °C, reconstitute in 1 mL mobile phase

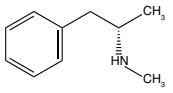
Material list	Cat. No.
Astec® CHIROBIOTIC® V2 Chiral HPLC Column 5 µm particle size, L × I.D. 15 cm × 4.6 mm	**15023AST**
Discovery® DSC-MCAX, 100 mg/1mL, SPE tube	**52782-U**
Acetonitrile hypergrade for LC-MS LiChrosolv®	**100029**
Methanol hypergrade for LC-MS LiChrosolv®	**106007**
Methanol gradient grade for liquid chromatography LiChrosolv® Reag. Ph Eur	**106035**
Ethanol gradient grade for liquid chromatography LiChrosolv®	**111727**
Water for chromatography (LC-MS Grade) LiChrosolv®	**115333**
Ultrapure water from Milli-Q® IQ 7 series water purification system	**ZIQ7005T0C**
Acetic acid 100% for LC-MS LiChropur™	**533001**
Ammonium acetate for mass spectrometry, LiChropur™, eluent additive for LC-MS	**73594**
Ammonium formate eluent additive for LC-MS, LiChropur™, ≥99.0%	**70221**
Ammonium Hydroxide Meets ACS Specifications, Meets Reagent Specifications for testing USP/NF monographs GR ACS	**AX1303**
Formic acid 98% - 100% for LC-MS LiChropur™	**533002**
Potassium dihydrogen phosphate anhydrous for HPLC LiChropur™	**543841**
Triethylammonium acetate buffer for HPLC, 0.98-1.02 M	**69372**
S(+)-Amphetamine (dextro-Amphetamine) solution 1.0 mg/mL in methanol, ampule of 1 mL, certified reference material, Cerilliant®	**A-008**
R(-)-Amphetamine (levo-Amphetamine) 1.0 mg/mL in methanol, ampule of 1 mL, certified reference material, Cerilliant®	**A-049**

Astec® CHIROBIOTIC® V2

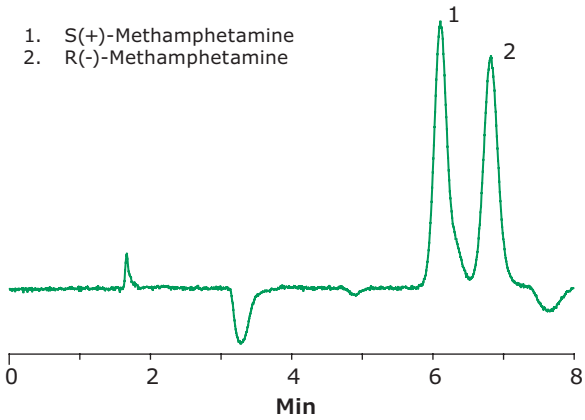
LC-MS Analysis of Methamphetamine Enantiomers on Astec® CHIROBIOTIC® V2 in Urine after SPE



R(-)-Methamphetamine



S(+)-Methamphetamine



Chromatographic conditions

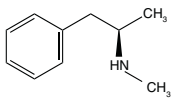
Column:	Astec® CHIROBIOTIC® V2, 15 cm x 4.6 mm I.D., 5 µm particles (**15023AST**)
Mobile phase:	[A] methanol; [B] water; [C] acetic acid; [D] ammonium hydroxide; (95:5:0.1:0.02, A:B:C:D)
Flow rate:	1.0 mL/min
Column temp.:	20 °C
Pressure:	1221 psi (84 bar)
Injection:	2 µL
Detector:	MS, ESI(+),SIR m/z 150.1
Sample:	1.0 mL urine
Sample preparation:	SPE (Solid Phase Extraction)
Sample/matrix:	1 mL of synthetic urine spiked with 25 ng/mL
Extraction process:	acidified to 3-4 pH with formic acid
SPE tube/ cartridge:	Discovery DSC-MCAX, 100 mg/1mL (**52782-U**)
Condition:	1 mL 1% formic acid in acetonitrile, then 1 mL water
Washing:	2 mL water, then 1 mL 25% methanol
Elution:	1 mL 10% ammonium hydroxide in acetonitrile
Eluate post-treatment:	evaporate to dryness under nitrogen at 40 °C, reconstitute in 1 mL mobile phase

Material list	Cat. No.
Astec® CHIROBIOTIC® V2 Chiral HPLC Column 5 µm particle size, L × I.D. 15 cm × 4.6 mm	15023AST
Discovery® DSC-MCAX, 100 mg/1mL, SPE tube	52782-U
Acetonitrile hypergrade for LC-MS LiChrosolv®	100029
Methanol hypergrade for LC-MS LiChrosolv®	106007
Methanol gradient grade for liquid chromatography LiChrosolv® Reag. Ph Eur	106035
Ethanol gradient grade for liquid chromatography LiChrosolv®	111727
Water for chromatography (LC-MS Grade) LiChrosolv®	115333
Ultrapure water from Milli-Q® IQ 7 series water purification system	ZIQ7005T0C
Acetic acid 100% for LC-MS LiChropur™	533001
Ammonium acetate for mass spectrometry, LiChropur™, eluent additive for LC-MS	73594
Ammonium formate eluent additive for LC-MS, LiChropur™, ≥99.0%	70221
Ammonium Hydroxide Meets ACS Specifications, Meets Reagent Specifications for testing USP/NF monographs GR ACS	AX1303
Formic acid 98% - 100% for LC-MS LiChropur™	533002
Potassium dihydrogen phosphate anhydrous for HPLC LiChropur™	543841
Triethylammonium acetate buffer for HPLC, 0.98-1.02 M	69372
Levmetamfetamine United States Pharmacopeia (USP) Reference Standard	1359506
R(-)-Methamphetamine solution 1.0 mg/mL in methanol, ampule of 1 mL, certified reference material, Cerilliant®	M-024
S(+)-Methamphetamine solution 1.0 mg/mL in methanol, ampule of 1 mL, certified reference material, Cerilliant®	M-020

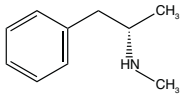
SPE

Astec® CHIROBIOTIC® V2

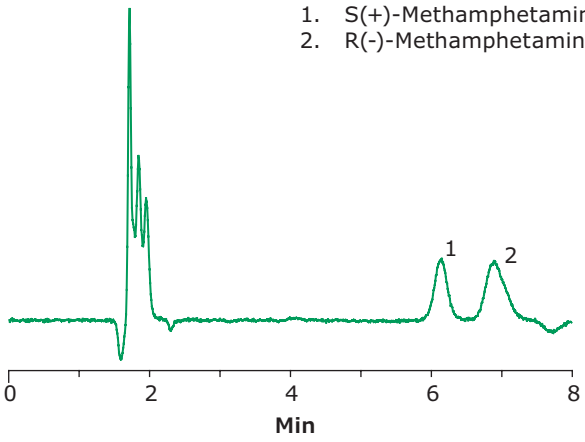
LC-MS Analysis of Methamphetamine Enantiomers on Astec® CHIROBIOTIC® V2 in Urine following Liquid/ Liquid Extraction



R(-)-Methamphetamine



S(+)-Methamphetamine

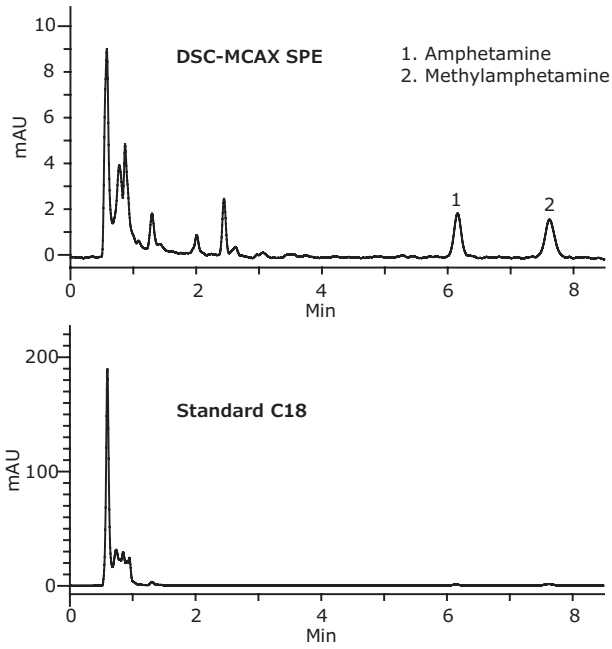


Column:	Astec® CHIROBIOTIC® V2, 15 cm x 4.6 mm I.D., 5 µm particles (**15023AST**)
Column temp.:	20 °C
Mobile phase:	[A] methanol; [B] water; [C] acetic acid; [D] ammonium hydroxide; (95:5:0.1:0.02, A:B:C:D)
Flow rate:	1.0 mL/min
Pressure:	1220 psi (84 bar)
Injection:	2.0 µL
Detector:	MS, ESI(+),SIR m/z 150.1
Sample preparation:	125 µL urine spiked at 125 ng/mL each enantiomer, add 1 mL diethyl ether, mix/ vortex 30 min, centrifuge at 10,000 rpm for 10 min. remove 500 µL aliquot of organic (upper) layer, (evaporate to dryness under nitrogen at 55 °C, reconstitute in 500 µL mobile phase, filter through 0.22 µm PVDF membrane (**Z227498**))

Material list	Cat. No.
Astec® CHIROBIOTIC® V2 Chiral HPLC Column 5 µm particle size, L × I.D. 15 cm × 4.6 mm	**15023AST**
Acetonitrile hypergrade for LC-MS LiChrosolv®	**100029**
Methanol hypergrade for LC-MS LiChrosolv®	**106007**
Methanol gradient grade for liquid chromatography LiChrosolv® Reag. Ph Eur	**106035**
Ethanol gradient grade for liquid chromatography LiChrosolv®	**111727**
Water for chromatography (LC-MS Grade) LiChrosolv®	**115333**
Acetic acid 100% for LC-MS LiChropur™	**533001**
Ammonium acetate for mass spectrometry, LiChropur™, eluent additive for LC-MS	**73594**
Ammonium formate eluent additive for LC-MS, LiChropur™, ≥99.0%	**70221**
Ammonium Hydroxide Meets ACS Specifications, Meets Reagent Specifications for testing USP/NF monographs GR ACS	**AX1303**
Formic acid 98% - 100% for LC-MS LiChropur™	**533002**
Potassium dihydrogen phosphate anhydrous for HPLC LiChropur™	**543841**
Triethylammonium acetate buffer for HPLC, 0.98-1.02 M	**69372**
Levmetamfetamine United States Pharmacopeia (USP) Reference Standard	**1359506**
R(-)-Methamphetamine solution 1.0 mg/mL in methanol, ampule of 1 mL, certified reference material, Cerilliant®	**M-024**
S(+)-Methamphetamine solution 1.0 mg/mL in methanol, ampule of 1 mL, certified reference material, Cerilliant®	**M-020**

Astec® CHIROBIOTIC® V2

HPLC Analysis of Amphetamines in Urine on Discovery® HS F5 after SPE using Discovery® DSC-MCAX and Standard C18



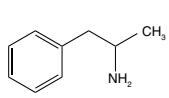
Note the Y-axis scale difference between DSC-MCAX and C18 SPE. DSC-MCAX SPE offered a maximum background height of ~9 mAU.

In contrast, standard C18 background levels were 20 times greater than DSC-MCAX.

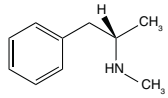
Also, on DSC-MCAX absolute recovery averaged at 100.3 and 101.7%, for amphetamine and methylamphetamine, respectively.

On standard C18, absolute recovery averaged at 48 and 79% for the two compounds.

Column:	Discovery® HS F5, 15 cm x 4.6 mm I.D., 5 µm particles (**567516-U**)
Mobile phase:	[A] 10 mM ammonium acetate, pH 4.5: [B] methanol; (35:65, A:B)
Flow rate:	2 mL/min
Column temp.:	40 °C
Injection:	10 µL
Detector:	UV, 210 nm
Sample preparation:	SPE (Solid Phase Extraction)
Sample/matrix:	human urine spiked amphetamine and methylamphetamine at with 2 mg/mL
SPE tube/cartridge:	Discovery® DSC-MCAX, 100 mg/3mL (**52783-U**)
Condition:	DSC-MCAX SPE tube: 1 mL methanol; 1 mL 50 mM ammonium acetate, pH 6.0; C-18 SPE tube: 1 mL methanol; 1 mL DI water
Sample addition:	1 mL
Washing:	DSC-MCAX SPE tube: 1 mL 50 mM ammonium acetate, pH 6.0; 1 mL 1M acetic acid; 1mL methanol; C-18 SPE tube: 1 mL DI water; 1 mL 20% methanol
Elution:	DSC-MCAX SPE tube: 1 mL 5% ammonium hydroxide in methanol; C-18 SPE tube: 1 mL methanol
Eluate post-treatment:	evaporate to dryness with nitrogen at room temperature, reconstitute in mobile phase
Sample Pre-Treatment:	1 mL human urine was spiked with 2 mg/mL amphetamine and methylamphetamine. The spiked sample was diluted 1:1 with 1:1 with 50 mM ammonium acetate, pH 6.0.



Amphetamine

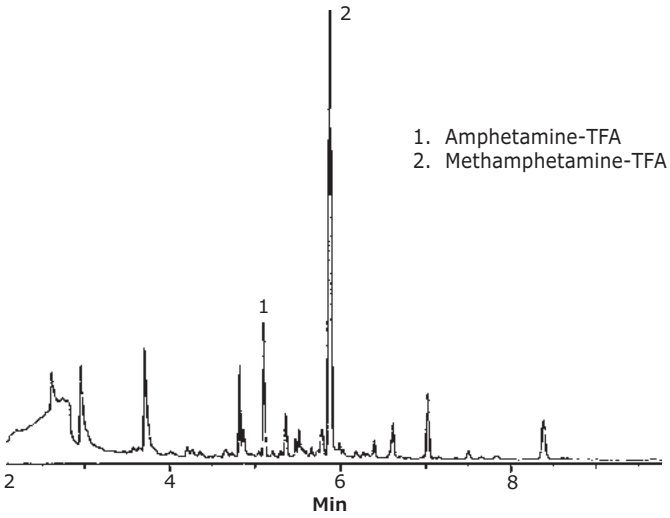


Methylamphetamine

Material list	Cat. No.
Discovery® HS F5 HPLC Column 5 µm particle size, L × I.D. 15 cm × 4.6	567516-U
Discovery® DSC-MCAX SPE Tube bed wt. 100 mg, volume 3 mL, pk of 5	52783-U
Acetonitrile hypergrade for LC-MS LiChrosolv®	100029
Ethanol gradient grade for liquid chromatography LiChrosolv®	111727
Methanol hypergrade for LC-MS LiChrosolv®	106035
Methanol gradient grade for liquid chromatography LiChrosolv® Reag. Ph Eur	106007
Water for chromatography (LC-MS Grade) LiChrosolv®	115333
Acetic acid 100% for LC-MS LiChropur™	533001
Ammonium acetate for mass spectrometry, LiChropur™, eluent additive for LC-MS	73594
Ammonium formate eluent additive for LC-MS, LiChropur™, ≥99.0%	70221
Formic acid 98% - 100% for LC-MS LiChropur™	533002
Potassium dihydrogen phosphate anhydrous for HPLC LiChropur®	543841
Triethylammonium acetate buffer for HPLC, 0.98-1.02 M	69372

Discovery® HS F5

GC-MS Analysis of Amphetamines in Urine after SPME



Column:	Equity®-1 Capillary GC Column, 12.5 m x 0.2 mm I.D., 0.33 µm (**28041-U**)
Oven:	60 °C (1 min) to 140 °C (4 min) at 30 °C/min, then to 276 C at 20 °C/min, 4 min
Inj. temp.:	splitless (closed 1 min), 270 °C
Detector:	MS, full scan
Sample:	1 mL urine + 0.7g potassium carbonate in 20 mL headspace vial, equilibrated at 80 °C, 30 min
Derivatization:	methyl bis-trifluoroacetamide (headspace, 0.5 min, ambient)
Desorption process:	1 min, 270 °C
Extraction:	headspace, 3-5 min, 80 °C
SPME fiber:	polydimethylsiloxane, 100 µm (**57300-U**)

Material list	Cat. No.
Equity®-1 Capillary GC Column, 12.5 m x 0.2 mm I.D., 0.33 µm	**28041-U**
SPME fiber assembly Polydimethylsiloxane (PDMS) df 100 µm, needle size 24 ga, for use with manual holder	**57300-U**

GC Columns

SPME

Alcohol

Alcohol is one of the oldest and most common recreational substances that causes intoxication

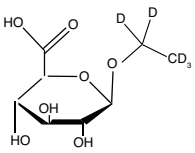
Among other effects, alcohol produces happiness and euphoria, decreased anxiety, increased sociability, sedation, impairment of cognitive, memory, motor, and sensory function, and generalized depression of central nervous system function.

Alcohol has been produced and consumed by humans for its psychoactive effects for almost 10,000 years. Drinking alcohol is generally socially acceptable and is legal in most countries, unlike with many other recreational substances. However, there are often restrictions on alcohol sale and use, for instance, a minimum age for drinking and laws against public drinking, and drinking and driving.

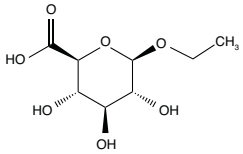
- LC-MS Analysis of Ethanol Metabolites in Diluted Urine on Ascentis® Express OH5 using Deuterated Internal Standards
- LC-MS Analysis of Phosphatidylethanol Metabolites on Ascentis® Express C18
- LC-MS/MS Determination of Ethyl glucuronide and Ethyl sulfate in Urine

LC-MS Analysis of Ethanol Metabolites in Diluted Urine on Ascentis® Express OH5 using Deuterated Internal Standards

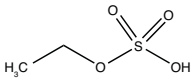
Ethyl sulphate (EtS) and ethyl glucuronide (EtG) are direct ethanol metabolites and may indicate recent alcohol consumption. The two compounds differ in their pathways for formation and degradation. Being polar compounds, they are poorly retained by C18 phases and elute early in the chromatogram along with matrix compounds. This aspect leads to poor or unreliable quantification. This application employs HILIC on an Ascentis® Express OH5 column to retain both analytes well, resulting in a robust and reliable, as well as highly MS-friendly. CRMs provided reliable quantification. The internal standard was needed for accurate quantification. External calibration resulted in a large excess of sulfate.



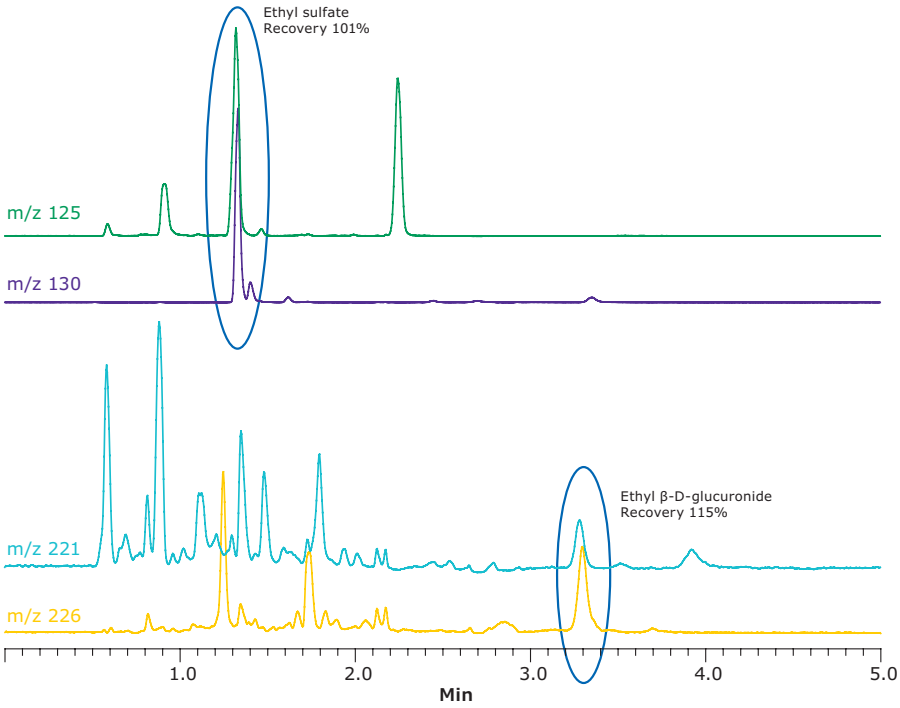
Ethyl-β-D-glucuronide-(ethyl-d5)



Ethyl-β-D-glucuronide



Ethyl sulfate (EtS)



Chromatographic conditions

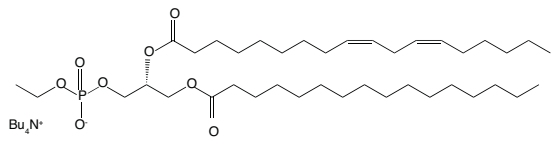
Column:	Ascentis® Express OH5, 10 cm x 2.1 mm I.D., 2.7 µm particles (**53757-U**)
Mobile phase:	[A] 5 mM ammonium formate and 0.1% formic acid in 95:5, acetonitrile:water; [B] 5 mM ammonium formate and 0.1% formic acid in 80:20, acetonitrile:water
Gradient:	0 to 100% B in 1 min, held at 100% B for 4 min, to 0% B, held for 5 min
Flow rate:	0.4 mL/min
Column temp.:	30 °C
Pressure:	1450 psi (100 bar)
Injection:	5 µL
Sample:	urine spiked with ethyl sulfate and ethyl-β-D-glucuronide, both at 50 ng/mL, diluted 20:1 in acetonitrile containing deuterated internal standards at 100 ng/mL

Ascentis® Express OH5

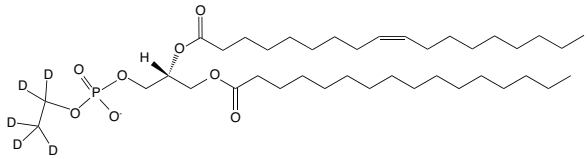
Material list	Cat. No.
Ascentis® Express OH5, 2.7 µm HPLC Column 2.7 µm particle size, L × I.D. 10 cm × 2.1 mm	53757-U
Acetonitrile hypergrade for LC-MS LiChrosolv®	100029
Methanol hypergrade for LC-MS LiChrosolv®	106035
Methanol gradient grade for liquid chromatography LiChrosolv® Reag. Ph Eur	106007
Ethanol gradient grade for liquid chromatography LiChrosolv®	111727
Water for chromatography (LC-MS Grade) LiChrosolv®	115333
Ultrapure water from Milli-Q® IQ 7 series water purification system	ZIQ7005T0C
Acetic acid 100% for LC-MS LiChropur™	533001
Ammonium acetate for mass spectrometry, LiChropur™, eluent additive for LC-MS	73594
Ammonium formate eluent additive for LC-MS, LiChropur™, ≥99.0%	70221
Formic acid 98% - 100% for LC-MS LiChropur™	533002
Potassium dihydrogen phosphate anhydrous for HPLC LiChropur™	543841
Triethylammonium acetate buffer for HPLC, 0.98-1.02 M	69372
Ethyl-β-D-glucuronide-(ethyl-d5) 1.0 mg/mL in methanol, ampule of 1 mL, certified reference material, Cerilliant®	E-063
Ethyl-β-D-glucuronide-(ethyl-d5) 100 µg/mL in methanol, ampule of 1 mL, certified reference material, Cerilliant®	E-048
Ethyl-β-D-glucuronide 1.0 mg/mL in methanol, ampule of 1 mL, certified reference material, Cerilliant®	E-015
Ethyl-β-D-glucuronide 100 µg/mL in methanol, ampule of 1 mL, certified reference material, Cerilliant®	E-016
Ethyl sulfate sodium salt 1.0 mg/mL in methanol (as ethyl sulfate), ampule of 1 mL, certified reference material, Cerilliant®	E-064
Ethyl sulfate sodium salt 10 mg/mL in methanol: water (1:1), certified reference material, ampule of 1 mL, Cerilliant®	E-116

LC-MS Analysis of Phosphatidylethanol Metabolites on Ascentis® Express C18

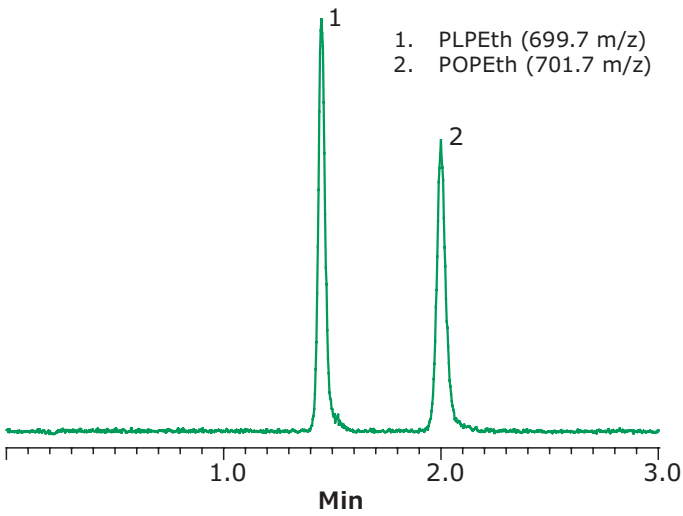
Phosphatidylethanol and its metabolites in blood are markers of ethanol consumption. Shown here is the rapid resolution of the metabolites on an Ascentis® Express C18 column.



PLPEth



POPEth



Chromatographic conditions

Column:	Ascentis® Express C18, 5 cm x 2.1 mm I.D., 2.0 µm particles (**50811-U**)
Mobile phase:	5 mM ammonium formate in [A] water; [B] methanol; [C] acetonitrile; (5:47.5:47.5, A:B:C)
Flow rate:	0.5 mL/min
Column temp.:	50 °C
Pressure:	2760 psi (190 bar)
Sample:	500 ng/mL in 50:50, water:acetonitrile
Injection:	2 µL
Detector:	MS, ESI-, combined SIR: 699.7, 701.7 m/z

Material list	Cat. No.
Ascentis® Express C18, 2 µm UHPLC Column 2 µm particle size, L × I.D. 5 cm × 2.1 mm	**50811-U**
Acetonitrile gradient grade for liquid chromatography LiChrosolv® Reag. Ph Eur	**100030**
Methanol gradient grade for liquid chromatography LiChrosolv® Reag. Ph Eur	**106007**
Water for chromatography (LC-MS Grade) LiChrosolv®	**115333**
Ultrapure water from Milli-Q® IQ 7 series water purification system	**ZIQ7005T0C**
Ammonium formate eluent additive for LC-MS, LiChropur™, ≥99.0%	**70221**

Ascentis® Express C18

LC-MS/MS Determination of Ethyl glucuronide and Ethyl sulfate in Urine on SeQuant® ZIC®-HILIC

Ethyl glucuronide (EtG) is a metabolite of ethyl alcohol that is formed in the body by glucuronidation following exposure to ethanol, for example by drinking alcoholic beverages. The usefulness of EtG as a recent alcohol consumption biomarker has been studied widely. A disadvantage of the test is that because EtG can be detected in samples at very low levels, it can also show positive results following exposure to alcohol from non-beverage sources, or incidental exposure, leading to false-positive results. Studies have found that EtG can only be formed after alcohol ingestion and is not formed endogenously. Therefore, the presence of EtG is definitive evidence of alcohol intake prior to post-mortem investigations. However, negative results of EtG should be interpreted with caution as false-negative results may be obtained. As there is a time lag between alcohol present in blood and EtG production, any results should be interpreted with caution as false-negative results may be obtained if death happened shortly after alcohol consumption. The stability of EtG has been brought into question after it was found that it can be degraded by bacteria. For this reason, ethylsulfate (EtS) has been introduced as a complementary marker with EtG due to its stable pattern and resistance to bacterial degradation; the presence of EtG and EtS provides strong evidence of recent alcohol consumption. An alternative marker for ethanol intake is phosphatidylethanols (PEth), a group of phospholipids formed only in the presence of ethanol via the action of phospholipase D.

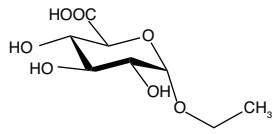
EtG and EtS are promising biomarkers because they are phase two ethanol metabolites and their excretion profiles have been studied and documented. The following method was aimed at developing and validating an LC-ESI-ion trap-MS/MS method for the identification and quantification of EtG and EtS as ethanol biomarkers from urine samples. The method provides good chromatographic separation, with adequate peak shapes for easy data interpretation. The method also provides baseline separation of the two biomarkers in less than seven minutes, and with low limit of quantitation (LOQ)

Chromatographic conditions

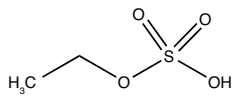
Column:	SeQuant® ZIC®-HILIC (3.5 µm, 200 Å) 150 × 2.1 mm ID (**150448**)
Mobile Phase:	A: Acetonitrile B: Ammonium acetate 5 mM, pH 6.8
Gradient:	

Time (min)	A (%)	B (%)	Flow rate (mL/min)
0	90	10	0.2
3	90	10	0.2
3.01	90	10	0.4
4	70	30	0.4
7	70	30	0.2
12	50	50	0.2
12.01	90	10	0.2
20	90	10	0.2

Flow rate:	See table
Temperature:	Column: 25 °C Autosampler: 4 °C
Injection volume:	5 µL
Diluent:	Mobile Phase
Detection:	LC-ESI ion trap MS/MS; see table for precursor and product ion information
Sample:	Urine samples treated according to in-house sample preparation protocol: 1. Following addition of pentadeuterated internal standards for EtG and EtS, 200 µL of acetonitrile was added to 0.1 mL of urine and centrifuged at 10000 rpm. 2. The supernatant was then evaporated before reconstituting with 100 µL of initial mobile phase prior to LC-MS/MS analysis. The full procedure can be found in the paper "Direct Determination of Ethyl Glucuronide and Ethyl Sulfate in Postmortem Urine Specimens Using Hydrophilic Interaction Liquid Chromatography-Electrospray Ionization-Tandem Mass Spectrometry " A.I. Al-Asmari et al. J. Anal. Tox. 34 (2010) 261-272

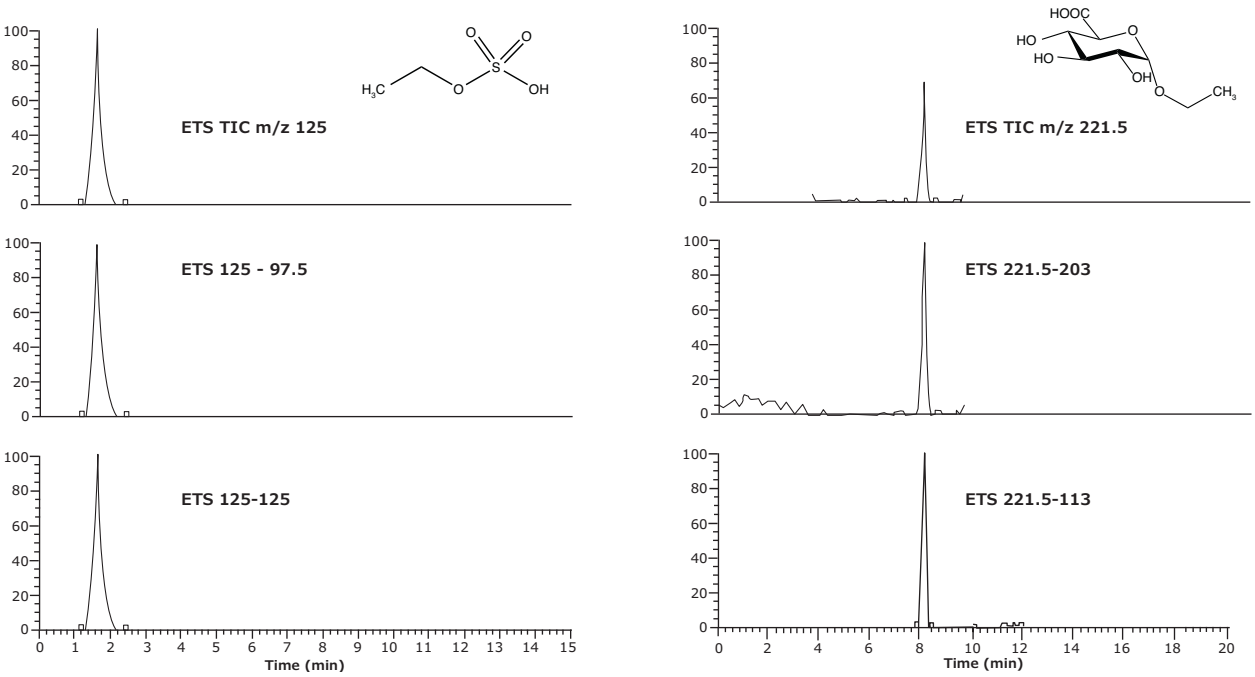


Ethyl glucuronide (EtG)



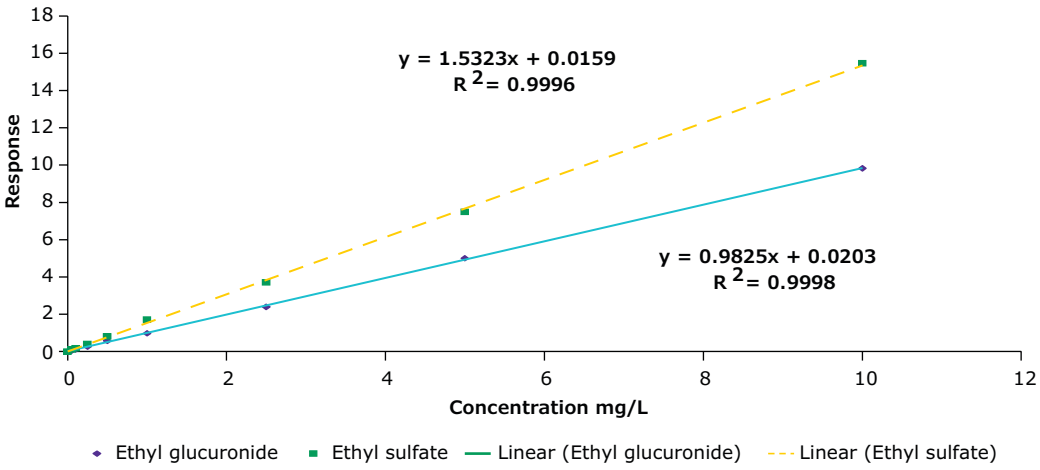
Ethyl sulfate (EtS)

SeQuant® ZIC®-HILIC



Chromatographic Data

	Compound	Retention Time (min)	Precursor ion (m/z)	Product ions (m/z)
	Void volume	1.3	-	
1	Ethyl sulfate (EtS)	1.7	125.5	97.5
2	Ethyl glucuronide (EtG)	6.7	221.5	103, 113



Parameter	Ethyl Glucuronide	Ethyl Sulfate
Precursor ion (m/z)	221.5	125.5
Product ion(s) (m/z)	103, 113	97.5
Quantifier ion (m/z)	221.5 -103	125.5 - 97.5
Qualifier ion (m/z)	221.5 -113	125.5 - 125.5
Internal standard (IS)	Ethyl glucuronide-d5	Ethyl sulfate-d5
IS precursor ion(s) (m/z)	226.5	130.5
Product ion (m/z)	208	98.5
Sheath gas (AU)	15	10
Auxiliary gas (AU)	10	
Capillary temperature (°C)	275	
Collision energy (%)	32	30
LLOQ (mg/L)	0.001	0.001
Cut off value (mg/L)	1	0.1

Material list	Cat. No.
SeQuant® ZIC®-HILIC 3.5 µm, 200Å 150 × 2.1 mm ID	**150448**
SeQuant® ZIC®-HILIC Guard Fitting 14 × 1.0 mm ID	**150434**
Acetonitrile hypergrade for LC-MS LiChrosolv®	**100029**
Water for chromatography (LC-MS grade) LiChrosolv®	**115333**
Ultrapure water from Milli-Q® IQ 7 series water purification system	**ZIQ7005T0C**
Ammonium acetate for mass spectrometry, LiChropur™, eluent additive for LC-MS, or in-situ prepared buffer from ammonia and acetic acid	**73594**
Ammonia solution 28-30% for analysis EMSURE® ACS, Reag. Ph Eur	**105423**
Acetic acid 96% for analysis EMSURE®	**100062**
Formic Acid 98%-100% for LC-MS LiChropur®	**533002**
Ethyl sulfate sodium salt, 1.0 mg/mL in methanol (as ethyl sulfate), ampule of 1 mL, certified reference material, Cerilliant®	**E-064**
Ethyl sulfate sodium salt, 10 mg/mL in methanol: water (1:1), certified reference material, ampule of 1 mL, Cerilliant®	**E-116**
Ethyl-d5 sulfate sodium salt 1.0 mg/mL in methanol (as ethyl sulfate), ampule of 1 mL, certified reference material, Cerilliant®	**E-066**
Ethyl-β-D-glucuronide 100 µg/mL in methanol, ampule of 1 mL, certified reference material, Cerilliant®	**E-016**
Ethyl-β-D-glucuronide 1.0 mg/mL in methanol, ampule of 1 mL, certified reference material, Cerilliant®	**E-015**
Ethyl-β-D-glucuronide-(ethyl-d5) 100 µg/mL in methanol, ampule of 1 mL, certified reference material, Cerilliant®	**E-048**
Ethyl-β-D-glucuronide-(ethyl-d5) 1.0 mg/mL in methanol, ampule of 1 mL, certified reference material, Cerilliant®	**E-063**

Z-drugs

Z-drugs or Nonbenzodiazepines are a class of psychoactive drugs that are very benzodiazepine-like in nature. These compounds are used in the treatment of sleep problems.

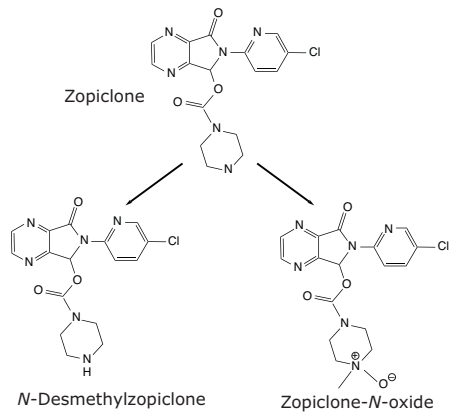
The Z-drugs are notable for producing side effects such as pronounced amnesia and more rarely hallucinations, especially when used in large doses.

It has been claimed that insomnia causes depression and hypothesized that insomnia medications may help to treat depression. In support of this claim, an analysis of data of clinical trials submitted to the Food and Drug Administration (FDA) concerning the drugs zolpidem, zaleplon, and eszopiclone found that these sedative hypnotic drugs more than doubled the risks of developing depression compared to those taking placebo pills. Z-drugs, have been associated with an increased risk of death.

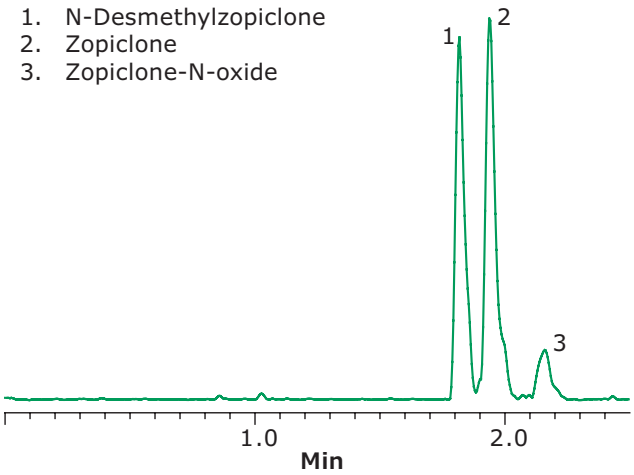
- LC-MS Analysis of Zopiclone and Metabolites in Urine on Ascentis® Express ES-Cyano 2.0 µm Column

LC-MS Analysis of Zopiclone and Metabolites in Urine on Ascentis® Express ES-Cyano 2.0 µm Column

Zopiclone is a nonbenzodiazepine hypnotic agent used in the treatment of insomnia. This drug is one of the so-called "Z-drugs." Because these drugs are additive and can be abused, their analysis in urine is necessary. Shown here is the rapid, sensitive analysis of zopiclone and its metabolites from urine on a Fused-Core® Ascentis® Express ES-Cyano Column with 2.0 µm particle size under UHPLC conditions.
CRMs provided reliable quantification.



1. N-Desmethylzopiclone
2. Zopiclone
3. Zopiclone-N-oxide



Column:	Ascentis® Express ES-Cyano, 10 cm x 3.0 mm I.D., 2.0 µm particles (**51732-U**)
Mobile phase:	[A] 10 mM ammonium formate pH 3.0 with formic acid; [B] acetonitrile; (70:30, A:B)
Flow rate:	0.4 mL/min
Column temp.:	25 °C
Pressure:	5300 psi (365 bar)
Injection:	10 µL
Sample:	1 ng/mL in urine:water:acetonitrile (10:80:10)
Detector:	MS, MRM, m/z 375/245, 389/245, 405/245
Sample preparation:	combine 100 µL urine spiked with zopiclone and metabolites at 10 ng/mL, 100 µL deuterated internal standards at 100 ng/mL in acetonitrile, and 800 µL water. centrifuge at 3000 rcf for 1 min.

Material list	Cat. No.
Ascentis® Express ES-Cyano, 2 µm UHPLC Column 2 µm particle size, L × I.D. 10 cm × 3 mm	51732-U
Acetonitrile hypergrade for LC-MS LiChrosolv®	100029
Methanol hypergrade for LC-MS LiChrosolv®	106035
Methanol gradient grade for liquid chromatography LiChrosolv® Reag. Ph Eur	106007
Ethanol gradient grade for liquid chromatography LiChrosolv®	111727
Water for chromatography (LC-MS Grade) LiChrosolv®	115333
Acetic acid 100% for LC-MS LiChropur™	533001
Ammonium acetate for mass spectrometry, LiChropur™, eluent additive for LC-MS	73594
Ammonium formate eluent additive for LC-MS, LiChropur™, ≥99.0%	70221
Formic acid 98% - 100% for LC-MS LiChropur™	533002
Potassium dihydrogen phosphate anhydrous for HPLC LiChropur™	543841
Triethylammonium acetate buffer for HPLC, 0.98-1.02 M	69372
Eszopiclone Related Compound A United States Pharmacopeia (USP) Reference Standard	1255861
Zopiclone	Z4900
Zopiclone solution 1.0 mg/mL in acetonitrile, ampule of 1 mL, certified reference material, Cerilliant®	Z-003
Zopiclone oxide European Pharmacopoeia (EP) Reference Standard	Z3001000
Zopiclone-N-oxide solution 100 µg/mL (Methanol with 1% 1 M HCl), ampule of 1 mL, certified reference material, Cerilliant®	Z-013

Ascentis® Express ES-Cyano

Recently published Scientific Reviews

1. Advances in drugs of abuse testing

Clinica Chimica Acta; 514 (2021) 40-47
Kenichi Tamama

2. After another decade: LC–MS/MS became routine in clinical diagnostics

Clinical Biochemistry, 82 (2020) 2-11
Christoph Seger and Linda Salzmann

3. Forensic applications of DART-MS: A review of recent literature

Forensic Chemistry, 22 (2021) 100294
Edward Sisco and Thomas P. Forbes

4. Determination of drugs and drug metabolites by ion mobility-mass spectrometry: A review

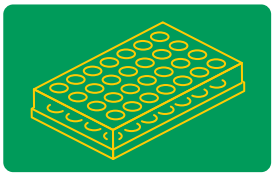
Analytica Chimica Acta, 1154 (2021) 338270
Dylan H.Ross and Libin Xu

Workflow

Sample Collection

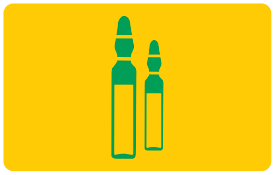
- Filtration Devices
- Liquid Handling
- Multi-well Plates
- Centrifuge Filters
- Analytical Vials

Sample Preparation



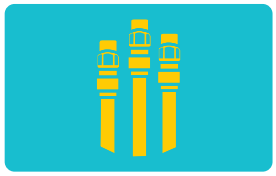
- Solid Phase Extraction (SPE) & QuEChERS
- Solid Phase Micro-Extraction (SPME) Fibers & Accessories
- Supported Liquid Extraction (SLE) Columns, Resins & Accessories
- Millex® Syringe Filters

Standardization and Calibration



- Certified Reference Materials
- Analytical Standards

Chromatographic Separation



- HPLC Columns & Accessories
- Solvents
- HPLC Buffers
- Chromatography standards
- TLC Plates & Adsorbents

SigmaAldrich.com/lcms

Supelco®

Analytical Products

MilliporeSigma
400 Summit Drive
Burlington, MA 01803

SigmaAldrich.com/LCMS

To place an order or receive technical assistance

Order/Customer Service: SigmaAldrich.com/order
Technical Service: SigmaAldrich.com/techservice
Safety-related Information: SigmaAldrich.com/safetycenter

SigmaAldrich.com

Lit. No. MS_AG8114EN Ver. 1.0
36597
03/2022