Determination and quantitation of drugs of abuse and designer opioids in serum

This study demonstrates a simple, rapid, and reliable method for the simultaneous quantitation of 71 basic drugs and drugs of abuse and their metabolites, including designer opioids, in serum using the Bruker Elute™ UHPLC coupled to the EVOQ™ LC-TQ Elite MS/MS system. Sample preparation was performed via solid phase extraction.

Introduction

Among the most common analytes in forensic toxicology are typical drugs of abuse (DOA), including cocaine, amphetamine and its derivatives, as well as conventional opioids such as morphine, tilidine, and fentanyl.

Increasing numbers of novel psychoactive substances (NPS) have recently appeared on the illicit drug market, including designer opioids such as fentanyl derivates (e.g., butyrylfentanyl, furanyl-fentanyl) and U-type opioids such as U-47700. These new analytes are analogues of existing drugs and are synthesized to circumvent evolving drug

Keywords:
Drugs of abuse, designer opioids, serum, quantitation

Authors: Rafaela Martin[1], Jürgen Kempf[2];
[1] Bruker Daltonik, Bremen, Germany; [2] Institute of Forensic Medicine, Medical Center, University of Freiburg, Freiburg, Germany.

prohibition laws and modern detection methods.

Successful forensic toxicology screening requires discriminatory power to confidently detect a wide range of drugs, including those recently emerging onto the drug market. Further, as many drugs are metabolized very quickly, it is important to include metabolites to enlarge the drug detection window.

The UHPLC-triple quadrupole mass spectrometry method described in this manuscript contains 71 analytes covering a broad range of classic DOA, designer opioids, and metabolites which are important in forensic cases such as driving under the influence of drugs (DUID)/driving while impaired (DWI), post-mortem analyses, or cases of intoxication with negative screening for classic DOA.

Experimental

Samples and Sample Preparation

Sample preparation was performed with solid phase extraction (SPE). 100 µL serum were loaded on a Chromabond DRUG cartridge (200 mg, 3 mL) and sequentially washed with water, acetic acid, and methanol. After elution with ethylene chloride/isopropanol/ammonia, the extracts were evaporated and reconstituted in 100 µL HPLC mobile phase A (2 mM ammoniumformate with 1% ACN + 0.1% formic acid) for analysis by UHPLC-MS/MS.

For quantitation, calibration curves in the range of 0.5 – 250 ng/mL serum were prepared, encompassing the toxicologically relevant range. Quality controls (QC) with a low (2.5 ng/mL), medium (25 ng/mL) and high concentration (100 ng/mL) were measured in triplicate as technical replicates to determine accuracy and precision.

Table 1: List of all 71 analytes included in the presented method.

4-Methoxybutyrfentanyl	Furanyl-Fentanyl	Norcodeine
6-Acetylcodeine	Hydrocodone	Norfentanyl
6-Acetylmorphine	Hydromorphone	Norketamine
Acetylfentanyl	Ketamine	Normorphine
Acrylfentanyl	Lidocaine	Noroxycodone
AH-7921	MDA	Nortilidine
Alfentanil	MDEA	Noscapine
Amphetamine	MDMA	Ocfentanil
Atropine	Meptazinol	O-Desmethyltramadol
Benzoylecgonine	Methadone	Oxycodone
Benzylfentanyl	Methamphetamine	Oxymorphone
Buprenorphine	Methene-U-47700	Papaverine
Butyryl fentanyl	Methoxetamine	Pentazocine
Carfentanil	Methoxyacetylfentanyl	Pethidine
Cocaethylene	Metoclopramide	Pholcodine
Cocaine	Mitragynine	Remifentanil
Codeine	Morphine	Sufentanil
Desomorphine	Nalbuphine	Tapentadol
Dextromethorphan	Naloxone	Tetrahydrofuranfentanyl
Dihydrocodeine	Naltrexone	Tilidine
Dihydromorphine	N-Desmethyltapentadol	Tramadol
Ecgoninmethylester	N-Ethylnorketamine	U-47700
EDDP	Norbuprenorphine	U-49900
Fentanyl	Norcocaine	

Retention times and MRM transitions for target analytes are available here:

For the determination of the limits of detection (LOD), samples with concentrations as low as 0.1 ng/mL were analyzed.

An overview of the 71 analytes included in the method is given in Table 1, and details of the UHPLC-MS/MS method conditions are given in Table 2.

Results and Discussion

Following the sample preparation requiring only 100 µL of serum, the chromatographic separation of the 71 analytes was performed within 11.0 minutes using the Elute UHPLC system. Figure 1 illustrates an overlay of the MRM traces for example analytes at the lowest calibrator concentration.

For the development of this screening assay, Bruker's MRM Builder was used (Figure 2). Optimization was performed by infusing mixtures of standard

Table 2: UHPLC-MS/MS method conditions

Liquid chromatography		
HPLC	Bruker Elute UHPLC	
Column	Intensity Solo C18-2, 100 x 2.1 mm, with 1.8 µm guard column	
Mobile Phase A	2 mM ammoniumformate with 1% ACN + 0.1 % formic acid	
Mobile Phase B	2 mM ammoniumformate with 99% ACN + 0.1 % formic acid	
Gradient	Time (min)	Mobile phase B (%)
	0.0	5
	1.0	5
	7.9	50
	8.0	99
	9.0	99
	9.1	5
	11.0	5
Flow Rate	500 µL/min	
Injection Volume	2 µL	
Column Oven	40°C	
Wash Solvent 1	Water	
Wash Solvent 2	30% each methanol, acetonitrile and isopropanol, 10% water	
Mass spectrometry		
Instrument	Bruker EVOQ LC-TQ Elite MS/MS system	
Ion Source	VIP H-ESI (Vacuum Insulated Probe)	
Spray Voltage	4500 V, positive mode	
Probe Gas	50 units at 400°C	
Cone Gas	25 units at 350°C	
Nebulizing Gas	50 units	
Active Exhaust	on	
Collision Gas	Argon, 1.5 mTorr	
Analysis mode	MRM (multiple reaction monitoring)	

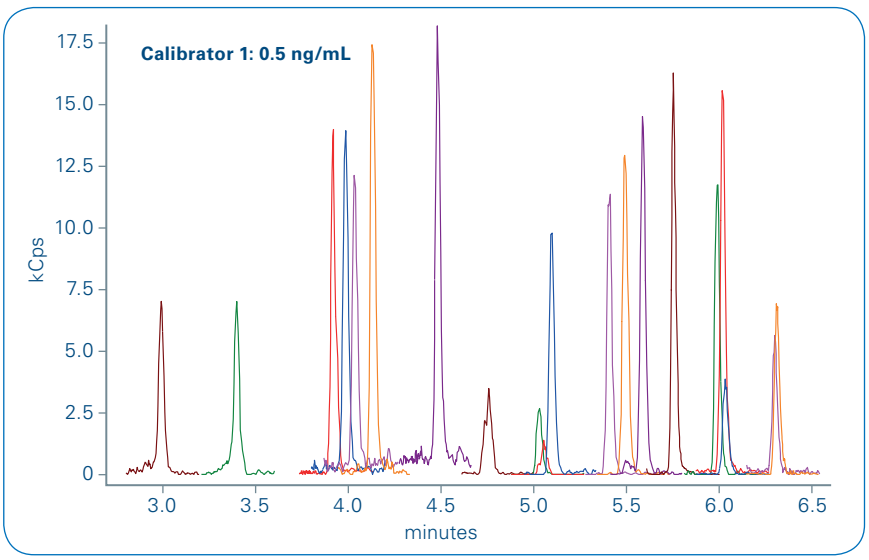

Figure 1: Overlaid MRM traces of example analytes of the lowest calibrator level (0.5 ng/mL)

solutions of the analytes. The MRM Builder identified the optimal MRM transitions and collision energies for each analyte which were then directly exported to the method and can also be added to a user library for future reference.

The quantitation of the analytes was performed using 21 isotopically labelled internal standards. Calibration curves included eight calibrator levels and provided excellent linearity with R^2 values from 0.9901 – 0.9999. The calibration curve of AH-7921 is shown as an example in Figure 3.

Results for accuracy and precision are shown in Figures 4 and 5, respectively. The study demonstrated a high accuracy as the calculated bias was within ±5% for two thirds of the QCs, demonstrating the high accuracy of the overall method. Bias ranged from 0.0 – 13.2%. The RSD was lower than 5% for more than 90% of the QCs and lower than 10% for 99% of the QCs (highest value, 12.6%), proving the very good precision of the instrumentation used. The values for precision and accuracy are well within the required range for quantitative forensic analyses.

Further, the method showed an excellent sensitivity. More than 75% of the analytes had a limit of detection (LOD) of 0.1 ng/mL or lower, as illustrated in Figure 6. The highest LODs equaled the lowest calibrator of 0.5 ng/mL.

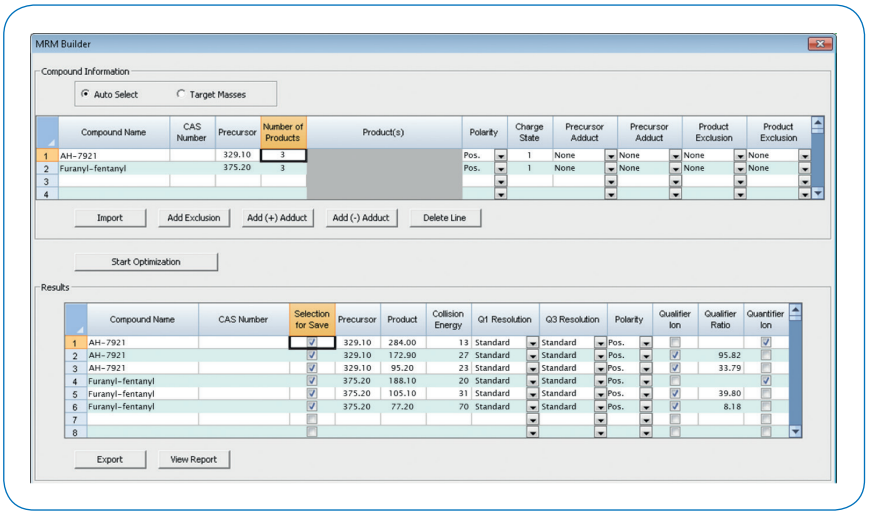

Figure 2: MRM Builder; top: define compound name, precursor and number of product ions desired; bottom: results of optimization with optional export to method and/or user library

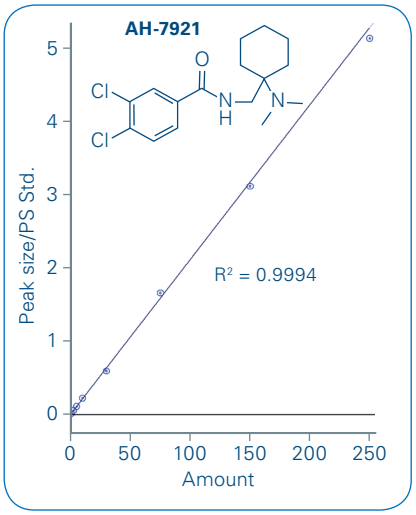

Figure 3: Calibration curve of AH-7921. Calibration range 0.5 – 250 ng/mL

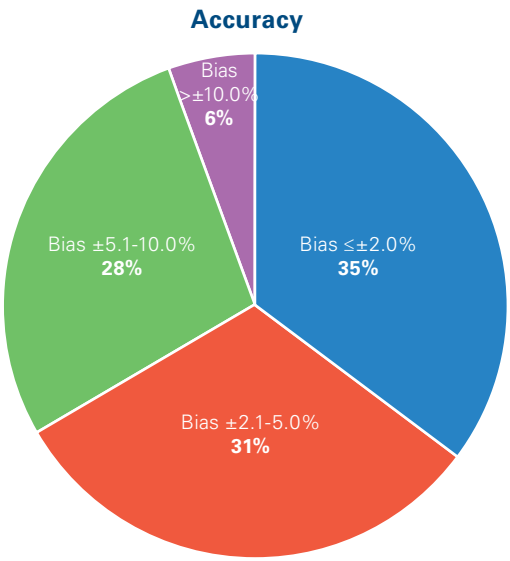

Figure 4: Distribution of bias for all QCs measurements (n=213); each QC analyzed in triplicate

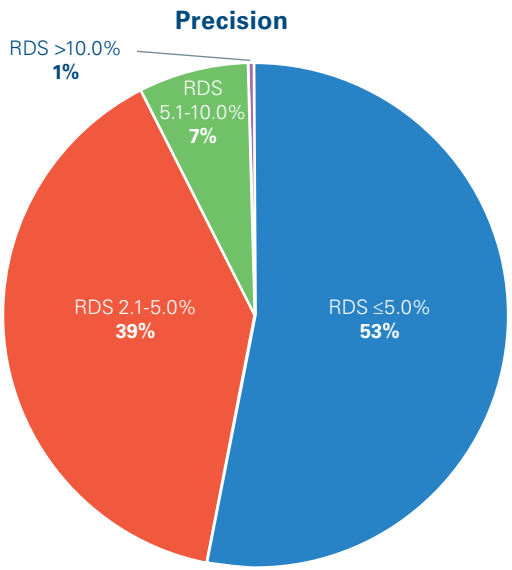

Figure 5: Distribution of RSD for all QCs measurements (n=213); each QC analyzed in triplicate

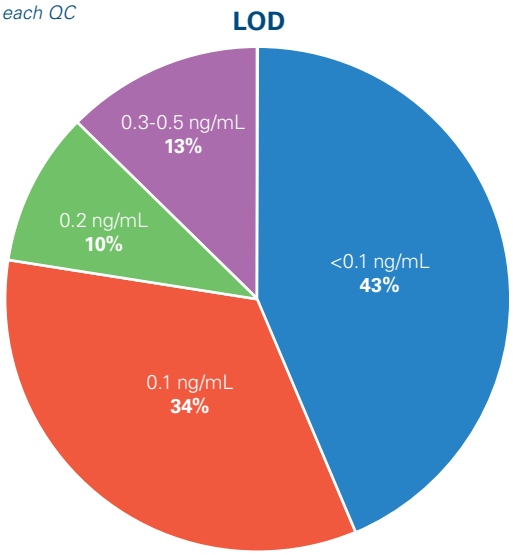

Figure 6: Distribution of LODs for all analytes

Related Reading

To learn more about the capabilities of these systems for the detection and quantitation of other compounds of forensic interest, including benzodiazepines, EtG/EtS, and synthetic cannabinoids, please follow the links below:

https://www.bruker.com/fileadmin/user_upload/8-PDF-Docs/Separations_MassSpectrometry/Literature/ApplicationNotes/LCMS-108_Benzodiazepine_09-2015_ebook.pdf

https://www.bruker.com/fileadmin/user_upload/8-PDF-Docs/Separations_MassSpectrometry/Literature/ApplicationNotes/1849820_LCMS-122_EtG-EtS.pdf

https://www.bruker.com/fileadmin/user_upload/8-PDF-Docs/Separations_MassSpectrometry/Literature/ApplicationNotes/1849846_LCMS-123_Cannabinoids_ebook.pdf

Conclusions

- The Bruker Elute UHPLC coupled to the EVOQ™ LC-TQ Elite triple quadrupole system provides a quick and reliable method to easily detect and quantitate 71 basic drugs and drugs of abuse, including designer opioids, in serum. All drug targets were detectable at sub ng/mL levels.
- The method requires very low volumes of serum (100 µL) for complete evaluation. This screening method can be easily expanded or customized – either by import from available libraries or direct addition with the MRM Builder – to include recently emerged NPS, over the counter (OTC) medications, and prescription medications. Linearity of calibration, precision, and accuracy were outstanding, making it suitable for many critical forensic applications, including DUID/DWI, cases of drug intoxication, or post-mortem analysis.

Learn More

You are looking for further Information?
Check out the link or scan the QR code for more details.

www.bruker.com

For Research Use Only. Not for Use in Clinical Diagnostic Procedures.

Bruker Daltonics GmbH & Co. KG

Bremen · Germany
Phone +49 (0)421-2205-0

Bruker Scientific LLC

Billerica, MA · USA
Phone +1 (978) 663-3660

ms.sales.bdal@bruker.com – www.bruker.com