LC-MS/MS and VAMS a well-coordinated team for toxicological analyzes

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Introduction

Accurate collection of a blood sample at the scene of an incident and its reliable analysis may seem like a challenging task. The classic venous blood collection has many disadvantages e.g. requires a place prepared for this purpose, trained medical person, an adequate protection and transport of the sample. The Mitra® device based on Volumetric Absorptive Microsampling (VAMS®) is an interesting alternative. Sampling in with VAMS® is safe, quick and convenience – a blood sample is taken from the finger. This can be done by a non-medical professional. Samples dry during shipping and as a dried matrix, they do not require controlled conditions before analysis which is the task of liquid chromatography-mass spectrometry. This well-coordinated effort accelerates, simplifies and increases availability while also lowering the cost of analysis.

Method development was focused on optimization of LC-MS/MS parameters to cover all 27 analytes (6-acetylmorphine, 7-aminoflunitrazepam, 7-aminoclonazepam, alprazolam, amphetamine, benzoylecgonine, diazepam, fentanyl, flunitrazepam, hydroxyzine, clonazepam, codeine, cocaine, lorazepam, MDA, MDEA, MDMA, methadone, methamphetamine, morphine, nordiazepam, oxazepam, THC, THC-COOH, tramadol, zolpidem, zopiclone) in one selective, sensitive and linear assay for quantitative determination that fulfills the requirements of DRUID guidelines using only 20 µL of blood collected using finger-prick method and a Mitra® device with VAMS® technology.

Methods

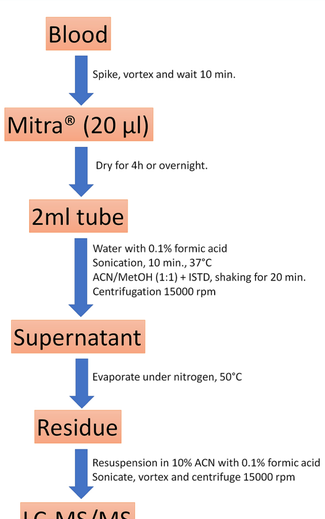

Figure 1. Procedure workflow.

A sample preparation procedure and quantitative LC-MS/MS method for determination of selected psychoactive compounds was developed, optimised and validated to meet DRUID project requirements.

Briefly - blood was collected with a Mitra® (Neoteryx LLC) microsampling device (20 µl), dried at room temperature, sonicated with 0.1% formic acid in water and extracted using acetonitrile:methanol solution (ACN:MetOH) 1:1. Extracts were dried under nitrogen, resuspended in 10% (v/v) ACN with 0.1% (v/v) formic acid prior LC-MS/MS analysis (Fig. 1).

All the tested compounds were separated using reversed-phase chromatography on Luna® Omega Polar C18 column (Phenomenex) and analyzed using ExionAC LC and QTRAP 5500+ MS/MS system (SCIEX) operating in positive and negative scheduled MRM mode. 2 MRM pairs (quantifier and qualifier ions) per tested compound and one for internal standards were used. Data processing were performed using SCIEX OS software.

Method validation included determination of lower limit of quantitation (LLOQ), linearity and intermediate precision of the method calculated on the basis of multiple repetitions ($p < 0.05$).

Results

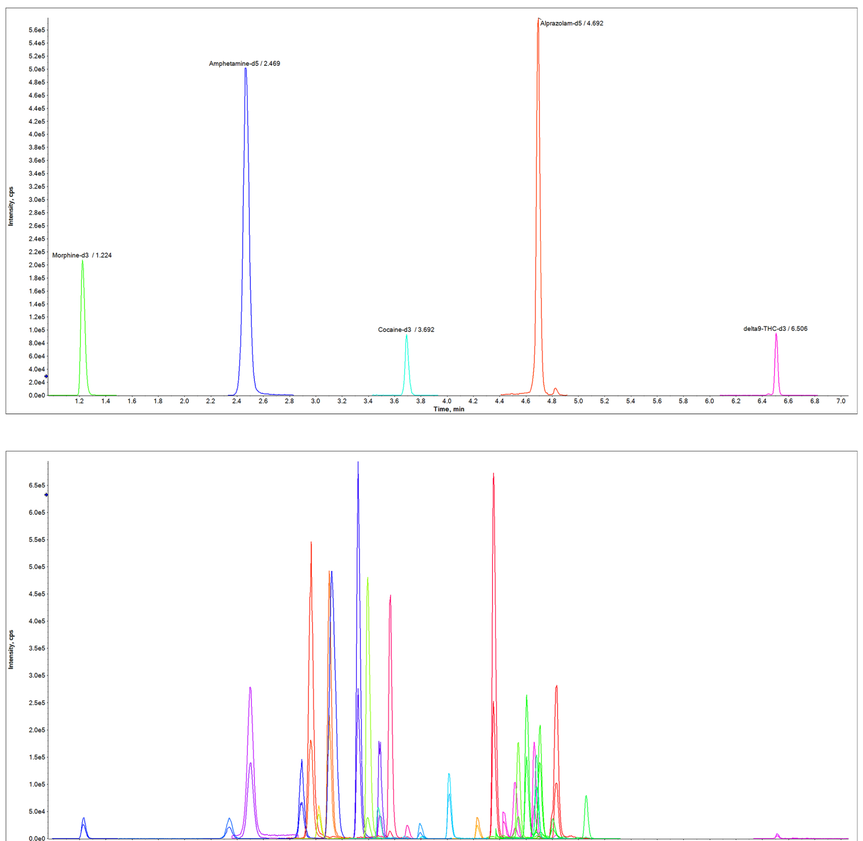

Figure 2. XIC chromatograms of the selected compounds - internal standards (top), analytes (bottom).

Table 1. Summary of the validation data - linearity, LLOQ and precision for all compounds in the assay.

Compound		Linearity		LLOQ		Intermediate precision [%]			
Analyte	ISTD	R	Working range [ng/mL]	LLOQ [ng/mL]	S/N	QC-1 [2.5 ng/ml]		QC-2 [25 ng/ml]	
						Accuracy	%CV	Accuracy	%CV
6-acetylmorphine	Alprazolam-D5	0.9994	0.1-50	0.1	18.5	97.9	10.62	94.3	8.59
7-aminoclonazepam	Alprazolam-D5	0.9975	0.5-50	0.5	14	109.16	10.63	99.65	10.13
7-aminoflunitrazepam	Alprazolam-D5	0.9990	0.5-50	0.5	30.6	112.9	8.75	98.83	9.88
Alprazolam	Alprazolam-D5	0.9993	0.1-50	0.1	14.2	101.63	7.12	95.97	5.91
Amphetamine	Amphetamine-D5	0.9987	0.1-50	0.1	8.1	103.31	6.91	95.68	3.72
Benzoylecgonine	Alprazolam-D5	0.9996	0.1-50	0.1	26.6	101.16	8.98	96.08	9.03
Clonazepam	Alprazolam-D5	0.9991	0.1-50	0.1	22.9	112.71	7.88	100.65	7.57
Cocaine	Cocaine-D3	0.9992	0.5-50	0.5	7.43	104	8.8	93.75	4.08
Codeine	Alprazolam-D5	0.9993	0.1-50	0.1	8	104.87	9.75	95.64	10.34
Diazepam	Alprazolam-D5	0.9977	0.5-50	0.5	13.3	109.29	10.03	100	11.07
Fentanyl	Alprazolam-D5	0.9960	0.1-50	0.1	33.9	113.74	12.38	111.59	12.83
Flunitrazepam	Alprazolam-D5	0.9980	0.1-50	0.1	9.25	104.9	8.44	97.22	6.13
Hydroxyzine	Alprazolam-D5	0.9992	0.1-50	0.1	22.37	114.31	7.67	107.07	9.32
Lorazepam	Alprazolam-D5	0.9991	0.1-50	0.1	25.03	109.55	8.29	99.85	6.51
MDA	Amphetamine-D5	0.9980	0.1-50	0.1	19.67	103.77	6.25	96.58	5.13
MDEA	Amphetamine-D5	0.9986	0.1-50	0.1	26.1	100.4	9.91	95.82	4.78
MDMA	Amphetamine-D5	0.9987	0.1-50	0.1	32.3	100.7	8.04	93.19	5.15
Metamphetamine	Amphetamine-D5	0.9984	0.1-50	0.1	35.77	103.09	7.09	94.25	4.55
Methadone	Alprazolam-D5	0.9976	0.1-50	0.1	26.67	113.83	7.73	103.36	7.84
Morphine	Morphine-D3	0.9991	0.1-50	0.1	9.93	103.04	6.06	92.9	4.26
Nordiazepam	Alprazolam-D5	0.9987	0.1-50	0.1	16.33	108.35	8.56	99.7	7.29
Oxazepam	Alprazolam-D5	0.9992	0.1-50	0.1	10	105.59	8.82	98.07	7.93
THC	delta9-THC-D3	0.9992	0.1-50	0.1	6.43	106.17	10.25	94.98	10.66
THC-COOH	delta9-THC-D3	0.9990	0.5-50	0.5	12.73	107.83	11.71	93.78	12.72
Tramadol	Cocaine-D3	0.9998	0.5-50	0.5	22.83	100.34	13.21	96.82	8.97
Zolpidem	Alprazolam-D5	0.9992	0.1-50	0.1	21.3	115.52	9.43	113.09	13.86
Zopiclone	Alprazolam-D5	0.9986	0.1-50	0.1	15.3	109.79	10.98	99.43	8.6

Acceptance criteria used for validation were:

Linearity - R ≥ 0.995
LLOQ - S/N ≥ 6 (using at least five measures of the chromatographic peak width for noise calculation, before the actual peak)
Working range - from LLOQ to highest concentration where R ≥ 0.995
Intermediate precision - 100±20% (for low and high QC)
%CV < 15 (for low and high QC)

Summary

Assay was validated with satisfactory results obtained for each tested parameter (Tab. 1) and depending on the tested compound demonstrated: LLOQ at the level: 0.1 to 0.5 ng/ml, good linearity in the working range of the method (regression coefficient in the range: 0.996 – 0.999), excellent precision (%CV: 3-13%) and accuracy in the range of 93-113%. Developed method meets DRUID project requirements and the attained LLOQ for each tested substance is well below analytical cut-off values established by European Monitoring Centre for Drugs and Drug Addiction within the project. Analysis of blood dried on Mitra® microsampling device is faster and less invasive method of proving a crime than taking a sample of whole blood from a driver using a syringe. The use of VAMS® in routine analysis will result in simplified handling, transport and storage. Considering that the volume of blood absorbed on Mitra® device does not depend on the hematocrit value, the VAMS® technology has a big advantage here over, for example, the Dried Blood Spots (DBS) technique. Obtained data showed that Mitra® devices with VAMS® coupled with optimized targeted LC-MS/MS analysis can be successfully applied for selected psychoactive compounds determination. We are quite confident that the use of Mitra® device will facilitate blood analysis in DRUID cases in the near future.