HR-MS/MS as a way to avoid false positive quantification of 27 psychoactive compounds in venous and VAMS-collected blood

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Modern technique of precise analysis of 27 psychoactive compounds from DRUID list consist of: optimized sample preparation; reversed-phase chromatography on ExionAC LC coupled with ZenoTOF 7600 (SCIEX) and a processing method in SciexOS software for multilevel confirmation of the obtained result.

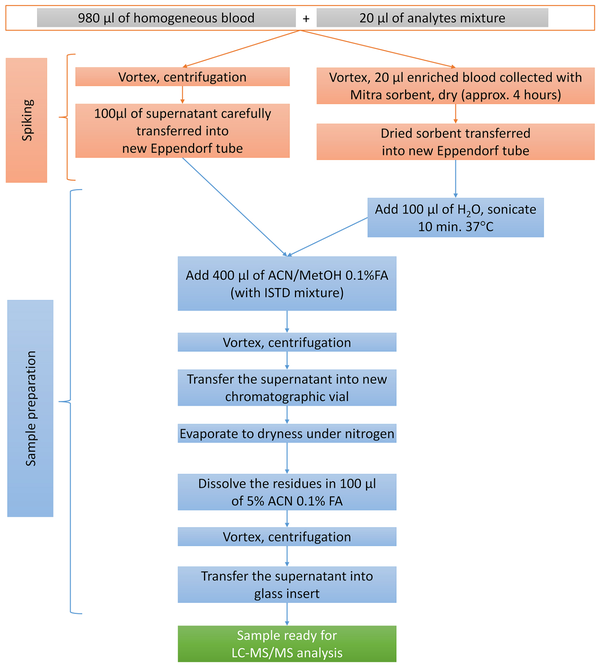


Figure 1. Sample preparation scheme.

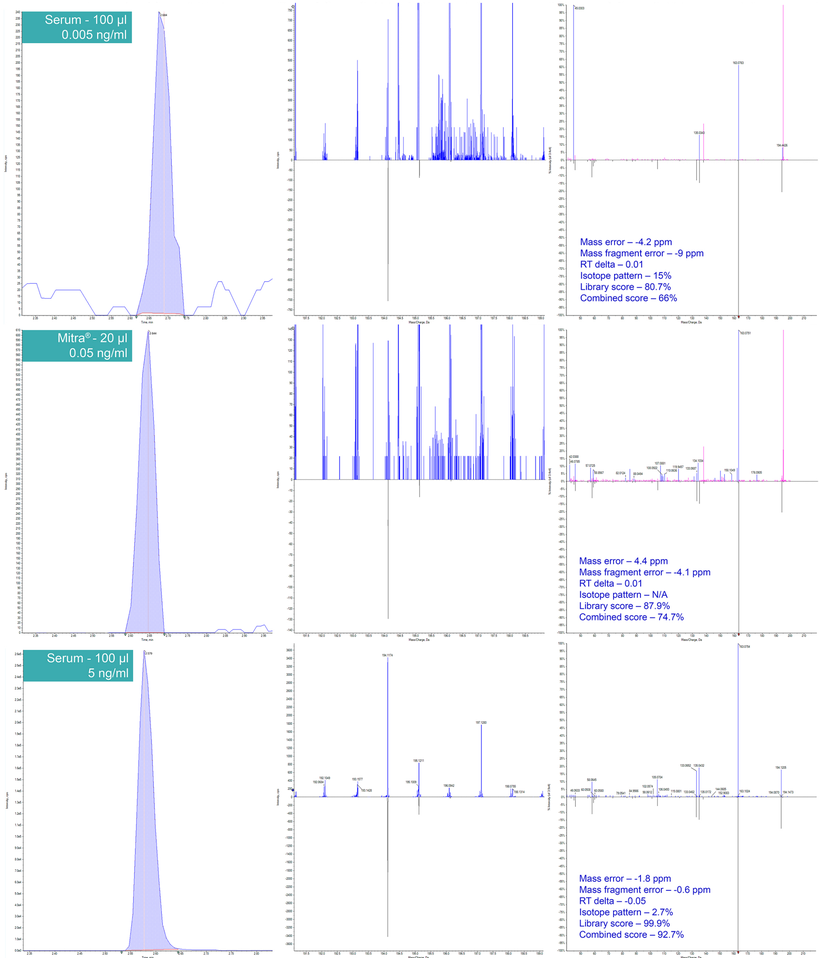


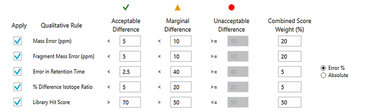
Figure 2. Examples of peak review and scoring data for MDMA in spiked samples.

Table 1. Limits of detection for analytes determined by the Druid method ZenoTOF LC-MS/MS system.

Analyte	LOD (blood) [ng/mL]	LOD (VAMS) [ng/mL]
MDA	0.050	0.100
MDEA	0.005	0.050
Zopiclone	0.005	0.050
Amphetamine	0.500	0.500
THC	0.500	0.500
Clonazepam	0.010	0.050
Cocaine	0.005	0.050
Tramadol	0.001	0.050
Methadone	0.001	0.010
Flunitrazepam	0.010	0.050
Methamphetamine	0.050	0.500
MDMA	0.005	0.050
Hydroxyzine	0.005	0.050
6-acetylomorphine	0.100	0.500
Benzoylecgonine	0.005	0.010
Oxazepam	0.050	0.100
Alprazolam	0.005	0.050
Lorazepam	0.010	0.050
Diazepam	0.010	0.050
Nordiazepam	0.005	0.050
7-aminoclonazepam	0.050	0.100
7-aminoflunitrazepam	0.005	0.500
Fentanyl	0.001	0.050
Zolpidem	0.001	0.500
Codeine	0.050	0.100
Morphine	0.050	0.500
THC-COOH	0.500	0.500

The method was validated separately for venous blood and blood collected on VAMS and fulfilled the following criteria for each analyte: linearity ($R \geq 0.995$), reproducibility (%CV ≤ 15%, accuracy 80 - 120%) and recovery (%CV ≤ 15%).

Developed processing method use multi-level confirmation and combined scoring based on HR MS and MS/MS data.



After series of analysis the cut-off point of Combined Score estimated at 60% allows flawless reporting of analytes from the DRUID list. The library has been updated with MS/MS spectra obtained from spiked at different levels matrix samples with optimized collision energies for each compound.

Table 2. Example of multi-level confirmation and scoring by the DRUID method in VAMS blood sample on ZenoTOF 7600 LC-MS/MS system.

Formula	Precursor Mass	Found At Mass	Mass Error (ppm)	Fragment Mass	Found At Fragment Mass	Fragment Mass Error (ppm)	Mass Error Confidence	Fragment Mass Error Confidence	RT Confidence	Isotope Confidence	Library Confidence	Library Hit	Library Score	Combined Score	Isotope Ratio Difference
$C_{10}H_{13}NO_2$	180.102	180.1015	-2.5	105.0699	105.0701	1.9	✓	✓	✓	✓	✓	MDA	99.5	92.628	0.8
$C_{10}H_{15}NO_2$	208.133	208.1329	-1.3	163.0754	163.0759	3.4	✓	✓	▲	✓	✓	MDEA	100.0	92.893	0.7
$C_{11}H_{15}NO_2$	194.118	194.1173	-1.2	163.0753	163.0759	3.8	✓	✓	✓	✓	✓	MDMA	99.8	93.573	1.1
$C_9H_{13}N$	141.143	141.1428	-0.8	91.0666	91.0670	4.3	✓	✓	▲	✓	✓	Amphetamine-D5 [Smart Confirmation]	84.1	73.291	10.2
$C_{21}H_{27}ClN_2O_2$	375.183	375.1829	-1.2	201.0465	201.0470	2.6	✓	✓	✓	✓	✓	Hydroxyzine [Smart Confirmation]	100.0	94.820	0.8
$C_{17}H_{17}ClN_4O_3$	389.112	389.1134	8.2	343.0334	343.0335	4.7	✓	✓	✓	▲	✓	Zopiclone [Smart Confirmation]	100.0	92.897	6.5
$C_{21}H_{27}NO$	310.217	310.2162	-1.0	265.1587	265.1598	4.1	✓	✓	✓	✓	✓	Methadone [Smart Confirmation]	100.0	93.101	3.0
$C_{16}H_{19}NO_4$	290.139	290.1389	0.9	168.1018	168.1025	4.1	✓	✓	✓	✓	✓	Benzoylecgonine [Smart Confirmation]	99.3	93.513	0.8
$C_{16}H_{25}NO_2$	264.198	264.1957	-0.5	58.0651	58.0653	3.8	✓	✓	✓	✓	✓	Tramadol	100.0	94.648	0.9
$C_9H_{13}N$	136.112	136.1116	-3.2	91.0542	91.0545	3.3	✓	✓	✓	✓	✓	Amphetamine [Smart Confirmation]	100.0	89.551	1.2
$C_{21}H_{20}O_3$	313.232	313.2314	-1.5	193.1221	193.1223	1.2	✓	✓	✓	✓	✓	THC Positive	98.4	94.141	1.8
$C_{15}H_{11}ClN_2O_2$	287.058	287.0580	-0.7	241.0529	241.0536	2.9	✓	✓	✓	✓	✓	Oxazepam [Smart Confirmation]	96.7	92.690	5.2
$C_{19}H_{21}NO_4$	328.154	328.1532	-2.4	165.0698	165.0705	4.1	✓	✓	✓	✓	✓	6-Monoacetylmorphine [Smart Confirmation]	99.4	92.848	1.2
$C_9H_{13}N$	150.128	150.1274	-3.5	91.0541	91.0545	5.1	✓	✓	✓	✓	✓	Methamphetamine [Smart Confirmation]	88.6	88.958	0.4
$C_{17}H_{18}ClN_3O_4$	307.173	307.1729	-0.8	185.1362	185.1365	1.7	✓	✓	✓	✓	▲	Cocaine-D3 [Smart Confirmation]	100.0	93.918	10.4
$C_{17}H_{21}NO_4$	304.154	304.1548	0.7	182.1174	182.1182	4.2	✓	✓	✓	✓	✓	Cocaine [Smart Confirmation]	100.0	94.002	0.7
$C_{17}H_{13}ClN_4$	309.090	309.0897	-1.4	281.0712	281.0729	6.2	✓	✓	✓	✓	✓	Alprazolam	98.7	88.744	6.3
$C_{21}H_{20}O_4$	345.206	345.2053	-2.2	327.1957	327.1956	-0.9	✓	✓	✓	✓	✓	THC-COOH Positive	99.8	92.073	1.8
$C_{16}H_{14}FNO$	284.119	284.1189	-1.8	135.0915	135.0918	2.1	✓	✓	✓	✓	✓	7-Aminoflunitrazepam	87.8	87.855	1.3
$C_{22}H_{28}N_2O$	337.227	337.2272	-0.8	188.1432	188.1441	4.7	✓	✓	✓	✓	✓	Fentanyl [Smart Confirmation]	100.0	93.049	1.5
$C_{16}H_{12}ClN_3O$	271.066	271.0659	-1.0	140.0261	140.0264	2.3	✓	✓	✓	✓	✓	Nordiazepam	95.3	91.775	5.8
$C_{16}H_{12}FN_3O$	314.094	314.0933	-0.7	239.0983	239.0985	0.9	✓	✓	✓	✓	✓	Flunitrazepam	94.7	94.377	2.8
$C_{21}H_{27}ClN_2O$	359.231	359.2498	-0.5	196.1414	196.1398	-8.2	✓	✓	✓	✓	✓	THC-x0 [Smart Confirmation]	85.7	78.136	3.8
$C_{16}H_{13}ClN_2O$	285.079	285.0787	-0.6	193.0888	193.0892	2.0	✓	✓	✓	✓	✓	Diazepam	96.5	94.580	1.4
$C_{15}H_{10}ClN_3O_3$	316.048	316.0480	-0.9	270.0556	270.0561	1.7	✓	✓	✓	✓	✓	Clonazepam [Smart Confirmation]	97.7	94.286	1.8
$C_{15}H_{12}ClN_3O$	286.074	286.0742	0.3	121.0784	121.0784	-0.4	✓	✓	✓	✓	✓	7-Aminoclonazepam [Smart Confirmation]	97.9	96.731	4.5
$C_{15}H_{10}ClN_3O_2$	301.019	301.0189	-1.0	275.0137	275.0146	3.5	✓	✓	✓	✓	✓	Lorazepam	99.8	93.337	3.8
$C_{17}H_{19}NO_3$	286.144	286.1437	-0.3	128.0623	128.0622	-1.1	✓	✓	✓	✓	✓	Morphine [Smart Confirmation]	99.1	96.840	4.4
$C_{19}H_{21}NO_3$	308.176	308.1757	0.0	225.1239	225.1244	6.1	✓	✓	✓	✓	✓	Zolpidem	100.0	93.627	0.5
$C_{17}H_{23}NO_3H$	314.122	314.1211	-1.5	210.1073	210.1073	0.1	✓	✓	✓	✓	✓	2D-Oxycodon [Smart Confirmation]	89.2	90.343	3.3
$C_{18}H_{21}NO_3$	300.159	300.1596	0.1	152.0621	152.0622	0.8	✓	✓	✓	✓	✓	Codeine [Smart Confirmation]	99.4	96.103	2.4
$C_{17}H_{19}NO_3H$	289.163	289.1634	-0.6	152.0621	152.0624	1.9	✓	✓	✓	✓	✓	Morphine-D3 [Smart Confirmation]	98.7	95.628	1.2

Compound presence confirmation is one of the key aspects of targeted and quantitative analysis in forensics applications. High resolution tandem mass spectrometry (HR-MS/MS) may become a major tool for this kind of analysis because of its mass accuracy resulting in unparallel specificity, all combined with broad linearity and scanning speed achieved in the newest hardware on the market

"Sensitive, specific and false positive resistant HR-MS/MS quantitation of 27 psychoactive compounds in blood and blood collected by VAMS."

Step 1 – sample preparation

Step 2 – LC-HR-MS/MS

Step 3 – quantitation, verification & confirmation