How sure you can be of the results of quantification of psychoactive compounds in blood?

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INTRODUCTION

The use of psychoactive substances by drivers is an increasingly common phenomenon observed in the European Union countries, contributing to expanded risk for road users. The DRUID (Driving Under the Influence of Drugs, Alcohol and Medicines) program was created to standardize regulations on the analysis of biological material collected from drivers. As a result of the work carried out under the DRUID program, a list of psychoactive substances subjected to control was created along with the recommended cut-offs.
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. In this work we show a modern HR-MS/MS method for a sensitive detection of 27 analytes with high confidence in venous blood and capillary blood collected on a volumetric absorptive microsampling (VAMS) probe.

MATERIALS AND METHODS

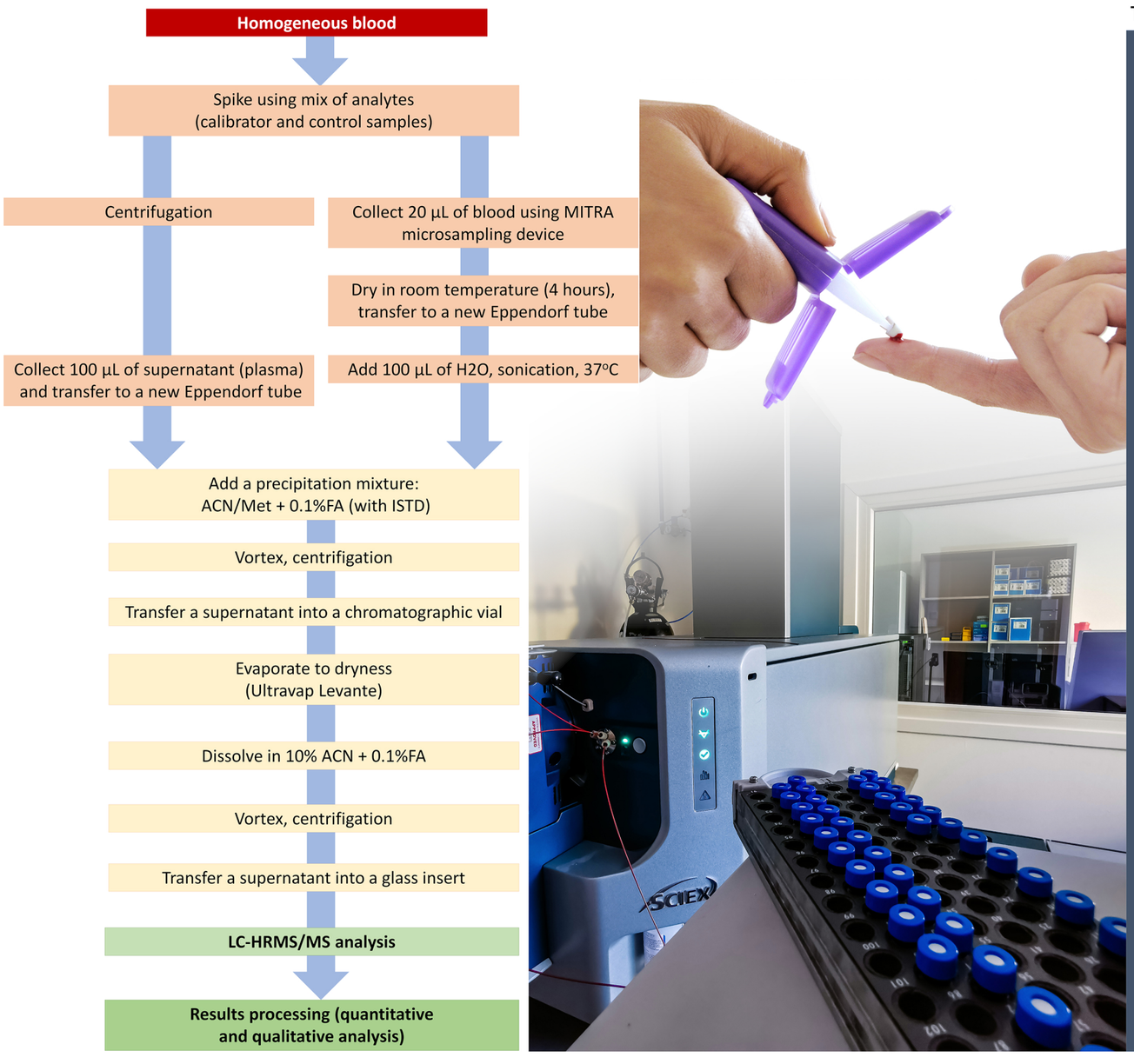


Figure 1. Procedure of blood samples preparation.

Table 1. Parameters applied in LC-MS/MS method

Method parameter	Applied value
Liquid chromatograph	ExionAC (Sciex)
Chromatographic column	Fortis H2O; 1.7 µm; 100x3mm
Mobile phases	A: 10% ACN + 0.1%FA B: ACN + 0.1%FA
Injection volume	20 µL
Analysis time	10 min
Mass spectrometer	ZenoTOF 7600 (Sciex)
Ion source	Turbo V™ Ion Source (ESI)
Ionization mode	Positive
Scanning mode	MRMHR
Fragmentation mode	CID

Ionization	Ion source parameters	
POS	CUR	35
	CAD	9
	IS	5000
	TEM	600
	GS1	60
	GS2	60

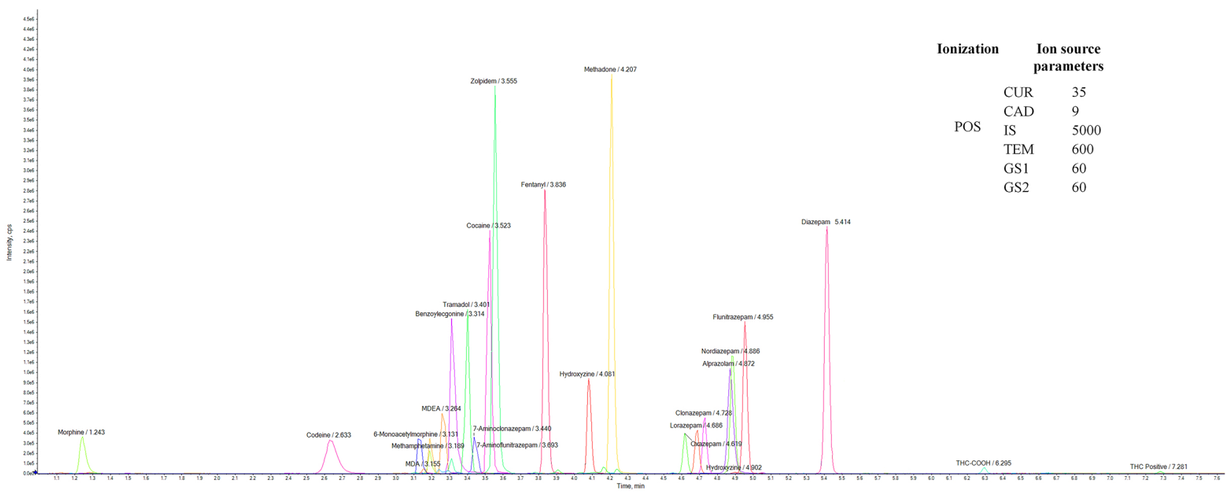
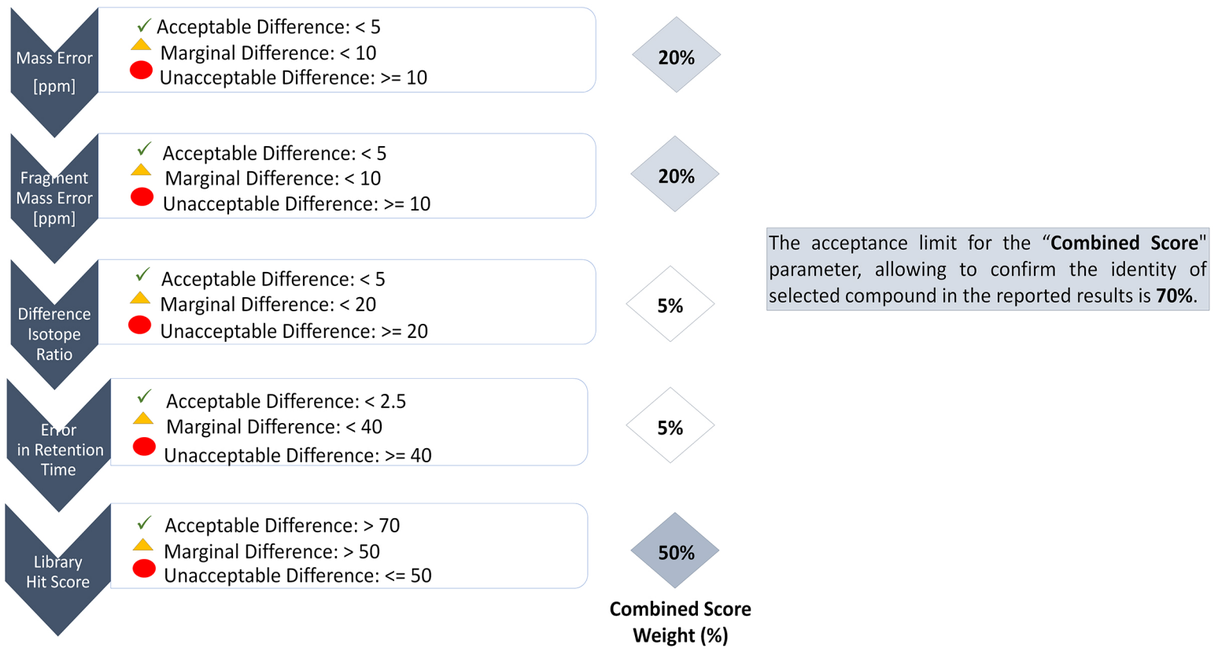


Figure 2. Chromatogram presenting LC-MS/MS analysis of 27 psychoactive compounds from DRUID list.



The acceptance limit for the "**Combined Score**" parameter, allowing to confirm the identity of selected compound in the reported results is **70%**.

Figure 3.Criteria applied in developed processing method using a multi-level confirmation and combined scoring based on HR data.

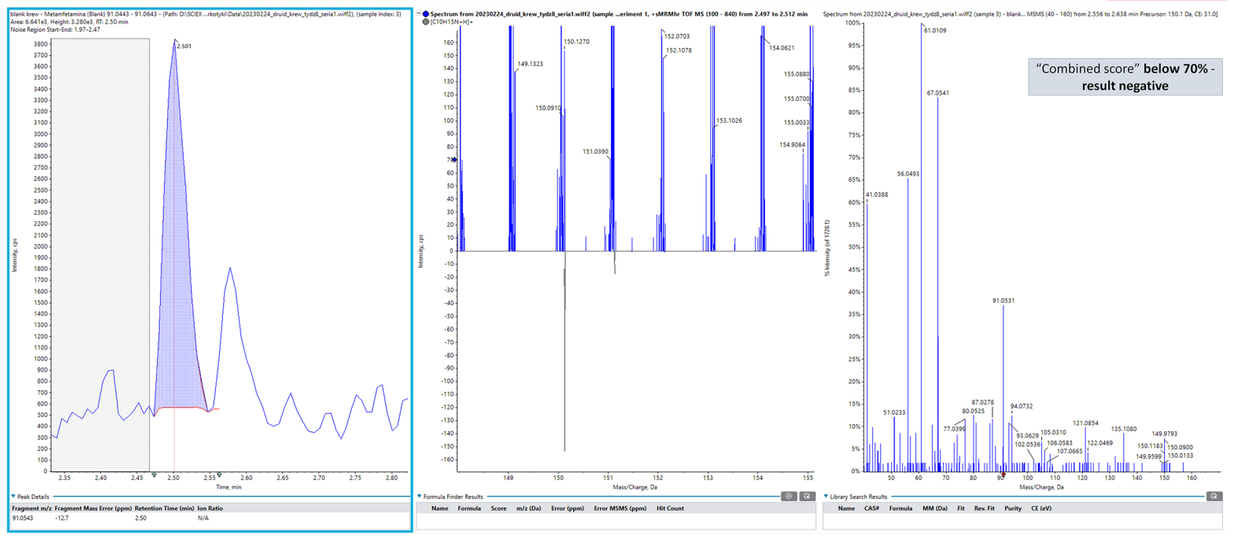
RESULTS

Table 2. Results of quantitative methods validation

Analyte	Analytical cut-off (DRUID program) [ng/ml]	Method range [ng/mL]		Limit of Detection (LOD) [ng/mL]		Precision CV[%] QC 1 (25 ng/mL)	
		Blood	Mitra	Blood	Mitra	Blood (n=6)	Mitra (n=6)
MDA	20	0.1-50	0.1-50	0.05	0.1	11.25	5.13
MDEA	20	0.1-50	0.1-50	0.005	0.05	9.60	4.78
Zolpiclone	10	0.1-50	0.1-50	0.005	0.05	6.60	8.60
Amphetamine	20	0.5-50	0.5-50	0.1	0.1	7.25	3.72
THC	1	0.5-50	0.5-50	0.1	0.1	8.04	10.66
Clonozepam	10	0.1-50	0.1-50	0.01	0.05	10.89	7.57
Cocaine	10	0.1-50	0.5-50	0.005	0.05	9.54	4.08
Tramadol	50	0.1-50	0.5-50	0.001	0.05	5.63	8.97
Methadone	10	0.1-50	0.1-50	0.001	0.01	3.95	7.84
Flunitrazepam	5.3	0.5-50	0.1-50	0.01	0.05	8.88	6.13
Metamphetamine	20	0.1-50	0.5-50	0.05	0.1	10.32	4.55
MDMA	20	0.1-50	0.1-50	0.005	0.05	10.00	5.15
Hydroxyzine	-	0.1-50	0.1-50	0.005	0.05	7.88	9.32
6-acetylomorphine	10	0.1-50	0.5-50	0.1	0.5	7.01	8.59
Benzoyloecgonine	50	0.1-50	0.1-50	0.005	0.01	5.46	9.03
Oxazepam	50	0.1-50	0.1-50	0.05	0.1	7.51	7.93
Alprazolam	10	0.1-50	0.1-50	0.005	0.05	6.40	5.91
Lorazepam	10	0.1-50	0.1-50	0.01	0.05	6.43	6.51
Diazepam	140	0.1-50	0.5-50	0.01	0.05	9.30	11.07
Nordiazepam	20	0.1-50	0.1-50	0.005	0.05	7.61	7.29
7-aminoclonazepam	10	0.1-50	0.5-50	0.05	0.1	12.59	10.13
7-aminoflunitrazepam	8.5	0.1-50	0.5-50	0.005	0.5	4.90	9.88
Fentanyl	-	0.1-50	0.1-50	0.001	0.05	5.71	12.83
Zolpidem	37	0.1-50	0.5-50	0.001	0.1	5.92	13.86
Codeine	10	0.1-50	0.1-50	0.05	0.1	5.19	10.34
Morphine	10	0.1-50	0.5-50	0.05	0.1	6.59	4.26
THC-COOH	-	0.5-50	0.5-50	0.5	0.5	12.21	12.72

Matrix blank – venous blood

Sample Name	Component Name	Mass Error Confidence	Fragment Mass Error Confidence	RT Confi...	Isotope Confidence	Library Confidence	Library Hit	Library Score	Combined Score
blank krew	Metamfetamina	✓	●	✓	▲	●	No Match	0.0	17.407



Calibrator [0.5 ng/ml] – spiked venous blood

Sample Name	Component Name	Mass Error Confidence	Fragment Mass Error Confidence	RT Confi...	Isotope Confidence	Library Confidence	Library Hit	Library Score	Combined Score
CAL 3	Metamfetamina	✓	✓	✓	✓	✓	Methamphetamine [Smart Confirmation]	98.6	91.536

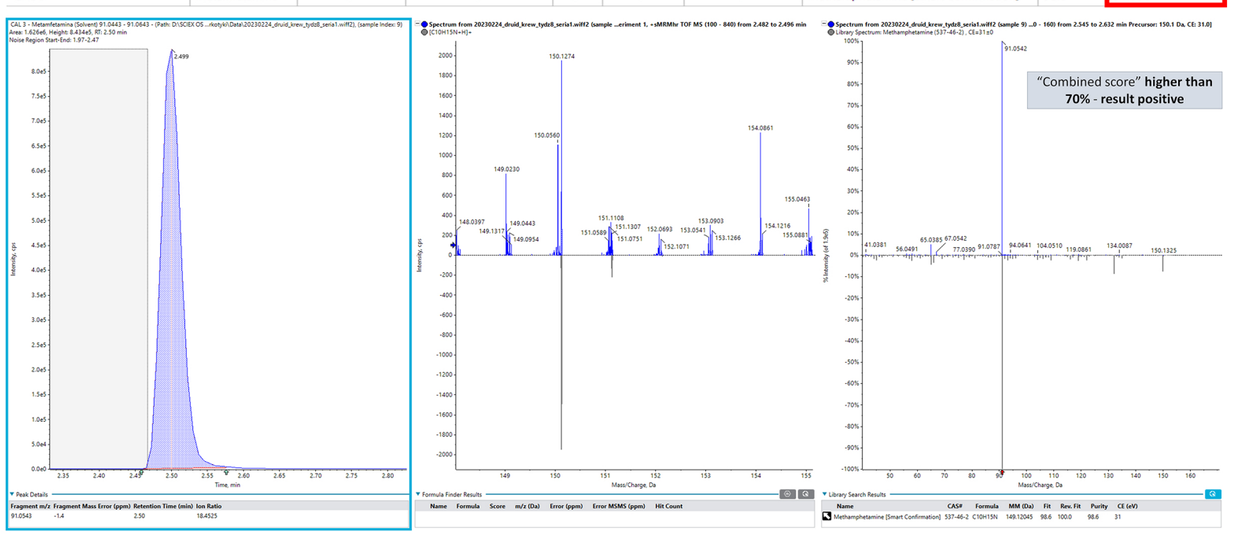


Figure 4. Example of false positive result exclusion for Metamphetamine in unspiked venuos blood sample due to multi-level confirmation method .

SUMMARY

- The developed procedure enabled to obtain a high sensitivity method of quantitative analysis of 27 psychoactive substances in venous and capillary blood (LOD far below DRUID cut-offs)
- High specificity of the developed targeted method was achieved through the use of high-resolution mass spectrometry and a multi-level system of the compound's identity confirmation.
- The following validation criteria were met: linearity (R ≥ 0.995), working range: 0.1/0.5 – 50 ng/mL, repeatability (%CV ≤ 15%), accuracy (80 - 120%).