UHPLC-MS/MS Methodology for Analysis of New Synthetic Opioids and Hallucinogens in Whole Blood

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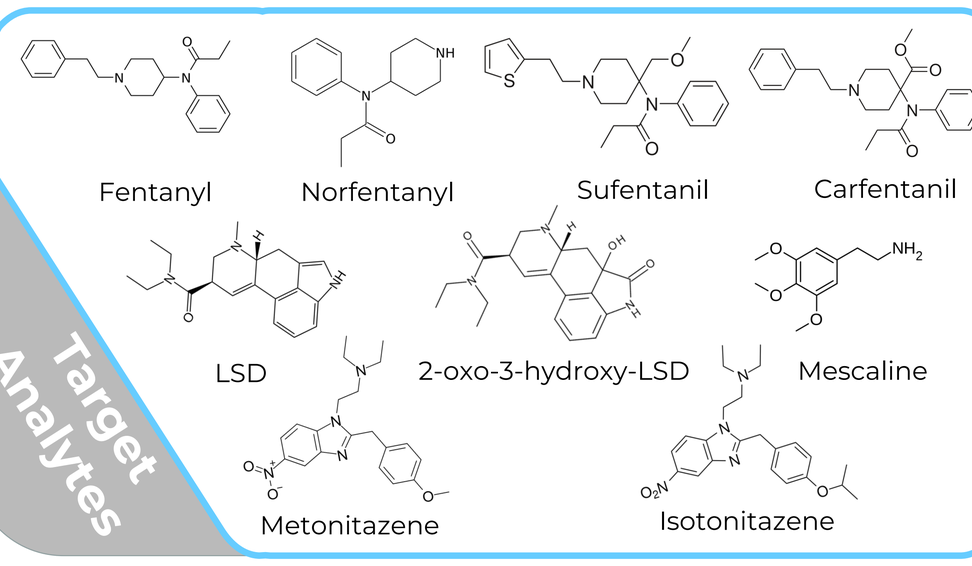
Introduction

New Psychoactive Substances (NPS) are a real contemporaneous threat, due to their potency, dangerousness, and lack of control/monitoring. The NPS group that has grown the most in the last years is the **synthetic opioids** group, where **fentanyl and its analogues** stand out. However, the emerging concerning synthetic opioids are **nitazenes**. Due to their high potency, even minimal consumption doses can lead to severe health effects or even fatal overdoses, making them a public health issue[1,2]. Notwithstanding, it is also important to remain vigilant towards more "traditional" psychoactive substances like **hallucinogens**, as they have been associated with poisonings/intoxications[3]. This is particularly relevant now that they are also being used for clinical purposes[1]. Therefore, it is essential to establish analytical methodologies for monitoring these compounds in biological matrices.

Target Analytes



Objective

The aim of this study was to **develop**, **optimize** and **validate** an **easy** to use, **fast**, **simple**, and **sensitive** method for **routine** analysis of **6** new synthetic opioids (fentanyls and nitazenes) and **3** hallucinogens in **postmortem and/or *in vivo* whole blood**.

Methodology

Optimized using Full Factorial Design of Experiments

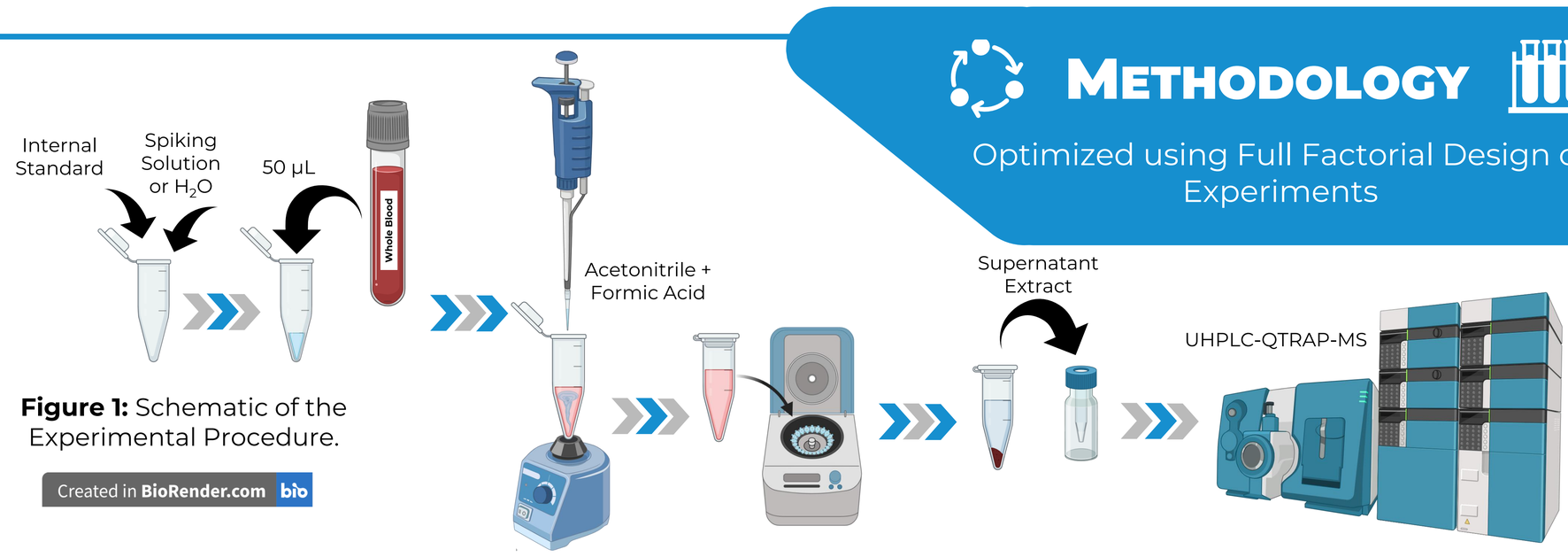


Figure 1: Schematic of the Experimental Procedure.

Main Results

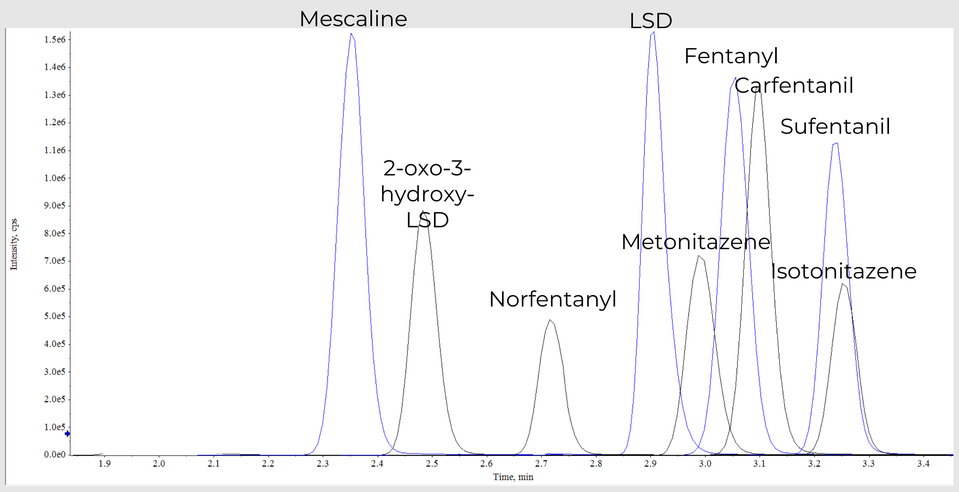


Figure 2: Overlaid extracted ion chromatograms of the target analytes of final method.

Precipitation Solvent Volume

- **100 µL**
- 150 µL
- 200 µL

% Formic Acid

- **0,1 %**
- 0,3 %
- 0,5 %

Centrifugation Time

- 2 min.
- **5 min.**
- 10 min.

Figure 3: Protein precipitation parameters optimized by full factorial design of experiments.

Selectivity:

No endogenous or exogenous (> 200 drugs) interferences

Validation according to ANSI/ASB Standard 036.

Ionization Suppression/Enhancement:

Between – 25 % and 25 %

CV % < 20 %

Table 1: LOD, LLOQ, and Calibration Model validation parameters obtained for each target analyte.

Substance	LOD (ng/mL)	LLOQ (ng/mL)	Linear Range (ng/mL)	Weighting Factor	Average r^2
Carfentanil	0.05	0.1	0.1 – 20	1/x	0.9951
Fentanyl	0.05	0.1	0.1 – 20	1/x	0.9958
Isotonitazene	0.05	0.1	0.1 – 20	1/x	0.9962
LSD	0.05	0.1	0.1 – 20	1/x	0.9955
LSD-OH	0.05	0.1	0.1 – 20	1/x	0.9963
Mescaline	1	2.5	2.5 – 500	1/x	0.9935
Metonitazene	0.1	0.1	0.1 – 20	1/x	0.9963
Norfentanyl	0.1	0.1	0.1 – 20	1/x	0.9949
Sufentanil	0.05	0.1	0.1 - 20	1/x	0.9962

Table 2: Maximum precision and accuracy values obtained for each target analyte (3 concentration levels).

Substance	Precision (CV %)		Accuracy (Bias %)	
	Intra-day (n = 3)	Inter-day (n = 15)	Intra-day (n = 3)	Inter-day (n = 15)
Carfentanil	8,42	14,67	15,34	5,88
Fentanyl	8,58	11,18	12,99	4,61
Isotonitazene	9,88	15,46	19,13	6,62
LSD	9,18	10,23	-15,73	-9,97
LSD-OH	11,74	13,43	19,42	-5,87
Mescaline	10,67	15,20	17,79	-9,87
Metonitazene	9,86	10,14	17,00	10,14
Norfentanyl	9,83	16,65	19,74	12,20
Sufentanil	8,89	17,79	19,17	10,43

Conclusion

- This is a valuable and powerful method for toxicology laboratories, enabling the simultaneous identification, confirmation, and quantification of two different families of psychoactive substances, whether in postmortem or *in vivo* samples.
- Its speed, simplicity, effectiveness, and reliability make it particularly advantageous for routine implementation.

References

[1] United Nations Office on Drugs and Crime. **World Drug Report 2024**. Vienna; 2024.

[2] European Monitoring Centre for Drugs and Drug Addiction. **European Drug Report 2024: Trends and Developments**. Lisbon; 2024.

[3] Schlag AK *et al* (2022) ***J Psychopharmacol* 36 (3)**: 258-272.

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