Extraction of Novel and Traditional Stimulants from Blood using Protein Precipitation and Dispersive Pipette XTRaction for LC-MS/MS Analysis

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ABSTRACT

This pilot study combined protein precipitation and in-tip dispersive solid phase extraction for the isolation of nine stimulants from blood for LC-MS/MS analysis. Recovery ranged from 68.7-78.8% and matrix effects from 90.9-106.3%. Calibration (1-100 ng/mL) achieved $R^2 > 0.99$ for most analytes with no carryover observed. Results demonstrate this approach offers a rapid and efficient sample preparation. Nevertheless, further method validation and application to authentic forensic specimens are needed.

INTRODUCTION

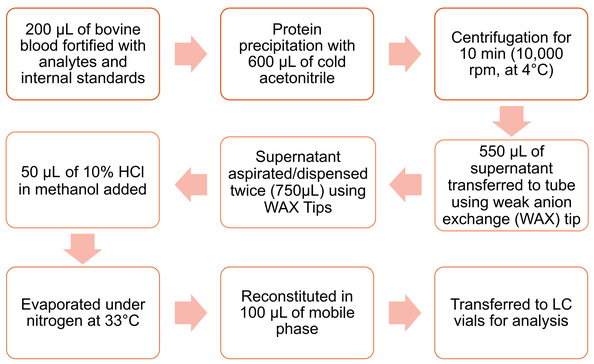
Dispersive Pipette XTRaction (DPX® Technologies) is a promising technique for forensic toxicology analysis, offering simple and rapid sample preparation requiring minimal sample and solvent volumes[1]. The technique uses sorbent materials contained within pipette tips. With the continued emergence of novel stimulants, forensic laboratories need efficient methods for the extraction of these substances from biological matrices.

The goal of this work was to explore the combination of dispersive pipette XTRaction (using the DPX® Technologies tips, Figure 1) and protein precipitation for LC-MS/MS analysis of stimulants in blood as a foundation for future method validation.

Figure 1. Dispersive pipette XTRaction tip (DPX® Technologies)

MATERIALS & METHODS

The sample preparation workflow was developed based on previously published literature[2] and is summarized below:



RESULTS & DISCUSSION

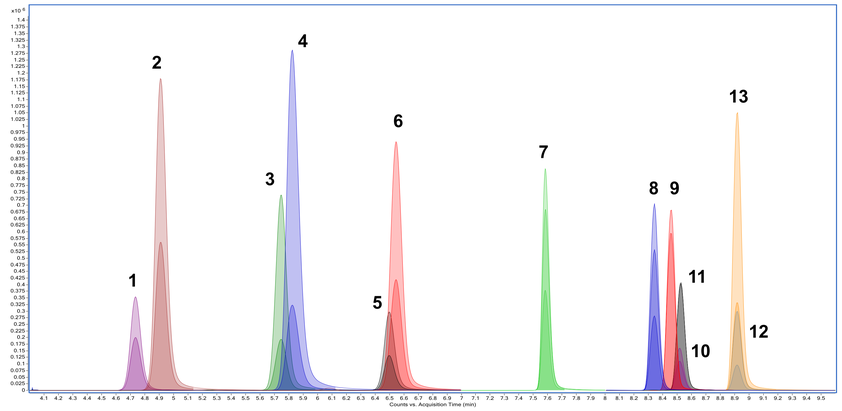


Figure 2. Chromatogram of a blood sample showing the separation of all target analytes (80 ng/mL).

	Analyte	Retention Time
1	Amphetamine-d_{11} (ISTD)	4.736 min
2	Amphetamine	4.911 min
3	MDA	5.748 min
4	Methamphetamine	5.825 min
5	MDMA-d_5 (ISTD)	6.499 min
6	MDMA	6.547 min
7	Eutylone	7.583 min
8	Pentylone	8.341 min
9	*N*-Propyl butylone	8.457 min
10	*N,N*-dimethylpentylone-d_6 (ISTD)	8.517 min
11	*N,N*-dimethylpentylone	8.524 min
12	Cocaine-d_3 (ISTD)	8.916 min
13	Cocaine	8.917 min

Chromatographic Separation

- The chromatographic method provided adequate separation and resolution (Figure 2).
- The isomers *N*-propyl butylone and *N,N*-dimethylpentylone exhibited retention times of 8.474 and 8.540 minutes. However, with different MRM transitions being monitored and the use of *N,N*-dimethylpentylone-d_6 as the internal standard, both isomers were properly distinguished.

Recovery and Matrix Effects

- Recovery was assessed in duplicate, at 80 ng/mL, and ranged from 68.7% to 78.8%.
- Matrix effects was assessed in duplicate, at 80 ng/mL, and ranged from 90.9% to 106.3%, which was considered acceptable.

Selectivity

- The extraction of a single blank bovine blood specimen exhibited no significant interferences to any analytes or ISTD.
- No carryover was observed after injecting a blank mobile phase three times after the 100 ng/mL calibrator.

Calibration Model

- A calibration model in the range of 1 - 100 ng/mL using a six-point calibration curve was assessed.
- A quadratic model with 1/x weight resulted in R^2 greater than 0.99 and acceptable calibrator accuracy for all analytes, except for cocaine (Figure 3).

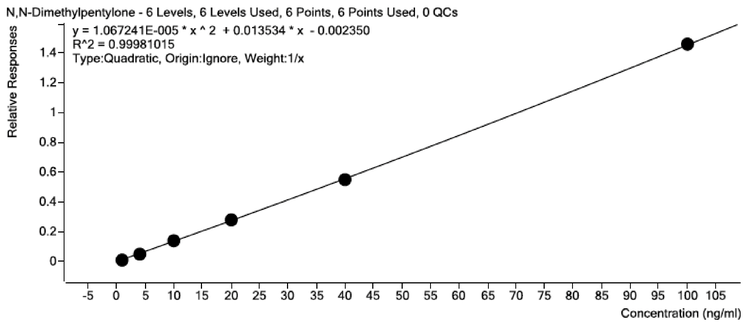


Figure 3. Representative six-point calibration curve for *N,N*-dimethylpentylone demonstrating linearity over the range of 1-100 ng/mL (quadratic model, 1/x weighting, $R^2 = 0.9998$).

REFERENCES

1. Bordin DC, Alves MN, de Campos EG, De Martinis BS. J Sep Sci. 2016 Mar;39(6):1168-72. doi: 10.1002/jssc.201500932.
2. Mata DC, Davis JF, Figueroa AK, Stanford MJ. J Anal Toxicol. 2016 Jan-Feb;40(1):58-63. doi: 10.1093/jat/bkv111.

MATERIALS & METHODS

Instrumental Analysis

- **Instrumentation:** Agilent 1290 Infinity II Liquid Chromatograph coupled to Agilent 6470 triple quadrupole MS
- **Column:** Agilent Poroshell 120 EC-C18 (2.1 x 100 mm, 2.7 µm) with matching guard column, at 35°C.
- **Mobile Phase:** Water (MPA) and acetonitrile (MPB) both containing 0.1% formic acid

	Time	A	B	Flow
1	5.00 min	90%	10%	0.300 mL/min
2	9.80 min	60%	40%	
3	10.00 min	5%	95%	
4	10.80 min	5%	95%	
5	10.90 min	95%	5%	
6	12.40 min	95%	5%	

- The mass spectrometer operated using positive electrospray ionization and multiple reaction monitoring mode

Preliminary Assessment of the Method

- Matrix effects, linearity and selectivity experiments were based on the ANSI/ASB 036 Standard.

CONCLUSIONS

- The combination of protein precipitation and DPX-WAX tips successfully extracted nine stimulants from blood with acceptable recovery (68.7-78.8%) and minimal matrix effects (90.9-106.3%)
- Method demonstrated good selectivity and linearity ($R^2 > 0.99$ for eight of nine analytes) across the forensic-relevant concentration range of 1-100 ng/mL.
- Further method validation is still required for assessment of the method's performance.

DISCLOSURE

The authors declare no conflicts of interest.

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