Development of an Analytical Method for Targeted Screening of Eutylone, *N,N*-Dimethylpentylone and Pentylone in Dried Blood Spots using Liquid Chromatography-Triple Quadrupole Mass Spectrometry (LC-MS/MS)

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INTRODUCTION

N,N-Dimethylpentylone (DMP), pentylone, and eutylone have been reported in forensic casework and listed in scope recommendations [1]. Methods capable of analyzing these unique analytes are important for screening toxicological samples suspected of containing synthetic cathinones. Dried blood spots (DBS) are a microsampling technique based on the application of a few microliters of blood (directly collected from the donor or from a previously collected blood sample) onto a paper card. Sample preparation is simplified compared to whole blood, typically consisting an extraction using organic solvents [2]. DBS offers multiple analytical advantages such as reduced sample volume, facilitated transportation and increased analyte stability [3].

OBJECTIVE

To develop and validate a targeted method for screening *N,N*-dimethylpentylone, eutylone and pentylone in DBS using LC-MS/MS.

MATERIALS & METHODS

DBS Extraction Optimization

- Incubation type: Sonication (35 kHz) or agitation (1000 rpm at 25°C)
- Solvent type: Methanol, 10% HCl in methanol, or methanol with 10 mM borate buffer pH 9
- Extraction time: 10 min, 20 min, or 30 min

Figure 1. A dried blood spot with 25 µL of bovine blood.

Sample Preparation

1. An aliquot of 25 µL of fortified or blank blood was pipetted onto pre-cut 10mm spots Whatman® 903 protein saver cards.
2. Spots were dried for 2 hours on a clean surface at room temperature, protected from ambient light.
3. Spots were extracted in 1 mL methanol containing pentylone-d_3 (ISTD) under sonication for 20 minutes.
4. The supernatant was transferred to a clean test tube and 50 µL of 10% HCl in methanol solution was added.
5. Extracts were evaporated to dryness under a nitrogen gas flow at 33°C.
6. Samples were reconstituted in 100 µL of 95:5 mobile phase A:mobile phase B and were centrifuged for 10 min at 10,000 rpm and 4°C.
7. Supernatants were transferred into LC vials and 10 µL was injected into the LC-MS/MS instrument

RESULTS & DISCUSSION

Method Development and Optimization

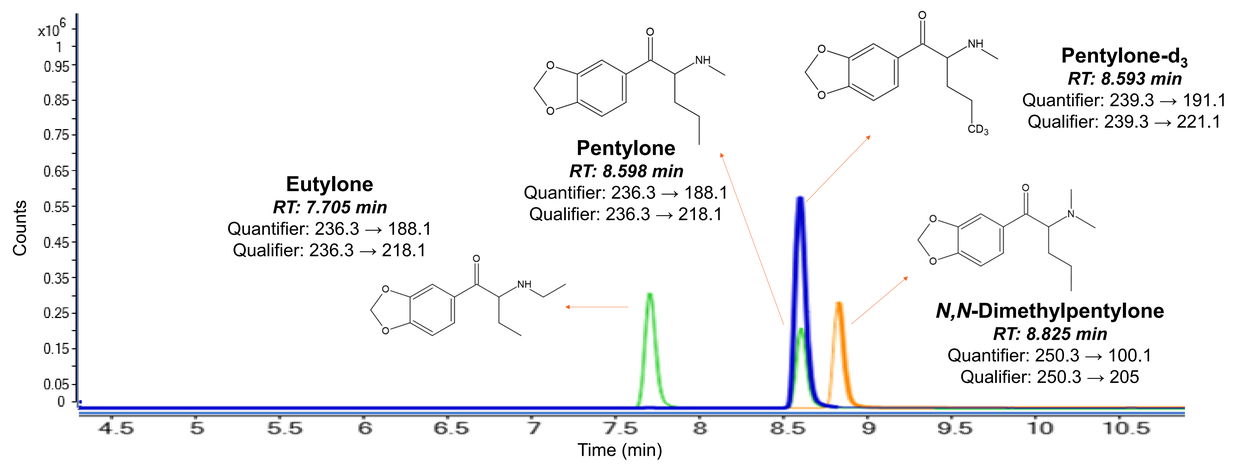

Figure 2. Example chromatogram of an extracted DBS sample depicting the MRM transitions and related structures of eutylone, pentylone, *N,N*-dimethylpentylone (HQC; 400 ng/mL) with pentylone-d_3 (ISTD; 25 ng/mL) in whole bovine blood.

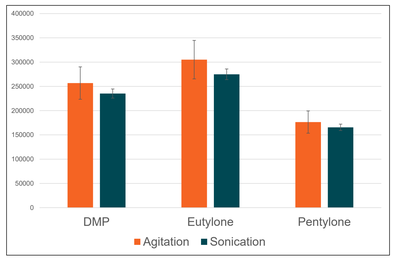

Figure 3. Average peak areas comparison between agitation or sonication as the method of extraction. Sonication demonstrated better peak reproducibility.

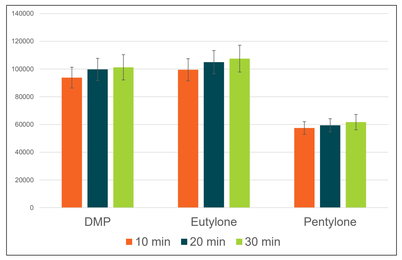

Figure 4. Average peak areas comparison between 10 min, 20 min, or 30 min extraction times. No significant difference was observed.

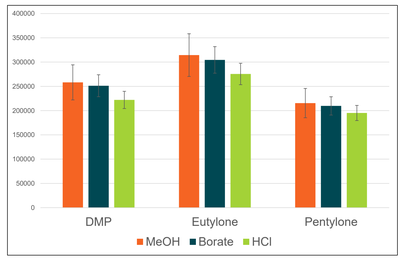

Figure 5. Average peak areas comparison between extraction solvents used. No significant differences were observed.

Method Validation

- **Matrix Effects:** Average ranged from 105% to 109% (LQC; 25 ng/mL) and 100 to 102% (HQC; 400 ng/mL) with a CV% less than 10%, over 2 days and using 6 sources.
- **Recovery:** Average ranged from 76% to 93% (LQC) with a CV% of 20%, and 74% to 84% (HQC) with a CV% of 7.5%, over 2 days and using 6 sources.
- **Limit of Detection:** Administratively set to 10 ng/mL and met all positive identification criteria.
- **Processed Sample Stability:** All analytes were stable in the autosampler up to 72 hours at 4°C with CV% less than 6%
- **Interference Studies:** No interferences from cannabinoids, MDA, MDMA, methamphetamine, PCP, phenylpropanolamine, benzoylecgonine, cocaethylene, cocaine, methadone, diazepam, nordiazepam, oxazepam and temazepam or 10 sources of bovine blood. A minor inference for *N,N*-dimethylpentylone observed from the ISTD, pentylone-d_3.
- **Carryover:** No carryover was observed in 3 consecutive mobile phase blanks after a 1000 ng/mL injection of the analytes

REFERENCES

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2. Jacques ALB et al. Forensic Sci Med Pathol. 2022;18(1):86–102. https://doi.org/10.1007/s12024-021-00434-5.
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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
Gradient Elution	Conditions start with 95% MPA, followed by a decrease to 90% MPA by 5 min and to 60% MPA by 11 min, then to 5% MPA by 11.10 min and held until 12.00 min. The gradient return to 95% MPA by 12.10 min and is held for until 14.10 min.
MS Parameters	The mass spectrometer operated in MRM mode using an Agilent Jet Stream source. The sheath gas was set to 12 L/min, at 400 °C. The collision gas was set to 200 °C and 9 L/min flow. The nebulizer operated at 35 psi, and the capillary was set to 4000 V.

Method Validation

A fit for purpose method validation was performed based on the ANSI/ASB 036 Standard.

CONCLUSIONS

A fast method for the identification of eutylone, pentylone, and *N,N*-dimethylpentylone in DBS using LC-MS/MS was developed. Acceptable sensitivity, selectivity, matrix effects and recovery were achieved. It is noteworthy that an interference from pentylone-d_3 and variability in the peak area in low concentration extracted samples was observed. This method can be applied to the analysis of the target cathinones using minimal volume of blood samples.

DISCLOSURE

The authors declare no conflicts of interest.

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