

Urine drug testing in a few drops: assessing dried urine spots for the detection of synthetic cathinones using liquid chromatography – tandem mass spectrometry

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INTRODUCTION

Dried urine spots (DUS) are a novel microsampling approach that provides an overall less complex matrix for the detection of analytes, and a longer detection window compared to blood sampling. DUS are simple to prepare, involving the application of a few microliters of urine to a paper card and sample preparation requires only a few steps compared to a liquid sample extraction^[1]. The implementation of DUS can facilitate sample storage and transportation. DUS can be beneficial to the toxicological analysis of synthetic cathinones, including eutylone, *N,N*-dimethylpentylone (DMP), and pentylone. These analytes are frequently reported in forensic casework and are listed in current scope recommendations^[2]. To the authors' knowledge, this is the first report on the analysis of the targeted synthetic cathinones in DUS.

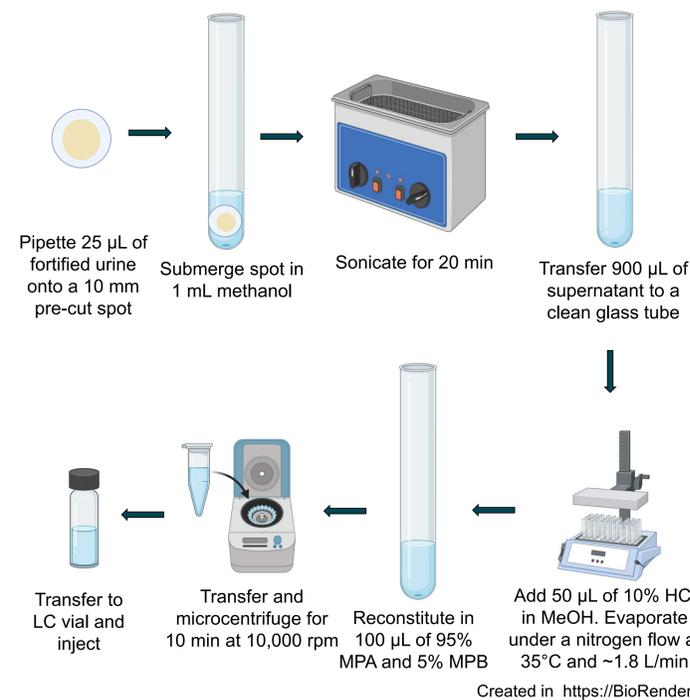
OBJECTIVE

To develop and assess a targeted method for screening *N,N*-dimethylpentylone, eutylone and pentylone in DUS via LC-MS/MS.

MATERIALS & METHODS

Sample Preparation

- The sample preparation method and instrumental analysis parameters are based off previous research and optimization with dried blood spots^[3]



RESULTS & DISCUSSION

Method Optimization and Assessment

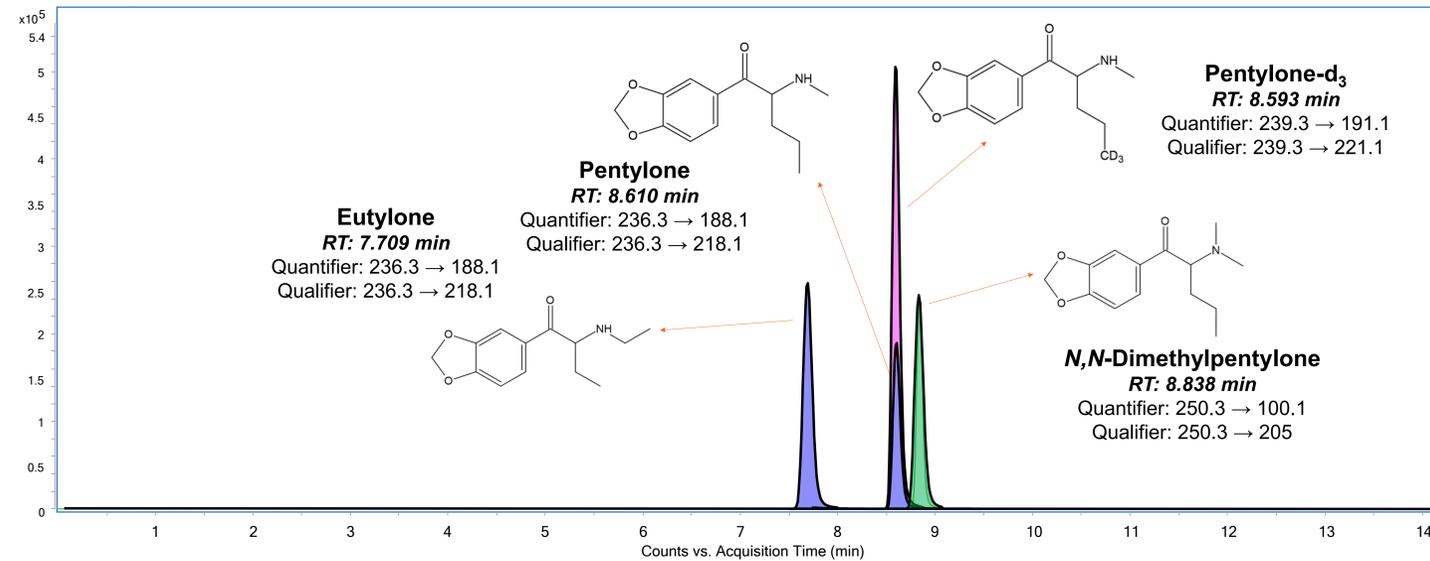


Figure 1. Example chromatogram of an extracted DUS sample (HQC; 400 ng/mL) depicting the MRM transitions and related structures of eutylone, pentylone, *N,N*-dimethylpentylone with pentylone- d_3 (ISTD; 20 ng/mL) in pooled human urine.

DUS Extraction Optimization

- Solvent type: Methanol, 10% HCl in methanol, or methanol with 10 mM borate buffer pH 9

DUS Extraction Optimization Results

- No significant differences between the three solvents tested were observed. A simple methanol extraction was selected.

Table 1. Method assessment summary

Parameter	Pentylone		Eutylone		<i>N,N</i> -Dimethylpentylone	
	LQC (25 ng/mL)	HQC (400 ng/mL)	LQC (25 ng/mL)	HQC (400 ng/mL)	LQC (25 ng/mL)	HQC (400 ng/mL)
Concentration level						
Matrix effects in 3 sources in duplicate	-41%	-40%	-35%	-29%	-43%	-47%
Recovery in 3 sources in duplicate	76%	90%	65%	84%	77%	89%
Processed sample stability for 24 and 48 hours	107 – 118%	101 – 122%	102 – 106%	98 – 114%	107 – 114%	102 – 121%
Limit of detection in 3 sources in triplicate	Administratively set to 10 ng/mL and met all identification criteria for peak shape, S/N, ion ratio, and retention time across 2 days					
Interference studies	No interferences for all analytes from 3 blank matrix sources Minor interferent peak for <i>N,N</i> -dimethylpentylone from pentylone- d_3					
Carryover	No carryover present for all three analytes from a 1000 ng/mL injection in three consecutive matrix blanks					

REFERENCES

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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 $^{\circ}\text{C}$.

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, 12 L/min, at 400 $^{\circ}\text{C}$. The collision gas was set to 200 $^{\circ}\text{C}$ and 9 L/min flow. The nebulizer operated at 35 psi, and the capillary was set to 4000 V.

Method Performance Assessment

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

CONCLUSIONS

DUS is a promising alternative microsampling approach for screening *N,N*-dimethylpentylone, eutylone and pentylone. Analytes were detected at a low concentration (10 ng/mL); however, significant ion suppression was observed for all compounds. Matrix effects and varying recoveries may be explained based on the high content of water/water-soluble substances in urine and the polarity of methanol. Additional studies are needed to further fully validate this method.

DISCLOSURE

The authors declare no conflicts of interest.

ACKNOWLEDGEMENTS

The authors would like to thank the Department of Forensic Science and the Graduate and Professional school at Sam Houston State University for the resources to conduct this research.