Differentiation of Synthetic Cathinone Isomers by GC-EI-MS and Multivariate Analysis

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ABSTRACT

Synthetic cathinones are phenylalkylamine derivatives synthesized in clandestine laboratories to intentionally circumvent legal regulations. The emergence of synthetic cathinone isomers has created a growing need for the expansion of the standard analytical approaches used in forensic laboratories. This project investigates the development of a multivariate analysis approach for the differentiation of synthetic cathinone isomers with gas chromatography-electron ionization-mass spectrometry (GC-EI-MS).

INTRODUCTION

Synthetic cathinones, commonly referred to as "bath salts", are designed to mimic the natural psychoactive chemical cathinone, which is derived from the leaves of the *Catha edulis* plant [1]. This plant, often referred to as "khat", produces stimulant-like effects when the leaves are chewed [2]. Synthetic cathinones are examples of novel psychoactive substances (NPS) due to their synthetic nature and stimulant-like effects. Figure 1 provides a comparison between cathinone, the natural psychoactive compound, and a general structure representing the common locations of substitution to the core synthetic cathinone structure.

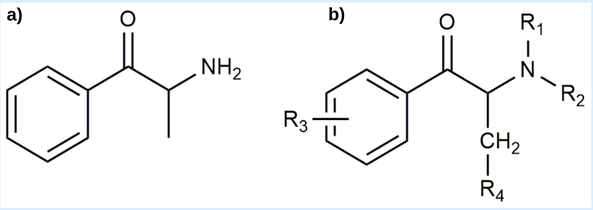

Figure 1. Comparison between the structure of cathinone (a) and the general structure of synthetic cathinones (b) modified from reference [2].

The emergence of highly similar synthetic cathinone isomers has necessitated research into the expansion of standard analytical approaches used in forensic laboratories. In this project, canonical discriminant analysis (CDA) is used for the differentiation of synthetic cathinone isomers. The central hypothesis of this project is that each synthetic cathinone isomer has a unique EI fragmentation pattern that will allow for the differentiation of synthetic cathinone isomers using multivariate classification. Traditionally, multivariate analysis has required relatively large data sets to develop robust statistical models [3]. This project investigates the use of multiple scans across the chromatographic peak and a reduction in the number of ions used to construct multivariate classification models to reduce the minimum number of replicate injections required to construct accurate multivariate models.

RESULTS AND DISCUSSION

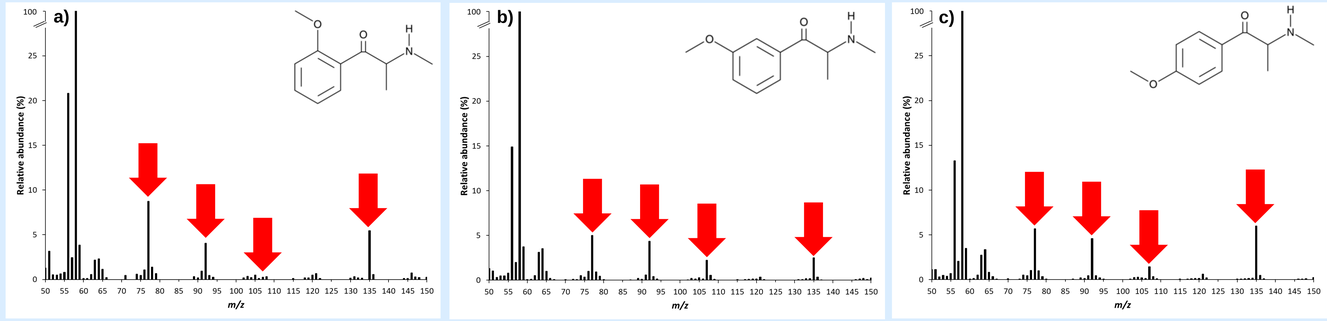

Figure 2. Exemplar EI mass spectra of 2-MeOMC (a), 3-MeOMC (b), and 4-MeOMC (c) and their chemical structures.

- ❖ Positional isomers have only subtle differences in EI fragmentation that become more apparent when comparing abundance ratios.
- ❖ CDA loadings highlight the importance of ions at *m/z* 77, 92, 107, and 135 for the differentiation of MeOMC positional isomers.

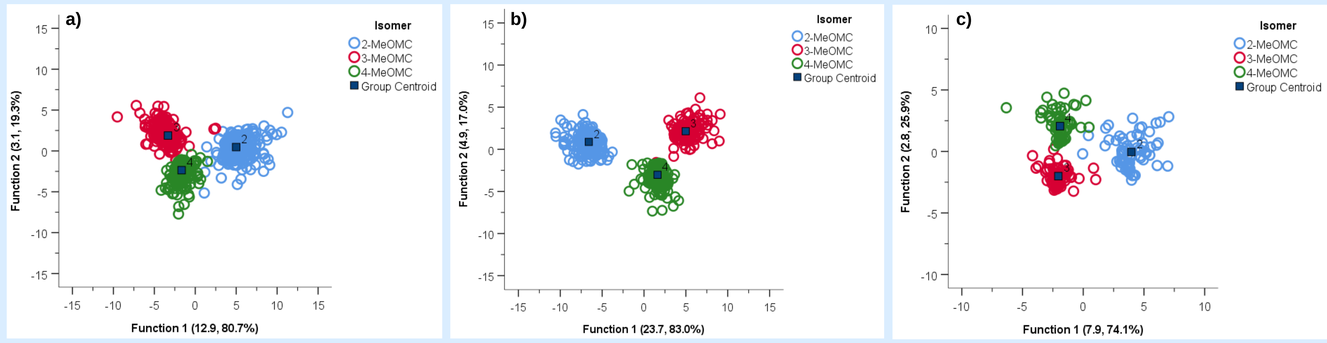

Figure 3. Canonical discriminant analysis (CDA) showing the classification of methoxymethcathinone positional isomers with 15 ions and 5 scans (N = 1125) (a), 10 ions and 3 scans (N = 675) (b), and 5 ions and 1 scan (N = 225) (c) from Table 1. The leave-one-out cross-validation (LOOCV) classification rates were 99.1%, 99.7%, and 98.7%, respectively.

- ❖ CDA provides multivariate separation based on the extracted relative ion abundances (Figure 3).
- ❖ Table 1 summarizes CDA prediction results for both original and LOOCV for all nine combinations of the number of scans across a chromatographic peak (1, 3, or 5) and the number of ions used to construct the CDA model (5, 10, or 15).

Table 1. Classification rates of CDA models for CEC, MeOMC and constitutional isomer sets with the original classification (1st %), cross-validation classification (2nd %) and sample size (N).

		CEC			MeOMC			Constitutional		
		Number of scans			Number of scans			Number of scans		
		1	3	5	1	3	5	1	3	5
Number of ions	5	(75.1%, 75.1%) N = 225	(73.3%, 72.3%) N = 675	(68.2%, 67.9%) N = 1125	(98.7%, 98.7%) N = 225	(98.8%, 98.7%) N = 675	(98.0%, 98.0%) N = 1125	(98.7%, 98.7%) N = 300	(98.6%, 98.4%) N = 900	(98.1%, 98.1%) N = 1500
	10	(91.6%, 88.0%) N = 225	(87.3%, 86.2%) N = 675	(82.2%, 81.6%) N = 1125	(100%, 100%) N = 225	(99.7%, 99.7%) N = 675	(99.3%, 99.2%) N = 1125	(99.7%, 99.7%) N = 300	(99.0%, 99.0%) N = 900	(99.1%, 98.9%) N = 1500
	15	(94.7%, 90.2%) N = 225	(89.3%, 88.6%) N = 675	(86.5%, 86.0%) N = 1125	(100%, 100%) N = 225	(99.7%, 99.7%) N = 675	(99.5%, 99.1%) N = 1125	(100%, 100%) N = 300	(100%, 100%) N = 900	(99.9%, 99.7%) N = 1500

- ❖ Increasing the number of ions and decreasing the number of scans increases the classification rate.
- ❖ The lowest LOOCV classification rate for each isomer set is 67.9% for CEC, 98.0% for MeOMC, and 98.1% for constitutional.
- ❖ This behavior reflects the degree of similarity within each isomer set based on possible different fragmentation pathways.

MATERIALS AND METHODS

Sample Preparation

The synthetic cathinone isomers chosen for this study include the constitutional isomers eutylone, dibutylone, pentylone, and the positional isomer of pentylone, 2,3-pentylone. The positional isomers chosen for this study include the 2-, 3-, and 4- chloroethcathinone (CEC) and methoxymethcathinone (MeOMC) positional isomers. All compounds were analyzed at concentrations of 50, 100, and 500 ppm.

Instrumentation and Data Analysis

An Agilent Technologies 7890A GC-5975C MS with an Agilent DB-5ms 30 m x 250 µm x 0.25 µm column was used for analysis. The SPSS software was used to generate the CDA models with relative ion abundances as continuous predictor variables.

CONCLUSIONS

- ❖Subtle differences in EI fragmentation result in multivariate differentiation.
- ❖CDA provides LOOCV classification rates ≥98% for MeOMC positional isomers and constitutional isomers.
- ❖CEC positional isomers produce the most similar EI spectra, thus the lowest classification rates.
- ❖CDA can identify characteristic ions useful for isomer differentiation.
- ❖Reducing the number of ions and increasing the number of scans shows potential as a method to reduce the required number of injections.
- ❖Overall CDA classification rates increased when the lowest concentration (50 ppm) samples were removed.

REFERENCES

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ACKNOWLEDGEMENTS

The authors thank the Department of Forensic Science at Sam Houston State University for providing the resources necessary to conduct this research and the FSF for the Student Affiliate Scholarship.