Synthetic Cathinone Isomer Differentiation Using GC-EI-MS and Multivariate Analysis

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1. Introduction

Synthetic cathinones are a class of phenylalkylamine derivatives that are designed to mimic cathinone, the natural psychoactive substance found in the leaves of the *Catha edulis* plant, often referred to as "khat" [1]. The scheduling of synthetic cathinones is compound specific, which means only slight chemical modifications are required to avoid legislative restrictions [2]. This places the burden on seized drug analysts to differentiate between non-scheduled and scheduled synthetic cathinone isomers.

Currently, forensic laboratories rely on gas chromatography-electron ionization-mass spectrometry (GC-EI-MS) for the differentiation of synthetic cathinone isomers. However, the differentiation of synthetic cathinone isomers is primarily dependent on slight differences in the EI mass spectra. Whereas skilled analysts may decipher these minor differences, a more robust approach is necessary for the reliable differentiation of synthetic cathinone isomers.

Canonical discriminant analysis (CDA) is one potential solution for this issue. CDA is a supervised technique that classifies an unknown into one of the known groups used to develop the model. However, multivariate analysis techniques often require relatively large datasets to develop robust statistical models [3], which is not easily incorporated into the traditional forensic laboratory approach.

2. Objectives

This study investigates the use of CDA for the differentiation of three synthetic cathinone isomer sets using GC-EI-MS. In addition, this study explores reduction strategies to reduce the number of replicate sample injections required to develop accurate multivariate models. Finally, this study compares two ion selection methods prior to CDA classification, which are the most abundant ions and the ions with the highest principal component analysis (PCA) loadings.

3. Methods

Samples

This study involved the analysis of two positional isomer sets and one constitutional isomer set. The two positional isomer sets involved the 2-, 3-, and 4-chloroethcathinone (CEC) and methoxymethcathinone (MeOMC) isomers. The constitutional isomer set was composed of dibutylone, eutylone, pentylone, and its positional isomer, 2,3-pentylone. All isomers were prepared at concentrations of 50, 100, and 500 ppm.

Instrumentation and Data Analysis

GC-EI-MS analysis was conducted using an Agilent Technologies 7890A GC-5975C MS with an Agilent DB-5ms 30 m x 250 µm x 0.25 µm column. Microsoft Excel was used to normalize the ion abundances to the base peak of each spectrum. The relative ion abundances were imported as variables into the SPSS software to generate the CDA models.

CDA Models

Initially, four sets of CDA models were generated for each of the isomer sets using the following conditions: 1) all three concentrations; 2) only the 100 and 500 ppm concentrations; 3) the constitutional isomer set without the 2,3-pentylone positional isomer; and 4) a combined dataset with all 10 isomers using all three concentrations. Two ion selection methods, referred to as the consensus and PCA loadings methods, were developed for the combined dataset with all 10 isomers due to no consensus for the 15 most abundant ions. The consensus method involved the 5 most abundant ions from all ten isomers and 10 abundant ions that are known to be structurally relevant for synthetic cathinones. In comparison, the PCA loadings method used the 15 ions with the highest PCA loadings based on the absolute sum of the first two principal components. The PCA loadings method was then applied to each of the isomer sets to determine the difference between the classification rates using only the 15 most abundant ions and the PCA loadings method.

4. Results

Table 1. CDA classification rates for the three isomer sets using all three concentrations.

CEC		Number of scans		
		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
	10	(91.6%, 88.0%) N = 225	(87.3%, 86.2%) N = 675	(82.2%, 81.6%) N = 1125
	15	(94.7%, 90.2%) N = 225	(89.3%, 88.6%) N = 675	(86.5%, 86.0%) N = 1125
MeOMC		**Number of scans**		
		1	3	5
Number of ions	5	(98.7%, 98.7%) N = 225	(98.8%, 98.7%) N = 675	(98.0%, 98.0%) N = 1125
	10	(100.0%, 100.0%) N = 225	(99.7%, 99.7%) N = 675	(99.3%, 99.2%) N = 1125
	15	(100.0%, 100.0%) N = 225	(99.7%, 99.7%) N = 675	(99.5%, 99.1%) N = 1125
Constitutional		**Number of scans**		
		1	3	5
Number of ions	5	(98.7%, 98.7%) N = 300	(98.6%, 98.4%) N = 900	(98.1%, 98.1%) N = 1500
	10	(99.7%, 99.7%) N = 300	(99.0%, 99.0%) N = 900	(99.1%, 98.9%) N = 1500
	15	(100.0%, 100.0%) N = 300	(100.0%, 100.0%) N = 900	(99.9%, 99.7%) N = 1500

The first percentage is the original classification, and the second percentage is the cross-validation classification. The N is the number of data points used to build the CDA model.

Table 2. CDA classification rates for the three isomer sets using only 100 and 500 ppm.

CEC		Number of scans		
		1	3	5
Number of ions	5	(86.7%, 86.0%) N = 150	(81.8%, 80.7%) N = 450	(75.7%, 75.3%) N = 750
	10	(98.7%, 95.3%) N = 150	(94.4%, 92.7%) N = 450	(90.0%, 89.3%) N = 750
	15	(98.7%, 98.7%) N = 150	(97.1%, 95.3%) N = 450	(92.5%, 91.7%) N = 750
MeOMC		**Number of scans**		
		1	3	5
Number of ions	5	(99.3%, 99.3%) N = 150	(99.3%, 99.3%) N = 450	(99.3%, 99.3%) N = 750
	10	(100.0%, 100.0%) N = 150	(100.0%, 100.0%) N = 450	(99.9%, 99.9%) N = 750
	15	(100.0%, 100.0%) N = 150	(100.0%, 100.0%) N = 450	(100.0%, 99.9%) N = 750
Constitutional		**Number of scans**		
		1	3	5
Number of ions	5	(99.5%, 99.5%) N = 200	(99.7%, 99.7%) N = 600	(99.4%, 99.4%) N = 1000
	10	(99.5%, 99.5%) N = 200	(99.5%, 99.5%) N = 600	(99.7%, 99.6%) N = 1000
	15	(100.0%, 100.0%) N = 200	(100.0%, 100.0%) N = 600	(100.0%, 99.9%) N = 1000

The first percentage is the original classification, and the second percentage is the cross-validation classification. The N is the number of data points used to build the CDA model.

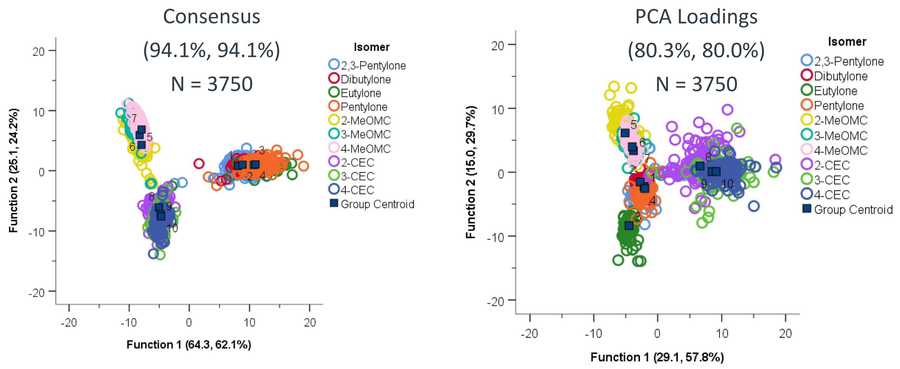

Figure 1. Combined isomer set CDA models for the consensus method and the PCA loadings method.

Table 3. Comparison of ion selection methods for each of the isomer sets with the CDA model using 15 ions and 5 scans across the chromatographic peak.

Ion selection method	Positional (CEC)	Positional (MeOMC)	Constitutional
Abundant	(86.5%, 86.0%) N = 1125	(99.5%, 99.1%) N = 1125	(99.9%, 99.7%) N = 1500
PCA Loadings	(86.1%, 85.7%) N = 1125	(99.6%, 99.5%) N = 1125	(99.9%, 99.9%) N = 1500

The first percentage is the original classification, and the second percentage is the cross-validation classification. The N is the number of data points used to build the CDA model.

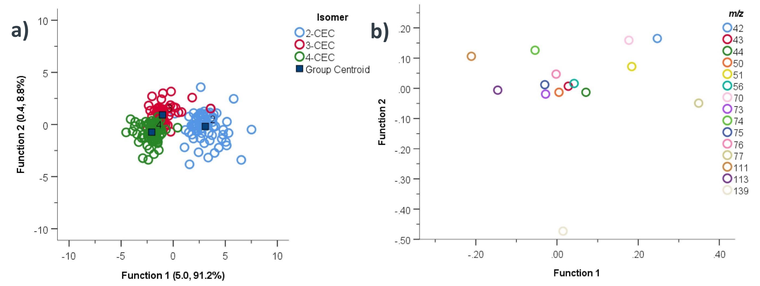

Figure 2. CEC CDA model with 15 ions and 1 scan (a) and the ion correlation plot of the model (b).

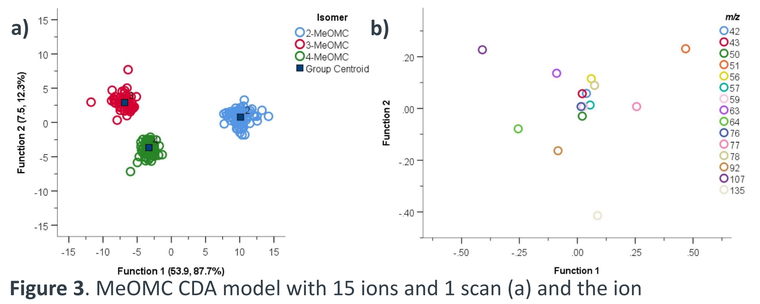

Figure 3. MeOMC CDA model with 15 ions and 1 scan (a) and the ion correlation plot of the model (b).

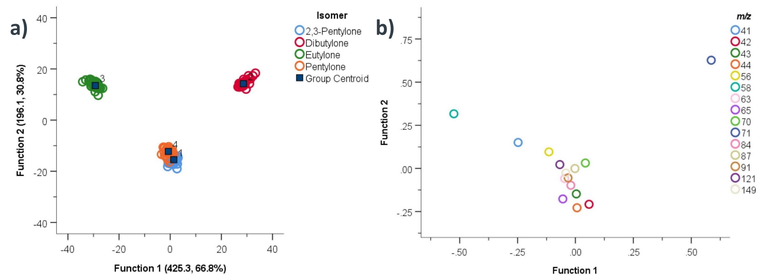

Figure 4. Constitutional CDA model with 15 ions and 1 scan (a) and the ion correlation plot of the model (b).

5. Conclusions

CDA Classification

- LOOCV classification rates of 90.2% and 100.0% with 15 ions and apex data and at least 67.9%, 98.0%, and 98.1% for the reduction strategies dataset

Improved CDA Classification

- Removal of the lowest concentration from each of the three isomer sets improved overall classification rates

Ion Selection Methods

- For each of the three isomer sets, using the highest PCA loadings ions resulted in similar classification rates as using the most abundant ions

Ion Correlation

- Ion correlation plots help explain why there are differences in the classification rates when removing ions from the dataset

6. Potential for Impact

- Developed an alternative technique for determining isomeric identity using a multivariate analysis approach conducted through commercial software
  - The data required for the technique is generated during a typical seized drug analytical scheme
- Using the most abundant ions produces similar results to the PCA loadings ions
  - Reduces the amount of time spent using the SPSS software

7. Limitations

- Did not apply this approach to authentic casework samples to assess the potential impact of impurities
- This approach may not work for other compounds, such as those that produce more fragment-rich mass spectra

References and Suggested Citation

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