# **Research Article**

# **Impurity Profiling of ADB-BUTINACA or MDMB-4en-PINACA e-Liquids via Multivariate Analysis**

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## Abstract

The analysis of impurities in seized drugs is also of great value. In the present study, impurity profiling was conducted by Gas Chromatography-Mass Spectrometry (GC-MS) and Inductively Coupled Plasma MS (ICP-MS) analysis of 211 e-liquids containing ADB-BU-TINACA or MDMB-4en-PINACA seized in the Yangtze River Delta and 68 commercial e-liquid samples. The impurities determined by GC-MS differed significantly between the seized and commercial e-liquid samples. The contents of 31 elements in seized and commercial e-liquids were detected by ICP-MS and analyzed by t-test comparison. The results of the t-test showed significant differences in the contents of Ca, V, Ni, Cu, Zn, Se, Sr, Zr, Mo, Ba, Ti, and Tl. Combined with the appearance, we basically ruled out the possibility that the currently popular e-liquids containing SCs were added secondarily by commercial e-liquids. One organic impurity associated with MDMB-4en-PINACA and four organic impurities associated with ADB-BUTINACA were identified. Combining the contents of 31 elements with k-means clustering indicated a certain aggregation among the seized e-liquid samples, which could provide a basis for establishing the association between e-liquid samples as well as a basis for inferring the route of drug trafficking.

**Keywords:** Impurity profiling; E-liquids; ADB-BUTINACA; MDMB-4en-PINACA

#### Introduction

The mining and utilization of drug-related intelligence plays a vital role in the detection of cases for anti-drug work. In seized drugs analysis, impurities can also often provide important clues or information. Sanggil Choe et al. [1] used Gas Chromatography-Mass Spectrometry (GC-MS) to analyze impurities in 609 methamphetamine crystals from a drug trafficking case seized in Korea and concluded that drug impurities can be used for similarity analysis and for monitoring changes in precursor chemicals or production sources. Cuimei Liu [2] used Inductively Coupled Plasma Mass Spectrometry (ICP-MS) to analyze 26 inorganic elements in 183 cocaine samples and found 21 groups of interrelated samples that provided intelligence for case articulation and revealing the distribution network.

Synthetic Cannabinoids (SCs) are a synthetic class of receptor agonists for the cannabinoids CB1 and CB2 [3]. When smoked, SC compounds can bind and agonize the body's cannabinoid receptors to produce hallucinogenic effects similar to those of natural cannabinoid tetrahydrocannabinol [4]. The SCs are highly addictive and toxic [5,6], and statistics [7] released by the United Nations Office on Drugs and Crime show them to be one of the most widely abused classes of new psychoactive substances. SCs come in many forms, are fashionable and cam-

Austin J Anal Pharm Chem Volume 10, Issue 1 (2023) www.austinpublishinggroup.com Xu Q © All rights are reserved ouflaged, and are already in common use in several countries. Vaping SCs via e-cigarettes is growing in popularity.

Our study compiled data from 210 seized e-liquid samples containing ADB-BUTINACA or MDMB-4en-PINACA and 68 commercial e-liquid samples, including data on impurities analyzed by GC-MS and ICP-MS. Through color analysis, compound and element composition analysis, and cluster analysis based on chemometrics, this study attempts to characterize whether the current popular e-liquids containing SCs in the Yangtze River Delta are prepared by the secondary addition of ordinary commercial e-liquids.

## **Materials and Methods**

#### Samples and Reagents

The 210 seized e-liquid samples were all from real cases. All samples were seized from the belongings of suspected users between 1 January and 31 December 2022, based on a trail to suspected drug users, following a legal inspection with full human rights safeguards. The inspection covered the Yangtze River Delta region. The study has been reported to the Ethics Committee Approval of the Academy of Forensic Science, China,

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and was analyzed under the framework of the forensic science international standardization ISO/TC 272. The 68 commercial eliquid samples were all purchased from tobacconists who have tobacco monopoly licenses.

# Acquire the Color of the E-liquid Samples

The e-liquid samples (100  $\mu$ L) were added dropwise to 96well plates, which were placed in the center of the proof station tabletop (JUST LED proof Station 30, Gurun Optoelectronic Technology, Guangzhou, China). A digital single lens reflex camera (D90, NIKON with AF-S Nikkor 35mm f/1.8G ED) was fixed 30 cm above the table, and the plates were photographed under a D65 light source using f8 ISO=100 automatic white balance with no exposure compensation.

Methanol was obtained from Sinopharm Chemical Reagent Co. (Shanghai, China). ICP multi-element standard solution, Mercury ICP standard solution, Gold ICP standard solution, and Cesium ICP standard solution were all purchased from Merck (America), as was the 65% HNO<sub>3</sub> solution. Deionized water was produced using a Milli-Q ultrapure water treatment system. Synthetic cannabinoid standards for N-[(2S)-1-amino-3,3-dimethyl-1-oxobutan-2-yl]-1-butylindazole-3-carboxamide (ADB-BUTINACA) and methyl (2S)-3,3-dimethyl-2-[(1-pent-4-enylindazole-3-carbonyl)amino]butanoate (MDMB-4en-PINACA) were purchased from Cayman Chemicals (Ann Arbor, MI, USA). Nicotine and lidocaine standards were also purchased from Cayman Chemicals (Ann Arbor, MI, USA).

# Screen by GC-MS

The chromatography and MS conditions were as previously described [8]. A 10 µL volume of the e-liquids was dissolved in 990 µL of methanol. If only a small amount of oily substance remained on the wall, it was diluted with an appropriate amount of methanol approximately at a 1:100 ratio (v/v). All the seized samples were prepared similarly and analyzed using GC-MS. The spectra were acquired using an Agilent 7890B GC and an MS5975 mass selective detector (Agilent Technologies, Palo Alto, USA). Separation was achieved on a capillary column (DB-5MS, 30 m length, 0.25 mm i.d., 0.25  $\mu$ m film thickness) with helium as the carrier gas at a constant flow rate of 1.2 mL/min. A 2 µL aliquot of each sample was injected with a split ratio of 4:1. The injection port temperature was 250°C. The oven ramp temperature was programmed as follows: initial temperature of 100°C held for 1.5 min and increased at 25°C/min to 280°C and held for 15 min. The MS ionization mode was electron ionization set at 70 eV, with an ion source temperature of 200°C and a transfer line temperature of 180°C. Ions were monitored using the SCAN mode, and the scan spectra were obtained between 42 and 550 amu. This method has a detection limit of 2µg/mL for ADB-BUTINACA and MDMB-4en-PINACA. The impurities of 211 seized e-liquid samples suspected of containing SCs and 68 commercial e-liquid samples were analyzed by GC-MS. The total ion chromatograms of the impurities were obtained and compared with the SWGDRUG Mass Spectral Library (version 3.10) and NIST 14 Spectral Library certified GC-MS libraries. Components with a confidence level higher than 80% were considered as identified, and the peak area normalization method was applied to calculate the relative contents of the impurities.

# ICP-MS

The ICP-MS conditions were as previously described [9]. A 100  $\mu$ L of aliquot the remaining e-liquid samples were added to polypropylene tubes, 100  $\mu$ L of 65% HNO<sub>3</sub>was added, and the

tubes were capped and placed on a heating plate at 80°C for 1 h. The tubes were cooled to room temperature and then 1.8 mL of deionized water was added and mixed well. The acquired samples were analyzed using a Thermo Scientific iCAP RQ ICP-MS. ICP-MS was calibrated using a 1µg/L tuning solution before use. The optimized instrument operating parameters were set as shown in Table 1, and the isotopes selected for determination of each element were <sup>7</sup>Li, <sup>9</sup>Be, <sup>11</sup>B, <sup>24</sup>Mg, <sup>43</sup>Ca, <sup>47</sup>Ti, <sup>51</sup>V, <sup>53</sup>Cr <sup>55</sup>Mn, <sup>57</sup>Fe, <sup>59</sup>Co, <sup>60</sup>Ni, <sup>63</sup>Cu, <sup>66</sup>Zn, <sup>69</sup>Ga, <sup>75</sup>As, <sup>82</sup>Se, <sup>85</sup>Rb, <sup>88</sup>Sr, <sup>90</sup>Zr, <sup>95</sup>Mo, <sup>107</sup>Ag, <sup>111</sup>Cd, <sup>118</sup>Sn, <sup>121</sup>Sb, <sup>133</sup>Cs, <sup>137</sup>Ba, <sup>197</sup>Au, <sup>202</sup>Hg <sup>205</sup>Tl, and <sup>208</sup>Pb. A t-test was conducted using GraphPad Prism 8 (2018) to determine whether a significant difference existed in the content of each element between the seized and commercial eliquid samples. The contents of the 31 elements in the seized eliquid samples were imported into Python (version 3.10, 2021) and subjected to Principal Component Analysis (PCA) using the PCA function in the Python Scikit-learn machine learning library. K-means clustering was conducted based on the data after dimensionality reduction, performed using the cluster function in Scikit-learn (2022).

Table 1: The operating parameters for ICP-MS.

Carrier gas flow rate	0.86 L/min
Auxiliary gas flow rate	0.22 L/min
Plasma gas flow rate	15 L/min
Radio Frequency Power	1500 w
Sampling depth	8 mm
Peristaltic pump flow rate	0.2 raps
Points time	0.1 s
Repeat times	3

 

 Table 2: The main impurities shown in the total ion chromatogram of the commercial e-liquid samples. (\*Labeled compounds are inferred from the database, and match accuracies are indicated.)

N⁰o	Retention Time	components	Fragments
1	3.947±0.078940	Benzoic acid*(93%–95%)	122/105/77
2	4.185±0.083700	Levomenthol*(89%–92%)	
3	4.277±0.085540	Ethyl maltol*(93%–96%)	
4	4.467±0.089340	1	103/43
5	4.848±0.096960	1	129/114/57
6	5.069±0.10138	Triacetin*(83%–86%)	43/103/145
7	5.281±0.10562	Nicotine	84
8	5.524±0.11048	Vanillin*(94%–97%)	151/123/109/81
9	6.547±0.13094	1	87/100/211
10	6.774±0.13548	1	203/157/115

**Table 3:** The main impurities shown in the total ion chromatogram of the seized e-liquid samples.(\*Labelled compounds are inferred from the database, and the match accuracies are indicated

N⁰o	Retention Time	components	Fragments
1	3.988±0.079760	Menthol*(89%–91%)	
2	4.525±0.090500	Docosane*(91%-93%)	
3	4.600±0.09200	/	129/114/57
4	5.281±0.10562	nicotine	
5	5.756±0.11512	n-heneicosane*(87%-89%)	
6	7.785±0.15570	methyl palmitate*(90%–91%)	
7	8.384±0.16768	/	217/174/131
8	8.481±0.16962	Acid methyl ester*(97%–99%)	
9	8.565±0.17130	methlyl stearate*(86%-89%)	
10	9.660±0.19320	/	247/215/159
11	10.671±0.21342	/	256/213/144

**Table 4:** Inorganic element concentration levels found in the seized e-liquids containing ADB-BUTINACA or MDMB-4en-PINACA and the commercial e-liquid samples.(A value of p<0.05 indicates a significant difference between the seized e-liquid samples and the commercial e-liquid samples.)

	Average concentration(ng/ml)		RSD		_	
	Seized e-liquid samples	Commercial e-liquid samples	Seized e-liquid samples	Commercial e-liquid samples	Р	
Li	91.89	26.91	5.05	34.75	0.2713	
Be	0.37	0.41	1.59	0.41	0.7166	
В	2359.55	2936.94	3.46	3816.70	0.8452	
Mg	3303.92	1763.84	2.28	1987.80	0.1507	
Ca	17707.33	9981.57	1.51	10133.99	<0.0001	
V	3.47	9.18	2.02	12.35	<0.0001	
Cr	817.60	3305.00	11.80	20384.10	0.0718	
Mn	75.25	108.31	2.81	347.19	0.1544	
Fe	3878.11	13714.27	10.07	81986.48	0.0751	
Со	74.65	17.82	3.13	55.74	0.1144	
Ni	7237.87	15322.87	3.21	28799.80	0.0279	
Cu	27479.19	90638.88	2.59	107998.08	<0.0001	
Zn	44548.98	115265.96	2.79	175486.51	0.0005	
Ga	1.56	2.95	2.64	3.61	0.053	
As	23.75	45.14	2.56	33.20	0.07	
Se	4.00	5.44	1.02	4.95	0.0282	
Rb	18.17	16.91	2.12	31.90	0.9679	
Sr	189.59	32.06	1.95	25.02	0.0017	
Mo	6.80	12.87	3.19	33.20	0.0453	
Ag	0.46	0.40	1.41	0.22	0.4914	
Cd	2.34	1.60	6.53	4.93	0.6577	
Cs	0.58	0.45	3.93	1.16	0.8204	
Ва	244.37	893.63	4.93	2449.63	0.0028	
Ti	0.21	0.41	1.95	0.64	< 0.0001	
Pb	1591.16	2533.01	6.41	9279.37	0.4625	
TI	127.70	272.52	1.08	157.50	< 0.0001	
Zr	20.93	63.71	2.44	88.19	< 0.0001	
Sn	821.01	469.86	6.89	736.17	0.5202	
Sb	47.91	64.62	3.14	132.03	0.4097	
Au	7.73	5.79	6.57	15.98	0.724	
Hg	0.33	0.18	2.31	0.39	0.346	

**Results and Discussion** 

In total, 211 seized e-liquid samples suspected of containing SCs and 68 commercial e-liquid samples were acquired. The color, volatile organic compounds, and inorganic compounds contained in the samples were analyzed separately and compared to provide references for anti-drug work.

#### Color

In terms of appearance, the seized e-liquid samples containing SCs were more likely to be disassembled, and a photograph of their typical appearance is shown (Figure 1). The color of the center of the wells in the 96-well plate was taken as the sample color data (Figure 2) using Photoshop 21.0.1 (Adobe, USA). The RGB values of the colors of the seized and commercial eliquid samples were compared separately based on the maximum variance discrepancy. A variable of 0.6014 was obtained for both groups, which was not greater than the parameter  $\kappa$ (κ=0.6480) of the distribution of the Von Mises-Fisher distribution within the group. Therefore, the seized e-liquid samples in this study were not considered to differ significantly from the commercial e-liquid samples in terms of color. However, from a visual perspective, some of the seized e-liquid samples containing ADB-BUTINACA or MDMB-4en-PINACA were brighter in color than the commercial samples, suggesting the possibility that drug cartels use brighter colors to facilitate labeling.

#### GC-MS

A total of 10 impurities were detected in the 68 commercial e-liquid samples, with each substance concentrated in the first 7 min of the GC-MS run. The retention time and major fragment ion information for each compound are shown (Table 2). The compound with the highest frequency of detection had a retention time of 4.848±0.09696 min, followed by nicotine. The main components of the detected substances were benzoic acid, levomenthol, ethyl maltol, triacetin, and vanillin, as extrapolated from the NIST 14 Spectral Library. The match accuracies are indicated (Table 2). Among them, ethyl maltol and vanillin are common flavor enhancers used in tobacco, food, beverages, and daily cosmetics. Triacetate is often used as a fragrance fixative and solvent. Benzoic acid [10] is used to modulate the nicotine salt content to simulate the rapid release of nicotine from cigarettes.



**Figure 1: (a)** Photograph of some of the seized e-liquid samples containing ADB-BUTINACA or MDMB-4en-PINACA; **(b)** Photograph of some of the commercial e-liquid samples.



**Figure 2: (a)** Color distribution of the seized e-liquid samples containing ADB-BUTINACA; **(b)** Color distribution of the seized e-liquid samples containing MDMB-4en-PINACA; **(c)** Color distribution of the seized e-liquid samples containing both ADB-BUTINACA and MDMB-4en-PINACA; **(d)** Color distribution of the commercial eliquid samples.







**Figure 4:** Excerpts of overlaid GC-MS base peak chromatograms for the seized e-liquid samples and the frequency of occurrence of the compounds.



**Figure 5: (a)** The association network diagram of each substance, centering on ADB-BUTINACA and MDMB-4en-PINACA (thicker lines represent stronger associations and higher probabilities of concomitant appearance); **(b)** The Electron Ionization-Mass Spectrometry (EI-MS) spectrum of compound with retention time at 9.660±0.1932; **(c)** The EI-MS spectrum of compound with retention time at 8.384±0.16768, **(d)** The EI-MS spectrum of compound with retention time at 10.671±0.21342; **(e)** The EI-MS spectrum of compound with retention time at 4.600±0.092.





Compounds with a retention time of 4.848±0.09696 and major fragment ions of 129, 114, and 57 were detected in the commercial e-liquid samples, as well as in the seized e-liquid samples containing ADB-BUTINACA or MDMB-4en-PINACA. The average value of the relative contents of these compounds in the commercial e-liquid samples was 50.6%, and in the seized e-liquids containing SCs, the average value was 21%.

Among the 211 seized e-liquid samples in which SCs were detected, 167 e-liquid samples contained a single psychoactive substance: 109 contained only ADB-BUTINACA, and the other 58 contained MDMB-4en-PINACA. Two psychoactive substances were detected in 35 e-liquid samples: 27 contained ADB-BUTINACA and MDMB-4en-PINACA, 6 contained MDMB-4en-PINACA and lidocaine, and the remainder contained ADB-BUTINACA, MDMB-4en-PINACA, and lidocaine. The distribution of the detected substances is shown (Figure 3). Lidocaine was the only adulterant found with MDMB-4en-PINACA, and no seized e-liquid samples contained only lidocaine and ADB-BU-TINACA. Lidocaine [11] is an amide-type local anesthetic that has a significant bidirectional inhibitory and excitatory effect on the central nervous system. Adulteration with local anesthetics, such as lidocaine, is a known practice to mimic the anesthetic effect of high-purity cocaine on mucous membranes.

In the present study, another 11 impurities were detected. The total ion chromatogram of the seized e-liquid samples containing ADB-BUTINACA or MDMB-4en-PINACA and the frequency of occurrence of impurities in each group are shown (Figure 4). The retention times and major fragment ion information for each compound are shown (Table 3). The association network diagram for each substance is shown (Figure 5). The substances that appeared with ADB-BUTINACA and MDMB-4en-PINACA were analyzed, with thicker lines representing stronger associations and higher probabilities of concomitant appearance.

The substances with retention times of 7.785±0.1557, 8.384±0.16768, and 8.565±0.1713 min were found in seized eliquid samples containing ADB-BUTINACA only. The substances with retention times of 7.785±0.1557 and 8.565±0.1773 min were identified as possibly methyl palmitate and methyl stearate, respectively, after comparison and analysis with the NIST spectral library. These substances are often used as emulsifiers. The main fragment ions of the substance with a retention time of 8.384 min were m/z 217, m/z 174 and m/z 131, indicating a parent nucleus composed of indazole and a side chain structure composed of butyl, and may be intermediates for the synthesis of ADB-BUTINACA. The substance with a retention time of 10.671±0.21342 was found more frequently in the e-liquid samples containing ADB-BUTINACA, and only once in the e-liquid samples in which MDMB-4en-PINACA was the main detectable component. The mass spectra consisted of fragments m/z 256, m/z 213, and m/z 144. This substance may be a synthetic intermediate of ADB-BUTINACA.

The substance with a retention time of  $9.66\pm0.1932$  min was found only in e-liquid samples containing MDMB-4en-PINACA. The mass spectra consisted of fragments m/z 303, m/z 247, m/z215, and m/z 159, corresponding to 1-tert-leucine methyl ester [12], a key reagent in the synthesis of MDMB-4en-PINACA. The role of 1-tert-leucine methyl ester is to obtain the substituent part of the target through the aminolysis reaction. N-trifluorobenzoyl-1-tert-leucine methyl ester has an amide bond that is highly susceptible to breakage due to the electron-absorbing effect of the halogen atom, and it induces a higher efficiency of the aminolysis reaction of 1-tert-leucine methyl ester during the synthesis reaction of MDMB-4en-PINACA. The synthetic pathway of drug processing reflected by N-trifluorobenzoyl-1tert-leucine methyl ester can be an important basis for establishing correlations between drug cases.

The substances that appeared more frequently in the seized e-liquid samples containing SCs were nicotine, menthol (inferred from the spectral library), and methyl oleate (inferred from the spectral library). Nicotine, a specific alkaloid component derived from tobacco, is also the primary psychoactive substance found in imitation tobacco products [13]. Although a common ingredient, nicotine is not always present, and it was detected in only 36.7% of the seized e-liquid samples containing SCs. Menthol is a common flavoring agent that imparts a cooling sensation with analgesic or counterirritant effects. Menthol also increases nicotine addiction and inhibits its metabolism [14]. Methyl oleate is a common emulsifier.

In general, there are significant differences in the categories and amounts of organic impurities between commercial and seized e-liquids. Meanwhile, not all the seized e-liquids have been detected nicotine. So we inferred that the seized e-liquids containing SCs were not added secondarily by commercial eliquids.

# **ICP-MS**

ICP-MS analysis provided the contents of 31 elements in the seized e-liquid samples and the commercial e-liquid samples. T-tests were conducted to determine significant differences in the content of each element between the seized and commercial e-liquid samples. The concentration ranges of the studied elements varied considerably. The mean contents and RSD of 31 elements in the seized e-liquid samples and the commercial e-liquid samples are shown (Table 4), together with the p-values. The results of the t-test showed significant differences in the contents of Ca, V, Ni, Cu, Zn, Se, Sr, Zr, Mo, Ba, Ti, and Tl. The contents of Ag and Sn were higher in the seized e-liquid samples than in the commercial e-liquid samples. Based on this, we can infer that the seized e-liquids containing SCs were not added secondarily by commercial e-liquids.

In addition, we further analyzed the contents of the 31elements in the seized e-liquids. For the seized e-liquid samples detected ADB-BUTINACA or MDMB-4en-PINACA, the element detected at the highest level was Zn, which ranged from 2.82–749656.3 ng/mL, the second highest level was for Cu, in the range of 2.6–458908.6 ng/mL, followed by Ni, in the range of 3.2–230052.5 ng/mL. We considered the characteristics of the 33 element contents together when conducting PCA and cluster analysis of the element contents in the seized e-liquid samples. The application of multidimensional statistical methods has shown potential for group classification of numerous samples in large datasets. The PCA results showed a cumulative contribution rate of the total variance of the first two principal components of 91.19% (i.e., the two extracted principal components can explain 91.19% of the variance in the experimental data). The contribution of Principal Component 1 was 84.19%. In the first principal component, the load coefficients of Zn, Cu, and Ni were 0.93, 0.33, and 0.14, respectively, which suggested that the contents of these three elements play a more important role in the aggregation of the samples. This may suggest that we can pay particular attention to the differences in the content of these three inorganic elements when inferring the source of the e-liquid samples.

Ca played a major role in the second principal component. The dispersion coefficients (standard deviation/mean) were calculated to measure the dispersion degree, and the dispersion coefficients were 3.99, 4.44, 4.26, and 1.48, for the contents of Zn, Cu, Ni, and Ca, respectively, indicating that these elements have a greater influence on the seized e-liquid samples. The results of K-means clustering based on the data carried out after dimensionality reduction of the PCA are presented (Figure 6), which clearly shows the similarities and differences between the seized e-liquid samples. The clustering results indicated a certain aggregation among the seized e-liquid sample points. A small distance between the sample points indicates that the seized e-liquid samples may be relatively close, there by providing an auxiliary strategy for combining drug cases and inferring the sources of drugs.

## Conclusions

GC-MS and ICP-MS were used to conduct impurity profiling of seized ADB-BUTINACA and MDMB-4en-PINACA e-liquid samples and common commercial e-liquid samples. Combined with the appearance, we basically ruled out the possibility that the currently popular e-liquids containing SCs were prepared by the secondary addition of ordinary commercial e-liquids. In addition to the appearance of the group, the high level of impurities was also analysed. In addition the high consistency of the appearance of impurities within the group suggests that there should be a specialised source of e-liquids provided, and the information provides important clues for investigation and antidrug crackdowns. The study demonstrates the importance and value of non-drug component analysis in anti-drug work as well.

## **Author Statements**

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