Analysis of cannabinoid-containing fluids in illicit vaping cartridges using a sequential TOF MS, DDA-MS/MS and DIA-MS/MS method

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Overview

- High resolution mass spectrometry has been applied to the analysis and identification of synthetic cannabinoid receptor agonists in electronic vaping devices and herbal extracts.
- The work included the analysis of three vaping cartridge fluids and herbal extracts resulting in the detection of the synthetic cannabinoid MDMB-4en-PINACA using a sequential high-resolution QTOF MS, DDA-MS/MS and DIA-MS/MS method for targeted and non-targeted analysis.
- Analysis was extended to verifying novel synthetic cannabinoid receptor agonists (SCRAs) synthesized as part of a growing need to expand SCRAs compound libraries.

1. Introduction

Synthetic cannabinoid receptor agonists (SCRAs) are a structurally diverse group of new psychoactive substances which are associated with significant acute toxicity and are increasingly used in electronic vaping devices. The diversity of SCRAs in the e-cigarette market has resulted in the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) monitoring 245 SCRAs with the likelihood that further synthetic cannabinoids with high potency will continue to be introduced into the market. This research describes a sequential high-resolution TOF MS, DDA-MS/MS and DIA-MS/MS method for the targeted and non-targeted analysis of known and novel SCRAs in electronic vaping devices.

2. Materials and Methods

A high resolution forensic toxicological screening workflow was applied to the analysis of drug samples. The method was designed to acquire data for targeted and non-targeted analysis.

Reversed phase LC Separation

- Column: Shim-pack Velox™ Biphenyl (100 x 2.1 mm, 2.7 µm); column temp. 40 ºC, flow rate: 0.3 mL/min, 17 min total analysis time.
- A: water + 2 mM ammonium formate + 0.002% formic acid.
- B: methanol + 2 mM ammonium formate + 0.002% formic acid.

QTOF analysis

- LCMS-9050 (Shimadzu Corporation, Japan).
- MS scan m/z 100-1000, 100 msec scan time.
- MS/MS DDA, up-to 4 dependent scans m/z 40-1000 CE 5-55 V, 40 msec; MS/MS DIA CE 5-55 V(1.1 sec total cycle time).

Non-targeted data processing

- LabSolutions Insight Discovery software was initially applied to the analysis of unknown drug samples. Discovery software requires no prior knowledge of a targeted compound list. The second-generation component detection algorithm was applied to the TOF MS scan event for peak detection and multiple HRMS MS/MS libraries were used for compounds identification (Shimadzu High Resolution Accurate Mass Library for Forensic Toxicology with over 2000 compounds and HighResNPS database).

Targeted data processing

- LabSolutions Insight quantitative software was used to quantitate the detected SCRA with an authentic standard (calibration range 0.01-1.0 mg/mL).

Method development for targeted and non-targeted analysis

- Sequential series of mass scans; TOF MS scan (for precursor quantitation and screening), DDA-MS/MS (targeted to known SCRAs delivering high confidence library searching) and DIA-MS/MS mass scans for retrospective screening.
- Data processing for targeted compound analysis used the Shimadzu High Resolution Accurate Mass Library for Forensic Toxicology. With over 1300 registered compounds to the biphenyl method and over 1400 to the ODS, totaling over 2000 compounds, every library entry was acquired using unit resolution targeted MS/MS and registered with Rt and compound structure. Spectra were mass corrected using structural assignment software Assign with noise ions excluded from registered library spectra.

3. Results

3.1 Analysis of vaping cartridge fluids and herbal extracts

Residual vaporizer fluid from 3 electronic vaping devices and 3 herbal extracts were analyzed by high resolution LC-MS/MS (LCMS-9050 QTOF, Shimadzu Corporation, Japan). The LC-MS/MS method was developed to specifically detect a panel of SCRAs including MDMB-4en-PINACA (formally risk assessed by the EMCDDA in December 2020).

Analysis of unknown vaping cartridges

- **Feature Detection**. A second-generation component detection algorithm has been enhanced and optimized to locate ions that behave as a recognized chromatographic feature (ion intensities rise and fall in abundance in a covariant manner) to give a simplified 'single component' output when multiple ion species are present. The algorithm assesses peak quality by considering jaggedness, symmetry and noise specifically at trace levels to report true components.
- **Library identification**. In all samples MDMB-4en-PINACA was detected.

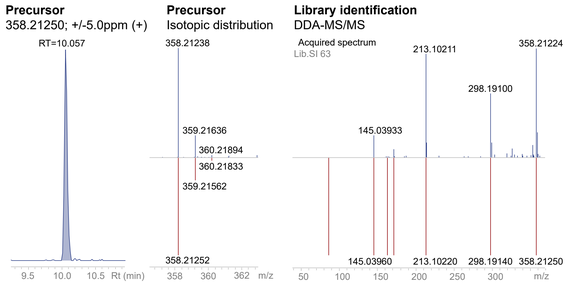

Figure 1. Analysis of electronic vaping devices and herbal extracts detected MDMB-4en-PINACA. The compound was reported with high confidence with agreement to precursor ion mass error, isotope distribution and MS-MS library identification (HighResNPS).

3.2 Targeted analysis

An authentic reference standard of MDMB-4en-PINACA was used to generate a library reference spectrum and quantify the SCRA in the drug samples which were found to range in concentration from 0.14 to 0.65 mg/mL (Table 1 and Figure 2).

Table 1. Quantitation of MDMB-4en-PINACA in herbal extracts and vape liquid.

Sample	Conc. (mg/mL)	Lib. Compound Name	Lib. SI	Found Rt	Rt diff	Mass Error (ppm)
Herbal Extract 1	0.163	MDMB-4en-PINACA	95	10.062	0.002	-1.228
Herbal Extract 2	0.151	MDMB-4en-PINACA	93	10.061	0.001	-0.754
Herbal Extract 3	0.140	MDMB-4en-PINACA	95	10.057	-0.003	-1.033
Vape liquid 1	0.159	MDMB-4en-PINACA	96	10.061	0.001	-1.005
Vape liquid 2	0.431	MDMB-4en-PINACA	96	10.065	0.005	-1.061
Vape liquid 3	0.647	MDMB-4en-PINACA	96	10.057	-0.003	-0.419
VG/PG	0.000	-	-	-	-	-

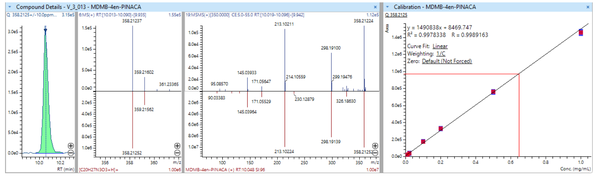

Figure 2. As one example, quantitation and library identification of MDMB-4en-PINACA in vape liquid using LabSolutions Insight software.

3.3 Expanding synthesized cannabinoid libraries

Synthetic cannabinoid receptor agonists (SCRAs), commonly referred to as 'spice' or 'K2', are the most rapidly growing class of recreational drugs. To meet a need for expanded compound libraries novel SCRAs including ADB-4en-PINACA, MMB-4en-PINACA, AB-4en-PINACA, MDMB-4en-PICA, ADB-4en-PICA, MMB-4en-PICA and AB-4en-PICA were synthesized as reference materials and verified using LC-MS and NMR.

Protonated precursor mass chromatograms
ADB-4en-PINACA, AB-4en-PINACA, ADB-4en-PICA AB-4en-PICA, MMB-4en-PICA, MDMB-4en-PICA, MMB-4en-PINACA and MDMB-4en-PICA

Generic LC conditions for forensic toxicology screening applied.

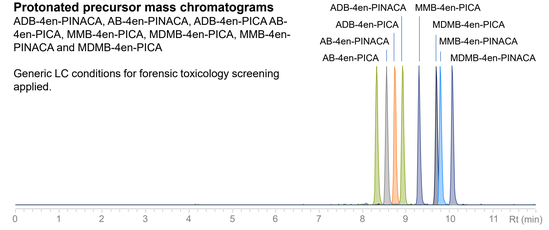

Figure 3. Mass chromatograms for novel synthesized cannabinoid receptor agonists (peak heights normalized to highlight retention times).

3.4 Synthesized cannabinoid LC-MS/MS

LC-MS/MS and NMR data were used to verify the chemical structure of novel synthesized cannabinoid receptor agonists. The structures and MS/MS spectra are shown in Figure 4.

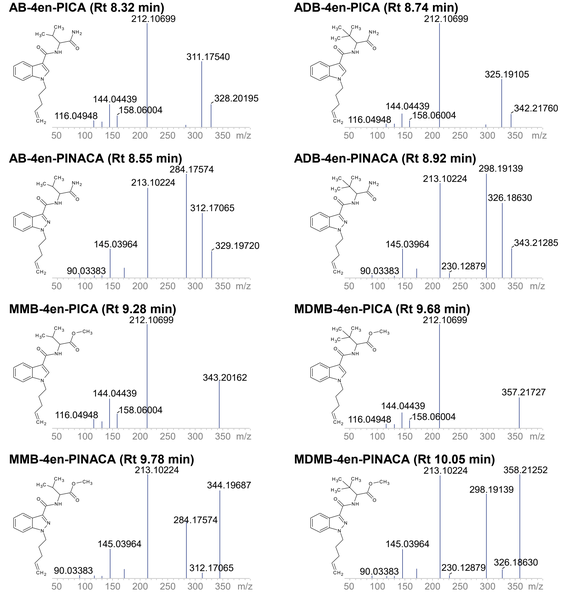

Figure 4. MS/MS spectra for a series of novel synthetic cannabinoid receptor agonists acquired with a collision energy spread of 5-55 V.

4. Conclusions

- The HRMS LC-MS/MS method with a sequential series of mass scans was applied to the analysis of synthetic cannabinoid receptor agonists in electronic vaping devices and herbal extracts.
- MDMB-4en-PINACA was detected in all seized drug samples using the Insight Discovery unknown analysis software application and quantified using an authentic reference standard.
- The HRMS LC-MS/MS method was used to authenticate the synthesis of novel synthetic cannabinoid receptor agonists with NMR and MS data to confirm novel reference materials for future screening.