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# Profiling of synthesis-related impurities of the synthetic cannabinoid Cumyl-5F-PINACA in seized samples of e-liquids via multivariate analysis of UHPLC/MS$^n$ data

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**Abstract**

Vaping of synthetic cannabinoids via e-cigarette increasingly grows in popularity. In the presented study, we tentatively identified twelve by-products found in a highly pure sample of the synthetic cannabinoid Cumyl-5F-PINACA (1-(5-fluoropentyl)-*N*-(2-phenylpropan-2-yl)-1*H*-indazole-3-carboxamide), a prevalent new psychoactive substance in e-liquids, via high-resolution mass spectrometry (HR-MS) fragmentation experiments. Furthermore, we developed a procedure to reproducibly extract this synthetic cannabinoid and related by-products from an e-liquid matrix via chloroform and water. The extracts were submitted to flash chromatography (F-LC) to isolate the by-products from the main component. The chromatographic impurity signature was subsequently assessed by ultra-high-performance liquid chromatography coupled to mass spectrometry (UHPLC-MS) and evaluated by automated integration. The complete sample preparation sequence (F-LC + UHPLC-MS) was validated by comparing the semi-quantitative signal integrals of the chromatographic impurity signatures of five self-made e-liquids with varying concentrations of Cumyl-5F-PINACA (0.1, 0.2, 0.5, 0.7 and 1.0 % (w/w)), giving an average relative standard deviation of 6.2 % for triplicate measurements of preparations of the same concentration and 10.5 % between the measurements of the five preparations with different concentrations. Lastly, the chromatographic signatures of fourteen e-liquid samples containing Cumyl-5F-PINACA from police seizures and internet test purchases were evaluated via hierarchical cluster analysis for potential links. For the e-liquid samples originating from test purchases, it was found that the date of purchase, the identity of the online shop and brand name are the critical factors for clustering of samples.

## 1 Introduction

Drug profiling can be defined as the quantitative assessment of the physical and chemical properties of a drug sample subsequent to an extraction procedure. It can be used to link seizures, identify trafficking and synthesis routes or provide information on precursor materials and their origin[1]. The manufacturing route of a drug is considered to have major influence on the chemical profile of samples, producing a distinct set of by-products or impurities depending on the used educts, coupling reagents, catalysts or overall reaction conditions[2-4]. Several methodologies of the analysis of organic impurities or by-products were developed for classic drugs like cocaine, heroin or amphetamine[5,6]. They mostly consisted of a straightforward liquid-liquid extraction (LLE) or solid phase extraction (SPE) procedure to remove the main active substance and to enrich related by-products, followed by analysis via gas chromatography (GC) or liquid chromatography (LC) coupled to different types of detectors[7-9]. The chromatographic signatures are then quantitatively evaluated via data base assisted chemometric models. In 2018, a new impurity profiling workflow aimed at highly pure drug substances, e.g. pure new psychoactive substances or methamphetamine, was developed by Münster-Müller et al.[10] with preparative flash-chromatography (F-LC) as central element. By selective fractionation of the chromatographic run, more than 99% of the main component could be removed from a sample while accumulating present by-products at the same time. This opened up new possibilities to profile even highly pure substances (> 98 % purity) for which the common extraction and enrichment methods (LLE, SPE) fail. The new workflow was successfully tested on different pure synthetic cannabinoids, a class of designer drug within the new psychoactive substance (NPS) phenomenon. Synthetic cannabinoids are of specifically high concern as they represent the largest group in terms of the overall number reported until the end of 2017 (251 synthetic cannabinoids amongst a total of 803 NPS of all classes[11]. The most popular form of consumption of these substances are so called "Spice-Products"[12], in which the pure synthetic cannabinoids are applied to the surface of an inactive herbal matrix. The new workflow with F-LC was also successfully adapted to these "Spice-Products"[10], by including a straightforward preceding extraction step to isolate the synthetic compounds from the surface of the herbal-matrix. It was proven that even though a synthetic cannabinoid was applied to and extracted from an herbal matrix, the original by-product profile could be maintained. A more thorough study of the impurity profile of the synthetic cannabinoid MDMB-CHMICA was recently published[13], including batch discrimination of a larger collective of pure samples, the characterization of single impurities via NMR and controlled synthesis using different coupling reagents and reaction conditions.

Our aim in the study presented here was to extend and validate the range of applications of the workflow to substances, in this case synthetic cannabinoids, dissolved in an e-liquid matrix. Different from the comprehensive study on MDMB-CHMICA, this work focuses on the more complex e-liquid matrices and the analytical challenges of reproducibly assessing the impurity profile of synthetic cannabinoids dissolved therein, in particular the prevalent compound Cumyl-5F-PINACA[14] (1-(5-fluoropentyl)-*N*-(2-phenylpropan-2-yl)-1*H*-indazole-3-carboxamide, **1,** Figure 1). Cumyl-5F-PINACA, also referred to as 'SGT-25'[15] belongs to the substance class of indazole carboxamides and is a liquid at room temperature, making it a prime candidate for mixing with a liquid matrix. With the increasing popularity of the e-cigarette,

more and more NPS distributors expand their products portfolio by e-liquids containing dissolved synthetic cannabinoids for several reasons. The products can be consumed in public places without raising suspicion by ominous odors and, although controversial discussion is still ongoing, vaping e-liquids is considered healthier in comparison to smoking products with a herbal matrix[16-18]. E-liquids generally consist of a polar mixture of propylene glycol (PG), vegetable glycerin (VG) and ethanol, aroma compounds and an active substance (e.g. nicotine or an NPS). Some reports are available concerning the analysis of e-liquids containing different types of classic cannabinoids and synthetic cannabinoids[19-22]. In comparison to the preparation of herbal formulations of synthetic cannabinoids such as "Spice-Products", the isolation of active component and related impurities from the polar matrix of E-liquids is more challenging. The samples could not be dissolved in eluent and directly injected into the F-LC for separation, as the polar matrix disrupts the chromatography. A more effortful sample preparation had to be developed in order to be able to perform the previously developed impurity profiling workflow[10].

As first step, it was necessary to assess and characterize "key-impurities" and their overall abundance in a pure sample of **1** via high-resolution mass spectrometry (HR-MS) and fragmentations experiments ($MS^n$) to provide a basis on which the sample preparation procedure and seized samples can be evaluated on. As only less than 100 mg of pure **1** were available for method development and all performed analytical studies, it was not possible to additionally isolate selected impurities in larger quantity for unequivocal structural identification via NMR. However, the HR-MS data, including systematic fragmentations experiments, were sufficient to identify characteristic structural elements already identified in impurities of MDMB-CHMICA with an acceptable grade of certainty. Indole and indazole based synthetic cannabinoids are expected to be synthesized by a modular design, following similar synthesis instructions. Thus, by comparing the tentatively identified impurities of **1** to the NMR-characterized impurities of MDMB-CHMICA, parallels between applied synthesis pathways might be found. As distinct from the procedure applied to "Spice-products" (herbal blends), an additional preceding rapid LLE procedure using a mixture of chloroform and water was developed and validated to remove the majority of the polar e-liquid matrix compounds to minimize impairment of the subsequent isolation of synthesis-related impurities by flash chromatography. To demonstrate the suitability of the profiling procedure, a small set of e-liquid samples from police seizures and internet test purchases containing **1** were evaluated for potential links using the complete workflow.

## 2 Materials and methods

The complete profiling workflow via F-LC, UHPLC-MS and the subsequent data processing method was already reported in our previous publication[10] and will not be stated again in full length. The main aim of the work presented here was to adapt the previously published profiling workflow [10] to the e-liquid matrix which has not yet been tested so far.

### *2.1 Chemicals and reagents*

Ethanol, chloroform and acetonitrile were purchased from Merck (Darmstadt, Germany), acetone was obtained by VWR Chemicals (Darmstadt, Germany), n-hexane and ammonium formiate were bought from Sigma Aldrich (Steinheim, Germany), ethyl acetate and formic acid

were purchased from Fluka (Steinheim, Germany) and propylene glycol and glycerine were obtained from ReiTrade (Vaihingen, Germany). Deionized water was prepared using a Milli-Q Synthesis A10 apparatus (Millipore, Schwalbach, Germany).

*2.2 E-liquid samples*

One pure sample of **1** (LJP) and four e-liquids (LJ01-LJ04) containing **1** as main active ingredient were available from a clandestine laboratory seized in Ljubljana/Slovenia in August 2015. Furthermore, ten e-liquids from internet test purchases (FR01-FR10) containing **1** were available from the University Medical Center in Freiburg/Germany.

To validate the extraction procedure and the overall sample preparation reproducibility, self-made e-liquids consisting of PG, VG and ethanol (40:40:20) (v/v) and containing 0.1, 0.2, 0.5, 0.7 and 1.0 % (w/w) of pure **1** (Ljubljana seizure), respectively, were employed.

*2.3 Micro extraction*

Before the cannabinoid compounds in the e-liquid samples could be separated into by-products and main component by means of F-LC, it was first necessary to perform a liquid-liquid micro extraction in order to remove the polar matrix components PG and VG as they disturb the normal phase chromatography. For that purpose, 200 µL of e-liquid, 4 mL of chloroform and 5 mL of deionized water were pipetted into a 15 mL glass vial and vortexed for 20 s. The chloroform phase was transferred to a 4 mL glass vial and dried under a steady stream of nitrogen. The resin-like residue was then dissolved in 1.5 mL ethyl acetate/hexane (1:2, v:v) for injection into the F-LC.

*2.4 Instruments*

Separation of impurities from the main component was achieved with a Sepacore F-LC system X50 from Büchi Labortechnik (Flawil, Switzerland) consisting of two pump modules (max. 50 bar pressure), a UV-VIS spectrometer (set to 284 nm), an automated fraction collector and a control unit. A prepacked 4 g silica gel HP column from Büchi (particle size 15-40 µm) was used and run with a gradient system of *n*-hexane and ethyl acetate. The complete 1.5 mL of dissolved sample after the micro extraction were loaded for each run. Separation was achieved with a flow rate of 20 mL/min starting with 40 s of 0 % eluent B, followed by an increase to 15 % B over 65 s, holding these 15% B for 25 s, followed by an increase to 30% B over 20 seconds. After holding for 50 s, eluent B was further increased to 100% over 20s and held there for another 160 s, giving an overall runtime of 380 s. **1** was collected from second 225 to 255, evaporated to dryness and weighed. The remaining fractions were combined, evaporated to dryness and again dissolved in 1 ml acetonitrile.

The combined impurity fractions were diluted according to the corresponding weight of the isolated main component as normalization step and measured on an UltiMate 3000 UHPLC system (Thermo Scientific, Waltham, MA, USA) coupled to an amaZon speed ion trap mass spectrometer (Bruker, Billerica, MA, USA) with electrospray ionization (ESI) source. The used eluents contained 98.9 % water, 1.0 % acetonitrile and 0.1 % formic acid (eluent A) and 1.0 % water, 98.9 % acetonitrile and 0.1 % formic acid (eluent B), respectively. The total run time was 12 min at a flow rate of 0.5 mL/min and the injection volume was 5 µL. The ESI was run

in positive mode with a voltage of 4.5 kV, the mass range was set to m/z 70 – 600 with a scan speed of 32500 m/z/s in Ultra-Scan mode. The dry gas flow rate was set to 10 L/min at a temperature of 320 °C. Fragmentation mode was Auto $MS^3$ using collision induced dissociation.

High-resolution data were obtained by an Accela HPLC system and an LTQ Orbitrap-MS (Thermo Scientific, Bremen, Germany). The eluents contained 94.9 % water, 5.0 % acetonitrile and 0.1 % formic acid (eluent A) and 5.0 % water, 94.9 % acetonitrile and 0.1 % formic acid (eluent B), respectively. The injection volume was 1 µL. The ESI was operated in positive mode with 3.75 kV and the mass range of the MS was set to 130-2000 m/z with a data dependent scan threshold for $MS^2$ of $10^6$ counts.

*2.5 Data processing*

The LC-MS data were processed via a rectangular bucketing algorithm (ProfileAnalysis, Bruker, Billerica, MA, USA), integrating the signals of all m/z values from 150 to 500 individually in intervals of 0.5 minutes from minute 2 to 9.5 of chromatographic runtime, forming so called buckets. Those buckets containing the required information about the assessed "key-impurities" were picked out and used for further interpretation. A more thorough description of the bucketing process can be taken from our previous work[10].

Hierarchical cluster analysis (HCA) via Ward's method was computed with the software Unscrambler X (Camo, Oslo, Norway).

## 3 Results and discussion

*3.1 Assessment and characterization of by-products found in a pure sample of Cumyl-5F-PINACA*

UHPLC-MS measurements of the pure cannabinoid majorly showed an intense signal for **1** with only few detectable by-product signals in the base line region of the chromatogram, insufficient for a reliable semi-quantitative profiling setup (Figure 2, top). Thus, the pure sample of **1** was submitted to F-LC and the combined impurity fractions of the chromatographic run were analysed again via UHPLC-MS, this time injected with a smaller dilution factor, as most of the main component was depleted from the sample. Figure 2 (bottom) shows the corresponding base peak chromatogram (BPC) of the combined impurity fractions after F-LC with the most abundant by-products of **1** highlighted from **I1** to **I12**. As stated before, the sample amount of pure **1** available for this was not sufficient for extensive isolation of single impurities for structural elucidation via NMR. Tentative structure elucidation of by-products was done on the basis of HR-$MS^3$-fragmentation experiments to verify the chemical relationship of detected chromatographic signals to the main component. The interpretation of corresponding product-ion spectra was based on the fragmentation pattern of **1**, since a certain structural similarity of by-products to the main product was expected. Neutral losses or product-ions with known exact masses were assigned to partial structural elements of **1** (in most cases the 5F-pentyl chain and the cumene residue).

With the described set of impurities, only hypothetical considerations regarding the synthesis pathway and reaction conditions can be made. Even though synthesis procedures for a variety

of synthetic cannabinoid compounds like **1** can be found in the original patent from Bowden et al.[15], the actual manufacturers have probably implemented their own synthesis procedures with higher economic efficiency or better suitability for upscaling. The patented synthesis conditions are summarized in the supplementary information in Figure S-14[15]. However, the synthesis procedures for synthetic cannabinoids with indole and indazole core structures are considered to be based on a modular design, only exchanging the building blocks for the side-chain and the linked residue. Similarities between characteristic structural elements of impurities for different synthetic cannabinoids might result from related synthesis procedures. Recently, we published an extensive study on impurity profiling for the synthetic cannabinoid MDMB-CHMICA including NMR and HR-MS characterization of fifteen impurities, including detailed discussion about their origin, based on series of controlled syntheses under varying conditions. In the work presented here, we aim at using this scientific basis for the interpretation of accordances between characteristic structural elements found in impurities of **1** and those identified in MDMB-CHMICA, with respect to conclusions about the synthesis pathway.

The corresponding analytical data and the proposed structures are summarized in Figure 1, a corresponding Table including analytical data and related HR-MS spectra including fragmentation experiments up to $MS^3$ can be found in the supplementary information. As only one sample of pure **1** was available, these by-products were selected as target impurities for the comparison of e-liquid samples without further knowledge, if they exhibit significant discriminating potential within a bigger set of pure samples.

The available literature suggests, that both, the formation of the amide bond or the *N*-alkylation, can be performed as last reaction step in the synthesis of synthetic cannabinoids[23], in the case of **1** the coupling of *N*-alkyl-3-indazole-carboxylic acid and cumylamine or the coupling of 1-bromo-5-fluoropentane (or the like) to *N*-(2-phenylpropan-2-yl)-1*H*-indazole-3-carboxamide[15]. However, we consider the amide bond formation as the last step, evidenced by the majority of impurities having the alkyl chain already attached to the indazole core (except for **I2** and **I3**) and the presence of **I6** in particular. With an additional 5-fluoropentyl chain coupled via an ester, it is likely that free 1*H*-indazole-3-carboxlic acid was present during the *N*-alkylation with 1-bromo-5-fluoropentane (or the like). The formation of **I7** with an ester bound $C_4H_8Cl$ residue cannot be explained, as both, the attached chlorine and the reduced chain length of four carbon atoms, do not fit any allegedly used educt. **I8** is expected to be the product of **1** after HF elimination, with either a double bond within the alkyl chain or formation of a ring. **I11** consist of a $C_6H_{11}$ aliphatic residue (as ring or with a double bond) attached to the indazole core, possibly formed by *N*-alkylation with impure 1-bromo-5-fluoropentane (or the like). **I5** showed the same exact mass but a slightly different fragmentation pattern and retention time compared to **1**. We expect this impurity to be a result of an annular tautomerization of the indazole core[24] with the 5F-pentyl chain attached to the 2-*H* position or the initial coupling of the 5-fluoropentyl to the 2-position of the 2*H*-indazole tautomer. In any case it is likely that for other indazole based synthetic cannabinoids also impurities in form of 2-alykl-2*H*-indazole analogues can be observed. **I12** is expected to be a coupling product of two 1-(5-fluoropentyl)-1*H*-indazole structures via an amide linker and **I3** appears to be a urea derivative with two *N*-linked cumyl residues, both possibly formed in the active reaction mixture.

For the remaining impurities **I1**, **I2**, **I4**, **I9** and **I10** several accordances in relation to the impurities of MDMB-CHMICA could be found[13], for example **I1** and **I4** with $C_2H_6$ and $C_4H_{10}$ aliphatic residues on the carboxamide linker group, identified as dimethyl and diethyl amides in the case of MDMB-CHMICA. Both of these impurities were also found in the controlled synthesis batches of MDMB-CHMICA using different types of coupling reagents and triethyl amine as base for amide bond formation. Possibly, they result from a side reaction of the activated carboxylic acid with primary or secondary amine contaminations of triethylamine. **I2** most probably is a carry-over product from a preceding reaction step with a failed *N*-alkylation of the pentyl chain in 1-*H* position of the indazole core. A structurally similar impurity was identified in seized samples of MDMB-CHMICA, a MDMB-ICA without the cyclohexyl methyl attached to the indole core. Lastly, two chlorinated derivatives of **1** were found with the chlorine attached to different positions of the indazole core. Without NMR confirmation, the exact position cannot be assessed. In the case of MDMB-CHMICA, a 2-Cl derivative was identified to be the most abundant impurity for the majority of seized samples. As source for the chlorine, an amide coupling reaction via acyl halide formation is proposed, using common reactants like oxalyl chloride or thionyl chloride for activation of the carboxylic acid instead of [dimethylamino(triazolo[4,5-b]pyridine-3-yloxy)methylidene]-dimethylazanium hexafluorophosphate (HATU) or *N,N'*-dicyclohexylcarbodiimide (DCC). Alternatively, but less probable, already chlorinated indazole derivatives could have been used from the beginning of the synthesis.

Regarding the batches of MDMB-CHMICA produced in the controlled synthesis series it is apparent that the highest accordance in impurity composition compared to **1** can be observed for the coupling step via thionyl chloride and oxalyl chloride, the use of which was also strongly indicated by the high concentration of the 2Cl-MDMB-CHIMCA impurity. Thus, it was expected that the activation of the carboxylic acid via acyl halide is the most probable synthesis procedure for MDMB-CHMICA. For **1** several impurity analogues to MDMB-CHMICA were found like **I1**, **I4** and especially **I9** and **I10**, suggesting again the pathway via acyl halide for amide bond coupling. However, based on our experimental data it is not possible to explain the presence of several other impurities of **1** without further knowledge of the reaction behaviour of the indazole core or the cumyl amine. A series of controlled syntheses would have to be carried out for a better understanding of the formation of the described reaction by-products, which, however, was not the scope of this work.

### 3.2 *Validation of the sample preparation process of micro extraction, flash chromatography and UHPLC-MS*

Before any real-life samples can be submitted to the profiling with the here presented combination of an extraction, F-LC and UHPLC-MS, the complete sample preparation procedure needs to be validated to ascertain that it does not have any impact on the by-product profile of **1**. We prepared self-made e-liquids with varying concentrations of **1** (0.1, 0.2, 0.5, 0.7 and 1.0 % of **1** in e-liquid matrix (w/w)) where the by-product composition is known from previous experiments with no e-liquid matrix. By comparing the by-product profile of pure **1** to the profiles obtained after the sample preparation procedure, any loss of signals or additional signals can easily be determined. The previously assessed impurity composition (relation of

impurity peaks to each other) serves as an "internal standard" to investigate potential changes in the profile throughout the extraction and enrichment process.

All self-made e-liquid samples were worked up via the micro extraction procedure, giving an average extraction yield of 72% for **1**. We found that in the organic phase (chloroform) a small, but constant portion of matrix is still present, which however had no measurable impact on the further workup with F-LC.

Each of the differently concentrated e-liquid samples were extracted and submitted to F-LC in triplicate. The corresponding by-product fractions were measured via UHPLC-MS and the signals of all previously assessed key-impurities were integrated. The overall profile of the pure sample of **1** was very similar to the profiles of the e-liquid extracts, although several additional matrix signals could be observed in the latter, which, however, were easily discriminable and non-interfering. For the triplicates an average relative standard deviation (RSD) of all key-impurity signals was found to be 6.2 %. Comparing the five differently concentrated e-liquids, an overall RSD of 10.5 % was calculated. These low values and the recovery of all expected impurities in the e-liquid samples indicate, that the sample preparation process has no major influence on the by-product composition and concentration.

Of course, in this validation study we only used PG and VE as matrix components. It is, however, commonly observed, that commercially available e-liquids contain a range of flavours which might appear as additional signals in the measurements of the isolated impurity fractions. That is why we chose to select only the previously assessed key impurities as target analytes to be assessed when comparing seized and on-line purchased e-liquids containing **1**. All other observed chromatographic peaks are excluded from the profiling procedure and will, therefore, have no impact on the actual impurity-profiling of the main component.

*3.3 Application of profiling workflow to seized and bought e-liquid samples containing Cumyl-5F-PINACA*

The previously selected target-impurities were used to compare seized samples of e-liquids containing **1**. Ideally, the chromatographic profiles of samples with **1** from the same production batch should show identical by-product patterns. Contrarily, samples from different batches are supposed to show variations in their by-product profile, caused by the choice of synthesis route, precursor chemicals and purification steps. In this small case study, we included the pure sample and four e-liquids available from a seized clandestine laboratory in Ljubljana/Slovenia. The aim was to investigate, if at least one of these e-liquids was produced with the available seized pure material, proven by the by-product signatures. Furthermore, ten e-liquid samples from internet test purchases were available from the University Medical Center in Freiburg. The samples were bought from different vendors and at different points of time.

All fifteen samples were processed according to the described sample preparation procedure and the pooled impurity fraction for each sample was measured via UHPLC-MS. The LC-MS data were automatically integrated and the intensity values of the targeted by-products (**I1** to **I15**) subsequently analyzed via HCA. A matrix of the available samples of **1** and the corresponding key-impurities was generated for better visualization of the data (Table in Figure 3). A greyscale, individual for each impurity, indicates the relative intensities (white: complete

absence of signal, black: the highest signal value). The last line of the table describes the average intensity ratios between the impurities, normalized to the least abundant. A hierarchical cluster analysis (HCA) using Ward's method (dendrogram in Figure 3) was carried out to group the 15 e-liquid samples according to their relative distance in impurity signatures. Additional information about the samples can be taken from Table 1.

Surprisingly, the pure sample of the seized laboratory in Ljubljana (LJP) showed no accordance to any of the four e-liquid samples from the same seizure with respect to the by-product signature. Especially the high relative abundance of **I9** to **I12** separated LJP from the remaining sample pool. Only LJ01 tends to be similar to LJP, but not to such an extent that a strong link between the two samples can be established. LJ03 was majorly different from the remaining sample set. LJ02 and LJ04 seem to be produced with aliquots of a single batch material of **1,** as they show very similar by-product patterns. Since these e-liquids had no label on them, it was impossible to evaluate the given data with any more background information like brand name. Three samples from an online test-purchase from Freiburg (FR01-FR03, bought in September 2014, same brand, same online shop, different flavors) clustered and were found to be relatively similar to LJ02 and LJ04, although the seizure and test purchase were approximately one year apart and no other connection between the samples can be drawn. All five samples showed an increased relative abundance of **I5** and **I8** and low relative abundance for all other impurities. To exclude false positive cluster assignment in this HCA model, the relative distance between the first two clusters seems to be sufficient to discriminate between samples consisting different batches of **1**.

Two other cluster formations could be observed for FR07-FR08 (bought in July and October 2015, same brand, same online shop, different flavors) and FR04-FR06 (bought in April 2015, same brand, same online shop, different flavors). The latter three samples were a repetition purchase of FR01-FR03, however bought approximately half a year later, showing majorly different relative abundances of **I1** to **I4** although all samples had the same brand and originate from the same online shop.

The given data suggest that the flavor is insignificant and the date of purchase, the identity of the online shop and the brand are critical factors for cluster formation. Based on this small case study the modus-operandi of the manufacturers can be hypothetically described as follows: One large batch of e-liquid matrix is prepared and pure material of **1** is dissolved. This large batch is then split into smaller aliquots and mixed with various flavors. These aliquots are filled into small bottles with equal brand names but different flavor types and sold. This process is repeated whenever an e-liquid runs out of stock as observed for the repetitive purchase of FR01-FR03 and FR-4-FR06, most certainly produced with different batches of **1**.

## 4 Conclusion

In this study, we developed and successfully applied a profiling procedure based on the isolation of related by-products from a pure sample of the synthetic cannabinoid Cumyl-5F-PINACA via flash-chromatography. To twelve of these by-products a tentative structure could be assigned to by high-resolution mass spectrometry and ion trap mass spectrometry fragmentation experiments. By implementing a straightforward liquid-liquid extraction procedure via chloroform and water in combination with flash-chromatography and UHPLC-

MS, the profiles of the targeted by-products of Cumyl-5F-PINACA could be also reproducibly assessed in an e-liquid matrix. This enabled the comparative analysis of a set of seized and bought e-liquid samples containing Cumyl-5F-PINACA for potential links. It was found that date of purchase, the identity of the online shop and brand name are the critical factors for differences in the by-product profiles of samples. Decomposition and pyrolysis products formed of active substances in e-Liquids when consumes in e-cigarettes and other vaping devices is a highly relevant and current topic for future research activities.

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**Supporting Information**

Summarizing Table with analytical information and HR-MS data up to $MS^3$ used for structural interpretation of **I1-I15.**

Figure showing the possible synthesis pathway of **1** according to patent WO2014167530[15]

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**Table 1: List of seized (labeled LJ) and online test purchased (labeled FR) e-liquids samples containing Cumyl-5F-PINACA used for this study to group samples according to their chromatographic impurity signatures, including the date of purchase/seizure, source, brand and flavor type of each e-liquid (if available).**

| Sample | Date | Source | Brand | Flavour |
|---|---|---|---|---|
| LJP LJ01-LJ04 | Aug 2015 | seizure | n.a. | n.a. |
| FR01 | Sep 2014 | Online-shop 1 | Brand 1 | Bubblegum |
| FR02 | | | | Blueberry |
| FR03 | | | | Vanilla |
| FR04 | Apr 2015 | Online-shop 1 | Brand 1 | Bubblegum |
| FR05 | | | | Blueberry |
| FR06 | | | | Vanilla |
| FR07 | Jul 2015 | Online-shop 2 | Brand 2 | Blueberry |
| FR08 | Oct 2015 | | | Bubblegum |
| FR09 | Oct 2015 | Online-shop 3 | Brand 3 | Blueberry |
| FR10 | Mar 2016 | Online-shop 1 | Brand 1 | Blackberry Menthol |

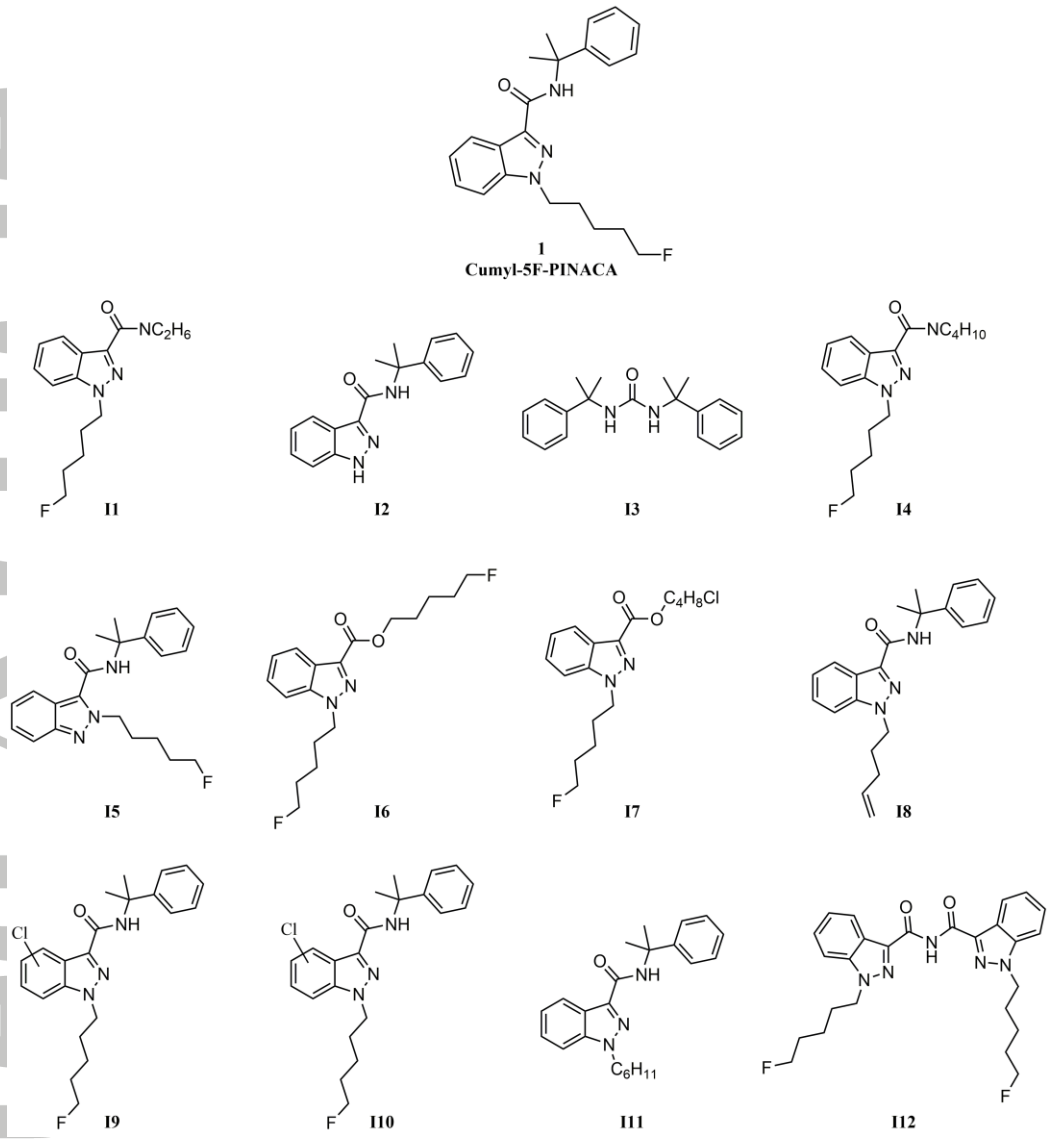


Figure 1:

Structural formula of Cumyl-5F-PINACA (1) and twelve related synthesis impurities. Tentative structural characterization of the impurities was carried out via HR-$MS^3$ experiments.

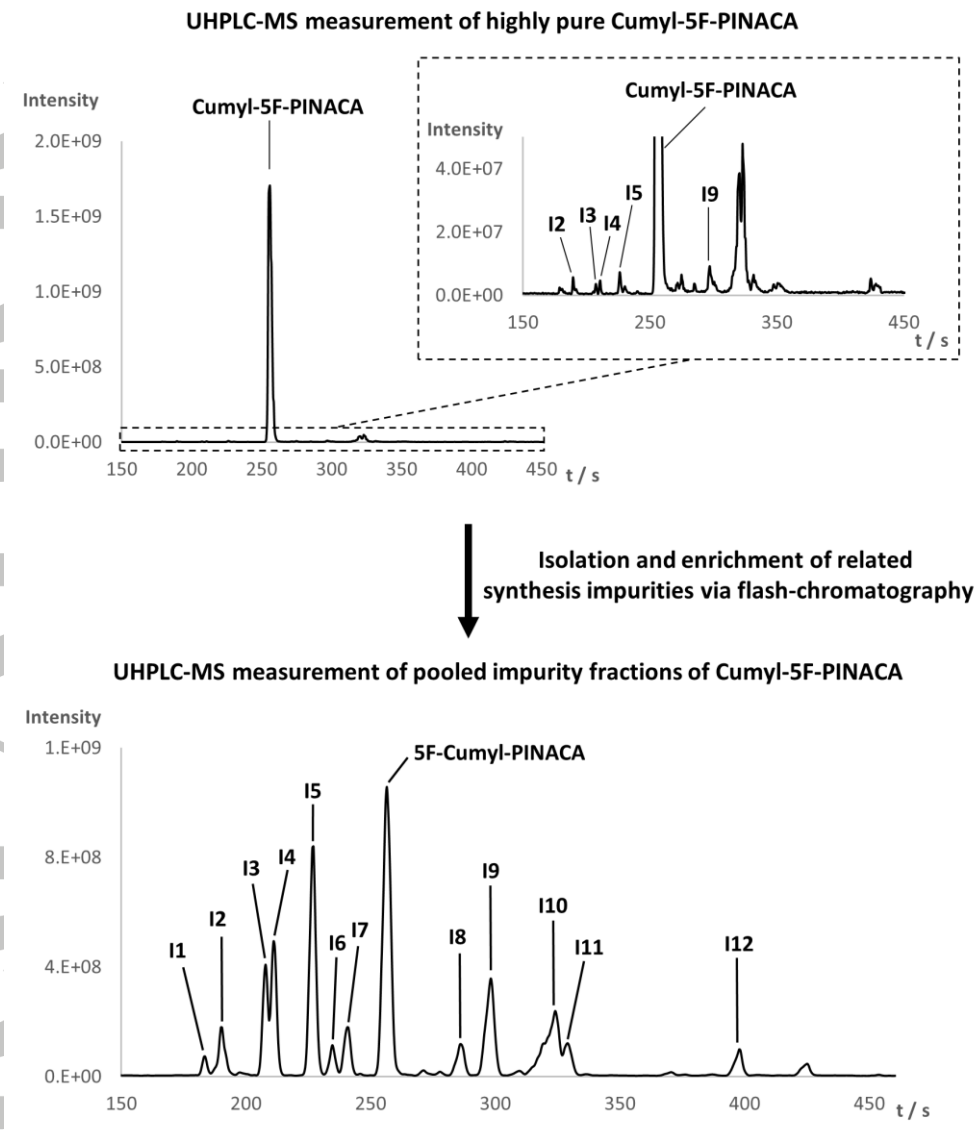


Figure 2:

Excerpts of two UHPLC-MS BPCs of a seized sample of Cumyl-5F-PINACA. (top) Without prior sample treatment. For related synthesis impurities, few signals can be observed in the low intensity range with small signal to noise ratios. (bottom) The same sample after isolation and enrichment of synthesis impurities via flash-chromatography. Being able to inject the sample with a lower dilution factor, previously absent and superimposed signals of impurities are now more intense. Related impurity peaks are numbered from I1- I12.

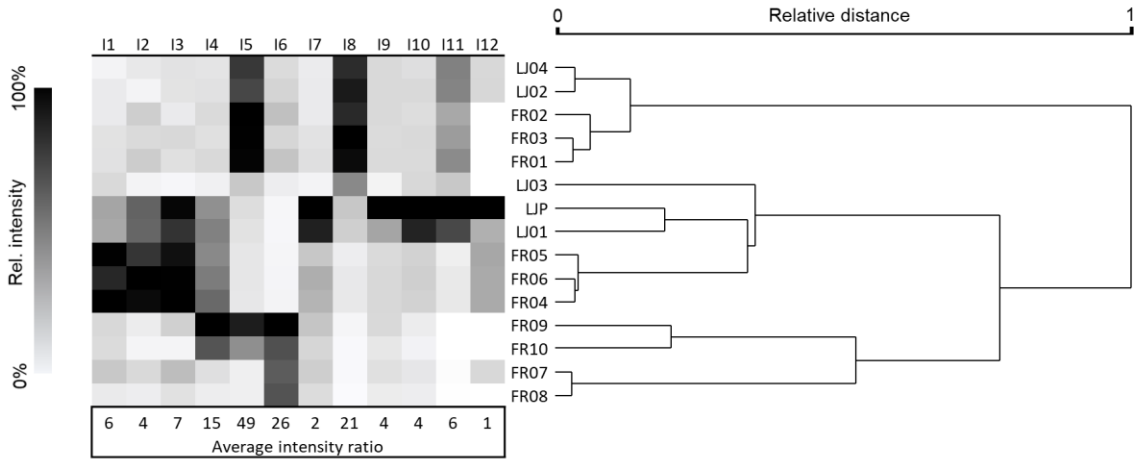


Figure 3:

On the left, a table showing the relative intensities for the assessed "key-impurities" I1 to I12 in seized and online test purchased e-liquid samples of Cumyl-5F-PINACA (labeled FR and LJ). For each impurity the relative intensities are shown in individual greyscales (white: complete absence of signal for this impurity, black: highest signal value for this impurity). The last line of the table states the average intensity ratio for each impurity normalized for I1 to I12. Grouping of samples based on the overall semiquantitative impurity signatures was done via HCA. The resulting dendrogram is attached next to the table for better visual interpretation of the data.

# Graphical Abstract

**Profiling of synthesis-related impurities of the synthetic cannabinoid Cumyl-5F-PINACA in seized samples of e-liquids via multivariate analysis of UHPLC/MS$^n$ data**

Sascha Münster-Müller*, Isabelle Matzenbach, Thomas Knepper, Ralf Zimmermann, Michael Pütz

We tentatively identified twelve by-products found in a pure sample of the synthetic cannabinoid Cumyl-5F-PINACA via HR-MS/MS experiments. A reproducible extraction procedure for this synthetic cannabinoid and related synthesis impurities was developed and validated. The chromatographic impurity signatures of fourteen e-liquid samples containing Cumyl-5F-PINACA from police seizures and internet test purchases were evaluated via hierarchical cluster analysis for potential links.