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The EU-project ´SPICE-profiling´ (2015-2017) - objectives and results of a first study on Spice products containing 5F-PB-22

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Aims: The project ´SPICE-profiling´, funded within EC´s ISEC 2013 programme (JUST/2013/ISEC/DRUGS/AG/ISEC/4000006421) will develop integrated and innovative approaches tackling the phenomenon of new psychoactive substances (NPS). The project builds on the results of the finalized EU-projects ´SPICE´ (JUST/2009/DPIP/AG/0948, 2010-2012) and ´SPICE II plus´ (JUST/2011-2012/DPIP/AG/4000003597, 2013-2015) which were more focused on health risks and prevention issues related to NPS. The core activities of the new project SPICE-profiling will employ and analytically characterize samples of NPS from internet test purchases, controlled laboratory syntheses, police and customs seizures as an information pool delivering details about the manufacturing procedures, the required key chemicals, the origin of adulterants and plant matrices and the chemical relations between different products. The comparative analysis of the samples will be based on the quantitative assessment of characteristic synthesis impurities, by-products and residual precursor chemicals by GC-MS/UHPLC-MS and of the carbon, nitrogen and hydrogen stable isotope ratios by EA- and GC-IRMS, assisted by multivariate data analysis. Results of a first model study on the general applicability of these analytical techniques to a range of recently seized ´Spice´-products with a focus on products containing the cannabimimetic aminoalkylindole 5F-PB-22 are presented. **Methods:** ´Spice´ products of eleven different brands from recent police seizures were extracted with acetonitrile and submitted to normal phase column chromatography to separate main active and side compounds in a preparative scale. ESI-MS-experiments were performed using an UHPLC coupled to an ion trap mass spectrometer. HR-MS experiments by Orbitrap-MS were conducted for exact mass determination of selected fragments and stable isotope ratio analysis was performed with an EA-IRMS instrument. **Results and Discussion:** The main active ingredient of the eleven different Spice products, the cannabimimetic 5F-PB-22, was separated from synthesis impurities and components of the herbal matrices. Even low concentrated synthesis side products of the main active substances were isolated and structure elucidation was performed by MS^n experiments. In each analyzed herbal brand the chemical structures of at least 15 different trace components including positional isomers were found and verified by HR-MS and NMR measurements. EA-IRMS was successfully applied to assess reliable carbon, nitrogen and hydrogen isotope ratio data of 5F-PB-22 samples isolated from Spice-products. **Conclusion:** The identification of cannabimimetics and synthesis impurities in herbal mixtures by state-of-the-art MS techniques is an important factor to stay on track with the ongoing introduction of NPS to the worldwide illicit drug market. The potential of IRMS for batch-to-batch linking of Spice-products based on stable isotope data was demonstrated.

1. Introduction

In 2008, several synthetic cannabinoid receptor agonists (further referred to as “synthetic cannabinoids” in this text) were detected in herbal smoking blends which were mainly sold via Internet and specialized head shops under a variety of brand names, “Spice gold” being

one of the most popular [1]. These professionally designed packages of products were specifically aimed at young consumers to present a “legal” alternative to Cannabis products. Actually they consisted of inherently inactive plant material that was laced with one or more highly potent synthetic cannabinoids. Since then, a dramatic increase of new psychoactive substances (NPS) on the European drug market has been observed by the EMCDDA via the European early warning system (EWS). Not only synthetic cannabinoids in ´Spice´ products appeared, but also a wide range of new stimulants, e.g. cathinone derivatives in so-called ´bath salt´ products (presenting an alternative to amphetamine and methamphetamine) or piperazine derivatives with psychotropic effects comparable to MDMA and other entactogens. The trend of steady increases in numbers of NPS reported to EMCDDA year-by-year is still intact. Compared to only 13 in 2008 the number was eightfold higher in 2014 (101 NPS), which was still a significant increase in relation to the already very high level (81 NPS) in 2013 [2]. The NPS phenomenon is, thus, one of the most urgent drug-related problems in the European Union, as substances that are included in the individual controlled substance acts of the member states are rapidly replaced by other NPS, many of them being highly potent and producing unpredictable effects in consumers. A broad range of aspects related to the NPS phenomenon including forensic, toxicological and socio-scientific studies have been addressed in the two finalized research projects within EC´s drug prevention and information programs ´SPICE´ (JUST/2009/DPIP/AG/0948, 2010-2012) and ´SPICE II plus´ (JUST/2011-2012/DPIP/AG/4000003597, 2013-2015) which were more focused on health risks and prevention issues related to NPS.

The knowledge about the production and the supply chain of NPS is still very limited to date and quite different from classic synthetic drugs (amphetamine-type-stimulants) which are typically clandestinely produced in European countries. Most of the NPS are presumably produced by specialized chemical companies in Asia, typically in China. The syntheses of aminoalkyl indoles and aminoalkyl indazoles, which are the most prevalent substance classes of synthetic cannabinoids found in Spice products, are not very complicated and can be achieved with comparatively inexpensive equipment and chemicals. However, details of the production, the employed synthesis routes, the sources and the nature of the precursor chemicals and reagents and the modus operandi of trafficking are often not known.

The main objective of the ongoing EU-project SPICE-profiling (2015-2017), which is more aimed at the needs of law enforcement agencies and legislative bodies, is to increase the knowledge base on the origin, production and supply chain of NPS by utilizing NPS samples from test purchases in internet shops, authentic samples from the chemical manufacturers and NPS samples from customs and police seizures as an information pool that is extracted by a wide range of analytical methods [3], especially by chemical impurity profiling with gas chromatography-mass spectrometry and isotopic profiling procedures by isotope ratio mass spectrometry. In addition, controlled syntheses of selected NPS with different batches of precursor chemicals will be conducted as reference and to fine-tune the profiling procedures to allow accurate and reliable batch-to-batch comparison of seized NPS samples of the same type.

The systematic test purchases of new products in the whole time-frame of the project will additionally allow precise observation and interpretation of the development of the NPS market, especially in dependence on changes in the legal treatment of single substances or substance classes in the narcotics or medicine acts of European member states, so the project will be able to assess the effectiveness of legal measure with respect to an actual reduction of supply or demand. Problems in the detectability of NPS in human bio samples, important for the proof of consumption of NPS related to driving or the surveillance of imprisoned persons or persons in drug control programs, will also be addressed in the project by metabolism studies of important NPS.

The following work streams will be performed in the project Spice-profiling by a consortium of forensic laboratories from police (German Bundeskriminalamt accompanied by the four State Criminal Police Offices of Bayern, Hessen, Rheinland-Pfalz and Schleswig-Holstein as associated partners and the French Police Forensic Science Institute, INPS), legal medicine (institute of legal medicine Freiburg) and customs (Finnish national customs, Helsinki):

Workstream 1:

- Product monitoring of products containing NPS by qualitative and quantitative analysis of products composition (internet test purchases). This includes identification of new compounds and particularly dangerous products and development of rapid detection strategies with portable instruments.

Workstream 2:

- Method development for clinical and forensic analysis of human bio samples and application of these methods to forensic case work and clinical emergency cases.

Workstream 3:

- Assessment of suitable synthesis impurities in unadulterated NPS samples by chemical analysis via GC-MS and UHPLC-MS and conducting controlled syntheses of synthetic cannabinoids.
- Development of flexible impurity profiling procedures for synthetic cannabinoids and synthetic cathinones via GC-MS.
- Application of the GC-MS profiling procedures to create a representative database and to harmonize the procedure and multivariate analysis of impurity profiling and isotopic profiling data.

Workstream 4:

- Development, validation and harmonization of extraction techniques and of methods to determine isotope ratios using EA-IRMS and GC-IRMS-MS of synthetic cannabimimetic substances.
- Evaluation of the impact of isotope ratio mass spectrometry (IRMS) techniques for the ability to establish links between different seizures and to link seizures to clandestine producers.

A frequently applied method for comparing drug samples is the quantitative analysis of a set of target synthesis impurities via GC/MS. Typically, prior to chromatographic profiling, the main active substance which is usually present in big excess, has to be removed by standardized extraction procedures. Examples are impurity profiling concepts for amphetamine or MDMA in seized powder drugs or Ecstasy pills [4, 5, 6, 7]. Only very few publications deal with the application of chromatographic methods for the comparative analysis of Spice products, two relevant papers are focused on the main compounds of the herbal products including the plant-based ingredients (e. g. from Damiana herb) and aroma compounds [8, 9]. To detect e. g. relations between different products manufactured in different clandestine drug laboratories, additionally IRMS-techniques have been used for the characterization of a wide range of illicit synthetic drugs [10, 11, 12, 13, 14]. As a specific isotopic signature of a product results from specific production factors like raw materials, synthetic method, batch size and manufacturer, this information can be used to infer the source of a trace.

This paper deals with the first profiling investigation of the synthetic cannabinoid receptor agonist 5F-PB-22 (1-Pentylfluoro-1*H*-indole-3-carboxylic acid-8-quinolinyl ester) in Spice products by using UHPLC-ESI-MS^n and isotope ratio mass spectrometry methods. 5F-PB-22, first reported by a Japanese researcher group [17] is one of the most frequently observed main active substances in Spice-products of various brands and producers in the last two years and is, thus, a relevant target substance for a model study on chromatographic and isotopic profiling of NPS in Spice products.

2. Material and Methods

2.1. Materials

2.1.1. 5F-PB-22

The aminoalkyl indole 5F-PB-22 (1-Pentylfluoro-1*H*-indole-3-carboxylic acid-8-quinolinyl ester) was extracted from 14 different Spice product brands including eleven product samples from a big police seizure in one internet shop. Additionally, one pure 5F-PB-22 sample from a different police seizure was available for analysis.

2.1.2. Sample preparation of 5F-PB-22 from herbal material

Stepwise extraction and purification: To extract 5F-PB-22 from the herbal material, ca. 200 mg of herbal blend is rinsed with 2 mL acetonitrile. The contact time between solvent and herbal material is minimized to reduce additional extraction of plant related matrix components. After removal of the extract, the solvent is evaporated to dryness under a steady stream of nitrogen. The dried material is overlaid with 1 mL methanol and gently pivoted until a spherical precipitation is visible. After 30 seconds of pivoting, the methanol is removed and evaporated to dryness, giving 5F-PB-22 in the form of white crystals (if necessary, crystallization can be facilitated by addition of small volumes of diethyl ether). The extracted material is subsequently submitted to UHPLC-ESI-MS and EA-IRMS measurements

Preparative column chromatography: To separate the main active substance from synthesis impurities and other side products, preparative column chromatography is used. A glass column with a length of 600 mm, a diameter of 30 mm and a PTFE stopcock (Lenz, Wertheim, Germany) was utilized. Silica gel 60 (not less than 0.063 mm) and fine granular quartz from Merck (Darmstadt, Germany) were used to pack the column. To achieve a sufficient separation, a mobile phase had to be established for all extracts. Different solvents were tested via TLC and a mixture of n-hexane:ethylacetate 2:1 (v:v) showed the best results.

Approximately 5 g of herbal material is extracted with 50 mL of acetonitrile. The extract is removed, evaporated to dryness and again dissolved in a sufficient amount of mobile phase. The sample is applied to the preparative column packed with 50g of silica gel. While running the chromatography, fractions of eluting mobile phase are collected in appropriate time periods. Each fraction is evaporated to dryness, again dissolved in acetonitrile and measured via UHPLC-ESI-MS.

2.2. Ultra high performance liquid chromatography mass spectrometry (UHPLC-MS)

The tertiary UHPLC system UltiMate 3000 by Dionex (Thermo Scientific, Waltham, MA, USA) was used for separation, consisting of a pump, auto sampler and column compartment. Elution was achieved by a gradient system with varying mixtures of Eluent A

(water:acetonitrile:formic acid 99:1:0.1) and B (water:acetonitrile:formic acid 1:99:0.1). The corresponding LC-program is shown in Table 1. Separation of 5F-PB-22 and related synthesis impurities was achieved using a Kinetex 2.6 µ C18 100A 100 x 2 x 10 mm column by Phenomenex (Aschaffenburg, Germany).

Tab. 1. UHPLC parameters for synthetic cannabinoid analysis with ion trap MS.

Oven temperature	40 °C
Gradient 0 min	20 % B
1 min	20 % B
2.5 min	60 % B
4 min	65 % B
5.5 min	65 % B
8 min	99 % B
10 min	99 % B
10.2 min	20 % B
12 min	20 % B
Overall runtime	12 min
Flow rate	0.5 mL/min
Pressure Max	512 bar
Injection volume	5 µL

An ion trap mass spectrometer AmazonSpeed by Bruker (Bruker, Billerica, MA, USA) with an ESI source was used. Ionization was achieved at +4.5 kV while the mass analyzer operated in Ultra Scan mode (32500 m/z per sec) with enabled SmartFrag to MS^3.

2.3. Elemental Analyzer - Isotope Ratio Mass Spectrometry (EA-IRMS)

0.5 mg of 5F-PB-22 was weighed into 3.3 mm x 5 mm tin capsules (IVA Analysentechnik e.K., Meerbusch, Germany). These were flash combusted on an Elemental Analyzer Flash EA 1112 (ThermoFisher, Bremen, Germany) coupled online to an isotope ratio mass spectrometer (Model delta V plus, ThermoFisher, Bremen, Germany) via a ConFlo interface (Model Conflo IV, ThermoFisher, Bremen, Germany). Catalytic oxidation and reduction beds were held at 1020°C and 650°C, respectively.

Dual measurement of $\delta^{15}N$ and $\delta^{13}C$ in the same analysis were performed. Pulses of working standard gas (purity at least 4.5 for CO_2 and 5.0 for N_2, Linde, München, Germany) were introduced via the ConFlo interface before and after the sample gases. The isotope ratios are expressed relative to the international standards (Vienna Pee Dee Belemnite (V-PDB) and AIR respectively) using the delta notation:

$$\delta[‰] = \left(\frac{R_{Sa}}{R_{St}} - 1 \right) * 1000$$

where R_{Sa} = ratio $^{13}C/^{12}C$ or $^{15}N/^{14}N$ for the sample and
R_{St} = ratio $^{13}C/^{12}C$ or $^{15}N/^{14}N$ for the standard.

For scale calibration (2-point end-member normalization) of the raw data, in-house standards, Peptone (Sigma-Aldrich, Steinheim, Germany, $\delta^{13}C_{V\text{-}PDB}$: -13.79 ‰; $\delta^{15}N_{AIR}$: 6.18 ‰) and Acetaminophen (>99% purity, Sigma-Aldrich, Steinheim, Germany, $\delta^{13}C_{V\text{-}PDB}$: -28.7 ‰; $\delta^{15}N_{AIR}$: -3.39 ‰) were used daily. These in-house standards and the working gases were calibrated against international referencing material as IAEA-CH6 ($\delta^{13}C_{V\text{-}PDB}$: -10.45 ‰) and IAEA-CH7 ($\delta^{13}C_{V\text{-}PDB}$: -32.15 ‰) for CO_2 and USGS-40 ($\delta^{15}N_{AIR}$: -4.52 ‰) and USGS-43 ($\delta^{15}N_{AIR}$: 8.44 ‰) for N_2.

Acetanilide p.a. (Merck, Darmstadt, Germany, $\delta^{13}C_{V\text{-}PDB}$: -34.21 ‰; $\delta^{15}N_{AIR}$: 1.80 ‰) was analysed as in-house standard to routinely check accuracy and long-time reproducibility of the system. Expanded measurement uncertainty (k=2) was determined to be 0.27 ‰ for $\delta^{13}C$ and 0.45 ‰ for $\delta^{15}N$ respectively.

3. Results and Discussion

3.1. Analytical workflow for NPS profiling

The analytical workflow applied for NPS profiling in the Spice-profiling project is depicted in Fig. 1. In a first step, the NPS product is analytically characterized by GC/MS (main and minor compounds including compounds from herbal matrices) and headspace-GC (volatile compounds, mainly solvent residues), optionally complemented by additional analytical techniques, e.g. UHPLC-DAD (for Spice products) or NMR (for bath salt and comparable products containing stimulant NPS) for quantification or ICP-MS for trace analysis of inorganic impurities (e.g. Pd, Pt or Ni as catalyst metals hinting to applied synthesis routes). In the second step, the main active substance (in this work exemplarily 5F-PB-22) is isolated from the sample matrix and in the same step an extract with combined and enriched synthesis impurities is prepared. In the model study described in this paper, column chromatography and a multistep extraction procedure have been applied for that purpose, in the further course of the project fully automated flash chromatography similar to the procedure described by Moosmann et al. [15] will be used to increase sample throughput. The purified active substance from the NPS product is submitted to EA-IRMS for stable isotope ratio analysis and can also serve as reference material e.g. for quantitative analysis of seized material. In a second phase of the project, after systematic evaluation, also GC-IRMS will be applied directly to extracts of the Spice products. The combined fractions with synthesis impurities are submitted to chromatographic profiling via GC/MS and UHPLC-ESI-MS. The chromatographic impurity data and the stable isotope ratio data will finally be interpreted via multivariate data analysis procedures (after data pre-treatment) to establish sample links. Together with data of samples produced in controlled laboratory syntheses (e.g. via synthesis routes published in relevant patents) comprehensive information on the origin and details of production of the NPS products can be retrieved.

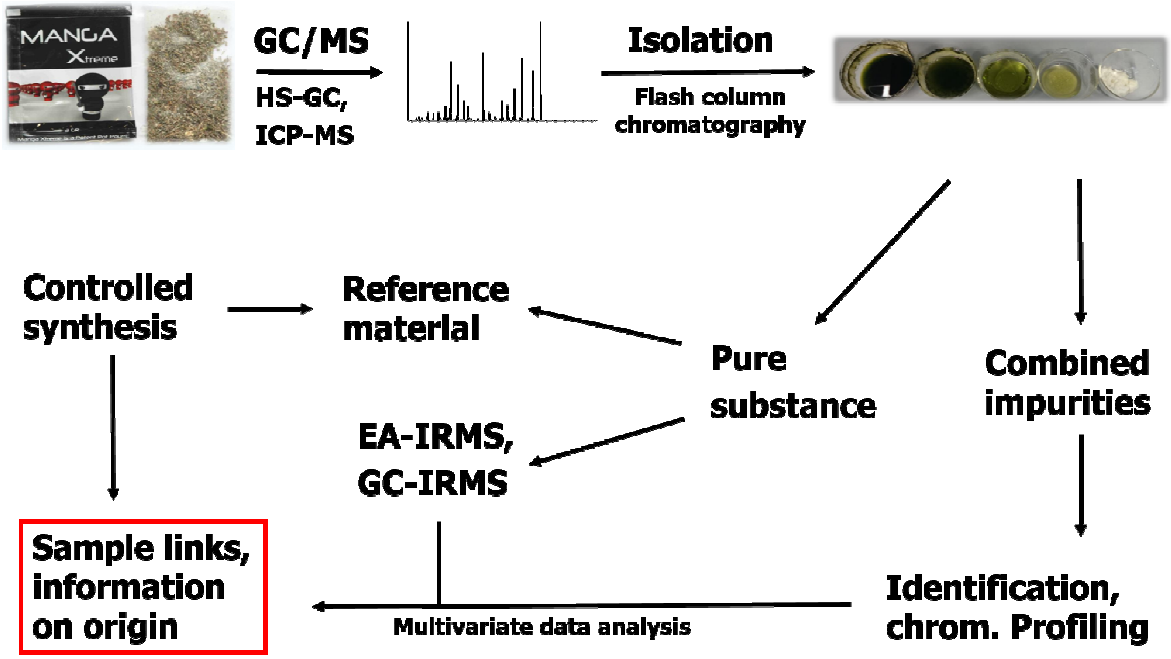

Fig. 1. Schematic workflow for the isolation of the main active substance and the combined synthesis impurities and subsequent profiling analyses by chromatography/mass spectrometry and stable isotope ratio mass spectrometry.

3.2. Assessment of 5F-PB-22 synthesis impurities via UHPLC-ESI-MS^n

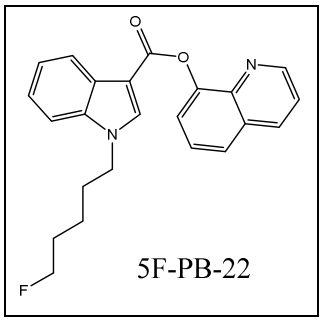

Fig. 2. 5F-PB-22 structure.

The synthetic cannabinoid receptor agonist 5F-PB-22 (1-Pentyl-fluoro-1*H*-indole-3-carboxylic acid-8-quinolinyl ester; Fig. 2) has been one of the most frequently observed main active substances in Spice-products of various brands and producers in the last two years and is, thus, a relevant target substance for a model study on chromatographic impurity profiling of synthetic cannabinoids. Similar to its parent compound PB-22, as already reported in [16], 5F-PB-22 is subject to thermal degradation (ester cleavage) when analyzed by gas chromatography. This can also be expected for many of the most relevant 5F-PB-22 synthesis impurities.

As a consequence, UHPLC-ESI-MS was selected as method for separation and identification of relevant synthesis impurities related to 5F-PB-22 in samples of seized Spice products of different brands. Among these, eleven samples originate from one police seizure from an Internet shop, presenting an interesting sample set-up for a model study on profiling.

Figure 3 demonstrates, that the direct analysis of solvent extracts of Spice products is not adequate for the assessment of the synthesis impurities related to 5F-PB-22 because of the large excess of 5F-PB-22 present in the sample (chromatogram in yellow color). Preparative normal-phase column chromatography was applied to isolate synthesis impurities from the sample extracts. The chromatogram in green color of Fig. 3 represents a fraction collected with preparative column chromatography containing some of the most relevant synthesis impurities (halogenated and methylated derivatives of 5F-PB-22). As the named synthesis impurities are structurally closely related to 5F-PB-22, a removal of the main active substance by a simple extraction is obviously not feasible which demonstrates the need for a chromatographic sample work-up with inherent enrichment of the impurities before the chromatographic impurity profiling can be conducted. To enhance performance and sample throughput, a fully automated flash chromatography system will be used for future profiling applications.

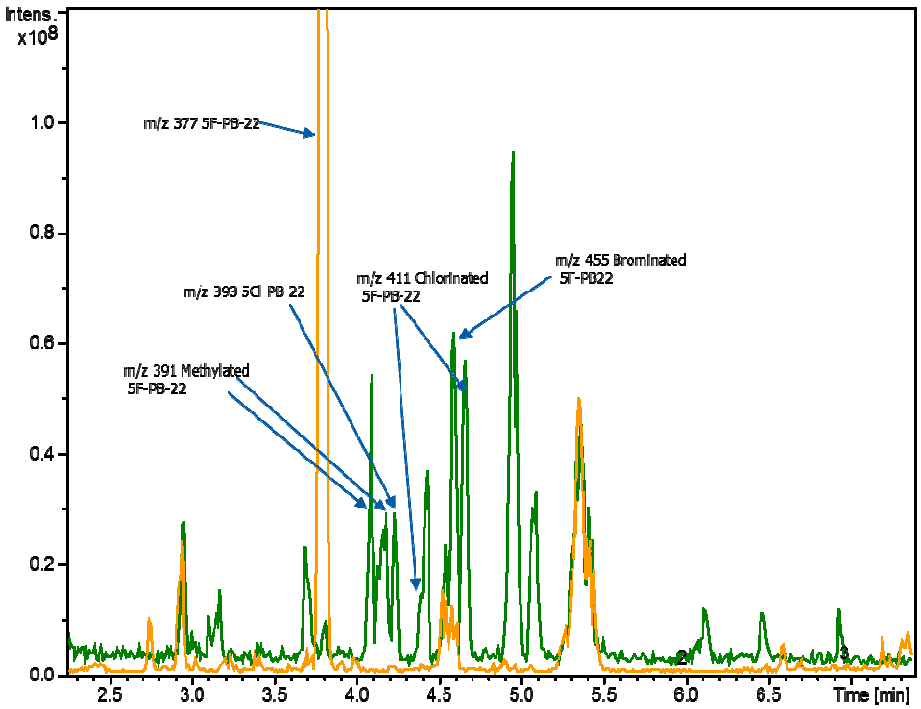

Fig. 3. UHPLC-ESI-MS base peak chromatograms for an extract of a Spice product in acetonitrile containing 5F-PB-22 as main active substance. Yellow: chromatogram of the sample extract without sample work-up. Green: chromatogram of one fraction containing discriminative synthesis impurities after removal of the main active substance from the same sample via column chromatography.

To evaluate the potential of chromatographic impurity profiling via UHPLC-ESI-MS for 5F-PB-22 related synthesis impurities, eleven Spice products from one seizure were comparatively analyzed. To save time for the sample work-up, instead of column chromatography, a rapid multi-step extraction procedure was tested for suitability. The most relevant impurities assigned in Fig. 3 were extracted, but in contrast to the column chromatographic sample work-up, also the main active compound 5F-PB-22 was extracted in significant amounts, somewhat limiting the chromatographic performance. Figure 4 exemplarily shows the results for two Spice products of different brands but from the same seizure that were clearly discriminable based on a set of characteristic synthesis impurities, one of them being the 5Cl-PB-22. For the development of a sophisticated, database-assisted profiling method stability tests for the most discriminative impurities would have to be carried out and the reproducibility of the sample pre-treatment procedure as well as of the quantitative determination of the target impurities´ chromatographic peak areas and of the related retention times would have to be assessed and ensured. In the light of the fast exchange cycle of NPS in the Spice products this effort seems to be justified only in rare cases of prevailing NPS that turn into establish drugs on the illegal market, as e.g. the case for mephedrone or MDPV in the area of stimulant NPS.

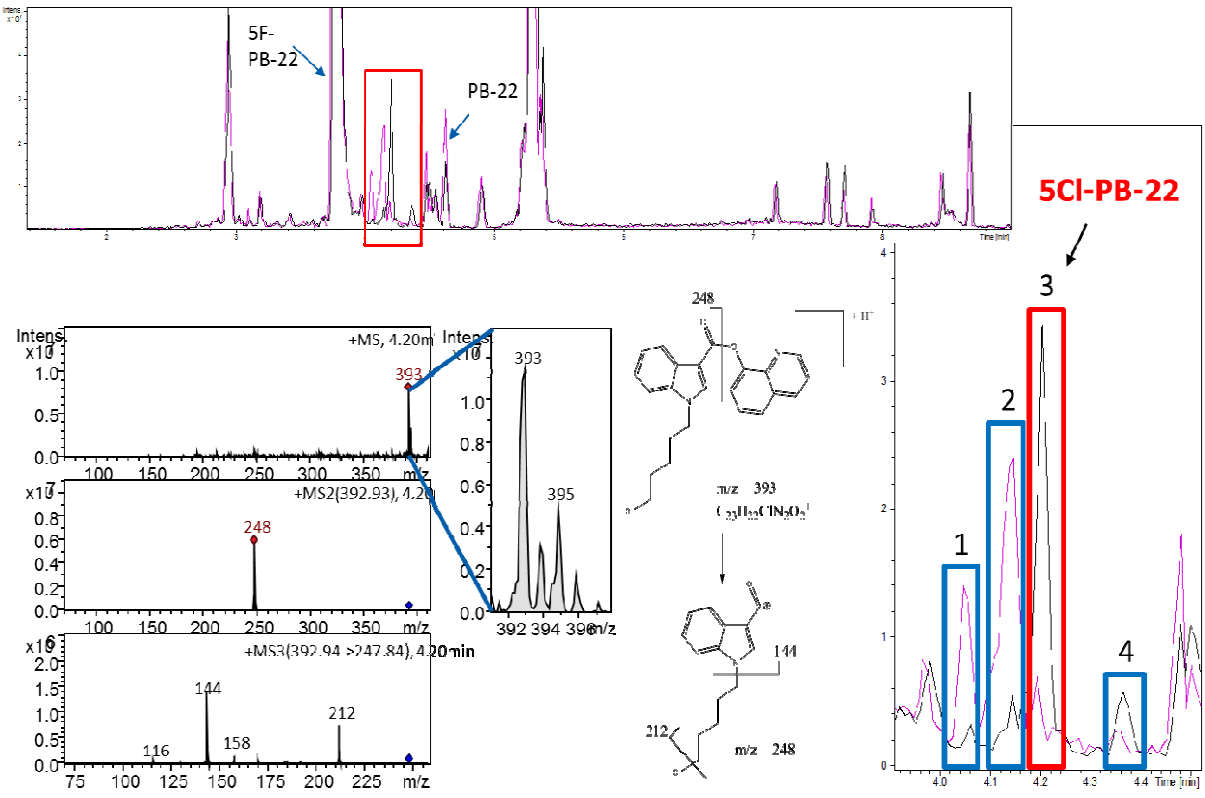

Fig. 4. UHPLC-ESI-MS analysis of two different Spice products containing 5F-PB-22 as main active substance (both products originating from the same police seizure). Top: UHPLC-ESI-MS chromatograms for extracts of the Spice products “Blaze” (pink) and “Jamaican Gold Extreme” (black). Right: Zoom of the UHPLC-ESI-MS chromatograms for extracts of the Spice products “Blaze” (pink) and “Jamaican Gold Extreme” (black) showing discriminative synthesis impurities of 5F-PB-22, tentatively identified via ion trap MS^n and Orbitrap HR-MS (1: mono methylated derivative of 5F-PB-22, possibly 6F-PB-22, 2: different mono methylated derivative of 5F-PB-22, 3: the chlorinated analog 5Cl-PB-22, 4: a chlorinated derivative of 5F-PB-22 with an additional Cl-atom in the indole ring). Left: MS^1, MS^2 and MS^3 spectra for impurity no. 3 (5Cl-PB-22) with a zoom showing the isotopic pattern of the $[M+H]^+$ molecular ion and the proposed fragmentation pathway.

3.3. Comparative analysis of 5F-PB-22 via stable isotope ratio analysis (EA-IRMS)

To evaluate the potential of EA-IRMS for batch-to-batch comparison of Spice products containing synthetic cannabinoids, 5F-PB-22, extracted from eleven Spice products from one seizure containing 5F-PB-22 were submitted to EA-IRMS analysis to yield isotope ratio data for carbon and nitrogen (the sample preparation has been described above). Additionally one seized sample of pure 5F-PB-22 and three further samples of 5F-PB-22 extracted from different Spice products were analyzed as reference samples to assess the discriminability. In Fig. 5 the nitrogen isotope ratio data are plotted against the carbon isotope ratio data for 5F-PB-22 from the named 14 samples. In Table 2 additionally the carbon and nitrogen data are listed together with the brand name of the product and its manufacturer (as stated on the packages) Interestingly, ten of the eleven Spice products can be assigned to two clusters of products that cannot be discriminated within the range of the measurement uncertainty, hinting that the products from each cluster contain 5F-PB-22 from the same production batch. Remarkably, two products from one manufacturer and three products from a different manufacturer are together in the "blue" product cluster. The two clusters, marked in Fig. 5 with a red and a blue ellipse, exhibit differences, indicating that different batches of 5F-PB-22 have probably been used for their production. One of the eleven named Spice products from one seizure (blue square data point) has a significantly lower carbon isotope ratio compared to the other ten products in the two clusters, pointing to a different origin of the contained 5F-PB-22. The impurity profiling of these ten Spice products based on a limited set of four relevant synthesis impurities related to 5F-PB-22 by UHPLC-ESI-MS yielded the same cluster assignment as the EA-IRMS analysis. Three of the four samples used as reference standards can be clearly distinguished from the two clusters of related products from one seizure, indicating a considerable variability of 5F-PB-22 batches in products from the drug market, indicating a sufficient potential for discriminability. It can be expected, that the inclusion of hydrogen isotope ratio data will further increase the potential of EA-IRMS to distinguish synthetic cannabinoids contained in Spice products.

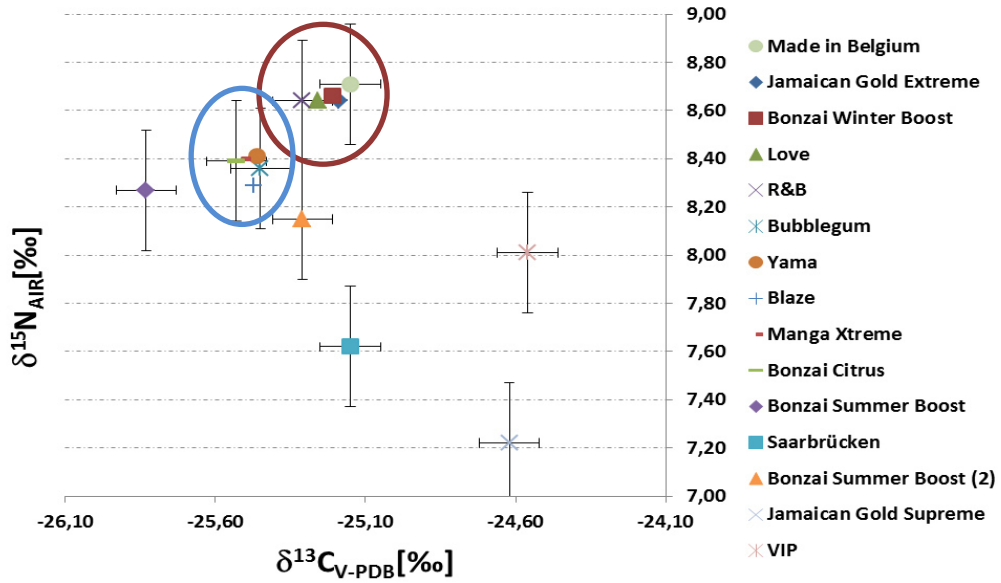

Fig. 5. Nitrogen isotope ratio data plotted against carbon stable isotope data for 5F-PB-22 samples isolated from eleven Spice products from one police seizure and for four reference samples of 5-F-PB-22 from different sources to demonstrate discriminability ("standards", one pure substance and three samples isolated from different Spice products).

Tab. 2. Carbon and nitrogen isotope ratio data for 5F-PB-22 samples presented in Fig. 5 (clustering of the product indicated by different coloring).

Producer	Product	$\delta^{13}C_{V\text{-}PDB}$[‰]	$\delta^{15}N_{AIR}$[‰]
Yama	Made in Belgium	**-25,15**	**8,71**
Yama	Jamaican Gold Extreme	**-25,19**	**8,64**
Yama	Bonzai Winter Boost	**-25,21**	**8,66**
Jural	Love	**-25,26**	**8,64**
Jural	R&B	**-25,31**	**8,64**
Yama	Bubblegum	**-25,45**	**8,36**
Yama	Yama	**-25,46**	**8,41**
Yama	Blaze	**-25,47**	**8,29**
Yama	Manga Xtreme	**-25,51**	**8,4**
Yama	Bonzai Citrus	**-25,53**	**8,39**
Yama	Bonzai Summer Boost	**-25,83**	**8,27**
	Standards		
pure subst.	Saarbrücken	**-25,15**	**7,62**
Yama	Bonzai Summer Boost (2)	**-25,31**	**8,15**
Yama	Jamaican Gold Supreme	**-24,62**	**7,22**
Yama	VIP	**-24,56**	**8,01**

4. Conclusions

The identification of cannabimimetics and related synthesis impurities in herbal mixtures by state-of-the-art MS techniques is an important factor to stay on track with the ongoing introduction of NPS to the worldwide illicit drug market. Spice products containing the synthetic cannabinoid 5F-PB-22 were submitted to different sample preparation techniques including column chromatography to deliver the pure active substance (for subsequent stable isotope ratio analysis) and a fraction of enriched synthesis impurities. A set of discriminative synthesis impurities was identified by UHPLC-ESI-MS^n and HR-MS and successfully implemented to distinguish different brands of Spice products. The potential of EA-IRMS for batch-to-batch linking of Spice-products based on stable isotope ratio data for carbon and nitrogen was successfully demonstrated by comparative analysis of 5F-PB-22 isolated from 14 samples of Spice products of which eleven were from one police seizure. The impurity profiling of ten of these eleven Spice products based on a limited set of four relevant synthesis impurities related to 5F-PB-22 by UHPLC-ESI-MS yielded the same cluster assignment as the EA-IRMS analysis based on carbon and nitrogen isotope ratios. Thus, the combined statistical evaluation of data from chromatographic impurity profiling and stable isotope ratio data will be a powerful concept for origin assessment assays of NPS.

5. Acknowledgements

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