**Combination of stable isotope ratio data and chromatographic impurity signatures as a comprehensive concept for the profiling of highly prevalent synthetic cannabinoids and their precursors**

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**Abstract**

In this study, we utilized elemental analyser (EA) and gas-chromatography (GC) isotope ratio mass spectrometry (IRMS) and ultra-high-performance liquid chromatography coupled to mass spectrometry (UHPLC-MS) in a comprehensive profiling approach assessing the chromatographic impurity signatures and δ13C and δ15N isotope ratios of synthetic cannabinoids from police seizures and internet test purchases. Main target of this study was the highly prevalent synthetic cannabinoid MDMB-CHMICA (methyl (2S)-2-([1-(cyclohexylmethyl)-1*H*-indol-3-yl]formamido)-3,3-dimethylbutaoate). Overall, 61 powder and 118 herbal blend (also called “Spice-Products”) samples were analysed using both analytical techniques and evaluated in a joint model to link samples from a common source. As a key finding, three agglomerates of Spice-product samples with similar dates of purchase were identified in the IRMS data, possibly representing larger shipments of MDMB-CHMICA, each produced with the same precursor material, successively delivered to the European market. The three agglomerates were refined into multiple sub-clusters based on the impurity profiling data, each representing an individual synthesis batch. One of the agglomerates identified in the IRMS data was found to consist two groups of four sub-clusters, respectively, with majorly different impurity profiles, demonstrating the necessity for both analytical techniques to extract the maximum amount of information from a limited sample pool. Additionally, 31 samples containing the recently surfaced synthetic cannabinoid Cumyl-PeGaClone (5-pentyl-2-(2-phenylpropan-2-yl)-2,5-dihydro-1*H*-pyrido[4,3-b]indol-1-one) were analysed for their and δ13C and δ15N isotope ratios to put the isotopic data recorded for MDMB-CHMNICA in a more global perspective. Three building blocks of precursor chemicals (indole, tert-leucine, cumylamine) potentially used for the synthesis of the two named synthetic cannabinoids were acquired from different global vendors and measured for their δ13C and δ15N isotope ratios to better understand variations in the isotopic composition of the synthetic cannabinoids and to trace their origin.

**1. Introduction**

Classic drugs like Cannabis, cocaine, heroin or amphetamine type stimulants (ATS) make up the largest amount of seized drugs of abuse worldwide [1]. Forensic profiling is a well-established method for law enforcement agencies to obtain basic information on the manufacturing and trafficking of these drugs. The two most frequently used analytical techniques are profiling of synthesis-related chemical impurities (impurity profiling) and stable isotope ratio analysis (SIRA), each providing orthogonal information on the history of a sample. Chemical profiling mainly targets the arising natural and synthetic by-products and impurities as a result of the synthesis pathway and chosen precursor material [2-6]. The analysis of the composition of stable isotopes for elements like carbon, nitrogen or hydrogen via isotope ratio mass spectrometry (IRMS) can provide information on the origin of precursor compounds of synthetic drugs like amphetamine [7, 8] or methamphetamine [9, 10] and was used to geographically narrow down the cultivation area of plant-based drugs like cocaine [11, 12] or heroin [13]. Buchanan et al. [14] published one of the few available literature sources where both techniques were used individually and as combination to discriminate between in-house produced samples of 3,4-methylenedioxymethamphetamine (MDMA), synthesized using two batches of piperonyl methyl ketone (PMK) and three different synthetic routes (Al/Hg, NaBH4 and Pt/H2). Via impurity-profiling it was possible to discriminate between the three synthesis routes due to differences in the chemical impurity composition. According to the authors, the IRMS data did not allow for unambiguous discrimination between neither the synthesis pathway, nor the starting material due to isotopic fractionation. However, a combination of impurity profiling and stable isotope analysis data allowed for the discrimination between both, the origin of the precursor material and the applied synthesis route, using pattern recognition techniques such as principle component analysis (PCA). In the work presented here, we investigated, to which extent the combination of these two analytical techniques can support the strategical evaluation, in particular the discrimination of batches and synthesis pathways, of new psychoactive substances (NPS). Since 2006, the appearance of new classes of NPS on the illicit drug market significantly increased in number of individually reported substances (overall 803 NPS in the period of 2009-2017) as well as in seized volume (22 tons in 2016), growing to a major drug-related issue of the twenty-first century [1]. Most of these substances, including synthetic cathinones, opioids or cannabinoids, were originally developed in legitimate pharmaceutical research and are now misused as recreational drugs [15-17]. The exact procedure of manufacturing, sourcing of precursor chemicals and trafficking of NPS are yet a topic of ongoing research and are expected to be majorly different to other synthetic drugs such as ATS, which are typically produced in clandestine laboratories in Europe. The majority of NPS are presumably produced by chemical companies in Asia, typically China, with professional instrumentation and trained chemists, reflected by the high purity of NPS on the market [18]. The pure drug substances are then shipped by air or sea to Europe where they are employed as active ingredients of designer drug products sold openly in internet-shops as so-called “legal-highs” or “research chemicals” (RC). In Germany, synthetic cannabinoids are the most prevalent NPS subclass and are mostly sold as herbal smoking blends, known as “Spice-Products” (SP) [19, 20]. The pure cannabinoids are sprayed onto an inactive dried plant matrix such as Damiana (*Turnera diffusa* Willd. Ex Schults. var *aphrodisica*) or strawberry leaf and packed into plastic sachets with professionally designed logos and recurrent branding. From the perspective of forensic investigation, tracing back the source of seized material of synthetic cannabinoids (either in pure form or laced onto herbal material) by analysis of the impurity composition or isotopic profile is very difficult, as usually no authentic reference materials from the original manufacturing sites are available. The history of each sample can be regarded as “black-box” which only enables the generation of links between individual seizures, but not reveals the original source. However, pure material from a single synthesis batch of synthetic cannabinoids carries a specific impurity profile and isotopic composition, which is maintained even though sprayed onto a herbal matrix [21, 22]. Multiple batches synthesized from the same precursor material also should exhibit a similar isotopic composition, when using the same synthesis pathway [23, 24]. Thus, by IRMS analysis it can be possible to link seized samples that were synthesized in different batches, but come from a single manufacturing site using the same precursor material for all syntheses. By analysis of the impurity composition, it can be possible to additionally discriminate between the individual synthesis batches even though the same precursor material was used. In this study, we aimed at interpreting both techniques individually for their discrimination potential and finally combine the information of both datasets to link Spice products from internet test purchases and obtain general information about underlying distribution channels and market structures. By implementing metadata into the data evaluation, for example the date of purchase of a specific SP, a time dependant correlation might be revealed.

IRMS profiling of three cathinone derivatives [25] and of the synthetic cannabinoid 5F-PB-22 [21] **3** (5F-QUPIC, 1-pentylfluoro-1*H*-indole-3-carboxylic acid-8-quinolinyl ester, Figure 1) were published, using the stable isotope ratios of δ2H, δ13C and δ15N to link seizures. Differences in isotopic composition for the presented sample pool of both the cathinone derivatives and the synthetic cannabinoid are expected to be a result of a different synthesis procedure or in the case of multiple consecutive batches with the same synthesis route, the use of multiple lots of a specific precursor material with altering isotopic composition. Recently, we demonstrated based on an impurity profiling study on the synthetic cannabinoids MDMB-CHMICA **1** (Methyl (2S)-2-([1-(cyclohexylmethyl)-1*H*-indol-3-yl]formamido)-3,3-dimethylbutanoate, Figure 1), that NPS manufacturers produce multiple synthesis batches to supply the demand on the drug market [22]. A large police seizure by Luxembourg customs in December 2014 of forty 1 kg bags of pure **1** stacked into two barrels was analysed for chromatographic impurity compositions. By comparative multivariate data analysis it was found, that the complete seizure comprised of at least six synthesis batches in sizes of 5 to 10 kg. Whether the manufacturers used the same precursor material for these syntheses is yet unknown, which is why we analysed samples of the named 40 bags of MDMB-CHMICA additionally via IRMS in the study presented here to obtain orthogonal information to the impurity data. The results from both techniques will provide a deeper insight into the manufacturing process for this specific sample pool and help to better interpret the correlation of isotopic and impurity composition of other seized samples. Although the Luxembourg seizure alone comprised for around 40 million single doses, still numerous RC and SP containing **1** could be found on the European market from 2014 to 2016, proving that other shipments of this synthetic cannabinoid have reached its destination in Europe. Considering the scenario of successive synthesis of large batches of **1**, over the period of two years it is likely that the manufacturer of **1** was forced to restock their depleted precursor material, which again might carry a different isotopic composition. This replacement cycle in the precursor material should be detectable in the isotopic data of the corresponding material of **1** found in street samples. Therefore, seized and online test-purchased pure samples and SPs containing **1** from the end of 2014 to the end of 2015were analyzed for their impurity composition and δ13C and δ15N isotope ratio data to identify potential links of samples from a common source. Although a large number of online-shops sell a variety of different SPs products with varying brands and logos, the synthetic cannabinoid material used to produce these SPs might come from a common source.

Cumyl-PeGaClone **2** (5-pentyl-2-(2-phenylpropan-2-yl)-2,5-dihydro-1H-pyrido[4,3-b]indol-1-one [26], Figure 1) was one of the highly prevalent synthetic cannabinoids in 2017 after the German NPS law was put into force, specifically launched to circumvent the generic submission of indole and indazole core structures of synthetic cannabinoids. Multiple SPs and one RC of **2** were available at that time, which is why we measured these samples via IRMS to assess their overall diversity in isotopic composition and put the isotopic data recorded for **1** in a more global perspective.

Additionally, we measured the δ13C and δ15N isotope ratios of precursor material potentially used in the synthesis of **1** (indole, tert-leucine and tert-leucine methyl ester) and **2** (cumylamine), acquired from different global vendors. Our aim was to assess the diversity of isotopic data in this precursor material, to better understand the variations in the final products and, thus, to validate the data interpretation and generated links. Only material in crystalline form was measured. Other potential precursors, such as cyclohexyl methyl bromide or pentyl bromide are liquids and were not included in this study.

This work combines the different analytical methodologies we developed in a series of previous publications targeting the impurity profiling and stable isotope analysis of synthetic cannabinoids. The experimental procedures will not be stated in their full length and can be taken from the corresponding literature [21, 22, 27].

**2. Materials and methods**

*2.1 Synthetic cannabinoids*

*2.1.1 MDMB-CHMICA*

Overall 61 pure samples of **1** were available, of which 40 were from one large seizure by Luxembourg customs in December 2014 (MDMB-01 to MDMB-40), 17 from seizures by the Finish customs (MDMB-41 to MDMB-57), 3 from online test-purchases by the University Medical Center in Freiburg (MDMB-58 to MDMB-60) and 1 from a seizure in Slovenia (MDMB-61).

Furthermore, 118 SP samples containing **1** as sole cannabinoid were available. 74 samples were from online test purchases by the University Medical Center in Freiburg between November 2014 and December 2015 (SP\_MDMB-001 to SP\_MDMB-74) and 44 from seizures by the Land Office of Criminal Investigation of Rhineland Palatine between September 2014 and June 2015 (SP\_MDMB-75 to SP\_MDMB-118).

*2.1.2 Cumyl-PeGaClone*

One pure sample of **2** from a police seizure by the Federal Criminal Institute (Peg-01) and 30 SPs from internet test-purchases containing **2** as sole cannabinoid by the University Medical Center in Freiburg between December 2016 and July 2017 (SP\_Peg-01 to SP\_Peg-30) were available.

*2.2 Precursor chemicals*

Indole was purchased from eight different global vendors: Sigma Aldrich (US), TCI (Japan), ABCR (Germany), Merck (Germany), Acros Chemicals (Belgium), Alfa Aeser (United Kingdom), BePharm (China) and MP Biomedicals (France). Tert-leucin was purchased once as (L) from and in triplicate as (D) form (same lot) from Alfa Aeser (United Kingdom). Tert-leucine methyl ester was purchased from two different Asian vendors BePharma (China) and TCI (Japan). Cumylamine was purchased from four different global vendors: ABCR (Germany), Acella (China), Enamine (Ukraine) and TCI (Japan) in liquid form and subsequently crystallized as cumylamine hydrochloride for measurements on EA-IRMS.

*2.3 Extraction procedure*

For both the seized and bought SPs, the material was poured onto a clean, plane surface and evenly divided into aliquot of ca. 500 mg. If less than 500 mg were available, the complete sample was extracted. The herbal material was rinsed twice with MeCN, once with 5 mL and again with 2 mL. The two extracts were combined and evaporated to dryness and dissolved again in 1.5 mL ethyl acetate/hexane (1:2, v:v) for injection into the flash-chromatography (F-LC).

Self-made SPs were prepared for **1** (MDMB-07, 97.5 ± 2 % purity [28]) and **2** (Peg-01 98.4 ± 2 % purity [28]) by dissolving 300 mg of pure substance in 10 mL Acetone and impregnating 3 g of damiana herb. After drying for 24 hours, each of these SPs were prepared according to the sample preparation procedure of seized SP. Each self-made SP was extracted and cleaned up in triplicate. The corresponding isotopic data can be taken from the supplementary material. A maximum difference of 0.15 ‰ for δ13C and 0.20 ‰ for δ15N between all measurements for **1**, and 0.12 ‰ for δ13C and 0.13 ‰ for δ15N between all measurements for **2** was calculated. Hence, neither the extraction from herbal material, nor the subsequent clean up via F-LC had influence on the isotopic composition of any synthetic cannabinoid.

*2.4 Isolation of the main component and assessment of impurity signatures*

In comparison to our previous IRMS measurements of synthetic cannabinoids [21], the manual preparative column chromatography was replaced with automated preparative F-LC as sample preparation tool as reported in a previous study [22]. By precise fractionation of the F-LC (Sepacore X50, Büchi Labortechnik), the main component fraction can be selectively cut out of the chromatographic run and is available for analysis via IRMS. The F-LC gradient program used for **1** to separate related synthesis impurities from the main component and **2** for main component clean-upcan be taken from the supplementary material. The purity (absence of by-products or matrix components from herbal material) of the main components were validated via UHPLC-MS and GC-MS prior to IRMS measurements.

The remaining fractions of the F-LC run containing related synthesis impurities are pooled again for analysis of the chromatographic impurity signatures via UHPLC-MS (Dionex 3000, Thermo Scientific; AmaZon Speed, Bruker). More detailed information about the UHPLC-MS method can be taken from the supplementary information.

*2.5 Data processing and multivariate data analysis*

The LC-MS data was processed via a rectangular bucketing algorithm (ProfileAnalysis, Bruker, Billerica, MA, USA), integrating the signals of all m/z values from 150 to 600 individually in intervals of 0.5 minutes from minute 1 to 9.5 of chromatographic runtime, forming so called buckets. The fifteen buckets of the previously assessed key-impurities for MDMB-CHMICA were extracted and analyzed via PCA and hierarchical cluster analysis (HCA) using Ward’s method with the software Unscrambler X (Camo, Oslo, Norway) to discriminate between individual synthesis pathways or production batches. The assessment of the corresponding key-impurities for **1** is described in reference [27].

*2.6 Isotope ratio mass spectrometry*

Stable isotope ratios were recorded on an EA-IRMS and GC-IRMS. Generally, EA-IRMS is the preferred technique as it is faster, easier to handle and less prone for malfunction compared to GC-IRMS. However, in some cases, the quantity of purified main component did not exceed 6-8 mg, which was the minimum sample amount for weighting enough solid material into the tin cups (in triplicate) for EA. These specific samples were dissolved as whole in acetonitrile and diluted adequately for subsequent analysis via GC-IRMS.

*2.6.1 Elemental Analyser - Isotope Ratio Mass Spectrometry (EA-IRMS)*

EA-IRMS analysis of pure and extracted material of synthetic cannabinoids was performed in triplicate for δ15N and δ13C, expressed as average ± SD, as reported in our previous study [21].

*2.6.2 Gas Chromatography - Isotope Ratio Mass Spectrometry (GC-IRMS)*

For GC-IRMS, the Isotope ratio mass spectrometer Delta V plus with gas chromatograph TRACE 1310 (including autosampler TriPLUS RSH), ISQ LT (Single Quadrupole Mass Spectrometer) and Interface Conflo IV (all Thermo Fisher Scientific, Bremen, Germany) was used.

All injections were conducted splitless into a hot injector (250°C) with constant flow (constant flow 1.5 mL/min). The analytical column was a Zebron ZB-5MSi (30 m x 0.32 mm ID x 0.50µm film thickness; phenomenex, Made in USA). The Combustion reactor for 13C- and 15N- measurements was a NiO tube/CuO-NiO reactor (Thermo Fisher Scientific, P/N 1255321). This reactor consists of a ceramic tube filled with a Ni-tube and NiO/CuO and Platinum wires. As it contains both an oxidation and a reduction unit in contrast to the EA, an additional reduction reactor was not necessary for nitrogen measurements (NOx reduction to N2). The operating temperature of the reactor was set to 1000°C. The reactor was initially oxidized for 6h at 600°C, 4h at 900°C, and 2h at 1000°C (as recommended by Thermo Fisher Scientific). Repeated oxidation was routinely performed at the beginning of each sequence [for 13C 30 min oxidation (60 min Backflush mode) and for 15N 1 to 5 min oxidation (30 min Backflush mode)].

As validation and quality control (QC) standards, MDMB-07 (previously isotopically characterized by EA-IRMS for comparative purposes), Methyl-N-methylanthranilat (M-MA) (for 15N) and Dodecane (for 13C) were analyzed. The injection volume is 10 µL for each run. The isotope ratios of carbon and nitrogen were measured in separate runs. Each sample was analysed at least four times and averaged. Cannabinoid samples were dissolved in 1 mL acetonitrile with concentration ranges of 80-100 µg mL-1 for δ13C and 600-700 µg mL-1 for δ15N. The GC temperature programs for the samples and QC standards are shown in Table 1.

**To validate that both IRMS instruments are able to measure isotopic compositions with equal results apart from the regularly checked QC standards,** thirteen randomly chosen herbal blend samples containing **1** were extracted, cleaned up and measured with both techniques**.** The corresponding isotopic data can be taken from the supplementary material. EA-IRMS shows a good precision for both elements with a maximum STD of 0.06 ‰ for δ13C and 0.11 ‰ for δ15N. The GC-IMRS measurements showed increased maximum STDs of 0.24 ‰ for δ13C and 0.40 ‰ for δ15N. The maximum Δ between the averaged values for GC-IRMS and EA-IRMS measurements were 0.18 ‰ for δ13C and 0.34‰ for δ15N. Despite the increased measurement uncertainty of the GC-IMRS, on average the results obtained with both instruments are comparable and in acceptable limits for the purposes of this work.

**3. Results and discussion**

*3.1 Isotope ratios of precursor material of MDMB-CHMICA and Cumyl-PeGaClone*

Since the identification of JWH-018 in SPs in 2008, the general structure of newly appearing synthetic cannabinoids has followed a recognizable pattern. They can be synthesized by a modular system consisting of a core structure, aliphatic residue, linker and linked residue, following similar synthesis procedures as published in various patents and publications by the pharmaceutical industry [16, 29]. Although most of the published syntheses start from the native core molecule like indole or indazole, it is yet unknown which exact precursor material the NPS manufacturers use. However, it is generally considered, that a minimum of two synthesis steps are taken: the coupling of the aliphatic residue to the N-atom of the core structure and the coupling of the linked residue to the linker (and thus the core), necessitating at least three individual precursor substances. Figure 2 shows the structural elements of **1** and **2** which are expected to be used in the course of their respective synthesis. In the case of **1**, these precursors should be indole or an indole derivative (indole-3-carboxylic acid, carboxylate or carbaldehyde), (bromomethyl)cyclohexane and tert-leucine methyl ester (TLME). In the case of **2**, a 2-methyl-indole or a derivative (2-methyl-indole-3-carboxylic acid, carboxylate or carbaldehyde), 1-bromopentyl and cumylamine.

Counting the number of carbon atoms in each precursor for **1**, their influence on the overall δ13C value is nearly equal (Indole: C8; Cyclohexyl methyl: C7; TLME: C7). For δ15N, only the indole and TLME, each with one nitrogen, have an impact on the corresponding isotope ratios. The synthesis of **2** is slightly more complex in comparison to synthetic cannabinoids with indole or indazole core structures. The gamma-carboline-1-one structure is expected to be synthesized by ring formation of a 2-methyl-indole derivative, following general synthesis instructions from Clark et al. [30] (Figure 3). Bristol-Meyer Squibb Co. patented the synthesis of a broad spectrum of gamma-carboline-1-one based cannabinoid receptor agonists similar to **2** [31]. This pathway is considered to be likely used by the NPS manufacturers as it is convenient to adapt the synthesis procedures of indole or indazole based core structures (such as Cuyml-5F-PICA) to 2-methyl-indoles with limited effort. Thus, in the stated synthesis, cumylamine (2-phenyl-2-propanamine) should be one of the precursors, contributing one of the two nitrogen and nine of the twenty-five carbon (36%) present in the molecule.

Jasper et al. analysed a set of active pharmaceutical ingredients (API) from different manufacturers and lots for their stable isotope ratios of δD, δ13C, δ15N and δ18O and found that each manufacture synthesized material with a specific isotopic composition, independent from the lot number [32]. It is generally agreed, that it is virtually impossible to artificially synthesize compounds with a target isotopic composition, considering the difficulty to even predetermine the ratio of a single isotope. Wokovitch et al. analysed the isotope ratios of the API Naproxen® and found a maximum inter-batch variation of 2.52 ‰ for δ13C for five different batches form a manufacturer in India [33]. It is not stated whether these differences are the result of an isotopically different precursor material or a poorly conducted or even different synthesis pathway. However, it was still possible to clearly discriminate all five batches from material of five other manufactures for Naproxen. With a global range of > 140 ‰ δ13C [34], 2.52 ‰ only make up for approximately 2 % of the naturally occurring range. Thus, we expect that two samples of synthetic cannabinoids with similar isotopic compositions to be produced by a single manufacturer, and material with indistinguishable isotopic compositions to be produced by a single manufacturer in a single batch or by the same combination of precursor material with distinct isotopic composition in multiple batches. A scenario, where two individual manufactures produce isotopically equal synthetic cannabinoids from precursor material of different sources is considered unlikely.

Indole was purchased from eight different global vendors. Tert-leucine was obtained in (L) and (D), both from AlfaAeser. (D)-tert-leucine was purchased three-fold, whereas the lot number on all three containers were equal, thus they came from a single synthesis or biotransformation batch. TLME was purchased from two different Asian vendors and cumylamine from four different global vendors. Table 2 shows the δ13C and δ15N measured via EA-IRMS for all available precursor substances. Whether the manufactures obtain their precursors from other wholesalers or produce them on their own is unknown.

The measured values of δ13C for all precursors of **1** ranged from -32.91 to -21.04 ‰ (Δ 11.87 ‰), which is the typical range of petro- or plant-based chemicals[35-37]. δ15N ranged from 2.00 to 9.66 ‰ (Δ 7.66 ‰) for the indoles, and from -2.34 to 0.74 ‰ (Δ 3.08 ‰) for the tert-leucines.

Indole can be synthesized by several pathways in industrial scale. Thus, the origin of the nitrogen cannot be assessed. Tert-leucine can be produced on industrial scale by reductive amination of ammonium trimethyl pyruvate by a semi-synthetic route involving leucine dehydrogenase as reducing enzyme, NADH as cofactor and format dehydrogenase for cofactor regeneration[38]. Ammoniac or ammonium is the source of nitrogen in this bio catalytic reaction, which itself is most probably manufactured by the Haber-Bosch process from atmospheric nitrogen, explaining the δ15N values close to zero (atmospheric N2 is defined as standard with 0.0 ‰ for δ15N). All three samples of (D)-tert-Leucine from the same lot exhibit indistinguishable isotopic composition, indicating batch homogeneity.

The measured values of cumylamine ranged from -26.43 to -40.77 ‰ (Δ 14.34 ‰) for δ13C and -19.05 to 8.34 ‰ (Δ 27.39 ‰) for δ15N, which is, compared to the other precursor substances, a significantly broader range. The low values for both elements are unexpected, however, agree with the measured values for **2** as listed in Table A-2 and shown in Figure 5.

*3.2 Isotopic composition of 40 kg pure MDMB-CHMICA from a large seizure by Luxembourg customs*

In a previously published profiling study, forty kilograms of **1** from a police seizure in Luxembourg in December 2014 (MDMB-01 to 40) were assigned into individual synthesis batches of 5-10 kg due to their organic impurity signatures [22]. For three of those forty kilograms, the impurity signatures were majorly different to the remaining sample complex, presumably the result of a differently conducted synthesis. After EA-IRMS analysis of the corresponding δ13C and δ15N values, the same three samples (MDMB-14, MDMB-21 and MDMB-37, batch 1 marked black in Figure 4) were outliers to the cluster of the remaining thirty-seven samples (batches 2 to 6 in Figure 4) ranging from -27.87 to -27.51 ‰ (Δ 0.36 ‰) for δ13C and 3.25 to 3.79 ‰ (Δ 0.54 ‰) for δ15N. A similar linear “dilution” pattern of MDMB-21 and MDMB-14 could be observed in the impurity profiling, with MDMB-37 showing the most dissimilar impurity composition to the remaining sample pool. The two “intermediate” samples MDMB-21 and MDMB-14 seem to be blends in varying composition of MDMB-37 with at least one of the thirty-seven other samples. Hypothetically, MDMB-37 was mixed with batch 4 (violet), indicated by the increased values for δ13C and δ15N of one of the violet samples in comparison to the remaining samples from batch 4. Mixing of batches, either with the same or a different isotopic composition might occur, e.g. when the finished products from multiple synthesis batches are stored in larger containers for interim storage, leading to a blurring of both the corresponding isotopic and impurity composition. Considering the isotope ratios of the measured precursor substances for **1** (Table 2), it is unlikely that material from alternative providence was used to synthesize the batches 2-6 (Figure 4), as already the exchange of the indole core would significantly influence the isotopic composition, either by the nitrogen or carbon or both values, and would let the corresponding samples stick out of the collective.

This seizure is of special interest as it represents a unique collection of samples with previously known connection. In this case, both the impurity profiling and the stable isotope analysis show their individual strengths to draw conclusions about the sample history. IRMS allows to conclude that thirty-seven of the samples were synthesized using the same combination precursor material and three samples seem to be different, either by their origin of precursor or by a different synthesis. However, no further information can be extracted at that point. Via impurity profiling, the same three samples were identified as outliers with distinct impurity signatures as the result of a different synthesis and the remaining thirty-seven samples could be divided into individual synthesis batches by minor but still significant variations in their impurity signatures. Structural identification of specific impurities can provide valuable information about the applied synthesis pathway[27]. However, no information about the provenance of the precursor material is obtained and thus no conclusions about a common origin of the different batches can be drawn, although the relatively similar impurity compositions of the thirty-seven samples are highly indicative. Combining the information obtained from both techniques in a larger forensic context, also considering the information about the shipment as such (e.g. packaging), provides unique insights into the manufacturing of this sample collective. As shown in Figure 4, IRMS data provides an overview of the precursor relationship, impurity profiling reveals the fine structure of the underlying synthesis batches. It validates our previous assumption that only one manufacturer synthesized and shipped the forty kg material of **1** seized by Luxembourg customs. The whole material was packed equally into 1 kg packages without any visual difference (apart from the colour of the powder). The clustering thirty-seven samples were synthesized in multiple batches, repeating a specific synthesis procedure, using the same combination of precursor material (Indole, TLME and cyclohexyl methyl). One batch was synthesized by another synthesis procedure and mixed with other batches, which again indicates a common location of storage and/or packaging and thus validates the idea of a common source for this material.

*3.3 Isotopic data for the complete sample pool of MDMB-CHMICA and Cumyl-PeGaClone*

No IRMS measurements were published for synthetic cannabinoids so far, apart from our early work on 5F-PB-22[21]. Thus, we collected a large sample pool of seized and online test-purchased pure samples and SPs of **1** (61 pure, 120 SP) and **2** (1 pure, 30 SP),two highly prevalent synthetic cannabinoids in Germany from 2014 to 2017, and assessed their isotope ratios for δ13C and δ15N via EA-IRMS and GC-IRMS with the intention to identify potential links between samples that share a common history or origin. Secondly, we wanted to generate an overview of the overall isotopic range for these two synthetic cannabinoids and investigate if there is a large scattering or tight clustering of samples from different sources and dates of receipt. In a previous chapter, we have proven that neither the extraction from the herbal matrix, nor the clean-up via F-LC had an influence on the δ13C and δ15N values of the synthetic cannabinoids.

Figure 5 shows the measured δ13C and δ15N values of all available samples of **1** and **2** and the previously assed values for 5F-PB-22 [21]. A clustering of the individual synthetic cannabinoids can be observed. The values for all samples of **1** ranged from -27.87 to -25.94 ‰ (Δ 1.93 ‰) for δ13C and 2.39 to 6.35 ‰ (Δ 3.97 ‰) for δ15N. All samples of **2** (December 2016 to July 2017) showed values ranging from -33.83 to -35.26 ‰ (Δ 1.43 ‰) for δ13C and -10.34 to -12.70 ‰ (Δ 2.36 ‰) for δ15N, clearly separating them from the other two synthetic cannabinoids.

Comparing the samples on the outer boarders of the respective clusters of **1** and **2**, their overall delta is higher than the measurement uncertainty, indicating multiple reaction batches with precursor material with different isotopic composition or, as already known for **1**, different synthesis. However, despite these significant intra-cluster variations, the individual clustering of the synthetic cannabinoids cannot be considered as pure coincidence. We expect that each of these cannabinoids were synthesized by one manufacturer, in the case of **1** the same that is responsible for the 40kg seizure by Luxembourg customs, who repeatedly uses bulk material of precursor substances from a specific provenance. Slight variations in isotopic composition are most probably the result of a different synthesis procedure leading to isotopic fractionation or the manufacturer supplementing his precursor stock. The latter is considered as very likely scenario. We already have proven that the 40 kg seizure by Luxembourg customs consisted of multiple reaction batches, indicating a successive production of this cannabinoid. As this delivery was taken from the European market, the manufacturers had to substitute the lost material. The high prevalence of **1** through 2015 is evidence for other deliveries reaching the European market, either as large shipments or small packages. The here presented values of precursor substances from different global vendors, especially those of carbon (e.g. the indoles with an overall Δ of 11.87 ‰ for δ13C), and the comparatively tight clustering of the individual synthetic cannabinoids are a clear indication that the precursor material for each cannabinoid is coming from a single producer, who synthesizes large batches with only minor isotopic varieties.

The isotopic composition for all samples of **1** fit the isotopic range of the corresponding precursor substances if no excessive isotopic fractionation occurs while synthesizing. The unusually low levels of δ15N for both **2** and three of the four cumylamines validate our assumed synthesis procedure involving this precursor, especially considering the low values of -40.77 ‰ for δ13C and -19.05 for δ15N of the cumylamine from Acella.

*3.4 Impurity profiling and isotope ratios and of “Spice-Products” of MDMB-CHMICA*

Via IRMS analysis of police seizures and online test-purchases of SPs, in our case with focus on **1**, links between samples with synthetic cannabinoids of a common source can be generated. These links do not necessary describe co-operations between specific internet-shops or single SP brands, but provide a bigger-picture of the relationships between all available SP samples of **1**.

All samples of **1** extracted from SP ranged from -27.83 to -26.37 ‰ (Δ 1.46 ‰) for δ13C and 3.29 to 5.39 ‰ (Δ 2.10 ‰) for δ15N (Table A.3, Figure 6). Three agglomerations of samples can be observed. The agglomerate nomenclature in the following discussion is dependent on which of the following Figures (Figure 6 or Figure 7) is referred to, as the combined information of both Figures might be discussed (A-C: The agglomerates as such, A1-C1: Figure 6 showing the IRMS data including the time correlation, A2-C2: Figure 7 showing the same IRMS data, this time including sub-clustering according to the impurity profiling). For all nomenclatures, the same set of samples are described.

The branding and origin of samples in each of these agglomerates are highly diverse, e.g. some of the SP were police seizures in mail items and search of persons and other were test-purchases in online shops. Thus, the complete distribution channel between online-shop and customer is displayed by our sample collective. Interpretation of isotopic data was aided by implementing the dates of online test-purchase or date of seizure. All SPs were labelled in intervals of five months, starting from September 2014 until December 2015. It should be kept in mind that this timely classification is dependent on the acquisition date of samples and is not a description of the actual date of synthesis. The online-market is regulated by demand and some products sell faster than others do, thus SP with “old” material of **1** might be sold month after the initial production of the SP or the corresponding synthesis. Agglomerate B1 in Figure 6 shows an isotopic composition within the range of the already identified clusters of the large seizure by Luxembourg customs with approximately 3.6 ‰ for δ15N. Aliquots of pure material of **1** with this distinct isotopic composition, most probably from the same respective manufacturer, might be shipped in larger quantities to Europe either before or after the seizure in Luxembourg was made. The majority of SPs in this isotopic range were bought or seized in the late 2014 and early 2015 (the Luxembourg seizure was December 2014). Agglomerate C1 primarily consists of samples seized or bought between March and July 2015, whereas agglomerate A1 mainly comprises samples from the period from August to December 2015. As mentioned before, this timely classification is not exact, however, a tendency of agglomeration of samples from similar periods can be observed. Possibly, each agglomerate represents one or multiple synthesis batches similar to the seizure in Luxembourg, which were delivered by a single manufacturer to the European market in intervals in the course of the years 2014 and 2015. This conclusion has several implications on larger scale: Keeping in mind that each agglomerate consists of samples from different internet-shops and seizures, including a large variability of product brands, single larger shipments would need to be distributed amongst the European SP producers, who then use this specific delivery/batch of **1** for their SP in a given period. The pre-packaging in 1 kg bags of the 40 kg from Luxembourg is an indication for the intention of reselling or distributing single bags of pure material to multiple SP producers. That again implies that the majority of SP producers have some kind of co-operation or internal distribution network, at least for ordering and distributing new pure material from the original manufacturer. Hypothetically, placing the delivery order of material in each agglomerate into a rough timeline according to the date of purchase or seizure for their respective samples, B1 would be the first delivery that reached Europe, C1 the second, and A1 the most recent. However, with this classification and no further data, it is difficult to explain the presence of samples from the period September 2014 to February 2015 in agglomerate A1.

Therefore, as for the seizure of 40 kg pure **1**, an impurity profiling was carried out for all samples shown here in order to determine the fine structure of the dataset, with special interest in agglomerate A. Considering the hypothesis that each agglomerate represents a large shipment like the Luxembourg seizure, this shipment should include multiple synthesis batches. Our target was the identification of the individual batches in the agglomerates of isotopically indistinguishable samples. No reference material of multiple reaction batches under controlled conditions from the original manufacturer were available which could provide a scientific basis to classify samples of unknown source into individual batches. Thus, we used the relative distance set for the batch discrimination of the Luxembourg seizure as “calibration” for the here presented pool of SPs. The chromatographic impurity signatures of the previously assessed 15 key-impurities for both the Luxembourg seizure (excluding the three outliers MDMB-14, MDMB-21 and MDMB-37, as they would disrupt the chemometric model) and the individual SPs were analysed via PCA and hierarchical cluster analysis. In this model, the relative distance at which the thirty-seven samples from the Luxembourg seizure were classified into their individual synthesis batches was also set as relative distance for the classification of SPs into individual batches. This way, fourteen sub-clusters were identified for the 118 SPs, which again were implemented into the IRMS data to reveal the fine structure of the three individual agglomerates, as shown in the three zooms of agglomerate A, B and C in Figure 7. The impurity-profiling sub-cluster are numbered consecutively from 1 to 14 and coloured differently for each zoom, respectively. The samples located in one of the agglomerates in the IRMS data (A, B or C) always clustered in the impurity profiling with other samples from the same agglomerates and never with a sample from one of the other two agglomerates. That again validates the IRMS measurement accuracy to some extent, although a few deviations between impurity and isotopic data can be found, for example the two intermediate samples between agglomerate A and B that are rated to B2 according to the impurity data or the single sample with 5.4 ‰ for δ15N in agglomerate A2 that is rated to samples with δ15N values of approximately 4.6 ‰. Logically speaking, the impurity profiling cannot suggest a batch relationship of two samples that are clearly distinguishable via IRMS as both samples should come from different reaction batches. As already described for the Luxembourg seizure before, a mixing of batches is always a possibility to consider, in addition to the measurement uncertainty, which leads to a blurring of both the isotope values and impurity signatures.

Agglomerate B2 as such is subdivided into four sub-clusters of four, five, nine and sixteen SP samples. Agglomerate C2 is divided into two sub-clusters of seven and twenty-two SP samples. The sub-clustering of samples in both B2 and C2 was due to minor differences in their overall impurity signatures, indicating individual synthesis batches with the same precursor material (evidenced by IRMS analysis) and the same synthesis procedure (evidenced by impurity profiling), similar to the thirty-seven samples from the Luxembourg seizure.

In agglomerate A1, eight sub-clusters were identified ranging between four and eighteen samples. However, compared to the agglomerates B2 and C2, a major difference in impurity composition was observed. Figure 8 shows a PCA (Scores and Loadings plot) of the chromatographic impurity signatures for all samples located in agglomerate A, clearly dividing the sample set in (at least) two sections with high and low scores on PC1, respectively. The corresponding Loadings plot shows, that majorly the two impurities **I11** (methyl 2-(1-(cyclohexylmethyl)-1H-indole-3-carboxamido)butanoate) and **I4** (tentatively identified in a previous work[27] as methyl 2-(1H-indole-3-carboxamido)-3,3-dimethylbutanoate with C2H5 attached to the indole) were responsible for this division. On the right part of Figure 8, the average relative intensity of **I11** and **I4** for the respective samples of both sub-cluster groups are shown, to visualize the corresponding difference in abundance.

We concluded, that the sub-cluster 1 to 4 and 5 to 8 belong to two separate synthesis series, although the IRMS data suggests a relationship between both sub-cluster groups. This conclusion is further validated by including the dates of purchase for the samples in each sub-cluster. All samples in agglomerate A1 from the early period of September 2014 to February 2015 (marked black in Figure 6) are included by sub-cluster 4. Furthermore, sub-cluster 1, 2 and 3 primarily consist of SP bought or seized in the period between March and July 2015 (marked red in Figure 6), and sub-cluster 5 to 8 consist of the majority of SPs from the period between August and December 2015. This implies that the previous placement of the agglomerates, presumably representing individual larger shipments of **1**, on the timeline was not 100% accurate. With the additional information from the impurity profiling, agglomerate A is expected to consist of two larger shipments of multiple successive synthesis batches, one reaching the European market in 2014, explaining the seized and bought SP from this period falling into agglomerate A, and another shipment in 2015, which was used to produce the majority of SP from the end of 2015.

**4. Conclusion**

IRMS analysis of a larger sample collective of the two synthetic cannabinoids MDMB-CHMICA and Cumyl-PeGaClone consisting of both Spice-product extracts and pure material suggested that for each of these cannabinoids only one manufacturer is responsible. This hypothesis is validated by the diverse isotope ratios of precursor substances, making it virtually impossible for two different manufacturers to synthesize material within the specific isotopic range as it was found for these two cannabinoids.

From the larger strategic perspective, the time dependant resolution of the data provided the most valuable implication for the supply and distribution of the synthetic cannabinoid MDMB-CHMICA. Larger batches of pure material seem to be delivered consecutively to the European market in intervals of multiple months. These deliveries are then distributed amongst the European Spice-Products producers, indicated by the variety of different sources for the samples (multiple internet-shops, street seizures) and individual products brands that are assigned into the respective agglomerates. On the basis of the synthesis batch discrimination carried out for the seizure of 40 kg pure MDMB-CHMICA by Luxembourg customs, the MDMB-CHMICA in Spice-product samples was assigned to corresponding synthesis batches. After implementation of this fine structure, two larger groups of sub-clusters were identified in one of the agglomerates observed in IRMS, which otherwise would be indistinguishable.

Combining IRMS and impurity profiling as analytical tools, generating orthogonal information, provides unique insights into the structure of a sample pool of unknown origin. Both techniques target a different part of the sample history, IRMS the origin of the precursor material and impurity profiling the corresponding synthesis pathway in which these precursors were used. By implementing the batch discrimination of the impurity profiling into the isotope ratio agglomerates or clusters, the fine-structure of the observed sample pool is visualized.

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Figure 1: Structural formulas of MDMB-CHMICA and Cumyl-PeGaClone



Figure 2: Potential precursor substances for MDMB-CHMICA (left) and Cumyl-PeGaClone (right)



Figure 3: Sketched synthesis of Cumyl-PeGaClone from a 2-methyl-indole derivative of Cumyl-PICA following the synthesis instructions from Clark et al. [30]



Figure 4: Measured δ13C and δ15N values for the 40 kg seizure (40 individual samples) of MDMB-CHMICA conducted by Luxembourg customs in December 2014. Each samples was coloured according to the batch discrimination by the impurity profiling conducted in a previous study [22].



Figure 5: δ13C and δ15N values for all samples of MDMB-CHMICA (green), Cumyl-PeGaClone (red) and 5F-PB-22 [21] (orange). All samples of synthetic cannabinoids of the same type clustered individually.



Figure 6: (Top) δ13C and δ15N values for all “Spice-Products” of MDMB-CHMICA, either measured via EA- or GC-IRMS. Colouring of samples is corresponding to the date of purchase or seizure of the “Spice-Products” in intervals of 5 month (● September 2014 to February 2014; ● March 2015 to July 2015; ● August 2015 to December 2015). (Bottom) Summarizing overview of the origin for samples located in each corresponding agglomerates A, B and C.



Figure 7: Zoom of the sample agglomerations A2, B2, and C2 in the IRMS data with implemented sub-clusters according to the organic impurity-profiling. The fourteen assessed sub-clusters were numbered from 1 to 14 and coloured differently in each zoom for better visualisation. Agglomerate A2 consist of eight sub-clusters, possibly representing individual synthesis batches, whereas the impurity signatures of sub-cluster 1-4 were majorly different from sub-cluster 5-8 (refer to Figure 8 for more information). In agglomerate B2, four sub-cluster were assigned, each possibly representing an individual synthesis batch. In agglomerate C2, two potential synthesis batches were found.



Figure 8: On the left a PCA (Scores and Loadings plot) of the chromatographic impurity signatures for the samples located in the IRMS agglomerate A (referring to Figure 6 and 7). The sample collective is subdivided into eight individual sub-cluster, each possibly representing an individual synthesis batch. However, between sub-cluster 1 to 4 and 5 to 8 a major difference in abundance of the two impurities I11 and I4 (as numbered and characterized in a previous study[27]) was found. On the right, the relative intensity of I11 and I4 for the respective samples of both sub-cluster groups are shown). Possibly, the samples assigned to sub-cluster 1 to 4 were synthesized by a different instrumentation, chemist or synthesis pathway than sub-cluster 5 to 8, although the IRMS data (referring again to Figure 6 and 7) suggests a relationship between both sub-cluster groups.

Table 1 GC temperature program for synthetic cannabinoids (in Acetonitrile), M-MA (in MTBE) and Dodecane (in hexane)

|  |  |  |  |
| --- | --- | --- | --- |
|  | Synthetic cannabinoid | M-MA | Dodecane |
|  | Rate (°C min-1) | T (°C) | Hold (min) | Rate (°C min-1) | T (°C) | Hold (min) | Rate (°C min-1) | T (°C) | Hold (min) |
| Initial |  | 70 | 2 |  | 40 | 2 |  | 80 | 2 |
| 1 | 15 | 270 | 0 | 15 | 280 | 0 | 15 | 250 | 1 |
| 2 | 5 | 320 | 8 | 50 | 40 | 4 | 70 | 40 | 3 |

Table 2: δ13C and δ15N values including standard deviation of triplicate measurements on EA-IRMS for potential precursor substances of MDMB-CHMICA and Cumyl-PeGaClone, purchased from different global vendors.

|  |  |  |  |
| --- | --- | --- | --- |
|  | Vendor | δ13CV-PDB[‰]average ± STD | δ15NAIR[‰]average ± STD |
| Indole | Sigma Aldrich | -21.04 ± 0.05 | 6.83 ± 0.04 |
| TCI | -26.80 ± 0.02 | 3.09 ± 0.13 |
| ABCR | -30.85 ± 0.04 | 2.08 ± 0.03 |
| Merck | -21.81 ± 0.02 | 8.78 ± 0.05 |
| Acros Chem. | -21.56 ± 0.03 | 9.15 ± 0.05 |
| AlfaAeser | -30.82 ± 0.04 | 2.00 ± 0.06 |
| BePharm | -22.25 ± 0.02 | 8.36 ± 0.03 |
| MP Biomed. | -28.99 ± 0.02 | 9.66 ± 0.05 |
| (D)-tert-Leucine | AlfaAeser(same lot) | -29.81 ± 0.02 | 0.37 ± 0.03 |
| -29.84 ± 0.03 | 0.35 ± 0.02 |
| -29.84 ± 0.03 | 0.34 ± 0.05 |
| (L)-tert-Leucine | AlfaAeser | -24.32 ± 0.02 | -2.34 ± 0.02 |
| (L)-TLME | TCI | -32.91 ± 0.02 | 0.74 ± 0.02 |
| BePharm | -24.02 ± 0.10 | -1.57 ± 0.05 |
| Cumyl-amine | ABCR | -26.43 ± 0.02 | -13.11 ± 0.03 |
| Acella | -40.77 ± 0.02 | -19.05 ± 0.07 |
| Enamine | -32.23 ± 0.01 | 8.34 ± 0.02 |
| TCI | -26.74 ± 0.02 | -9.20 ± 0.04 |

Table A.1 δ13C and δ15N values including standard deviation of triplicate measurements for all pure samples of MDMB-CHMICA

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | δ13CV-PDB[‰]average ± STD | δ15NAIR[‰]average ± STD |  |  | δ13CV-PDB[‰]average ± STD | δ15NAIR[‰]average ± STD |
| MDMB-01 | -27.76 ± 0.01 | 3.64 ± 0.08 |  | MDMB-32 | -27.76 ± 0.03 | 3.32 ± 0.08 |
| MDMB-02 | -27.51 ± 0.01 | 3.60 ± 0.10 |  | MDMB-33 | -27.55 ± 0.03 | 3.55 ± 0.07 |
| MDMB-03 | -27.75 ± 0.05 | 3.56 ± 0.14 |  | MDMB-34 | -27.82 ± 0.02 | 3.57 ± 0.04 |
| MDMB-04 | -27.61 ± 0.02 | 3.71 ± 0.03 |  | MDMB-35 | -27.69 ± 0.05 | 3.58 ± 0.13 |
| MDMB-05 | -27.77 ± 0.02 | 3.71 ± 0.08 |  | MDMB-36 | -27.67 ± 0.01 | 3.51 ± 0.00 |
| MDMB-06 | -27.77 ± 0.03 | 3.71 ± 0.08 |  | MDMB-37 | -26.90 ± 0.02 | 5.02 ± 0.09 |
| MDMB-07 | -27.64 ± 0.01 | 3.45 ± 0.05 |  | MDMB-38 | -27.56 ± 0.03 | 3.48 ± 0.10 |
| MDMB-08 | -27.68 ± 0.02 | 3.47 ± 0.09 |  | MDMB-39 | -27.74 ± 0.03 | 3.25 ± 0.04 |
| MDMB-09 | -27.53 ± 0.03 | 3.64 ± 0.09 |  | MDMB-40 | -27.66 ± 0.03 | 3.44 ± 0.09 |
| MDMB-10 | -27.71 ± 0.03 | 3.41 ± 0.04 |  | MDMB-41 | -26.65 ± 0.12 | 2.39 ± 0.30 |
| MDMB-11 | -27.64 ± 0.04 | 3.64 ± 0.10 |  | MDMB-42 | -27.69 ± 0.03 | 5.02 ± 0.06 |
| MDMB-12 | -27.59 ± 0.01 | 3.60 ± 0.03 |  | MDMB-43 | -27.45 ± 0.01 | 5.23 ± 0.08 |
| MDMB-13 | -27.55 ± 0.01 | 3.59 ± 0.02 |  | MDMB-44 | -27.56 ± 0.04 | 4.72 ± 0.07 |
| MDMB-14 | -27.43 ± 0.21 | 4.08 ± 0.27 |  | MDMB-45 | -27.56 ± 0.04 | 4.72 ± 0.04 |
| MDMB-15 | -27.65 ± 0.01 | 3.62 ± 0.10 |  | MDMB-46 | -27.53 ± 0.03 | 4.82 ± 0.07 |
| MDMB-16 | -27.71 ± 0.03 | 3.42 ± 0.07 |  | MDMB-47 | -27.55 ± 0.02 | 4.72 ± 0.16 |
| MDMB-17 | -27.73 ± 0.01 | 3.57 ± 0.04 |  | MDMB-48 | -27.57 ± 0.04 | 4.82 ± 0.21 |
| MDMB-18 | -27.52 ± 0.19 | 3.79 ± 0.29 |  | MDMB-49 | -27.66 ± 0.01 | 3.57 ± 0.10 |
| MDMB-19 | -27.67 ± 0.01 | 3.74 ± 0.15 |  | MDMB-50 | -27.61 ± 0.02 | 3.59 ± 0.02 |
| MDMB-20 | -27.65 ± 0.07 | 3.65 ± 0.05 |  | MDMB-51 | -26.95 ± 0.04 | 6.22 ± 0.04 |
| MDMB-21 | -27.18 ± 0.07 | 4.61 ± 0.22 |  | MDMB-52 | -26.98 ± 0.01 | 6.25 ± 0.05 |
| MDMB-22 | -27.64 ± 0.00 | 3.66 ± 0.05 |  | MDMB-53 | -26.95 ± 0.02 | 6.20 ± 0.06 |
| MDMB-23 | -27.83 ± 0.04 | 3.51 ± 0.08 |  | MDMB-54 | -27.48 ± 0.01 | 4.90 ± 0.05 |
| MDMB-24 | -27.70 ± 0.03 | 3.51 ± 0.03 |  | MDMB-55 | -27.47 ± 0.01 | 5.01 ± 0.09 |
| MDMB-25 | -27.71 ± 0.04 | 3.61 ± 0.05 |  | MDMB-56 | -27.65 ± 0.02 | 5.36 ± 0.00 |
| MDMB-26 | -27.87 ± 0.04 | 3.61 ± 0.02 |  | MDMB-57 | -25.94 ± 0.01 | 6.35 ± 0.01 |
| MDMB-27 | -27.59 ± 0.00 | 3.52 ± 0.06 |  | MDMB-58 | -27.71 ± 0.01 | 3.63 ± 0.18 |
| MDMB-28 | -27.86 ± 0.01 | 3.58 ± 0.07 |  | MDMB-59 | -27.67 ± 0.04 | 3.66 ± 0.04 |
| MDMB-29 | -27.86 ± 0.02 | 3.62 ± 0.06 |  | MDMB-60 | -27.46 ± 0.03 | 4.88 ± 0.08 |
| MDMB-30 | -27.70 ± 0.01 | 3.49 ± 0.02 |  | MDMB-61 | -27.04 ± 0.01 | 4.62 ± 0.04 |
| MDMB-31 | -27.65 ± 0.03 | 3.49 ± 0.05 |  |  |  |  |

Table A.2 δ13C and δ15N values including standard deviation of triplicate measurements for one pure sample and all “Spice-Products” of Cumyl-PeGaClone

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | δ13CV-PDB[‰]average ± STD | δ15NAIR[‰]average ± STD |  |  | δ13CV-PDB[‰]average ± STD | δ15NAIR[‰]average ± STD |
| Peg-01 | -34.65 ± 0.09 | -11.83 ± 0.05 |  | SP\_Peg-16 | -34.73 ± 0.19 | -10.80 ± 0.12 |
| SP\_Peg-01 | -34.21 ± 0.01 | -12.69 ± 0.07 |  | SP\_Peg-17 | -34.74 ± 0.03 | -10.91 ± 0.01 |
| SP\_Peg-02 | -34.23 ± 0.27 | -12.70 ± 0.22 |  | SP\_Peg-18 | -34.98 ± 0.21 | -10.87 ± 0.11 |
| SP\_Peg-03 | -34.69 ± 0.16 | -10.54 ± 0.12 |  | SP\_Peg-19 | -34.64 ± 0.01 | -10.56 ± 0.02 |
| SP\_Peg-04 | -34.43 ± 0.28 | -10.77 ± 0.10 |  | SP\_Peg-20 | -34.25 ± 0.03 | -12.58 ± 0.04 |
| SP\_Peg-05 | -34.39 ± 0.15 | -10.34 ± 0.02 |  | SP\_Peg-21 | -34.89 ± 0.08 | -10.91 ± 0.06 |
| SP\_Peg-06 | -33.88 ± 0.01 | -10.68 ± 0.01 |  | SP\_Peg-22 | -35.21 ± 0.02 | -10.59 ± 0.02 |
| SP\_Peg-07 | -34.49 ± 0.13 | -10.51 ± 0.13 |  | SP\_Peg-23 | -35.01 ± 0.10 | -10.58 ± 0.03 |
| SP\_Peg-08 | -34.77 ± 0.29 | -10.85 ± 0.08 |  | SP\_Peg-24 | -34.98 ± 0.16 | -10.98 ± 0.02 |
| SP\_Peg-09 | -34.67 ± 0.22 | -10.97 ± 0.08 |  | SP\_Peg-25 | -34.75 ± 0.12 | -10.46 ± 0.01 |
| SP\_Peg-10 | -34.57 ± 0.09 | -10.62 ± 0.08 |  | SP\_Peg-26 | -34.86 ± 0.03 | -12.04 ± 0.11 |
| SP\_Peg-11 | -34.34 ± 0.23 | -10.38 ± 0.02 |  | SP\_Peg-27 | -35.26 ± 0.02 | -10.63 ± 0.02 |
| SP\_Peg-12 | -34.43 ± 0.14 | -10.46 ± 0.02 |  | SP\_Peg-28 | -35.11 ± 0.13 | -10.51 ± 0.16 |
| SP\_Peg-13 | -34.54 ± 0.08 | -10.49 ± 0.06 |  | SP\_Peg-29 | -35.00 ± 0.03 | -11.47 ± 0.07 |
| SP\_Peg-14 | -34.41 ± 0.13 | -10.87 ± 0.03 |  | SP\_Peg-30 | -35.26 ± 0.02 | -10.83 ± 0.05 |
| SP\_Peg-15 | -33.83 ± 0.14 | -11.36 ± 0.11 |  |  |  |  |

Table A.3 δ13C and δ15N values including standard deviation of triplicate measurements for all “Spice-Products” of MDMB-CHMICA

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | δ13CV-PDB[‰]average ± STD | δ15NAIR[‰]average ± STD |  |  | δ13CV-PDB[‰]average ± STD | δ15NAIR[‰]average ± STD |
| SP\_MDMB-01 | -27.67 ± 0.02 | 3.54 ± 0.05 |  | SP\_MDMB-60 | -27.54 ± 0.01 | 4.89 ± 0.02 |
| SP\_MDMB-02 | -27.62 ± 0.04 | 3.51 ± 0.06 |  | SP\_MDMB-61 | -27.54 ± 0.02 | 4.82 ± 0.02 |
| SP\_MDMB-03 | -27.65 ± 0.04 | 3.58 ± 0.09 |  | SP\_MDMB-62 | -27.54 ± 0.06 | 4.79 ± 0.05 |
| SP\_MDMB-04 | -27.65 ± 0.04 | 3.53 ± 0.04 |  | SP\_MDMB-63 | -27.61 ± 0.04 | 4.67 ± 0.07 |
| SP\_MDMB-05 | -27.63 ± 0.03 | 3.51 ± 0.06 |  | SP\_MDMB-64 | -27.63 ± 0.03 | 4.71 ± 0.00 |
| SP\_MDMB-06 | -27.65 ± 0.02 | 3.69 ± 0.07 |  | SP\_MDMB-65 | -27.65 ± 0.02 | 4.85 ± 0.02 |
| SP\_MDMB-07 | -27.70 ± 0.04 | 3.66 ± 0.12 |  | SP\_MDMB-66 | -27.30 ± 0.07 | 4.48 ± 0.29 |
| SP\_MDMB-08 | -27.72 ± 0.02 | 3.40 ± 0.10 |  | SP\_MDMB-67 | -26.71 ± 0.02 | 4.10 ± 0.07 |
| SP\_MDMB-09 | -27.71 ± 0.02 | 3.56 ± 0.04 |  | SP\_MDMB-68 | -27.56 ± 0.04 | 4.86 ± 0.07 |
| SP\_MDMB-10 | -27.59 ± 0.05 | 4.01 ± 0.01 |  | SP\_MDMB-69 | -27.51 ± 0.04 | 4.66 ± 0.08 |
| SP\_MDMB-11 | -27.66 ± 0.01 | 3.63 ± 0.04 |  | SP\_MDMB-70 | -27.58 ± 0.01 | 4.74 ± 0.07 |
| SP\_MDMB-12 | -27.74 ± 0.03 | 3.41 ± 0.10 |  | SP\_MDMB-71 | -27.69 ± 0.03 | 4.66 ± 0.03 |
| SP\_MDMB-13 | -27.61 ± 0.03 | 3.70 ± 0.13 |  | SP\_MDMB-72 | -26.42 ± 0.02 | 4.04 ± 0.14 |
| SP\_MDMB-14 | -27.69 ± 0.01 | 3.43 ± 0.06 |  | SP\_MDMB-73 | -27.47 ± 0.02 | 3.63 ± 0.15 |
| SP\_MDMB-15 | -27.77 ± 0.02 | 4.75 ± 0.08 |  | SP\_MDMB-74 | -26.47 ± 0.08 | 4.30 ± 0.31 |
| SP\_MDMB-16 | -27.81 ± 0.03 | 4.59 ± 0.09 |  | SP\_MDMB-75 | -27.52 ± 0.10 | 4.75 ± 0.30 |
| SP\_MDMB-17 | -27.76 ± 0.05 | 4.73 ± 0.10 |  | SP\_MDMB-76 | -27.39 ± 0.07 | 4.86 ± 0.27 |
| SP\_MDMB-18 | -27.37 ± 0.07 | 3.47 ± 0.19 |  | SP\_MDMB-77 | -27.29 ± 0.04 | 5.01 ± 0.17 |
| SP\_MDMB-19 | -27.49 ± 0.05 | 3.61 ± 0.05 |  | SP\_MDMB-78 | -27.65 ± 0.02 | 4.63 ± 0.06 |
| SP\_MDMB-20 | -27.49 ± 0.13 | 3.51 ± 0.16 |  | SP\_MDMB-79 | -27.23 ± 0.08 | 5.03 ± 0.21 |
| SP\_MDMB-21 | -27.70 ± 0.02 | 3.73 ± 0.11 |  | SP\_MDMB-80 | -27.44 ± 0.14 | 4.72 ± 0.15 |
| SP\_MDMB-22 | -27.66 ± 0.04 | 3.58 ± 0.13 |  | SP\_MDMB-81 | -27.46 ± 0.08 | 4.93 ± 0.10 |
| SP\_MDMB-23 | -27.70 ± 0.04 | 3.41 ± 0.12 |  | SP\_MDMB-82 | -26.47 ± 0.11 | 4.08 ± 0.39 |
| SP\_MDMB-24 | -27.70 ± 0.02 | 4.40 ± 0.09 |  | SP\_MDMB-83 | -26.50 ± 0.10 | 4.04 ± 0.24 |
| SP\_MDMB-25 | -27.73 ± 0.02 | 3.39 ± 0.10 |  | SP\_MDMB-84 | -26.37 ± 0.09 | 3.92 ± 0.25 |
| SP\_MDMB-26 | -27.83 ± 0.06 | 4.45 ± 0.07 |  | SP\_MDMB-85 | -27.64 ± 0.06 | 4.35 ± 0.09 |
| SP\_MDMB-27 | -26.72 ± 0.01 | 4.02 ± 0.01 |  | SP\_MDMB-86 | -27.67 ± 0.10 | 3.66 ± 0.13 |
| SP\_MDMB-28 | -27.63 ± 0.04 | 4.69 ± 0.01 |  | SP\_MDMB-87 | -27.56 ± 0.08 | 4.50 ± 0.14 |
| SP\_MDMB-29 | -26.64 ± 0.06 | 3.99 ± 0.03 |  | SP\_MDMB-88 | -26.78 ± 0.05 | 4.19 ± 0.14 |
| SP\_MDMB-30 | -26.79 ± 0.07 | 4.06 ± 0.06 |  | SP\_MDMB-89 | -27.60 ± 0.02 | 3.76 ± 0.05 |
| SP\_MDMB-31 | -26.78 ± 0.04 | 4.08 ± 0.05 |  | SP\_MDMB-90 | -27.54 ± 0.06 | 4.11 ± 0.40 |
| SP\_MDMB-32 | -26.74 ± 0.03 | 4.09 ± 0.13 |  | SP\_MDMB-91 | -27.50 ± 0.08 | 3.83 ± 0.27 |
| SP\_MDMB-33 | -26.76 ± 0.07 | 4.15 ± 0.12 |  | SP\_MDMB-92 | -26.52 ± 0.11 | 4.34 ± 0.19 |
| SP\_MDMB-34 | -26.76 ± 0.05 | 3.99 ± 0.01 |  | SP\_MDMB-93 | -27.51 ± 0.17 | 3.84 ± 0.11 |
| SP\_MDMB-35 | -26.76 ± 0.02 | 4.05 ± 0.15 |  | SP\_MDMB-94 | -27.53 ± 0.10 | 4.95 ± 0.10 |
| SP\_MDMB-36 | -27.67 ± 0.02 | 4.76 ± 0.09 |  | SP\_MDMB-95 | -27.55 ± 0.07 | 3.58 ± 0.22 |
| SP\_MDMB-37 | -26.80 ± 0.02 | 3.89 ± 0.06 |  | SP\_MDMB-96 | -27.61 ± 0.07 | 4.91 ± 0.20 |
| SP\_MDMB-38 | -26.64 ± 0.03 | 4.19 ± 0.09 |  | SP\_MDMB-97 | -27.46 ± 0.08 | 5.08 ± 0.18 |
| SP\_MDMB-39 | -26.72 ± 0.02 | 4.05 ± 0.02 |  | SP\_MDMB-98 | -27.57 ± 0.10 | 4.81 ± 0.14 |
| SP\_MDMB-40 | -27.15 ± 0.04 | 4.55 ± 0.07 |  | SP\_MDMB-99 | -26.44 ± 0.21 | 4.05 ± 0.24 |
| SP\_MDMB-41 | -27.54 ± 0.01 | 4.82 ± 0.02 |  | SP\_MDMB-100 | -27.40 ± 0.16 | 3.82 ± 0.32 |
| SP\_MDMB-42 | -27.27 ± 0.01 | 4.67 ± 0.06 |  | SP\_MDMB-101 | -26.43 ± 0.22 | 4.42 ± 0.27 |
| SP\_MDMB-43 | -26.85 ± 0.04 | 4.14 ± 0.07 |  | SP\_MDMB-102 | -26.64 ± 0.22 | 4.33 ± 0.04 |
| SP\_MDMB-44 | -27.44 ± 0.05 | 4.76 ± 0.05 |  | SP\_MDMB-103 | -27.42 ± 0.05 | 3.80 ± 0.35 |
| SP\_MDMB-45 | -27.41 ± 0.01 | 4.79 ± 0.07 |  | SP\_MDMB-104 | -26.59 ± 0.11 | 3.89 ± 0.21 |
| SP\_MDMB-46 | -27.50 ± 0.31 | 3.40 ± 0.11 |  | SP\_MDMB-105 | -27.38 ± 0.11 | 4.84 ± 0.14 |
| SP\_MDMB-47 | -27.21 ± 0.02 | 4.57 ± 0.03 |  | SP\_MDMB-106 | -27.18 ± 0.09 | 4.62 ± 0.20 |
| SP\_MDMB-48 | -27.16 ± 0.02 | 4.57 ± 0.07 |  | SP\_MDMB-107 | -26.65 ± 0.15 | 4.09 ± 0.10 |
| SP\_MDMB-49 | -27.56 ± 0.03 | 4.79 ± 0.12 |  | SP\_MDMB-108 | -26.61 ± 0.06 | 4.15 ± 0.15 |
| SP\_MDMB-50 | -27.56 ± 0.03 | 4.66 ± 0.05 |  | SP\_MDMB-109 | -26.75 ± 0.04 | 4.37 ± 0.23 |
| SP\_MDMB-51 | -27.60 ± 0.03 | 4.79 ± 0.03 |  | SP\_MDMB-110 | -26.71 ± 0.18 | 3.86 ± 0.04 |
| SP\_MDMB-52 | -27.58 ± 0.01 | 4.60 ± 0.10 |  | SP\_MDMB-111 | -27.56 ± 0.08 | 3.73 ± 0.23 |
| SP\_MDMB-53 | -27.41 ± 0.01 | 4.85 ± 0.04 |  | SP\_MDMB-112 | -27.67 ± 0.06 | 3.59 ± 0.05 |
| SP\_MDMB-54 | -27.53 ± 0.01 | 4.85 ± 0.02 |  | SP\_MDMB-113 | -27.57 ± 0.09 | 4.77 ± 0.13 |
| SP\_MDMB-55 | -27.55 ± 0.03 | 4.74 ± 0.03 |  | SP\_MDMB-114 | -26.54 ± 0.08 | 3.96 ± 0.16 |
| SP\_MDMB-56 | -27.36 ± 0.07 | 5.39 ± 0.06 |  | SP\_MDMB-115 | -27.52 ± 0.09 | 4.53 ± 0.39 |
| SP\_MDMB-57 | -27.52 ± 0.03 | 4.85 ± 0.03 |  | SP\_MDMB-116 | -27.52 ± 0.07 | 3.69 ± 0.14 |
| SP\_MDMB-58 | -27.47 ± 0.02 | 4.77 ± 0.11 |  | SP\_MDMB-117 | -27.21 ± 0.07 | 4.98 ± 0.09 |
| SP\_MDMB-59 | -27.50 ± 0.01 | 4.81 ± 0.10 |  | SP\_MDMB-118 | -27.14 ± 0.10 | 4.82 ± 0.19 |