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Synthetic cannabinoids in e-liquids: a proton and fluorine NMR analysis from a conventional spectrometer to a compact one

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Abstract

The ^1H NMR profiles of 13 samples of e-liquids supplied by French customs were obtained with high-field and low-field NMR. The high-field ^1H NMR spectra allowed the detection of matrix signals, synthetic cannabinoids, and flavouring compounds. Quantitative results were obtained for the five synthetic cannabinoids detected: JWH-210, 5F-MDMB-PICA, 5F-ADB, 5F-AKB48, and ADB-FUBINACA. Conventional GC-MS analysis was used to confirm compound identification. Fluorine-19 NMR was proposed for the quantification of fluorinated synthetic cannabinoids and was successfully implemented on both 400 MHz and 60 MHz NMR spectrometers. This study based on few examples explored the potentiality of low-field NMR for quantitative and quantitative analysis of synthetic cannabinoids in e-liquids.

Keywords: e-liquid, e-cigarette, qNMR, compact NMR, ^{19}F NMR, cannabinoid

Introduction

Electronic cigarettes or e-cigarettes have spread rapidly in recent years as an alternative method for nicotine delivery. Compared to traditional tobacco, they are inexpensive, easy to use, and described as safer for public health [1, 2] although they have been associated with a variety of health effects, particularly the exposure to some constituents of aerosols that might result in respiratory complications [3, 4]. Electronic liquids or e-liquids are formulations used in e-cigarettes which can vaporize aerosol for inhalation when the e-cigarette is activated. E-liquids usually contain a mixture of propylene glycol (PG) and glycerol as the main matrix but also flavours and in some case a pharmaceutical and/or herbal agent inside [5, 6].

Synthetic cannabinoids (SCs) are the most rapidly growing class of recreational designer drugs used in e-liquids [6-8]. Their chemical structures differ from naturally occurring cannabinoids, but they have similar pharmacological activities by binding with CB1 and/or CB2 receptors [9]. SCs could enhance the excitatory effect by promoting the ability to pass the blood-brain barrier because of chemical structure modifications reducing the molecular polarity [10]. SCs were originally used as therapeutic drugs for their anti-convulsant, anti-nociceptive, and anti-psychotic properties, but long time inhalation or high dose intake can induce addiction, cardiovascular disease, mental confusion, high blood pressure, seizures, acute kidney injury, cancer, and even death [11]. SCs usually exhibit more potency than natural cannabis as they are designed to bind more strongly to CB1/CB2

receptors in the neuronal system [12], and they also may interact with other receptors in the brain that natural cannabis does not [13]. Due to the fast generation of SCs, their structures are quite diverse and a large number of new psychoactive SCs have appeared on sale, which makes it even more difficult to detect and distinguish them [14]. Over the past decade, various studies have described the detection of SCs, most of them focusing on the identification and structural characterization of new cannabinoids [15-21]. In addition to these researches, the use of different analytical methods for the detection or/and quantification of SCs in commercial herbal mixture blends has become a vibrant area of research [22-29]. However, only a few studies on screening for SCs or natural cannabinoids in e-liquids have been reported. Peace and co-workers have identified SCs and other components in commercially available e-liquid formulations using direct analysis in real time mass spectrometry (DART-MS) and gas chromatography-mass spectrometry (GC-MS), as well as naturally occurring cannabinoids quantified by high-performance liquid chromatography-triple quadrupole mass spectrometry (HPLC-MS/MS) [5, 7, 30]. Poklis et al. evaluated 5F-ADB in 9 cannabidiol e-liquids from the same manufacturer by DART-MS and GC-MS in 2019 [8].

Nuclear Magnetic Resonance (NMR) spectroscopy has already been described as a powerful method for the analysis of complex mixtures and recent review articles summarized the promising capabilities of NMR in the fields of forensic and fraud analysis for both qualitative and quantitative purposes [31-33]. Concerning SCs, NMR was used for their structural identification and for the analysis of herbal blends [18, 22, 25-27, 34]. Although high-field (HF) NMR is not yet considered as a routine tool in analytical chemistry mainly because of its high cost, benchtop cryogen-free low-field (LF) NMR which uses much cheaper instruments can partially solve this cost issue and be implemented in forensic or customs laboratories. Indeed, compact NMR spectroscopy has been applied recently for the quality control of various matrices, including meat [35], edible oils [36], drugs [37], dietary supplements [38, 39] and herbal blends containing cannabinoids [24].

The aim of the present study was to assess the capacity of HF and LF NMR for the characterization of e-liquid contents. Proton (^1H) and fluorine (^{19}F) NMR analyses were carried out with both HF (400 MHz for ^1H) and LF (60 MHz for ^1H) spectrometers and GC-MS was used as a confirmation and reference method. To our knowledge, this study is the first to use compact LF NMR for the characterization of SCs and flavouring agents in e-liquids.

2. Experimental

2.1. Materials

13 e-liquid samples from customs seizures were provided by the SCL laboratory (French Customs). The standard SCs JWH-210, 5F-AKB48, and 5F-MDMB-PICA were obtained from Cayman Chemical (Ann Arbor, MI, USA). 3-(trimethylsilyl)propionic-2,2,3,3-d₄ acid sodium salt (TSP, purity $\geq 99\%$, isotopic purity 98 atom % D), α,α,α -trifluorotoluene (TFT, purity $\geq 99\%$), vanillin, ethyl maltol, limonene, anisyl alcohol, raspberry ketone (4-(4-hydroxyphenyl)butan-2-one), menthol, linalool and chromium(III) acetylacetonate ($\text{Cr}(\text{acac})_3$) were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.2. NMR analysis

2.2.1. Sample preparation for NMR

200 μl of each e-liquid withdrawn with a glass precision syringe were diluted with 400 μl of methanol-d₄ (MeOD) and mixed with 30 μl of a TSP solution (10 mM in MeOD) as reference for ^1H NMR experiments. ^{19}F NMR experiments were run for the 10 e-liquid samples **4-13** containing the fluorinated SCs 5F-MDMB-PICA, 5F-ADB, 5F-AKB48, and ADB-FUBINACA. For these ^{19}F NMR experiments, 200 μl of sample were diluted with 400 μl of MeOD and then mixed with 9 μl of a TFT solution (1M in MeOD; final concentration 15 mM). Two mg of $\text{Cr}(\text{acac})_3$ as a relaxation agent were added into the NMR tube. The HF and LF ^{19}F NMR experiments were recorded on the same NMR tube. The exact concentrations of the TSP and TFT solutions used were determined relative to that of a solution of yohimbine hydrochloride (European Pharmacopoeia Reference Standard). All quantitative experiments were done in triplicate from sampling the e-liquid to processing the NMR spectrum.

2.2.2. HF NMR analysis

HF ^1H NMR experiments were performed on a Bruker Avance 400 spectrometer (Bruker Biospin AG, Fallanden, Switzerland) equipped with a 5 mm triple resonance probe (TXO) with ^{19}F direct detection. Typical acquisition parameters optimized for quantitative experiments were as follows: number of scans (NS) 128, pulse width 4.52 μs (flip angle 30°), acquisition time 3.40 s, spectral width 12 ppm, 32K data points, and an additional relaxation delay of 10 s; the recording time was thus ca. 29 min. The signal of TSP set at 0 ppm was used as an internal reference for chemical shift measurement and quantitative analysis. Three samples with lower concentrations of SCs (**1-3**) were recorded by increasing the NS to 1024

for obtaining a correct signal-to-noise (S/N) ratio. HF ^1H NMR data were processed using the Bruker TopSpin 4.0.8 software with one level of zero-filling and Fourier transformation after multiplying FIDs by an exponential line-broadening function (LB) of 0.3 Hz.

HF ^{19}F NMR experiments were recorded with the same spectrometer and probe at 376 MHz. First, a basic zg sequence allowed to acquire ^1H -coupled ^{19}F NMR spectra, then quantitative experiments were carried out using an inverse gated decoupling pulse sequence with the following parameters: NS 32, pulse width 4.52 μs (flip angle 30°), acquisition time 1.57 s, spectral width 221 ppm, 256K data points, and an additional relaxation delay of 3 s for complete longitudinal relaxation; the recording time was thus less than 3 min. The signal of TFT set at -63.72 ppm with respect to CFCl_3 (0 ppm) was used as an internal reference for chemical shift measurement and quantitative analysis.

2.2.3. LF NMR analysis

Spectra were acquired on a PulsarTM benchtop NMR spectrometer (Oxford Instruments, Abingdon, UK) operating at a frequency of 59.7 MHz for ^1H and 56.17 MHz for ^{19}F . The temperature inside the spectrometer was 310 K. Acquisition was performed with the SpinFlow 1.2.0.1 software (Oxford Instruments) and data processing with the MNova 11.0 software (Mestrelab Research, Santiago de Compostela, Spain).

LF ^1H NMR spectra were recorded with a flip angle of 90° (12.5 μs), a spectral width of 5000 Hz, and 8K data points (acquisition time of 1.64 s). The relaxation delay was set at 2 s, and 1024 transients were recorded leading to a total acquisition time of ≈ 1 h. For data processing, the FIDs with one level of zero-filling were apodized with an exponential (0.3 Hz) filter and an automatic Whittaker smoother baseline correction was applied.

LF ^{19}F NMR spectra were recorded with a flip angle of 90° , a spectral width of 20833 Hz, and 32K data points (acquisition time of 1.57 s). The relaxation delay was set at 1 s, and 2048 transients were recorded leading to a total acquisition time of 1 h 27 min. For data processing, the FIDs were apodized with an exponential (3.0 Hz) filter and an automatic baseline correction was applied.

2.2.4. T_1 measurements

T_1 s of protons targeted for HF quantification (written in bold in Table 1) and of ^{19}F nuclei (HF and LF) were measured by the inversion-recovery pulse sequence with 20 inversion delays from 0.001 to 25 s. For these T_1 measurements, the acquisition parameters were as follows: 64K data points, acquisition time 6.81 s, spectral width 12 ppm, number of

scans 32, and relaxation delay 40 s for HF ¹H NMR; 128K data points, acquisition time 1.75 s, spectral width 100 ppm, number of scans 16, and relaxation delay 5 s for HF ¹⁹F NMR; 16K data points, acquisition time 2.05 s, spectral width 133 ppm (8000 Hz), number of scans 96, and relaxation delay 5 s for LF ¹⁹F NMR.

2.2.5. NMR quantification

Concentrations were measured by comparing the areas of convenient signals (written in bold in Table 1) of targeted compounds with that of the reference signal, the area of each NMR peak being directly proportional to the number of nuclei giving rise to it.

After phasing and baseline correction of the spectra, the targeted signals were integrated. The amount of each compound expressed in mg/ml of e-liquid sample was determined using the following general equation:

$$Amount (mg / ml) = \frac{A_{SCs}}{N_{SCs}} \times [REF] \times \frac{N_{REF}}{A_{REF}} \times V_{total} \times \frac{Mw_{SCs}}{V_{e-liquid}}$$

[REF]: concentration of the reference (TSP or TFT),

A_{SCs} and A_{REF} : signal areas of SCs and reference signals, respectively,

N_{SCs} and N_{REF} : number of nuclei giving rise to the signal considered,

V_{total} : total volume of solution in the NMR tube,

Mw_{SCs} : molecular weight of SCs,

$V_{e-liquid}$: volume of e-liquid used for the NMR assay.

Linear regression and the Wilcoxon signed-rank test were used to compare the amounts of fluorinated SCs measured by HF ¹⁹F NMR versus HF ¹H NMR and versus LF ¹⁹F NMR. The significance of the slope and the fit of the regression line were tested by t-test and F-test respectively.

2.3. GC-MS analysis

Each sample was vortexed for 30 s before aliquoting. 250 µl of each e-liquid sample were then mixed with 500 µl of CHCl₃. The analyses were performed using an Agilent 6890 N Gas Chromatograph coupled with a GCT Premier CAB109 TOF mass spectrometer (Waters Corporation, Milford, MA, USA). An Agilent HP-5MS capillary column (30 m × 0.25 mm, 0.25 µm film thickness) was used to perform the chromatographic separation. Helium as the carrier gas was delivered at a constant flow of 1.0 ml/min. The temperature program was as follows: initial temperature 70 °C for 2 min, increase to 200 °C at 5 °C/min

then to 280 °C at 10 °C/min, 24 min holding time at 280 °C for a total run time of 60 min. Injections were performed with a 1:20 split ratio and injection volumes of 1.0 µL. Mass spectra were recorded in the range m/z 30-650. Compounds were identified using the SWGDRUG 3.3 (www.swgdrug.org) and NIST 08 databases.

3. Results and discussion

3.1. HF ^1H NMR qualitative analysis of e-liquids

In the first step of this study, the 13 e-liquid samples were screened by HF ^1H NMR in order to characterize their contents in SCs and other chemicals. GC-MS was used as the gold standard method to confirm the identification of SCs as well as flavouring components.

The HF ^1H NMR spectra of all 13 samples allowed the detection of major signals of propylene glycol (PG) and glycerol which constitute the matrix of e-liquids. As an illustration, Figure 1A shows the complete HF ^1H NMR spectrum of one e-liquid sample (**3**). Matrix signals of PG and glycerol have the most prominent signals in the upfield area. For PG, a doublet (d; CH_3) was detected at 1.11 ppm ($J = 6.4$ Hz), a multiplet at 3.77 ppm ($<\text{CH}$), an ABd system centered at 3.41 ppm ($J = 11.1, 6.5, 4.8$ Hz) ($-\text{CH}_2$), and broad singlets for hydroxyl protons at 4.60 and 5.24 ppm. The methine and methylene groups of glycerol led to a multiplet at 3.66 ppm, an ABd system at 3.58 and 3.51 ppm ($J = 11.3, 6.1, 4.8$ Hz) whereas hydroxyl groups resonated as doublet and triplet at 5.29 and 5.19 ppm respectively (depending on the rate of hydroxyl proton exchange, these signals were more or less broad). Moreover, ethylene glycol and/or polyethylene glycol were identified in samples **9-12** from their characteristic intense broad signals around 3.6 ppm. At last, ethanol was detected in samples **6, 8, 10, and 11** by its methyl signal at 1.2 ppm whereas the quadruplet of its methylene group overlapped with the signal of glycerol at 3.59 ppm. The presence of ethanol in e-liquid increases the solubility of SCs and flavors and can significantly lower the viscosity of glycerol, which improves the formation of the aerosol for inhalation. Peace and co-workers previously evaluated alcohol content in e-liquid by headspace gas chromatography with flame ionization detector (HS-GC-FID) [7].

In addition to the signals of the matrix, the resonances of psychoactive SCs were detected in the NMR spectra. Five SCs were identified: JWH-210 was detected in samples **1-3**, 5F-MDMB-PICA in samples **4-6**, 5F-ADB in samples **7-9**, ADB-FUBINACA in samples **10-12**, and 5F-AKB48 in sample **13**. Structures of these SCs (Table 1) are based on indole or indazole rings with differently substituted carboxamide and alkyl side chains, four SCs being

fluorinated. The HF ^1H NMR profiles (5.6-10.0 ppm) of samples **3**, **4**, **8**, **11**, and **13** are reported in Figure 1B and the assignments of SCs signals are given in Table 1. Characteristic signals with close chemical shifts and multiplicity are observed for the indazole protons (H4, H5, H6, and H7) of 5F-ADB, ADB-FUBINACA and 5F-AKB48 whereas signals of the indole ring (H2, H4, H5, H6, and H7) in JWH-210 and 5F-MDMB-PICA have a different spectral signature. For SCs bearing fluorine on the alkyl chain (5F-MDMB-PICA, 5F-ADB, and 5F-AKB48), a doublet of triplet is observed for H5" due to both ^2J coupling with fluorine nucleus and $^3\text{J}_{\text{HH}}$ with neighboring methylene. Protons from the methylene groups H1" adjacent to the nitrogen atom of indazole or indole ring give rise to characteristic deshielded triplets (≈ 4.3 -4.5 ppm). Some signals could not be detected due to their overlap with matrix resonances; for instance, the singlet of methyl ester (5F-ADB or 5F-MDMB-PICA) overlaps with the signal of propylene glycol around 3.7-3.8 ppm. NMR characteristics for SCs are in accordance with previously published data [15, 16, 19, 24].

In addition to matrix and SCs signals, resonances of other chemicals were detected in e-liquid HF ^1H NMR spectra. Indeed, flavouring ingredients are widely added in e-liquids to give fruit aromas or other pleasant scents. Flavouring compounds like limonene, vanillin, ethyl maltol, raspberry ketone, anisyl alcohol, linalool, and menthol were successfully identified by spiking with standard compounds and their HF ^1H NMR assignments are reported in Table 2. Vanillin, the most common flavor agent, was detected in 8 out of 13 samples. Its more significant signal is that of the aldehyde proton which leads to a deshielded singlet at 9.74 ppm (Figure 1B); protons on the aromatic ring resonate in the range 6.9-7.5 ppm whereas the methoxy group singlet is detected at 3.92 ppm. Ethyl maltol which has sweet caramelized sugar smell was detected in six samples. It has two characteristic doublets at 8.00 and 6.42 ppm corresponding to aromatic protons, whereas protons from the ethyl group are detected at 2.74 ppm (q, H2) and 1.22 ppm (t, H1). Characteristic signals of raspberry ketone were observed in three samples with doublets of the para-substituted aromatic ring at 7.00 and 6.69 ppm and singlet of the methyl group at 2.11 ppm. Linalool was found in samples **10** and **11** from its characteristic doublet of doublet at 5.89 ppm as shown in Figure 1B. The characteristic upfield signals of menthol allowed its detection in sample **13**. Anisyl alcohol and limonene were also each detected in only one sample.

Besides NMR analysis, GC-MS was performed for verification of SCs and flavouring compounds identification. Retention times, exact masses and characteristic fragments are reported in Table S1 for SCs and Table 2 for flavours. These data are also supported by previous GC-MS characterization of e-liquids [5, 30].

3.2. LF ^1H NMR profiling

All samples were then analysed on the benchtop 60 MHz ^1H NMR spectrometer using the same solutions in the same NMR tubes than for HF recording. As an illustration, the HF and LF ^1H NMR spectra of sample **8** are shown in Figure 2A. Compared to the HF spectrum, signals are much broader with more overlap due to the lower resolution at LF. The resonances are especially crowded in the low frequency region (0.5-4.5 ppm) where targeted signals are erased by intense matrix signals, but some clues can nevertheless be drawn from the ethylenic/aromatic region i.e. above 5.6 ppm. Figure 2B shows the five typical LF profiles of samples **3**, **4**, **8**, **11**, and **13** with the same zoom than for their HF spectra illustrated in Figure 1B. The SC JWH-210 being at a low concentration in sample **3** could not be detected under the chosen experimental conditions. For the four other samples, even if signals are broad and similarities appear, the LF profile of each sample differs from the others and gives specific spectral signatures. For instance, singlets detected at 8.0 and 5.7 ppm in samples **4** and **11** are characteristic of 5F-MDMB-PICA (H2) and ADB-FUBINACA (H1'') respectively. The profiles of 5F-ADB and 5F-AKB48 in samples **8** and **13** are close even if the multiplet at ≈ 7.2 ppm is more spread out in sample **13**. Concerning flavouring compounds, the singlet of vanillin is observed at 9.74 ppm in samples **4** and **8**, likewise for the two characteristic doublets of ethyl maltol at 8.0 and 6.4 ppm in sample **8**.

From LF ^1H NMR analyses of all samples, it appears that despite intense matrix signals, some characteristic signals of SCs can be detected if their concentration is not too low. These LF ^1H NMR experiments constitute a proof of concept that direct profiling of e-liquids containing SCs could be relevant and should be applied on a larger number of samples for considering a chemometrics treatment as recently proposed in agrifood and pharmaceutical applications [39-41]. However, we did not further investigate the ^1H NMR experiments on the compact spectrometer because the method was not suitable for quantitative purposes in this study.

3.3. ^{19}F NMR analysis

Because of their enhanced potency on CB1 receptors, an increasing number and rate of emergence of fluorinated SCs have been reported over the last decades [42, 43]. In this context, it is therefore of interest to propose a specific method for detecting fluorinated molecules. Insofar the ^{19}F nucleus has favourable NMR characteristics (nuclear spin of 1/2, relatively narrow lines, 100% natural abundance, good sensitivity (83% that of proton) and

large chemical shift range), the fluorine NMR analysis can be used to study these fluorinated cannabinoids as reported recently by Naqi et al. who applied HF ^{19}F NMR spectroscopy to quantify the amounts of two fluorinated SCs, AM-694 and 5F-ADB, in herbal incense packages [43].

^{19}F NMR spectra were recorded for all samples at both HF and LF, i.e. 376 and 56.2 MHz respectively. Typical spectra without proton decoupling are presented in Figure 3, with HF and LF spectra stacked. TFT was added as a reference for chemical shift (-63.72 ppm with respect to CFCl_3) and quantification. The fluorine atom of ADB-FUBINACA linked to the aromatic ring leads to a singlet at -116.0 ppm. Fluorine nuclei at the end position of the alkyl chains of 5F-AKB48, 5F-MDMB-PICA, and 5F-ADB give rise to signals at \approx -220 ppm. The chemical shifts of 5F-AKB48 and 5F-ADB are almost identical ($\Delta\nu < 1$ Hz at HF), while that of 5F-MDMB-PICA slightly differs with $\Delta\nu \approx 37$ Hz at HF and ≈ 6 Hz at LF. They appear as triplet of triplet at HF due to the coupling of the ^{19}F nucleus with the neighbouring methylene protons H5'' ($^2J_{\text{HF}} = 47.5 \pm 0.1$ Hz) and H4'' ($^3J_{\text{HF}} = 25.5 \pm 0.2$ Hz) while, at LF, an apparent septuplet ($^3J_{\text{HF}} = 23.7 \pm 0.4$ Hz) is observed because signals are much more spread out as coupling constants are independent of the applied magnetic field.

Unlike LF proton NMR analyses of e-liquids that have limitations for quantitative purposes and cannot be implemented due to strong signal overlap, ^{19}F NMR opens up the possibility of setting up quantitative experiments.

3.4. Quantitative analysis

3.4.1. Setting up of quantitative conditions

The aim of the quantitative study was to consider HF proton and fluorine NMR as reference quantitative methods to evaluate the ability of quantification of LF ^{19}F NMR. Indeed, HF NMR is recognized as a powerful method for quantification of compounds in various mixtures for both ^1H [24, 25, 43-45] and ^{19}F [43, 44, 46, 47] nuclei.

To accurately quantify SCs in e-liquids, MeOD was chosen as the solvent due to its good miscibility with e-liquids, and T_1 relaxation times were determined by the classical inversion-recovery pulse sequence method in order to define the relevant acquisition parameters for quantitative ^1H and ^{19}F NMR experiments. HF T_1 values of protons selected for the quantification of SCs and internal reference were measured in samples **3**, **4**, **7**, **11** and **13** (Table 3). All ^1H resonances of SCs were considered as fully relaxed since 99.9% of the signal intensity of the proton with the longest T_1 (2.7 s) was recovered in the recording

conditions used (flip angle of 30° and repetition time of 13.4 s). For ^{19}F NMR quantifications, the relaxation agent $\text{Cr}(\text{acac})_3$ was added into the NMR tube to shorten the relaxation times of the ^{19}F nuclei. The T_1 s of the ^{19}F of the four fluorinated SCs in e-liquids are reported in Table 3. The longest T_1 for SCs was found for 5F-ADB in sample **7** (0.14 s and 0.18 s at HF and LF respectively) and the T_1 of the internal reference TFT was measured at 0.27 s at LF. For both HF and LF, spectra recording conditions were chosen to gain full relaxation of the ^{19}F nuclei. Repetition times of 4.57 s and 2.57 s were used for HF and LF NMR with flip angles of 30° and 90°, respectively. All ^{19}F nuclei were thus completely relaxed since more than 99.9% of the ^{19}F magnetization was recovered.

Recording times were set at 2.5 min (32 scans) and 90 min (2048 scans) for HF and LF ^{19}F NMR experiments leading to S/N ratios of 200 and 50, respectively. The limits of detection (LOD) and quantitation (LOQ) of the LF analyses were estimated by recording diluted solutions of standard SCs (5F-AKB48 and 5F-MDMB-PICA) of known concentrations. The LODs were evaluated at 0.33 and 0.25 mg/ml and the LOQs at 0.92 and 1.0 mg/ml for 5F-MDMB-PICA and 5F-AKB48, respectively, i.e. significantly lower than the concentrations measured in e-liquids (Table 4). We did not measure the LOQ of 5F-ADB which bears a fluorinated chain similar to 5F-AKB48, nor that of ADB-FUBINACA as the absence of multiplicity of the signal (singlet) enhances the S/N ratio of the resonance.

3.4.2. Quantitative analyses of SCs in e-liquids

The amounts of SCs in each e-liquid sample analysed in triplicate are reported in Table 4. For the HF ^1H NMR quantification, a characteristic signal could be integrated for each SC despite some overlaps. The ^{19}F NMR quantification was straightforward as the NMR signals of the fluorinated SCs and the reference TFT are easily integrated at both HF and LF.

HF proton and fluorine NMR are recognized as quantitative methods in forensic [25, 31, 43] but have never been applied to e-liquids to our knowledge. The results reported in Table 4 show that, even if a matrix effect could bias the ^1H NMR quantification, data obtained by both techniques are in good agreement. Indeed, the linear regression equation of the HF ^1H versus ^{19}F values display a slope of 0.99 ± 0.03 (p-value = 0), a y-intercept of -0.34 ± 0.46 and a correlation coefficient of 0.98 (p-value 1.1×10^{-22}). The p-value of 0.55 given by the Wilcoxon signed-rank test demonstrates the absence of significant differences between these two data sets. The same observation can be done when comparing LF ^{19}F versus HF ^{19}F quantifications: a slope of 0.90 ± 0.03 (p-value = 0), a y-intercept of 1.22 ± 0.47 , a correlation coefficient of 0.97 (p-value = 1.6×10^{-21}) and a p-value of 0.74 for the Wilcoxon signed-rank

test. These results demonstrate that SCs in e-liquids can be successfully quantified by NMR and that an accurate ^{19}F NMR quantification of fluorinated SCs can be implemented on a compact LF NMR spectrometer without requiring any specific standard compound.

As shown in Table 4, the 13 e-liquids contained variable concentrations of SCs: samples **1-3** contained the lowest amount of SCs with JWH-210 at around 1 mg/mL, whereas sample **13** contained more than 20 mg/mL of 5F-AKB48. The contents in 5F-MDMB-PICA, 5F-ADB, and ADB-FUBINACA ranged from ≈ 7 to ≈ 18 mg/ml in samples **4-12**. To our knowledge, there are no published quantitative data on SCs in e-liquids for discussion of these results. However, even if the toxicological incidence is not the direct purpose of this study, we must highlight the high CB1/CB2 binding potency of the third generation of SCs which include the fluorinated SCs detected in this study [10, 15, 48, 49]. For instance, ADB-FUBINACA is a highly potent agonist of CB1 ($\text{EC}_{50} = 1.2$ nM) [15] by comparison to JWH 210 ($\text{EC}_{50} = 25.3$ nM) [49] or THC ($\text{EC}_{50} = 172$ nM) [15]. This high affinity of SCs for cannabinoids receptors along with their significant concentration in e-liquids obviously poses a threat to the health of consumers.

Conclusion

In this study, 13 commercial e-liquid formulations were screened by ^1H and ^{19}F NMR. HF ^1H NMR allowed a holistic analysis of SCs and flavouring compounds in the e-liquids. In addition, the ability of benchtop LF ^1H and ^{19}F NMR spectroscopy was evaluated. To the best of our knowledge, this is the first study dealing with the analysis of e-liquids containing SCs by LF NMR. Although LF ^1H NMR has shown limitations for quantitative applications, in particular due to (i) the huge difference in concentrations between the matrix and the active compounds, and (ii) signal overlaps, some characteristic signals could be distinguished. The interesting novelty of this study is that NMR and in particular LF ^{19}F NMR can easily allow the quantification of fluorinated SCs in e-liquids without complex sample preparation and with no need for specific standard compounds. Thanks to the detection specificity of LF fluorine NMR, this proof-of-concept study opens the way to routine screening for the detection of illicit fluorinated SCs in various matrices such as e-liquids, herbal blends, or bath salts in forensic or customs laboratories.

Captions for Figures

Figure 1. Proton NMR spectra of e-liquids (400 MHz, in MeOD): (A) Whole NMR spectrum of sample **3**, (B) Overlap of 5 spectra of e-liquids containing different SCs (5.6-10.0 ppm). EM: ethyl maltol, V: vanillin, RK: raspberry ketone, Lin: linalool, AA: anisyl alcohol.

Figure 2. Low-Field (60 MHz) proton NMR spectra of e-liquids: (A) Comparison of the ^1H NMR spectra of sample **8** recorded in MeOD on the high-field (400 MHz) and low-field (60 MHz) spectrometers, (B) Overlap of 5 spectra (60 MHz) with different SCs (5.6-10.0 ppm). EM: ethyl maltol, V: vanillin, RK: Raspberry Ketone, Lin: Linalool.

Figure 3. ^{19}F NMR spectra of e-liquid samples recorded in MeOD at 376 MHz (HF) and 56.17 MHz (LF).

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Figure 1

(A)

Sample 3

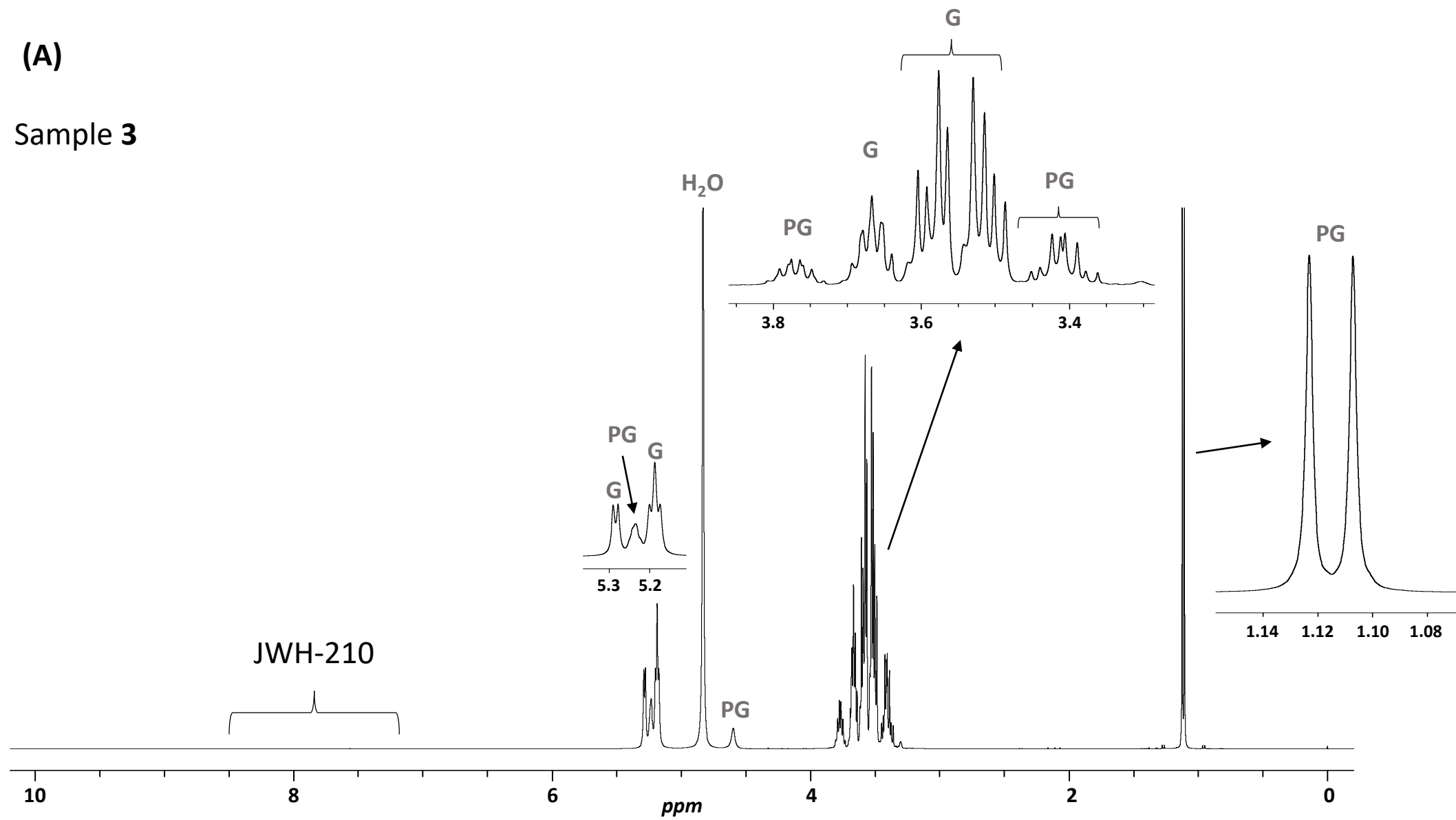


Figure 1

(B)

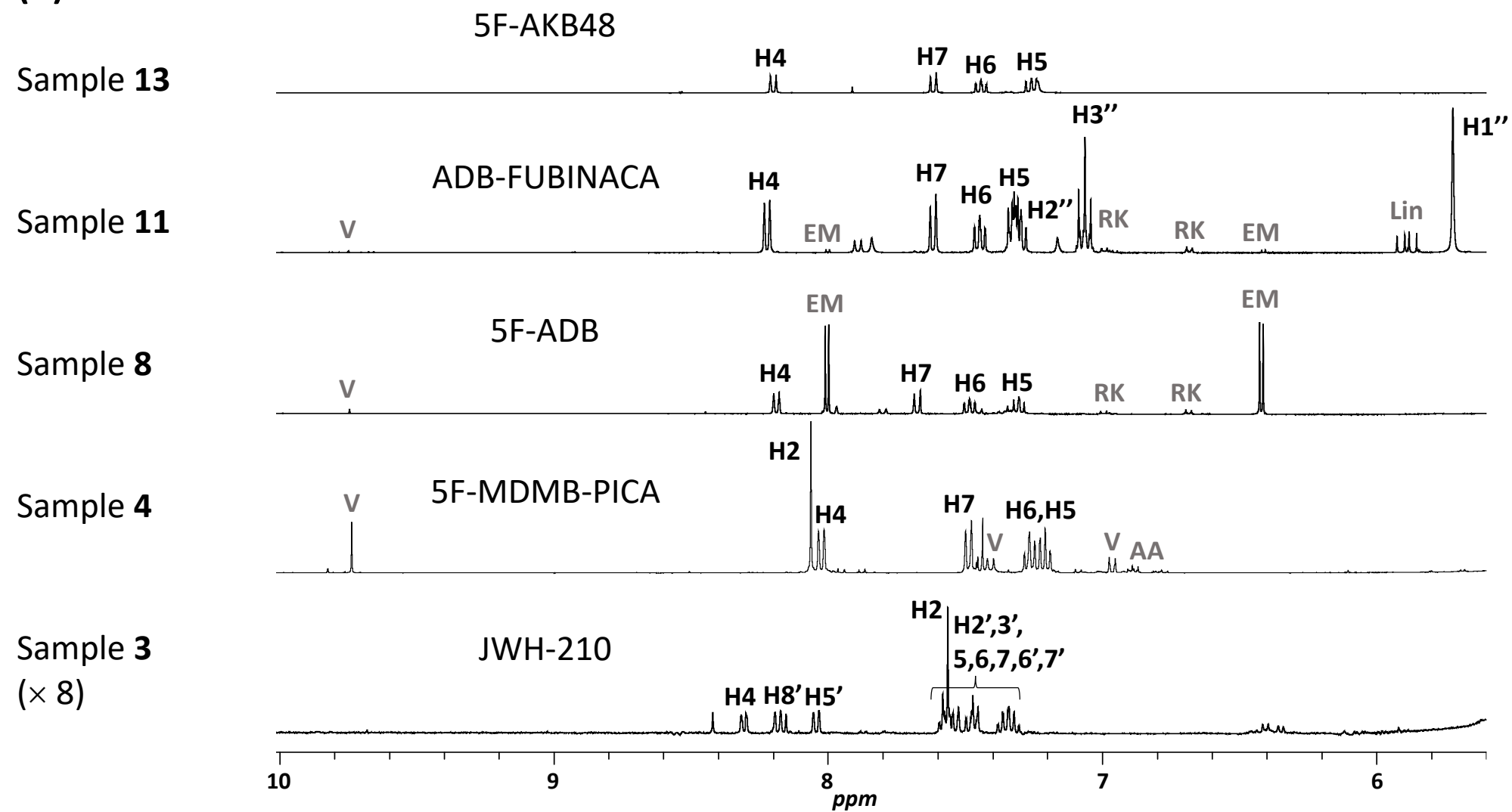
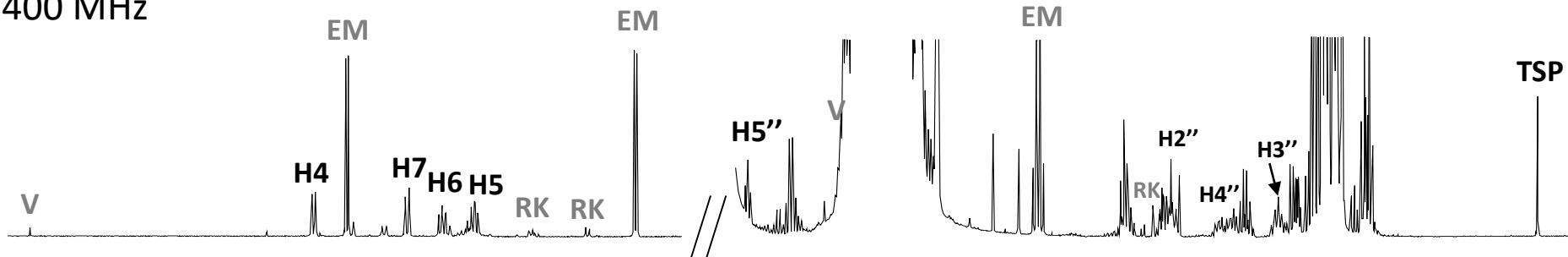


Figure 2

(A)

Sample **8** (5F-ADB)

400 MHz



60 MHz

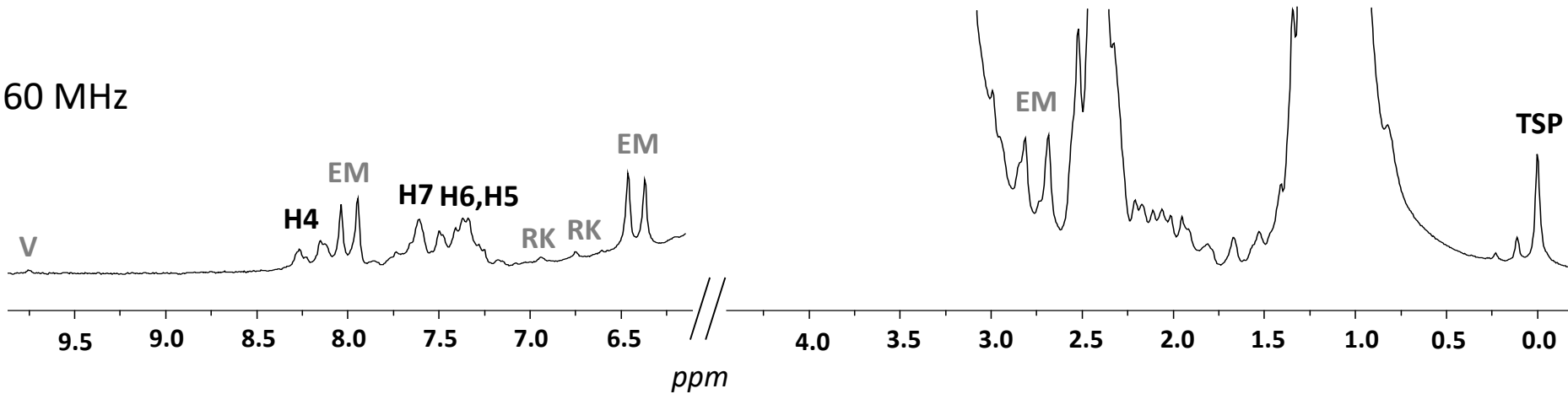


Figure 2

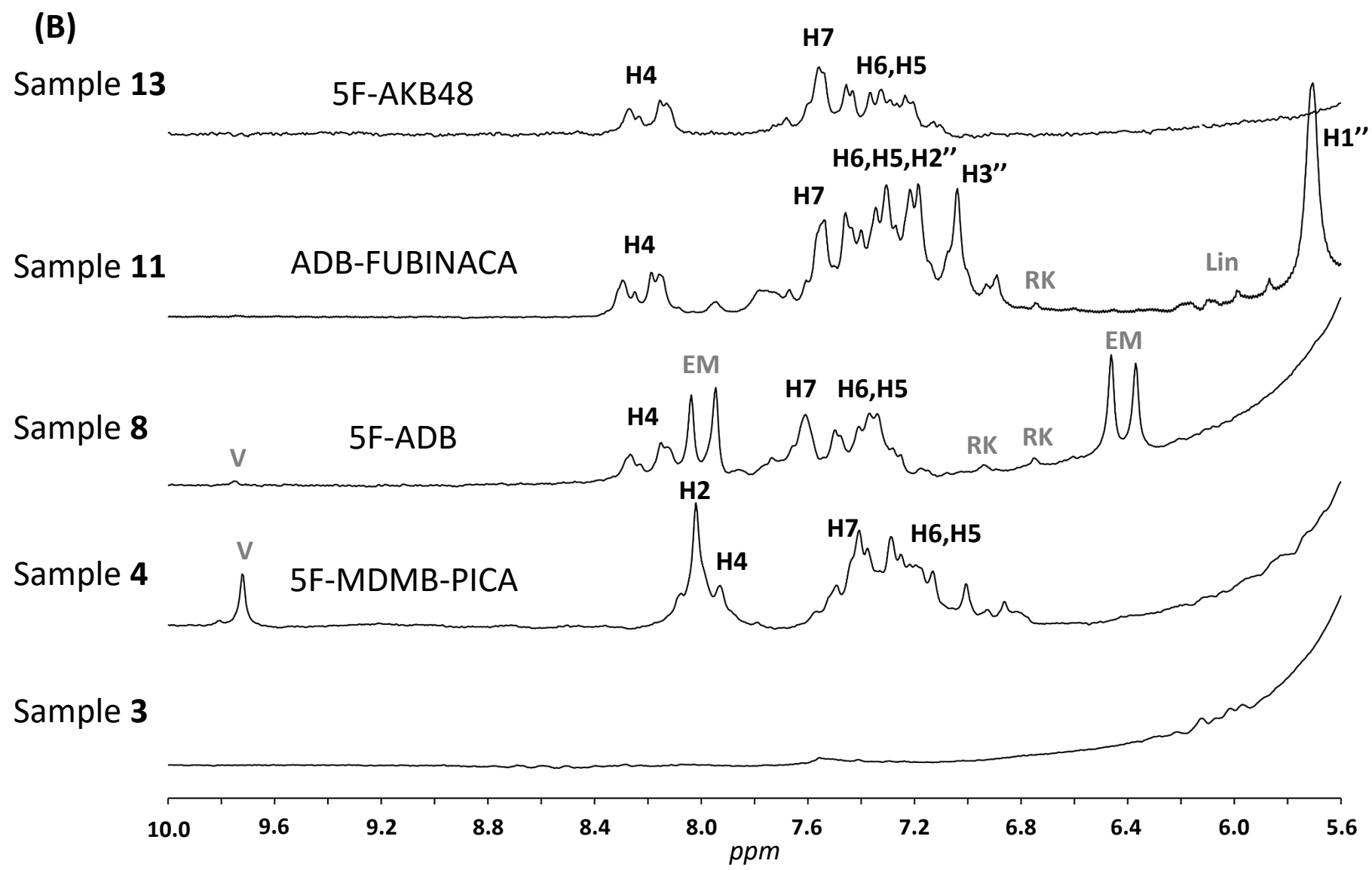


Figure 3

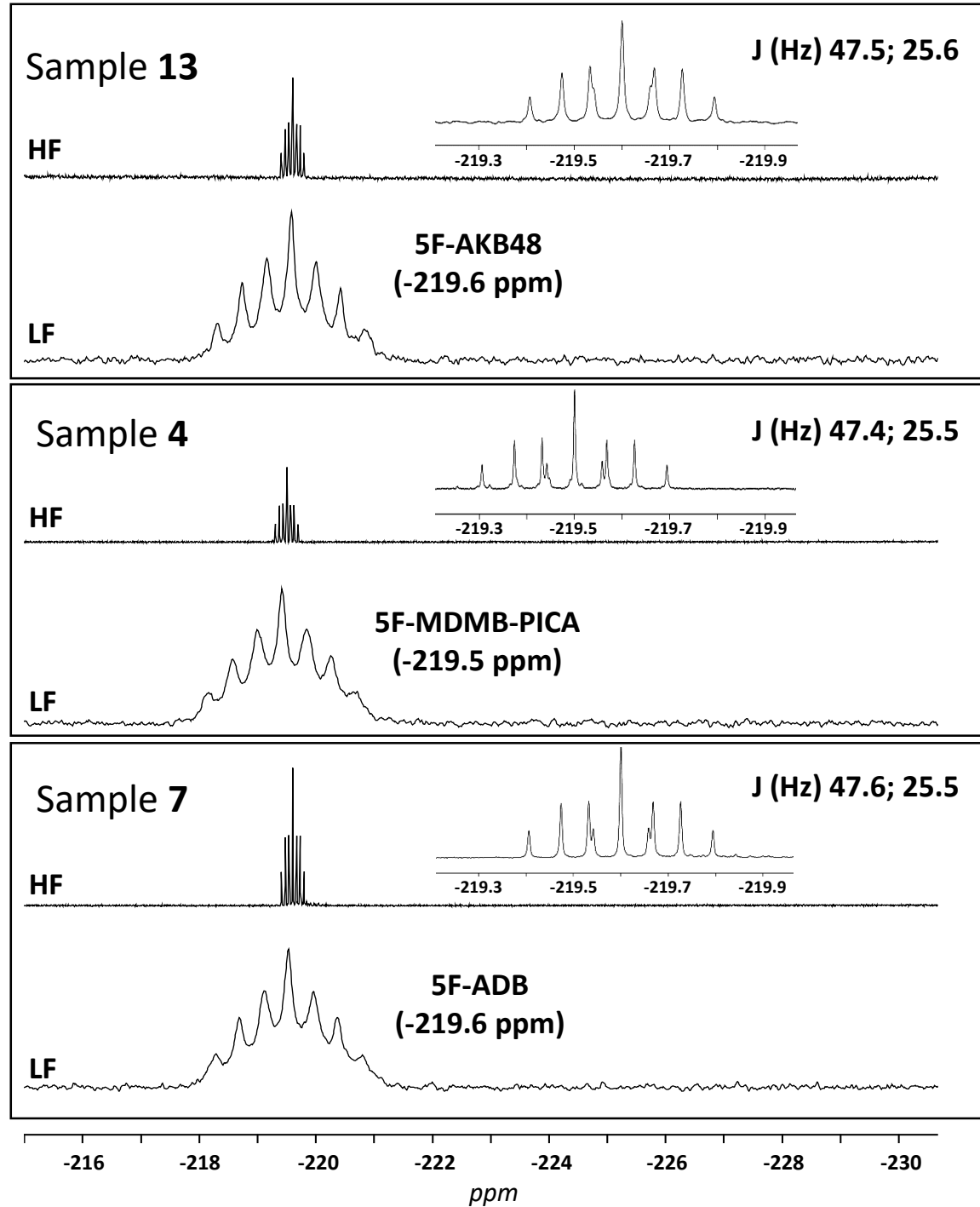
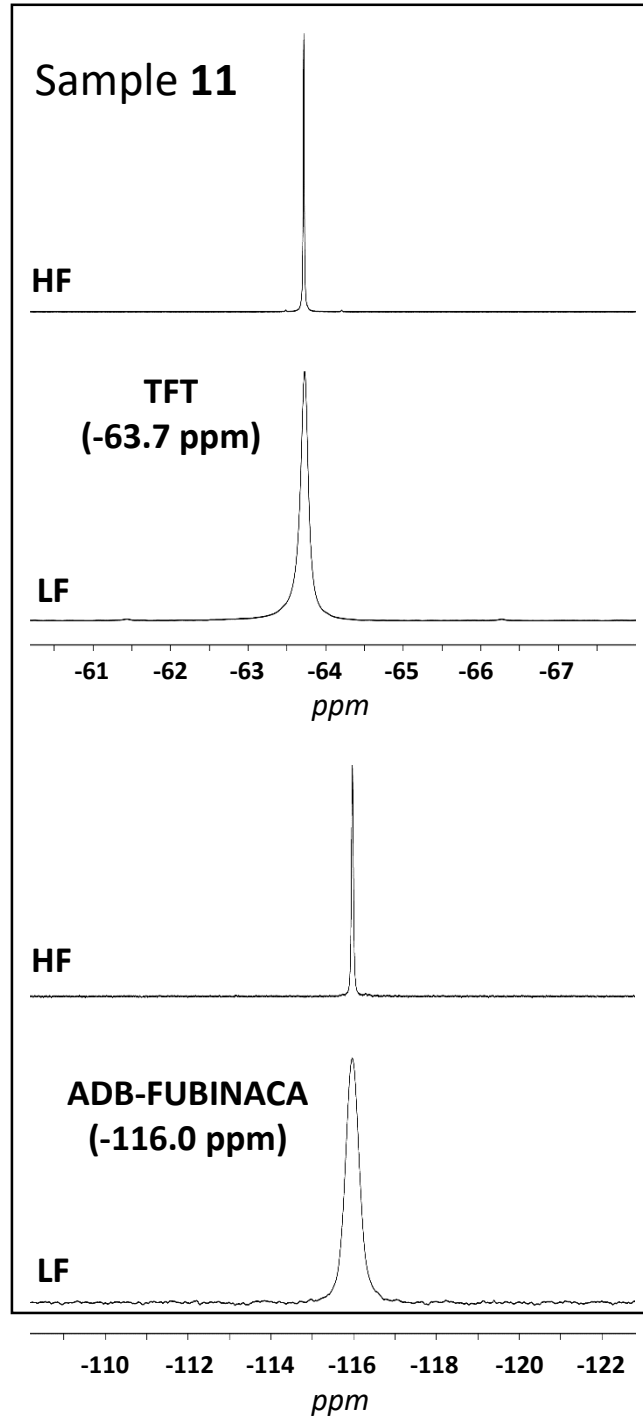


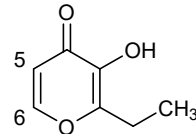
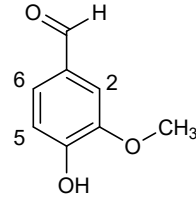
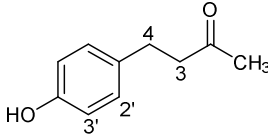
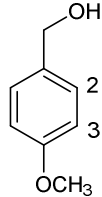
Table 1. High-field ¹H NMR (400 MHz) characteristic signals of synthetic cannabinoids detected in e-liquid samples recorded in MeOD. Signals in bold were used for quantification.

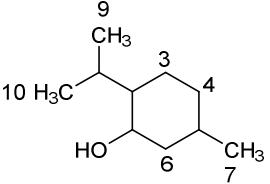
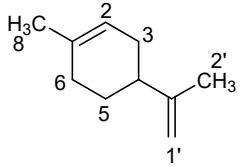
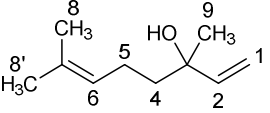
	JWH-210			5F-MDMB-PICA			5F-ADB			ADB-FUBINACA			5F-AKB48		
¹ H label ^a	δ (ppm)	M ^b	J (Hz)	δ (ppm)	M ^b	J (Hz)	δ (ppm)	M ^b	J (Hz)	δ (ppm)	M ^b	J (Hz)	δ (ppm)	M ^b	J (Hz)
2	7.56	s		8.06	s										
4	8.31	dd	7.0/1.3	8.02	d	7.8	8.20	d	8.2	8.22	d	8.2	8.20	d	8.2
5	7.30-7.60	m		7.21	m		7.31	m		7.31	m		7.26	m	
6	7.30-7.60	m		7.27	m		7.48	m		7.44	ddd	8.6 7.0 1.1	7.44	ddd	8.6 6.9 1.1
7	7.30-7.60	m		7.49	d	8.2	7.68	d	8.6	7.61	d	8.5	7.62	d	8.5
2'	7.44-7.60	m		Overlap			Overlap			Overlap			2.20	m	
3'	7.44-7.60	m		/			/			/			2.13	br s	
4'	/			/			/			/			1.78	br s	
5'	8.04	d	8.4	/			/			/			/		
6'	7.30-7.60	m		/			/			/			/		
7'	7.30-7.60	m		/			/			/			/		
8'	8.18	d	8.6	/			/			/			/		
1''	Overlap			4.26	t	7.1	4.54	t	7.0	5.72	s		4.48	t	7.0
2''	1.78	quin	7.5	1.92	quin	7.5	2.01	quin	7.5	7.31	m		1.98	quin	7.5
3''	Overlap			1.42	m		1.43	m		7.06	app t	8.8	1.42	m	
4''	Overlap			1.69	m		1.72	m		/			1.72	m	
5''	0.82	t	7.0	4.40	dt	47.5/6.0	4.39	dt	47.5/6.0	/			4.39	dt	47.5/6.0
CH ₃ -CH ₂	1.42	t	7.5	/			/			/			/		
(CH ₃) ₃	/			1.09	s		1.08	s		1.09	s		/		

^a Proton signals mentioned as “overlap” or missing (CH₃-OCO-, CH₃-CH₂) could not be readily detected due to their overlap with other signals.

^b M: multiplicity; s: singlet; d: doublet; dd: doublet of doublet; t: triplet; dt: doublet of triplet; quin: quintuplet; m: multiplet; app t: apparent triplet; br s: broad signal.

Table 2. High-field ^1H NMR (400 MHz, MeOD) and GC-MS data of flavouring compounds detected in the e-liquids analyzed. Only detected ^1H NMR signals are reported.

Compound	Structure	¹ H NMR	GC-MS	Detected in samples		
		δ (ppm), multiplicity ^a (J (Hz)), attribution	Retention time (min)	Formula Experimental accurate mass m/z (mass error in ppm)	m/z of major characteristic fragment ions observed	
Ethyl maltol		8.00, d (5.5), H6 6.42, d (5.5), H5 2.74, q (7.6), CH ₂ 1.22, t (7.6), CH ₃	9.89	C ₇ H ₈ O ₃ 140.0467 (0.7)	55, 69, 71, 97, 139	7-12
Vanillin		9.74, s, CHO 7.46, m, H6 7.44, s, H2 6.96, d (8.5), H5 3.92, s, -O-CH ₃	15.00	C ₈ H ₈ O ₃ 152.0462 (3.9)	81, 109, 123, 151	4-9, 11, 12
Raspberry ketone		7.00, d (8.5), H2' 6.69, d (8.5), H3' 2.11, s, CH ₃	19.18	C ₁₀ H ₁₂ O ₂ 164.0834 (1.2)	107, 131, 149	8, 10, 11
Anisyl alcohol		6.88, d (8.7), H3 4.52, s, CH ₂	12.04	C ₈ H ₁₀ O ₂ 138.0676 (0.7)	77, 109, 121	4

Menthol		1.61, m, H ₃ (1H) 0.92, d (7.1), CH ₃ 9 0.91, d (6.6), CH ₃ 7 0.84, m, H ₃ (1H) 0.79, d (6.9), CH ₃ 10	9.65	C ₁₀ H ₂₀ O 156.1519 (3)	71, 81, 95, 123, 138	13
Limonene		4.68, m, H1' 1.62, broad s, CH ₃ 8	5.50	C ₁₀ H ₁₆ 136.1245 (1.5)	68, 79, 93	6
Linalool		5.89, dd (17.4, 10.8), H2 5.01, dd (10.8, 1.6), H1 (1H) 1.65;1.58, two broad s, CH ₃ 8,8' 1.23, broad s, CH ₃ 9	7.19	C ₁₀ H ₁₈ O 154.1360 (2)	71, 80, 93, 121, 136	10, 11

^a s: singlet; d: doublet; dd: doublet of doublet; t: triplet; q: quadruplet; m: multiplet.

Table 3. T₁ values measured in e-liquid samples.

Cannabinoids (sample number)	HF NMR		LF NMR
	¹ H NMR	¹⁹ F NMR ^c	¹⁹ F NMR ^c
	T ₁ (s)	T ₁ (s)	T ₁ (s)
JWH-210 (3)	1.5 (H5')	-	-
5F-MDMB-PICA (4)	0.5 (H2'')	0.13	0.17
5F-ADB (7)	1.1 (H5)	0.14	0.18
ADB-FUBINACA (11)	0.4 (H1'')	0.09	0.14
5F-AKB48 (13)	2.0 (H5'')	0.14	0.16
TSP ^a	2.7	-	-
TFT ^b	-	0.20	0.27

^a Mean values from samples **3**, **4**, **7**, **11** and **13**.

^b Mean values from samples **4**, **7**, **11** and **13**.

^c ¹⁹F NMR measurements were done on samples containing the relaxation agent Cr(acac)₃.

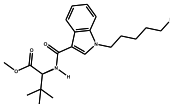
Table 4. Amounts of synthetic cannabinoids (SC) in 13 e-liquids measured with high-field ^1H and ^{19}F NMR and low-field ^{19}F NMR. All experiments were done in triplicate from sampling the e-liquid to processing the NMR spectrum.

Sample	Cannabinoid	Amount (mg/ml) \pm SD		
		HF NMR		LF NMR
		^1H NMR	^{19}F NMR	^{19}F NMR
1	JWH-210	0.9 ± 0.1	- ^a	-
2		0.9 ± 0.1	-	-
3		1.1 ± 0.1	-	-
4	5F-MDMB-PICA	18.4 ± 0.3	19.3 ± 0.3	18.4 ± 0.3
5		17.2 ± 0.3	18.0 ± 0.2	17.3 ± 0.3
6		15.2 ± 0.6	14.9 ± 0.4	14.3 ± 1.3
7	5F-ADB	15.6 ± 0.3	15.6 ± 0.3	16.0 ± 0.7
8		7.3 ± 0.1	7.7 ± 0.3	8.0 ± 0.2
9		10.9 ± 0.4	11.7 ± 0.5	11.8 ± 0.6
10	ADB- FUBINACA	15.1 ± 0.3	15.4 ± 0.1	15.2 ± 0.5
11		16.6 ± 0.4	17.8 ± 0.5	17.5 ± 0.5
12		15.7 ± 0.3	16.3 ± 0.3	15.0 ± 0.2
13	5F-AKB48	24.1 ± 0.1	24.1 ± 0.1	22.7 ± 0.5

^a JWH-210 is not fluorinated.



Profiling



Q-NMR

