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

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21

## 1 **Abstract**

2 The <sup>1</sup>H NMR profiles of 13 samples of e-liquids supplied by French customs were obtained  
3 with high-field and low-field NMR. The high-field <sup>1</sup>H NMR spectra allowed the detection of  
4 matrix signals, synthetic cannabinoids, and flavouring compounds. Quantitative results were  
5 obtained for the five synthetic cannabinoids detected: JWH-210, 5F-MDMB-PICA, 5F-ADB,  
6 5F-AKB48, and ADB-FUBINACA. Conventional GC-MS analysis was used to confirm  
7 compound identification. Fluorine-19 NMR was proposed for the quantification of fluorinated  
8 synthetic cannabinoids and was successfully implemented on both 400 MHz and 60 MHz  
9 NMR spectrometers. This study based on few examples explored the potentiality of low-field  
10 NMR for quantitative and quantitative analysis of synthetic cannabinoids in e-liquids.

11 **Keywords: e-liquid, e-cigarette, qNMR, compact NMR, <sup>19</sup>F NMR, cannabinoid**

12

## 13 **Introduction**

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

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

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

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

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

## 65 **2. Experimental**

## 66 2.1. Materials

67 13 e-liquid samples from customs seizures were provided by the SCL laboratory  
68 (French Customs). The standard SCs JWH-210, 5F-AKB48, and 5F-MDMB-PICA were  
69 obtained from Cayman Chemical (Ann Arbor, MI, USA). 3-(trimethylsilyl)propionic-2,2,3,3-  
70 d4 acid sodium salt (TSP, purity  $\geq 99\%$ , isotopic purity 98 atom % D),  $\alpha,\alpha,\alpha$ -trifluorotoluene  
71 (TFT, purity  $\geq 99\%$ ), vanillin, ethyl maltol, limonene, anisyl alcohol, raspberry ketone (4-(4-  
72 hydroxyphenyl)butan-2-one), menthol, linalool and chromium(III) acetylacetonate ( $\text{Cr}(\text{acac})_3$ )  
73 were purchased from Sigma-Aldrich (St. Louis, MO, USA).

## 74 2.2. NMR analysis

### 75 2.2.1. Sample preparation for NMR

76 200  $\mu\text{l}$  of each e-liquid withdrawn with a glass precision syringe were diluted with 400  
77  $\mu\text{l}$  of methanol-d4 (MeOD) and mixed with 30  $\mu\text{l}$  of a TSP solution (10 mM in MeOD) as  
78 reference for  $^1\text{H}$  NMR experiments.  $^{19}\text{F}$  NMR experiments were run for the 10 e-liquid  
79 samples **4-13** containing the fluorinated SCs 5F-MDMB-PICA, 5F-ADB, 5F-AKB48, and  
80 ADB-FUBINACA. For these  $^{19}\text{F}$  NMR experiments, 200  $\mu\text{l}$  of sample were diluted with 400  
81  $\mu\text{l}$  of MeOD and then mixed with 9  $\mu\text{l}$  of a TFT solution (1M in MeOD; final concentration  
82 15 mM). Two mg of  $\text{Cr}(\text{acac})_3$  as a relaxation agent were added into the NMR tube. The HF  
83 and LF  $^{19}\text{F}$  NMR experiments were recorded on the same NMR tube. The exact  
84 concentrations of the TSP and TFT solutions used were determined relative to that of a  
85 solution of yohimbine hydrochloride (European Pharmacopoeia Reference Standard). All  
86 quantitative experiments were done in triplicate from sampling the e-liquid to processing the  
87 NMR spectrum.

### 88 2.2.2. HF NMR analysis

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

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

100 HF  $^{19}\text{F}$  NMR experiments were recorded with the same spectrometer and probe at 376  
101 MHz. First, a basic zg sequence allowed to acquire  $^1\text{H}$ -coupled  $^{19}\text{F}$  NMR spectra, then  
102 quantitative experiments were carried out using an inverse gated decoupling pulse sequence  
103 with the following parameters: NS 32, pulse width 4.52  $\mu\text{s}$  (flip angle  $30^\circ$ ), acquisition time  
104 1.57 s, spectral width 221 ppm, 256K data points, and an additional relaxation delay of 3 s for  
105 complete longitudinal relaxation; the recording time was thus less than 3 min. The signal of  
106 TFT set at -63.72 ppm with respect to  $\text{CFCl}_3$  (0 ppm) was used as an internal reference for  
107 chemical shift measurement and quantitative analysis.

### 108 2.2.3. LF NMR analysis

109 Spectra were acquired on a Pulsar<sup>TM</sup> benchtop NMR spectrometer (Oxford  
110 Instruments, Abingdon, UK) operating at a frequency of 59.7 MHz for  $^1\text{H}$  and 56.17 MHz for  
111  $^{19}\text{F}$ . The temperature inside the spectrometer was 310 K. Acquisition was performed with the  
112 SpinFlow 1.2.0.1 software (Oxford Instruments) and data processing with the MNova 11.0  
113 software (Mestrelab Research, Santiago de Compostela, Spain).

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

119 LF  $^{19}\text{F}$  NMR spectra were recorded with a flip angle of  $90^\circ$ , a spectral width of 20833  
120 Hz, and 32K data points (acquisition time of 1.57 s). The relaxation delay was set at 1 s, and  
121 2048 transients were recorded leading to a total acquisition time of 1 h 27 min. For data  
122 processing, the FIDs were apodized with an exponential (3.0 Hz) filter and an automatic  
123 baseline correction was applied.

### 124 2.2.4. $T_1$ measurements

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

129 scans 32, and relaxation delay 40 s for HF <sup>1</sup>H NMR; 128K data points, acquisition time 1.75  
130 s, spectral width 100 ppm, number of scans 16, and relaxation delay 5 s for HF <sup>19</sup>F NMR;  
131 16K data points, acquisition time 2.05 s, spectral width 133 ppm (8000 Hz), number of scans  
132 96, and relaxation delay 5 s for LF <sup>19</sup>F NMR.

133

### 134 2.2.5. NMR quantification

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

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

$$141 \text{ Amount (mg / ml)} = \frac{A_{SCs}}{N_{SCs}} \times [REF] \times \frac{N_{REF}}{A_{REF}} \times V_{total} \times \frac{MW_{SCs}}{V_{e-liquid}}$$

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

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

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

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

146  $MW_{SCs}$ : molecular weight of SCs,

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

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

### 152 2.3. GC-MS analysis

153 Each sample was vortexed for 30 s before aliquoting. 250  $\mu$ l of each e-liquid sample  
154 were then mixed with 500  $\mu$ l of CHCl<sub>3</sub>. The analyses were performed using an Agilent 6890  
155 N Gas Chromatograph coupled with a GCT Premier CAB109 TOF mass spectrometer  
156 (Waters Corporation, Milford, MA, USA). An Agilent HP-5MS capillary column (30 m  $\times$   
157 0.25 mm, 0.25  $\mu$ m film thickness) was used to perform the chromatographic separation.  
158 Helium as the carrier gas was delivered at a constant flow of 1.0 ml/min. The temperature  
159 program was as follows: initial temperature 70  $^{\circ}$ C for 2 min, increase to 200  $^{\circ}$ C at 5  $^{\circ}$ C/min

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

### 164 3. Results and discussion

#### 165 3.1. HF <sup>1</sup>H NMR qualitative analysis of e-liquids

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

169 The HF <sup>1</sup>H NMR spectra of all 13 samples allowed the detection of major signals of  
170 propylene glycol (PG) and glycerol which constitute the matrix of e-liquids. As an illustration,  
171 Figure 1A shows the complete HF <sup>1</sup>H NMR spectrum of one e-liquid sample (**3**). Matrix  
172 signals of PG and glycerol have the most prominent signals in the upfield area. For PG, a  
173 doublet (d; CH<sub>3</sub>) was detected at 1.11 ppm (J = 6.4 Hz), a multiplet at 3.77 ppm (<CH), an  
174 ABd system centered at 3.41 ppm (J = 11.1, 6.5, 4.8 Hz) (-CH<sub>2</sub>), and broad singlets for  
175 hydroxyl protons at 4.60 and 5.24 ppm. The methine and methylene groups of glycerol led to  
176 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  
177 hydroxyl groups resonated as doublet and triplet at 5.29 and 5.19 ppm respectively  
178 (depending on the rate of hydroxyl proton exchange, these signals were more or less broad).  
179 Moreover, ethylene glycol and/or polyethylene glycol were identified in samples **9-12** from  
180 their characteristic intense broad signals around 3.6 ppm. At last, ethanol was detected in  
181 samples **6, 8, 10, and 11** by its methyl signal at 1.2 ppm whereas the quadruplet of its  
182 methylene group overlapped with the signal of glycerol at 3.59 ppm. The presence of ethanol  
183 in e-liquid increases the solubility of SCs and flavors and can significantly lower the viscosity  
184 of glycerol, which improves the formation of the aerosol for inhalation. Peace and co-workers  
185 previously evaluated alcohol content in e-liquid by headspace gas chromatography with flame  
186 ionization detector (HS-GC-FID) [7].

187 In addition to the signals of the matrix, the resonances of psychoactive SCs were  
188 detected in the NMR spectra. Five SCs were identified: JWH-210 was detected in samples **1-3**,  
189 5F-MDMB-PICA in samples **4-6**, 5F-ADB in samples **7-9**, ADB-FUBINACA in samples **10-**  
190 **12**, and 5F-AKB48 in sample **13**. Structures of these SCs (Table 1) are based on indole or  
191 indazole rings with differently substituted carboxamide and alkyl side chains, four SCs being

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

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

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

### 226 3.2. LF <sup>1</sup>H NMR profiling

227 All samples were then analysed on the benchtop 60 MHz <sup>1</sup>H NMR spectrometer using  
228 the same solutions in the same NMR tubes than for HF recording. As an illustration, the HF  
229 and LF <sup>1</sup>H NMR spectra of sample **8** are shown in Figure 2A. Compared to the HF spectrum,  
230 signals are much broader with more overlap due to the lower resolution at LF. The resonances  
231 are especially crowded in the low frequency region (0.5-4.5 ppm) where targeted signals are  
232 erased by intense matrix signals, but some clues can nevertheless be drawn from the  
233 ethylenic/aromatic region i.e. above 5.6 ppm. Figure 2B shows the five typical LF profiles of  
234 samples **3**, **4**, **8**, **11**, and **13** with the same zoom than for their HF spectra illustrated in Figure  
235 1B. The SC JWH-210 being at a low concentration in sample **3** could not be detected under  
236 the chosen experimental conditions. For the four other samples, even if signals are broad and  
237 similarities appear, the LF profile of each sample differs from the others and gives specific  
238 spectral signatures. For instance, singlets detected at 8.0 and 5.7 ppm in samples **4** and **11** are  
239 characteristic of 5F-MDMB-PICA (H2) and ADB-FUBINACA (H1") respectively. The  
240 profiles of 5F-ADB and 5F-AKB48 in samples **8** and **13** are close even if the multiplet at ≈7.2  
241 ppm is more spread out in sample **13**. Concerning flavouring compounds, the singlet of  
242 vanillin is observed at 9.74 ppm in samples **4** and **8**, likewise for the two characteristic  
243 doublets of ethyl maltol at 8.0 and 6.4 ppm in sample **8**.

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

### 252 3.3. <sup>19</sup>F NMR analysis

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

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

262  $^{19}\text{F}$  NMR spectra were recorded for all samples at both HF and LF, i.e. 376 and 56.2  
263 MHz respectively. Typical spectra without proton decoupling are presented in Figure 3, with  
264 HF and LF spectra stacked. TFT was added as a reference for chemical shift (-63.72 ppm with  
265 respect to  $\text{CFCl}_3$ ) and quantification. The fluorine atom of ADB-FUBINACA linked to the  
266 aromatic ring leads to a singlet at -116.0 ppm. Fluorine nuclei at the end position of the alkyl  
267 chains of 5F-AKB48, 5F-MDMB-PICA, and 5F-ADB give rise to signals at  $\approx$ -220 ppm. The  
268 chemical shifts of 5F-AKB48 and 5F-ADB are almost identical ( $\Delta\nu < 1$  Hz at HF), while that  
269 of 5F-MDMB-PICA slightly differs with  $\Delta\nu \approx 37$  Hz at HF and  $\approx 6$  Hz at LF. They appear as  
270 triplet of triplet at HF due to the coupling of the  $^{19}\text{F}$  nucleus with the neighbouring methylene  
271 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  
272 septuplet ( $^3J_{\text{HF}} = 23.7 \pm 0.4$  Hz) is observed because signals are much more spread out as  
273 coupling constants are independent of the applied magnetic field.

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

### 277 3.4. Quantitative analysis

#### 278 3.4.1. Setting up of quantitative conditions

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

283 To accurately quantify SCs in e-liquids, MeOD was chosen as the solvent due to its  
284 good miscibility with e-liquids, and  $T_1$  relaxation times were determined by the classical  
285 inversion-recovery pulse sequence method in order to define the relevant acquisition  
286 parameters for quantitative  $^1\text{H}$  and  $^{19}\text{F}$  NMR experiments. HF  $T_1$  values of protons selected  
287 for the quantification of SCs and internal reference were measured in samples **3**, **4**, **7**, **11** and  
288 **13** (Table 3). All  $^1\text{H}$  resonances of SCs were considered as fully relaxed since 99.9% of the  
289 signal intensity of the proton with the longest  $T_1$  (2.7 s) was recovered in the recording

290 conditions used (flip angle of 30° and repetition time of 13.4 s). For <sup>19</sup>F NMR quantifications,  
291 the relaxation agent Cr(acac)<sub>3</sub> was added into the NMR tube to shorten the relaxation times of  
292 the <sup>19</sup>F nuclei. The T<sub>1</sub>s of the <sup>19</sup>F of the four fluorinated SCs in e-liquids are reported in Table  
293 3. The longest T<sub>1</sub> for SCs was found for 5F-ADB in sample 7 (0.14 s and 0.18 s at HF and LF  
294 respectively) and the T<sub>1</sub> of the internal reference TFT was measured at 0.27 s at LF. For both  
295 HF and LF, spectra recording conditions were chosen to gain full relaxation of the <sup>19</sup>F nuclei.  
296 Repetition times of 4.57 s and 2.57 s were used for HF and LF NMR with flip angles of 30°  
297 and 90°, respectively. All <sup>19</sup>F nuclei were thus completely relaxed since more than 99.9% of  
298 the <sup>19</sup>F magnetization was recovered.

299 Recording times were set at 2.5 min (32 scans) and 90 min (2048 scans) for HF and  
300 LF <sup>19</sup>F NMR experiments leading to S/N ratios of 200 and 50, respectively. The limits of  
301 detection (LOD) and quantitation (LOQ) of the LF analyses were estimated by recording  
302 diluted solutions of standard SCs (5F-AKB48 and 5F-MDMB-PICA) of known  
303 concentrations. The LODs were evaluated at 0.33 and 0.25 mg/ml and the LOQs at 0.92 and  
304 1.0 mg/ml for 5F-MDMB-PICA and 5F-AKB48, respectively, i.e. significantly lower than the  
305 concentrations measured in e-liquids (Table 4). We did not measure the LOQ of 5F-ADB  
306 which bears a fluorinated chain similar to 5F-AKB48, nor that of ADB-FUBINACA as the  
307 absence of multiplicity of the signal (singlet) enhances the S/N ratio of the resonance.

### 308 3.4.2. Quantitative analyses of SCs in e-liquids

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

313 HF proton and fluorine NMR are recognized as quantitative methods in forensic [25,  
314 31, 43] but have never been applied to e-liquids to our knowledge. The results reported in  
315 Table 4 show that, even if a matrix effect could bias the <sup>1</sup>H NMR quantification, data obtained  
316 by both techniques are in good agreement. Indeed, the linear regression equation of the HF <sup>1</sup>H  
317 versus <sup>19</sup>F values display a slope of 0.99 ± 0.03 (p-value = 0), a y-intercept of -0.34 ± 0.46  
318 and a correlation coefficient of 0.98 (p-value 1.1×10<sup>-22</sup>). The p-value of 0.55 given by the  
319 Wilcoxon signed-rank test demonstrates the absence of significant differences between these  
320 two data sets. The same observation can be done when comparing LF <sup>19</sup>F versus HF <sup>19</sup>F  
321 quantifications: a slope of 0.90 ± 0.03 (p-value = 0), a y-intercept of 1.22 ± 0.47, a correlation  
322 coefficient of 0.97 (p-value = 1.6×10<sup>-21</sup>) and a p-value of 0.74 for the Wilcoxon signed-rank

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

326 As shown in Table 4, the 13 e-liquids contained variable concentrations of SCs:  
327 samples **1-3** contained the lowest amount of SCs with JWH-210 at around 1 mg/mL, whereas  
328 sample **13** contained more than 20 mg/mL of 5F-AKB48. The contents in 5F-MDMB-PICA,  
329 5F-ADB, and ADB-FUBINACA ranged from  $\approx 7$  to  $\approx 18$  mg/ml in samples **4-12**. To our  
330 knowledge, there are no published quantitative data on SCs in e-liquids for discussion of these  
331 results. However, even if the toxicological incidence is not the direct purpose of this study, we  
332 must highlight the high CB1/CB2 binding potency of the third generation of SCs which  
333 include the fluorinated SCs detected in this study [10, 15, 48, 49]. For instance, ADB-  
334 FUBINACA is a highly potent agonist of CB1 ( $\text{EC}_{50} = 1.2$  nM) [15] by comparison to JWH  
335 210 ( $\text{EC}_{50} = 25.3$  nM) [49] or THC ( $\text{EC}_{50} = 172$  nM) [15]. This high affinity of SCs for  
336 cannabinoids receptors along with their significant concentration in e-liquids obviously poses  
337 a threat to the health of consumers.

## 338 **Conclusion**

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

## 352 **Captions for Figures**

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

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

360 **Figure 3.** <sup>19</sup>F NMR spectra of e-liquid samples recorded in MeOD at 376 MHz (HF) and  
361 56.17 MHz (LF).

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529

Figure 1

(A)

Sample 3

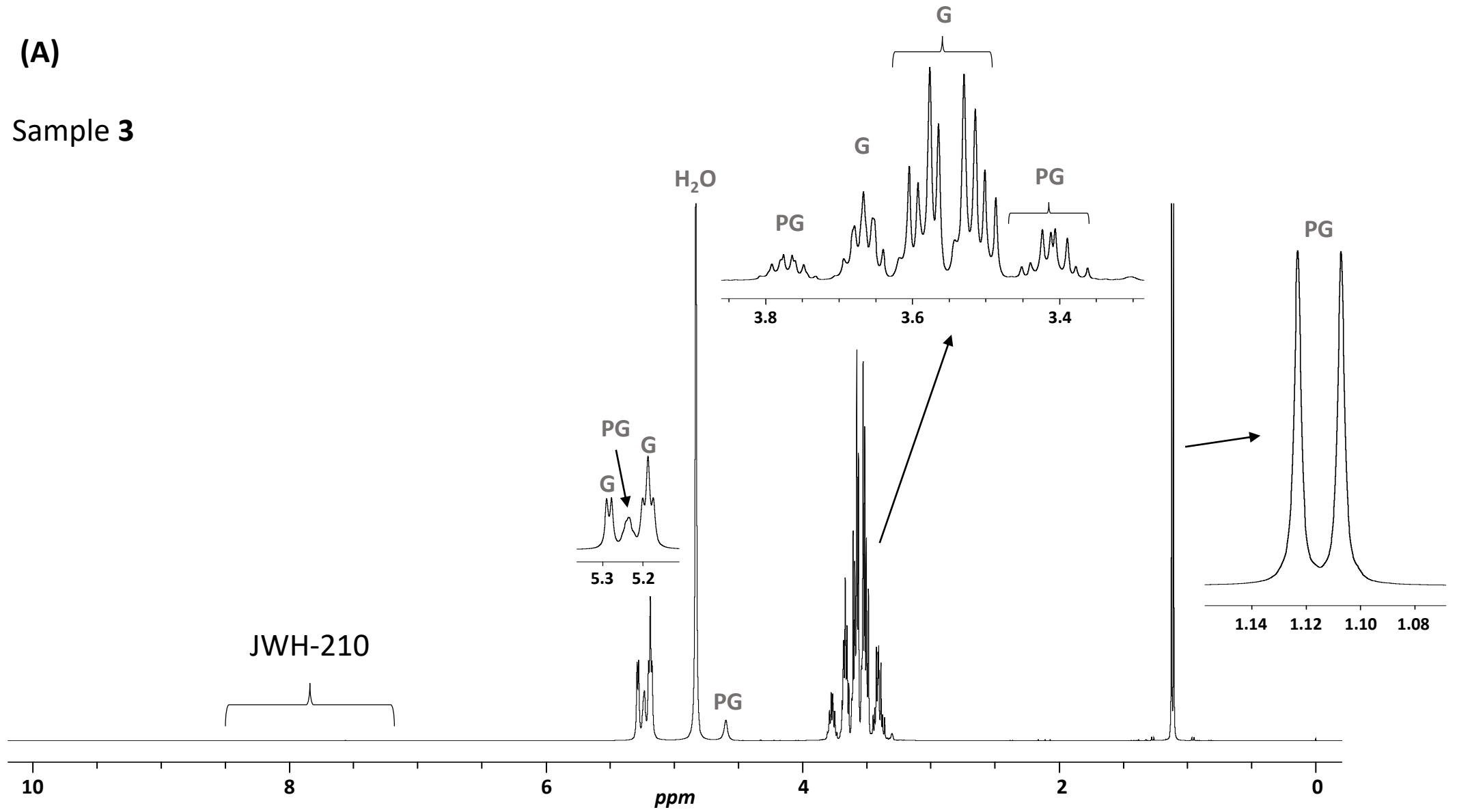


Figure 1

(B)

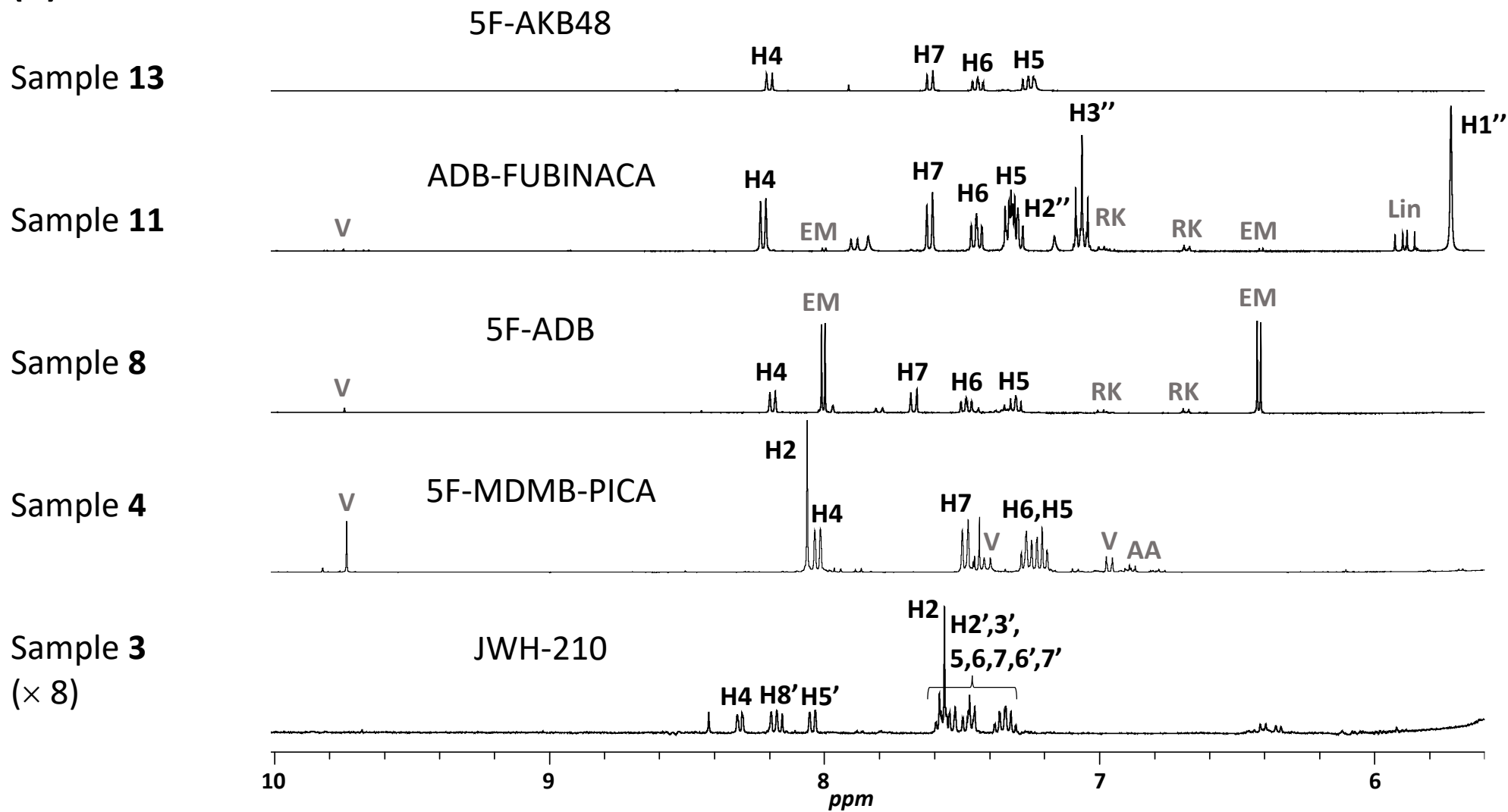
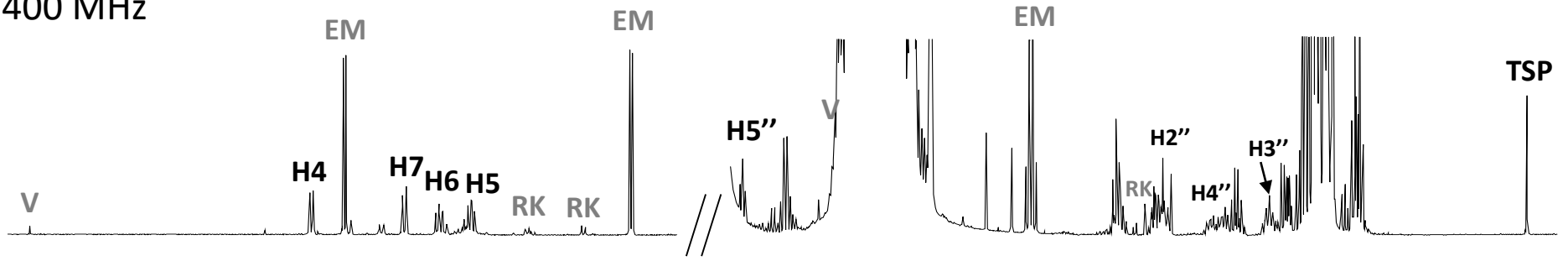


Figure 2

(A)

Sample 8 (5F-ADB)

400 MHz



60 MHz

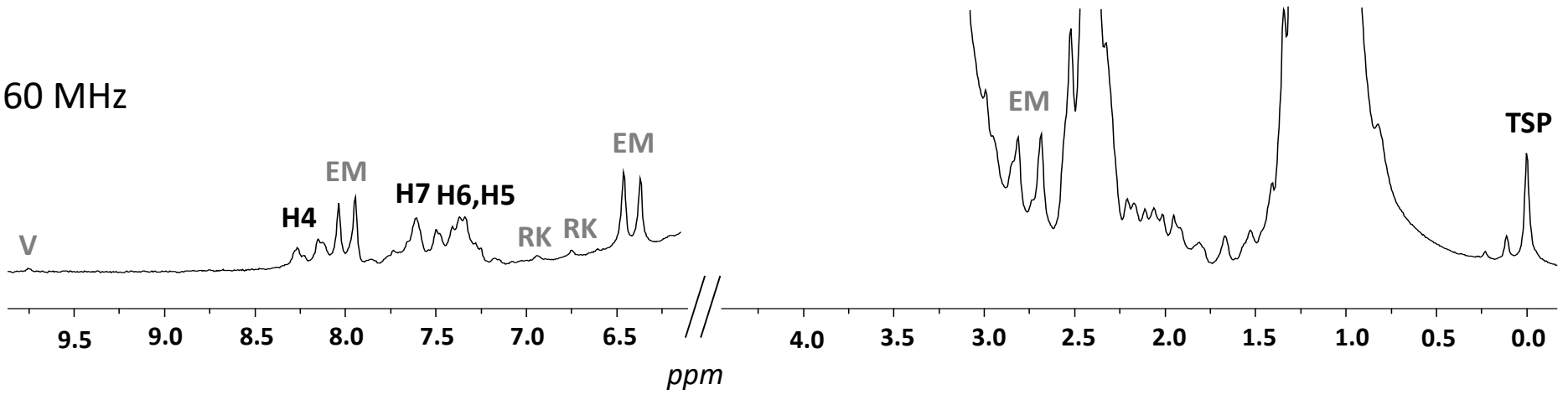


Figure 2

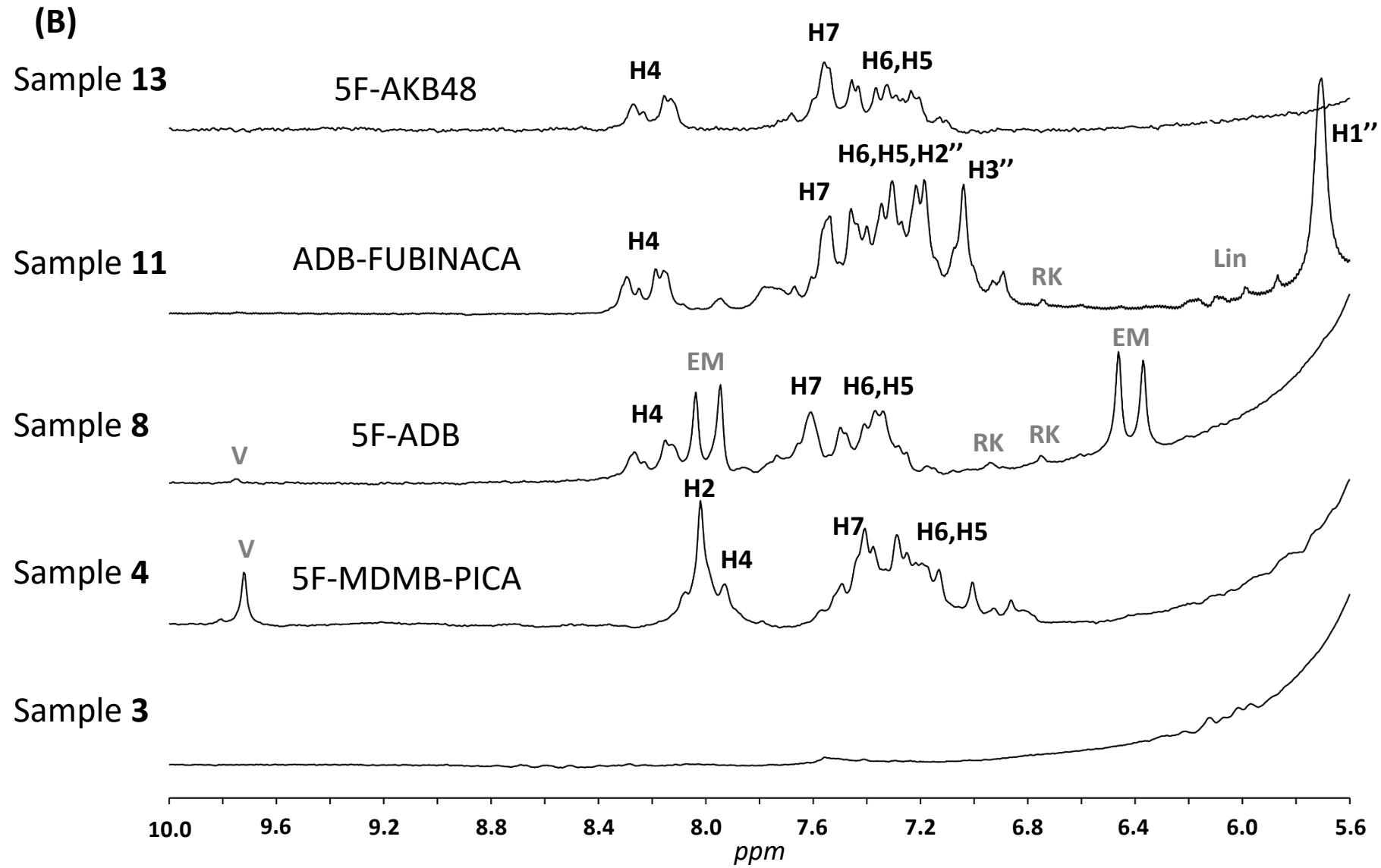
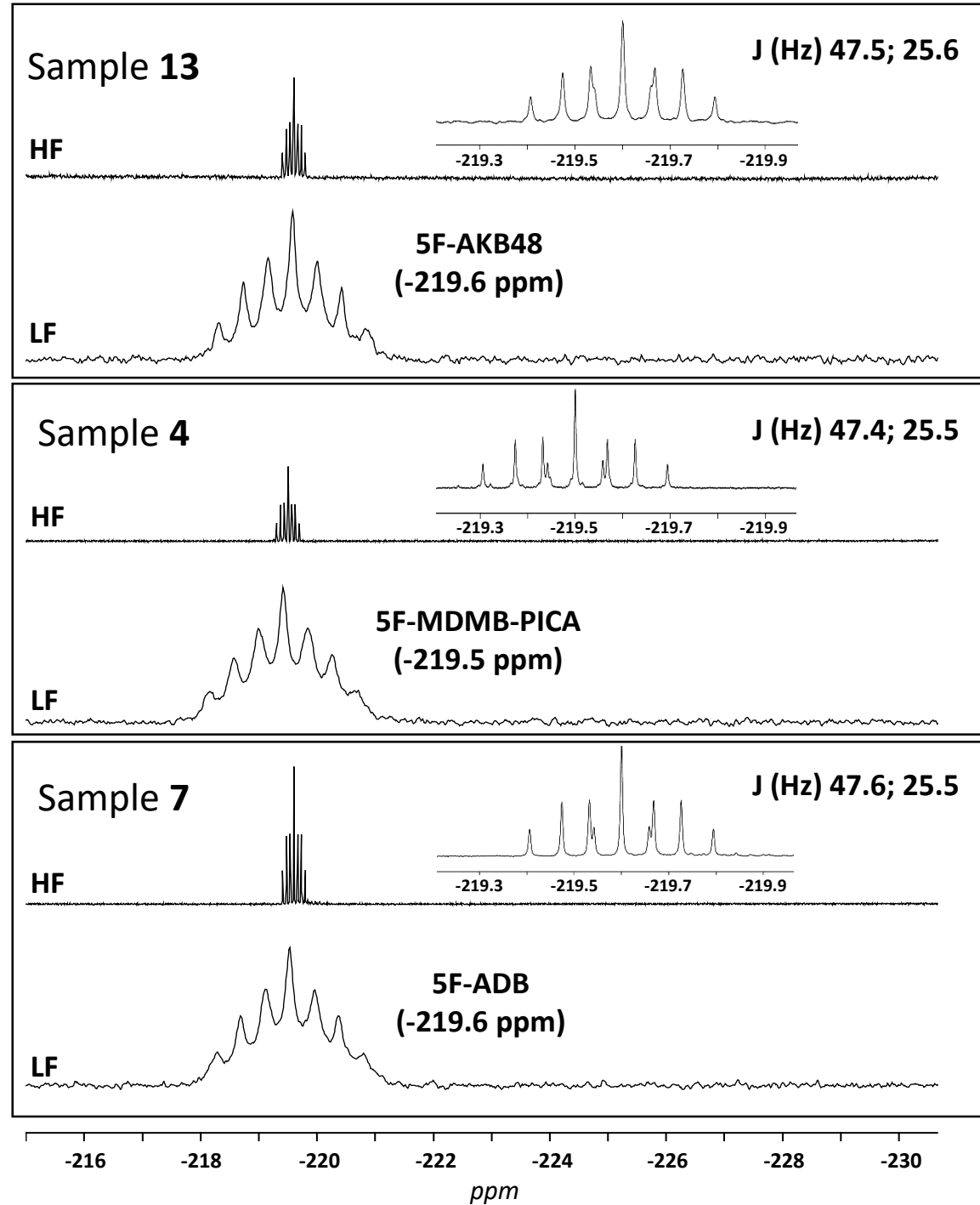
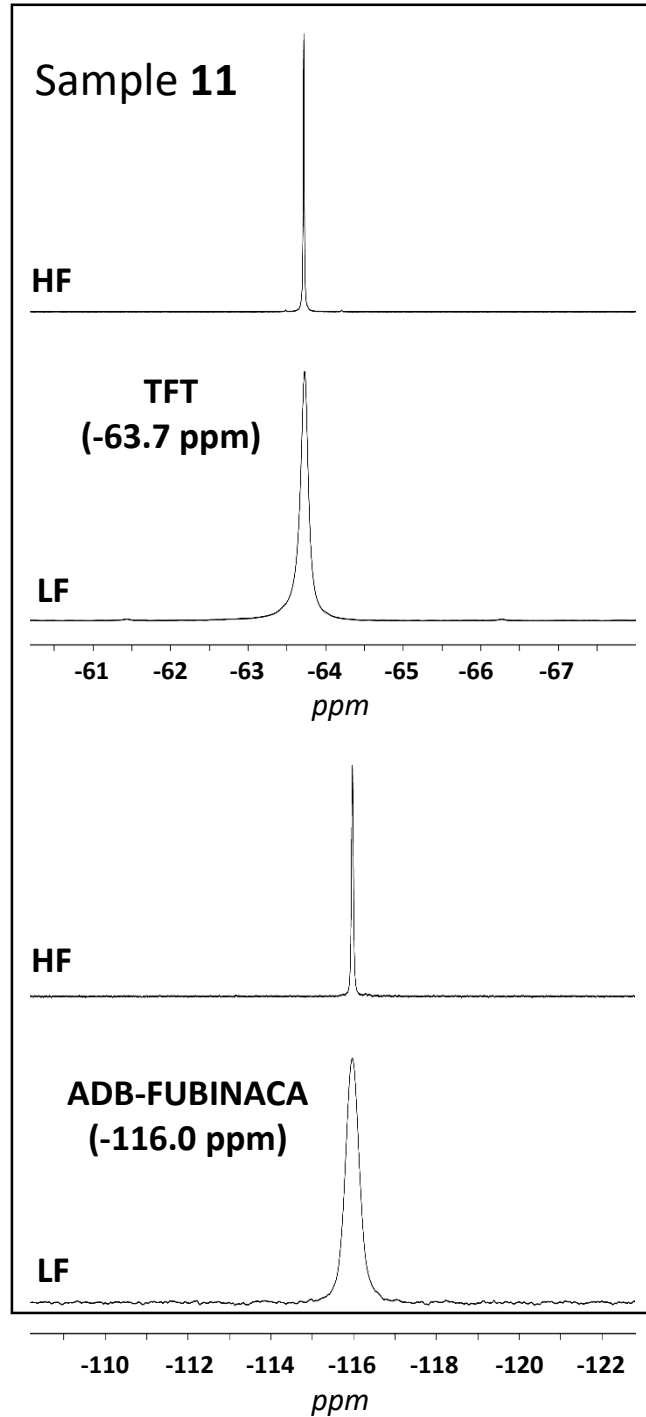


Figure 3



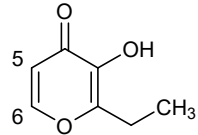
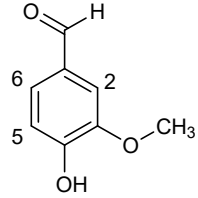
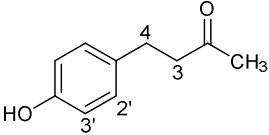
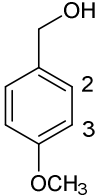
**Table 1.** High-field <sup>1</sup>H NMR (400 MHz) characteristic signals of synthetic cannabinoids detected in e-liquid samples recorded in MeOD. Signals in bold were used for quantification.

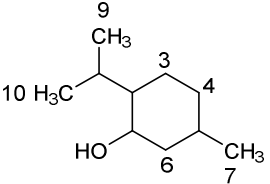
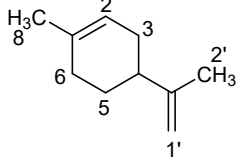
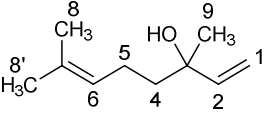
	<b>JWH-210</b>			<b>5F-MDMB-PICA</b>			<b>5F-ADB</b>			<b>ADB-FUBINACA</b>			<b>5F-AKB48</b>		
<sup>1</sup> H label <sup>a</sup>	δ (ppm)	M <sup>b</sup>	J (Hz)	δ (ppm)	M <sup>b</sup>	J (Hz)	δ (ppm)	M <sup>b</sup>	J (Hz)	δ (ppm)	M <sup>b</sup>	J (Hz)	δ (ppm)	M <sup>b</sup>	J (Hz)
2	7.56	s		8.06	s										
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		<b>7.31</b>	<b>m</b>		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	<b>7.68</b>	<b>d</b>	<b>8.6</b>	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'	<b>8.04</b>	<b>d</b>	<b>8.4</b>	/			/			/			/		
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	<b>5.72</b>	<b>s</b>		4.48	t	7.0
2''	1.78	quin	7.5	<b>1.92</b>	<b>quin</b>	<b>7.5</b>	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	/			<b>4.39</b>	<b>dt</b>	<b>47.5/6.0</b>
CH <sub>3</sub> -CH <sub>2</sub>	1.42	t	7.5	/			/			/			/		
(CH <sub>3</sub> ) <sub>3</sub>	/			1.09	s		1.08	s		1.09	s		/		

<sup>a</sup> Proton signals mentioned as “overlap” or missing (CH<sub>3</sub>-OCO-, CH<sub>3</sub>-CH<sub>2</sub>) could not be readily detected due to their overlap with other signals.

<sup>b</sup> 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  $^1\text{H}$  NMR (400 MHz, MeOD) and GC-MS data of flavouring compounds detected in the e-liquids analyzed. Only detected  $^1\text{H}$  NMR signals are reported.

Compound	Structure	$^1\text{H}$ NMR		GC-MS		Detected in samples
		$\delta$ (ppm), multiplicity <sup>a</sup> (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 <sub>2</sub> 1.22, t (7.6), CH <sub>3</sub>	9.89	C <sub>7</sub> H <sub>8</sub> O <sub>3</sub> 140.0467 (0.7)	55, 69, 71, 97, 139	<b>7-12</b>
Vanillin		9.74, s, CHO 7.46, m, H6 7.44, s, H2 6.96, d (8.5), H5 3.92, s, -O-CH <sub>3</sub>	15.00	C <sub>8</sub> H <sub>8</sub> O <sub>3</sub> 152.0462 (3.9)	81, 109, 123, 151	<b>4-9, 11, 12</b>
Raspberry ketone		7.00, d (8.5), H2' 6.69, d (8.5), H3' 2.11, s, CH <sub>3</sub>	19.18	C <sub>10</sub> H <sub>12</sub> O <sub>2</sub> 164.0834 (1.2)	107, 131, 149	<b>8, 10, 11</b>
Anisyl alcohol		6.88, d (8.7), H3 4.52, s, CH <sub>2</sub>	12.04	C <sub>8</sub> H <sub>10</sub> O <sub>2</sub> 138.0676 (0.7)	77, 109, 121	<b>4</b>

Menthol		1.61, m, H3 (1H) 0.92, d (7.1), CH <sub>3</sub> 9 0.91, d (6.6), CH <sub>3</sub> 7 0.84, m, H3 (1H) 0.79, d (6.9), CH <sub>3</sub> 10	9.65	C <sub>10</sub> H <sub>20</sub> O 156.1519 (3)	71, 81, 95, 123, 138	<b>13</b>
Limonene		4.68, m, H1' 1.62, broad s, CH <sub>3</sub> 8	5.50	C <sub>10</sub> H <sub>16</sub> 136.1245 (1.5)	68, 79, 93	<b>6</b>
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 <sub>3</sub> 8,8' 1.23, broad s, CH <sub>3</sub> 9	7.19	C <sub>10</sub> H <sub>18</sub> O 154.1360 (2)	71, 80, 93, 121, 136	<b>10, 11</b>

<sup>a</sup> s: singlet; d: doublet; dd: doublet of doublet; t: triplet; q: quadruplet; m: multiplet.

**Table 3.** T<sub>1</sub> values measured in e-liquid samples.

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

<sup>a</sup> Mean values from samples **3**, **4**, **7**, **11** and **13**.

<sup>b</sup> Mean values from samples **4**, **7**, **11** and **13**.

<sup>c</sup> <sup>19</sup>F NMR measurements were done on samples containing the relaxation agent Cr(acac)<sub>3</sub>.

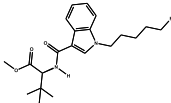
**Table 4.** Amounts of synthetic cannabinoids (SC) in 13 e-liquids measured with high-field  $^1\text{H}$  and  $^{19}\text{F}$  NMR and low-field  $^{19}\text{F}$  NMR. All experiments were done in triplicate from sampling the e-liquid to processing the NMR spectrum.

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

<sup>a</sup> JWH-210 is not fluorinated.



Profiling



Q-NMR

