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► To cite this version:

Nao Wu, Saïda Danoun, Stéphane Balayssac, Myriam Malet-Martino, Véronique Gilard, et al.. Synthetic cannabinoids in e-liquids: A proton and fluorine NMR analysis from a conventional spectrometer to a compact one. *Forensic Science International*, 2021, 324, pp.110813. 10.1016/j.forsciint.2021.110813 . hal-03245107

HAL Id: hal-03245107

<https://hal.science/hal-03245107v1>

Submitted on 24 May 2023

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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 ^1H 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 ^1H 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, ^{19}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 (^1H) and fluorine (^{19}F) NMR analyses were
61 carried out with both HF (400 MHz for ^1H) and LF (60 MHz for ^1H) 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), α,α,α -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 μl of each e-liquid withdrawn with a glass precision syringe were diluted with 400
77 μl of methanol-d4 (MeOD) and mixed with 30 μl of a TSP solution (10 mM in MeOD) as
78 reference for ^1H NMR experiments. ^{19}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}F NMR experiments, 200 μl of sample were diluted with 400
81 μl of MeOD and then mixed with 9 μl 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}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 ^1H 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}F direct detection. Typical acquisition parameters optimized for quantitative
92 experiments were as follows: number of scans (NS) 128, pulse width 4.52 μs (flip angle 30°),
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 ^1H 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}F NMR experiments were recorded with the same spectrometer and probe at 376
101 MHz. First, a basic zg sequence allowed to acquire ^1H -coupled ^{19}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 μs (flip angle 30°), 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 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 PulsarTM benchtop NMR spectrometer (Oxford
110 Instruments, Abingdon, UK) operating at a frequency of 59.7 MHz for ^1H and 56.17 MHz for
111 ^{19}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 ^1H NMR spectra were recorded with a flip angle of 90° (12.5 μs), 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 ≈ 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}F NMR spectra were recorded with a flip angle of 90° , 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}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 ¹H 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 ¹⁹F 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 ¹⁹F 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 ¹⁹F NMR versus HF ¹H NMR and versus LF ¹⁹F
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 μ l of each e-liquid sample
154 were then mixed with 500 μ l of CHCl₃. 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 μ 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 ¹H NMR qualitative analysis of e-liquids

166 In the first step of this study, the 13 e-liquid samples were screened by HF ¹H 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 ¹H 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 ¹H 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; CH₃) 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) (-CH₂), 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 ^1H 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 ^2J 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 (≈ 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 ^1H 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 ^1H 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 ^1H NMR profiling

227 All samples were then analysed on the benchtop 60 MHz ^1H NMR spectrometer using
228 the same solutions in the same NMR tubes than for HF recording. As an illustration, the HF
229 and LF ^1H 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 ^1H 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 ^1H 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 ^1H NMR experiments on the
250 compact spectrometer because the method was not suitable for quantitative purposes in this
251 study.

252 3.3. ^{19}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 ^{19}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}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}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 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 ≈ 6 Hz at LF. They appear as
270 triplet of triplet at HF due to the coupling of the ^{19}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}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}F NMR.
281 Indeed, HF NMR is recognized as a powerful method for quantification of compounds in
282 various mixtures for both ^1H [24, 25, 43-45] and ^{19}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 ^1H and ^{19}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 ^1H 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 ¹⁹F NMR quantifications,
291 the relaxation agent Cr(acac)₃ was added into the NMR tube to shorten the relaxation times of
292 the ¹⁹F nuclei. The T₁s of the ¹⁹F of the four fluorinated SCs in e-liquids are reported in Table
293 3. The longest T₁ 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 T₁ 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 ¹⁹F 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 ¹⁹F nuclei were thus completely relaxed since more than 99.9% of
298 the ¹⁹F magnetization was recovered.

299 Recording times were set at 2.5 min (32 scans) and 90 min (2048 scans) for HF and
300 LF ¹⁹F 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 ¹H NMR quantification, a characteristic signal could be integrated for
311 each SC despite some overlaps. The ¹⁹F 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 ¹H NMR quantification, data obtained
316 by both techniques are in good agreement. Indeed, the linear regression equation of the HF ¹H
317 versus ¹⁹F 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 1.1×10⁻²²). 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 ¹⁹F versus HF ¹⁹F
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 = 1.6×10⁻²¹) 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}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 ≈ 7 to ≈ 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 ^1H and ^{19}F NMR.
340 HF ^1H NMR allowed a holistic analysis of SCs and flavouring compounds in the e-liquids. In
341 addition, the ability of benchtop LF ^1H and ^{19}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 ^1H 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}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 ¹H
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.** ¹⁹F 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

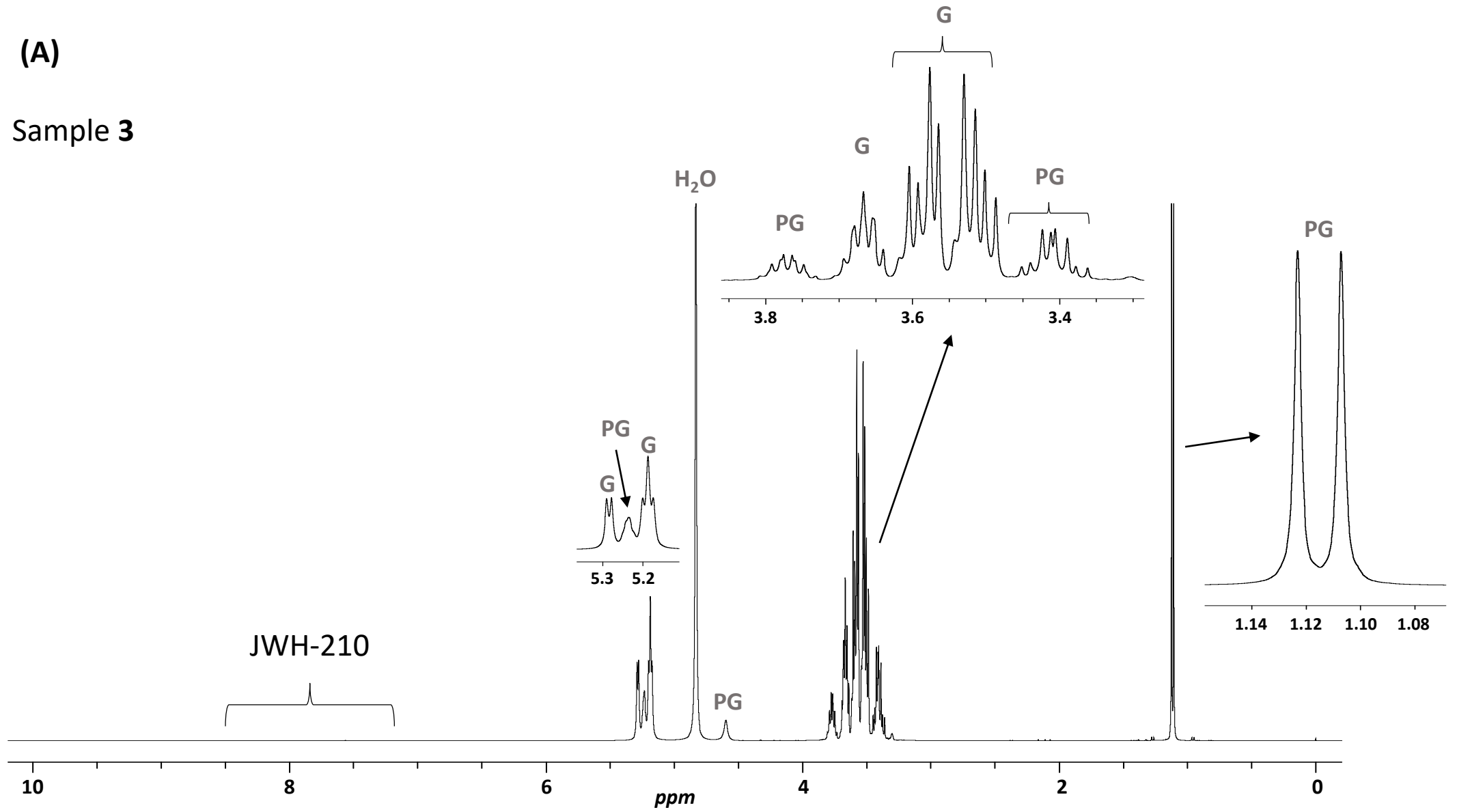


Figure 1

(B)

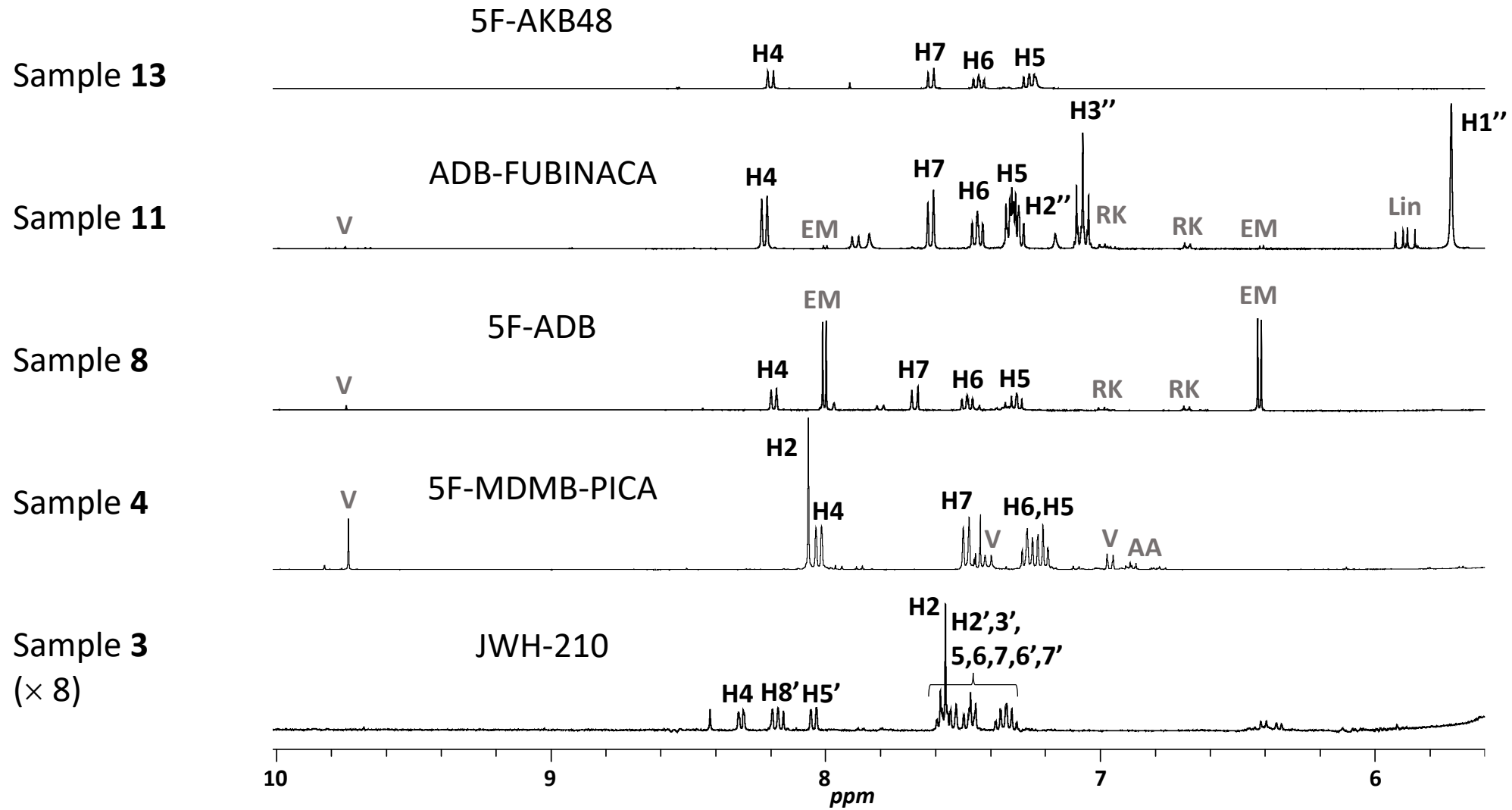
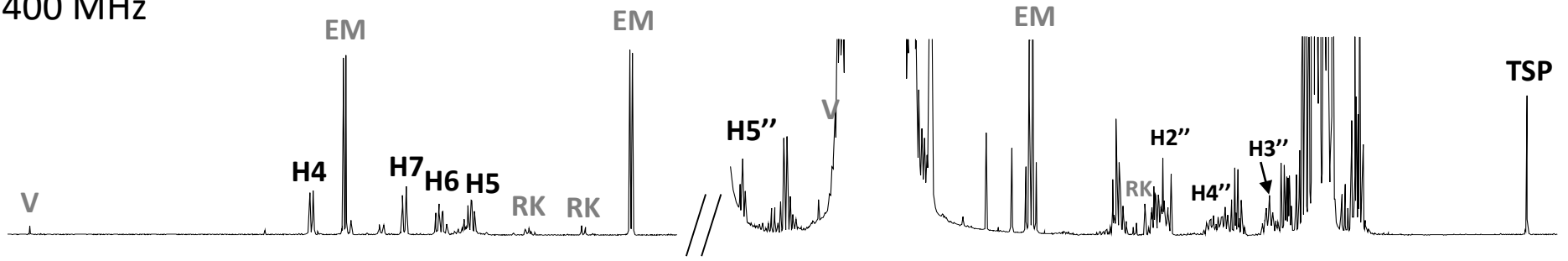


Figure 2

(A)

Sample 8 (5F-ADB)

400 MHz



60 MHz

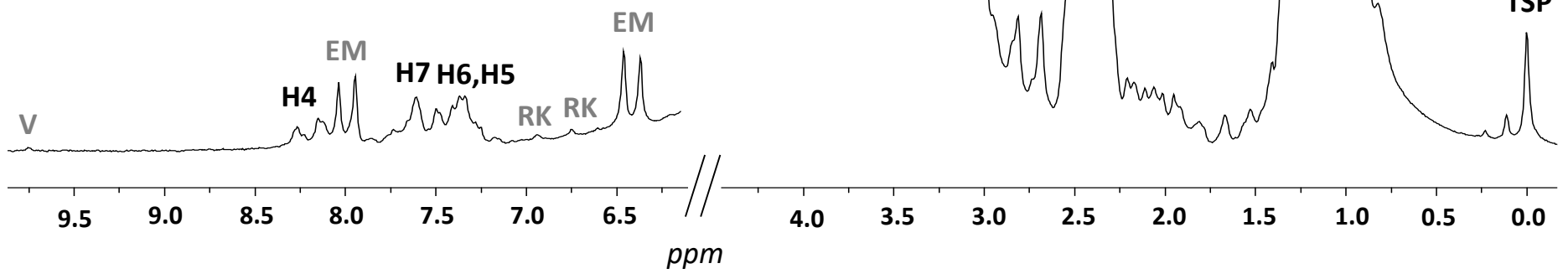


Figure 2

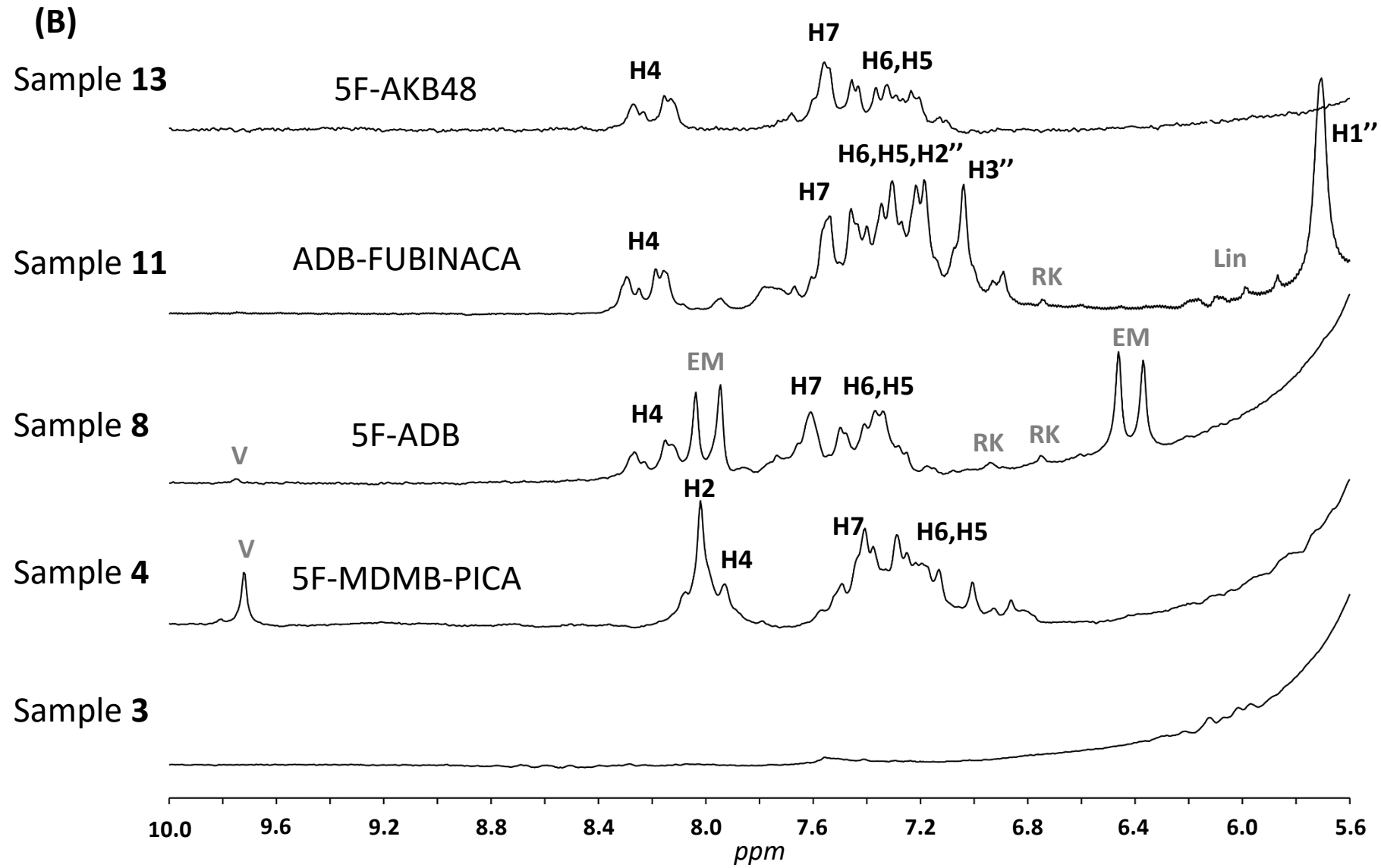


Figure 3

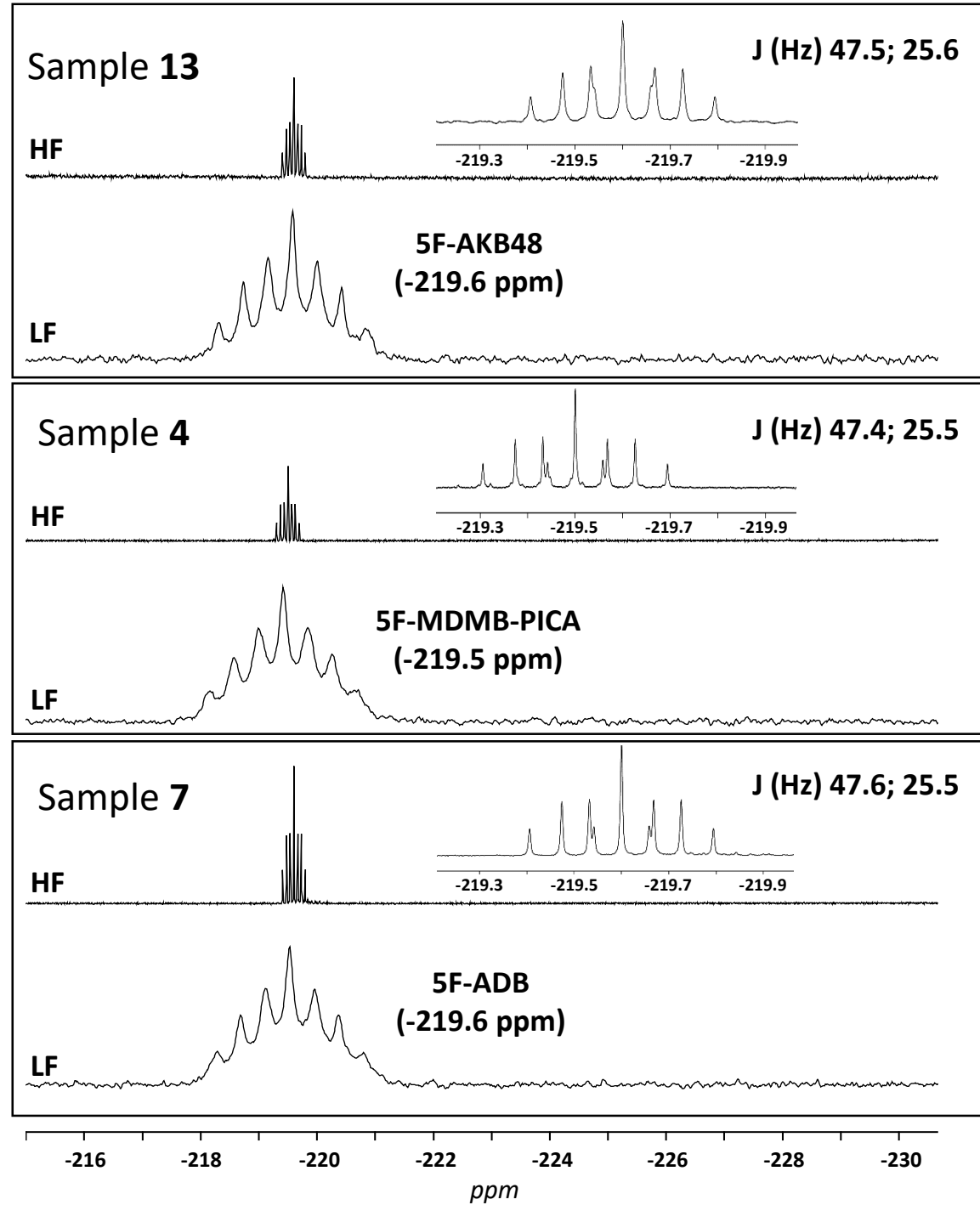
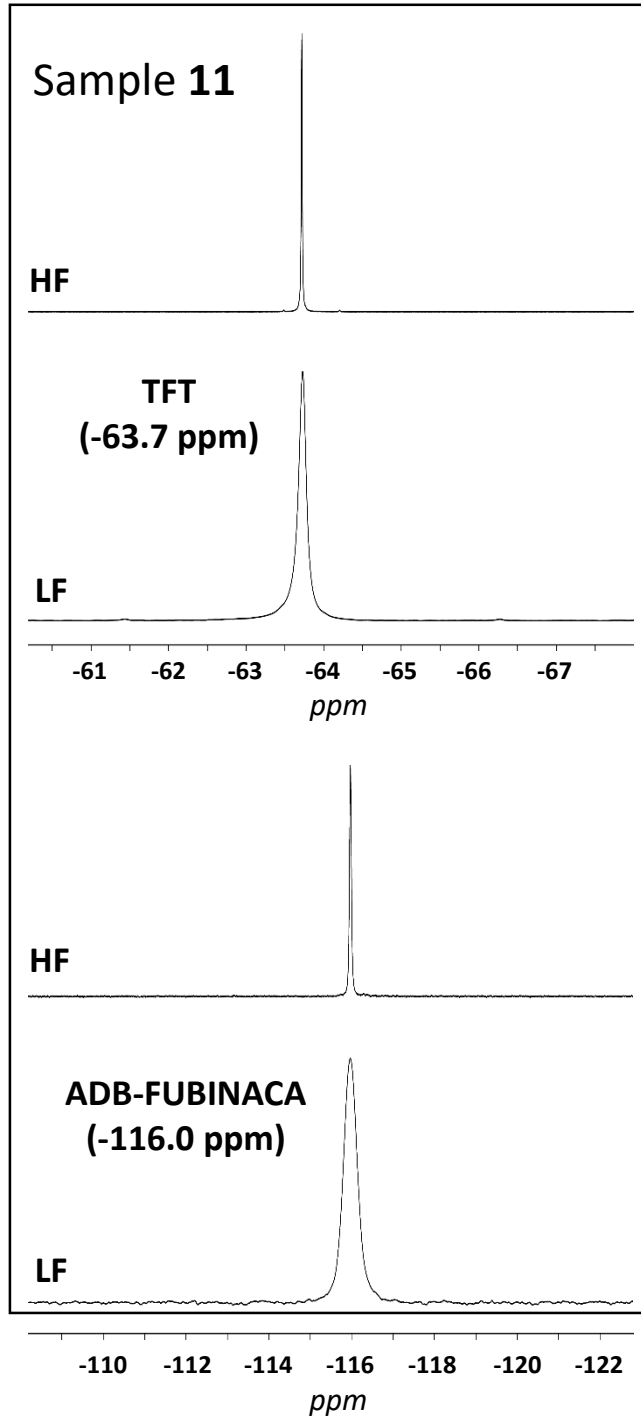


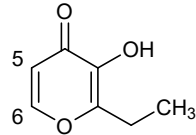
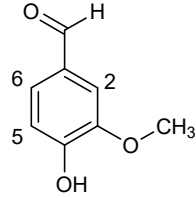
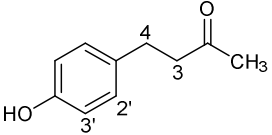
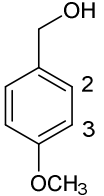
Table 1. High-field ¹H NMR (400 MHz) characteristic signals of synthetic cannabinoids detected in e-liquid samples recorded in MeOD. Signals in bold were used for quantification.

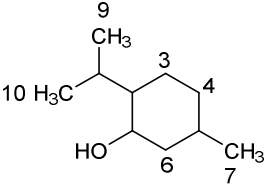
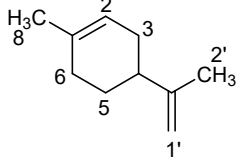
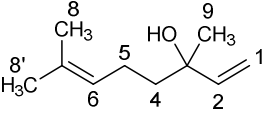
	JWH-210			5F-MDMB-PICA			5F-ADB			ADB-FUBINACA			5F-AKB48		
¹ H label ^a	δ (ppm)	M ^b	J (Hz)	δ (ppm)	M ^b	J (Hz)	δ (ppm)	M ^b	J (Hz)	δ (ppm)	M ^b	J (Hz)	δ (ppm)	M ^b	J (Hz)
2	7.56	s		8.06	s										
4	8.31	dd	7.0/1.3	8.02	d	7.8	8.20	d	8.2	8.22	d	8.2	8.20	d	8.2
5	7.30-7.60	m		7.21	m		7.31	m		7.31	m		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	^1H 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, H3 (1H) 0.92, d (7.1), CH ₃ 9 0.91, d (6.6), CH ₃ 7 0.84, m, H3 (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 T ₁ (s)	¹⁹ F NMR ^c T ₁ (s)	¹⁹ F NMR ^c 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.

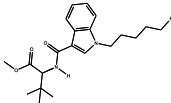
Table 4. Amounts of synthetic cannabinoids (SC) in 13 e-liquids measured with high-field ^1H and ^{19}F NMR and low-field ^{19}F NMR. All experiments were done in triplicate from sampling the e-liquid to processing the NMR spectrum.

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

