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Discriminating different Cannabis sativa L. chemotypes using attenuated total reflectance - infrared (ATR-FTIR) spectroscopy: A proof of concept

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(Article begins on next page)

1 **Discriminating different *Cannabis Sativa* L. chemotypes using attenuated total**
2 **reflectance - infrared (ATR-FTIR) spectroscopy: a proof of concept**

3

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25 **Abstract**

26

27 An original, innovative, high-throughput method based on attenuated total reflectance -
28 Fourier's transform infrared (ATR-FTIR) spectroscopy has been developed for the
29 proof-of-concept discrimination of fibre-type from drug-type *Cannabis Sativa* L.
30 inflorescences. The cannabis sample is simply placed on the instrument plate and
31 analysed without any previous sample pretreatment step. In this way, a complete
32 analysis lasts just a few seconds, the time needed to record an ATR-FTIR spectrum. The
33 method was calibrated and cross validated using data provided by liquid
34 chromatography - tandem mass spectrometry (LC-MS/MS) analysis of the different
35 cannabis samples and carried out the statistical assays for quantification. During cross-
36 validation, complete agreement was obtained between ATR-FTIR and LC-MS/MS
37 identification of the cannabis chemotype. Moreover, the method has proved to be
38 capable of quantifying with excellent accuracy (75-103% vs. LC-MS/MS) seven neutral
39 and acidic cannabinoids (THC, THCA, CBD, CBDA, CBG, CBGA, CBN) in
40 inflorescences from different sources. The extreme feasibility and speed of execution
41 make this ATR-FTIR method highly attractive as a proof-of-concept for a possible
42 application to ~~on-the-spot~~ quality controls during ~~cultivation and~~ pharmaceutical
43 product manufacturing, as well as on-the-street cannabis controls and user counselling.
44 ~~also opening the opportunity to use by non scientifically trained personnel.~~

45

46 **Keywords:** *Cannabis sativa* L., ATR-FTIR, LC-MS/MS, cross-validation

47

48 1. INTRODUCTION

49 *Cannabis sativa* L. is currently believed to be one of the first plants to be cultivated by
50 man, probably since at least eight millennia ago. Since then, it has been used all over the
51 world as a source of textile, building, writing and food/feed material, as well as for
52 religious and recreational purposes.

53 In relatively recent times, this plant has been subjected to increasingly restricting
54 legislation, due to the psychoactive effects [1] and possible neurotoxicity [2] of some of
55 its cannabinoid constituents, mainly Δ^9 -tetrahydrocannabinol (THC, Figure 1a) and its
56 precursor Δ^9 -tetrahydrocannabinolic acid (THCA, Figure 1b). THCA is easily
57 decarboxylated to THC by heat, light and other stress sources.

58 However, since the 1970s several countries have begun reducing their restricting
59 regulations, also due to the fact that scientifically-based medical uses for cannabis
60 products have been found, in the treatment of emesis, muscle spasms [3] and some
61 forms of seizures [4]. Other medical applications are still under investigation, for
62 example against neurodegenerative disorders [5], with very promising results. The
63 muscle-relaxing effects of cannabis are largely attributed to both THC and another, non-
64 psychoactive cannabinoid, namely cannabidiol (CBD, Figure 1c) [6], which is in turn
65 formed by decarboxylation of cannabidiolic acid (CBDA, Figure 1d). Putative
66 neuroprotective activities are currently linked mainly to CBD [7].

67 In most countries, authorities have generally classified three chemotypes of cannabis,
68 according to their respective “total CBD” / “total THC” ratio, i.e., $([CBD] + [CBDA]) /$
69 $([THC] + [THCA])$ ratio (C/T): THC-predominant, drug-type or “medical cannabis”
70 $(C/T \leq 0.005)$, CBD-predominant type, fibre-type or “industrial hemp” $(C/T \geq 15)$ and
71 intermediate type $(0.5 \leq C/T \leq 3)$ [8]. However, these numbers can vary according to

72 the specific source: for example, the United Nations Office on Drugs and Crime
73 (UNODC) [9] and the American Herbal Pharmacopoeia (AHP) [10] define “fibre-type”
74 cannabis as having $C/T < 1$, “drug-type” cannabis as having $C/T > 1$ and intermediate
75 cannabis as having $C/T \approx 1$. Moreover, specific laws usually prescribe maximum
76 absolute THC levels for cannabis products to be considered non-narcotic and thus its
77 farming to be considered legal. This concentration varies according to the specific State,
78 but it is usually in the tenths of percent range (for example, in the EU it is set at 0.2%
79 [11], in the USA it is 0.3% [12]).

80 Given this legislative frame, reliable and accurate methods for the determination of
81 THC, CBD and their acidic precursors in cannabis plant part samples and derived
82 products are obviously needed, also due to the relatively high cannabinoid content
83 variability in the plant material, which finally reverberates in the inconsistency of the
84 finished product quality parameters [13]. Moreover, more comprehensive information is
85 also desirable in addition to simple THC content and C/T values, for example with the
86 determination of other main cannabinoids contained in the plant: e.g., cannabigerol
87 (CBG), cannabigerolic acid (CBGA), cannabinol (CBN) and others still (chemical
88 structures in Figure 1e-g). These cannabinoids seem to have a significant role in the
89 modulation of many cannabis product effects, with important repercussions on safety
90 and efficacy [14].

91 Due to forensic requirements, most analytical methods for cannabinoids (in particular
92 for THC) rely on highly specialised and expensive equipment [15,16], mainly based on
93 liquid chromatography with tandem mass spectrometry (LC-MS/MS) [17-20] and gas
94 chromatography (GC)-MS [21,22], although other methods are also available, such as
95 electrochemical sensors [23], HPLC with photodiode array (PDA) detection [24] and

96 thin layer chromatography (HPTLC)-MS [25]. These methods also require highly
97 trained personnel to perform accurate and reliable analyses and they can mainly be
98 applied in high-level laboratory settings.

99 On the other hand, faster and high-throughput methods for cannabinoid analysis would
100 be attractive, e.g. for on-the-spot quality controls of their products by farmers,
101 intermediaries and manufacturers, and especially for products intended for medical use
102 and also for user counselling in public spaces and for possible screening application on
103 the street, during controls by law enforcement agencies. Only recently spectroscopic
104 techniques have been considered fit for this purpose, mainly due to their relative lack of
105 selectivity coupled to the highly complex composition of cannabis plant material. To the
106 best of our knowledge, just two papers of this kind can be found in literature. the first
107 one carries out the determination of THC, THCA, CBD and CBDA by mid-IR
108 spectroscopy [13], thus it does not include CBN, CBG and CBGA like the present
109 method does. The second one determines eight cannabinoids by near infrared (NIR)
110 spectroscopy but is not able to discriminate acidic from neutral forms of cannabinoids
111 [26]. ¹³C-qNMR has also been successfully applied to the quantitative analysis of non-
112 psychoactive cannabinoids in different samples fibre-type inflorescences [27].

113 In the following paper, we are presenting the proof of concept of an original method for
114 quantitative analysis of the main seven cannabinoids in cannabis inflorescences, based
115 on attenuated total reflectance – Fourier’s transform infrared (ATR-FTIR) spectroscopy,
116 using cross-validation with four calibration groups. ATR-FTIR is particularly attractive,
117 since it can be directly applied to the intact cannabis sample, which is simply laid on the
118 instrument sample plate and pressed by a calibrated screw. No sample pretreatment is
119 needed, effectively increasing potential throughput by orders of magnitude. Moreover,

120 this kind of analysis can easily be carried out by using portable instrumentation ~~and by~~
121 ~~non-scientifically trained personnel~~. Thus, it is an ideal candidate for use during on-the-
122 spot quality checks by manufacturers, as well as for law enforcement controls, subject
123 to subsequent confirmation by MS methods.

124

125 **2. EXPERIMENTAL**

126

127 **2.1. Chemicals and Plant Material**

128 THC, THCA, CBD, CBDA and CBN standard solutions in acetonitrile (1 mg/mL), and
129 the corresponding deuterated standard solutions (used as internal standards, ISs), MS-
130 grade methanol, acetonitrile, acetone were purchased from Sigma Aldrich (Darmstadt,
131 Germany). CBG and CBGA standards were provided by Prof. Federica Pollastro's
132 research group as solid compounds purified from *C. sativa* inflorescences according to
133 the method described in a previously published method [28].

134 A total of 36 samples of *C. sativa* inflorescences were used in this study. Eight of them
135 were drug-type cannabis from police evidence, 14 of them were fibre-type cannabis
136 samples purchased on the free market from authorized sellers and 14 were "light"
137 cannabis samples at low THC concentration. No details were available on the specific
138 cultivar of each sample.

139

140 **2.2. Instrumentations and methods**

141 **2.2.1. ATR-FTIR Sample Pretreatment and Analysis**

142 Whole flowers were used as such for ATR-FTIR analysis, and no treatment (drying,
143 grinding, extraction) was carried out on them beforehand.

144 For ATR-FTIR analysis, a Thermo Scientific (Waltham, MA, USA) Nicolet iS5
145 instrument was used, equipped with an iD5 ATR cell and controlled by Thermo
146 Scientific Omnic software. Nicolet iS5 FTIR Spectrometer, equipped with an iD5
147 Diamond crystal ATR cell accessory (single-bounce ATR; crystal type: diamond with
148 ZnSe lens; sampling area: 1.5 mm) and controlled by Thermo Scientific Omnic 9
149 software (Version 9.9.473). Each spectrum was recorded as the average of 20
150 subsequent scans.

151 For preliminary assays, ATR-FTIR spectra in the 5000-400 cm^{-1} region for THC,
152 THCA, CBD, CBG, CBGA and CBN were obtained; The spectrum for pure CBDA was
153 taken from a published source [29], since the pure compound was not available (Figure
154 S1). These spectra were compared among them and with spectra from different kinds of
155 cannabis inflorescences, including fibre-type cannabis, from drug-type cannabis and
156 also from other non-cannabis plant material (such as *Humulus lupulus* L. belonging to
157 the Cannabaceae family as well). Other fibre-type plant spectra still were obtained from
158 the Toronto Forensic database.

159 For the analysis of cannabis inflorescences, each individual floret was placed on the cell
160 plate and squashed with the calibrated screw for spectrum acquisition in the 4000-400
161 cm^{-1} range with a resolution of 4 cm^{-1} . Three spectra were acquired from different
162 points of the same floret and averaged for quantitative analysis.

163 THC quantification was carried out using the first derivative of the signal in the 1415-
164 1485 cm^{-1} range; THCA quantification was carried out using the direct signal in the
165 1391-1414 cm^{-1} range; CBD was carried out using the first derivative of the signal in
166 the 3085-3060 cm^{-1} range; CBDA was carried out using the first derivative of the signal
167 in the 982-959 cm^{-1} range; CBG quantification was carried out using the direct signal in

168 the 844-830 cm^{-1} range; CBGA was carried out using the first derivative of the signal in
169 the 820-807 cm^{-1} range; CBN was carried out using the first derivative of the signal in
170 the 910-872 cm^{-1} range.

171 ATR-FTIR spectra of pure solid cannabinoids were obtained from the Toronto Forensic
172 database running in TQ Analyst 9 software (Version 9.7.0.27). QCheck software was
173 used for quantitative analytical data treatment. PLS regression models were developed
174 by using the TQ Analyst 9 software. For spectra comparison and quantitative analytical
175 data treatment, the QCheck function of the Omnic software was exploited.

176

177 **2.2.2. LC-MS/MS Sample Pretreatment and reference analysis**

178 The same cannabis samples previously analysed were extracted and analysed a second
179 time by means of a published, validated method [19]. In order to extract the analytes, 50
180 mg of plant material, finely cut, were pretreated by the addition of 10 mL of MeOH
181 (containing ISs at the concentration of 10 ng/mL) and subject to an ultrasonic bath for
182 30 min. Then, the solution was agitated for 1 min by means of vortex and subject again
183 to ultrasonic bath for another 15 min. After extraction, the supernatant was filtered
184 through an Agilent (Palo Alto, USA) membrane filter (13 mm membrane, 0.20 μm ,
185 nylon) and then the MeOH was evaporated through a rotary evaporator to obtain the dry
186 extract. Before injection into the analysis system, it was dissolved into a 50:50 (v/v)
187 mixture of ACN and H_2O (1 mL).

188 The LC instrumentation consisted in a Waters Alliance e2695 chromatographic pump
189 system equipped with an autosampler. Chromatographic conditions for analyte
190 separation were: a reverse phase Waters Cortecs C18+ column (100 mm \times 2.1 mm i.d.,
191 2.7 μm), equipped with a guard column, as the stationary phase; a mixture of 0.1% FA

192 in ACN (solvent A) and 0.1% FA in H₂O (solvent B) as the mobile phase, kept at a
193 constant flow rate of 0.3 mL/min. The analysis was carried out at room temperature and
194 the mobile phase composition gradient program was the following: starting at 50% of
195 solvent A, then linearly ramping up to 95% of solvent A over 5 min. This composition
196 was maintained for 5 min before returning to the starting conditions over 1 min
197 followed by 2 min of constant composition to re-equilibrate the system. The total run
198 time was 13 min, and injection volume was 10 μ L.

199 The acquisition was performed by the means of Waters Micromass Quattro Micro triple
200 quadrupole mass spectrometer, set in multiple reaction monitoring (MRM) mode. For
201 the acquisition of analyte signals both the positive (ESI+) and negative (ESI-) ion mode
202 were used, with ionization polarity switching. The following optimized settings were
203 used for the acquisition: ion source voltage, 4.0 kV; ion source temperature, 1250 $^{\circ}$ C;
204 desolvation temperature, 250 $^{\circ}$ C; desolvation gas (nitrogen) flow, 250 L/h; argon was
205 used as the collision gas. For each analyte and IS the dwell times per channel were set at
206 300 ms. Table 1 reports the precursor and product ions for each analyte and IS, with the
207 relative values of cone voltage and collision energy. Data were processed by using
208 Waters MassLynx 4.1 software.

209

210 **2.3. Data processing and calibration development**

211 The spectra from cannabis inflorescences obtained from ATR-FTIR analysis were fitted
212 to the content of cannabinoids determined by LC-MS/MS to develop the correspondent
213 calibration equations. Partial least squares (PLS) regression method was used to develop
214 predictive models. As for the PLS model construction, the best model performance
215 results automatically detected by the TQ Analyst software were obtained either without

216 pre-processing in the defined spectral ranges or with first derivative (FD) pre-treated
217 spectra for each analyte, as reported in section 2.2.1, while the number of components
218 (latent variables) automatically selected for each analyte were 3 for THCA, CBN,
219 CBDA and CBG, 4 for THC and CBGA, 6 for CBD. Possible disadvantages of
220 multivariate regression in terms of over-fitting of the calibration models has been
221 prevented by applying the “leave-one-out” internal cross-validation validation process,
222 in which the calibration set was split into four groups and PLS model calculations were
223 performed using all the calibration sample sets except one at a time [30]. Each group
224 was predicted using a calibration developed on the other samples, until all samples have
225 been used for validation and all the four sample group have been predicted.

226 Elimination of outlier samples (samples with abnormally high residuals of predicted
227 versus reference values) was not used, since no sample showed this characteristic in a
228 significant amount.

229 The performance of the calibration models was evaluated using the root mean square
230 error of cross-validation (RMSECV), the coefficient of determination for calibration
231 (r^2), the root mean square error of calibration (RMSEC), and the coefficient of
232 determination for cross validation (r^2_{CV}).

233 The predictive ability of the equations was assessed by external validation, by means of
234 a regression between ATR-FTIR predicted values and reference data: the models
235 developed with the strategies described above were applied to the validation samples
236 (never used for calibration). Comparison of real (LC-MS/MS) validation sample data
237 with predicted data according to calibrations provided the SEP value (standard error of
238 prediction) and slope and regression coefficients. Paired *t*-test was performed to

239 evaluate the significance of the differences between laboratory and predicted results of
240 models in the validation sets.

241

242 **2.4. Sample Inhomogeneity Evaluation**

243 A complete ATR-FTIR analysis was carried out on 5 different locations of each single
244 floret for six florets in the same sample, in order to evaluate sample inhomogeneity at
245 the floret level (intra-floret inhomogeneity); the results from each single floret were
246 then compared to those obtained from five other florets of the same sample, to evaluate
247 inter-floret inhomogeneity. This procedure was carried out for one randomly selected
248 sample of each class: drug-type, fibre-type and “light” *Cannabis*.

249

250

251 **3. RESULTS AND DISCUSSION**

252 **3.1. Preliminary assays**

253 It was decided from the beginning that no pretreatment whatsoever would be applied to
254 the plant inflorescences, so that ATR-FTIR analysis would be carried out on intact
255 samples, in the most similar fashion to possible real-world application of the method.

256 However, since the samples were of different origins and had already been stored in
257 different conditions and for variable amounts of time a successful method would be
258 demonstrated to be resilient to all these sources of variability and error.

259 The results of preliminary spectra comparisons showed that most fibre-type plant
260 spectra, including fibre-type cannabis spectra, had several zones lacking significant
261 specific absorption bands in the 3100-800 cm^{-1} range, whereas drug-type cannabis had
262 significant IR absorption bands in the same zones. Moreover, by comparing the same

263 spectra as above with those of pure analytes, it was possible to observe that the latter
264 had significant absorption signals specifically in those zones where fibre-type plants
265 lacked them.

266 These observations led us to hypothesise the possibility of using the spectral zones thus
267 identified for the qualitative identification of drug-type cannabis (and thus for its
268 discrimination from its fibre-type counterpart). Details of the spectral zones thus
269 individuated, and of the corresponding wavelengths used for analyte identification and
270 quantification, are found in section 3.2 and in Table 2. ~~This led us to also set up the
271 procedures to use IR absorption values (direct or in derivative) for the quantitative
272 determination of different cannabinoids.~~

273

274 **3.2. Discrimination of *Cannabis Sativa* L. Chemotypes and Setup of Quantitative** 275 **Analysis Procedures**

276 Fibre-type cannabis can usually be easily discriminated from drug-type cannabis even
277 by a simple visual IR spectra comparison. Specific bands can also be used for this
278 purpose: for example, between 1230 and 1300 cm^{-1} THCA produces a single band while
279 THC sports a double band in the same range. Non psychoactive CBD and CBDA do not
280 produce any significant signal in this range. Other cannabinoids, having notably lower
281 concentrations, do not usually interfere.

282 However, intermediate-type cannabis specimens and mixture of different cannabis types
283 could, at least theoretically, lead to difficulties in this regard. Thus, we embarked in the
284 study of the conditions needed to use ATR-FTIR to reliably quantify THC and THCA,
285 which are of course the psychoactive principles legally involved in the differentiation
286 between cannabis types. However, in order to obtain as much information as possible on

287 the samples, we also set out to quantify other, non-psychoactive and mildly-
288 psychoactive cannabinoids, including CBD, CBDA, CBG, CBGA and CBN from the
289 same spectra. In fact, being CBN the final product of THC degradation by heat, light
290 and humidity over time, concentration ratios of CBN to THC could serve as a guide in
291 determining the approximate age and storage conditions of a given sample.

292 This detailed analysis of IR absorption bands led us to identify seven of them, peculiar
293 or especially intense for each of these analytes. A list of the specific band(s)
294 corresponding to each analyte is reported in Table 2. Examples of the spectra from
295 which these data were extracted are reported in Figure 2.

296 The calibration samples were then analysed both by ATR-FTIR and, immediately
297 thereafter, by LC-MS/MS. The cannabinoid amount found in each sample by LC-
298 MS/MS was then used as the calibration amount for calibration curves set up in ATR-
299 FTIR. In total, 6 calibration samples were analysed in this way, and the parameters of
300 the corresponding calibration curves are reported in Table 3. As one can see, excellent
301 r^2 values were obtained for all analytes; those for the two most critical compounds
302 (THC and THCA) are especially good. RMSEC values are also satisfactory, being in the
303 range $1.62 \times 10^{-9} - 2.40 \times 10^{-1}$. As expected, given the good calibration parameters, back-
304 calculated concentrations were very close to the real concentrations, in the 75-103%
305 range. For the evaluation of method sensitivity, the limit of quantitation (LOQ) for each
306 analyte was calculated as $LOQ = 10 \sigma/S$, where σ is the standard deviation of the
307 response and S is the slope of the calibration curve. The calculated LOQ values,
308 expressed as mg of analyte/g of sample, were as follows: 0.3 mg/g for THC and THCA,
309 0.7 mg/g for CBD, 0.8 mg/g for CBDA and CBG, 1.0 mg/g for CBGA and 0.5 mg/g for
310 CBN.

311

312 **3.3. Cross-Validation**

313 Having set up calibration equations, validation samples were used to cross-validate the
314 method. A total of 36 validation samples were analysed in this way, and the results of
315 cannabinoid concentrations calculated from the calibrations were compared to those
316 obtained by using the LC-MS/MS method. Very close agreement was observed in all
317 cases, always within $\pm 6.8\%$ from reference values. Moreover, Bland-Altman difference
318 plot for this comparison was obtained for all analytes and is shown in Figure 3a. As one
319 can see, all ATR-FTIR results are always within the 95% confidence limit range.

320 Linear correlation plot was also obtained and is shown in Figure 3b: both slope (mean
321 value: 1.0284) and regression (mean value: 0.9995) parameters are really satisfactory.

322

323 **3.4. Sample Inhomogeneity Evaluation**

324 Results of sample inhomogeneity evaluation tests for the two most relevant analytes
325 (THC and CBD, whose respective concentrations dictate the assignment of each sample
326 to one of the three *Cannabis* types) are reported in Supplementary Materials, Table S1.

327 As one can see, sample inhomogeneity within each floret was relatively small for all
328 tested florets, of the order of magnitude usually allowed for precision assays during
329 analytical method validation. On the other hand, some inhomogeneity was detected
330 between different florets of the same sample, but this did not significantly impact
331 sample attribution to one of the three *Cannabis* types outlined in this study (drug-type,
332 fibre-type, light). In any case, even when relatively large sample inhomogeneity would
333 be found, multiple analyses carried out on sample positions as diverse as possible,

334 followed by averaging, would largely overcome the problem, while also retaining the
335 additional information regarding sample inhomogeneity.

336 **4. CONCLUSIONS**

337

338 A very fast, reliable and robust ATR-FTIR method was developed for the proof-of-
339 concept routine discrimination between fibre-type and drug-type cannabis samples.

340 The method does not require, and indeed does not include, any sample pretreatment step
341 and it proves to be a much faster and more feasible alternative when compared to
342 classic, reference instrumental methodologies. Moreover, it does not require solvents
343 and reagents, ultimately leading to no waste production, thus providing a more user-
344 friendly and environmentally sound analytical platform.

345 As a consequence, a complete analysis only lasts less than a minute, leading to the
346 possibility of extremely high throughput even in real-world application, on-the-spot
347 quality control during cultivation and pharmaceutical product manufacturing, as well as
348 on-the-street cannabis controls and user counselling. In addition, the developed
349 analytical workflow could be successfully applied by sub-optimally trained, possibly
350 non-scientist, personnel.

351 Very good calibration and cross-validation results were obtained, and a 100%
352 discrimination rate was achieved for the 36 samples analysed in this way.

353 Thus, despite its extreme feasibility and the lack of any sample pretreatment step, the
354 method has proved to be selective and sensitive enough to reliably quantify 7
355 cannabinoids, including of course the two diagnostic cannabinoids THC and THCA.

356 More assays are in progress in order to increase the number of cross-validation samples
357 to whom to apply the method, as well as to extend its application to different cannabis
358 products, such as leaves, stems, unidentifiable plant parts and oils.

359

360 **Declaration of Competing Interest**

361 The authors declare that they have no known competing financial interests or personal
362 relationships that could have appeared to influence the work reported in this paper.

363

364 **CRedit authorship contribution statement**

365 **Marco Cirrincione:** Methodology, Investigation, Formal analysis, Validation, Writing
366 - original draft. **Bruno Saladini:** Methodology, Investigation, Formal analysis,
367 Validation. **Virginia Brighenti:** Resources, Funding acquisition. **Stefano Salamone:**
368 Conceptualization, Resources, Data curation. **Roberto Mandrioli:** Conceptualization,
369 Resources, Data curation, Writing - original draft, Writing - review & editing. **Federica**
370 **Pollastro:** Writing - review & editing, Resources, Funding acquisition, Project
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372 Funding acquisition. **Michele Protti:** Methodology, Investigation, Data curation,
373 Writing - original draft, Writing - review & editing, Visualization. **Laura Micolini:**
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383 University of Bologna.

384

385 **FIGURE AND TABLE LEGEND**

386 **Figure 1.** Chemical structures of the cannabinoids considered in this study. (a) THC, (b)
387 THCA, (c) CBD, (d) CBDA, (e) CBG, (f) CBGA, (e) CBN.

388 **Figure 2.** ATR-FTIR spectra (direct signal and first derivative) of intact, untreated
389 cannabis samples: (a) fibre-type, (b) drug-type, (c) “light” cannabis

390 **Figure 3.** (a) Bland-Altman difference plots and (b) linear correlation plots for the
391 comparison between ATR-FTIR and LC-MS/MS determination of all analytes in
392 cannabis samples

393

394 **Table 1.** Analyte-dependent MRM MS/MS parameters

395 **Table 2.** Main ATR-FTIR absorption spectra bands selected for the quali-quantitative
396 analysis of the 7 cannabinoids considered for this study.

397 **Table 3.** Parameters of the calibration curves obtained from the ATR-FTIR analysis of
398 cannabinoids in cannabis inflorescences.

399 **Table 4.** Linear regression parameters of ATR-FTIR analysis vs. LC-MS/MS analysis
400 result comparison.

401

402 6. REFERENCES

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Highlights

- A very fast and reliable ATR-FTIR method was developed for quantitative analysis of cannabinoids in cannabis.
- It is a proof-of-concept for the discrimination between fibre-type and drug-type cannabis samples.
- The method does not require any sample pretreatment step.
- The method has proved to be selective and sensitive enough to reliably quantify 7 cannabinoids.

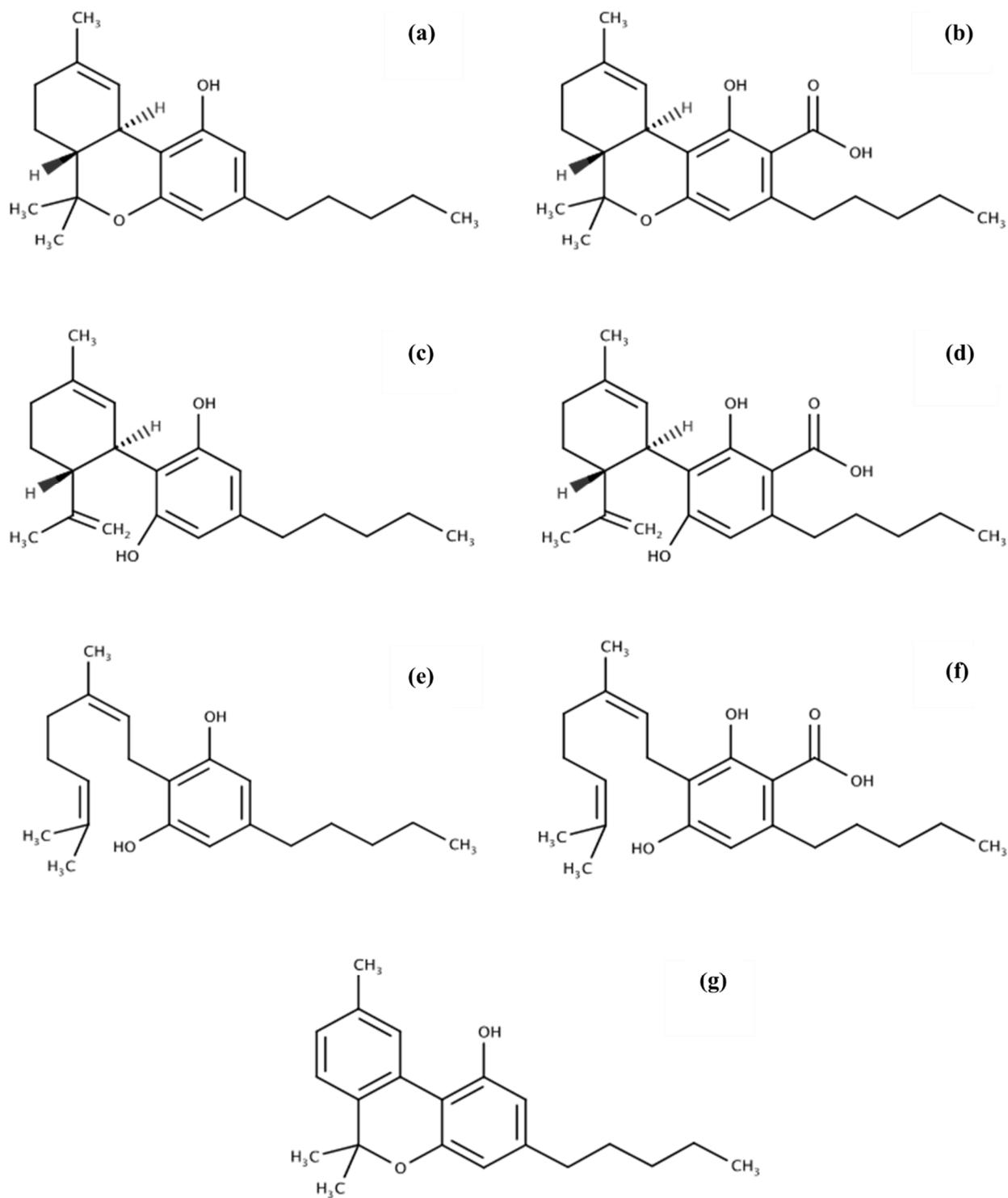


Figure 1. Chemical structures of (a) THC, (b) THCA, (c) CBD, (d) CBDA, (e) CBG, (f) CBGA, (e) CBN.

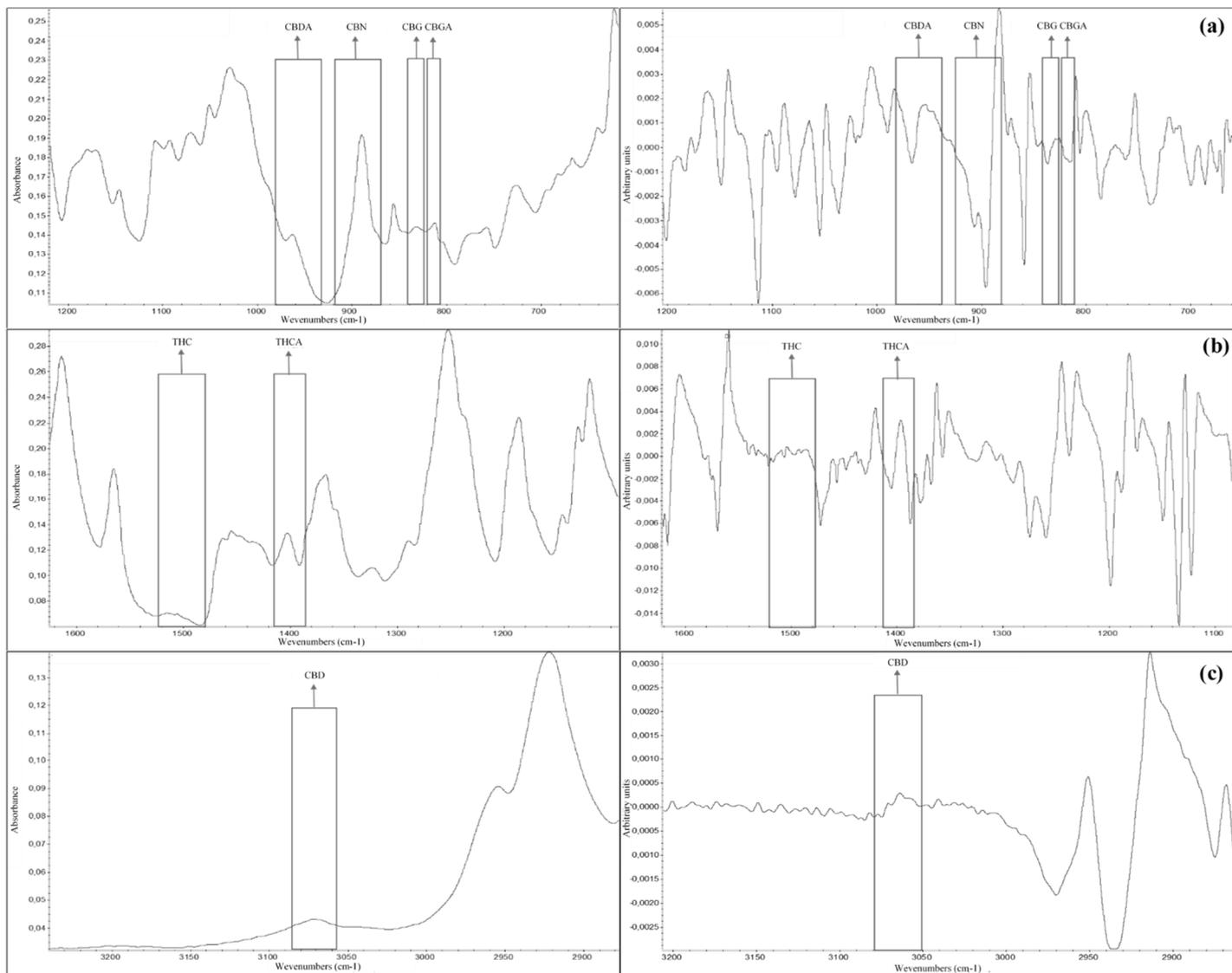


Figure 2. ATR-FTIR spectra (direct signal and first derivative) of intact, untreated *Cannabis* samples: (a) fibre-type, (b) drug-type, (c) “light” *Cannabis*

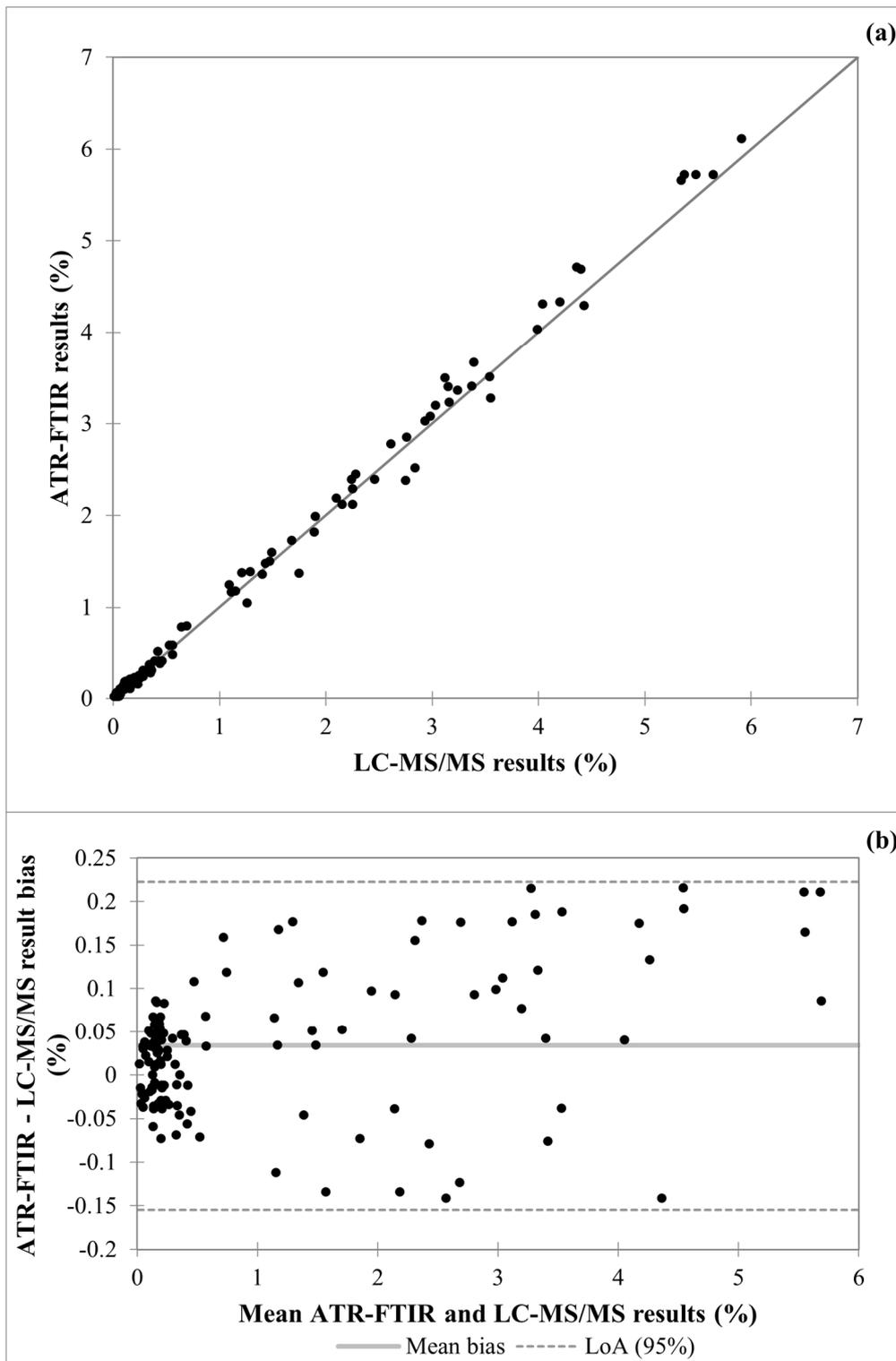


Figure 3 - Linearity correlation (a) and Bland Altman (b) plots for the comparison between ATR-FTIR and LC-MS/MS analysis of *Cannabis* samples

Table 1. Analyte-dependent MRM MS/MS parameters

Analyte	Precursor ion (<i>m/z</i>)	Product ion (<i>m/z</i>)	Collision energy (eV)	Cone voltage (V)
THC	315.52	193.25	45	45
		122.87	50	41
THCA	357.46	245.21	41	40
		191.33	45	36
CBD	315.42	246.28	35	50
		180.11	33	46
CBDA	357.22	245.23	32	46
		338.25	29	42
CBG	317.15	193.06	37	42
		123.01	35	38
CBGA	359.01	219.40	35	40
		261.12	31	36
CBN	311.19	280.14	55	51
		172.08	52	48
THC-d3	318.45	196.22	43	40
CBD-d3	318.39	249.30	40	36
CBN-d3	314.21	283.17	53	48

Table 2. Main ATR-FTIR absorption spectra bands selected for the quali-quantitative analysis of the 7 cannabinoids considered for this study.

Analyte	ATR-FTIR band (cm⁻¹)
THC	1514-1485
THCA	1415-1392
CBD	3085-3060
CBDA	982-959
CBG	844-830
CBGA	820-807
CBN	910-872

Table 3. Parameters of the calibration curves obtained from the ATR-FTIR analysis of cannabinoids in *Cannabis* samples

Analyte	r^2	Equation	RMSEC	RMSECV
THC	0.9993	$y = 1.0292x - 0.0238$	0.053	0.020
THCA	0.9984	$y = 0.9985x + 0.0143$	0.141	0.065
CBD	0.9999	$y = 1.0059x - 0.0194$	0.533×10^{-7}	0.375
CBDA	0.9910	$y = 0.9911x + 0.0288$	0.238	0.310
CBG	0.9930	$y = 0.9895x + 0.0031$	0.163×10^{-7}	0.038
CBGA	0.9988	$y = 0.9471x + 0.0195$	0.163×10^{-8}	0.041
CBN	0.9946	$y = 0.9679x + 0.0018$	0.787×10^{-3}	0.163