Short communication

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Direct analysis of cannabis samples by desorption atmospheric pressure photoionization-mass spectrometry

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Fast analysis of cannabis samples without prior sample preparation or chromatography was performed using desorption atmospheric pressure photoionization-mass spectrometry (DAPPI-MS). The MS^2 spectra of the molecular ions of tetrahydrocannabinol (THC) and cannabidiol (CBD) formed in DAPPI-MS showed distinct product ions, unlike the protonated molecules formed with other ambient mass spectrometry techniques, making possible the reliable identification of THC from cannabis samples.

Supporting information may be found in the online version of this article.

Keywords: desorption atmospheric pressure photoionization; ambient mass spectrometry; cannabis; tetrahydrocannabinol; cannabidiol

Introduction

Cannabis sativa derived products, such as marijuana and hashish, are the most widely produced and consumed illicit drugs worldwide.[1] In 2009, the number of cannabis users in the world was estimated to be between 125 and 203 million and the total amount of cannabis herb and resin seizures by the world's police and customs was reported to be 7200 tons. The large amounts seized put forensic drug laboratories under a lot of pressure, since the results are used as evidence in the justice process, and analytical techniques used to analyze the drug samples should be fast and reliable. In case of cannabis herb, the techniques should also be able to differentiate between the drug and the fibre-type cannabis; the latter may be cultivated in some countries with permission, if the tetrahydrocannabinol content in the hemp is sufficiently low ($<$ 0.2 % in EU).[2]

The most commonly used analysis methods for cannabis samples include gas chromatography (GC) with mass spectrometry (MS)[3,4] or flame ionization detector (FID),[5] and high performance liquid chromatography (HPLC) with ultraviolet (UV)[5,6] or MS.[6,7] The analysis of plant samples is typically time-consuming, since prior to the actual chromatographic and MS analysis, the plant samples chosen for analysis need to be dried, homogenized, sieved, and extracted.[8]

The recent innovation of ambient MS techniques, for example desorption electrospray ionization (DESI),[9] direct analysis in real time (DART),[10] easy ambient sonic spray ionization (EASI),[11] and desorption atmospheric pressure photo ionization (DAPPI),[11] has speeded up the analysis of confiscated drugs in forensic laboratories. These techniques make possible the direct analysis of confiscated drugs in their solid forms, such as tablet, resin, or plant, completely without sample preparation or chromatographic separation.[12–17] The analyses can typically be performed in seconds instead of hours or days, as with traditional methods. Reliable identification of the forbidden compounds in the samples can be done most of the time based on the typical fragments of the compounds formed by collision-induced dissociation (CID) in tandem mass spectrometry (MS^2). However, there is a serious problem related to the analysis of cannabis: most ambient MS techniques cannot differentiate between the active ingredient tetrahydrocannabinol (THC) and a non-active ingredient, cannabidiol (CBD), which have the same molecular mass (314.2 g/mol). This is because with most ambient techniques THC and CBD form protonated molecules with similar CID fragmentation patterns.[12] DAPPI is an exception, since it can also be used to form molecular ions. Here, we show that this feature can be utilized in cannabis analysis, since the molecular ions of THC and CBD have characteristic CID fragmentation patterns.

Experimental

The cannabis herb samples analyzed were confiscated samples from the National Bureau of Investigation (Vantaa, Finland). The herb samples were placed on a glass microscope slide using a Fissaforte double-sided PE foam tape (Sicad group, Uboldo, Italy) and analyzed as such, using a home-made DAPPI source. In DAPPI, hot solvent vapour and nebulizer gas were delivered towards the sample using a heated nebulizer microchip, causing

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the thermal desorption of the analytes from the sample surface. Nitrogen was used as the nebulizer gas and toluene as the spray solvent. A krypton discharge VUV lamp was used to initiate the ionizing reactions. The DAPPI set-up and the instrumentation were identical to those described by Kauppila *et al*.[17] More details about the experimental conditions are given in Supplemental Information.

Results and discussion

Figure 1 shows the analysis of THC and CBD 10 μg/ml standards using μAPPI and toluene and acetone as the solvents. With toluene as the solvent, both compounds showed molecular ions at *m/z* 314 as the main ion, while with acetone as the solvent both compounds showed protonated molecules at *m/z* 315. The product ion spectra of the molecular ions of THC and CBD are clearly different from each other, whereas the product ions of the protonated molecules are exactly the same. The formation of similar product ion spectra from the protonated molecules of THC and CBD has been noted earlier with thermospray ionization and DESI.[12,18]

Figure 2 shows suggestions for the structures of the observed product ions of *m/z* 314 of THC and CBD. Many of the ions have previously been reported in EI-MS studies, although the relative intensities of the product ions obtained with DAPPI-MS^2 somewhat differ from those obtained by EI.[3,19] The most important product ions for the differentiation of THC and CBD molecular ions were thought to be the ions at *m/z* 299 and 272 in THC and CBD spectra (see Supplemental Information for principal component analysis using these ions as variables), and ions at *m/z* 193 and 208, observed in CBD spectrum only. The product ion at *m/z* 299 was more abundant in the spectrum of THC, probably because the geminal methyl group is detached more easily from the pyran ring present in THC structure.[3] The ion at *m/z* 272, on the other hand was less abundant for THC than CBD, and again this was thought be because of the pyran ring of THC, since it has to be opened before the reaction can go further. The ions at *m/z* 208 and 193, which were observed only in the CBD spectrum, were also thought to be more probable for the open-ring structure of CBD than the closed pyran ring structure of THC.

For analysis with DAPPI, a set of 10 cannabis herb samples with different, known proportions of THC and CBD was chosen. The proportions of THC and CBD in the samples, measured using GC-FID, and the THC/CBD ratios, are shown in Table 1. The samples were placed on a moving stage outside the mass spectrometer and analyzed as such. The full-scan mass spectra of all samples showed an intense ion at *m/z* 314, which can be the $M^{+\cdot}$ of either THC or CBD. Figure 3 shows the product ion spectra of the ion at *m/z* 314 from all the samples. The product ion spectra from samples A–D were highly similar to the product ion spectra of the molecular ion of THC recorded with μAPPI (Figure 1). This correlates well with the GC-FID data, since it indicates that these samples contained mainly THC (Table 1). On the contrary, the product ion spectra from samples I-J were very similar to the product ion spectra of the molecular ion of CBD. Again, the GC-FID data show that these samples mainly contained CBD. The product ion spectra of samples E-H show features from both THC and CBD, since the proportions of THC and CBD in the samples were close to each other. The DAPPI spectra show thus directly whether the sample contains substantial amounts of THC.

Conclusions

DAPPI-MS has been shown to make possible the mass spectrometric analysis of cannabis samples directly from the sample surface, without any elaborate sample preparation or chromatography, or the danger that a false positive signal is caused due to the presence of CBD. The results were clearest for the drug type cannabis samples with very high THC contents. Since the majority of the cannabis samples confiscated by the police are drug type, the work load in the forensic laboratories could be significantly reduced using DAPPI-MS, since only the samples containing smaller amounts of THC would need to be analyzed by the slower GC-MS or GC-FID methods.

Supporting information

Supporting information may be found in the online version of this article.

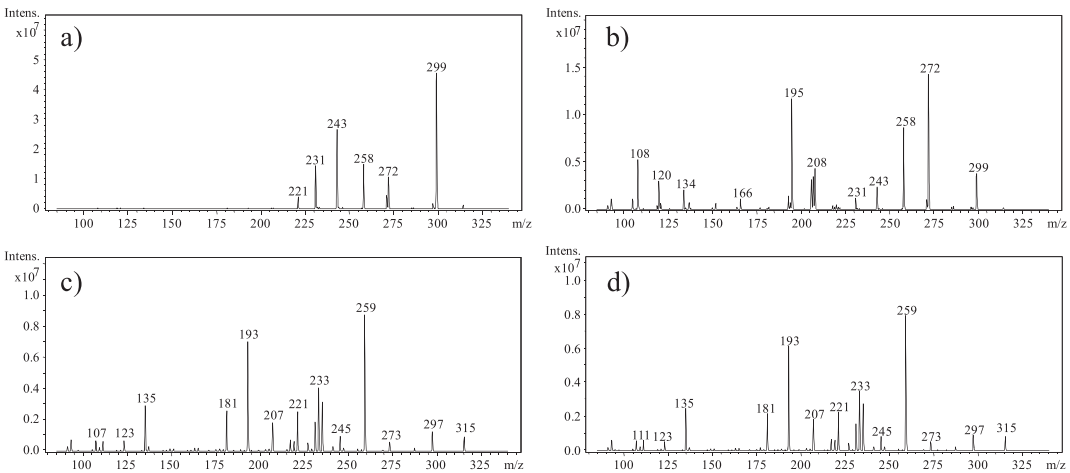

Figure 1. Product ions of (a) *m/z* 314.2 of THC (solvent toluene), (b) *m/z* 314.2 of CBD (solvent toluene), (c) *m/z* 315.2 of THC (solvent acetone) and (d) *m/z* 315.2 of CBD (solvent acetone). All measurements were done using continuous flow μAPPI. The fragmentation voltages were 0.35 and 0.30 for *m/z* 314 and *m/z* 315, respectively, and the isolation width was 1.2 *m/z*.

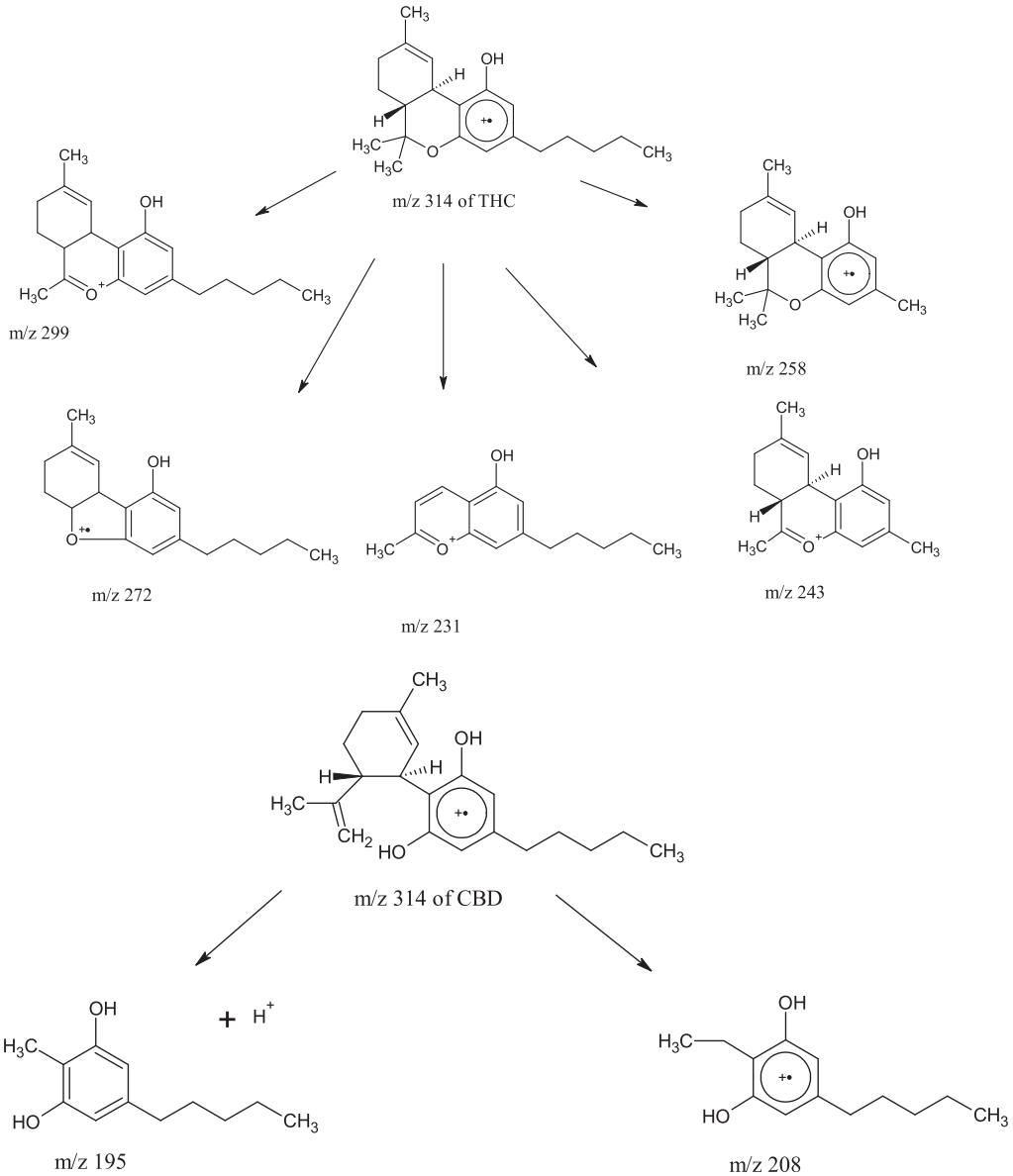

Figure 2. Suggested structures of the main product ions for *m/z* 314 of THC and CBD. Product ions at *m/z* 231, 243, 258, 272 and 299 were observed also in CBD spectra and they were thought to correspond to those of THC at the same *m/z*.

Table 1. The proportions of THC and CBD in the studied cannabis samples as determined by GC-FID and the ratio of THC and CBD

Sample nr	THC (mass-%)	CBD (mass-%)	THC/CBD
A	6.12	0.13	48.17
B	6.97	0.22	31.53
C	0.42	0.00	-
D	1.00	0.27	3.73
E	2.57	1.65	1.56
F	0.24	0.58	0.41
G	1.15	3.53	0.33
H	0.20	0.63	0.32
I	0.07	2.07	0.03
J	0.00	0.35	0.00

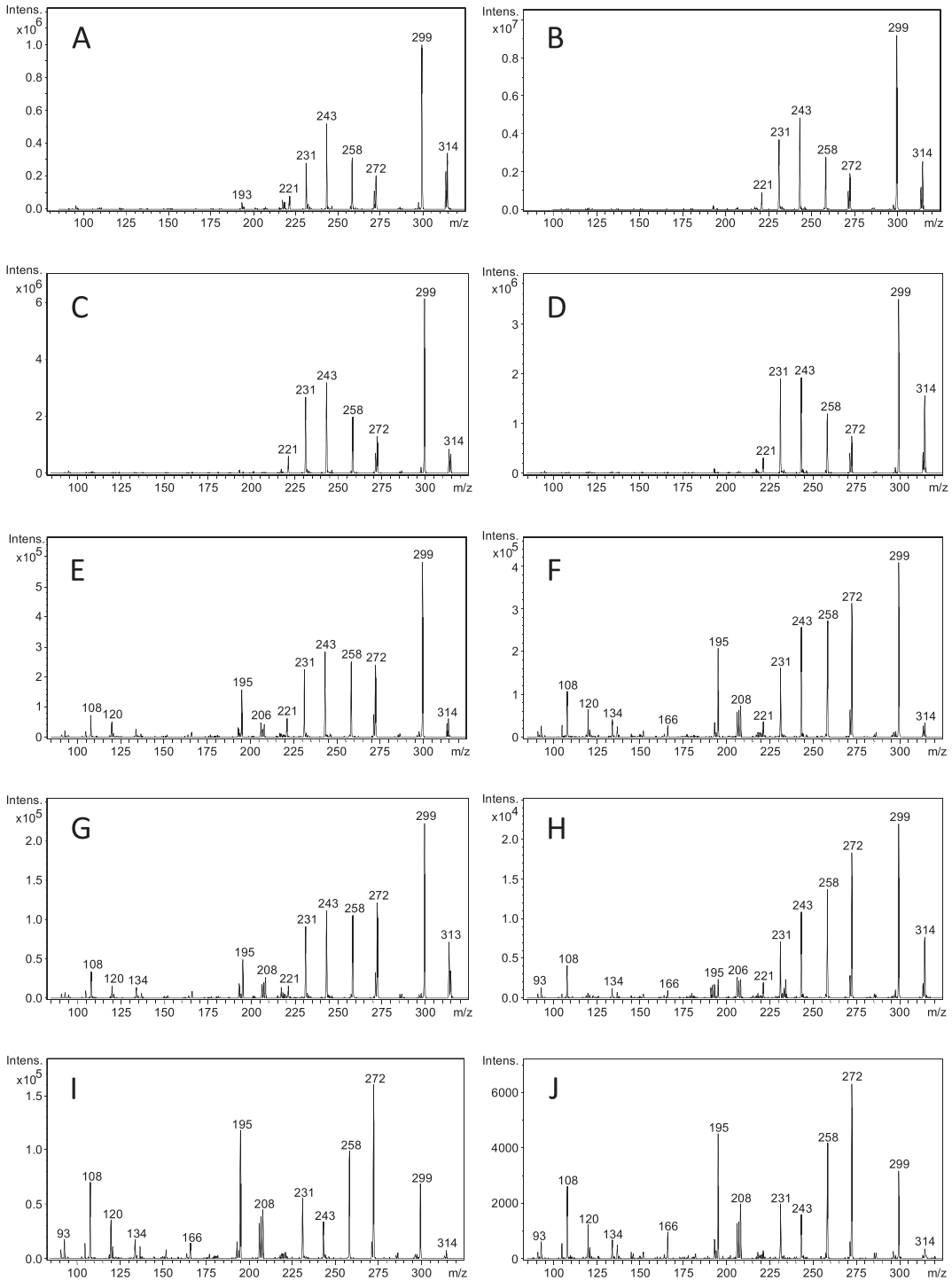

Figure 3. Product ion spectra of the studied cannabis samples. The samples are shown in the decreasing order of THC/CBD as shown in Table 1.

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References

[1] United Nations Office for Drug Control and Crime Prevention. World Drug Report 2011.
[2] Article 52 of Regulation (EC) No 1782/2003. *Official Journal of the European Union, Brussels*, **2008**.
[3] T.B. Vree. Mass spectrometry of cannabinoids. *J. Pharm. Sci.* **1977**, *66*, 1444.

[4] Y. Ilias, S. Rudaz, P. Mathieu, P. Christen, J.-L. Veuthey. Extraction and analysis of different cannabis samples by headspace solid-phase microextraction combined with gas chromatography-mass spectrometry. *J. Sep. Sci.* **2005**, *28*, 2293.
[5] V. Gambaro, L. Dell'Acqua, F. Farè, R. Froldi, E. Saligari, G. Tassoni. Determination of primary active constituents in cannabis preparations by high-resolution gas chromatography/flame ionization detection and high-performance liquid chromatography/UV detection. *Anal. Chim. Acta* **2002**, *468*, 245.
[6] C. Rustichelli, V. Ferioli, F. Vezzalini, M. Rossi, G. Gamberini. Simultaneous separation and identification of hashish constituents by coupled liquid chromatography-mass spectrometry (HPLC-MS). *Chromatographia* **1996**, *43*, 129.
[7] A.A.M. Stolker, J. van Schoonhoven, A.J. de Vries, I. Bobeldijk-Pastorova, W.H. Vaes, R. van den Berg. Determination of cannabinoids in cannabis products using liquid chromatography–ion trap mass spectrometry. *J. Chromatogr. A* **2004**, *1058*, 143.
[8] T.J. Raharjo, R. Verpoorte. Methods for the analysis of cannabinoids in biological materials: A review. *Phytochem. Anal.* **2004**, *15*, 79.
[9] Z. Takáts, J.M. Wiseman, B. Gologan, R.G. Cooks. Mass spectrometry sampling under ambient conditions with desorption electrospray ionization. *Science* **2004**, *306*, 471.
[10] R.B. Cody, J.A. Laramee, H.D. Durst. Versatile new ion source for the analysis of materials in open air under ambient conditions. *Anal. Chem.* **2005**, *77*, 2297.
[11] R. Haddad, R. Sparrapan, M.N. Eberlin. Desorption sonic spray ionization for (high) voltage-free ambient mass spectrometry. *Rapid Commun. Mass Spectrom.* **2006**, *20*, 2901.
[12] S.E. Rodríguez-Cruz. Rapid analysis of controlled substances using desorption electrospray ionization mass spectrometry. *Rapid Commun. Mass Spectrom.* **2006**, *20*, 53.
[13] T.J. Kauppila, V. Arvola, M. Haapala, J. Pól, L. Aalberg, V. Saarela, *et al*. Direct analysis of illicit drugs by desorption atmospheric pressure photoionization. *Rapid Commun. Mass Spectrom.* **2008**, *22*, 979.
[14] R.R. Steiner, R.L. Larson. Validation of the direct analysis in real time source for use in forensic drug screening. *J. Forensic Sci.* **2009**, *54*, 617.
[15] W. Romão, P.M. Lalli, M. F. Franco, G. Sanvido, N.V. Schwab, R. Lanaro, *et al*. Chemical profile of meta-chlorophenylpiperazine (m-CPP) in ecstasy tablets by easy ambient sonic-spray ionization, X-ray fluorescence, ion mobility mass spectrometry and NMR. *Anal. Bioanal. Chem.* **2011**, *400*, 3053.
[16] L.A. Leuthold, J.-F. Mandscheff, M. Fathi, C. Giroud, M. Augsburger, E. Varesio, *et al*. Desorption electrospray ionization mass spectrometry: Direct toxicologial screening and analysis of illicit Ecstacy tablets. *Rapid Commun. Mass Spectrom.* **2006**, *20*, 103.
[17] T.J. Kauppila, A. Flink, M. Haapala, U.-M. Laakkonen, L. Aalberg, R.A. Ketola, *et al*. Desorption atmospheric pressure photoionization–mass spectrometry in routine analysis of confiscated drugs. *Forensic Sci. Int.* **2011**, *210*, 206.
[18] K. Ndjoko, J.L. Wolfender, K. Hostettmann. Analysis of cannabinoids by liquid chromatography - thermospray mass spectrometry and liquid chromatography - tandem mass spectrometry. *Chromatographia* **1998**, *47*, 72.
[19] A. Hazekamp, A. Peltenburg, R. Verpoorte, C. Giroud. Chromatographic and spectroscopic data of cannabinoids from cannabis sativa L. *J. Liq. Chromatogr. R T* **2005**, *28*, 2361.