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JMS Letter

Dear Sir,

'Spice' and other herbal blends: harmless incense or cannabinoid designer drugs?

Since 2004, herbal mixtures such as 'Spice' are sold in Switzerland, Austria, Germany and other European countries mainly via Internet shops. Although declared as incense, they are smoked as 'bio-drugs' by the consumers. In corresponding blogs, drug users reported cannabis-like effects after smoking. These products enjoy great popularity particularly among younger people, as up to now the mixtures are sold in headshops and via internet in many countries without age restriction. Moreover, commonly used drug tests are not able to detect use of these drugs so far.

The list of ingredients indicates potentially bioactive herbs containing alkaloids (e.g. aucubin in *Pedicularis densiflora*, declared as 'Indian Warrior' or leonurine in *Leonotis leonurus* declared as 'Wild Dagga' or 'Lion's Tail'), which could in general produce cannabis-like effects. However, the suspicion was raised that synthetic compounds added underhandedly may be the main cause for pharmacological activity.

To prove pharmacological activity and to gain drug positive blood and urine samples, a self-experiment was conducted by two of the authors. One cigarette containing 0.3 g of 'Spice diamond' was smoked together and several blood and urine samples were collected. Approximately 10 min post-application the first noticeable effects occurred in the form of considerably reddened conjunctivae, significant increase of pulse rates, xerostomia and an alteration of mood and perception. In objective psychomotor tests, no abnormalities were detected, although the subjects had the impression of being moderately impaired. The effects continued for about 6 h under slow attenuation. The whole next day, some minor after-effects were still noticeable. These findings were consistent with the majority of reports available on the Internet and confirmed the presence of pharmacologically active compounds.

For isolation of active compounds, ethanolic extracts of the materials (0.5 g of 'Spice silver', 'Spice gold', 'Spice diamond', 'Smoke', 'Sence', 'Skunk' and 'Yucatan Fire' each, with 5 mL ethanol) were prepared. Routine qualitative drug analysis procedures, including GC-MS-screening with library search, multi-target screening by LC-MS/MS[1] and immunological screening procedures, revealed no evidence for the presence of any illegal drug or known active pharmaceutical ingredient. Only α-, β- and γ-tocopherol, as well as some phytosterols, were identified. Furthermore, three abundant signals were detected in GC-MS with unknown mass spectra (Fig. 1). The signals at 21.39 and 21.56 min showed similar EI mass spectra with a nominal molecular mass of 332 amu (compounds **1** and **2**). The third signal (compound **3**) was detected at 26.21 min. Compound **3** was identified by a laboratory in Frankfurt as JWH 018, a synthetic cannabimimetic aminoalkylindole (news item dpa, 15 December 2008) with an about fourfold affinity to the CB_1 receptor and about tenfold affinity to the CB_2 receptor compared with Δ9-tetrahydrocannabinol (THC) (**6**).[2,3] The fragmentation as proposed in Fig. 1 explains the main fragments seen in the EI/MS spectrum.

Thin layer chromatography (TLC) of the extracts of three commercially available variants of 'Spice' (silver, gold and diamond) showed an increase in intensity of the spot corresponding to compounds **1** and **2** after dyeing (Fast Blue RR Salt, NaOH), in parallel to an increase in price of product (Fig. 2). The UV spectrum confirmed the presence of a phenolic chromophore. High-accuracy mass measurement (monoisotopic mass of $[M + H]^+$: 333.2787 Da $\pm$ 2 ppm) led to the molecular formula $C_{22}H_{36}O_2$ (calculated monoisotopic mass of $[M + H]^+$: 333.2788 Da). For isolation of compounds **1** and **2**, 3 g of 'Spice diamond' were extracted with *n*-heptane and separated on a preparative silica gel column (400 mm $\times$ 40 mm i.d., silica 60, 0.06–0.2 mm particle size; solvent for elution: cyclohexane : ethyl acetate from 20 : 1 (v/v) to 1 : 1 (v/v)). The fractions containing **1** and/or **2** were dried under a nitrogen stream. Approximately 30 mg of raw product were obtained, corresponding to a concentration of roughly 1%. NMR studies and interpretation of EI-MS spectra (underivatised, trimethylsilylated and acetylated, cp. Figures 1 and 3) and ESI-IonTrap-MS^n experiments (Fig. 4) lead to the structure of the synthetic cannabinoid **2** (cp. Figure 5). The isotopic patterns of molecular ions and main fragments of all GC-MS spectra were in good agreement with the proposed structures, too. Compound **2** is a homolog of a non-classical cannabinoid called CP 47,497 (compound **4**) and is formally derived from **4** by prolonging the dimethylheptyl side chain to dimethyloctyl. ^{13}C-NMR data of **4** from Xie *et al.*[4] are shown together with data of compound **2** in Table 1. Both **2** and **4** are potent CB_1 and CB_2 agonists with **2** being the most potent in the row of homologs.[5] Compound **1** was found to be the *trans*-diastereomer of **2**, which is a much weaker cannabinoid receptor agonist and may be a side product of the synthesis procedure, which according to Melvin *et al.*[6] includes a reduction of a racemic ketone precursor with $NaBH_4$. The analgesic potency of CP 47,497 (**4**) in various mouse models is about 5 to 10-fold higher compared with THC.[7] Compton *et al.*[8] found compounds **2** and **4** to have similar pharmacological effects as THC, starting at considerably lower doses. A further study showed that **4** qualitatively

Table 1. ^{13}C-NMR chemical shift assignments of CP 47,497 (75 MHz, data taken from Xie *et al.*[4]) and compound **2** (100.6 MHz, isolated from 'Spice diamond') in $CDCl_3$ solution at 295 K

Carbon (cp. Figure 5, compound **4**)	Chemical shift (ppm) for CP 47,497	Carbon (cp. Figure 5, compound **2**)	Chemical shift (ppm) for compound **2**
1	152.22	1	152.50
2	113.08	2	113.12
3	149.18	3	149.02
4	118.54	4	118.20
5	126.36	5	126.27
6	128.72	6	128.82
7	35.28	7	35.26
8	41.88	8	41.84
9	71.16	9	71.32
10	35.51	10	35.34
11	24.54	11	24.48
12	31.80	12	31.89
1′	37.30	1′	37.23
2′	44.58	2′	44.56
3′	24.66	3′	24.69
4′	31.80	4′	31.59
5′	30.04	5′	30.34
6′	22.68	6′	29.23
7′	14.11	7′	22.63
8′	28.87	8′	14.08
9′	28.87	9′	28.84
–	–	10′	28.84

Chemical shifts are reported in ppm relative to $CDCl_3$ (δ = 77.0) as internal standard.

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Table 2. List of compounds found in the different 'herbal mixtures'

Product	Compounds **1** and **2**	Compound **3**	Compound **4**	Compound **6**	Other substances
'Spice silver'	+	+ (?)*	–	–	Ethylvanillin
'Spice gold'	++	+ (?)*	–	–	Ethylvanillin
'Spice diamond'	+++	+ (?)*	–	–	Ethylvanillin
'Yucatan Fire'	+++	++	–	–	Eucalyptol
'Smoke'	–	+++	–	++	Eugenol
'Skunk'	–	+++	–	++	Eugenol
'Sence'	+++	–	+	–	Benzophenone

The number of '+' indicates relative content of the respective compound as estimated from signal intensities in the corresponding GC-MS chromatograms.
* Compound **3** (JWH 018) was only found in some of the investigated packets and in much lower quantity compared to 'Yucatan Fire', 'Smoke' and 'Skunk'.

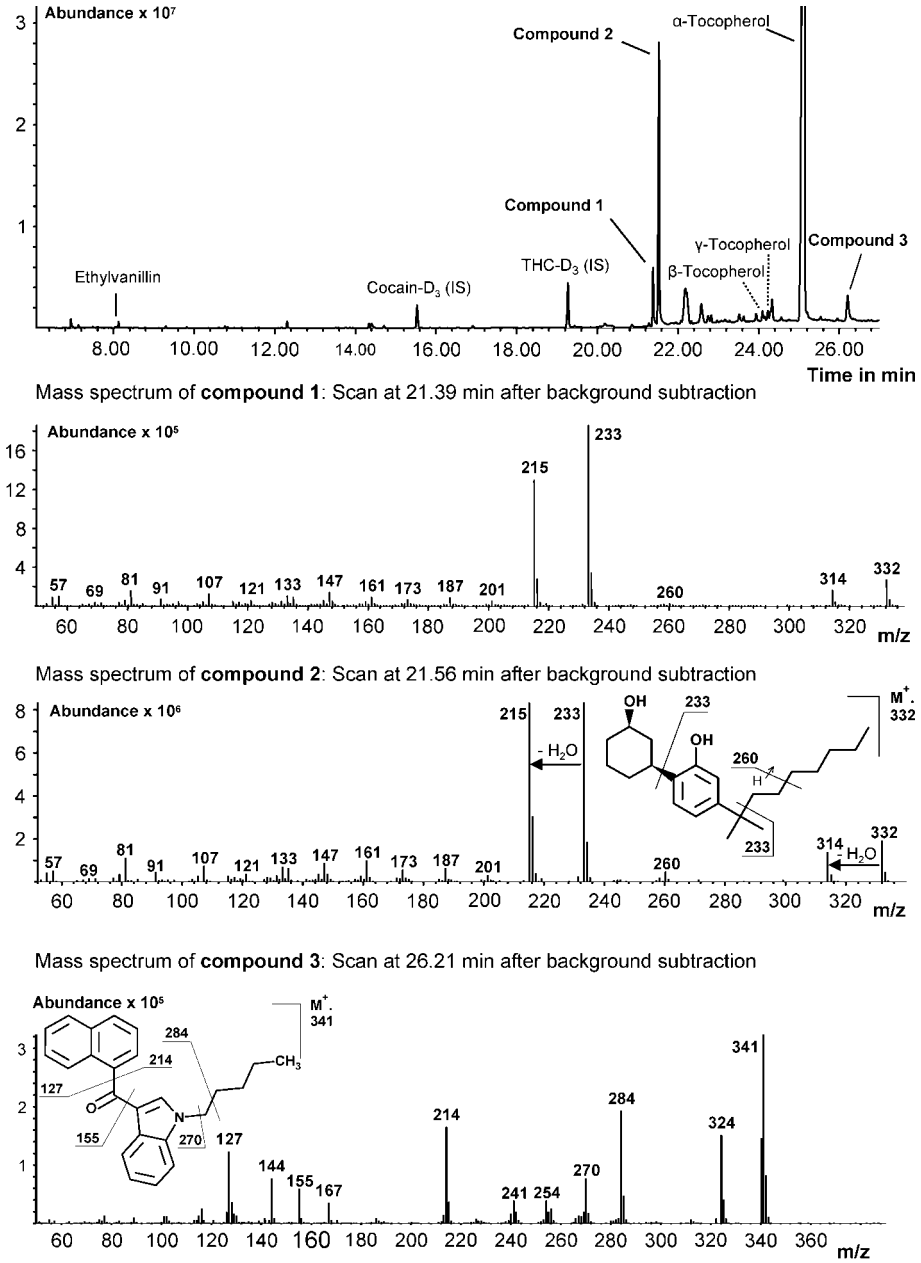

Figure 1. GC-MS chromatogram (TIC) of an ethanolic 'Spice' extract with mass spectra of the suspicious peaks. Main fragments are indicated for compounds **2** and **3**.

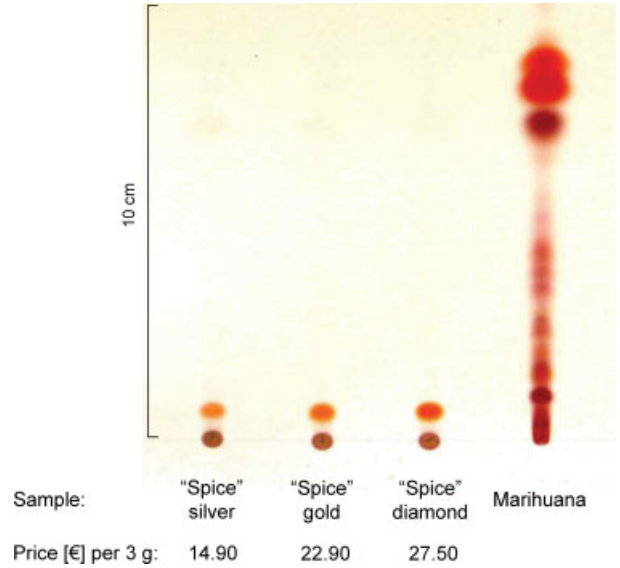

Figure 2. TLC plate (silica gel 60, *n*-hexane: diethylether [2 : 1(v/v)]) with extracts of three variants of 'Spice' and a cannabis control. The upper spots (orange) show compounds **1** and **2** after dyeing with Fast Blue RR Salt. Quotation of prices from http://www.kauf-spice.de on October 6th 2008.

resembles THC pharmacology, showing a 3–28 times greater potency compared with THC depending on the applied model.[9]

In Table 2, the compounds found in the different 'herbal mixtures' are listed. Structures are shown in Fig. 5. In the preparations named 'Smoke' and 'Skunk', besides JWH 018 (**3**), a larger quantity of oleamide (**6**) was found. Oleamide exhibits cannabinoid-like behavioural responses when ingested[10] and may be an additional adulterant in these products. 'Sence' contained primarily compounds **1** and **2**, but small amounts of **4** and its less potent *trans*-diastereomer were also detected alongside with benzophenone. The different 'Spice' variants and 'Yucatan Fire' contained mainly **1** and **2,** only in some of the tested packets was JWH 018 also detectable. Further products are currently under investigation in our labs.

Compound **2** was detected in the blood samples obtained from the self-experiment ('Spice diamond') using C_{18} solid phase extraction, trimethylsilylation and GC-EI/MS (modified routine method for cannabinoid analysis in blood[11]). All samples showed signals in selected ion monitoring (SIM) mode (targets ions *m/z* 476, 377, 289) at the expected retention time and with the correct relative ion intensities. Three blank serum samples were analysed in the same series and showed negative results. Figure 6 shows typical extracted ion chromatograms (XIC's) for a positive and a negative sample.

Screening with an immunoassay based on an antibody specific for 11-nor-9-carboxy-delta9-THC (major oxidative metabolite of THC) was negative for all blood and urine samples. Obviously, compounds **1**, **2** and **3** and their metabolites do not show significant cross-reactivity to the used assay.

This is so far the first time cannabinoid-like designer drugs were used as adulterants in commercially available products designed for inhalative application. Compounds **2** and **3** are strong cannabinoid receptor agonists and therefore account for the cannabis-like effects of 'Spice' and further 'herbal blends'. So far nothing is known about the metabolism of these compounds. Some of the metabolites may be toxic and/or pharmacologically active. Furthermore, differences from batch to batch in the kind and amount of applied drugs result in the risk of accidental overdosing, which in the last weeks occurred several times in our region with hospitalisation of the patients. There are hundreds of further compounds with cannabinoid receptor activity and it can be assumed that further substances will appear on the market soon, which will be an ongoing challenge for toxicologists as well as for law enforcement.

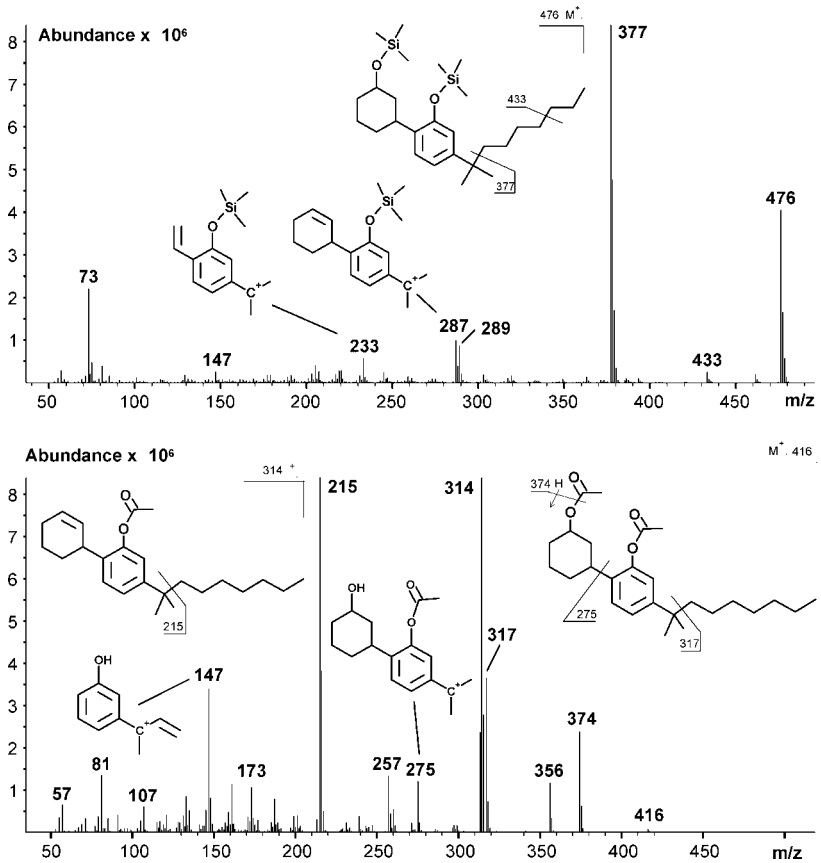

Figure 3. EI/MS spectra of compound **2** after derivatisation (chromatograms not shown) with illustration of the main fragments. The upper mass spectrum corresponds to compound **2** after silylation, lower after acetylation.

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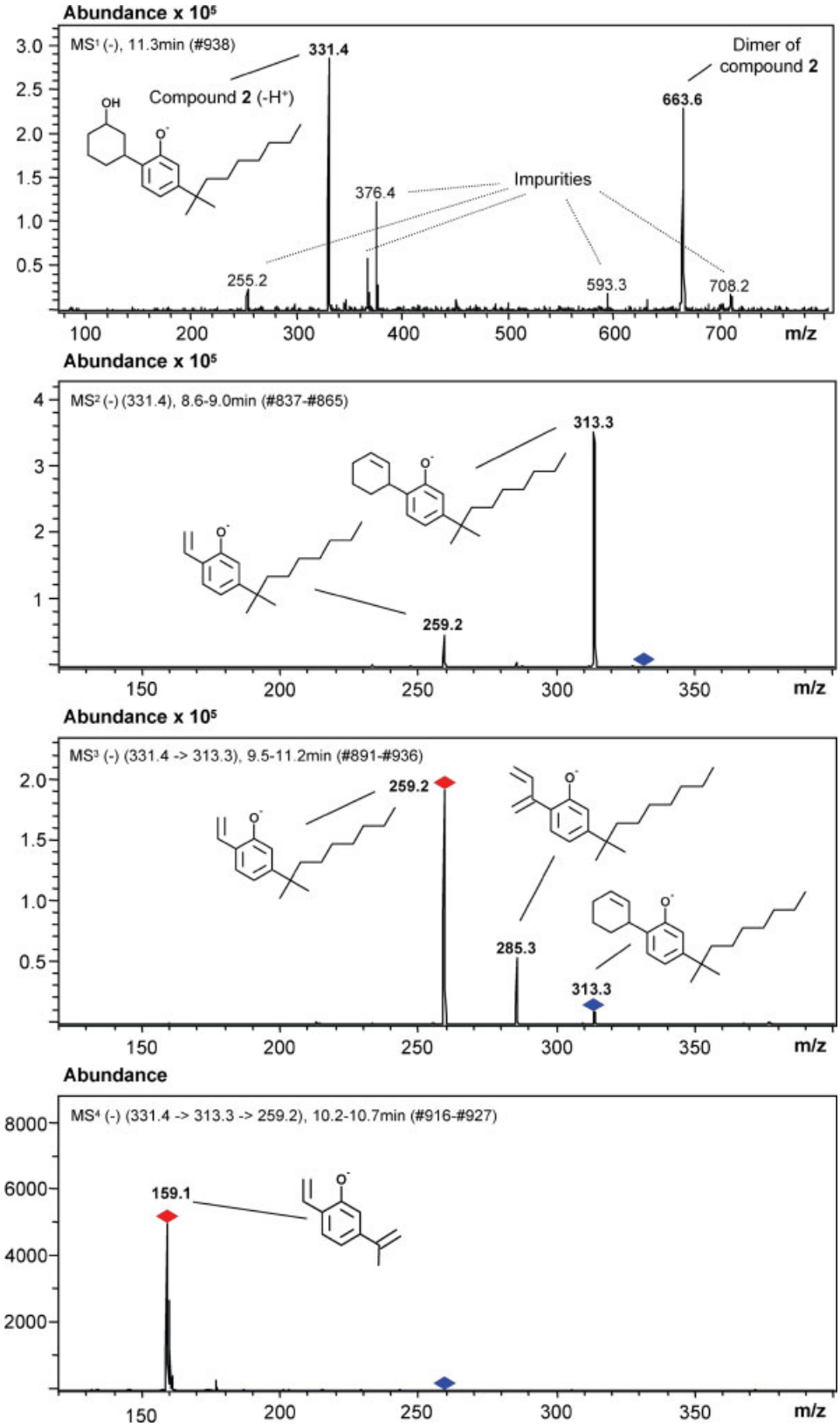

Figure 4. ESI-IonTrap-MS^n spectra of compound **2** after TLC of an extract of 'Spice diamond' with indicated fragmentation. Instrument: Bruker HCTplus Iontrap with sheath liquid coaxial ESI interface (Agilent Technologies), sheath liquid isopropanol: H_2O [1 : 1 (v/v)], flow 3 µL/min.

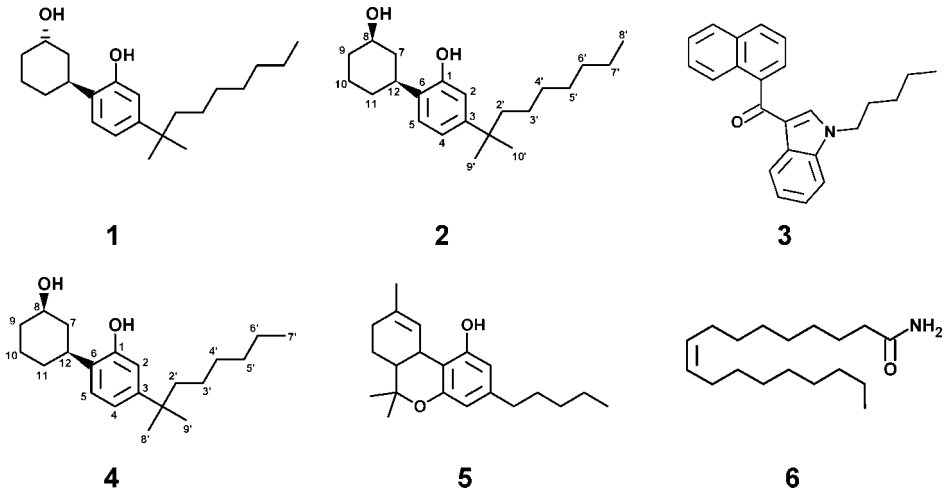

Figure 5. Chemical structures of cannabinoid receptor ligands. **3**: JWH 018, **4**: CP 47,497, **5**: Δ9-THC, **6**: oleamide.

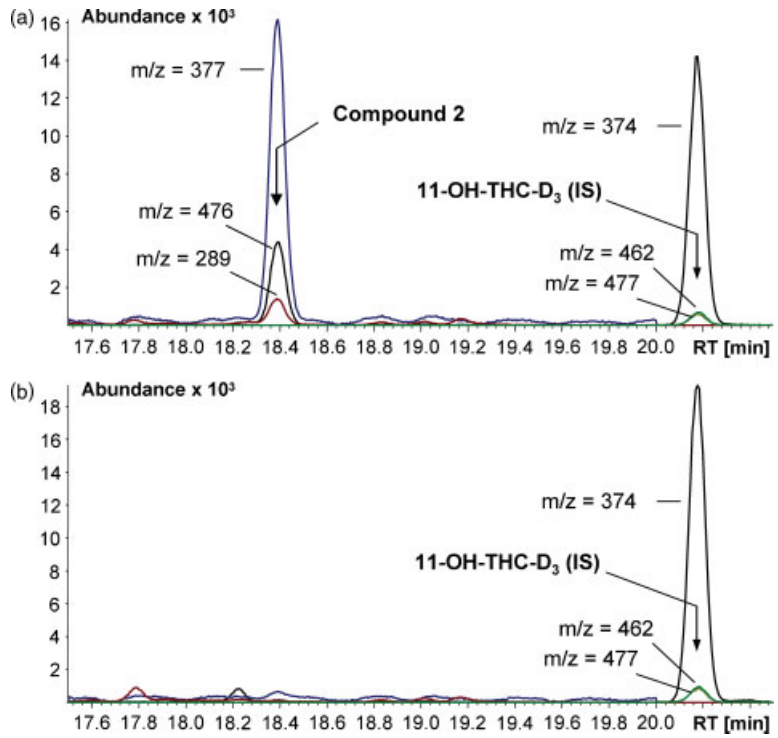

Figure 6. Extracted ion chromatograms (GC-EI/MS) for a drug positive sample from the self-experiment (a) and a blank serum sample (b). SIM mode with *m/z* 476, 377 and 289 for detection of compound **2**, internal standard 11-hydroxy-Δ9-THC-D_3.

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