Investigation on the consumption of synthetic cannabinoids among teenagers by the analysis of herbal blends and urine samples

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Abstract

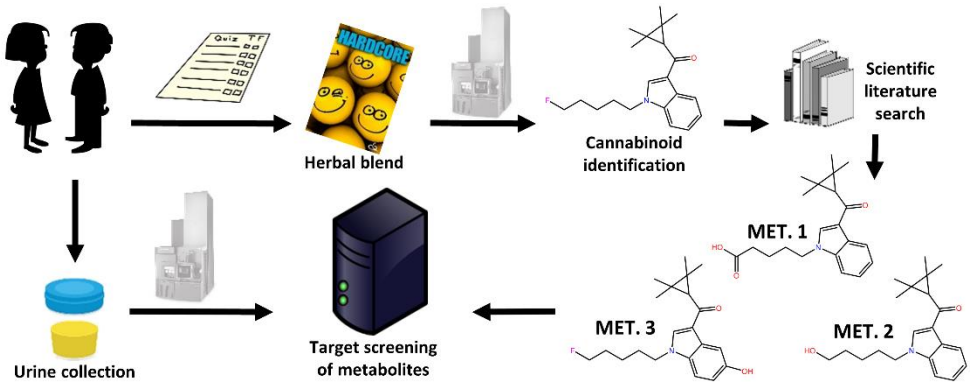
The use of synthetic cannabinoids (SCs), which escape conventional detection systems, may be a good alternative to elude routine drug analysis for cannabis. The detection of these drugs in urine is unusual due to their complete and fast metabolism, therefore requiring alternative strategies. In this work, an investigation has been made on SCs consumption by minors (less than 18 years old) in juvenile offenders' centres. 667 urine samples (from 127 minors) were collected after their permits with stay at home. We also studied the SCs from 7 herbal blends available at the smartshop frequented by the minors. Both, urine and herbal blends, were analysed by liquid chromatography coupled to high resolution mass spectrometry. The analysis of urine confirmed the absence of more than 200 SCs investigated. Thus, the focus was made on metabolites reported for those SCs identified in the herbal blends collected from the smart-shop. The major metabolites of XLR-11 and UR-144 (N-pentanoic acid and N-(5-hydroxypentyl)) were found in several urine samples. Apart from the main metabolites included in the initial searching, a thorough investigation of more metabolites for these SCs was additionally performed, including MS/MS experiments for the tentative identification of compounds detected in the urine samples. The 16 samples positive to the XLR-11 metabolites were assigned to 6 minors, only 2 of which had recognized consumption. On the basis of the results obtained, preventive and therapeutic interventions must be implemented to reduce the consumption of psychoactive substances and to improve the risk-perception of these substances by minors.

Keywords: juvenile offenders' centres, synthetic cannabinoids, drug analysis, XLR-11, UR-144.

Highlights

- The consumption of synthetic cannabinoids among teenagers was studied.
- 667 urine samples from 127 teenagers from 5 juvenile offender centres were collected.
- Different herbal blends potentially consumed by teenagers were analysed.
- The main metabolites of the detected cannabinoids in herbs were searched.
- 6 teenagers had consumed the cannabinoids XLR-11, found in one herb sample.

Graphical Abstract



1. Introduction

The consumption of cannabis has gradually increased in Spain, as in most other countries, over the last decade. In Europe, cannabis is consumed by up to 30.4% of the population in Spain and accounts for 29.9% of related admissions for treatment [1]. The legal consequences of consumption vary depending on the judicial system: cannabis use is legalised in some areas with no repercussions while, in others, its use can have civil or even criminal repercussions. At this point, synthetic cannabinoids (SCs) can be used as a replacement of cannabis. In addition to the low perception of risk [2–4] and high availability and low cost of these substances, the detection of SCs and their metabolites in humans is difficult, due to the high number of existing compounds and the variability in their chemical structures. All these aspects surely contribute to the increased use of SCs.

According to the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA), more than 160 SCs have been identified in herbal blends or spices since 2008. These herb mixtures, sold as 'legal' cannabis substitutes, are readily available in smartshops and via the internet. As stated by this organism, 80,000 products containing new psychoactive substances (NPS) were seized in Europe in 2015 and SCs accounted for 29% of these. Moreover, many novel SCs are identified in herbal mixtures every year, illustrating the ongoing appearance of new substances [5–7]. High-resolution mass spectrometry (HRMS) is much useful for identifying SCs in herbal blends [8,9] but standardized analytical strategies are required for identification of SCs in forensic samples, especially in urine, to advance in the knowledge of this topic. This would first require metabolism studies to establish appropriate urinary consumption-markers for SCs. Several studies have shown that it is near impossible to detect unaltered SCs in urine samples [10–13]. Thus, urine analysis of potential

SC consumers should focus on the major metabolites [14], using powerful techniques, such as ultra-high performance liquid chromatography (UHPLC) coupled to HRMS [12,15,16] or tandem mass spectrometry (MS/MS) [17–19].

The prevalence of SC use in juvenile detention centres is commonly unknown, and data about its consumption is obtained only via self-reports [20], a standard procedure in the early stages of substance-use detection [21]. Given the suspect use of SCs in juvenile detention centres and the difficulties associated to their detection, we present a comprehensive strategy to reveal the consumption of SC by minors through the analysis of herbal blends presumably consumed. In parallel, a searching of the main metabolites of the SCs identified was performed in urine samples. Considering the high number of compounds investigated, HRMS with hybrid quadrupole-time of flight (QTOF) mass analyser was applied for analysis given the useful information (accurate-mass full-spectrum data) provided by this technique.

2. Materials and methods

2.1. Reagents and chemicals

HPLC-grade water was obtained by purifying demineralised water using a Milli-Q system from Millipore (Bedford, MA, USA). LC-MS grade methanol, acetone, and hydrochloric acid (HCl 37%) were purchased from Scharlau (Scharlab, Barcelona, Spain). Diammonium hydrogen phosphate ($(NH_4)_2HPO_4$) was purchased from Merck (Darmstadt, Germany). *β*-glucuronidase from *E. Coli K12* (80 U/mL at 25 ºC) was purchased from Roche (Indianapolis, IN, USA).

1 M $H_2PO_4^-/HPO_4^{-2}$ buffer was prepared by dissolving the corresponding amount of $(NH_4)_2HPO_4$ in HPLC-grade water and adjusting the pH to 7 with HCl.

105 **2.2. Study design, herbal blends purchase and urine samples collection**

106 127 teenagers, aged less than 18 years and linked to one of the five participant juvenile
107 offenders' centres in the Valencian region (Spain) were included in this study. The inclusion
108 criterion was to have therapeutic permits with stay at home, which entailed providing a urine
109 sample for toxicological evaluation upon their return to the centre. The subjects participated
110 in a group interview, where they were asked about their use of NPS; if they mentioned their
111 own consumption, we asked them about the commercial name of the NPS and how they
112 acquired it.

113 Up to seven herbal blends suspicious to contain synthetic cannabinoids were acquired
114 in a smartshop located near the juvenile offenders' centres participating in this study: *Oro*
115 *Fantastico*, *Mazazo*, *Sonrisa Absoluta*, *Placaje*, *Sonrisa*, *Hardcore*, and *Tio Tieso* (**Figure 1**
116 shows the packaging of these products). These herbal blends were purchased by the time of
117 collecting urine samples from the teenagers (this is, in 2016).

118 A total of 667 urine samples were collected between May and October 2016 from all
119 the participants in the study. Individual urine samples were kept at -23 ºC after collection.

120 **2.3. Sample treatment**

121 Synthetic cannabinoids in the herbal blends were identified by HRMS, as described
122 in literature [9]. Briefly, 0.1 g of herbal blend was extracted with 2 mL of acetone in a 2 mL
123 propylene tube and introduced in an ultrasonic bath for 15 min. After centrifugation at 12000
124 rpm for 10 min, the supernatant was 1000-fold diluted with ultrapure water. Finally, the
125 extract was transferred to a glass vial and 20 µL were injected into the UHPLC-HRMS
126 system.

127 Urine samples were processed using an enzymatic hydrolysis procedure adapted from
128 the literature [22,23], thus releasing the unconjugated compound. This process has been

shown to be effective for cleaving NPS-derived glucuronides found in mice urine samples [24]. Briefly, 1 mL of urine was buffered with 0.4 mL of phosphate buffer; 16 µL of β-glucuronidase from E. coli strain K12 was added and the sample was incubated for 1 hour at 55 ±2 °C. Samples were then frozen at -18 °C for at least 3 hours, thawed, and centrifuged at 12000 rpm for 15 min to remove any lipids and proteins. Finally, 20 µL were injected into the UHPLC-HRMS system.

2.4. Instrumentation

The herbal blends and urine samples were analysed using an ACQUITY UPLC ultra-high performance liquid chromatography (UHPLC) system (Waters Corp, Mildford, MA, USA) coupled to a XEVO G2 QTOF hybrid quadrupole time-of-flight (QTOF) mass spectrometer (Waters Corp, Manchester, UK) with an orthogonal Z-spray electrospray ionization (ESI) interface operating in positive ionization mode.

Chromatographic separation was performed using a Cortecs C18 100 x 2.1 mm 2.7 µm particle size analytical column (Waters Corp, Wexford, Ireland) at a flow rate of 0.3 mL/min. Mobile phases were H_2O (A) and methanol (B), both with 0.01% formic acid. The mobile phase gradient was performed as follows: 10% of B at 0 min, 90% of B at 14 min linearly increased, 90% of B at 16 min, and finally 10% B at 18 min in order to return to initial conditions. The injection volume was 20 µL. The column temperature was set to 40ºC. The TOF resolution was ~20000 at FWHM at *m/z* 556 in positive ionisation mode. The range acquired by the MS system was from *m/z* 50 to 1000. A capillary voltage of 0.7 kV and a cone voltage of 20 V were used during all the chromatographic run. Nitrogen (Praxair, Valencia, Spain) was used as desolvation and nebulizing gas. The desolvation gas flow was set at 1000 L/h, while cone gas was set to 80 L/h. Argon 99.995% (Praxair) was used as a collision gas. The interface temperature was set to 600ºC and the source temperature to

120ºC. For MS^E experiments, two acquisition functions with different collision energies were created. Further details about the instrumental conditions can be found in literature [24], as well as the selection of the UHPLC-HRMS parameters for detecting NPS metabolites in urine samples from NPS consumers [25].

MS data were acquired in continuum mode using MassLynx software version 4.1 (Waters Corp, Manchester, UK), and processed with UNIFI scientific information system version 1.8 (Waters Corp, Manchester, UK).

2.5. Ethical consideration

This study was approved by the Research and Ethics Committee at the Consorcio Hospitalario Provincial (Castellon) on 26 September, 2014 (ref. 20141113), by the Conselleria de Benestar Social at the Generalitat Valenciana on 1 June, 2015 (registration 3 June, 2015, ref. 32833), and the Office of the Children's Justice Prosecutor, following the principles and requirements established in the Declaration of Helsinki and the European Council Convention for research on humans. The confidentiality of the participants and their data was guaranteed according to Organic Law 15/1999 on the Protection of Personal Data, and the subjects and their legal guardians signed their informed consent to their participation in the study.

3. Results

Given the extensive and fast metabolism of SCs in humans, a realistic approach to reveal the consumption of SCs is to monitor their major metabolites in urine [14]. In the present work, we included more than 200 SCs in our database, but we did not consider all their metabolic pathways due to the lack of information in some cases, particularly for the newest SCs. Therefore, we devised an alternative strategy of searching parent SCs in the herbal blends

referred to by the minors participating in this study. After identifying the SCs present in those herbal blends, we investigated their main reported metabolites in urine samples.

3.1. UHPLC-HRMS screening strategy and herbal blends analysis

During the group interviews, 59 participants recognised to have ever consumed a SC marketed as *Hardcore* during therapy periods to avoid being caught by conventional urine analyses. They described acquiring this product in a smartshop close to the centres involved. On this way, we purchased all seven herbal blends available in the smartshop via its webpage (**Figure 1**).

Of the seven herbal blends acquired for this study, we had already analysed *Oro fantastico*, *Mazazo*, *Placaje*, and *Sonrisa absoluta* (**Figure 1A**), and had identified four SCs in them: JWH-081, JWH-250, JWH-203, and JWH-019 [8]; our repeated analysis in this study found no differences in their composition. We also analysed the new herbal products, *Hardcore*, *Sonrisa*, and *Tio tieso* (**Figure 1B**) by UHPLC-HRMS and cross-referenced the suspect peaks against our SC database [9]. Based on the observed accurate-mass data on fragmentation and information in the literature, we tentatively identified four additional SCs: XLR-11, UR-144, an UR-144 *N*-(5-chloropentyl) analogue, and 5F-AKB48 (5F-APINACA), as shown in **Table 1**. The identity of the SCs could not be unequivocally confirmed by comparison with the analytical reference standards as they were not available in our laboratory. Nevertheless, the fragmentation observed, its compatibility with the chemical structures of the NPS, and the agreement with HRMS data reported in literature, provided a high degree of reliability in the tentative identification of the synthetic cannabinoids found in herbal blends.

As an illustrative example, the analysis of the herb mixture *Hardcore* was as follows: three chromatographic peaks were observed in the base peak intensity chromatogram (BPI)

of the low-energy function (**Figure 2B**), at 13.40 min ($[M+H]^+$ *m/z* 330.2222), 13.99 min ($[M+H]^+$ *m/z* 346.1931), and 14.54 min ($[M+H]^+$ *m/z* 312.2327). These were tentatively identified as XLR-11, an UR-144 *N*-(5-chloropentyl) analogue, and UR-144, based on the accurate-mass collision-induced dissociation (CID) fragments observed in the high-energy function (**Figure 2A**). The fragments observed for XLR-11 and UR-144 also coincided with fragmentation profiles reported in the literature [26,27], while fragmentation of the UR-144 *N*-(5-chloropentyl) analogue was justified based on the XLR-11 and UR-144 fragments. After CID ion evaluation, a plausible fragmentation pathway was proposed for the three SCs found in the *Hardcore* herbal blend (**Figure S1** in **Supplementary material**). At the time of performing this study and collecting herbal blend samples from the smartshops (i.e. in 2016), there was a notable spread of XLR-11 and UR-144 in Spain.

3.2. Detection of SC metabolites in urine samples

Once identified several SCs in the herbal blends, a suspect list containing the major metabolites (from two to four) for each compound was built [12,18]. No metabolites were found for the UR-144 *N*-(5-chloropentyl) analogue, a fact that was considered not much relevant because this substance was only found in *Hardcore* at very low abundance in comparison to the other two SCs (XLR-11 and UR-144) present in this product. **Table 2** shows the 19 metabolites to be investigated in urine samples based on the literature search. It can be seen that UR-144 and XLR-11, the two main components of *Hardcore*, share two metabolites, and only XLR-11 presented an additional specific one.

Two SC metabolites were found in 16 urine samples: N-pentanoic acid and N-(5-hydroxypentyl). **Figure 3** shows the tentative identification of both metabolites (**A**, N-pentanoic acid; **B**, N-(5-hydroxypentyl)) based on the accurate-mass fragmentation observed.

The presence of these metabolites suggested the consumption of XLR-11 and/or UR-144, being not possible to stablish which of these was consumed as both share the same metabolites. The extracted-ion chromatogram at the exact mass of N-(6-hydroxyindole), another metabolite of XLR-11, showed a chromatographic peak, but the fragmentation pattern did not fit with that reported in previous works, with the hydroxylation point appearing to be on the tetramethylcyclopropane ring (**Figure S2**). However, the potential consumption of this substance was supported by the tentative identification of the remaining two metabolites. This is in agreement with previous findings, where the main metabolites reported in urine samples from XLR-11 consumers were N-pentanoic acid and N-(5-hydroxypentyl) [28].

3.3. Confirmation of the consumption of XLR-11 or UR-144

The 16 urine samples positive to the major metabolites of XLR-11/UR-144 were re-processed, searching for additional minor metabolites described in urine from XLR-11 consumers [28]. This step was performed in order to confirm whether the cannabinoid consumed was XLR-11 or UR-144. In total, 12 phase-I metabolites were searched in positive urine samples. In some cases, the same biotransformations occurred on different moieties of the molecule, and therefore two (or more) metabolites had the same elemental composition. For example, four metabolites corresponded to hydroxylations on different carbon atoms of the tetramethylcyclopropane ring. **Table 3** shows the XLR-11 metabolites selected for the screening of individual urine samples.

Up to six XLR-11 metabolites were found in the 16 urine samples (**Table 3**).. To obtain cleaner spectra and enhance reliability in the metabolites tentative identification, additional MS/MS experiments were performed to obtain the accurate-mass product ion spectra, comparing the fragmentation observed with that described in the literature [28]. In

the particular case of M5, two chromatographic peaks were observed corresponding to two hydroxylation metabolites. According to literature [28], the structures of these metabolites would correspond to hydroxylation in different points of the degraded tetramethylcyclopropane ring. However, with only the HRMS data available, it is not possible to determine either the exact position of the hydroxyl group, or if the hydroxylation occurred in the degraded tetramethyl cyclopropane ring or in the intact ring. To obtain such information, both compounds should be synthetized and analysed by UHPLC-HRMS in order to unequivocally identify the exact structure of the metabolite. The MS/MS spectra of the detected metabolites at 10, 20, 30, and 40 eV collision energies, and the fragment-structure justifications are detailed in the **Supplementary material** (**Figure S3-S8**). For fragment structure justification, the biotransformation is placed in the structure to facilitate the fragmentation interpretation.

With all information obtained after a comprehensive analysis by HRMS, the 16 urine samples positives to XLR-11 metabolites could be assigned to 6 minors based on the anonymous urine sample codification. Only two adolescents had recognized consumption in the administered questionnaires, while the remaining 4 did not recognize any SCs consumption.

4. Discussion

The consumption of SCs seems not very common in the juvenile offenders' centres from the Valencian region, but a few cases have been found in this work. In the present study, 29 out of 127 participants admitted having ever used SCs, although our survey did not record when the consumption had occurred. Therefore, indication of SC consumption did not necessarily imply that the urine sample collected would produce a positive result, or maybe

the opposite case: some consumers did not recognise consumption in the survey, thus should be cross-referenced with toxicological analysis. Analysis of the herbal blends reported to have been consumed by the participants allowed the detection of several SCs. Subsequent urine analysis demonstrated the presence of major metabolites of XLR-11 and UR-144, supporting the consumption of the suspect products by some participants. In our study, SC consumption was only detected in 6 of the 29 self-referred cases. Considering the high number of SCs reported until now and the much higher number of potential metabolites, some of them being still unknown, it seems wise to focus the investigation on major metabolites of the active compounds identified in the products (e.g. herbal blends) within the "distribution area" of the minors. To increase the detection rate, the analysis could be focused on the appropriate target compounds. Both, toxicological and consumption information, can then be obtained from users in a synchronised way [29]. The application of advanced analytical techniques, such as UHPLC-HRMS, allows performing wide-scope screening of large number of suspect compounds, without the need of reference standards available. The use of appropriate databases containing as many metabolites reported as possible is a good strategy to increase the number of compounds under investigation. This makes feasible to detect more SC consumers via their urines analysis from among those who do not recognise their consumption via surveys or interviews. In our study, we detected 4 cases of minors who, in the group interviews and questionnaires, denied the use of synthetic cannabinoids. The final, unequivocal identification of the detected metabolites in urine would have required the acquisition of the corresponding analytical reference standards. The main limitation when investigating NPS metabolites is the non-commercial availability for many of them. However, in the present work the careful interpretation of mass spectra, their compatibility

with the chemical structure, and the agreement with previous data reported gave a high degree of confidence to their identification.

5. Conclusions

In this work, SC consumption among teenagers confined in juvenile offenders' centres has been investigated through the analysis of 667 urine samples collected from 127 participants, and the analysis of the herbal blends potentially consumed by the minors. Based on the metabolic behaviour of SC, the screening strategy applied by UHPLC-HRMS to urine samples was focused on the major metabolites reported for the SC that were identified in the herbal blends potentially consumed by the teenagers. In this way, the main metabolites of XLR-11 and UR-14 were identified in 16 urines, corresponding to 6 teenagers.

These two substances, XLR-11 and UR-144, were banned in Europe a few years ago (e.g. in Germany the maximum spread was reached between 2012 and 2015), and other synthetic cannabinoids, such as 5F-ADB, AB-FUBINACA and MDMB-CHMICA, replaced them. However, at the time of performing this work, both compounds were still in use in Spain, and in fact they were identified in the herbal blends sold in the local smartshop nearby the juvenile offenders centre. In September 2018, the herbal blends containing these two SCs were removed from the market, being replaced by a new one containing 5F-ADB (see [30]).

The results from our study demonstrate that SCs are occasionally consumed in juvenile offenders' centres in the Valencian region. The different approaches to reveal SCs consumption must be efficiently synchronised, so that information obtained from interviews and questionnaires must be matched to the composition of the products from the smartshops where the consumers acquire these substances, as well as to the urine analysis results within few hours of their last consumption.

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Declaration of interest

No potential conflict of interest was reported by the authors.

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Tables

Table 1 SCs identified in the herbal blend samples analysed in this work.

Herbal blend sample	Synthetic cannabinoids found
Oro Fantastico	JWH-081[a]
Sonrisa Absoluta	JWH-081[a]
	JWH-250[a]
Placaje	JWH-081[a]
	JWH-250[a]
	JWH-019[a]
	JWH-203[a]
Mazazo	JWH-081[a]
	JWH-250[a]
	JWH-019[a]
	JWH-203[a]
Hardcore	XLR-11[b]
	UR-144[b]
	UR-144 *N*-(5-chloropentyl) analog[b]
Sonrisa	5F-AKB48[b]
	XLR-11[b]
	UR-144[b]
Tio Tieso	5F-AKB48[b]
	XLR-11[b]
	UR-144[b]

[a] Compound identified with reference standard.

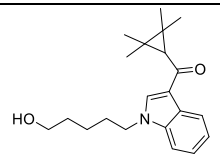
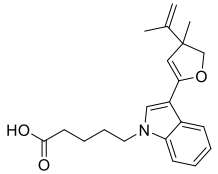
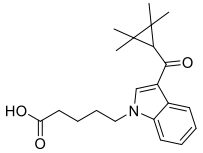
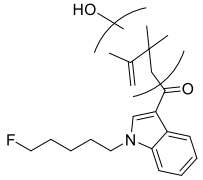
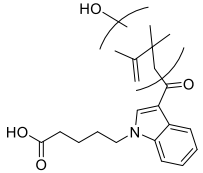
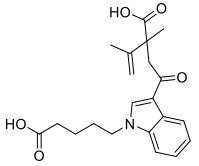
[b] Compound tentatively identified based on the accurate-mass fragmentation observed and information available on literature.

Table 2 Synthetic cannabinoid metabolites selected for the suspect screening of urine samples.

Synthetic cannabinoid	Target metabolites
JWH-081	JWH-081 *N*-(5-hydroxypentyl) metabolite
	JWH-081 *N*-pentanoic acid metabolite
	JWH-081 5-hydroxyindole metabolite
	JWH-081 4-hydroxynaphthyl metabolite
JWH-250	JWH-250 *N*-(5-hydroxypentyl) metabolite
	JWH-250 *N*-pentanoic acid metabolite
	JWH-250 5-hydroxyindole metabolite
JWH-203	JWH-203 *N*-(5-hydroxypentyl) metabolite
	JWH-203 *N*-pentanoic acid metabolite
	JWH-203 5-hydroxyindole metabolite
JWH-019	JWH-019 *N*-(6-hydroxyhexyl) metabolite
	JWH-019 *N*-pentanoic acid metabolite
	JWH-019 5-hydroxyindole metabolite
XLR-11	XLR-11/UR-144 *N*-(5-hydroxypentyl) metabolite*
	XLR-11 6-hydroxyindole metabolite
	XLR-11/UR-144 *N*-pentanoic acid metabolite*
UR-144	XLR-11/UR-144 *N*-(5-hydroxypentyl) metabolite*
	XLR-11/UR-144 *N*-pentanoic acid metabolite*
5F-AKB48	AKB48 *N*-(5-hydroxypentyl) metabolite
	AKB48 *N*-pentanoic acid metabolite
	5F-AKB48 *N*-(4-hydroxypentyl) metabolite

*Common metabolites for XLR-11 and UR-144

463 **Table 3** XLR-11 Phase I metabolites selected for the suspect screening of individual urine samples (based on [28])

Metabolite	Biotransformation	Elemental composition	Proposed Structure
M1[1]	Defluorination to hydroxylation	$C_{21}H_{29}NO_2$	
M2	Defluorination to carboxylic acid + hydroxylation + dehydration (2 metabolites described: M2-1, M2-2)	$C_{21}H_{25}NO_3$	
M3[2]	Defluorination to carboxylic acid (2 metabolites described: M3-1, M3-2)	$C_{21}H_{27}NO_3$	
M4[3]	Hydroxylation	$C_{21}H_{28}FNO_2$	
M5	Defluorination to carboxylic acid + hydroxylation (4 metabolites described: M5-1, M5-2, M5-3, M5-4)	$C_{21}H_{27}NO_4$	
M6	Defluorination to carboxylic acid + carboxylation (2 metabolites described: M6-1, M6-2)	$C_{21}H_{25}NO_5$	

[1] XLR-11/UR-144 N-(5-hydroxypentyl) metabolite
[2] XLR-11/UR-144 N-pentanoic acid metabolite

[3] XLR-11 degradant hydroxylated metabolite

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Figures

Fig. 1 Front of the different herbal blends products purchased on the smartshop. **A** Products previously analysed and reported in literature. **B** Products recently analysed.

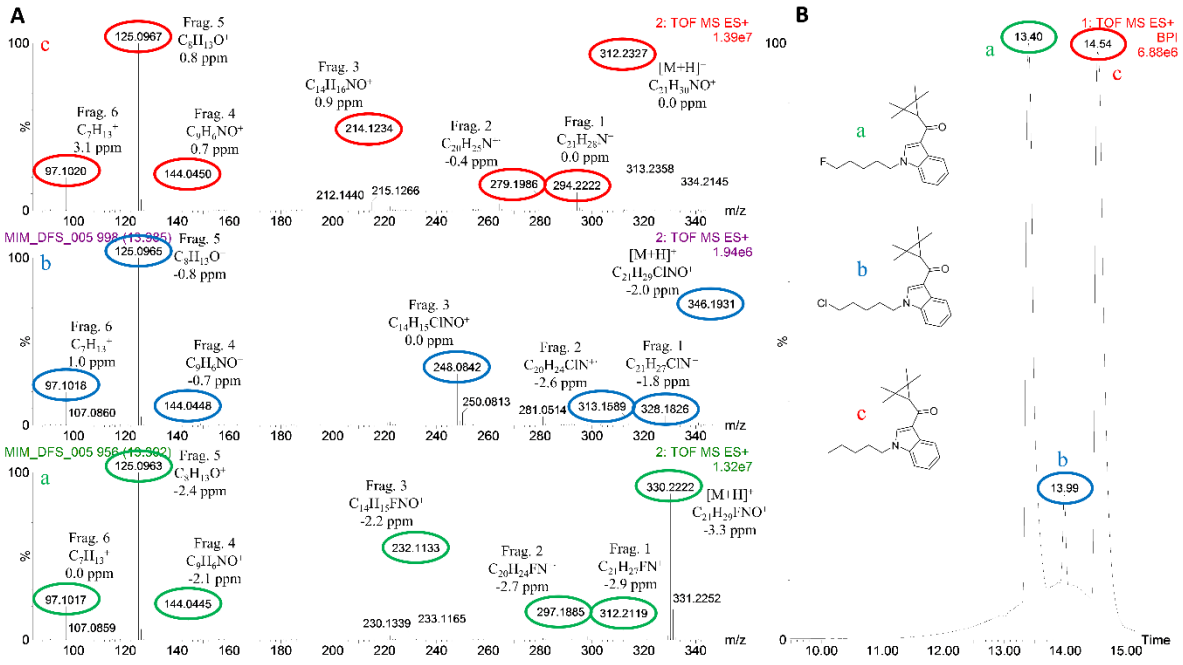
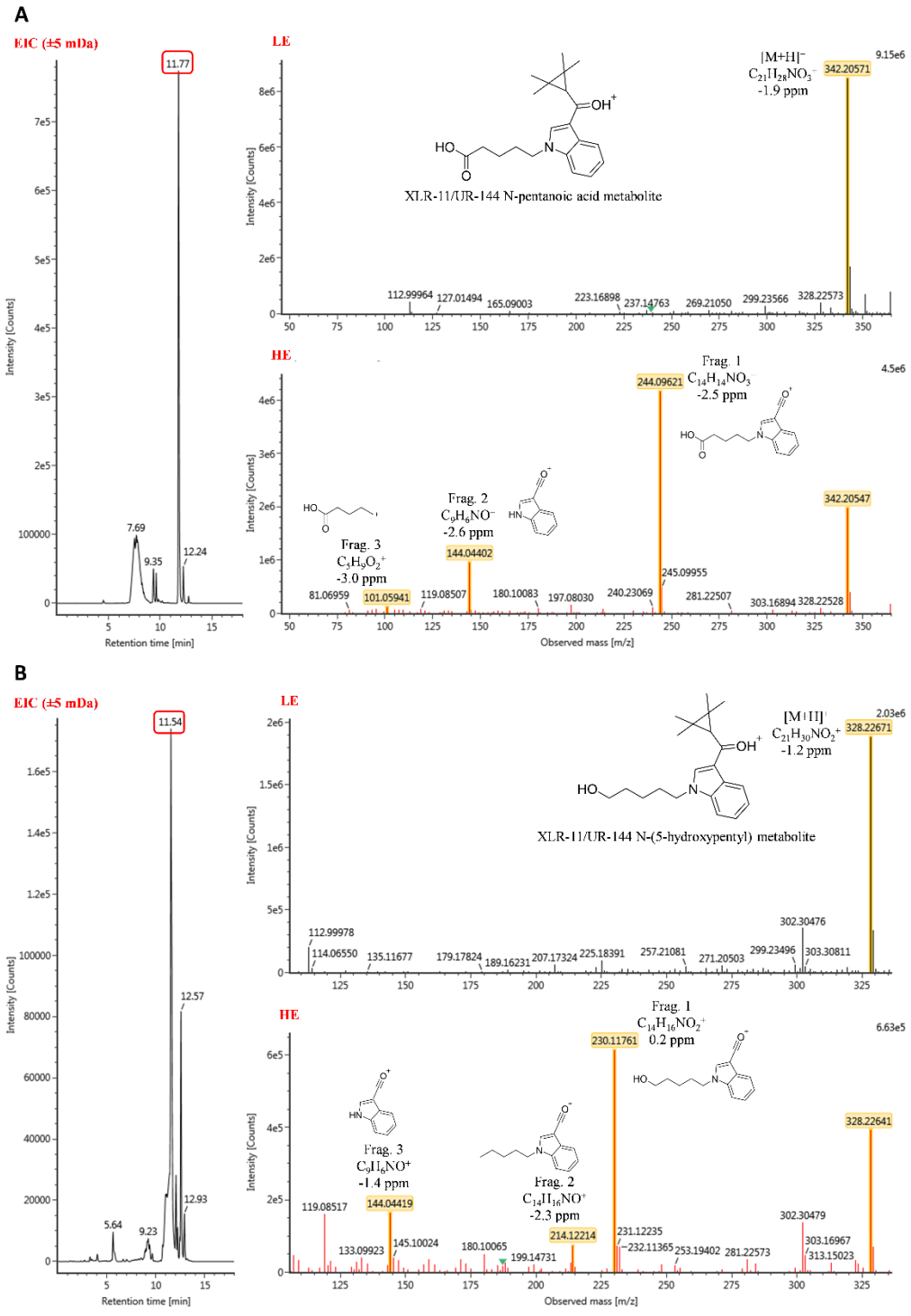


Fig. 2 Tentative identification of three SCs (a: XLR-11, b: UR-144 *N*-(5-chloropentyl) analog, c: UR-144) in *Hardcore* herbal blend by UHPLC-HRMS. **A** Accurate-mass fragmentation observed for the three SCs. **B** BPI chromatogram of the herbal blend extract.



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476 **Fig. 3** Tentative identification of the two major XLR-11 human metabolites in a urine sample.

477 EIC (left) and accurate-mass fragmentation (right). **A** N-pentanoic acid metabolite. **B** N-(5-

478 hydroxypentyl) metabolite.

Supplementary information

Investigation on the consumption of synthetic cannabinoids among teenagers by the analysis of herbal blends and urine samples

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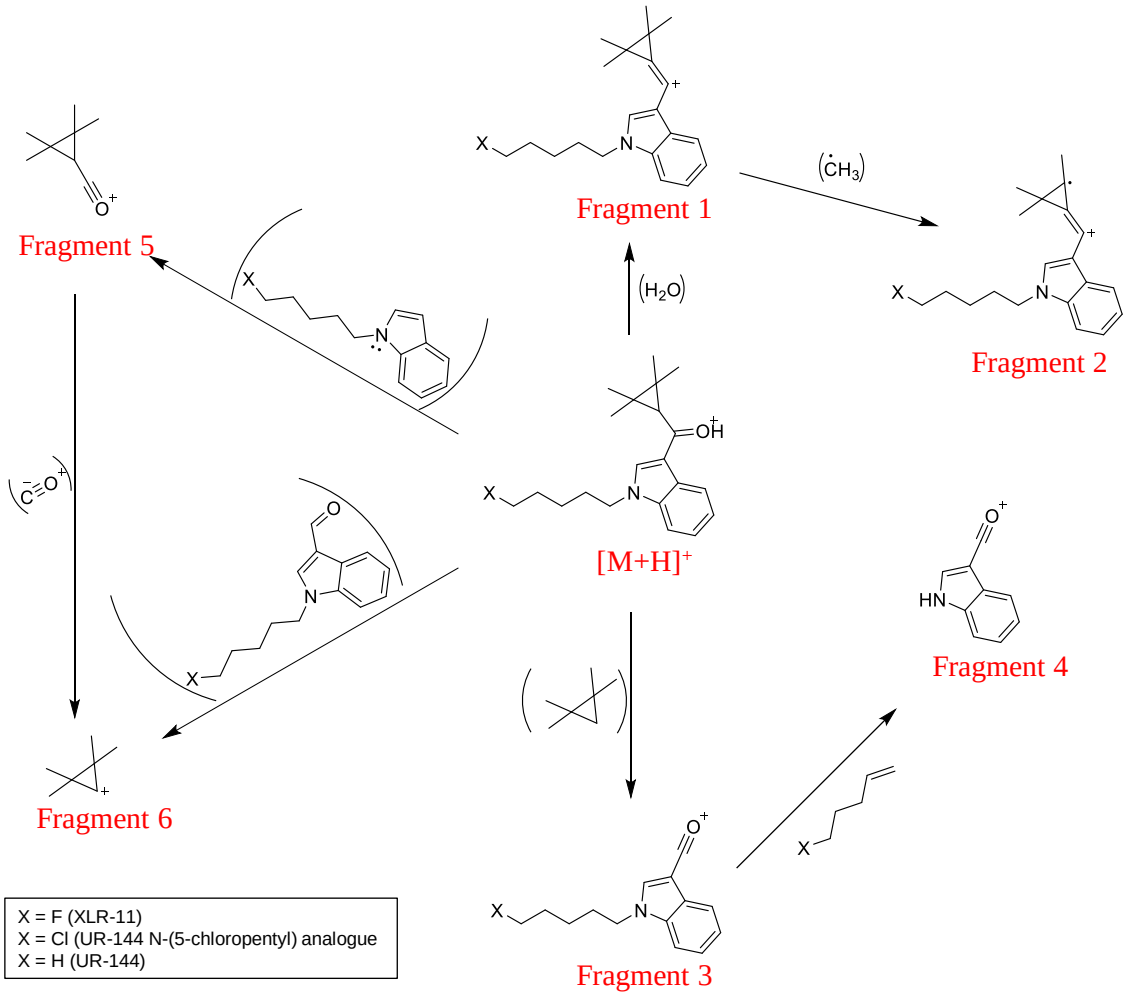

Fig.S1 Proposed fragmentation pathway for the three SCs found in *Hardcore* herbal blend.

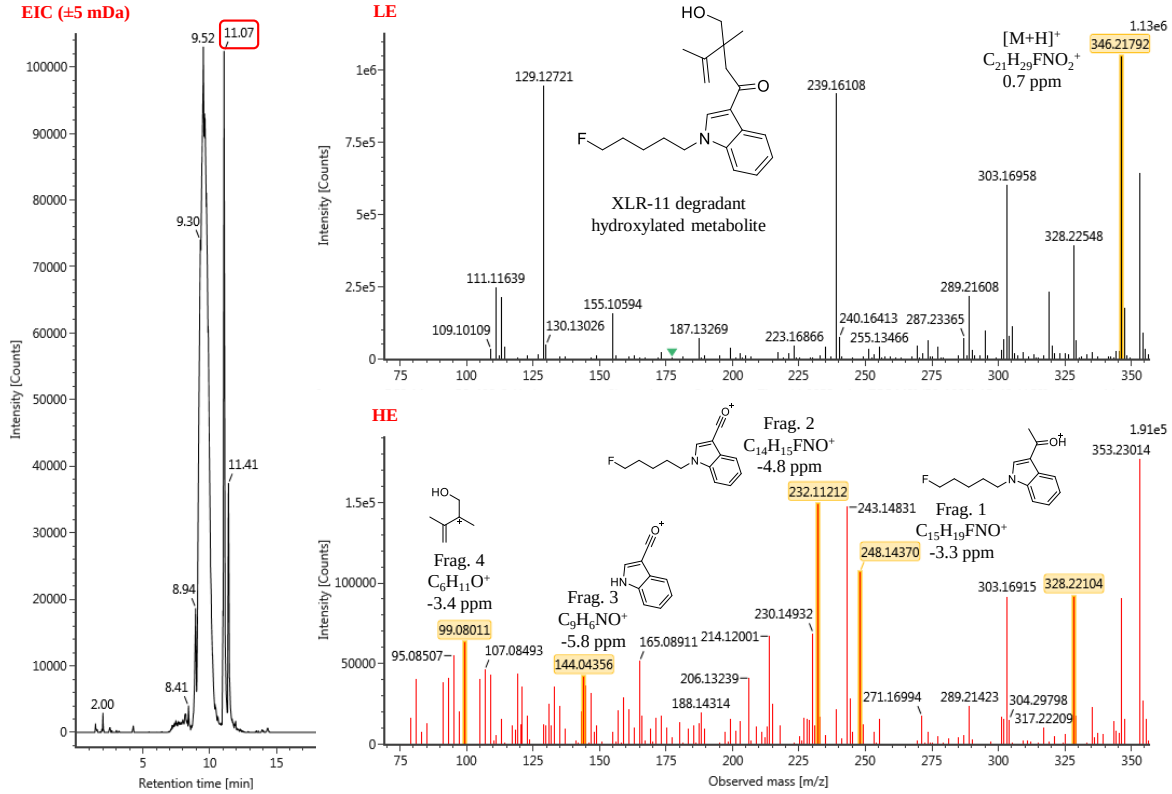

Fig.S2 Tentative identification of the third XLR-11 found in a pooled urine sample. EIC (left) and accurate-mass fragmentation (right).

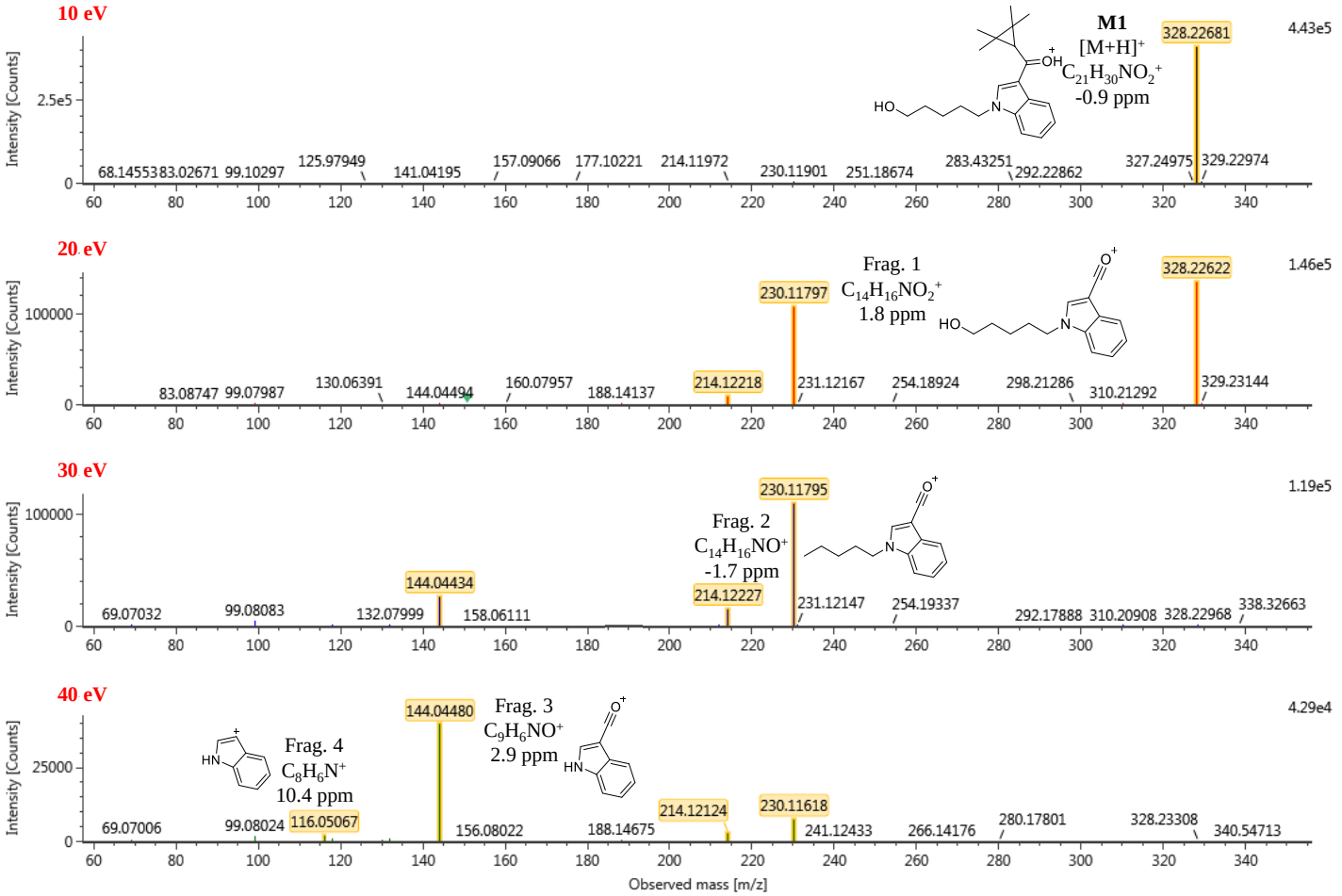

Fig. S3 MS/MS spectra at different collision energies for the metabolite M1 of the XLR-11.

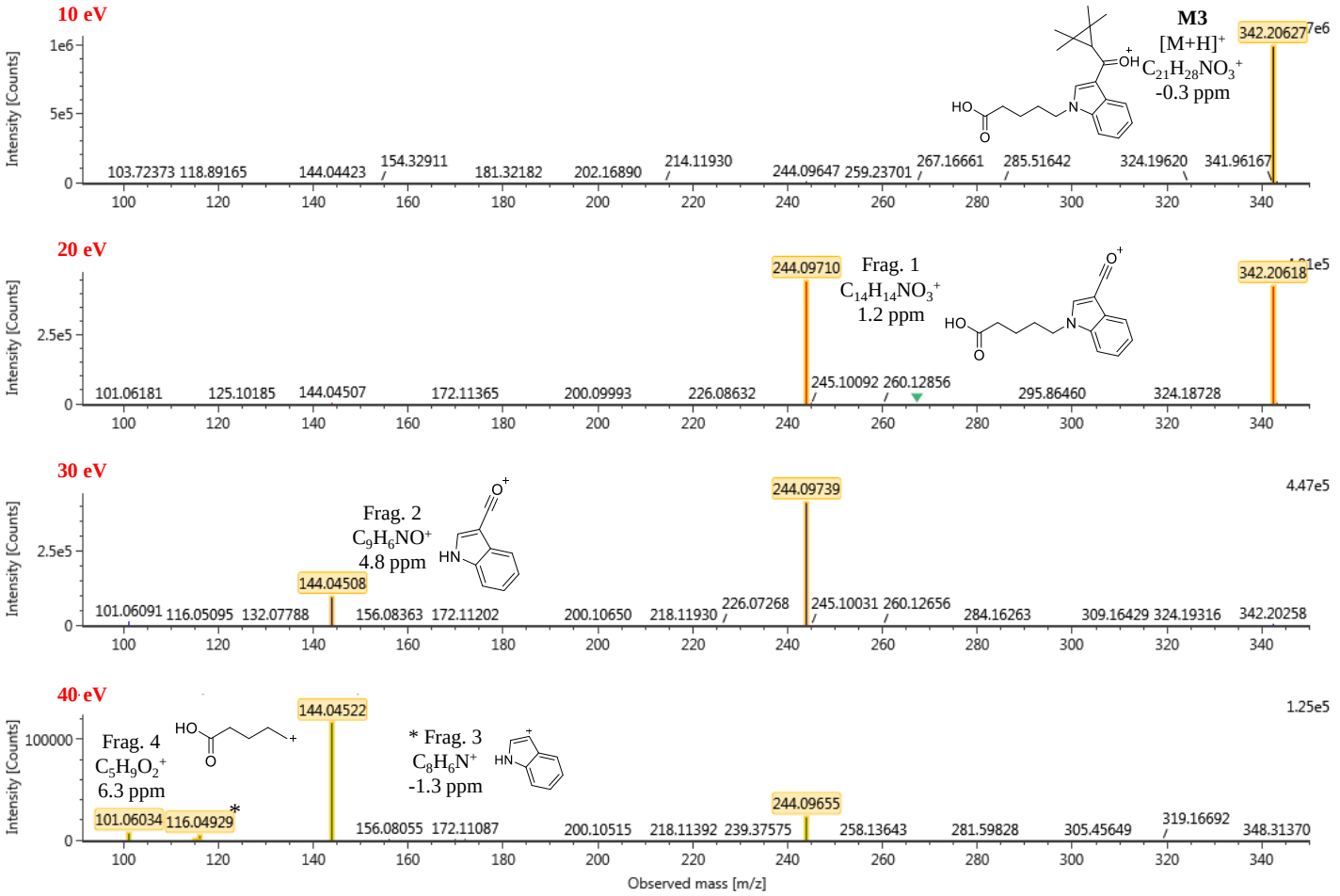

Fig. S4 MS/MS spectra at different collision energies for the metabolite M3 of the XLR-11.

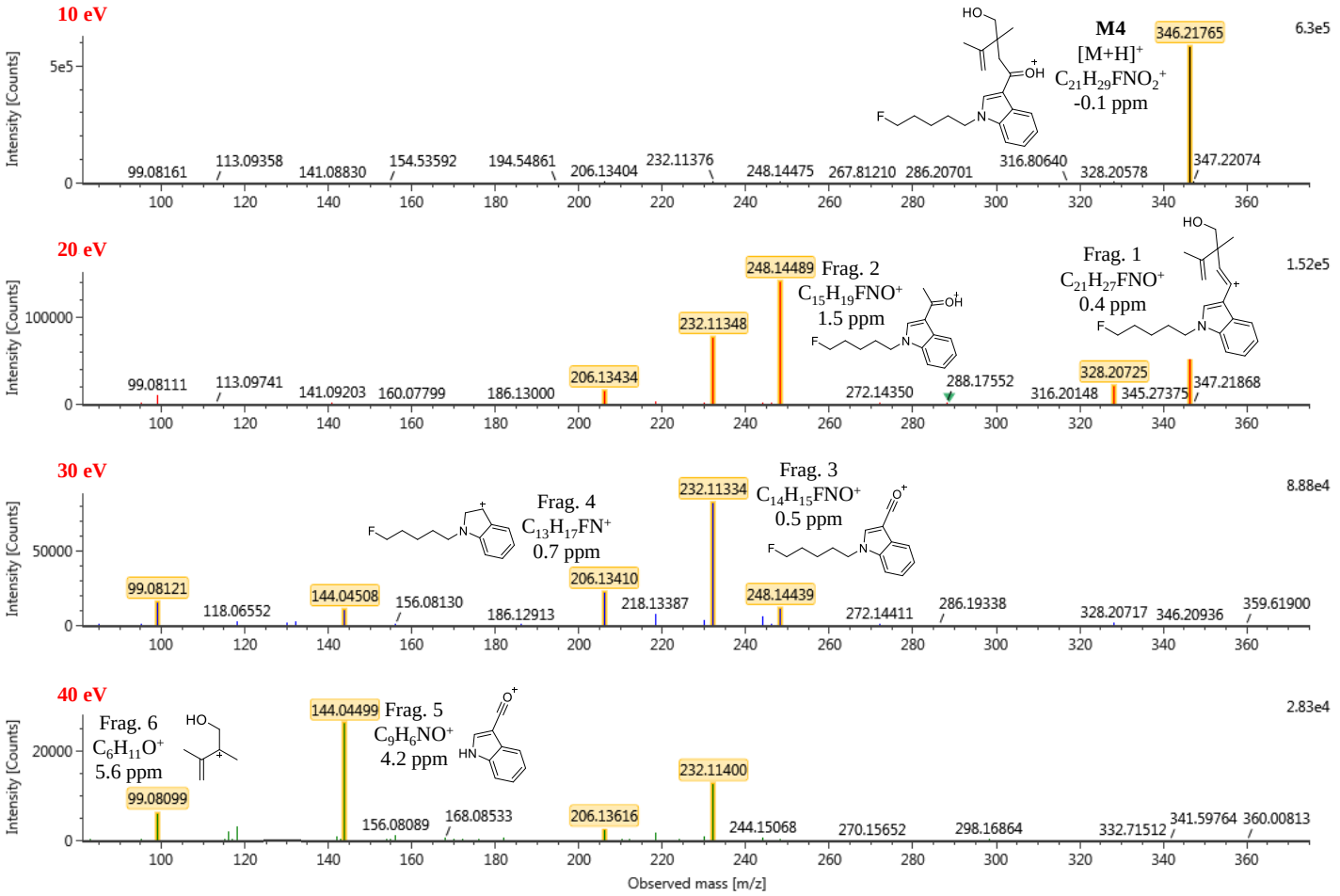

Fig. S5 MS/MS spectra at different collision energies for the metabolite M4 of the XLR-11.

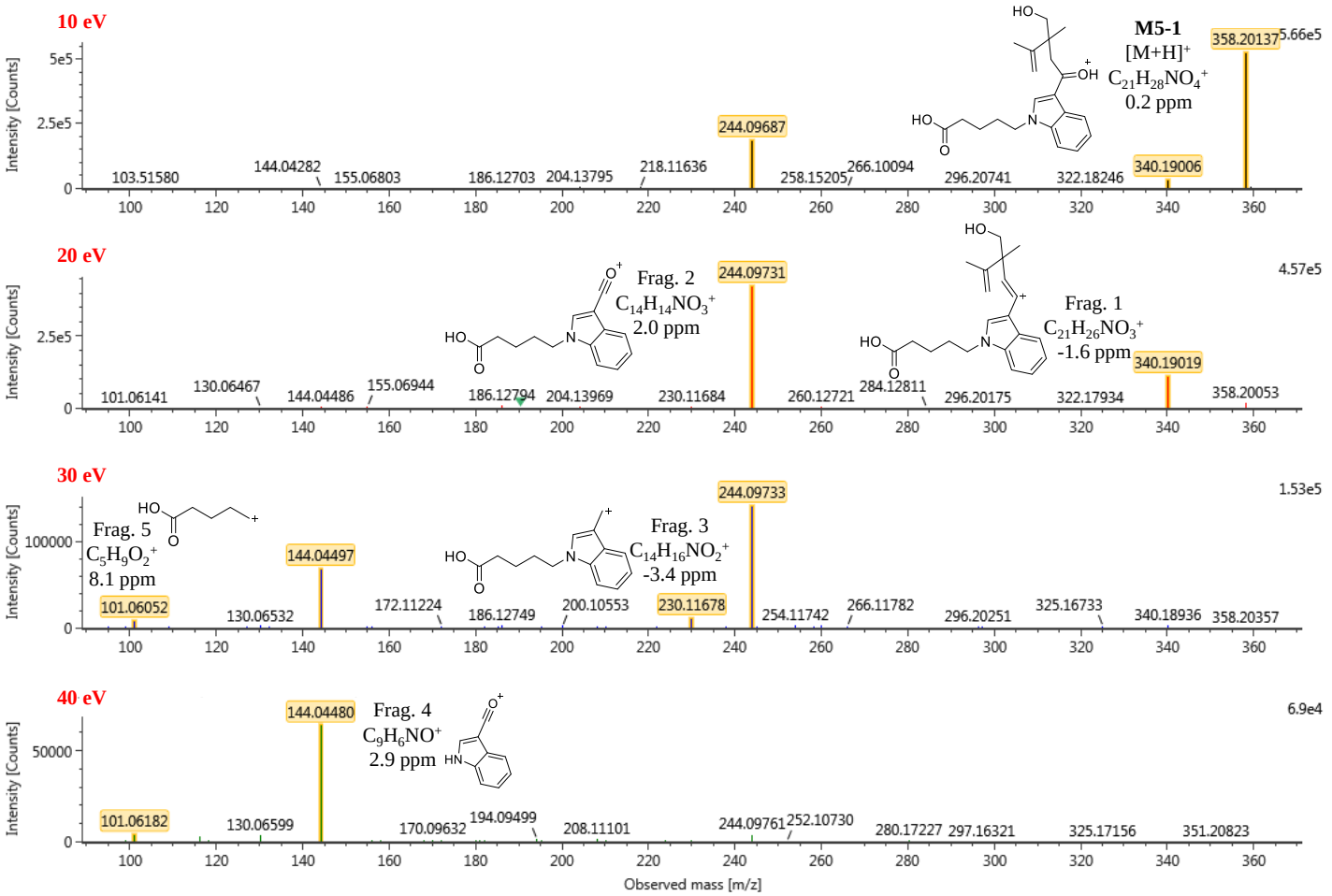

Fig. S6 MS/MS spectra at different collision energies for the metabolite M5-1 of the XLR-11.

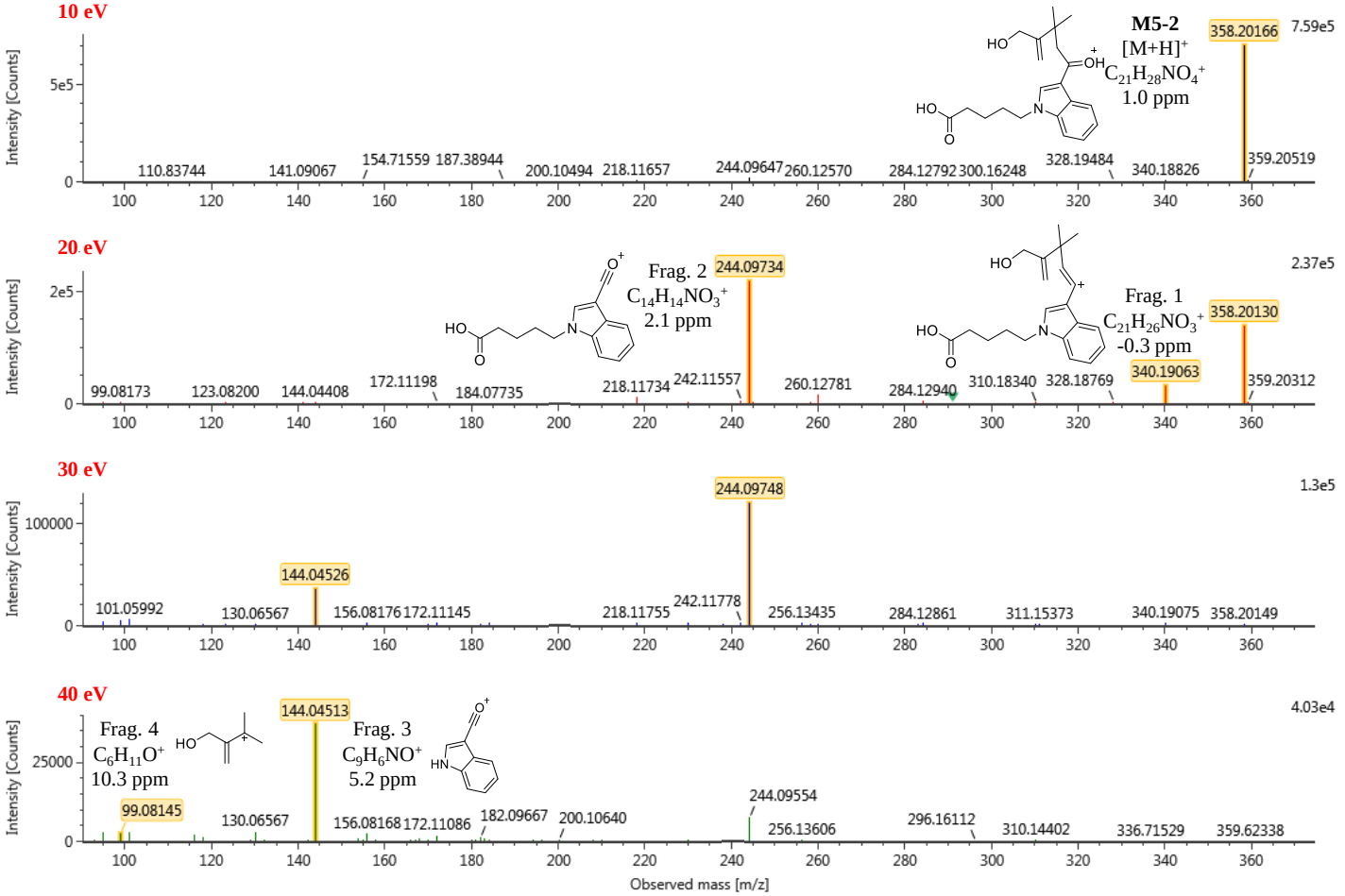

Fig. S7 MS/MS spectra at different collision energies for the metabolite M5-2 of the XLR-11.

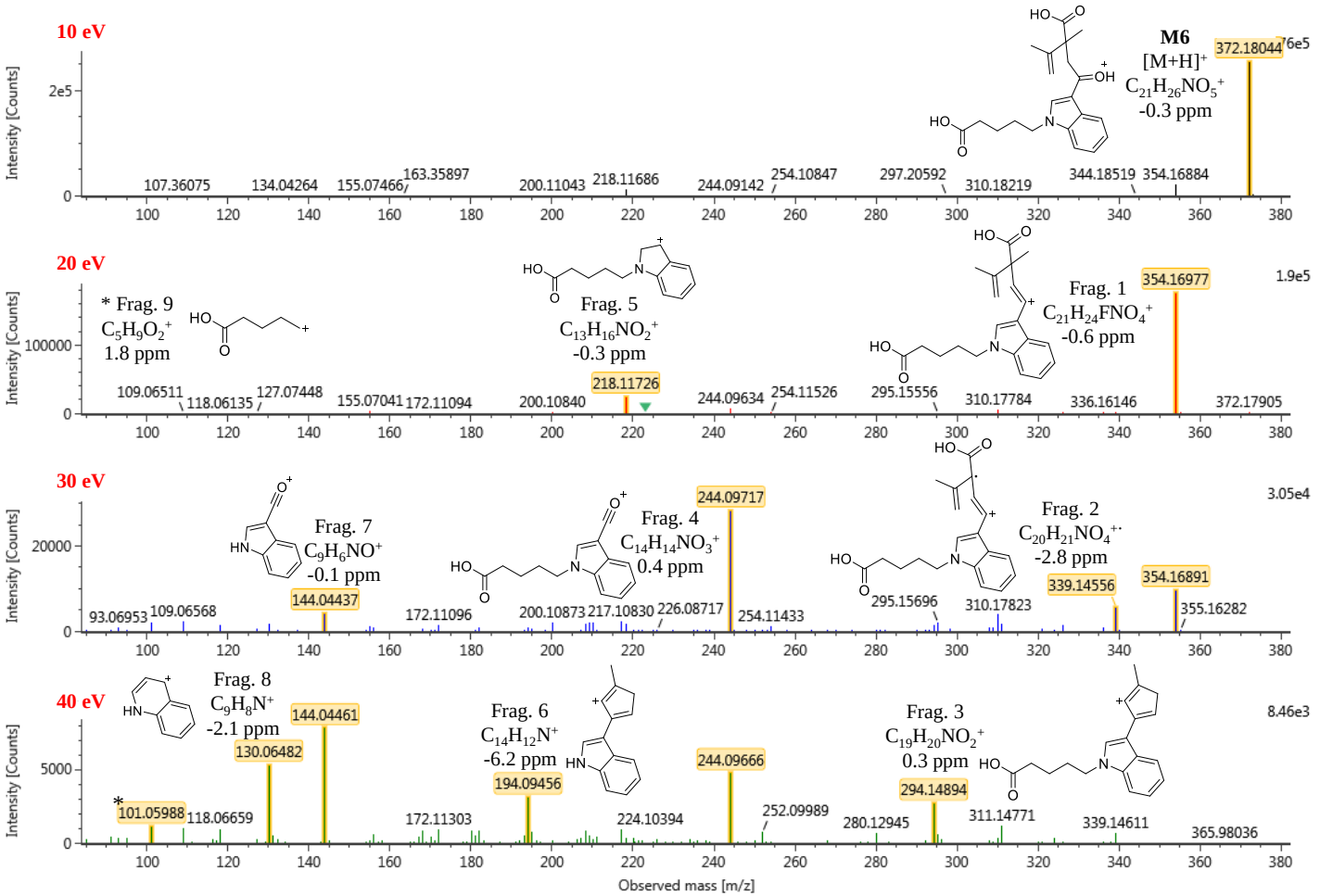

Fig S8 MS/MS spectra at different collision energies for the metabolite M6 of the XLR-11.