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Analytical characterization of some synthetic cannabinoids, derivatives of indole-3-carboxylic acid

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A B S T R A C T

By means of gas chromatography with high resolution mass spectrometry (GC–HRMS), ultra-high performance liquid chromatography in combination with high resolution tandem mass spectrometry (UHPLC–HRMS), nuclear magnetic resonance spectroscopy (NMR) and Fourier transform infrared spectroscopy (FT-IR), structure of a series from a novel synthetic cannabinoids, derivatives of indole-3-carboxylic acid, was established. Methods for determination of the compounds in mixtures, involving chromatographic separation with mass-spectroscopic determination, were elaborated. Analytical data obtained in the paper will make reliable identification of synthetic cannabinoids of the new type during forensic examination possible.

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1. Introduction

In illegal market of psychoactive compounds, synthetic cannabinoids [1–23] became world-wide spread. To get round legislative prohibition of known compounds, drug dealers offer for consumption compounds with similar (modified) structure. That is why efficient determination of the structure of new psychoactive compounds occurring in illegal turnover becomes the most acute challenge, for forensic experts.

Beginning in October 2012, new type of synthetic cannabinoids, a structural modification of compounds from 3-naphthoylindole series [3–17] has begun to be seized by police regularly, on the territory of Russia and Belarus.
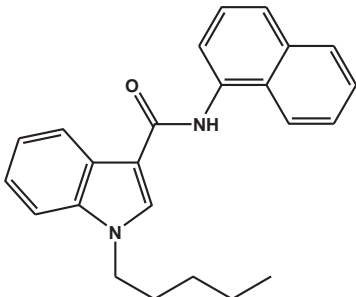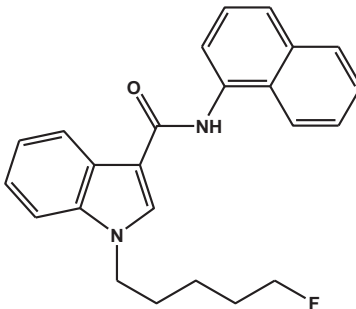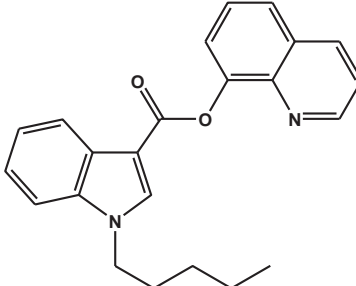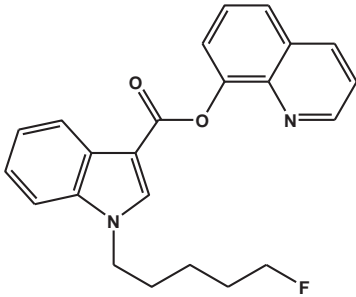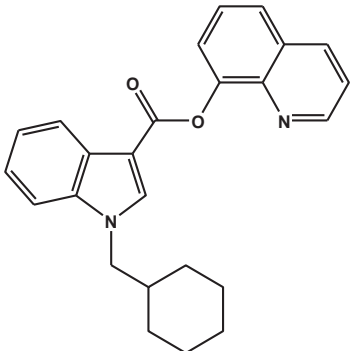
Most important – both in the sense of chemical classification and in juridical aspect – is that structural changes have effected carbonyl group in position 3 of indole ring resulting in new type of compounds, amides and esters of indole-3-carboxylic acid. Structures, chemical names and authors' conventional names for compounds under discussion are given in Table 1. For compounds **3–5**, short names used by Internet-dealers are also given (in brackets).

Changing of keto group in position 3 of indole ring for amide one with the aim of preparation of new types of synthetic cannabinoids, was used already e.g. for the synthesis of narcotics in 3-adamantoylindole and 3-adamantoylindazole series [19,20]. Moreover for compound **1**, method of synthesis and biological activity were described in the literature [24], which made its emergence in illegal market of synthetic cannabinoids quite predictable. However identification of compound **1** – when is arose as a subject of expertise – was complicated by the absence of its analytical characteristics (except for ^{1}H NMR spectrum), in scientific literature. On contrary though compound **2** was not described, in the literature, it was an example of typical modification of compound **1**.

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Table 1
Structures and chemical names of compounds 1–5.

Compound	Conventional name	Structure	Chemical name
Indole-3-carboxamides			
1	CBM-018 (NNEI; MN-24)		*N*-(naphthalen-1-yl)-1-pentyl-1*H*-indole-3-carboxamide
2	CBM-2201 (MN-24F; 5F-MN-24)		1-(5-fluoropentyl)-*N*-(naphthalen-1-yl)-1*H*-indole-3-carboxamide
Indol-3-carboxylates			
3	QCBL-018 (PB-22; MN-25; LTI-696; QUPIC)		quinolin-8-yl 1-pentyl-1*H*-indole-3-carboxylate
4	QCBL-2201 (PB-22F; 5F-PB-22; MN-25F)		quinolin-8-yl 1-(5-fluoropentyl)-1*H*-indole-3-carboxylate
5	QCBL-CHM (BB-22; QUCHIC)		quinolin-8-yl 1-(cyclohexylmethyl)-1*H*-indole-3-carboxylate

As for 8-oxiquinoline esters of *N*(1)-alkylindole-3-carboxylic acids (**3–5**), they are a result of absolutely new type of structural modification, and no data on their synthesis or biological activity could be found, in the literature. Some analytical characteristics of compounds **3** and **5** were published in the paper [25] recently.

It is worth noting that in compounds **1–5** only substituents in position 3 of indole were changed (in comparison with 3-naphthoylindoles), while *N*-alkyl substituents were quite traditional for forbidden synthetic cannabinoids.

We have detected compounds **1–5** in numerous real evidences brought for examination to the forensic laboratories of Ekaterinburg (Russian Federation) and Minsk (Belarus) both in form of powders and in ready for use herbal mixtures.

In the course of elaboration of methods for sample preparation, we have noticed that the use of water-containing solvents *e.g.* 96% ethanol lead to detection, during GC–HRMS analyses of compounds **3–5**, a peak of 8-hydroxyquinoline. Its intensity was the higher the longer the solution was kept at room temperature before being analyzed. Most probably that was a result of partial hydrolysis of ester moiety in the presence of water. We assume that similar process takes place *in vivo* conditions as well. On contrary (as expected) there was no evidence of hydrolysis of amides **1** and **2** in the course of sample preparation.

In current paper, we report on determination of above-mentioned compounds basing on analytical properties *i.e.* data of electron ionization (EI) and electrospray ionization (ESI) mass-spectra (including MS and MS/MS high resolution spectra), NMR spectroscopy and FT-IR spectra as well as on generalized log-linear retention indices (GI) and retention times (RT) obtained in chromatographic conditions applied. These would help forensic experts to determine composition of seized psychoactive compounds.

2. Materials and methods

2.1. Materials

Subject substances were sampled in gram amounts from criminal evidences brought for examination. The samples for analysis were microcrystal white or pale beige powders, soluble in alcohols, acetone, chloroform and acetonitrile, and hardly soluble in water. Original crude samples containing compounds **1** and **2** had slight odor of naphthylamine.

Preliminary control by GC–HRMS and UHPLC–HRMS has revealed unidentified impurities in all samples. Therefore for NMR investigations and before recording FT-IR spectra, compounds **1–5** were additionally purified by re-crystallization from ethyl acetate until no more contaminants were detected.

2.2. Solvents and reagents

Methanol ('for liquid chromatography' grade) was purchased from Merck KGaA (Darmstadt, Germany), water ('for GC, HPLC and Spectrophotometry' grade) – from Honeywell Burdick and Jackson (Muskegon, USA), acetonitrile ('HPLC-gradient' grade) – from Panreac (Barcelona, Spain), formic acid (98.0% min.) – from Sigma-Aldrich (Steinheim, Germany), ethyl acetate ('analytical grade') – from Komponent-Reaktiv (Moscow, Russia). For recording NMR spectra, DMSO-d$_6$ with isotopic purity 99.8% min ('A' grade, by TU 95-1893-89, St. Petersburg, Russia) was used. For determination of chromatography GI, a set of individual *n*-alkanes (Fluka, Sigma–Aldrich, Steinheim, Germany) was applied as a standard. For recording FT-IR spectra, potassium bromide (IR grade, Panreac, Barcelona, Spain) was used.

2.3. Preparation of sample solution

For qualitative analyses, *ca.* 1 mg (for GC–MS) or *ca.* 0.5 mg (for GC–HRMS) of each product was dissolved in 1 ml methanol. After dissolving, solution was passed through a cellulose membrane filter (5064-8222, Agilent Technologies, USA) to serve as sample solution for analysis. For UHPLC–HRMS, the solution thus obtained was diluted with additional methanol to attain 5 μg/ml. For all analyses, the solution was diluted with methanol to a suitable concentration before instrumental analysis if necessary.

2.4. Instruments and Methods

GC–MS Analysis of compounds in their solutions in methanol was performed with 'Agilent 7820A' gas chromatograph equipped with 'Agilent 5975' quadrupole mass selective detector and with GC-HRMS 'Agilent 7890A' connected with tandem quadrupole time-of-flight (QTOF) accurate mass detector 'Agilent 7200 Accurate-Mass Q-TOF GC/MS' (all – from Agilent Technologies, USA). The conditions were: capillary column HP-5ms with chemically immobilized (5%-biphenyl)-95%-dimethylpolysiloxane (30.0 m × 0.25 mm × 0.25 μm, 19091 S-433, Agilent Technologies, USA), injector temperature was 280 °C, the transfer line temperature was maintained at 290 °C, MSD was operated in the EI mode (70 eV), a mass range of total ion current of 30–550 amu, TOF detector in extended dynamic range mode (EDR, 2 GHz) with acquisition rate 5 spectra/sec. Calibration of detectors and additional tuning of TOF detector before analysis was performed using perfluorotributylamine standard (PFTBA, 99.8%, Agilent Technologies, USA). The oven temperature program used consisted of an initial temperature of 100 °C; held for 2.0 min; followed by a ramp to 290 °C with 20 °C/min. The final temperature was held for 25 min. Total run time was 36.5 min.

The GC was operated in split mode (1:50 for GC–MS and 1:150 for GC–HRMS) with sample volume 1 μl and with a helium as a carrier gas at flow rate 1.0 ml/min (constant flow).

UHPLC–HRMS was used with QTOF accurate mass detector 'Agilent 6540 UHD Accurate-Mass Q-TOF LC/MS' (Agilent Technologies, USA). Chromatographic separation was performed using a 'Agilent 1290 Infinity' ultra-high performance liquid chromatography system at 50 °C with an Zorbax Extend-C18 RRHT, 2.1 × 50 mm, 1.8 μm, column (727700-902, Agilent Technologies, USA). For gradient elution, the mobile phases 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B) were used with the time program: 0 min 10% B, linear to 15% B at 1 min, linear to 100% B at 19 min, constant 100% B to 20 min, back to 10% B and equilibration for 2 min. The flow rate was 0.5 ml/min. The spectra were measured by flow injection of 5 ng of each substance (1 μl of 5 μg/ml solution in methanol).

QTOF detector was used with electrospray ion source (DualESI) in positive ion mode. Nitrogen at 350 °C with flow rate 10 l/min was used as a drying gas; nebulizer pressure 40 psi. Capillary voltage 3500 V, skimmer voltage 65 V, fragmentor voltage 100 V. In MS mode, quadrupole was adjusted for total ion current, but in MS/MS mode quadrupole was adjusted to isolate precursor ions only with isolation width $\Delta m/z = 1.3$. MS/MS spectra were obtained using collision-induced dissociation (CID) of precursor ions with collision energies 10 and 20 eV in hexapole collision cell with nitrogen as collision gas. MS spectra were registered within mass range 100–1700 amu and MS/MS spectra – within 50–500 amu. TOF detector was operated in EDR (2 GHz) mode, acquisition rate 1 spectra/sec. Spectra were internally mass corrected in real time using an automatically introduced reference mass solution containing two compounds: purine ([M + H]$^+$ = 121.050873) and HP-921 – hexakis (1*H*,1*H*,3*H*-tetrafluoropropoxy) phosphazine ([M + H]$^+$ = 922.009798).

^{1}H and ^{13}C NMR spectra were recorded on Bruker 'Avance II' spectrometer (Bruker, Switzerland) in DMSO-d$_6$ (400 and 100 MHz, respectively) using TMS as an internal standard. ^{13}C NMR spectra were recorded in *J*-mode (APT). Final assignment of the spectra was made by two-dimensional experiments ^{1}H–^{13}C Heteronuclear Multiple Bond Correlation (HMBC) and Heteronuclear Single Quantum Correlation (HMQC).

FT-IR spectra were recorded in KBr matrix by means of 'Avatar 370' (Nicolet, USA) Fourier transform IR (FT-IR) spectrometer in range 4000–400 cm^{-1}, with resolution 4 cm^{-1}, number of scans 16 – for the sample and 64 – for background.

3. Results and discussions

3.1. Gas chromatography with MS detection

EI HRMS for compounds **1–5** as well as their RT and GI [26] are given in Fig. 1.

It is worth to note that EI HRMS registered with TOF detector are rather similar to low resolution EI MS obtained with the use of quadrupole detector. The spectra are similar not only in the set of ions but also in their relative intensity. To estimate the similarity, low resolution spectra for compounds **1–5** were converted by means of 'NIST MS Program' and imported into the user MS library. Comparison of HRMS and the library MS spectra gave Match- and R.Match-factors in the range 900–920.

Analysis of MS spectra for compounds **1–5** shows that their fragmentation is similar to that for synthetic cannabinoids of 3-phenylacetylindole series such as JWH-250 or JWH-203 [6,9,12,23]. This could lead (basing only on automatic search within libraries of MS – without visual control by an expert) to
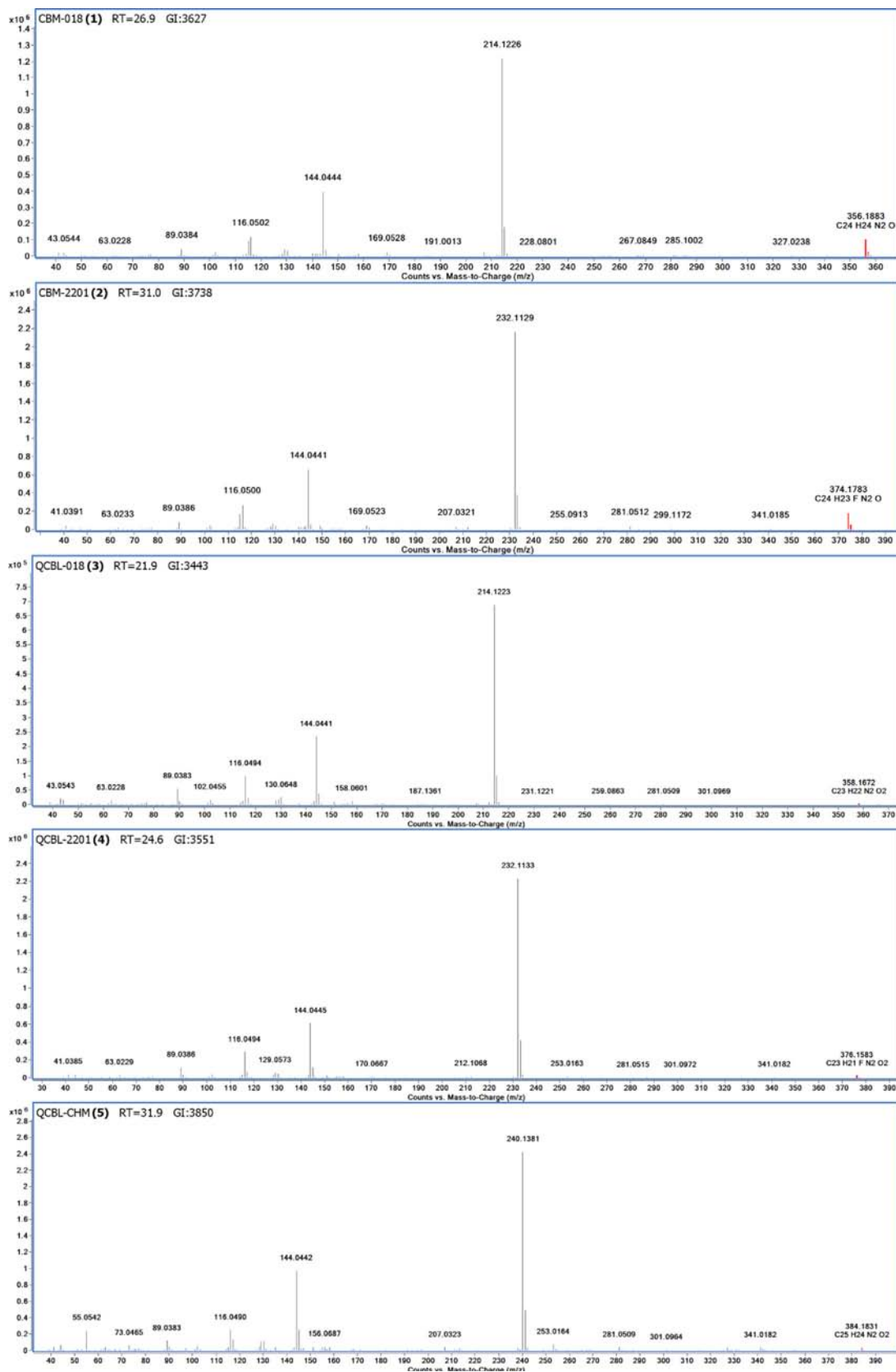
Fig. 1. EI HRMS, RT and GI for compounds 1–5.

Table 2
UHPLC–HRMS data for compounds 1–5.

Compound	RT (min)	Molecular formula	Accurate mass (amu)	Exact mass (amu)	Δm (ppm)
1	10.50	$C_{24}H_{24}N_2O$	356.1888	356.1889	0.07
2	9.14	$C_{24}H_{23}FN_2O$	374.1795	374.1794	−0.20
3	10.48	$C_{23}H_{22}N_2O_2$	358.1678	358.1681	0.86
4	8.91	$C_{23}H_{21}FN_2O_2$	376.1588	376.1587	−0.19
5	11.35	$C_{25}H_{24}N_2O_2$	384.1835	384.1838	0.79

Table 3
HRMS/MS for compounds **1–5** in CID, ESI conditions.

Compound	Collision energy (eV)	m/z (relative intensity)
1	20	357,1956 → 214,1223 (100); 144,0440 (3); 188,1428 (2); 116,0494 (<1); 132,0807 (<1); 215,1262 (<1); 118,0648 (<1); 158,0605 (<1); 89,0385 (<1); 71,0856 (<1)
2	20	375,1862 → 232,1131 (100); 144,0439 (3); 206,1338 (2); 116,0498 (<1); 233,1158 (<1); 118,0651 (<1); 89,0391 (<1); 69,0696 (<1); 132,0807 (<1); 212,1061 (<1)
3	10	359,1752 → 144,0443 (1); 116,0502 (<1); 215,1254 (<1); 158,0566 (<1); 214,6753 (<1); 274,0530 (<1); 301,1385 (<1); 89,0383 (<1); 298,1276 (<1)
4	10	377,1656 → 232,1128 (100); 144,0439 (1); 116,0494 (<1); 233,1169 (<1); 89,0387 (<1); 69,0691 (<1); 61,0435 (<1); 212,1078 (<1); 155,0706 (<1); 130,0644 (<1)
5	10	385,1903 → 240,1377 (100); 144,0440 (1); 55,0546 (<1); 241,1403 (<1); 97,1012 (<1); 116,0501 (<1); 69,0704 (<1); 89,0379 (<1); 74,0971 (<1); 63,0223 (<1)

erroneous attributing compounds of new class to cannabinoids of 3-phenylacetylindole series.

Thus main direction of fragmentation of compounds **1–5** is α-cleavage which is typical for 3-acylindoles leading to formation of corresponding N-alkylindolylacylium ions. Subsequent elimination of olefine from N-alkyl substituents and lose of C=O lead to ions with m/z 144.0444 ($C_9H_6NO^+$) and 116.0495 ($C_8H_6N^+$) which are characteristic for fragmentation of 3-acylindole compounds. At the same time fragment ions corresponding to substituents at carbonyl group are either not detected or could hardly be deduced because of possible masking due to overlapping with the other peaks. In this connection, analytical importance of molecular ions becomes higher. Moreover, for pairs **1** and **3**, **2** and **4** values of molecular ions are the only distinguishing parameter.

3.2. Ultra-high performance liquid chromatography mass-spectroscopy detection

UHPLC–HRMS with QTOF detection enabled us to get additional data for identification of compounds **1–5** and to determine the nature of substituents in positions 1 and 3 of indole ring. When we used 100 eV voltage on the ion source fragmentor, the only signals detected were those of protonated molecular ions, which made determination of accurate molecular masses of the compounds with minimal inaccuracy possible. Using software of MassHunter Qualitative Analysis B.05.00 instrument, these masses were calculated to give molecular formulae for compounds **1–5**.

To study fragmentation of protonated molecules of compounds **1–5** induced by their collisions with neutral nitrogen molecules, we have carried out MS/MS experiments. The spectra thus obtained can serve stable analytical characteristic of compounds **1–5**, which does not depend on ionization source condition and thus could be used for reliable identification of these compounds in seized samples.

Accurate and exact molecular masses, calculated molecular formulae as well as RT for compounds **1–5** are given in Table 2. High resolution MS/MS spectral data in form of mass and relative intensity of ten most intensive peaks are shown in Table 3. HRMS/MS for compounds **1–5** as well as their RT are presented in Fig. 2.

In CID conditions, fragmentation of protonated molecules from compounds **1–5** basically involves cleavage of C–C-bonds and formation (as in EI) of corresponding indolylacylium ions (Fig. 3). Further fragmentation thereof leads to characteristic ions with lower m/z value. Amide groups in compounds **1** and **5** make alternative direction of fragmentation (cleavage of bond between carbonyl function and indole ring) possible which leads to formation of corresponding alkyl-1H-indolium ions.

Cyclohexylmethyl substituent in compound **5** is proved not only with molecular formula, but also with ions $C_7H_{13}^+$ (m/z = 97.1012) and $C_4H_7^+$ (m/z = 55.0542) in its MS/MS.

3.3. NMR spectroscopy[1]

Chemical shifts and attributing of signals in NMR spectra for compounds **1–5** are shown in Tables 4 and 5. Final proof of structure for compounds **1–5** was made basing on their ^1H and ^{13}C NMR spectra using 2D experiments and data from reference literature [27]. Full NMR analysis including 2D experiments was performed only for two representative compounds, **1** (indole-3-carboxamide) and **4** (indol-3-carboxylate). The assignment of signals in NMR spectra for compounds **2**, **3** and **5** was made by analogy with compounds **1** and **4**.

Integral intensity, chemical shifts and multiplicity of signals in ^1H NMR spectrum of compound **1** completely correspond to N-(naphthalen-1-yl)-1-pentyl-1H-indole-3-carboxamide. In its ^{13}C NMR spectra recorded in J-mode (APT), number of opposite-phase signals is also in accordance with the structure of compound **1**.

In upfield part of proton spectrum for compound **1**, signals of H-1b, H-2b, H-3b, H-4b, H-5b which differ significantly in chemical shifts due to electron-withdrawing character of indole core are observed. This makes their unambiguous assessment possible. In downfield, singlets at 9.90 and 8.48 ppm are characteristic for NH and H-2 protons correspondingly. Multiplicity and spin-spin coupling constants for signals at 8.23, 7.60, 7.25 and 7.18 ppm are characteristic for protons H-4 – H-7 from *ortho*-substituted
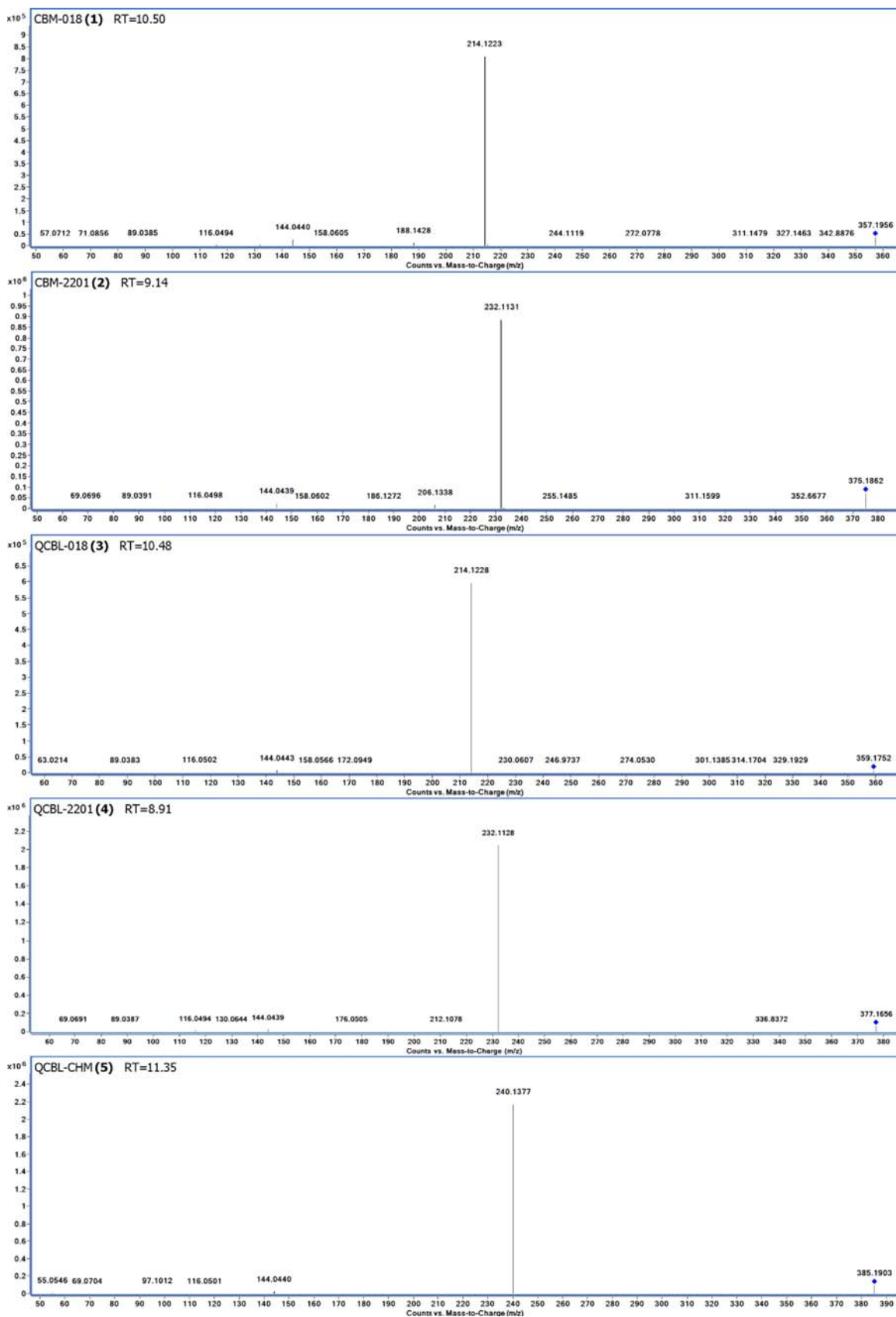

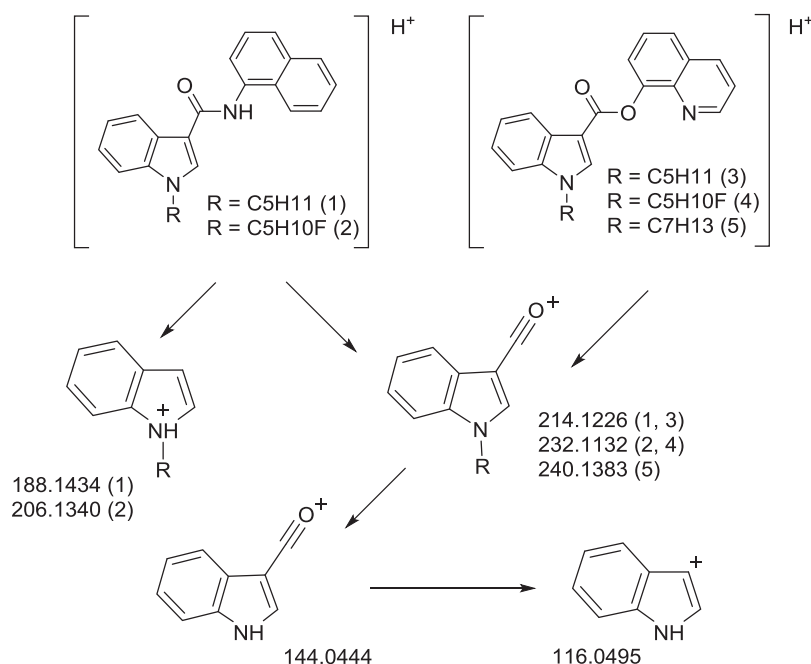
Fig. 2. HRMS/MS, RT for compounds 1–5.

Fig. 3. Scheme of formation of main characteristic ions in MS/MS spectra for compounds 1–5.

Table 4
NMR spectra for compounds 1 and 2.

Number of C atom	CBM-018 (1)		CBM-2201 (2)	
	^{1}H	^{13}C	^{1}H	^{13}C
2	8,48 (s., 1H)	131.6	8.48 (s., 1H)	131.7
3	–	109.3	–	109.4
3′	–	126.9	–	126.9
4	8,23 (d., 1H, J = 8.0 Hz)	121.3	8.21 (d., 1H, J = 8.0 Hz)	121.3
5	7,18 (t., 1H, J = 7.2 Hz)	120.8	7.19 (t., 1H, J = 7.2 Hz)	120.9
6	7,25 (t., 1H, J = 7.2 Hz)	122.1	7.26 (t., 1H, J = 7.2 Hz)	122.2
7	7,60 (d., 1H, J = 8.0 Hz) overlapping	110.3	7.62 (d., 1H, J = 8.0 Hz)	110.5
7′	–	136.2	–	136.2
1a	–	163.5	–	163.6
2a	9.90 (s., 1H)	–	9.91 (s., 1H)	–
1c	–	134.1	–	134.2
2c	7.49–7.58 (m., 1H) overlapping	125.5	7.52–7.59 (m., 1H) overlapping	125.6
3c	7.49–7.58 (m., 1H) overlapping	125.5	7.52–7.59 (m., 1H) overlapping	125.6
4c	7.49–7.58 (m., 1H) overlapping	125.8	7,52–7,59 (m., 1H) overlapping	125.9
5c	–	133.7	–	133.8
6c	7.70 (d., 1H, J = 7.2 Hz)	123.0	7.70 (d., 1H, J = 7.2 Hz)	123.1
7c	7.93–8.00 (m., 1H)	127.9	7.93–8.00 (m., 1H)	128.0
8c	8.07–8.14 (m., 1H)	123.3	8.06–8.13 (m., 1H)	123.4
9c	7.81 (d., 1H, J = 8.0 Hz)	125.2	7.82 (d., 1H, J = 8.0 Hz)	125.3
10c	–	128.9	–	129.0
1b	4.27 (t., 2H, J = 7.2 Hz)	45.9	4.31 (t., 2H, J = 7.2 Hz)	45.8
2b	1.86 (quin., 2H, J = 7.2 Hz)	29.2	1.91 (quin., 2H, J = 7.6 Hz)	29.2
3b	1.26–1.42 (m., 2H) overlapping	28.3	1.42 (quin., 2H, J = 8.0 Hz)	22.1 (d., $^3J_{C-F}$ = 5.1 Hz)
4b	1.26–1.42 (m., 2H) overlapping	21.7	1.63–1.79 (d.m., 2H)	29.3 (d., $^2J_{C-F}$ = 19.1 Hz)
5b	0.88 (t., 3H, J = 7.2 Hz)	13.8	4.51 and 4.39 (d.t., 2H, ^2J = 6.0 / ^3J = 47.6 Hz)	83.6 (d., $^1J_{C-F}$ = 160.7 Hz)

Table 5
NMR spectra for compounds 3–5.

Number of C atom	QCBL-018 (3)		QCBL-2201 (4)		QCBL-CHM (5)
	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H
1	–	162.4	–	162.3	–
2	8.49 (s., 1H)	136.1	8.50 (s., 1H)	136.0	8.42 (s., 1H)
3	–	104.5	–	104.5	–
3′	–	126.4	–	126.4	–
4	8.07 (d., 1H, J = 8.0 Hz)	120.7	8.07 (d., 1H, J = 8.0 Hz)	120.7	8.06 (d., 1H, J = 8.0 Hz)
5	7.26 (t., 1H, J = 8.0 Hz)	122.0	7.26 (t., 1H, J = 8.0 Hz)	122.0	7.25 (t., 1H, J = 8.0 Hz)
6	7.32 (t., 1H, J = 8.0 Hz)	122.6	7.32 (t., 1H, J = 8.0 Hz)	122.6	7.31 (t., 1H, J = 8.0 Hz)
7	7.64–7.73 (m., 1H) overlapping	111.1	7.64–7.73 (m., 1H) overlapping	111.0	7.64–7.72 (m., 1H) overlapping
7′	–	136.4	–	136.4	–
1a	–	147.2	–	147.2	–
2a	7.64–7.73 (m., 1H) overlapping	121.7	7.64–7.73 (m., 1H) overlapping	121.7	7.64–7.72 (m., 1H) overlapping
3a	7.91–7.98 (m., 1H)	125.7	7.91–7.98 (m., 1H)	125.7	7.91–7.97 (m., 1H)
4a	7.64–7.73 (m., 1H) overlapping	121.9	7.64–7.73 (m., 1H) overlapping	121.9	7.64–7.72 (m., 1H) overlapping
5a	–	129.1	–	129.0	–
6a	8.46 (d.d., 1H, ^3J = 8.0 Hz / ^4J = 1.6 Hz)	136.5	8.45 (d.d., 1H, ^3J = 8.0 Hz / ^4J = 1.6 Hz)	136.5	8.46 (d.d., 1H, ^3J = 8.0 Hz / ^4J = 1.6 Hz)
7a	7.59 (d.d., 1H, ^3J = 8.0 Hz / 4.3 Hz)	126.4	7.58 (d.d., 1H, ^3J = 8.0 Hz / 4.3 Hz)	126.4	7.58 (d.d., 1H, ^3J = 8.0 Hz / 4.3 Hz)
8a	8.86 (d.d., 1H, ^3J = 4.3 Hz / ^4J = 1.6 Hz)	150.4	8.86 (d.d., 1H, ^3J = 4.3 Hz / ^4J = 1.6 Hz)	150.3	8.86 (d.d., 1H, ^3J = 4.3 Hz / ^4J = 1.6 Hz)
9a	–	141.1	–	141.1	–
1b	4.34 (t., 2H, J = 7.2 Hz)	46.1	4.31–4.43 (m., 2H) overlapping	46.0	4.20 (d., 2H, J = 7.2 Hz)
2b	1.85 (quin., 2H, J = 7.2 Hz)	29.2	1.89 (quin., 2H, J = 7.2 Hz)	29.1	1.83–1.97 (m., 1H)
3b	1.23–1.40 (m., 2H) overlapping	28.3	1.39 (quin., 2H, J = 8.0 Hz)	22.0 (d., $^3J_{C-F}$ = 5.2 Hz)	0.98–1.11 (m., 1H) and 1.52–1.65 (m., 1H) overlapping
4b	1.23–1.40 (m., 2H) overlapping	21.7	1.61–1.78 (d.m., 2H)	29.3 (d., $^2J_{C-F}$ = 19.2 Hz)	1.11–1.25 (m., 1H) and 1.65–1.75 (m., 1H) overlapping
5b	0.87 (t., 3H, J = 6.8 Hz)	13.8	4.31–4.43 (m., 1H) overlapping and 4.49 (t., 1H, ^2J = 6.0 Hz)	83.5 (d., $^1J_{C-F}$ = 160.8 Hz)	1.11–1.25 (m., 1H) and 1.52–1.65 (m., 1H) overlapping
6b	–	–	–	–	1.11–1.25 (m., 1H) and 1.65–1.75 (m., 1H) overlapping
7b	–	–	–	–	0.98–1.11 (m., 1H) and 1.52–1.65 (m., 1H) overlapping

benzene system. Signals at 8.07, 7.93, 7.81 and 7.55 ppm could be assigned to protons H-2c – H-4c and H-6c – H-9c of naphthalene ring.

Cross-peaks between protons of α-CH$_2$ group of *N*-1-substituent and H-2 in HMBC spectrum (H-1b/C-7′, H-1b/C-2 and H-2/C-3′, H-2/C-3, H-2/C-1a, H-2/C-1b, H-2/C-7′) play a key role in assessment of signals of protons and carbons of indole ring. Further HMBC analysis of cross-peaks between C-3′, C-3 and C-7′ carbons and protons brought us to unambiguous assessment of indole core protons. Signals of CH-carbons of indole cycle were assigned basing on the data of HSQC spectrum.

We have found that NH-2a-proton gives cross peaks not only with C-1a carbonyl carbon but with another three carbons, C-1c,

C-2c and C-10c as well. Analysis of cross-peaks between these carbons and naphthalene protons H-2c – H-4c, H-6c – H-9c enabled us to assign signals of protons and carbons from naphthalene fragment.

Similar approach was used to assignment of signals in NMR spectra for compound **4**. Thus integral intensity, chemical shifts and multiplicity of signals in ^{1}H NMR spectrum of compound **4** completely correspond to quinolin-8-yl 1-(5-fluoropentyl)-1*H*-indole-3-carboxylate. In its ^{13}C NMR spectra recorded in *J*-mode (APT), number of opposite-phase signals is also in accordance with the structure of compound **4**.

Pattern of signals of H-1b, H-2b, H-3b, H-4b and H-5b protons in upfield of ^{1}H NMR spectrum of compound **4** is complicated with

their interaction with fluorine atom. In ^{13}C spectrum, signals of fluoroalkyl substituents are shifted downfield significantly due to electron-withdrawing character of fluorine and appear as doublets with characteristic spin-spin coupling constants ($^1J_{C-F}$ = 160.8 Hz, $^2J_{C-F}$ = 19.2 Hz, $^3J_{C-F}$ = 5.2 Hz).

In downfield part of proton spectrum for compound **4**, singlet at 8.50 ppm is characteristic for H-2 proton. Multiplicity and spin-spin coupling constants for signals at 8.07, 7.32 and 7.26 ppm are characteristic for protons H-4 – H-6 from *ortho*-substituted benzene system. Signals at 8.86, 8.45, 7.94 and 7.58 ppm could be assigned to protons of quinoline ring. Multiplet at 7.64–7.73 ppm is formed by overlapping of signals of protons from quinoline (H-2a and H-4a) and indole cycles (H-7).

Cross-peaks between protons of α-CH$_2$ group of *N*-1-substituent and H-2 in HMBC spectrum for compound **4** (H-1b/C-7′, H-1b/C-2 and H-2/C-3′, H-2/C-3, H-2/C-1a, H-2/C-1b, H-2/C-7′) play a key role in assessment of signals of protons and carbons of indole ring. Further HMBC analysis of cross-peaks between C-3′, C-3 and C-7′ carbons and protons brought us to unambiguous assessment of indole core protons. Signals of CH-carbons of indole cycle were assigned basing on the data of HSQC spectrum.

According to general concepts in NMR spectroscopy and in compliance with calculations by means of a packet of programs from ACDLabs, C-8a-proton should be most downfield – among the protons of quinoline cycle and was actually found as doublet of doublets at 8.86 ppm (d.d., 1H, ^3J = 4.3 Hz, ^4J = 1.6 Hz). Signals of carbon atom in bridge (C-9a) and in proton-bearing (C-7a and C-6a) carbons were identified by characteristic cross-peaks between them and C-8a-proton. Consecutive analyses of cross-peaks between fixed carbons and the other protons in quinoline lead to total assignment of the rest of signals of quinoline protons and carbons.

3.4. FT-IR spectroscopy

FT-IR spectra for compounds **1–5** are shown in Figs. 4 and 5. Strong absorbance bands corresponding to valence vibrations of carbonyl group around 1635 cm^{-1} (for amides **1** and **2**) or in the range 1716–1724 cm^{-1} (for esters **3–5**) are characteristic for such compounds [27] and are in concordance with suggested structures. Intense bands of valence vibrations of C–O-bond at 1083–1086 cm^{-1} in FT-IR spectra for compounds **3–5** serve an additional evidence for ester function in their structure.
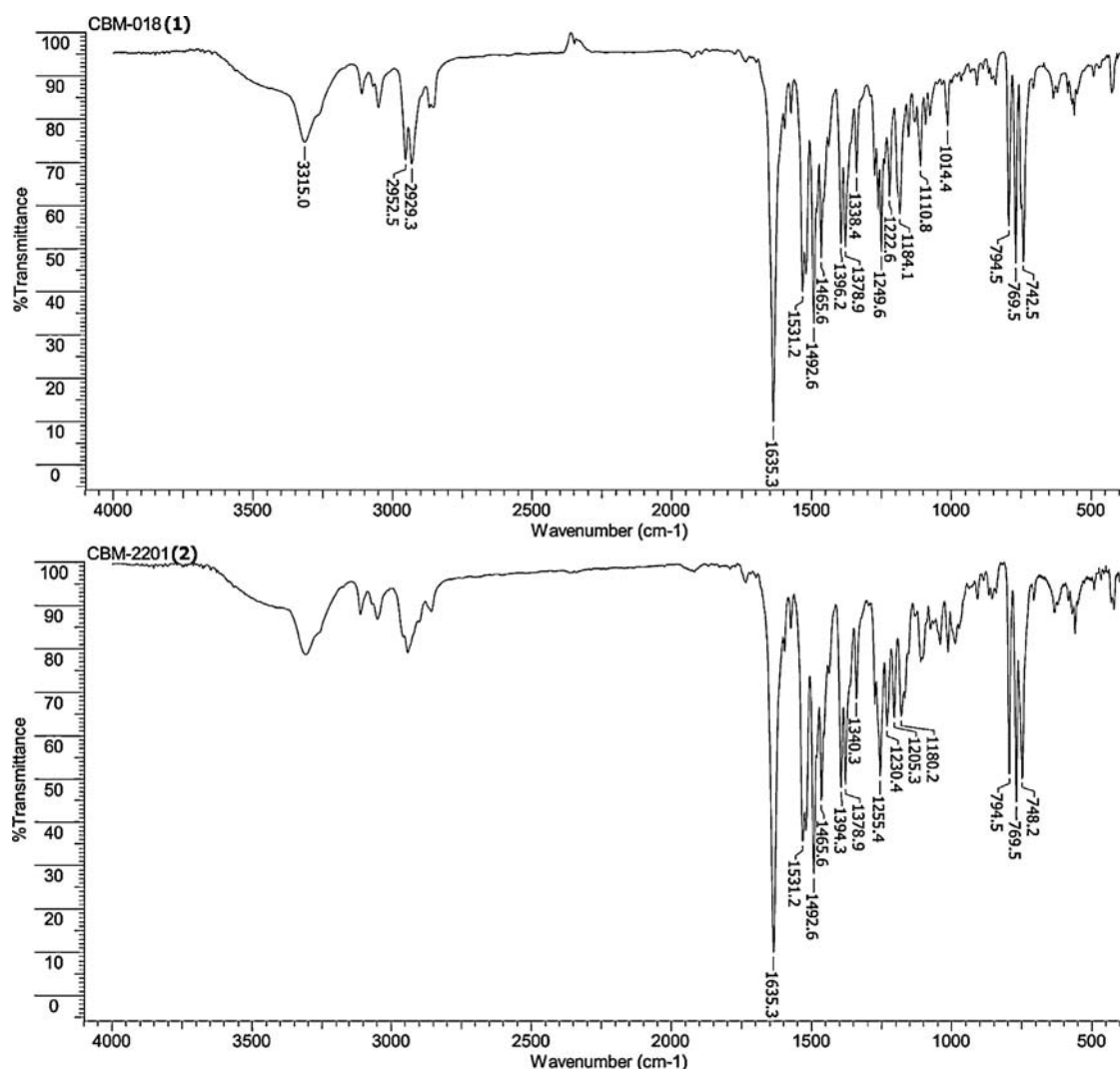

Fig. 4. FT-IR spectra for compounds 1, 2.

4. Conclusion

Derivatives of indol-3-carboxlic acid, a novel type of synthetic cannabinoids could be used as narcotics and are found in illegal turnover. In current study, the structure of a series of such compounds was determined. Analytical characteristics obtained for the compounds from this novel type of synthetic cannabinoids will make their reliable identification during forensic examination by means of chromatographic and mass-spectrometric methods possible.

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