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ANALYTICAL FEATURES OF SYNTHETIC MDMB(N)-073F CANNABIMIMETICS AND ITS MARKERS IN BIOLOGICAL MATERIAL

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The aim of the research is to study both analytical features of synthetic MDMB(N)-073F cannabimimetics of indazole carboxamides group by gas chromatography methods combined with tandem mass spectrometry (GC-MS) and high performance liquid chromatography with high-resolution mass spectrometry (HPLC-HRMS) as well as characteristics of the major MDMB(N)-073F metabolite, its glucuronide and derivatives, using gas chromatography with mass-spectrometric (GC-MS) detection and high-performance liquid chromatography (HPLC) with MS/MS mass spectrometry (HPLC-MS/MS) in urine samples to be applied in expert practice, chemical-toxicological and forensic and chemical analyses.

Materials and methods. To carry out the study, the following materials were used: plant-based objects with narcotic drugs withdrawn from illegal trafficking and applied to them;. urine samples to be studied under chemical-toxicological and forensic and chemical analyses. For solid-phase epitaxy, SampliQ EVIDEX TFE cartridges – 200 mg – 3 ml (Agilent, USA) were used for sample preparation; β-glucuronidase, Type HP-2, From Helix Pomatia, 100000 UA/ml (Sigma-ALDRICH CHEMI, Germany) was used for enzymatic hydrolysis. GC-MS/MS analysis was made using Agilent 7890 gas chromatograph with a tandem quadrupolar mass-spectrometer Agilent 7000 (Agilent, США); GC-MS analysis was carrid out using gas chromatograph Agilent 7820 with mass-selective detector Agilent 5975 (Agilent, USA); HPLC-HRMS research was made on liquid chromatograph Agilent 1260 with tandem hybrid high-resolution quadrupole-time-of-flight detector Agilent 6540 (Agilent, США); liquid chromatograph Agilent 1260 with Agilent 6460 (Agilent, USA) with tandem mass-spectrometer were used for making HPLC-MS/MS research.

Results. The structure of MDMB(N)-073F compound has been confirmed and an exact mass of the protonated molecule corresponding to the chemical formula $C_{19}H_{27}FN_3O_3$ fixed by GC-MS/MS and HPLC-HRMS methods. Spectral characteristics of MDMB(N)-073F have been given. One of the branches in MDMB(N)-073F biotransformation in the human body found out by GC-MS and HPLC-MS/MS methods, is the ester decomposition with further conjugation of the resulting acid. The product interacting with glucuronic acid, is found to be the conjugate of major MDMB(N)-073F metabolite of the Ist phase in biotransformation. Metabolites appearing due to the ester decomposition and its conjugate with glucuronic acid, are recommended to be used as markers for synthetic MDMB(N)-073F cannabimimetics in the analysis by chromatographic methods; they can be used for regular screening of biological samples.

Conclusion. The research results presented here, are the following: the analytical features characteristic for synthetic MDMB(N)-073F cannabimimetics found out by gas chromatography methods combined with tandem mass spectrometry (GC-MS/MS) and liquid chromatography of hybrid high-resolution quadrupole-time-of-flight mass spectrometry (HPLC-HRMS), as well as characteristics of major MDMB(N)-073F metabolite, its glucuronide and derivatives with the use of gas chromatography with mass-spectrometric detection (GC-MS) and liquid chromatography combined with tandem mass spectrometry (HPLC-MS/MS) in urine samples to be applied in expert practice, chemical-toxicological, forensic and chemical analyses.

Keywords: MDMB(N)-073F, cannabimimetics, gas chromatography – mass spectrometry (GC-MS), high performance liquid chromatography (HPLC), high-resolution mass spectrometry (HRMS), hybrid high-resolution quadrupole-time-of-flight mass spectrometry

Abbreviations: MRM – multiple reaction monitoring, GC – gas chromatography, MS mass spectrometry, HPLC – high performance liquid chromatography, HRMS – high-resolution mass spectrometry, a.u. – antitoxic unit.

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АНАЛИТИЧЕСКИЕ ХАРАКТЕРИСТИКИ СИНТЕТИЧЕСКОГО КАННАБИМИМЕТИКА MDMB(N)-073F И ЕГО МАРКЕРОВ В БИОЛОГИЧЕСКОМ МАТЕРИАЛЕ

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Целью исследования является изучение аналитических характеристик синтетического каннабимиметика группы индазолкарбоксамидов MDMB(N)-073F методами газовой хроматографии с тандемной масс-спектрометрией (ГХ-МС/МС) и жидкостной хроматографии с гибридной квадруполь-времяпролетной масс-спектрометрией высокого разрешения (ВЭЖХ-МСВР), а также характеристик главного метаболита MDMB(N)-073F, его глюкуронида и дериватов с использованием газовой хроматографии с масс-спектрометрическим детектированием (ГХ-МС) и жидкостной хроматографии с тандемной масс-спектрометрией (ВЭЖХ-МС/МС) в моче для целей экспертной практики, химико-токсикологического и судебно-химического анализа.

Материалы и методы. Объекты растительного происхождения с нанесенными на них наркотическими средствами, изъятые в нелегальном обороте. Образцы мочи, поступившие на химико-токсикологическое и судебно-химическое исследование. Для пробоподготовки использовались патроны для ТФЭ *SampliQ EVIDEX* – 200 мг-3 мл (*Agilent*, США), для ферментативного гидролиза использовалась β-глюкуронидаза, *Type HP-2, From Helix Pomatia*, 100000 ЕД/мл (*Sigma-ALDRICH CHEMI*, Германия). ГХ-МС/МС анализ проводили на газовом хроматографе Agilent 7890 с тандемным квадрупольным масс-спектрометром Agilent 7000 (Agilent, США); ГХ-МС анализ выполнен на газовом хроматографе Agilent 7820 с масс-селективным детектором Agilent 5975 (Agilent, США); ВЭЖХ-МСВР исследование проводили на жидкостном хроматографе Agilent 1260 с тандемным гибридным квадруполь – времяпролетным детектором высокого разрешения Agilent 6540 (Agilent, США); ВЭЖХ-МС/МС исследование выполнено на жидкостном хроматографе Agilent 1260 с тандемным масс-спектрометром Agilent 6460 (Agilent, США).

Результаты. В результате исследования, проведенного методами ГХ-МС/МС и ВЭЖХ-МСВР, подтверждена структура соединения MDMB(N)-073F, определена точная масса протонированной молекулы, соответствующая брутто-формуле $C_{19}H_{27}FN_3O_3$. Приведены спектральные характеристики MDMB(N)-073F. Методами ГХ-МС и ВЭЖХ-МС/МС установлено, что одним из направлений биотрансформации MDMB(N)-073F в организме человека является гидролиз сложноэфирной связи с последующей конъюгацией образующейся кислоты. Установлено, что конъюгатом главного метаболита MDMB(N)-073F фазы I биотрансформации является продукт взаимодействия с глюкуроновой кислотой. Метаболиты, образующиеся в результате гидролиза сложноэфирной связи, и его конъюгат с глюкуроновой кислотой рекомендованы в качестве маркеров употребления синтетического каннабимиметика MDMB(N)-073F при анализе хроматографическими методами они могут быть использованы при систематическом аналитическом скрининге биологических образцов.

Заключение. Приведены аналитические характеристики синтетического каннабимиметика MDMB(N)-073F методами газовой хроматографии с тандемной масс-спектрометрией (ГХ-МС/МС) и жидкостной хроматографии гибридной квадруполь-времяпролетной масс-спектрометрией высокого разрешения (ВЭЖХ-МСВР), а также характеристики главного метаболита MDMB(N)-073F, его глюкуронида и дериватов с использованием газовой хроматографии с масс-спектрометрическим детектированием (ГХ-МС) и жидкостной хроматографии с тандемной масс-спектрометрией (ВЭЖХ-МС/МС) в моче для целей экспертной практики, химико-токсикологического и судебно-химического анализа.

Ключевые слова: MDMB(N)-073F, каннабимиметики, метаболизм, газовая хроматография – масс-спектрометрия, высокоэффективная жидкостная хроматография, тандемная масс-спектрометрия, гибридная квадруполь-времяпролетная масс-спектрометрия высокого разрешения

Список сокращений: ГХ-МС/МС – газовая хроматография с тандемной масс-спектрометрией, ВЭЖХ-МСВР – жидкостная хроматография с гибридной квадруполь-времяпролетной масс-спектрометрией высокого разрешения, ГХ-МС – газовая хроматография с масс-спектрометрическим детектированием

INTRODUCTION

The analysis of toxicants' properties and processes of metabolism in the human body, methods of their extraction and identification is the main part in both forensic and toxicological chemistry. This is particularly true for new hazardous psychoactive substances including synthetic cannabimimetics, regularly appearing in illegal drug market.

All the factors – various equipment in expert institutions, various approaches to analyzing synthetic cannabimimetics to be found in biological materials, lack of analytical standards – make it difficult to interpret and compare the results obtained from different sources.

Gas chromatography with mass-spectrometric (GC/MS) detection is widely applied in laboratory practice in the Russian Federation, with the application thereof being combined with labor-intensive and time-consuming sample preparation to make qualitative sampling. In this case metabolite conjugation, extraction and derivatization are to be made when testing urine for cannabimimetics metabolites [1, 2].

Liquid chromatography combined with with a tandem mass-spectrometry (HPLC-MS/MS) method is considered to be the best, as it makes it possible to simplify the sample preparation process to detect metabolites of synthetic cannabimimetics as no deconjugation and derivatization are required [3-5].

In this scientific work, the results of the research of analytical features of synthetic MDMB(N)-073F cannabimimetics found out by gas chromatography methods combined with MS/MS mass spectrometry (GC-MS/MS) and liquid chromatography of hybrid high-resolution quadrupole-time-of-flight mass spectrometry (HPLC-HRMS), as well as characteristics of major MDMB(N)-073F metabolite and glucuronide and derivatives thereof using gas chromatography with mass-spectrometric detection (GC-MS) and liquid chromatography with MS/MS mass spectrometry (HPLC-MS/MS) in the urine samples to be applied in expert practice, chemical-toxicological, forensic and chemical analyses have been presented here.

OBJECTS OF RESEARCH

To carry out the study, the following materials were used: plant-based objects with narcotic drugs withdrawn from illegal trafficking and applied to them;. urine samples to be studied under chemical-toxicological and forensic and chemical analyses.

MATERIALS AND METHODS

Synthetic cannabimimetics in plant-based objects were detected and identified by gas-liquid chromatography methods with tandem mass-spectrometry and high performance liquid chromatography with high resolution MS/MS quadrupole-time-of-flight detector.

Gas-liquid chromatography methods with tandem quadrupole mass-spectrometric detector and high performance liquid chromatography with tandem hybrid high-resolution quadrupole-time-of-flight mass spectrometry (HPLC-HRMS) were applied to detect cannabimimetics markers and metabolites in biological material.

Equipment

- Gas chromatograph Agilent 7890 (capillary column DB-5MS, similar to (5% phenyl)-methylpolysiloxane), ID = 0.25mm, length = 30m, thickness of stationary phase film = 0.25 μm) with tandem quadrupole mass-spectrometer MS/MS Agilent 7000 (Agilent, USA);
- Gas chromatograph Agilent 7820 (capillary column HP-5MS (5% phenyl)- methylpolysiloxane), ID = 0.25 mm, length = 30m, thickness of film=0.25 μm) with mass-selective detector Agilent 5975 (Agilent, USA);
- Liquid chromatograph Agilent 1260 (chromatographic column Zorbax Extend C-18 2.1*50 mm, sorbent grain diameter = 1.8 μm) with tandem hybrid high-resolution quadrupole-time-of-flight detector Agilent 6540 (resolution at least 40000) (Agilent, USA);
- Liquid chromatograph Agilent 1260 (chromatographic column 3*150 mm with reversed-phase sorbent Poroshell 120 EC-C18, grain size = 2.7 μm) with tandem mass-spectrometer Agilent 6460 (Agilent, USA);
- 12 position SPE Vacuum Manifold System (Supelco);
- low vacuum pump (KNF lab LABOPORT (France);
- thermal block PE-4030 (Ecros, Russia);
- single-channel vaporizer PE-2300 (“Ecros”, Russia);
- microshaker PE-2 (“Ecros”, Russia);
- microwave Supra MWS-1824SW (Russia);
- solid-phase extraction cartridges SampliQ EVIDEX (200 mg / 3 ml) (Agilent, USA);
- semi-automatic dispensers with 4–40, 40–200 μl and 0.2–1, 1–5 ml range.

Materials

Bis-trimethylsilyl-trifluoroacetamide (BSTFA) containing 1% trimethylchlorosilane; 2,2,3,3,3-pentafluoropropanol, 2,2,3,3,3-pentafluoropropionic anhydride, methyl iodide, β-glucuronidase, *Type HP-2, From Helix Pomatia,* 100,000 EU / ml *(Sigma-ALDRICH CHEMI,* Germany). The chemicals used in the study are of the “chemically pure” brand. The storage of the urine samples before the study was carried out at + 4°C.

Sample preparation

Plant-based objects preparation (for GC-MS/MS and HPLC-HRMS analyses)

A weighed quantity of 10 mg of the plant-based object was extracted with 10ml of ethanol for 5 minutes. The resulting extract was separated from the plant matrix by centrifugation, diluted with ethanol by 10 times and analyzed by GC-MS/MS and HPLC-HRMS methods.

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Preparation of urine samples (applying enzymatic hydrolysis, for GC-MS analysis)

50 µl of an internal standard alcohol solution (0.2 mg/ml Hexenal, 250 µl of 1/15 M pH 6 phosphate buffer and 50 µl of β-glucuronidase) was added to 1 ml of the urine sample. Then the vial was corked up and exposed to 45°C for 2 hours. After cooling down, 2 ml 1/15 M phosphate buffer (pH 4,8) was added. The contents of the vials were centrifugated at 3000 rpm for 10 minutes, the centrifugate was separated from the residue.

For extraction, SPE cartriges SampliQ EVIDEX (200 mg/3 ml) with a mixed phase were used. Conditioning of a sorbent was conducted via succesive transfer of 2 ml of 95% ethanol and 2 ml of 1/15 M phosphate buffer (pH 4.8) through the cartridge. After that, the sample was downloaded at the speed of 1 ml/min. Flushing was conducted in a successive manner: first 1 ml of 1/15 M phosphate buffer (pH 4.8) and then 1 ml of 10% ethanol. Drying the cartridge was carried out in vacuum for 20 minutes. Eluate was derived via double transfer of 2 ml of n-hexane – ethylacetate (2:1) concoction through the cartridge. Eluate was vaporized in the nitrogen flow at 45°C.

Derivatization

Methylation

500 µl of anhydrous acetone, 40 µl of iodomethane and 20–25 mg of anhydrous potassium carbonate were added to the dry residue of eluate I. The vial was corked up and heated at 60°C in the thermal block for 60 minutes. Then the vial was cooled down, the fluid fraction of the reactive concoction was separated and transferred into a clean vial, then vaporized at 40 °C in the nitrogen flow. The dry residue was dissolved in 100 µl of anhydrous ethylacetate and 1 µl of this solution was put into the gas chromatograph evaporator.

Esterification with 2,2,3,3,3-pentafluoropropanol

20µl of 2,2,3,3,3-pentafluoropropanol and 60µl of 2,2,3,3,3-pentafluoropropionic anhydride (washing off the vial walls) were added to the dry residue of eluate, the vial was sealed and MW-irradiated in the 560W microwave oven for 5 minutes. After cooling down, the vial was opened and excess reagents were vaporized in the nitrogen flow (not above 40°C). The dry residue was dissolved in 100µl of anhydrous ethylacetate, and 1µl thereof was added to chromate-mass-spectrometer's evaporator.

Trimethylsilyl esters acquisition

100 µl of BSTFA containing 1% of trimethylchlorsilane was added to the dry residue of eluate, the vial was corked up, shaken with the microshaker and heated at 80°C in the thermal block for 60 minutes. The vial was cooled down and 2 µl was put into the chromato-mass-spectrometer's evaporator.

Preparation of urine samples (without hydrolysis, for HPLC analysis)

0.45ml of internal standard mixture in acetonitrile (with 0.03µm/ml concentration of ethylmorphine and cyclizine *ana*) was added to the urine sample (0.05ml) in Eppendorf tube. The tube was centrifuged at 10000 rpm for 15 minutes, the supernatant layer was transferred to the vial for autosampling, and 2ml of the resulting solution was added to the chromatograph.

Operation mode for GC-MS/MS analysis (gas chromatograph Agilent 7890 with MS/MS quadrupole mass-spectrometer Agilent 7000)

- Chromatograph vaporizer temperature – 280ºC;
- vaporizer operation mode: split/splitless (15:1 split ratio, with 1 min delay after the sample injection);
- detector interface temperature – 280°C;
- initial temperature of the column heating oven – 220°C;
- final temperature of the column heating oven – 300°C;
- variation of the column temperature – 20 degrees/min;
- exposure at the final temperature – 5 min;
- carrier gas – helium, column flow rate – 1 ml/min;
- injection volume – 1 ml;
- collision cell gas – nitrogen, 1.5ml/min;
- «cooling gas» – helium, flow rate – 2.25 ml/min;
- collision energy – 10–20eV.

Operation mode for HPLC-HRMS analysis (liquid chromatograph Agilent 1260 with high resolution MS/MS hybrid quadrupole-time-of-flight detector Agilent 6540)

- Gradient eluation with phase A (a 0.1% formic acid solution in deionized water) and phase B (acetonitrile) at the increase of the content of phase B from 1% to 100% for 10 minutes;
- volume of injected sample – 1 ml;
- flow rate – 0.3 ml/min;
- column temperature – 45°C;
- electrospray ionization in positive ion mode;
- drying gas temperature (nitrogen) – 350°C;
- drying gas flow rate (nitrogen) – 8 l/min;
- nebulizer gas pressure (nitrogen) – 20 psi;
- capillary voltage – 3500 V;
- fragmentor voltage – 100 V and 180 V;
- mass-spectrometer operation mode: Auto MS/MS;
- equipment calibration and accuracy correction of

mass measuring in the course of the analysis were made by standard calibrators recommended by the equipment manufacturers.

A part of the previously obtained alcoholic extract to be analyzed was diluted with water and studied under the above conditions.

Operation mode for GC-MS analysis (gas chromatograph Agilent 7820 with mass-selective detector Agilent 5975)

- Flow rate of the carrier gas (helium) through the column – 1.5 ml/min;
- working mode of vaporizer split/splitless (low-splitting – 15:1 with the impulse delay of 1 minute after the sample injection);
- the temperatures of the injection port and the line connecting to the mass spectrometer were 250°C and 280°C, respectively;
- the initial temperature of the column was 70°C for 2 minutes; then, the column was heated up to 280°C at the programming speed of 20 degrees/min. and kept at the final temperature for 8 minutes;
- the temperatures of the ion source and the quadrupole were at 230 and 150°C, respectively;
- voltage of the multiplier of the mass-spectrometric detector was set equal to that of the automatic routine adjustment of the detector.
- The registration of mass spectrum for methyl derivatives in the full ion scanning mode was in mass range of 42–450 a.u. The registration of mass spectrum for trimethylsilyl and pentafluoropropyl derivatives in the full ion scanning mode was in the mass range of 43–650 a.u.

The conjugation degree of the major MDMB(N)-073F metabolite of biotransformation phase I in the urine was determined by the ratio of the peak area of methyl ethers for the ion with m/z value 219 and the peak area of the ion m/z 235 for *N*-methylhexenal (internal standard) in eluate I of the urine with enzymatic hydrolysis and by similar procedure without hydrolysis.

Operation mode for HPLC-MS/MS analysis (liquid chromatograph Agilent 1260 with MS/MS mass-spectrometer Agilent 6460)

- Gradient eluation with phases A (a 10 mM solution of ammonium formate and 0.1% formic acid in deionized water) and B (0,01% formic acid in methanole);
- eluent flow rate was 0.6 ml/min;
- column temperature – 50ºC;
- gradient mode: 0 – 1.0 min – reaching 95% of phase A, by the 5th minute reducing to 50% of phase A, by the 15th – by 2%, by the 17th – by 2%, by the 17.1 – by 95% and the column regeneration within 3 minutes was 95% of phase A;
- injection volume – 2 mcl;
- electrospray ionization in positive ion mode;
- flow of desiccant gas (nitrogen) to the ion source 6 l/min;
- spray gas pressure (nitrogen) 40 psi;
- temperature of desiccant gas – 300°C;
- capillary voltage – 3500 V;
- fragmentor voltage – 125 V;
- mass-spectrometer operation modes: dynamic MRM and Product Ion Scan (mass range: 100–550 Da).

Processing of the chromatograms in order to identify the components of the samples was carried out using *MSD ChemStation E.02.01.1177* (*Agilent*), *MassHunter B.08.02 (Agilent)* and *AMDIS* (The Automatic Mass Spectral Deconvolution and Identification System, NIST) software.

RESULTS AND DISCUSSION

As has been previously shown on the basis of the relative metabolite content in the urine samples, the principal pathway for MDMB(N)-073F cannabimimetics metabolism is MDMB(N)-073F ester decomposition with further conjugation of the resulting product (Fig.1). This metabolite of biotransformation phase I has both the maximum signal intensity in chromatograms of MDMB(N)-073F users and a characteristic mass-spectrum thereby making it possible to be used as a marker for this cannabimimetics [6, 7]. It should be also considered, that in the urine, this metabolite of biotransformation phase I is significantly associated with the conjugated form (Table 1), so in case of applying GC-MS research methods, hydrolysis of conjugates is required.

Table 1 – Marker conjugation values in 10 urine samples of MDMB(N)-073F cannabimimetics users

Sample	561	663	717	721	722	224	705	752	754	756	Median, %
Marker conjugation, %	96	0	49	98	97	97	99	99	99	100	97.5

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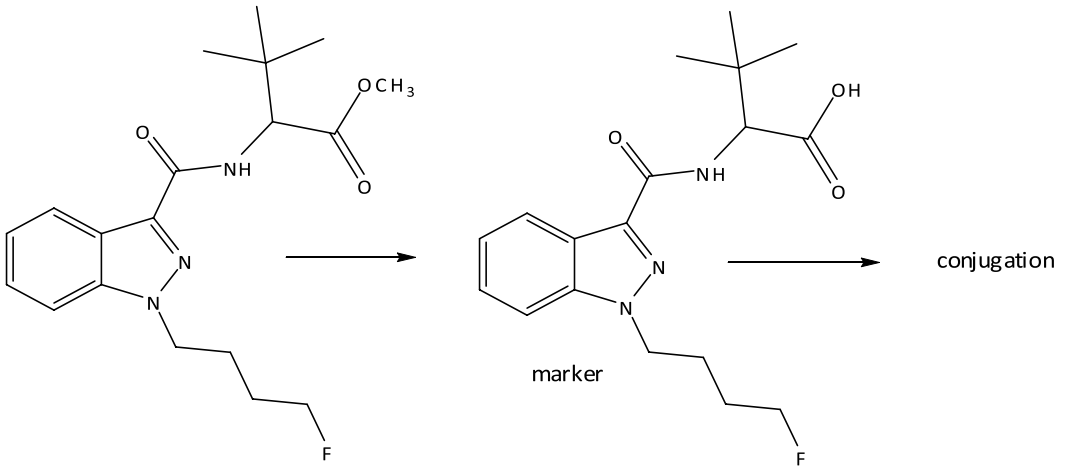

Figure 1 – Principal MDMB(N)-073F metabolic pathway

Obtaining–methyl, trimethylsilyl and 2,2,3,3,3-pentafluoropropyl esters is considered the most common variant of derivatization in cannabimimetics markers screening in the biological material based on gas chromatography with a quadrupole mass-spectrometric detector.

The formation of methyl ester MDMB(N)–073F marker corresponding to the original compound (Fig. 2), takes place during methylation, thereby simplifying the compound identification. Mass-spectra, retention indices and structures of trimethylsilyl and 2,2,3,3,3-pentafluoropropyl esters of MDMB(N)-073F marker, are given in Fig. 3 and 4, respectively.

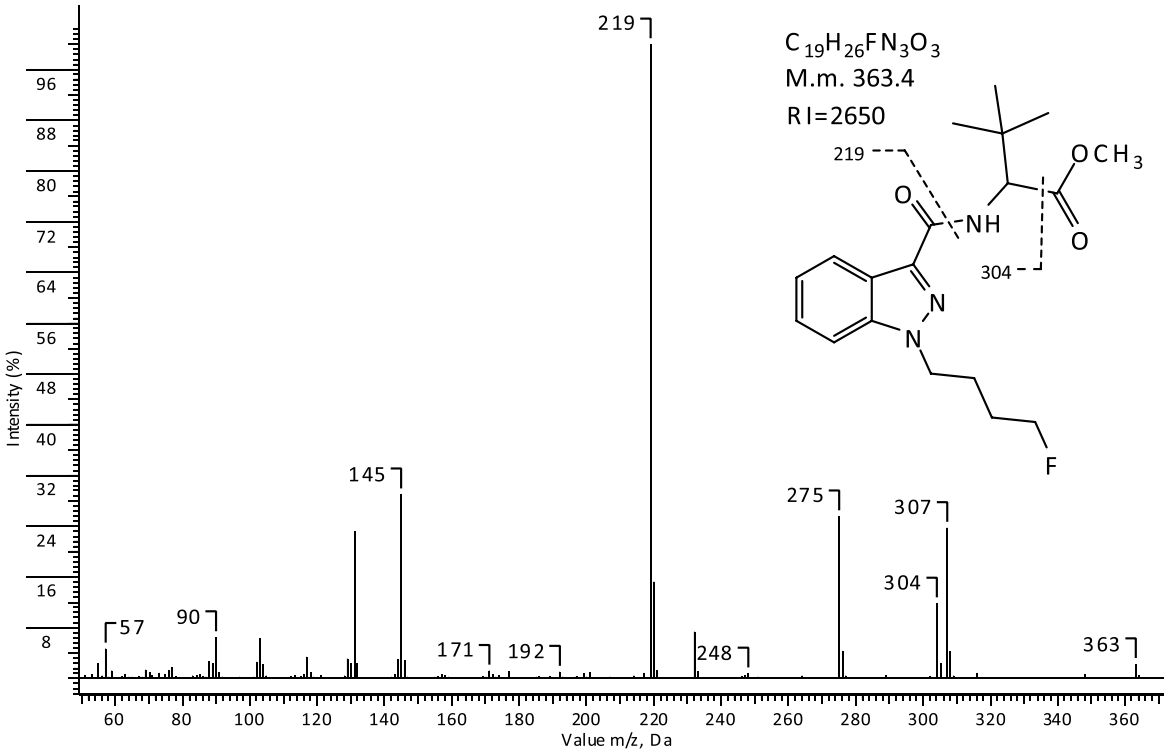

Figure 2 – Mass-spectrum, retention index and structure of methyl ester of MDMB(N -073F marker

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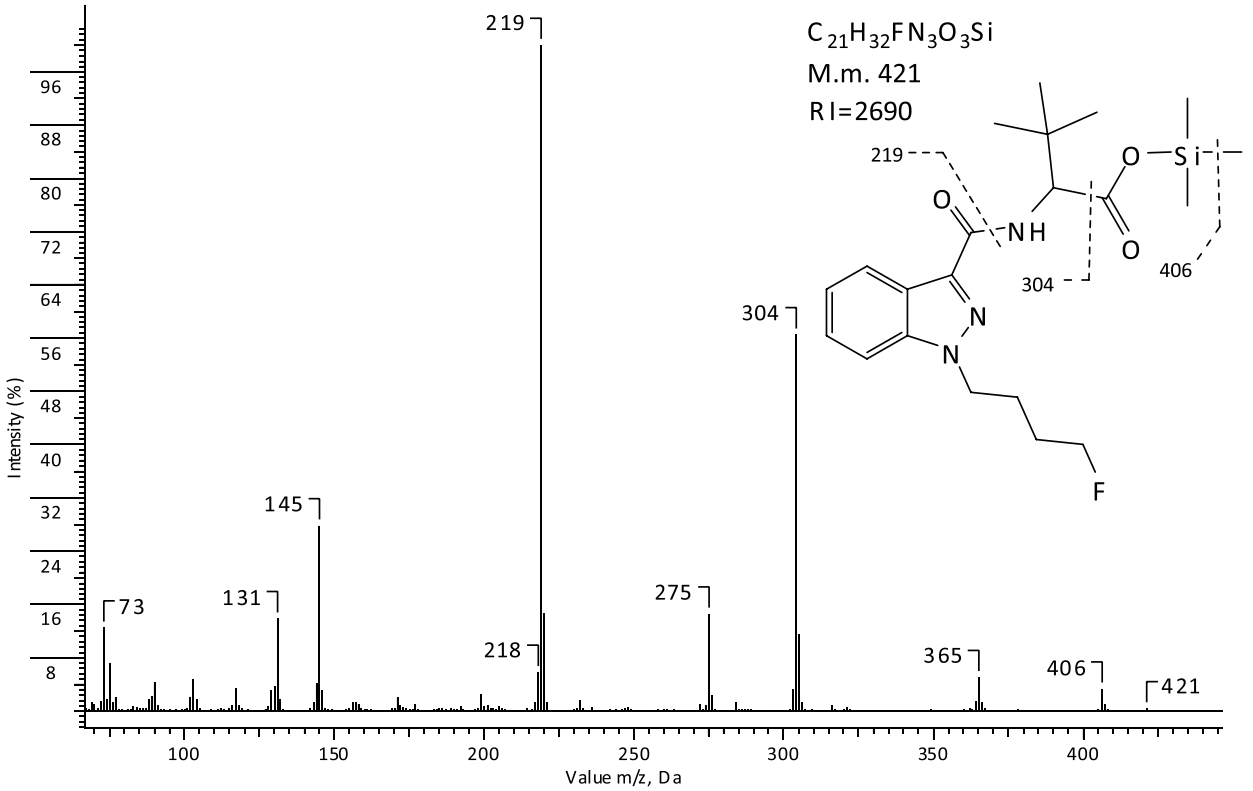

Figure 3 – Mass-spectrum, retention index and structure of trimethylsilyl ester of MDMB(N)-073F marker.

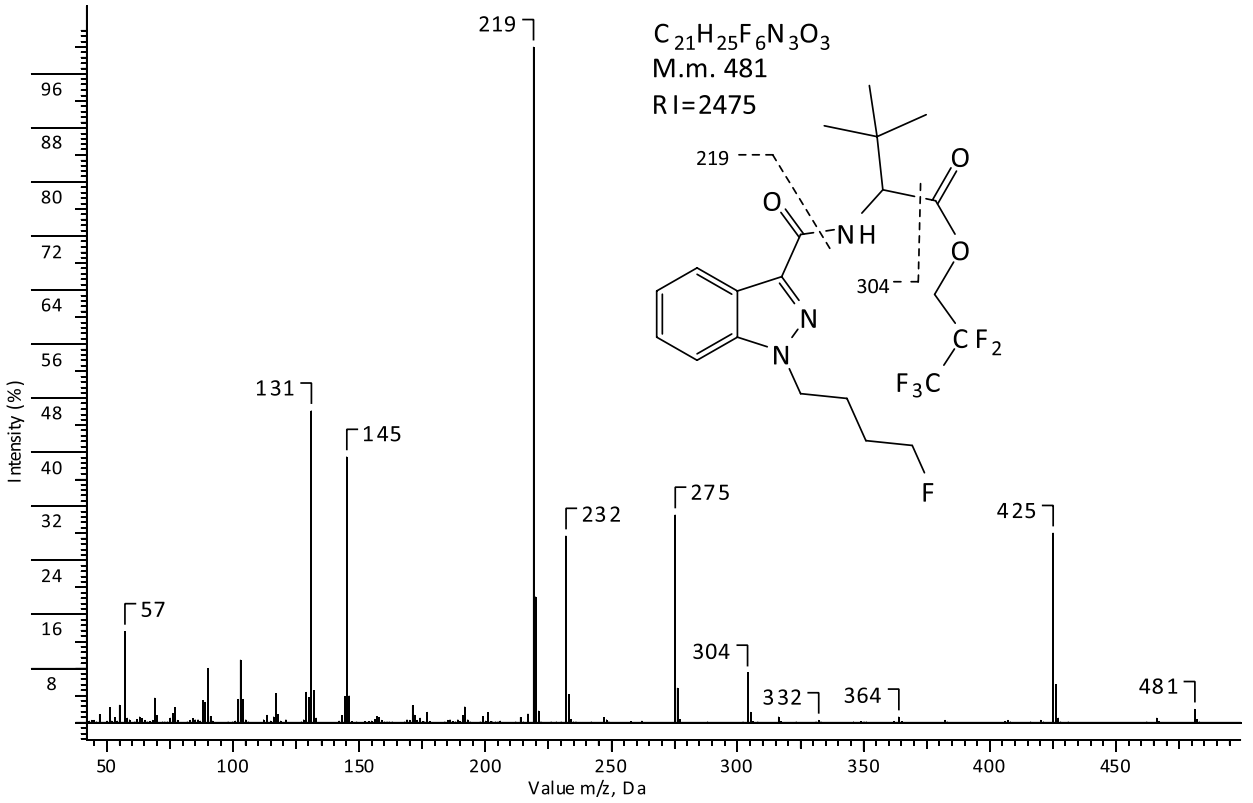

Figure 4 – Mass-spectrum, retention index and structure of 2,2,3,3,3-pentafluoropropyl ester of MDMB(N)-073F marker.

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Taking into account the identical structures of MDMB (N)-073F and the methyl derivative of its main metabolite (Fig. 1), to study the properties of MDMB(N)-073F, the analysis of the original cannabimimetic MDMB (N) -073F was carried out on the basis of the methods of GC-MS / MS and HPLC-HRMS**.** When applying gas chromatography method with a tandem mass-spectrometric triple quadrupole detector, the fragmentation of basic ions being formed under the electron impact ionization from MDMB(N)-073F was analyzed (Fig. 5–10).

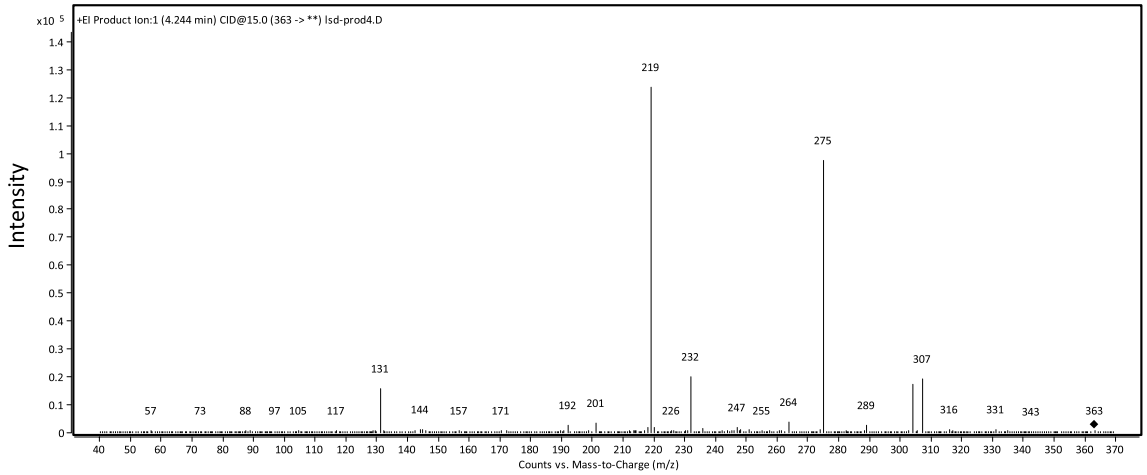

Figure 5 – MS/MS ion spectrum with m/z 363 (collision energy – 15 eV).

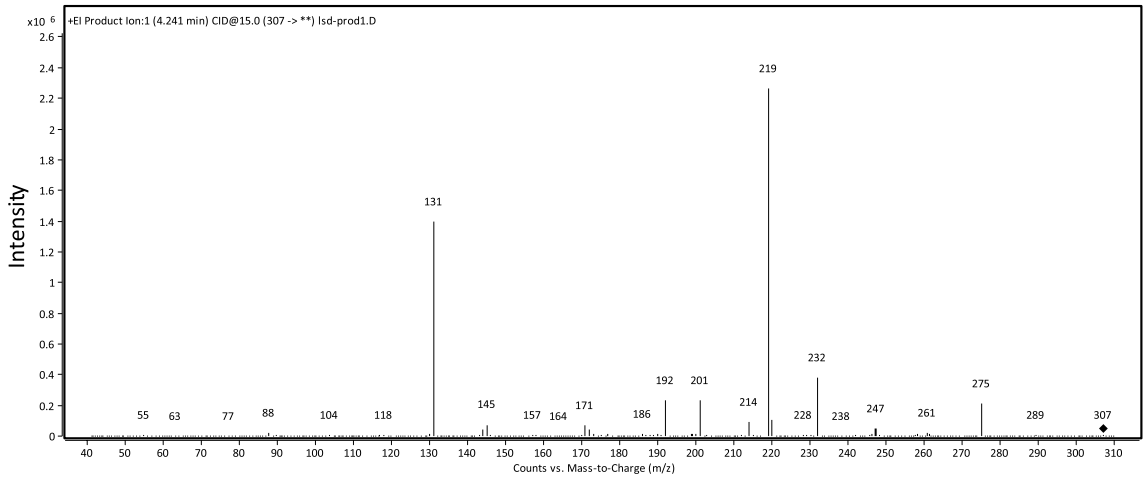

Figure 6 – MS/MS ion spectrum with m/z 307 (collision energy – 15 eV).

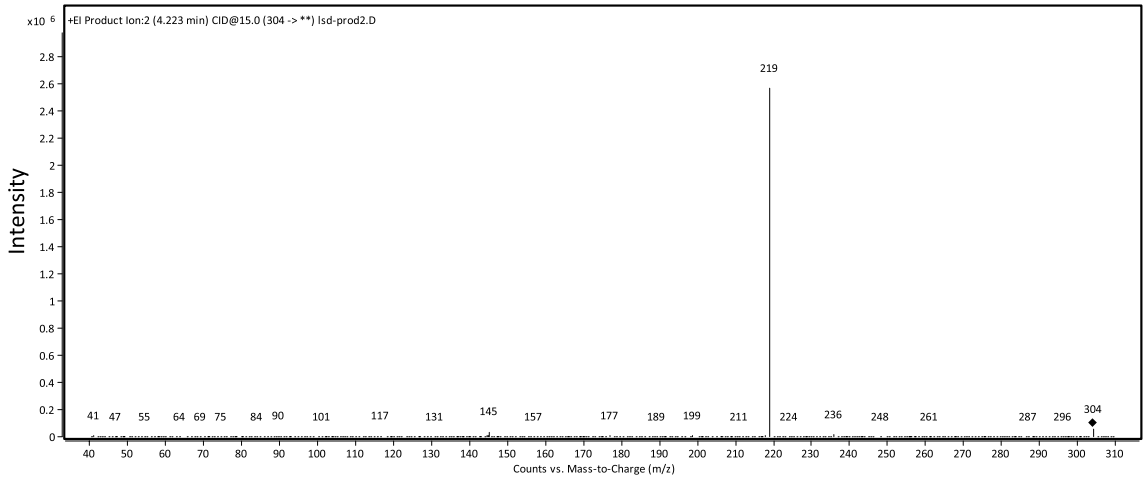

Figure 7 – MS/MS ion spectrum with m/z 304 (collision energy – 15 eV).

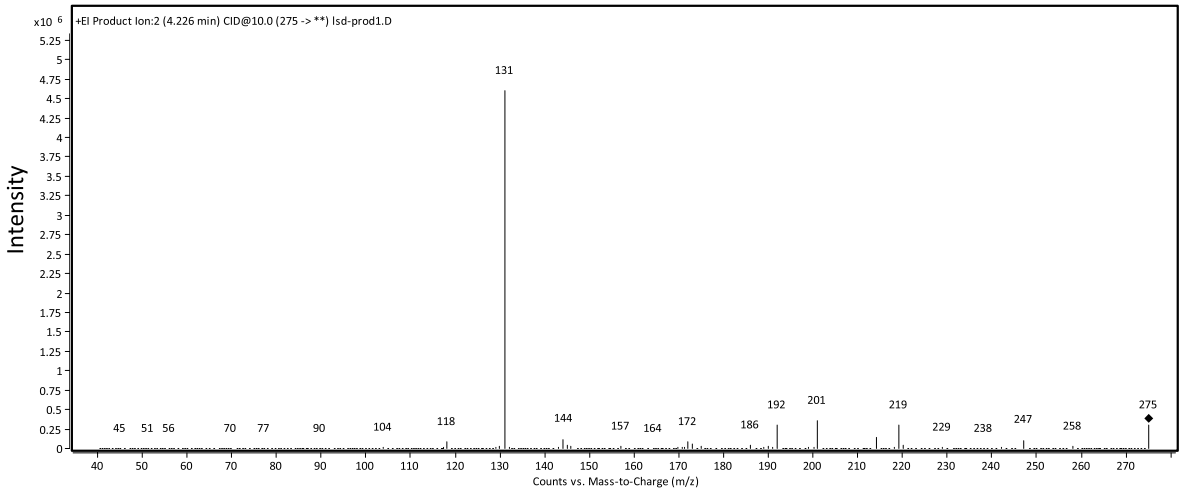

Figure 8 – MS/MS ion spectrum with m/z 275 (collision energy – 10 eV)

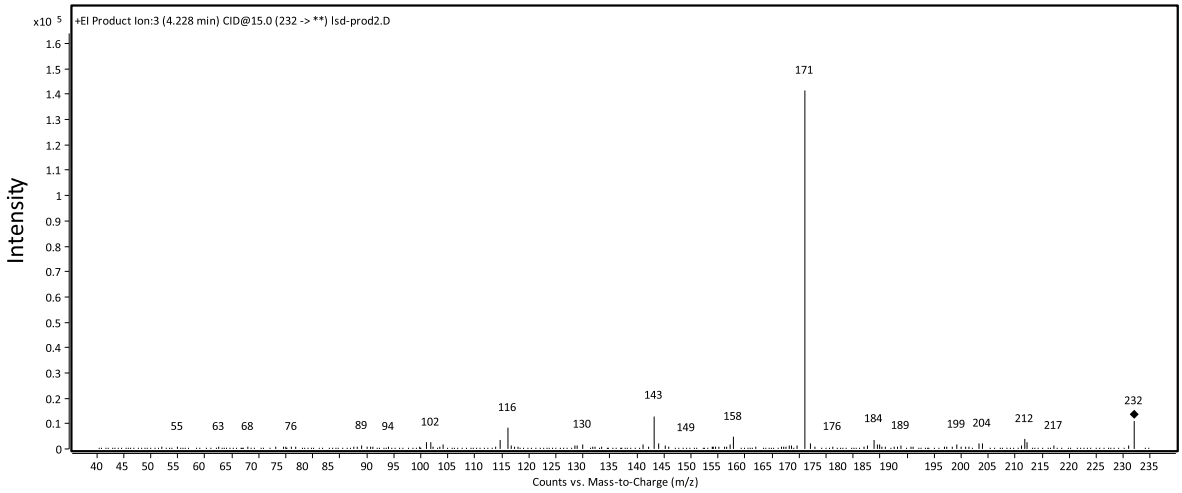

Figure 9 – MS/MS ion spectrum with m/z 232 (collision energy – 15 eV).

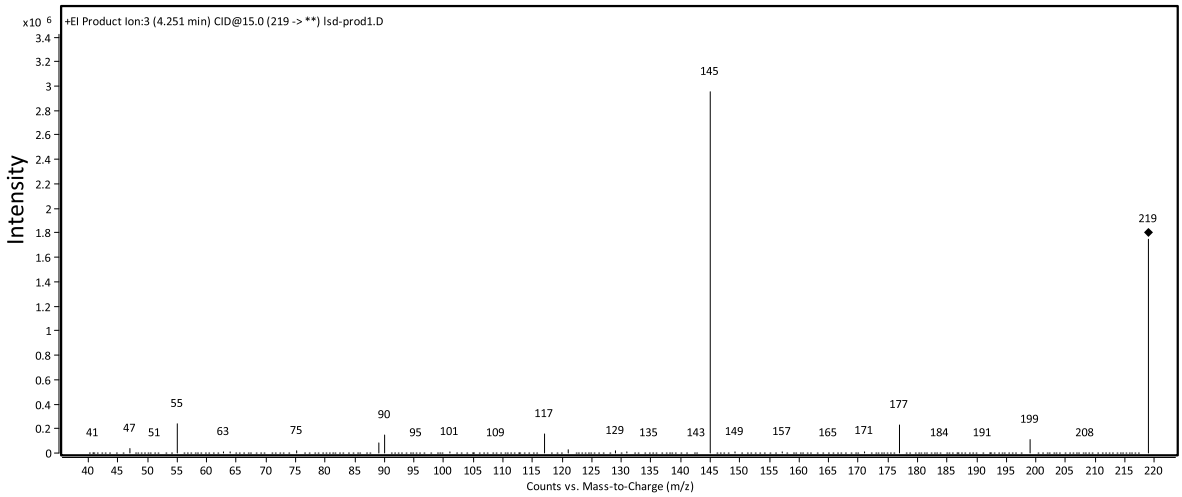

Figure 10 – MS/MS ion spectrum with m/z 219 (collision energy – 15 eV).

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According to the mass spectra of individual ions presented in Fig. 5–10, all the ions are seen to be structurally bound together. So, ion with m/z value of 304 a.e.m., comprises ions with m/z values of 219 and 145 a.e.m., and ion with m/z value of 219 a.e.m. comprises ion 145 a.e.m., and ion with m/z value of 307 comprises ions with m/z values of 232, 275 and 131 a.e.m. The obtained results presented below, comply with the fragmentation structure impacted by electron impact (Fig. 11).

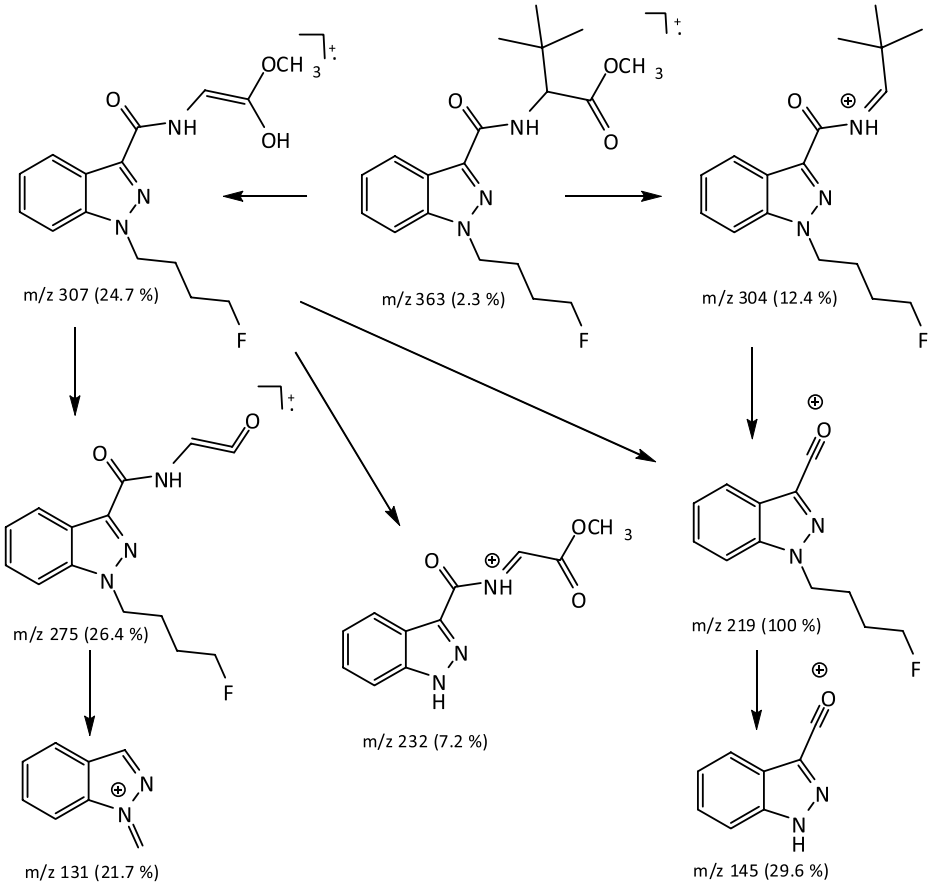

Figure 11 – Proposed structure of MDMB(N)-073F fragmentation.

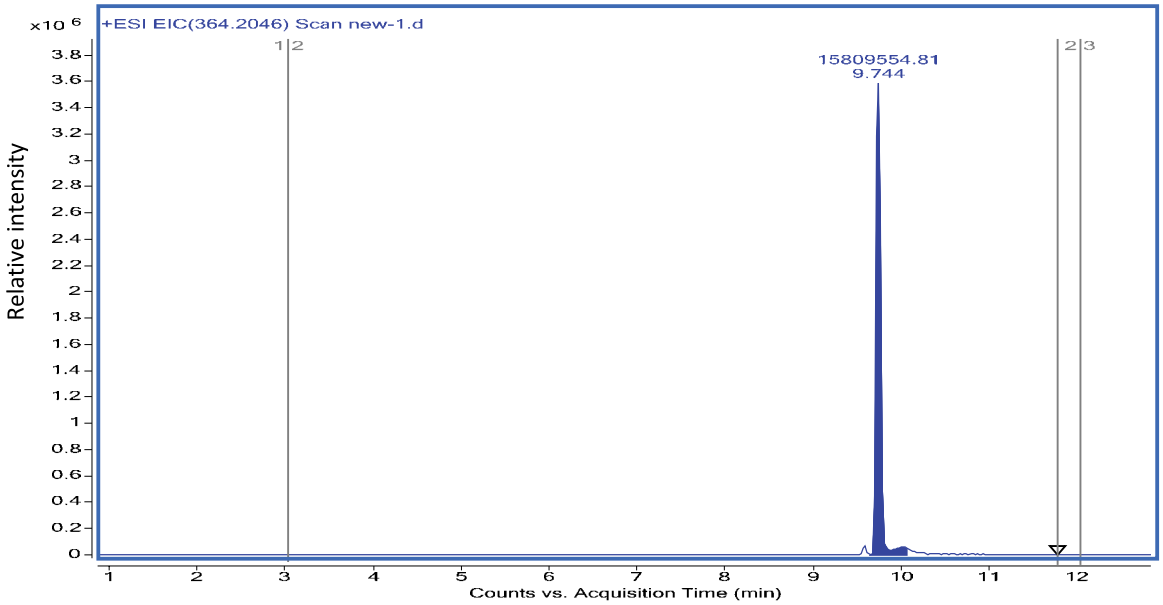

Figure 12 – Chromatogram of the plant-based MDMB(N)-073F object (HPLC-HRMS, m/z 364.203, image range ±5 mDa).

The data on the fragmentation of the main ions, obtained in the investigations of MDMB(N)-073F were found in the plant-based objects. The analysis was carried by HPLC-HRMS methods, taking into account the exact masses of the main ions. The chromatogram and spectrum of MDMB(N)-073F obtained in its analysis, are given in Fig.12 and 13, accordingly. Both theoretical and experimentally found exact masses of MDMB(N)-073F protonated molecule and fragment ions are presented in Table 2. The computed error is also given there.

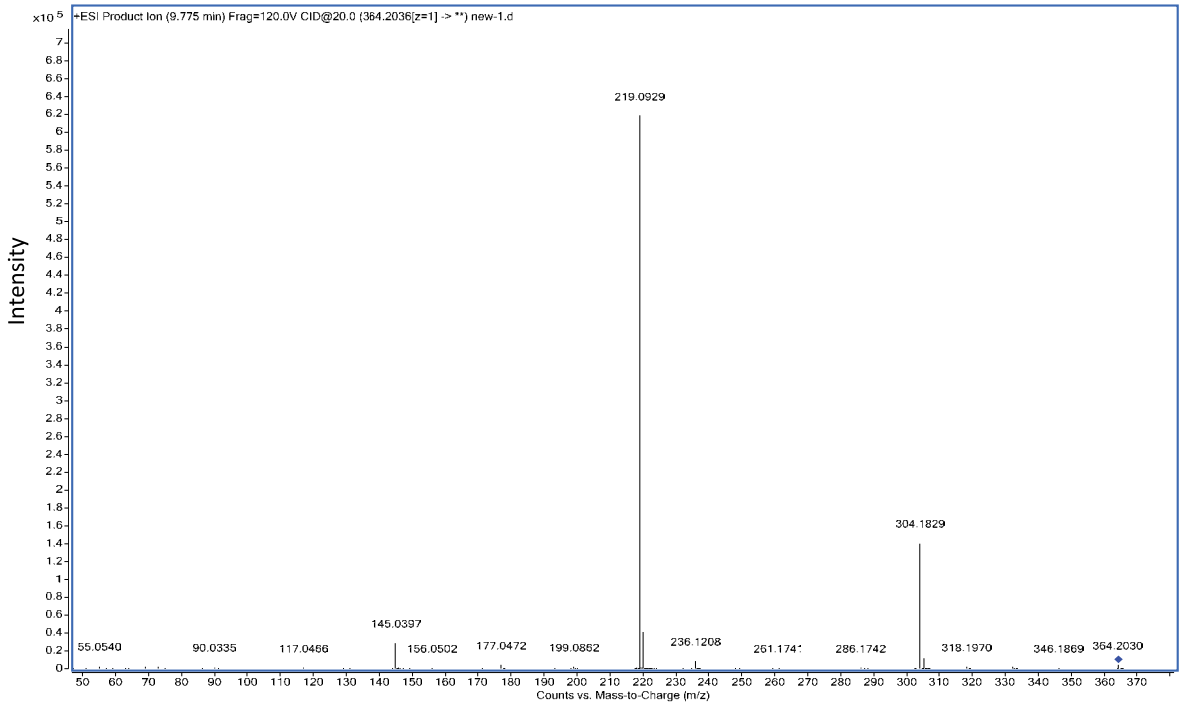

Figure 13 – MS/MS ion spectrum with m/z 364, 203.

Ions corresponding to protonated molecules of the original substance, are known to be within positive electrospray conditions. The ion with the chemical formula of $C_{19}H_{27}FN_3O_3$ and the exact mass of 364, 2031 Da is to be used in the combination with MDMB(N)-073F structure. The exact molecular mass of the ion measured in the experiment, is different from the mass calculated for 0.27 ppm, thereby proving the accuracy of the proposed chemical formula.

Table 2 – Determination of exact masses of protonated molecule and fragment ions of MDMB(N)-073F

Ion formula	Theoretical mass, Da	Measured mass, Da	Error, ppm
$C_{19}H_{27}FN_3O_3$	364.2031	364.2030	0.27
$C_{17}H_{23}FN_3O$	304.1852	304.1829	7.56
$C_{12}H_{15}FN_3O$	236.1193	236.1208	6.35
$C_{12}H_{12}FN_2O$	219.0928	219.0929	0.46
$C_8H_5N_2O$	145.0396	145.0397	0.69
C_4H_7	55.0542	55.0540	3.63

A study of the consumer's urine, containing MDMB (N)-073F, by HPLC-MS/MS methods and using multiple reaction registration (MRR) showed the following: apart from the marker, the conjugate with glucuronic acid are excreted together with the urine. The chromatograms are presented in Fig. 14–17.

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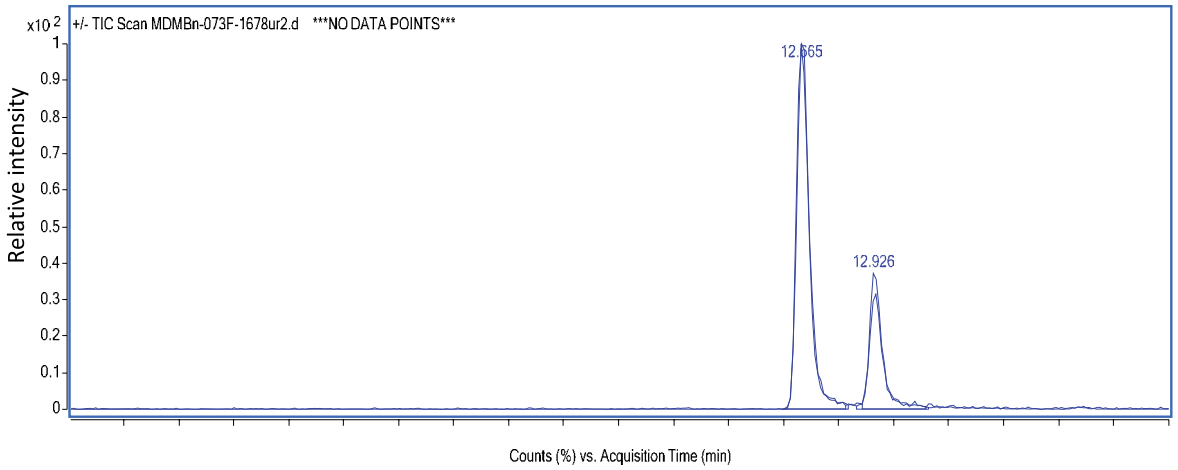

Figure 14 – Chromatogram of MDMB(N)-073F user's urine (HPLC-MS/MS, the total ion current for MRM transitions 350.1 > 145.0 and 350.1 > 219.0). Retention time for MDMB(N)-073F marker and its glucuronide is thereof 12.926 and 12.665 min., respectively

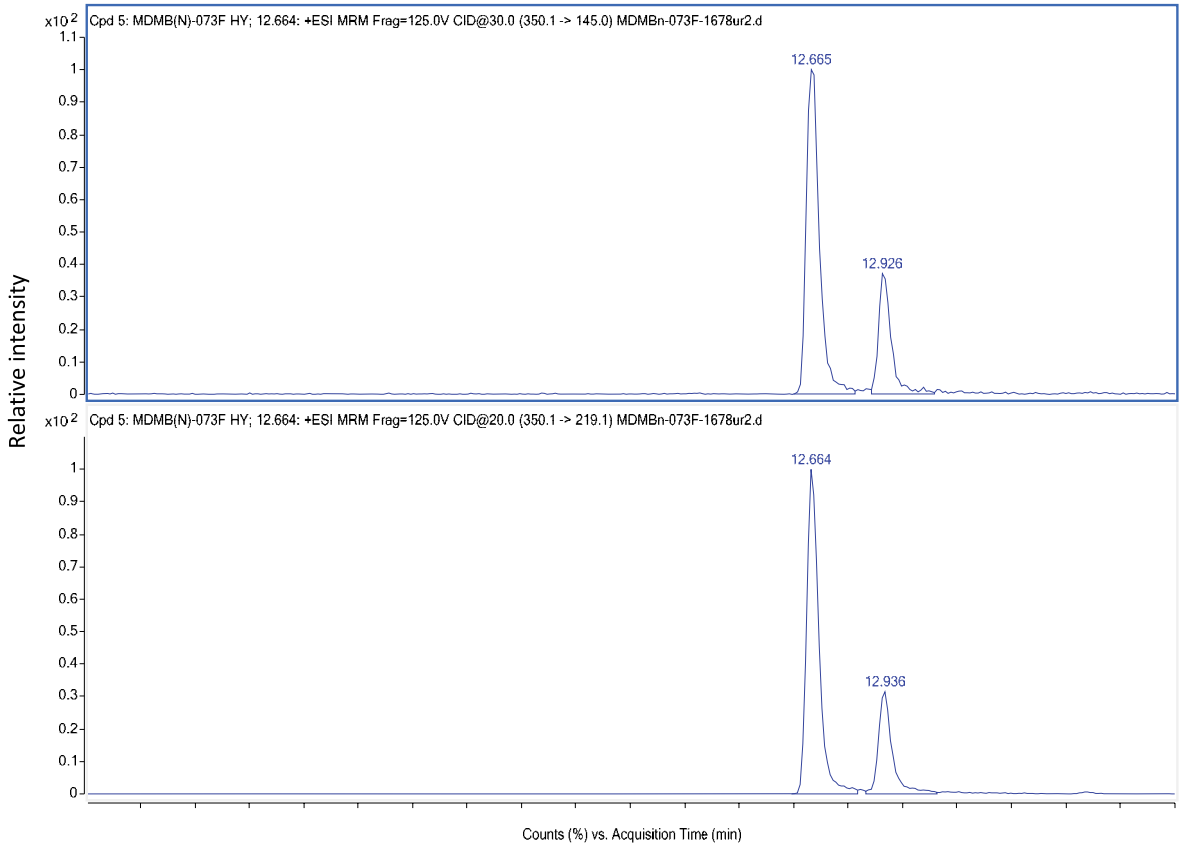

Figure 15 – Chromatogram of MDMB(N)-073F user's urine (HPLC-MS/MS; MRM transitions: above 350.1 > 145.0, below 350.1 > 219.0)

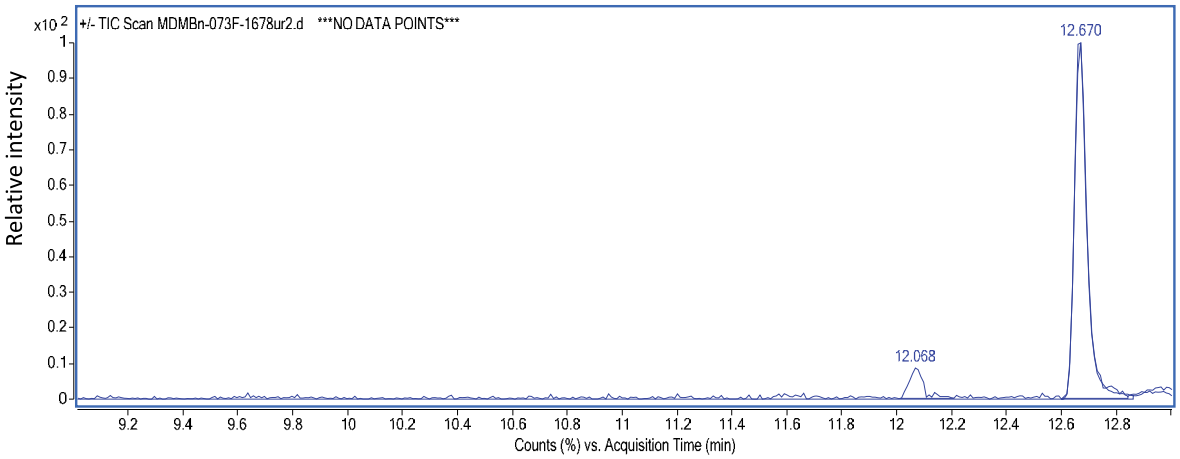

Figure 16 – Chromatogram of MDMB(N)-073F user's urine (HPLC-MS/MS, total ion current for MRM transitions 526.3 > 145.0 and 526.3 > 219.0). Retention time for glucuronide marker of MDMB(N)-073F– 12.670 min.

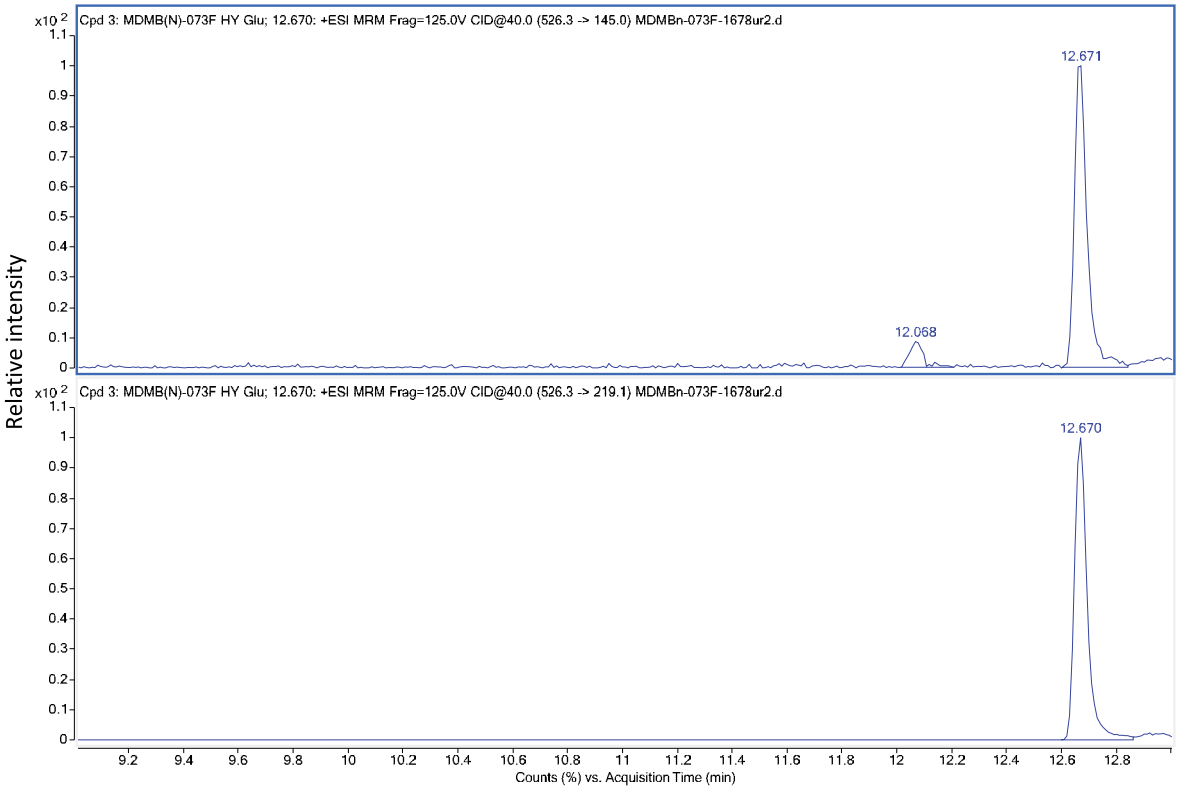

Figure 17 – Chromatogram of MDMB(N)-073F user's urine (HPLC-MS/MS; MRM transitions: above 526.3 > 145.0, below 526.3 > 219.0).

Mass-spectrum of ions – products of MDMB(N)-073F marker with m/z value of 350 for a protonated molecule – is similar to the spectrum of the unchanged compound (Fig. 13),; the ions with m/z values of 219 and 145 are presented therein (Figures 14, 15). The spectrum of ions – glucuronide products of MDMB(N)-073F marker (m/z 526 for the protonated molecule) – also contains these ions (Fig. 16, 17), thereby making it possible to use them in the registration of MRM transitions for both compounds.

Glucuronide of MDMB(N)-073F marker is a compound ester. This leads to its partial fragmentation in the ion source of liquid mass spectrometers with positive ionization [3]. Fragmentation of glucuronide in the source is basically due to the elimination of glucuronic acid residue. The resulting ion with m/z value of 350 corresponds to the protonated molecule of this MDMB(N)-073F marker itself. Glucuronide instability of MDMB(N)-073F marker makes it possible to use ion with m/z value of 350 as a precursor when finding both compounds (Fig. 14, 15).

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The data presented herein connfirm that the major part of MDMB(N)-073F marker is found in the urine of its users as a conjugate with glucuronic acid.

CONCLUSIONS

The structure of MDMB(N)-073F compound has been confirmed by methods of gas chromatography with MS/MS mass-spectrometry and liquid chromatography with hybrid high-resolution quadrupole-time-of-flight mass spectrometry. Mass-spectral characteristics of MDMB(N)-073F have been given herein.

Ester decomposition with further conjugation of the resulting acid has been found one of MDMB(N)-073F biotransformation in the human body. The product interacting with glucuronic acid, is the conjugate of MDMB(N)-073F metabolite of phase I in biotransformation.

Metabolite formed as a result of ester decomposition and its conjugate with glucuronic acid, are recommended to be applied as markers for synthetic MDMB(N)-073F cannabimimetics in the analysis by chromatographic methods; they can be used in regular analytical screening of biological samples.

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AUTHOR CONTRIBUTIONS

All authors had equally contributed to the research work.

CONFLICTS OF INTEREST

The authors and peer reviewers of this paper report no conflicts of interest.

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