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Identification of a synthetic cannabinoid 5F-NPB-22 (indazole analog 5F-PB-22) by gas chromatography/mass spectrometry (GC/MS) method

Summary

This article presents the problem of transesterification of a synthetic cannabinoid 5F-NPB-22 and other structurally related indazole and indole-based compounds, such as NPB-22, 5F-PB-22, BB-22 and PB-22, caused by methanol – a solvent commonly applied in gas chromatography/mass spectrometry (GC/MS) analysis. The above process can result in drawing incorrect conclusions about the composition of analyzed product, due to the formation of ester compounds, which mainly affects the reliability of results. In the present study, in addition to the mixture of methanol and toluene, as well toluene alone was used to evaluate the effect of both these solvents on the results obtained with GC/MS. The quality of analyses was additionally confirmed by quadrupole-time-of-flight (Q-TOF) mass spectrometry with direct injection of sample into the ion source. The final results indicate that in the case of transesterification-sensitive compounds, the selection of an adequate extraction-solvent is of major importance. It is recommended to use toluene and exclude transesterification-supporting solvents such as low molecular weight alcohols (methanol.ethanol). Moreover, a prompt analysis of the extracted substances should be ensured in order to eliminate their potential esterification.

Keywords 5F-NPB-22, esterification, cannabinoids, methyl ester, GC/MS

Study objective

The study aims at presenting the problem of transesterification of a synthetic cannabinoid 5F-NPB-22 and other structurally related indazole and indole-based compounds, such as NPB-22, 5F-PB-22, BB-22 and PB-22, caused by methanol – a solvent commonly applied in gas chromatography/mass spectrometry (GC/MS) analysis. The above process can result in drawing incorrect conclusions about the composition of analyzed product, due to the formation of ester compounds. Finally it affects the reliability of results and, in consequence, may contribute to reaching erroneous conclusions by experts issuing opinions in the field of forensic chemistry. The issue raised in this article emerged while performing at the Central Forensic Laboratory of the Police partial analyses of new psychoactive substances and other substances having similar effect (as defined in the Act of Counteracting Drug Addiction) ordered by external customers.

Introduction

5F-NPB-22 (1-(5-fluoropentyl)-8-quinolinyl ester-1H-indazole-3-carboxylic acid) is a synthetic cannabinoid belonging to the group of indazoles, structurally similar to NPB-22, carrying an additional fluorine atom at the end of an aliphatic carbon chain (Fig. 1a, b). The pure 5F-NPB-22 assumes crystalline form and it is soluble in common organic solvents such as methanol, ethanol, toluene, DMSO (dimethyl sulphoxide) or DMF (dimethylformamide). The solubility in the two last mentioned solvents amounts to 10 mg/ml [1]. Due to structural similarities between NPB-22 and 5F-NPB-22, pharmacological activity of these compounds should be similar, albeit it remains largely unknown. NPB-22 is a structural analog of JWH-018 (Fig. 1c), thus it can be concluded that both compounds exhibit similar mechanism of action. JWH-018 is a strong antagonist of cannabinoid receptors with affinity constants K_i 9,00 ± 5,00 nM for CB_1 receptor and K_i 2,94 ± 2,65 nM for CB_2 receptor;

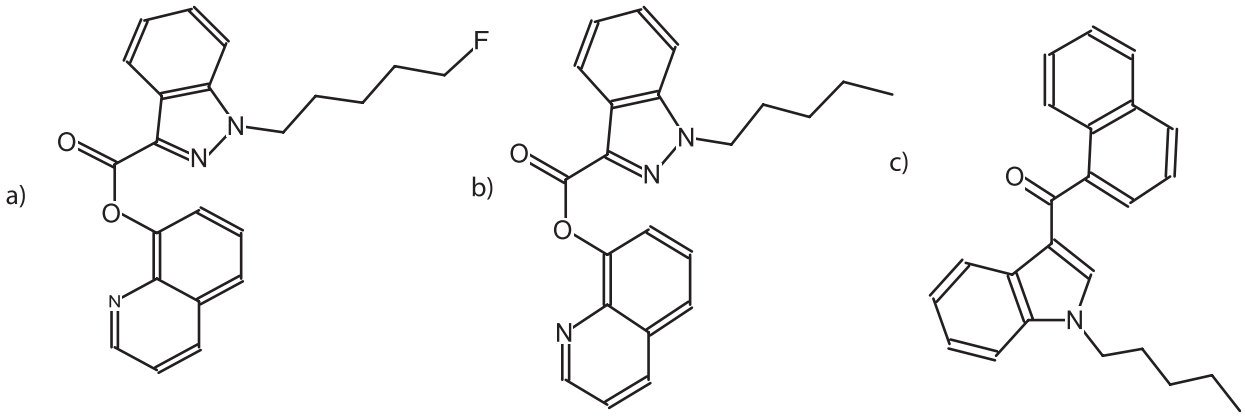


Fig. 1. Structural formulas of 5F-NPB-22 (a), NPB-22 (b) and JWH-018 (c).

compared to Δ^9-THC (Δ^9-tetrahydrocannabinol), JWH-018 exhibits stronger psychoactive properties, due to a 4-fold and 10-fold higher affinity for CB_1 and CB_2 receptor, respectively. However, its duration of action is shorter (approx. 1-2 hours) [2].

Fig. 2. Pouch with dried plant products.

Sample preparation

The study focused on the selected preparation belonging to the group of so called "designer drugs" [3] in the form of a homogeneous, green, dried plant product contained in a zip-lock plastic pouch (fig. 2).

1 g of test dried product was grounded in an agate mortar in order to obtain homogenized material. Next, two aliquots of 30 mg were transferred into separate tubes. One sample was treated with 1.5 ml of 1:1 ratio mixture of methanol and toluene with nonadecane as an internal standard, whereas the second with 1.5 ml of pure toluene. Both tubes were closed with plastic screw caps and placed on a rotator for approx. 30 min. Subsequently, the solutions were passed through a PTFE membrane syringe filter (dim. 13 mm, pore size 0.45 µm), thereby generating two homogeneous solutions, which were transferred into glass vials typically used for gas chromatography (GC) measurements. The vials were closed with disposable caps containing a PTFE membrane.

Equipment used and measurement conditions

For the purpose of the study was used a Single Quad 7890 A/MSD-5975 C gas chromatography/mass spectrometry (GC/MS) system from Agilent Technologies, equipped with a Rxi-5Sil MS capillary column (L: 30 m; ID: 0.25 mm; DF: 0.25 µm). The following measurement method was applied:

a. temperature program: 100°C – 0.50 min; 100°C ÷ 300°C x 12°/min – 16.67 min; 300°C – 18.00 min;
b. injection port temperature: 230°C;
c. injection volume: 1 µl;
d. injection mode: split 80:1;
e. detector type: MS Quad;
f. detector temperature: 230°C;
g. mass range: 30 ÷ 550 m/z;
h. carrier gas: helium.

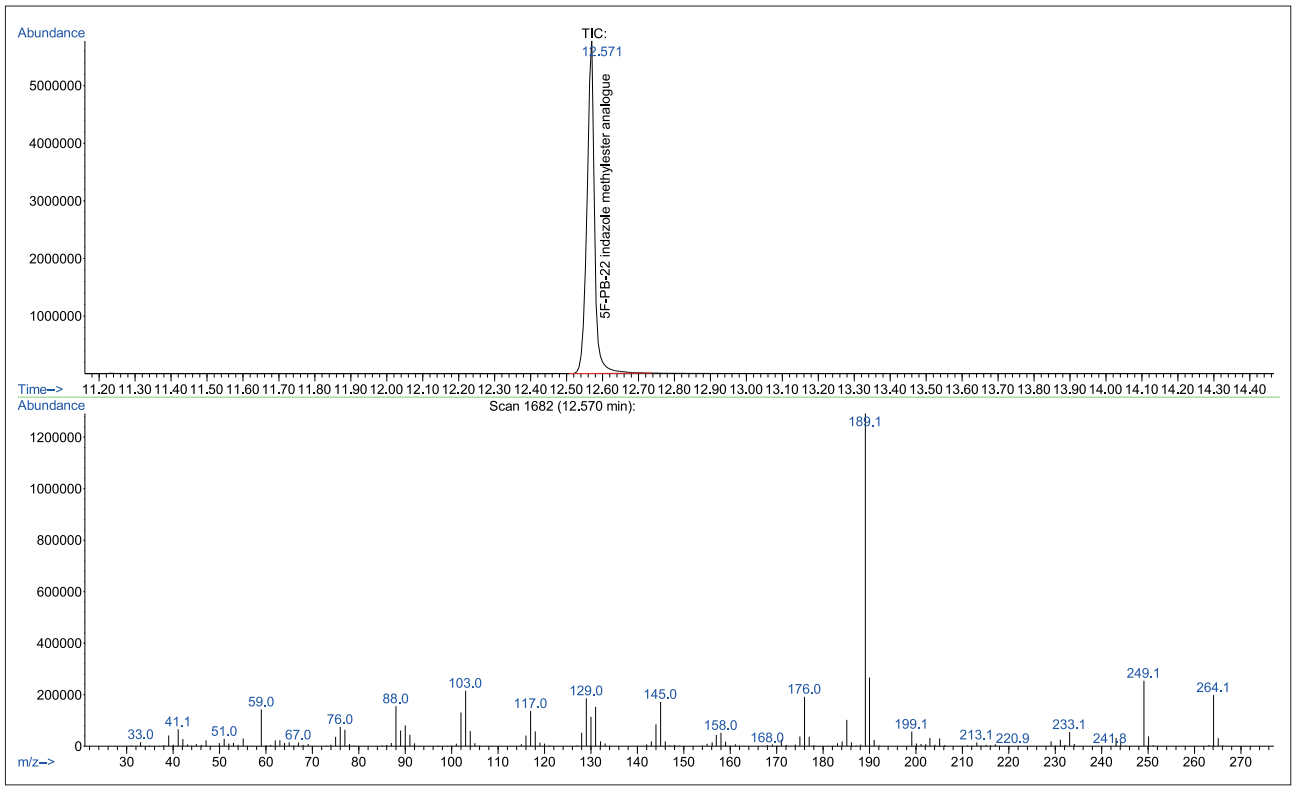


Fig. 3. Chromatogram of methyl ester 5F-NPB-22; extraction solvent: mixture of methanol and toluene (1:1) with an admixture of nonadecane as an internal standard.

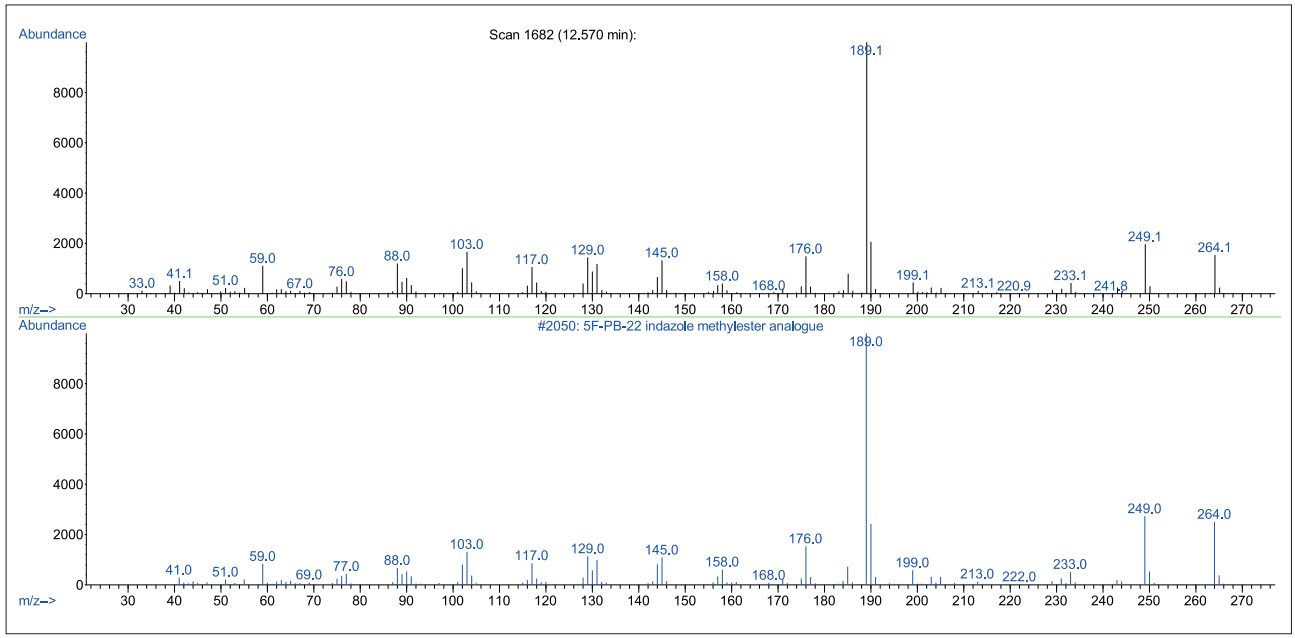


Fig. 4. Mass spectrum of a sample containing methyl ester 5F-NPB-22 compared against a GC/MS reference library.

Results

1. Solvent used: a 1:1 ratio mixture of methanol (LC-MS grade, min. purity 99.95%, from Witko, Poland) and toluene (GC-MS, MS SupraSolv grade, min. purity 99.8%, from Merck, Germany) with addiition of an internal standard: nonadecane (min. purity 99%, from ACROS Organics, US)

For the above measurement conditions, the main peak at the retention time Rt = 12.57 min and with a dominant mass spectrum m/z = 189 was observed on the chromatogram (fig. 3). Next, the mass spectrum

obtained was compared against the SWGDRUG (Ver. 3.0) mass spectral library [4]. The comparison result indicated the presence of 5F-NPB-22 methyl ester (fig. 4). Neither the chromatographic peak, nor the mass spectrum characteristic of 5F-NPB-22 were obtained.

2. Solvent used: toluene (GC-MS, MS Suprasolv grade, min. purity 99.8%, from Merck KGaA, Germany)

For the same measurement conditions as set out in point 1, the main peak at the retention time Rt = 22.40 min and with a dominant mass spectrum m/z = 233 was observed on the chromatogram (fig. 5). Next, as previously, the obtained mass spectrum was compared against the SWGDRUG (Ver. 3.0) mass spectral library [4]. The comparison result indicated the presence of 5F-NPB-22 (fig. 6). Neither the chromatographic peak, nor the mass spectrum characteristic of 5F-NPB-22 methyl ester were obtained.

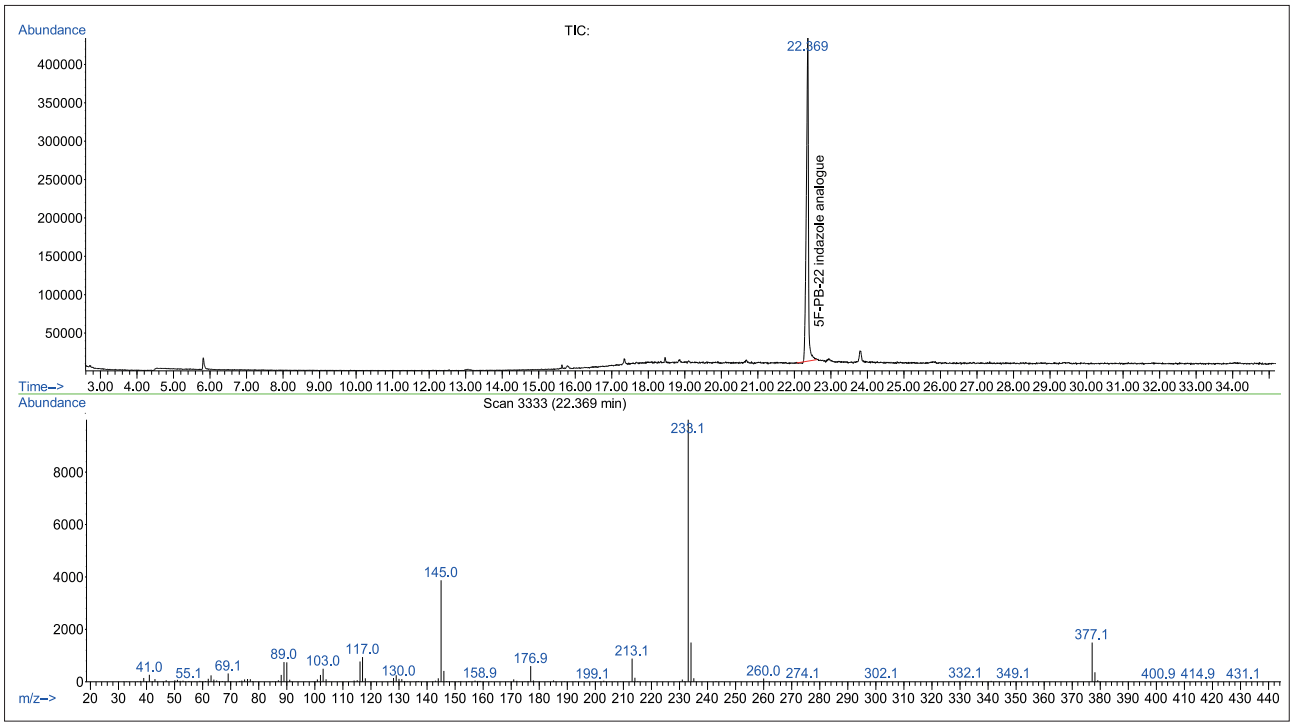


Fig. 5. Chromatogram of 5F-NPB-22; extraction solvent: toluene.

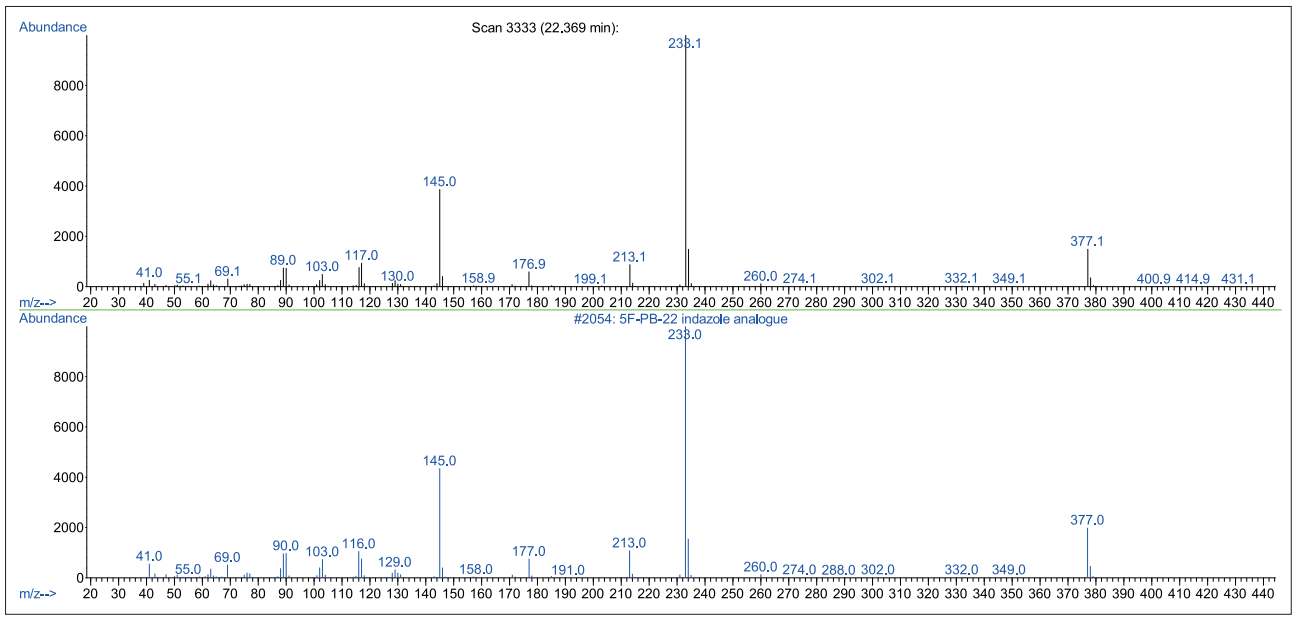


Fig. 6. Mass spectrum of a sample containing 5F-NPB-22 compared against a GC/MS reference library.

Confirmation of products identification using a quadrupole time-of-flight (Q-TOF) mass spectrometer and direct injection into the ion source

An aliquot of approx. 20 mg of the fragmented dried plant product was placed in a glass tube and admixed with a mixture of acetonitrile (LC-MS grade, min. purity 99.99%, from POCh, Poland) and deionized water at a 60:40 ratio. Next, similarly as in the case of sample preparation for GC/MS analysis, the tube was closed with a plastic screw cap and placed on a rotator for approx. 30 min. Subsequently, the solution was passed through a PTFE membrane syringe filter (dim. 13 mm, pore size 0.45 µm), thereby generating a homogeneous solution ready for injection.

The analyses were carried out using a TripleTOF 4600 quadrupole time-of-flight (Q-TOF) mass spectrometer from AB SCIEX equipped with a *duo-spray* ion source (ESI/APCI). Direct injection was applied using a 1 ml syringe, at a constant flow rate of 10 µl/min without liquid chromatography and using an ESI (electrospray ionization) ion source. The following measurement modes were applied:

1. TOF MS – mass range: 30 – 800 Da; measurement time: 1 min; mass measurement mode: TIC (total ion current).
2. Product Ion – mass range: 30 – 400 Da; measurement time: 1.30 min; precursor mass: 378 Da.

The TOF MS measurement yielded a mass spectrum with a visible mass M = 378.1594 Da (Fig. 7), corresponding to 5F-NPB-22 increased by one proton as a result of positive polarization applied during the measurement.

The Product Ion mode involved a 378 Da mass precursor, which was fragmented inside the spectrometer's collision cell to yield fragment ions listed in Table 1, corresponding to the particular fragments of 5F-NPB-22 compound (fig. 8).

Table 1.

Mass [Da]	Intensity [%]	Fragment
233.1093	100.00	$C_{13}H_{14}N_2OF^+$
213.1030	84.74	$C_{13}H_{13}N_2O^+$
185.1076	3.35	$C_9H_{14}N_2OF^+$
177.0463	23.54	$C_9H_6N_2OF^+$
145.0399	46.62	$C_8H_5N_2O^+$
69.0702	14.21	$C_5H_9^+$

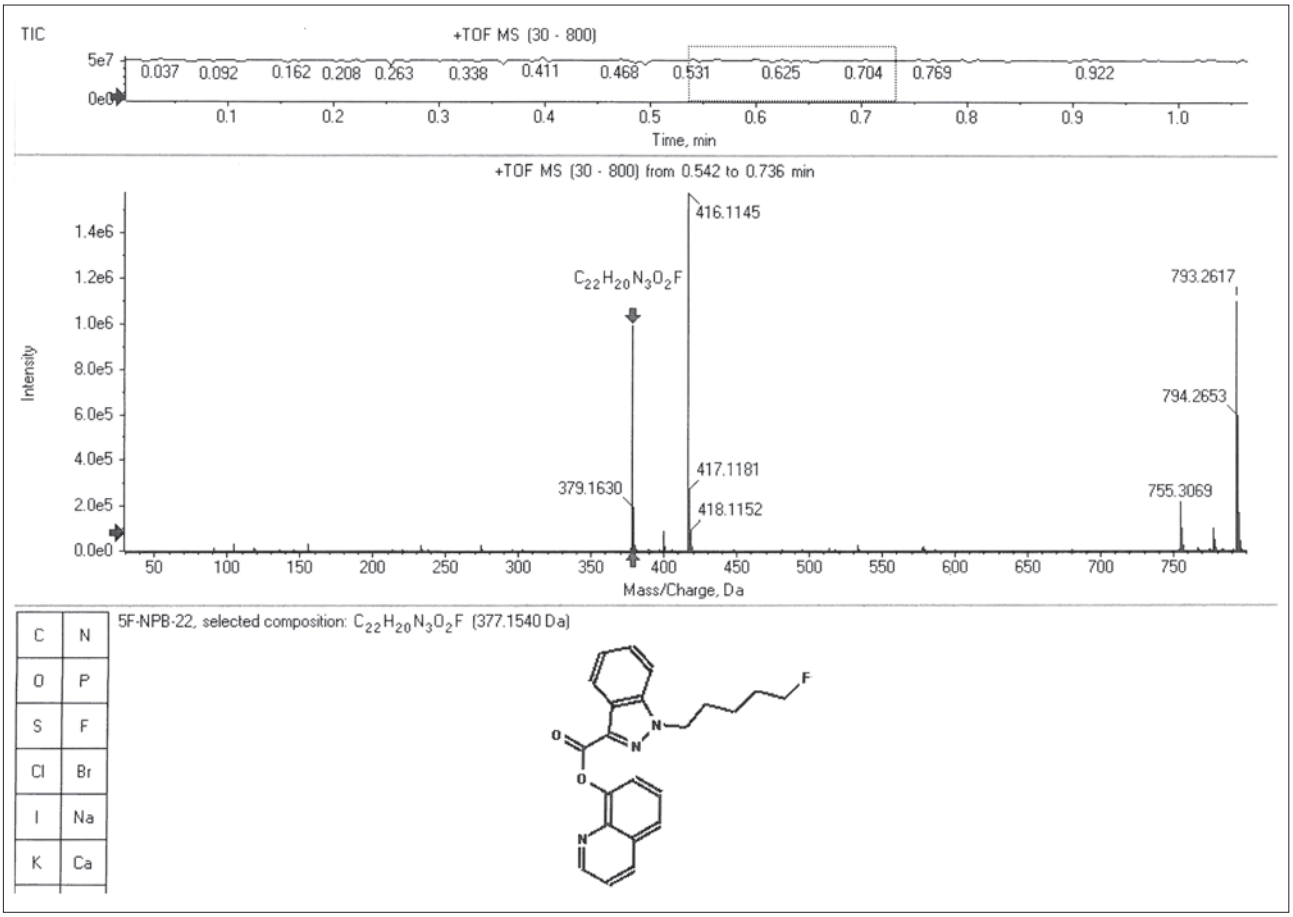


Fig. 7. Result of a Q-TOF analysis in the TOF MS mode of a sample containing 5F-NPB-22.

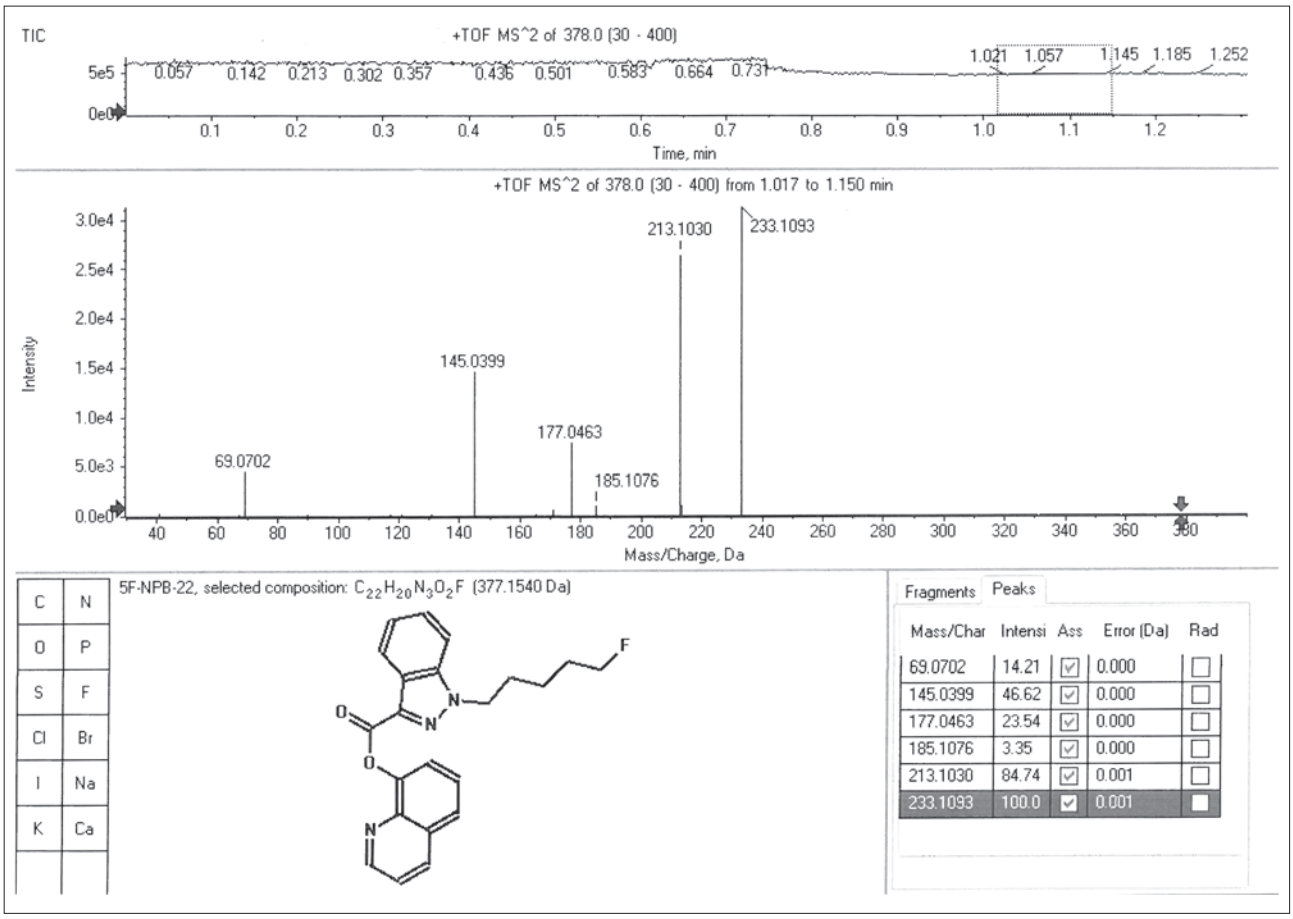


Fig. 8. Result of a Q-TOF analysis in the Product Ion mode of a sample containing 5F-NPB-22.

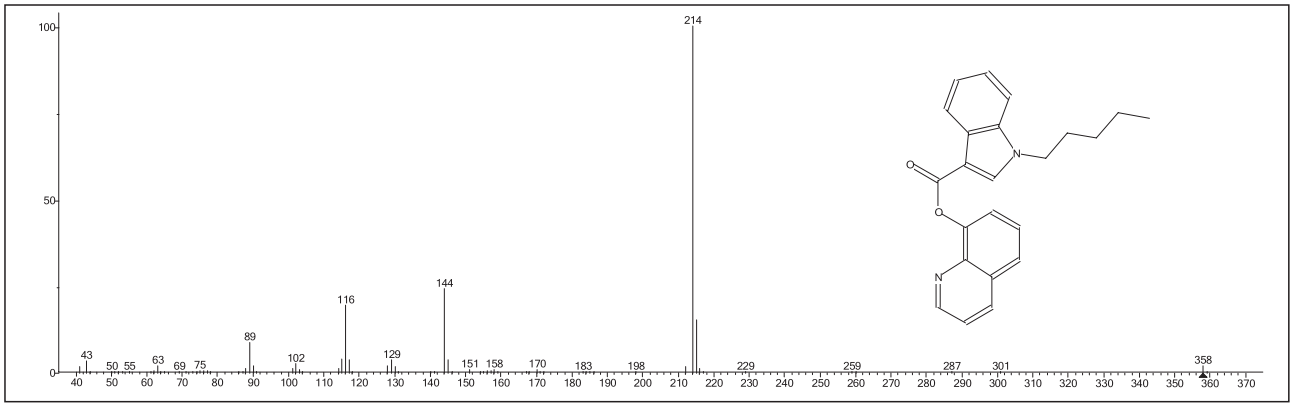
The above described analysis with the use of a quadrupole time-of-flight (Q-TOF) mass spectrometer has clearly confirmed that the tested compound was identified as 5F-NPB-22, whereas 5F-NPB-22 methyl ester identified by GC/MS measurement was merely a product of 5F-NPB-22 transesterification in the presence of methanol as extraction solvent.

Transesterification of 5F-NPB-22 – related compounds (synthetic cannabinoids of the group of indazoles and indoles)

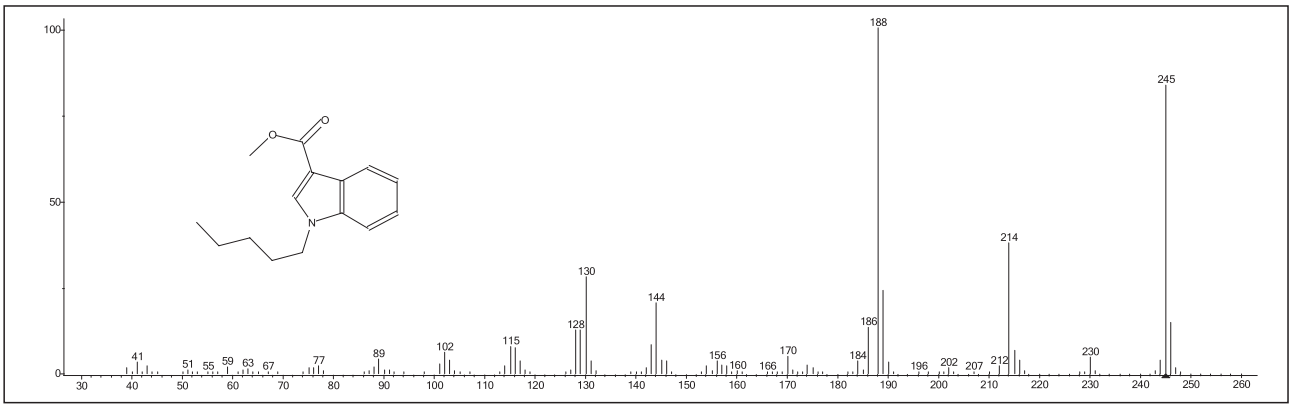
Due to the structural similarity with 5F-NPB-22, such compounds as PB-22; 5F-PB-22; BB-22 and PB-22 indazole analog, also undergo transesterification upon the treatment with methanol and some other

Fig. 9. Summary of 5F-NPB-22 and its derivatives' transesterification products resulting from the use of methanol, ethanol, n-butanol and pentafluoropentanol as extraction solvents.

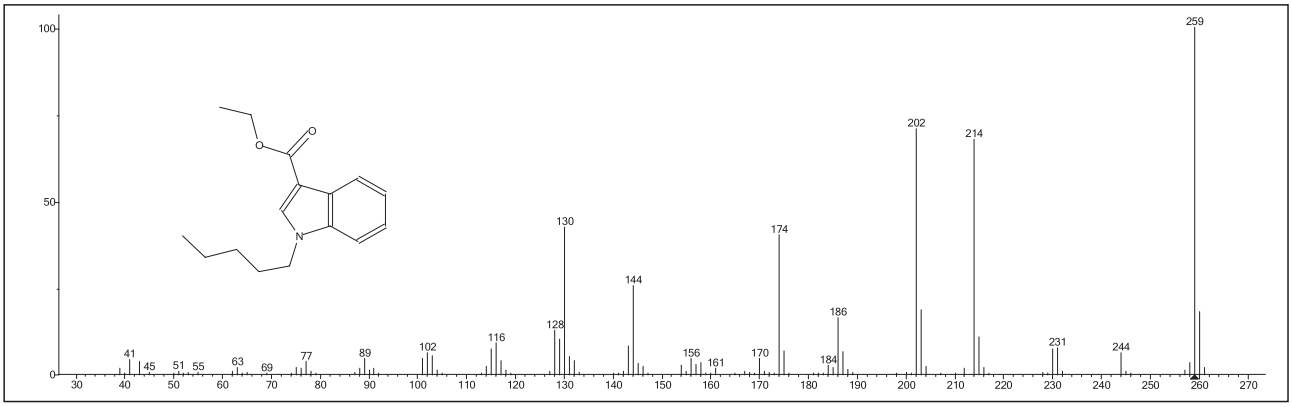
PB-22



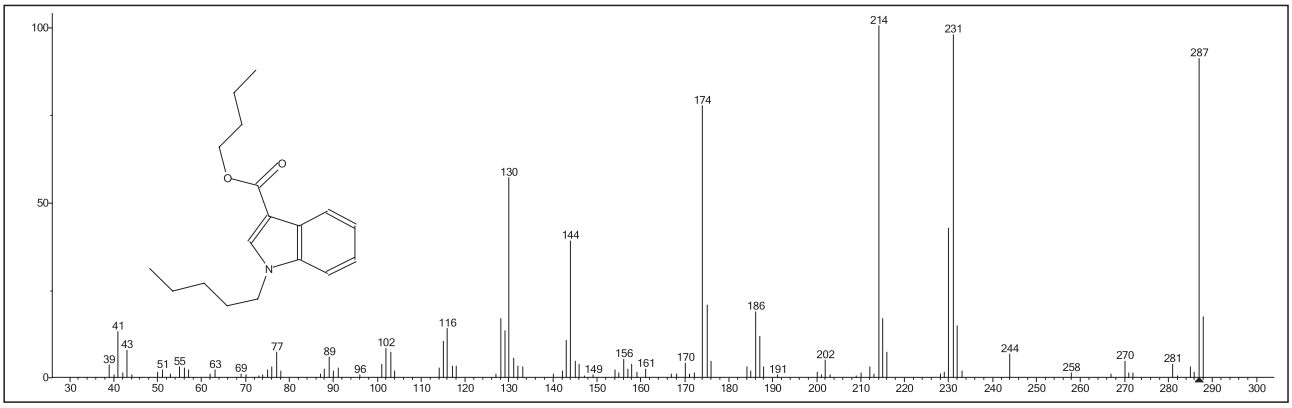
ester PB-22; used solvent: Methanol



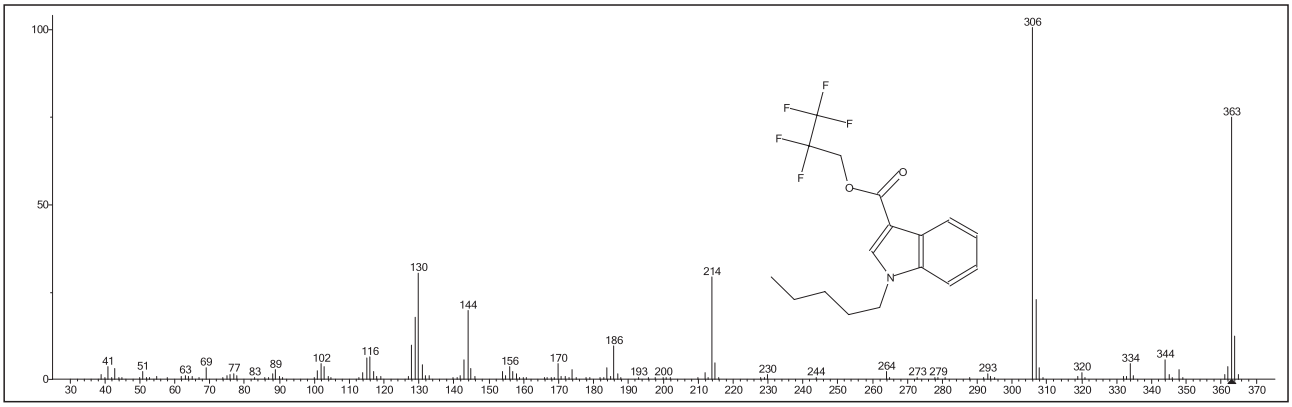
ester PB-22; used solvent: Ethanol



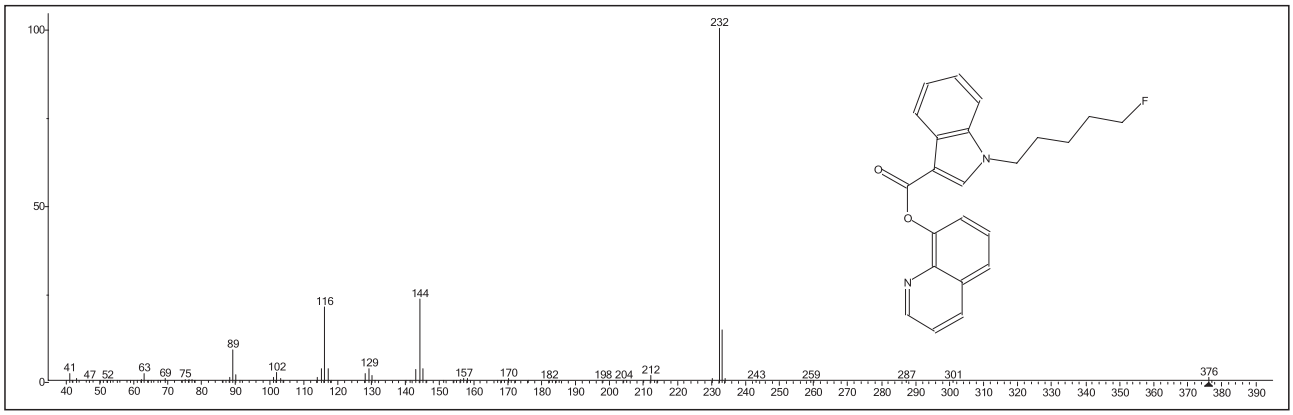
ester PB-22; used solvent: n-Butanol



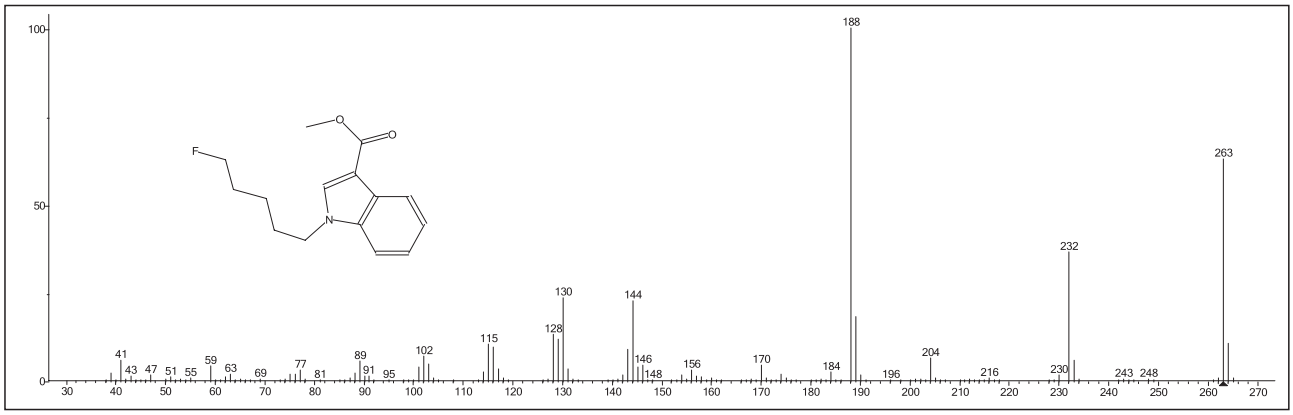
ester PB-22; used solvent: Pentafluoropentanol



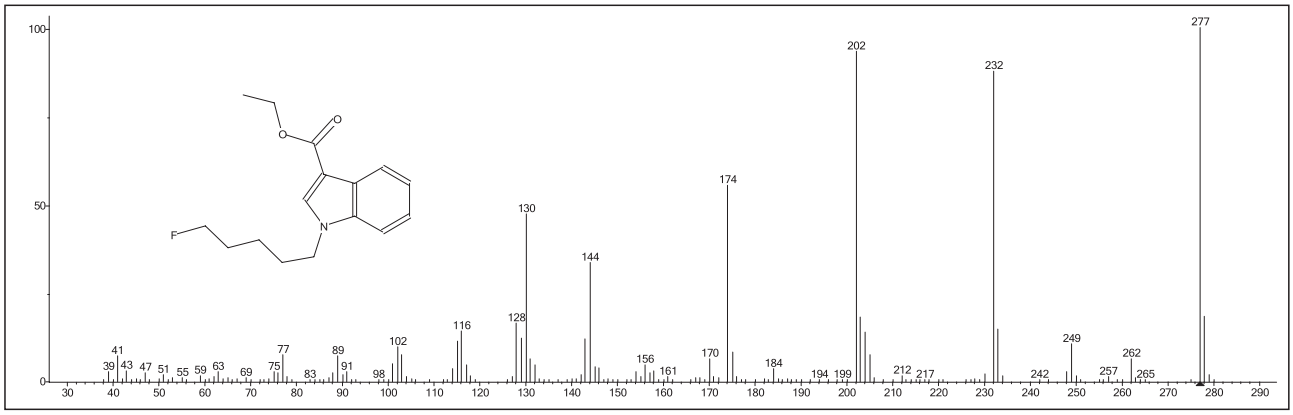
5F-PB-22



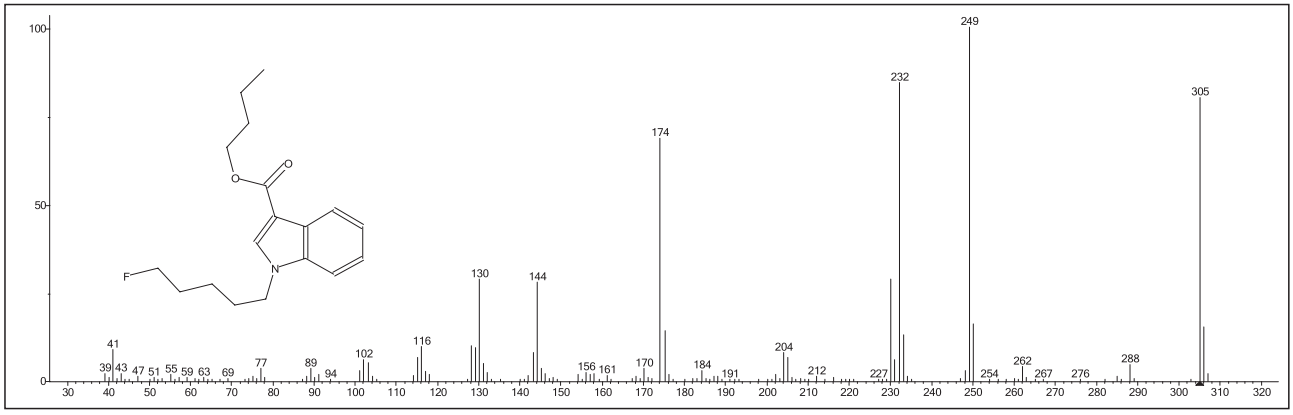
ester 5F-PB-22; used solvent: Methanol



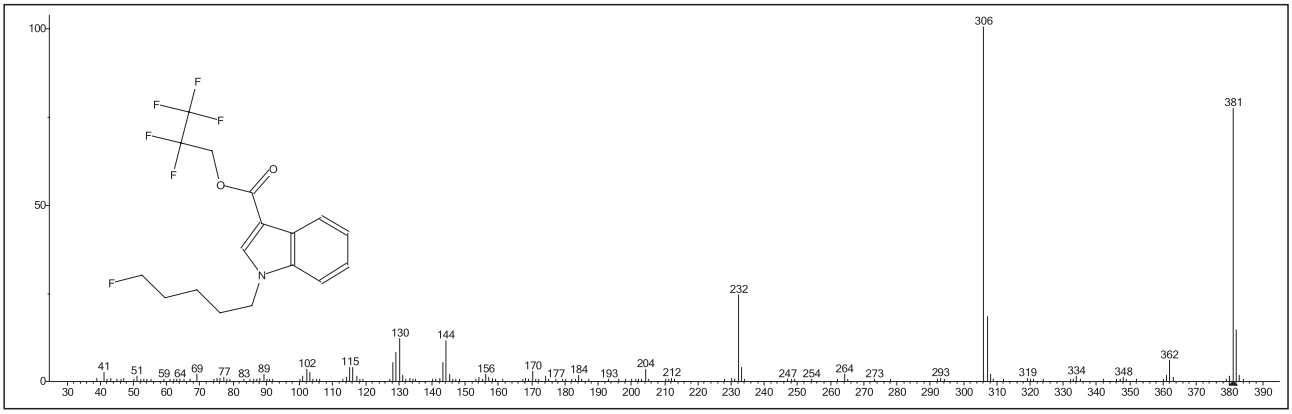
ester 5F-PB-22; used solvent: Ethanol



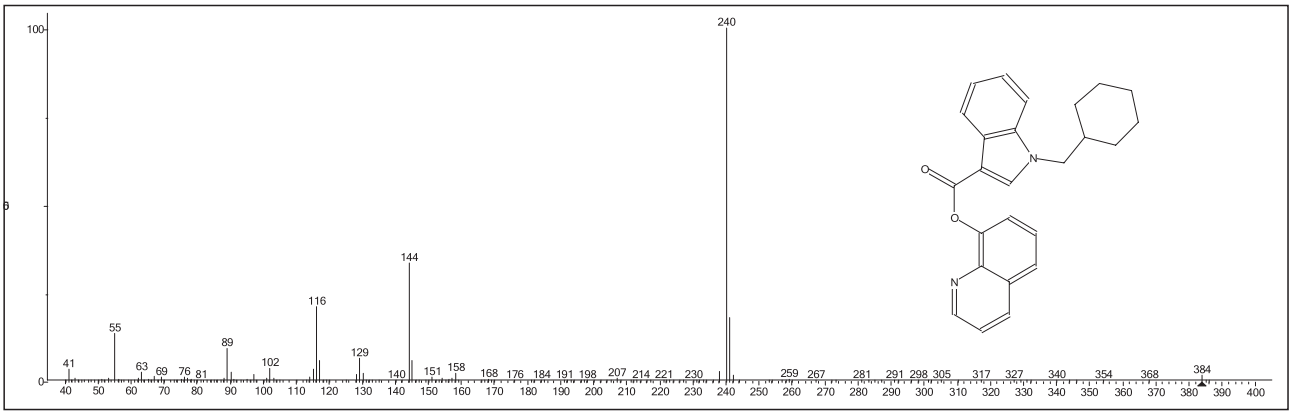
ester 5F-PB-22; used solvent: n-Butanol



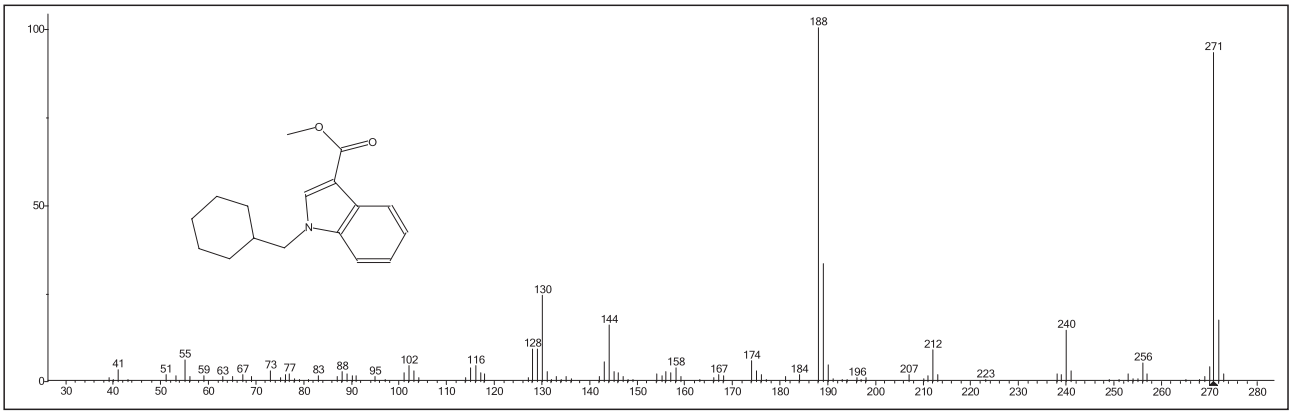
ester 5F-PB-22; used solvent: Pentafluoropentanol



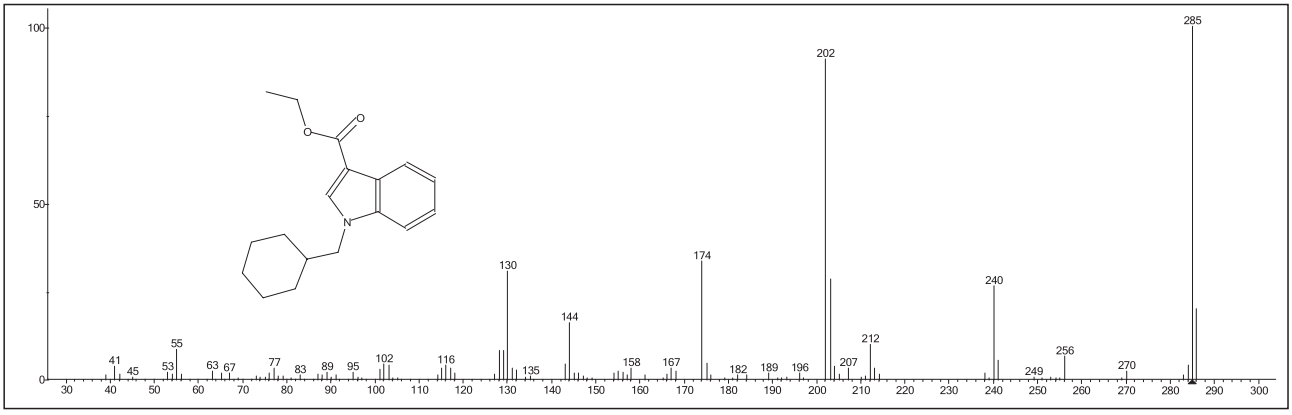
BB-22



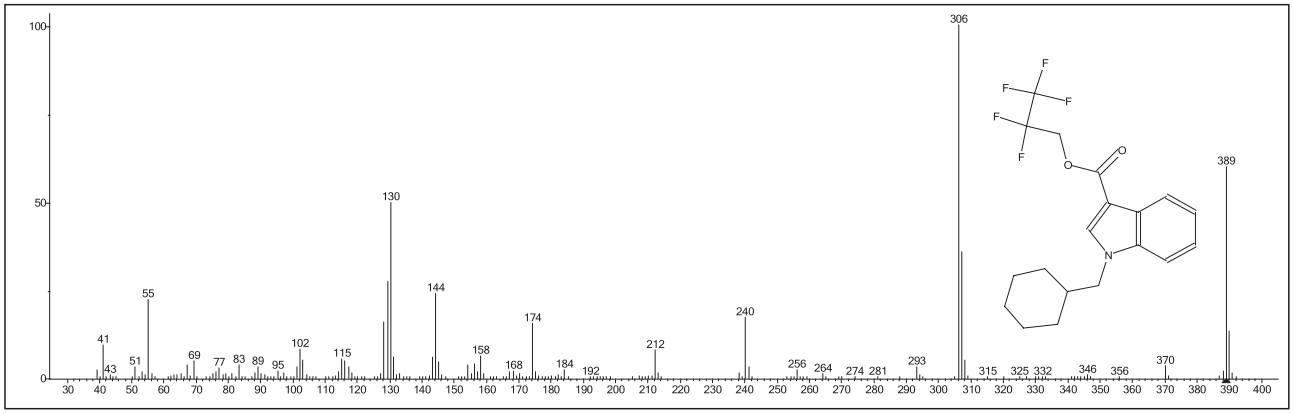
ester BB-22; used solvent: Methanol



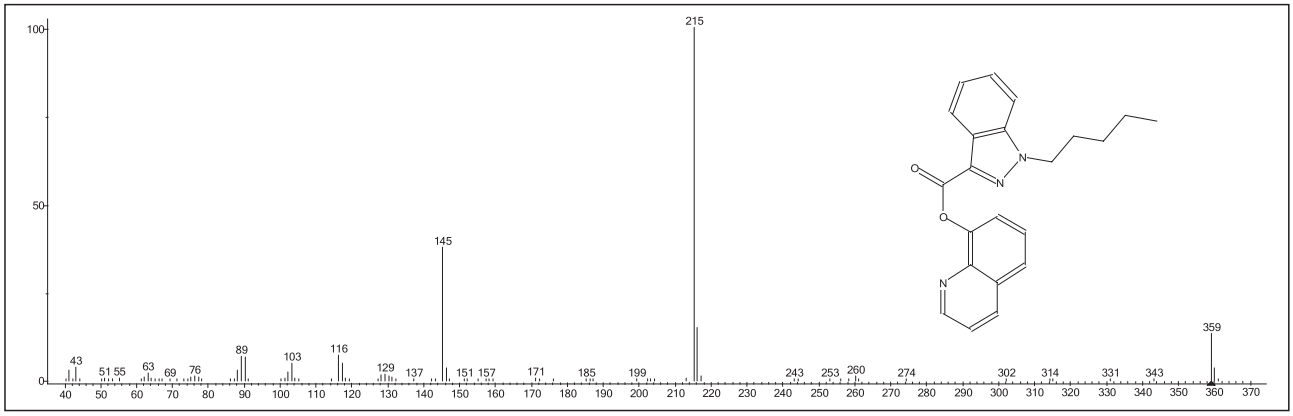
ester BB-22; used solvent: Ethanol



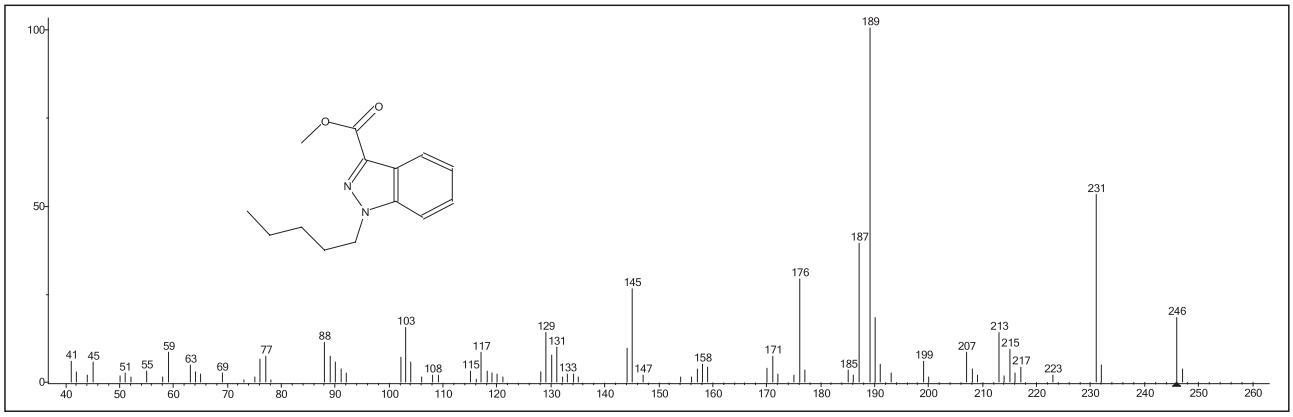
ester BB-22; used solvent: Pentafluoropentanol



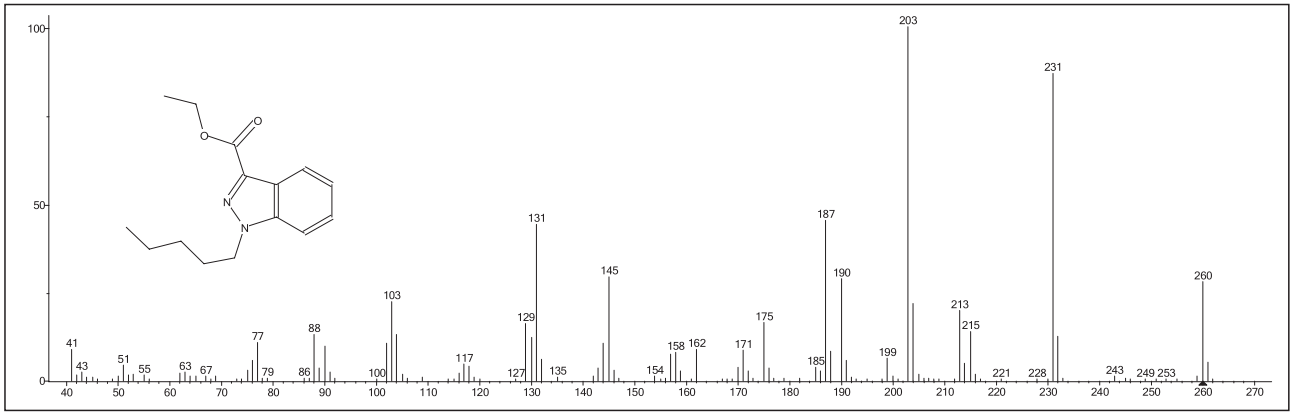
PB-22 indazole alalogue



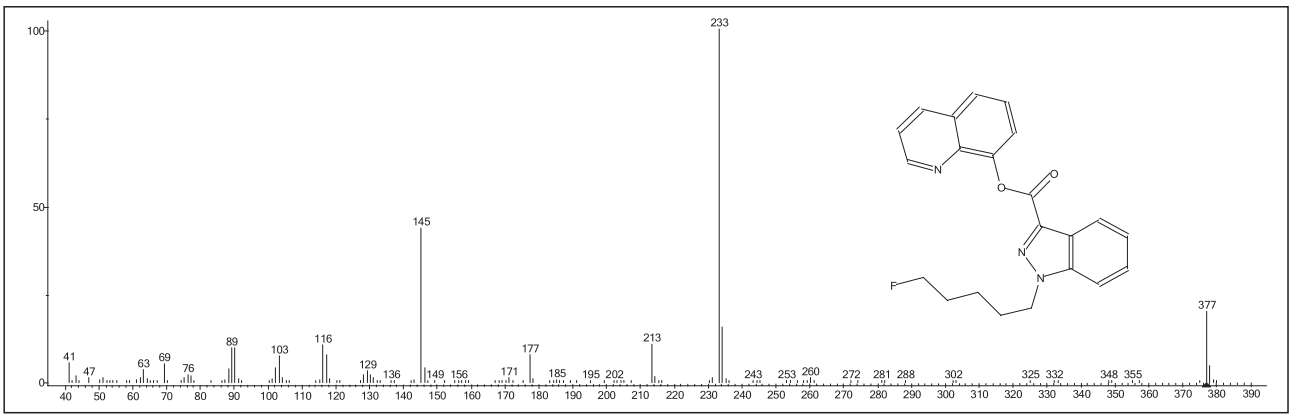
ester PB-22 indazole analogue; used solvent: Methanol



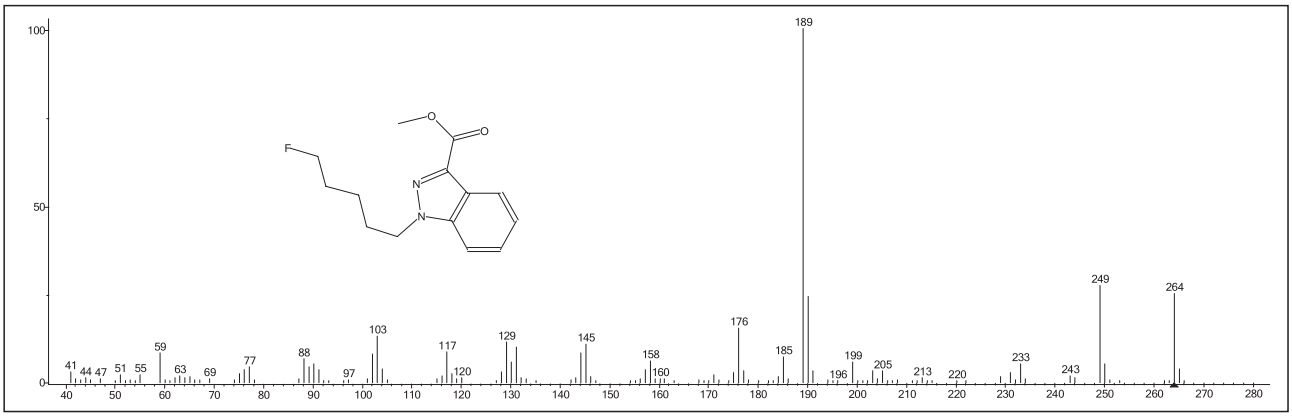
ester PB-22 indazole analogue; used solvent: Ethanol



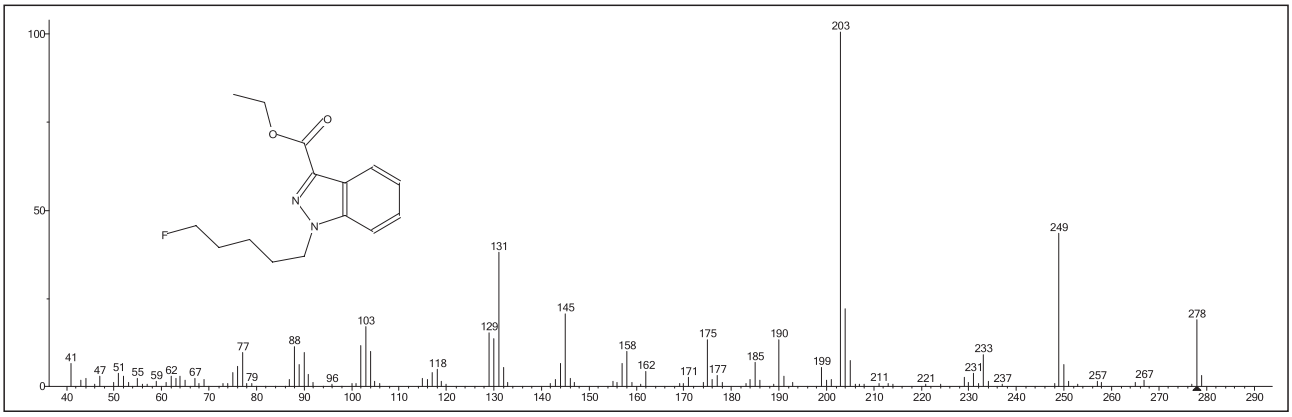
5F-NPB-22



ester 5F-NPB-22; used solvent: Methanol



ester 5F-NPB-22; used solvent: Ethanol



solvents [5], [6]. The results of the study conducted at the Hungarian Institute for Forensic Science indicated that the transesterification reaction takes place at room temperature, a few hours after addition of methanol or ethanol to any of the compounds listed above (including 5F-NPB-22) with methanol or ethanol. Aside from these two solvents, also the use of n-butanol for the extraction of PB-22 and 5F-PB-22 and pentafluoropropanol for PB-22, 5F-PB-22 and BB-22 facilitates the generation of esters [4]. The corresponding substrates and products are summarized in figure 9.

Further research at the Hungarian Institute for Forensic Science revealed that the use of isopropanol and isobutanol for the extraction does not cause the transesterification phenomenon in any of the analyzed compounds, even when heating at 90°C for several hours [4].

Summary and conclusions

GC/MS analysis of a synthetic cannabinoid 5F-NPB-22 (1-(5-fluoropentyl)-8-quinolinyl ester-1H-indazole-3-carboxylic acid) indicated that the use of methanol/toluene mixture at a 1:1 ratio for the extraction facilitated the generation of the 5F-NPB-22 methyl ester final

product, instead of the anticipated test cannabinoid. When toluene alone was applied to GC/MS analysis as the solvent, the non-reacted compound was extracted.

The above phenomenon is a transesterification of 5F-NPB-22 in the presence of methanol, proceeding after a prolonged period (several hours) of the methanol action on the analyzed compound at room temperature.

Based on the results obtained, it is recommended to avoid using methanol for 5F-NPB-22 extraction, and to use other solvents, such as toluene or acetonitrile, that do not facilitate the transesterification reaction.

A similar transesterification phenomenon was observed for the combinations of methanol and ethanol with the following compounds: PB-22; 5F-PB-22; BB-22 and PB-22 indazole analog [5]. In this case, in order to avoid esterification, the following extraction solvents can be used: isopropanol, isobutanol [4] or toluene, which has been tested by the authors.

Furthermore, the authors propose to perform a very thorough analysis of the GC/MS results in terms of the possibility of transesterification occurrence. The analysis of any of the above-described compounds (or derivatives thereof) should not be carried out with the use of a low molecular weight alcohol such as methanol or ethanol, but in the presence of one of other solvents, e.g. toluene or acetonitrile. Even trace quantities of a low molecular weight alcohol can lead to partial esterification. Moreover, the analysis of the extracted substances should be performed as fast as possible, since an extended period of measurement and too long remaining of the above esters in dissolved phase can facilitate partial transesterification.

Sources of figures and table: authors

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