Forensic Science International 252 (2015) 168–176

Contents lists available at ScienceDirect

Forensic Science International

journal homepage: www.elsevier.com/locate/forsciint

4F-PBP (4′-fluoro-α-pyrrolidinobutyrophenone), a new substance of abuse: Structural characterization and purity NMR profiling

Helena Gaspar [a,*], Soraia Bronze [a], Sara Ciríaco [a], Cláudio Rafael Queirós [a,b], Ana Matias [a,b], João Rodrigues [b], Cristina Oliveira [c], Carlos Cordeiro [a,d], Susana Santos [a]

[a] Centro de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal
[b] Laboratório de Polícia Cientifica da Polícia Judiciária, Novo edifício-sede da Polícia Judiciária, Rua Gomes Freire, 169-007 Lisboa, Portugal
[c] Centro de Química Estrutural, Faculdade de Ciências, Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal
[d] Laboratório de FTICR e espectrometria de massa estrutural, Faculdade de Ciências, Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal

ARTICLE INFO

Article history:
Received 19 February 2015
Received in revised form 27 April 2015
Accepted 3 May 2015
Available online 12 May 2015

Keywords:
Designer drugs
Cathinones
Pyrolidinophenone
4F-PBP
Fluoropyrolidinobutyrophenone
Quantification
NMR

ABSTRACT

The rapidly growing problem of new psychoactive substances (NPS) makes the time management for international control a real challenge, with the traditional detection methods becoming increasingly inadequate. NPS screening technologies, such as NMR, which allows multiple substances to be detected, characterized and quantified simultaneously from a single sample, offers a rapid solution to this problem. This study describes the application of NMR to the simultaneous detection, characterization and quantification of samples of white powders seized by the Portuguese Police. 4F-PBP (4′-fluoro-α-pyrolidinobutyrophenone) a new synthetic psychoactive cathinone cut with *myo*-inositol was found in two seized products. The structural characterization of 4F-PBP was elucidated in the mixture, and confirmed after isolation from the matrix by ^{1}H, ^{13}C, ^{19}F NMR and MS. *Myo*-inositol was found for the first time as a cutting agent of cathinones. Furthermore another seized product was characterized as being MDPBP, with a high degree of purity, and its spectroscopic elucidation enabled the correction of ^{13}C NMR literature assignments.

1. Introduction

A "designer drug" is a synthetic compound that mimics the rising effects of an original illegal drug, but with a slightly altered chemical structure, to circumvent legislation restrictions against illegal substances [1]. Designer drugs include psychoactive substances that have been designated by the European Union [2] as new psychoactive substances (NPS), intended as "new narcotic or psychotropic drugs, that are not listed in the Single Convention on Narcotic Drugs of 1961[3] or the Convention on Psychotropic Substances of 1971, but which may pose a public health threat comparable to that posed by substances listed in those conventions" [4].

In recent years, on average, one NPS was detected every week in the EU and the numbers are expected to increase in the coming years. The rapid and unprecedented rate of evolution and spread of NPS makes the time management for international control a real challenge. The EU Early Warning System (EWS), a monitoring system administred by the European Monitoring Center for Drugs and Drug Addiction (EMCDDA) and EUROPOL was conceived, precisely, as a way to respond quickly to the increase of NPS in the European space. Since 1997, more than 350 substances were reported trough the EWS, and just between 2009 and 2013 the number of monitored NPS more than tripled [5].

Among these NPS, "synthetic cathinones" burst into the market at an explosive rate, with no signs of slowing. "Synthetic cathinones" are β-keto phenethylamines, the structural analogs of the natural occurring cathinone, the psychoactive stimulant found naturally in the khat plant (*Catha edulis*) [6]. These substances are sold as powders, tablets and capsules, masked as "bath salts" and "plant feeders" under different names. They are commercialized over the Internet and in retail establishments, such as "head shops" and "smartshops". Based on their amino groups, cathinones can be divided in two types: alkylamine and pyrrolidine cathinones [7]. Pyrrolidine cathinones (pyrrolidinophenones, also called pyrovalerones) are related to pyrovalerone, the first commercially available drug from the α-pyrrolidinophenones class, introduced in the market in the 1960s as a stimulant

* Corresponding author. Tel.: +351 217500563.
E-mail address: hmgaspar@ciencias.ulisboa.pt (H. Gaspar).

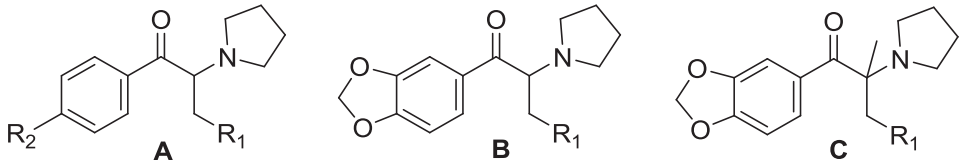
http://dx.doi.org/10.1016/j.forsciint.2015.05.003
0379-0738/

drug [8], having its clinical use being largely discontinued due to its association with risks of abuse and addiction. Alkylamine cathinones are structurally related to *N*-alkylamphetamines, differing only by the presence of β-keto group in the aliphatic chain. Synthetic cathinones made their largest first appearance on the market in the mid-2000s, with methylone (an analogue of MDMA, 3,4-methylenedioxy-methamphetamine, the scientific name of ecstasy) being the first one reported to the EMCDDA [9]. Since then, more than 50 different cathinones have appeared on the market [5]. In the EU, 22 pyrrolidinophenones were reported since 2004 [Table 1].

Pyrrolidinophenones found in the European Union can be grouped in three subclasses, according to the substitution pattern of the aryl moiety and the α-carbon substitution. In the first subclass, the aromatic ring is unsubstituted or *para* substituted with an alkyl group or halogen atom and the α-carbon is a tertiary one, with a C1 to C6 alkyl chain. A second class gathers compounds possessing a 3′,4′-methylenedioxyphenyl substitution, being the α-carbon, as well, a tertiary one with a C1 to C4 alkyl chain. Finally, the third class differs from the previous one on the fact that the α-carbon is quaternary holding an extra methyl group.

Few reports on the toxicity of synthetic cathinones exist to date, but it is known that cathinones, like many psychostimulants, exert their action interacting with monoamine neurons in the central nervous system (CNS) [10]. These neurons synthesize, store and release at least one of the neurotransmitters norepinephrine, dopamine and serotonin. The regulation of the extracellular concentration of these neurotransmitters is executed by the so-called monoamine transporters (MATs), integral plasma membrane proteins located outside the synaptic cleft, which transports

Table 1
Pyrrolidinophenones reported to EMCDDA as NPS, until December 2014.



Compound	Chemical name (common name, IUPAC name)	Molecular formula	Structure			EU	Portugal
				R_1	R_2		
PPP	α-pyrrolidinopropiophenone (*R,S*)-1-phenyl-2-(pyrrolidin-1-yl) propan-1-one	$C_{13}H_{17}NO$	**A**	H	H	Dec. 2008 Denmark	–
MPPP	4′-methyl-α-pyrrolidinopropiophenone (*R,S*)-2-(pyrrolidin-1-yl)-1-(*p*-tolyl)propan-1-one	$C_{14}H_{19}NO$	**A**	H	CH_3	Aug. 2010 UK	–
MOPPP	4′-methoxy-α-pyrrolidinopropiophenone (*R,S*)-1-(4-methoxyphenyl)-2-(pyrrolidin-1-yl)propan-1-one	$C_{14}H_{19}NO_2$	**A**	H	OCH_3	June 2004 Germany	–
ClPPP	4′-chloro-α-pyrrolidinopropiophenone (*R,S*)-1-(4-chlorophenyl)-2-(pyrrolidin-1-yl)propan-1-one	$C_{13}H_{16}ClNO$	**A**	H	Cl	Mar. 2013 Poland	–
MDPPP	3′,4′-methylenedioxy-α-pyrrolidinopropiophenone (*R,S*)-1-(benzo[*d*][1,3]dioxol-5-yl)-2-(pyrrolidin-1-yl)propan-1-one	$C_{14}H_{17}NO_3$	**B**	H	–	June 2004 Germany	–
MDMPP	3′,4′-methylenedioxy-α-methylpyrrolidinopropiophenone (*R,S*)-1-(benzo[*d*][1,3]dioxol-5-yl)-2-methyl-2-(pyrrolidin-1-yl)propan-1-one	$C_{15}H_{19}NO_3$	**C**	H	–	Aug. 2010 Switzerland	–
PBP	α-pyrrolidinobutiophenone (*R,S*)-1-phenyl-2-(pyrrolidin-1-yl)butan-1-one	$C_{14}H_{19}NO$	**A**	CH_3	H	Dec. 2011 Finland	Oct. 2014
MPBP	4′-methyl-α-pyrrolidinobutiophenone (*R,S*)-2-(pyrrolidin-1-yl)-1-(p-tolyl)butan-1-one	$C_{15}H_{21}NO$	**A**	CH_3	CH_3	July 2010 Bulgaria	–
MDPBP	3′,4′-methylenedioxy-α-pyrrolidinobutiophenone (*R,S*)-1-(benzo[*d*][1,3]dioxol-5-yl)-2-(pyrrolidin-1-yl)butan-1-one	$C_{15}H_{19}NO_3$	**B**	CH_3	–	May 2010 UK	Mar. 2013
MOPBP	4′-methoxy-α-pyrrolidinobutiophenone (*R,S*)-1-(4-methoxyphenyl)-2-(pyrrolidin-1-yl)butan-1-one	$C_{15}H_{21}NO_2$	**A**	CH_3	OCH_3	June 2014 Sweden	–
PVP	α-pyrrolidinovalerophenone (*R,S*)-1-phenyl-2-(pyrrolidin-1-yl)pentan-1-one	$C_{15}H_{21}NO$	**A**	C_2H_5	H	Feb. 2011 France	Mar. 2013
MOPVP	4′-methoxy-α-pyrrolidinovalerophenone (*R,S*)-1-(4-methoxyphenyl)-2-(pyrrolidin-1-yl)pentan-1-one	$C_{16}H_{23}NO_2$	**A**	C_2H_5	OCH_3	Dec. 2012 Finland	–
MDPV	3′,4′-methylenedioxy-α-pyrrovalerone (*R,S*)-1-(benzo[*d*][1,3]dioxol-5-yl)-2-(pyrrolidin-1-yl)pentan-1-one	$C_{16}H_{21}NO_3$	**B**	C_2H_5	–	Nov. 2008 UK/Finland	Feb. 2011
FPVP	4′-fluoro-α-pyrrolidinovalerophenone (*R,S*)-1-(4-fluorophenyl)-2-(pyrrolidin-1-yl)pentan-1-one	$C_{15}H_{20}FNO$	**A**	C_2H_5	F	Dec. 2013 Sweden	–
PHP	α-pyrrolidinohexanophenone (*R,S*)-1-phenyl-2-(pyrrolidin-1-yl)hexan-1-one	$C_{16}H_{23}NO$	**A**	C_3H_7	H	July 2013 Poland	–
MPHP	4′-methyl-α-pyrrolidinohexanophenone (*R,S*)-2-(pyrrolidin-1-yl)-1-(p-tolyl)hexan-1-one	$C_{17}H_{25}NO$	**A**	C_3H_7	CH_3	June 2004 Germany	Nov.2012
MDPHP	3,4-methylenedioxy-α-pyrrolidinohexiophenone (*R,S*)-1-(benzo[*d*][1,3]dioxol-5-yl)-2-(pyrrolidin-1-yl)hexan-1-one	$C_{17}H_{23}NO_3$	**B**	C_3H_7	–	Nov. 2014 Sweden	–
PHPP	α-pyrrolidinoheptanophenone (*R,S*)-1-phenyl-2-(pyrrolidin-1-yl)heptan-1-one	$C_{17}H_{25}NO$	**A**	C_4H_9	H	July 2013 Sweden	–
MOPEP	4-methoxy-α-pyrrolidinoenanthophenone (*R,S*)-1-(4-methoxyphenyl)-2-(pyrrolidin-1-yl)heptan-1-one	$C_{18}H_{27}NO_2$	**A**	C_4H_9	OCH_3	Nov. 2014 France	–
FPEP	4-fluoro-α-pyrrolidinoenanthophenone (*R,S*)-1-(4-fluorophenyl)-2-(pyrrolidin-1-yl)heptan-1-one	$C_{17}H_{24}FNO$	**A**	C_4H_9	F	Sept. 2014 Hungary	–
POP	α-pyrrolidinooctanophenone (*R,S*)-1-phenyl-2-(pyrrolidin-1-yl)octan-1-one	$C_{18}H_{27}NO$	**A**	C_5H_{11}	H	Sept. 2014 Germany	–
FPOP	4-fluoro-α-pyrrolidinooctanophenone (*R,S*)-1-(4-fluorophenyl)-2-(pyrrolidin-1-yl)octan-1-one	$C_{18}H_{26}FNO$	**A**	C_5H_{11}	F	Sept. 2014 Hungary	–

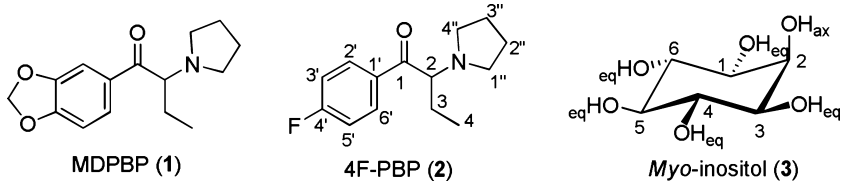


Fig. 1. Compounds identified in seized products in Portugal.

the monoamine transmitter from the synaptic cleft back to the cytoplasm of the pre-synaptic neuron, influencing the extent and duration of signaling between neurons [1,11]. Three major classes of MATs are responsible for the reuptake of their associated amine neurotransmitter: NET, DAT and SERT (norepinephrine, dopamine and serotonin transporter, respectively). Blocking the action of the MATs causes an increase of extracellular concentration of the neurotransmitters and, therefore, an increase in the corresponding neurotransmission.

Preliminary research on the pharmacological action of cathinones showed that they increase the synaptic concentration of monoamines either by inhibiting their corresponding transporters, although their selectivity for the SERT, NET and DAT varies considerably, or by increasing the pre-synaptic release of the neurotransmitters [12]. Recent studies classified cathinones in three groups according to their relative potency as SERT, NET and DAT inhibitors and their action as substrate releasers [13,14]: (a) cocaine-MDMA-mixed cathinones (mephedrone, methylone, ethylone, butylone and naphyrone), which inhibit dopamine and serotonin (5-HT) reuptake with a potency similar to cocaine, being also (with the exception of naphyrone) seretonin and dopamine releasers; (b) methamphetamine-like cathinones (cathinone, methcathinone and flephedrone) which act as dopamine and norepinephrine pharmacological reuptake inhibitors and as dopamine releasers and are similar to amphetamine and methamphetamine; (c) pyrrolidinophenones (pyrovalerone and MDPV), which act as very potent and selective dopamine and norepinephrine reuptake blockers, with little effect upon serotonin trafficking, and do not act as substrate releasers [15].

Additional information about pharmacological action of several pyrrolidinophenones (3,4-methylenedioxypyrovalerone, pyrovalerone, pyrrolidinovalerophenone, 3,4-methylenedioxypyrrolidinopropiophenone, and 3,4-methylenedioxypyrrolidinobutyrophenone) was recently published [16], showing that all pyrrolidinophenones are potent NET and DAT inhibitors. Additionally, the *para*- phenyl substituted compounds exhibit enhanced direct and indirect serotonergic agonist properties. Consequently, these compounds are likely to be associated with MDMA toxicity and may pose a high risk of addiction.

The legal status of NPS varies between countries and depends on the substance. In Portugal, the production, possession and commercialization of 159 NPS (34 of which are synthetic cathinones and 7 of them are pyrrolidinophenones) is illegal under legislation published on April 17th, 2013 [17]. Although only some substances are listed in the Portuguese legislation, it also takes into account derivatives of such substances, making it possible to control new emerging substances that may arise without the need for further legislation.

Due to the warnings issued by numerous public health authorities and poison control centers describing the adverse health effects associated with the use of synthetic cathinones, these substances are closely monitored by forensic laboratories. For instance in the EU, since its entry into the market, MDPV was linked to 107 non-fatal intoxications and 99 deaths, particularly in Finland and the United Kingdom [5].

This work describes and discusses the analytical assays performed to identify the components of three products seized in Portugal: one sample (A) was identified as MDPBP (1) with a purity higher than 99% and the other two (B and C) contained a NPS, named 4F-PBP, 4′-fluoro-α-pyrolidinobutyrophenone (2), cut with *myo*-inositol (3) in a percentage around 60% (Fig. 1). To the best of our knowledge this is a new drug of abuse in the EU, reported by us to the EMCDDA on January 28th, 2015.

2. Materials and methods

2.1. Chemicals

All solvents and reagents were obtained from commercial suppliers with an analytical grade and were used without further purification. Water was grade I quality.

2.2. Samples

Three different seized products (A, B and C) analyzed in this work were provided by the Forensic Science Laboratory of Portuguese Criminal Police and were part of originally seized white powder batches. An authentic sample of MDPBP as hydrochloride salt was synthetized as previously reported [8].

2.3. Isolation of 4F-PBP

Pure 4F-PBP as hydrochloride salt was isolated by filtration through cotton of a solution of 55 mg of seized product C dissolved in 1.5 mL of deuterated chloroform ($CDCl_3$). NMR characterization of the compound was also performed in $CDCl_3$, DMSO-d_6 and D_2O (deuterium oxide).

2.4. Isolation of 4F-PBP free base

Product C (100 mg) was dissolved, under sonication, in 2 mL of diethyl ether. The solution was cooled in an ice bath and 2 mL of a cold 1% aqueous solution of $NaHCO_3$ were added, resulting in yellowish oil. The phases were separated and the aqueous one was re-extracted twice with ethyl ether (2 × 2 mL), under sonication. The combined organic layers were washed with de-ionized water (5 × 2 mL), and evaporated under reduced pressure, affording 15 mg of pure 4F-PBP free base (η_{ext} = 15%). NMR characterization of the compound was performed in $CDCl_3$.

2.5. GC-MS analysis

Approximately 5–10 mg of the seized powders were extracted in a methanol/chloroform mixture (50:50) up to 2.5–5 mg/mL. 1 μL of the extracts was injected into the GC-MS system.

A gas chromatographer (Agilent® GC System 6890 Series) coupled to a mass spectrometer (Agilent® 5973 Network) with a HP-5MS column (30m × 0.25 mm × 0.25 μm) was used. Samples were injected in split mode 1:40. Helium was the carrier gas. Initial temperature set to 150 °C, held for 1 min, then increased to 270 °C at 12 °C/min and held for 8 min. The injector temperature was 280 °C and the GC-MS transfer line temperature was 230 °C. Electron ionization (EI) energy was 70 eV. Scan range was 40–550 *m*/*z*.

2.6. FTICR-MS analysis

High resolution mass spectra (HRMS) were recorded in an Apex Qe FTICR mass spectrometer with an Apollo II ESI/MALDI Combi source. Spectra were acquired by ESI in positive mode. Samples were dissolved in water (1 mg/mL) and diluted 1:1000 in ESI solution (water/methanol/formic acid 50/50/1%).

2.7. NMR analysis

An aliquot of each seized product (10 to 15 mg) was dissolved in DMSO-d_6 to run NMR structural analysis or in D_2O for qNMR quantification. Both solvents allowed collection of NMR spectra of 4F-PBP and *myo*-inositol in the mixture. ^{1}H NMR (400.1 MHz), ^{13}C NMR (100.6 MHz) and ^{19}F spectra (376.3 MHz) were recorded on a Bruker Avance spectrometer; chemical shifts were expressed as δ values and referenced to the residual solvent peak (DMSO-d_6, δH = 2.50, δC = 39.5; $CDCl_3$, δH = 7.26, δC = 77.00) or to the signal of maleic acid (D_2O, δH = 6.42, δC = 132.16) coupling constants were reported in units of Hertz (Hz). NMR spectra ran for structure characterization used standard pulse programs. The identification of structures with the corresponding assignments of the proton and carbon signals was based on the analysis of NMR spectra obtained by 1D (^{1}H, ^{13}C, APT, ^{19}F) and 2D (including COSY, HMBC and HSQC) techniques.

For quantification, seized samples (ca. 10 mg) were dissolved in 500 μL of a standard solution of maleic acid (CRM for quantitative NMR, Fluka, purity 99%) in deuterated water (D_2O). This standard solution was prepared dissolving 100.50 mg of maleic acid in 10 mL of D_2O. The ^{1}H qNMR spectra were acquired with a zg 90° pulse program, with 64 scans using a fid with 48,000 data points, a digital resolution of 10 points/Hz, a spectral width of 12 ppm and a recovery delay of 45 s. The protons' chemical shift was calculated using as reference the maleic acid resonance signal at 6.42 ppm. The ^{1}H signal integration for each compound (MDPBP at 0.84 ppm; *myo*-inositol at 3.69 ppm; 4F-PBP at 0.82 ppm) was calculated calibrating for 100 the area of maleic acid resonance peak. The absolute quantity (mol) was determined through the ratio between the integration of the target protons of each compound and the signal of maleic acid (internal standard, IS). The purity of the products was assessed calculating the mass fraction (% m/m) of each compound in the seized products using the following equation:

$$\%\text{compound} = \frac{I_X}{I_{IS}} \times \frac{n_{IS}^H}{n_X^H} \times \frac{V_2}{V_1} \times \frac{M_X}{M_{IS}} \times \frac{m_{IS}^W}{m_{sample}^W} \times P_{IS} \times 100 \quad (1)$$

where m_{sample}^W and m_{IS}^W are the masses (weights in g), M_X and M_{IS} are the molecular weights in g/mol (for cathinones is the mass of hydrochloride salt), n_X^H and n_{IS}^H are the numbers of protons generating the selected signals for integration, I_X and I_{IS} are the integrals and P_{IS} is the purity of maleic acid; V_2 is total standard solution volume and V_1 is the standard solution volume added to each sample.

2.8. Ion chromatography analysis

Aqueous solution samples of seized products were analyzed by ion chromatography (IC) for the confirmation of the presence of ion chloride. IC measurements were performed in a Dionex® DX500 system with conductivity detection (CD20), equipped with Peaknet® software. The chromatograph was equipped with an isocratic pump IP20, an anion pre-column IonPack AG14 4 × 50 mm, an analytical column IonPack AS14 4 × 250 mm, an anion suppressor ASRSR–II Ultra 4 mm and a loop of 25 μL. The eluent was a carbonate-bicarbonate buffer solution (3.5 mmol/L Na_2CO_3 + 1 mmol/L $NaHCO_3$).

3. Results and discussion

In Portugal, commercialization of synthetic cathinones has been outlawed since 2013 and subject to strict control by the police and, therefore, samples of the seized substances are regularly submitted to the forensic laboratory for identification. Recently, GC-MS analysis of three seized products (A, B and C), presented as white powders, revealed chromatograms with one major peak, with the corresponding EI spectra showing no molecular peak and the same fragment base peak at *m/z* 112. However, sample B and C shared a compound with a common retention time (RT = 4.796 min) different from the one of sample A (RT = 8.289 min) (Fig. 2). Comparison of these data with GC/MS databases preliminarily identified the three samples as MDPBP (**1**), which clearly could not be the case due to the different retention times. In order to obtain a standard of MDPBP, synthesis of this cathinone was undertaken, following literature procedures for other α-pyrolidinophenones [8].

The ^{1}H and ^{13}C NMR spectral data of compound **1** in DMSO-d_6 showed identical signals to the synthesized MDPBP and were both consistent with literature values [18,19]. However, we found that some of the NMR aromatic carbon signals were incorrectly assigned in the first report of MDPBP analytical data [18]. Table 2 shows the correct assignments of all MDPBP (hydrochloride salt) NMR signals taken in DMSO-d_6, D_2O and $CDCl_3$. The characterization of compound **1** as MDPBP was confirmed by the EI mass spectrum (Figs. 2 and 3) that displayed the characteristic pattern of the α-pyrrolidinobutyrophenones, being the major striking feature the base peak at *m/z* 112, which corresponds to the immonium peak produced by the α-cleavage of the benzyl bond. The minor peak at *m/z* 149 is characteristic of the methylenodioxybenzoyl moiety [20].

Products B and C contain a major compound, which shares the same base peak fragment (*m/z* 112) with MDPBP, a fact that can be indicative that they are probably α-pyrrolidinobutyrophenones (Fig. 3).

The analysis of NMR data in DMSO-d_6 of products B and C showed that they were both similar mixtures of two compounds, **2** and **3**, and allowed their discrimination and structural identification (Tables 3–5). The four well resolved proton signals (3.69, 1H, t; 3.34, 2H, t; 3.11, 2H, dd; 2.89 1H t) in the ^{1}H NMR spectrum and the value of the corresponding *J*, together with the corresponding carbon signals (75.30, 72.78, 72.66, 71.90 ppm) allowed to identify *myo*-inositol (**3**) in seized product C (Table 3). The NMR comparison between an authentic sample of *myo*-inositol and the one present in sample C is shown in Fig. 4. Although in the ^{1}H NMR high field region (1.5-4 ppm) there is some partial overlapping between signals from compounds **2** and **3**, all the expected COSY connectivity's for *myo*-inositol can be observed. Further evidence was obtained from the HSQC correlations in the clean zone of the spectrum around 70 ppm (Fig. 5). NMR analysis of the seized products proved to be a valuable tool to identify *myo*-inositol that is not detected in GC analysis due to its low volatility.

The NMR spectra pattern of compound **2** confirmed the MS findings that it should be structurally related with MDPBP, the only significant differences being in the downfield aromatic region. In this region, the pseudo-triplet at 7.11 ppm (2H, *J* = 8.5 Hz) and the doublet of doublets at 8.20 (2H, *J* = 8.8 and 5.6 Hz) with additional J_{HF} ($^3J_{HF} \approx {}^3J_{HH}$ and $^4J_{HF} > {}^3J_{HH}$), embody an AA'XX' system characteristic of a 4-fluor substituted moiety. The ^{1}H decoupled ^{13}C NMR spectrum showed the splitting of all aromatic carbon signals as doublets, with coupling constant ranging from 2.9 to

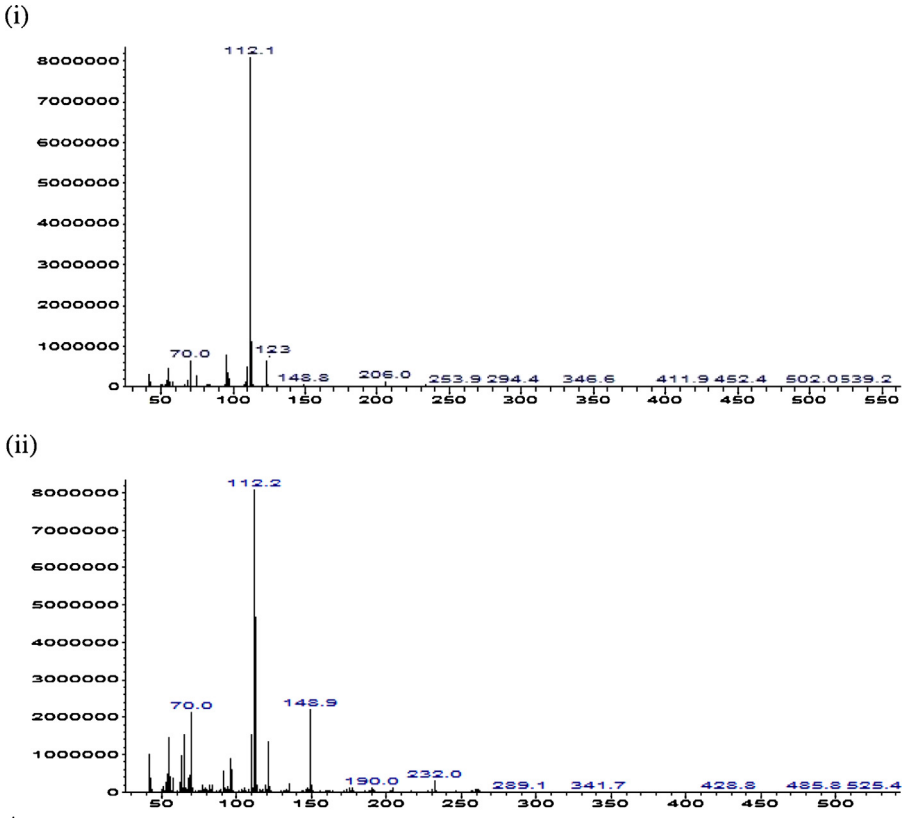


Fig. 2. Mass spectra of 4F-PBP in product C (i) and MDPBP in product A (ii).

259 Hz ($^4J_{CF}$, $^3J_{CF}$, $^2J_{CF}$, $^1J_{CF}$) a feature characteristic of *para*- fluoro substituted phenones, like flephedrone [21,22]. The presence of fluor in the aromatic ring was also confirmed by the presence of one only peak in the ^{19}F NMR spectrum, and by the minor peak in the mass spectrum at *m/z* 123 (Fig. 3), typical of a fluorobenzoyl ion [20,21]. The structure of compound **2** was established as 4F-PBP (IUPAC name: 1-(4-fluorophenyl)-2-(pyrrolidin-1-yl)butan-1-one) on the basis of extensive 1D and 2D NMR (Tables 4 and 5).

In order to obtain NMR spectra of neat 4F-PBP, and confirm its characterization, an aliquot of sample C was dissolved in deuterated chloroform, the insoluble *myo*-inositol was filtered-off and the NMR characterization in several deuterated solvents was performed (Tables 3 and 4). Basic extraction of sample C, followed by ethyl ether extraction, has allowed to remove *myo*-inositol and to obtain the 4F-PBP free base, which was also fully characterized by NMR (Tables 3 and 4). The complete match of all the NMR signals of pure 4F-PBP and *myo*-inositol with the signals observed in the mixture is a proof that compounds were reliably identified by this technique without need of the time consuming extraction from the matrix.

The presence of 4F-PBP and *myo*-inositol in seized products B and C was confirmed by the accurate mass spectrum obtained by FTICR, that displays two ion peaks, at *m/z* 236.14450 and *m/z* 203.05250, corresponding respectively to the protonated

Table 2
NMR assignments of MDPBP (**1**), as hydrochloride salt, found in product A.

Position	DMSO-d_6		D_2O		$CDCl_3$	
	δ ^{13}C	δ ^{1}H, m, *J* (Hz)	δ ^{13}C	δ ^{1}H, m, *J* (Hz)	δ ^{13}C	δ ^{1}H, m, *J* (Hz)
1	194.23	–	195.93	–	194.28	–
2	68.13	5.46, 1H, m	70.59	5.18, 1H, m	63.18	5.00, 1H, m
3	23.19	1.98, 2H, m	24.29	2.17, 2H, m	24.44	2.09, 1H, m; 2.32, 1H, m
4	8.48	0.75, 3H, t, 7.4	8.17	0.84, 3H, t, 7.4	10.78	1.00, 3H, t, 7.6
1′	128.88	–	128.88	–	130.84	–
2′	107.87	7.56, 1H, brs	108.68	7.47, 1H, brs	107.76	7.42, 1H, brs
3′	148.31	–	149.37	–	149.06	–
4′	153.06	–	154.70	–	153.76	–
5′	108.61	7.16, 1H, d, 8.4	109.49	7.03, 1H, d, 8,4	108.48	6.92, 1H, d, 8.4
6′	126.10	7.75, 1H, brd 8.4	127.63	7.70, 1H, d, 8.4	125.83	7.59, 1H, d, 8.4
7′	102.62	6.20, 2H, s	103.59	6.14, 2H, s	102.49	6.10, 2H, s
NH	–	10.44, 1H, brs	–	–	–	12.47, 1H, brs
1″	51.77	3.19, 1H, m; 3.56, 1H, m	52.67	3.32, 1H, m; 3.76, 1H, m	48.96	3.64, 1H, m; 3.82, 1H, m
2″	22.87	1.94, 1H, m; 2.04, 1H, m	23.52	2.00–2.16, 2H, m	23.70	2.05–2.22, 2H, m
3″	22.87	1.93, 1H, m; 1.99,1H, m	23.71	2.00–2.16, 1H, m; 2.46, 1H, m	23.91	1.99, 1H, m; 2.32, 1H, m
4″	53.79	3.00, 1H, m; 3.46, 1H, m	55.99	3.05, 1H, m; 3.69, 1H, m	52.79	2.83 m, 1H, m; 3.76, 1H, m

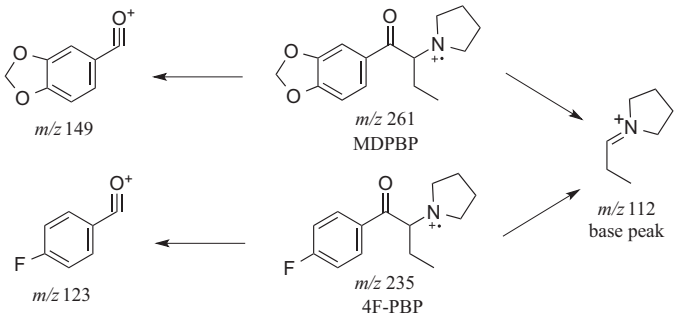


Fig. 3. Important fragments in the EIMS spectra of PBP derivatives **1** and **2**.

molecular ion of 4F-PBP ($C_{14}H_{18}FNO^+$; calcd. 236.14452) and to the sodium adduct of *myo*-inositol ($C_6H_{12}O_6Na^+$; 203.05261).

In order to identify the anion present in the seized samples, as often synthetic cathinones can be obtained as hydrochloride or hydrobromide salts, as is the case of 4-methylethcathinone [23], or even nitrate salts [18], ion chromatography was undertaken. The results showed that 4F-PBP was in the form of hydrochloride salt and also confirmed its relative amount in samples B and C, as the concentration of the chloride ion is equimolar to the concentration of 4F-PBP.

The absolute quantification of several components of a mixture without analyte standards' is one of the great advantages of ^{1}H qNMR spectroscopy when compared to other quantitative techniques such as GC-MS or HPLC-UV [24]. Indeed, the purity of several standard drugs and the analysis of illicit drugs and adulterants was already determined by NMR [25]. Using our previously ^{1}H qNMR validated methodology that allows the evaluation of the purity of cathinones with low uncertainty [26,27], we were able to determine the compositions of seized products and the purity of synthetized MDPBP. NMR conditions were optimized for the simultaneous determination of the analytes: we found that water was the best solvent, enabling the complete solubilization of the seized samples, that

Table 3
NMR assignments of *myo*-inositol (**3**).

Position	DMSO-d_6[a]		DMSO-d_6[b,c]		D_2O[a]		D_2O[b]	
	δ ^{13}C	δ ^{1}H, m, *J* (Hz)	δ ^{13}C	δ ^{1}H, m, *J* (Hz)	δ ^{13}C	δ ^{1}H, m, *J* (Hz)	δ ^{13}C	δ ^{1}H, m, *J* (Hz)
1,3	71.90	3.11, 2H, dd, 2.8;9.6	71.87	3.11, 2H, ddd, 2.8; 5.2; 8.4	72.12	3.53, 2H, dd, 2.8; 10.0	72.12	3.49, 2H, dd, 3.2; 10.0
2	72.66	3.69, 1H, t, 2.6	72.66	3.69 1H, dt, 3.2; 2.8	73.16	4.06, 1H, t, 2.8	73.18	4.02, 1H, t, 2.8
4,6	72.78	3.34, 2H, t, 9.2	72.76	3.34, 2H, td, 9.6; 4.4	73.39	3.62, 2H, t, 9.8	73.39	3.58, 2H, t, 9.8
5	75.30	2.89, 1H, t, 9.0	75.26	2.89, 1H, td, 9.1; 3.9;	75.34	3.27, 1H, t, 9.4	75.34	3.23, 1H, t, 9.2

[a] Signals of *myo*-inositol in the seized product C.
[b] Signals of *myo*-inositol standard.
[c] $OH_{eq\text{-}5}$ δ4.58, 1H, 4.0; $OH_{eq\text{-}4,6}$ δ4.51, 2H, 4.4; $OH_{ax\text{-}2}$ δ4.48, 1H, 3.2; $OH_{eq\text{-}1,3}$ δ4.37, 2H, 5.6.

Table 4
^{1}H NMR assignments of 4F-PBP (**2**).

Position	Free base $CDCl_3$	$CDCl_3$[a]	D_2O[a]	D_2O[b]	DMSO-d_6[a]	DMSO-d_6[b]
	δ_H, m, *J* (Hz)	δ_H, m, *J* (Hz)	δ_H, m, *J* (Hz)	δ_H, m, *J* (Hz)	δ_H, m, *J* (Hz)	δ_H, m, *J* (Hz)
1	–	–	–	–	–	–
2	3.69, 1H, dd, 9.0, 4.5	5.30, 1H, m	5.25, 1H, m,	5.26, 1H, m	5.56, 1H, m	5.55, 1H, m
3	1.84, 1H, m 1.91, 1H, m	2.14, 1H, m 2.21, 1H, m	2.17, 2H, m	2.18, 2H, m	2.04, 2H, m	2.04, 2H, m
4	0.82, 1H, t, 7.6	1.01, 3H, t, 7.6	0.82, 3H, t, 7.5	0.82, 3H, t, 7.6	0.76, 3H, t, 7.5	0.75, 3H, t, 7.6
1′	–	–	–	–	–	–
2′	8.20, 2H, dd, 8.8, 5.6[c]	8.08, 2H, dd, 8.6, 5.4[c]	8.11, 2H, dd, 8.5, 5.6 [c]	8.11, 2H, dd, 8.8, 5.6 [c]	8.18, 2H, dd, 8.7, 5.5[c]	8.17, 2H, dd, 8.4, 5.6[c]
3′	7.11, 2H, t, 8.5[d]	7.23, 2H, t, 8.4[d]	7.34, 2H, t, 8.5 [d]	7.34, 2H, t, 8.8[d]	7.48, 2H, t, 8.8 [d]	7.49, 2H, t, 8.4 [d]
4′	–	–	–	–	–	–
5′	7.11, 2H, t, 8.5[d]	7.23, 2H, t, 8.4[d]	7.34, 2H, t, 8.5 [d]	7.34, 2H, t, 8.8[d]	7.48, 2H, t, 8.8 [d]	7.49, 2H, t, 8.4 [d]
6′	8.20, 2H, dd, 8.8, 5.6 [c]	8.08, 2H, dd, 8.6, 5.4 [c]	8.11, 2H, dd, 8.5, 5.6 [c]	8.11, 2H, dd, 8.8, 5.6 [c]	8.18, 2H, dd, 8.7, 5.5 [c]	8.17, 2H, dd, 8.4, 5.6[c]
NH	–	12.13, 1H, brs	–	–	10.50, 1H, brs	10.41, 1H, brs
1″	2.50–2.70, 2H, m	3.68, 2H, m	3.33, 1H, m; 3.75, 1H, m	3.32, 1H, m; 3.76, 1H, m	3.21,1H, m; 3.60, 1H, m	3.21, 1H, m; 3.60, 1H, m
2″	1.76, 2H, m	2.05–2.33, 2H, m	1.96–2.29, 2H, m	1.96–2.27, 2H, m	1.89–2.12, 2H, m	1.85–2.12, 2H, m
3″	1.76, 2H, m	2.05–2.33, 2H, m	1.96–2.29, 2H, m	1.96–2.27, 2H, m	1.89–2.12, 2H, m	1.85–2.12, 2H, m
4″	2.50–2.70, 2H, m	3.02, 1H, m; 3.83, 1H, m	3.07, 1H, m; 3.71, 1H, m	3.06, 1H, m; 3.71, 1H, m	3.07, 1H, m; 3.50, m	3.06, 1H, m; 3.50, 1H, m

[a] Proton signals of purified 4F-PBP.
[b] Proton signals of 4F-PBP in the seized product C.
[c] Coupling $^3J_{HH} > {}^4J_{HF}$.
[d] Coupling $^3J_{HH} \approx {}^3J_{HF}$.

Table 5
^{13}C NMR assignments of 4F-PBP (**2**).

Position	Free base $CDCl_3$	$CDCl_3$[a]	D_2O[a]	D_2O[b]	DMSO-d_6[a]	DMSO-d_6[b]
	δ_C, J_{CF} (Hz)	δ_C, J_{CF} (Hz)	δ_C, J_{CF} (Hz)	δ_C, J_{CF} (Hz)	δ_C, J_{CF} (Hz)	δ_C, J_{CF} (Hz)
1	199.40	194.89	196.75	196.73	194.93	195.00
2	71.61	65.05	70.92	70.98	68.46	68.60
3	23.75	24.22	23.99	24.02	22.90	22.90
4	10.28	10.45	8.05	8.05	8.30	8.27
1′	133.12, d, 3.1	131.91, d, 2.9	130.87, d, 2.7	130.88, d, 2.9	130.97, d, 2.8	130.93, d, 2.4
2′	131.40, d, 9.3	131.65, d, 9.8	132.98, d, 10.2	133.02, d, 10.3	132.07, d, 9.2	132.15, d, 9.9
3′	115.48, d, 21.7	116.70, d, 22.2	117.44, d, 22.6	117.49, d, 22.5	116.41, d, 22.3	116.50, d, 22.2
4′	165.62, d, 254.5	166.84, d, 259.3	167.85, d, 256.2	167.90, d, 256.6	165.96, d, 254.2	166.02, d, 254.7
5′	115.48, d, 21.7	116.70, d, 22.2	117.44, d, 22.6	117.49, d, 22.5	116.41, d, 22.3	116.50, d, 22.2
6′	131.40, d, 9.3	131.65, d, 9.8	132.98, d, 10.2	133.02, d, 10.3	132.07, d, 9.2	132.15, d, 9.9
1″	51.32	50.48	52.76	52.77	51.77	51.82
2″	23.34	23.74	23.74	23.79	22.90	22.90
3″	23.34	23.91	23.57	23.62	22.90	22.90
4″	51.32	52.48	56.17	56.20	53.87	54.00

[a] Carbon signals of purified 4F-PBP.
[b] Carbon signals of 4F-PBP in the seized product C.

afterwards can be easily recovered, and affording spectra with very good peak resolution. Product B was evaluated as a mixture of 38% of 4F-PBP and 61% of inositol, while product C showed a content of 42% of 4F-PBP and 57% of inositol (Fig. 6).

The seizure of a sample of cut 4F-PBP was not surprising. Indeed, it is documented that 'bath salts' and powders, tablets or capsules containing cathinones consist typically of one or several synthetic cathinones, often mixed with other substances such as caffeine, topical anesthetics, binding and cutting agents, and even other illicit drugs [28]. For instance, a study of street mephedrone seized in South Wales over two years showed a decrease in sample's purity after the UK ban, with the most common cutting agents being sodium monoglutamate, sucrose and creatine [29]. Benzocaine was also reported as a cutting agent for the synthetic cathinone flephedrone [22]. Studies on the composition of legal highs' marketed in Portugal, prior to being illegal, reported that products were usually mixtures of synthetic cathinones and several other ingredients such as glucose, lactose and caffeine [30,31].

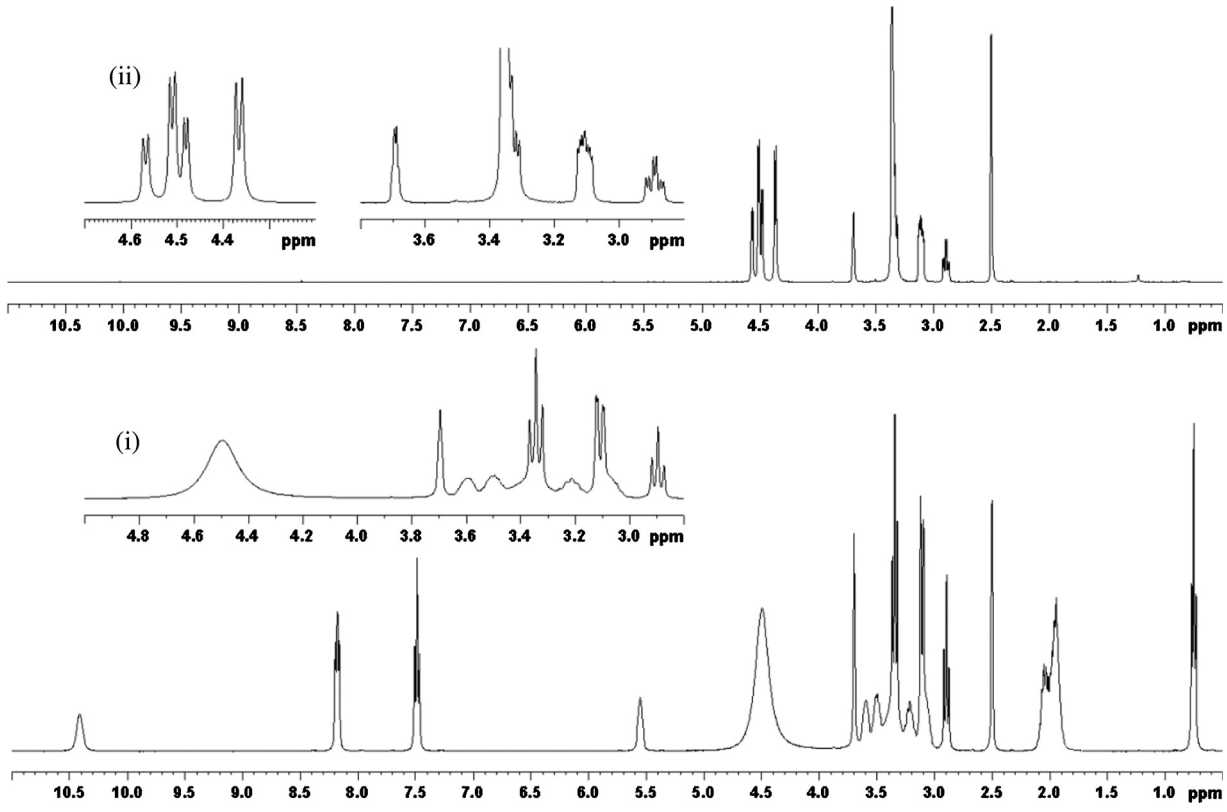


Fig. 4. ^1H NMR (DMSO-d_6) spectra of seized product C (i) and *myo*-inositol (ii).

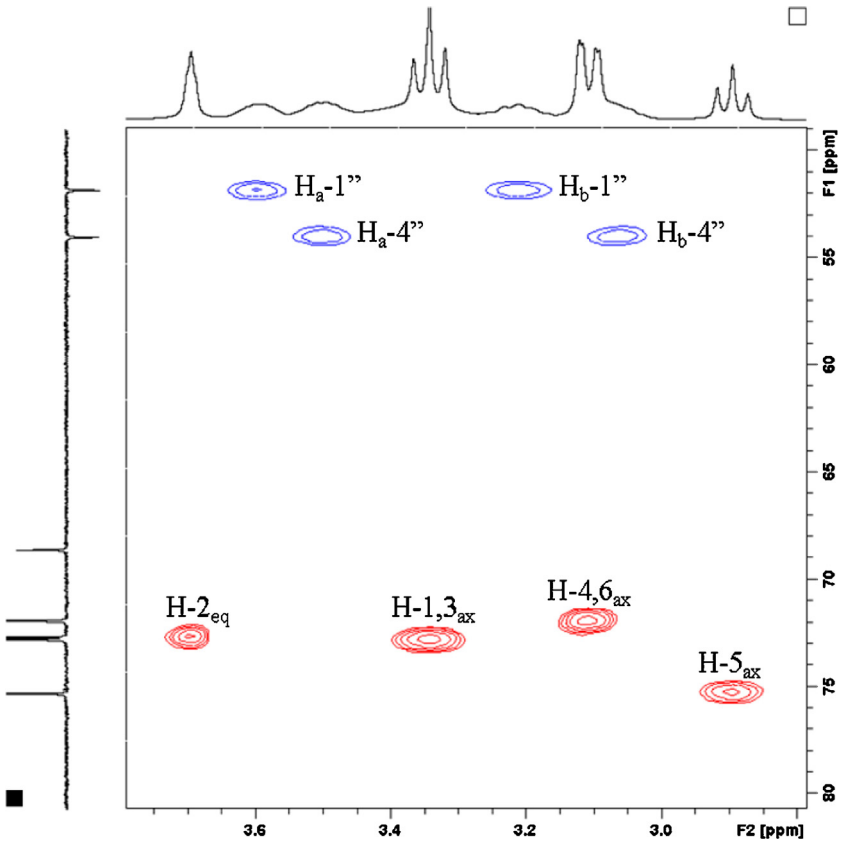


Fig. 5. Expansion of gHSQC NMR experiment of seized product C in the region 3.7-2.8; the correlations $^{13}C/^{1}H$ of *myo*-inositol methine groups are shown in red and the ones of NCH_2 group of 4F-PBP pyrrolidine ring are shown in blue.

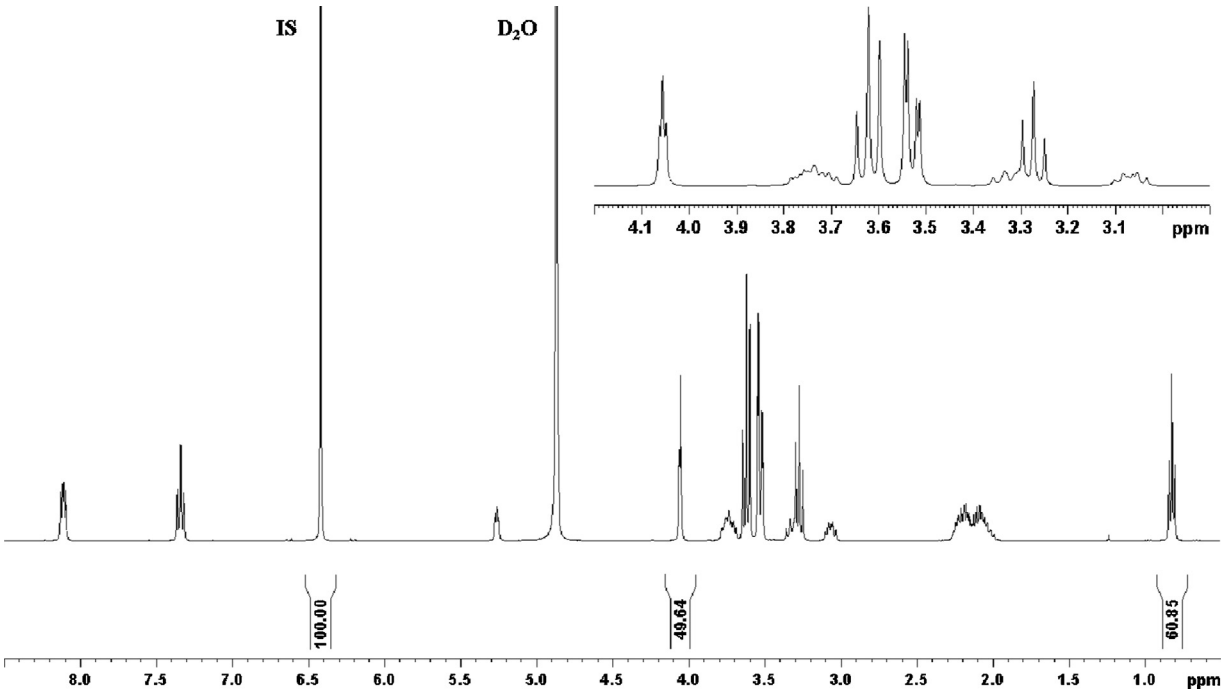


Fig. 6. ^{1}H qNMR (D_2O) spectrum of seized product C, using maleic acid as internal standard.

4. Conclusion

The structure of the new designer drug 4F-PBP, was elucidated by ^{1}H, ^{13}C and ^{19}F NMR as well as MS spectrometry. This cathinone was detected in batches recently seized in Portugal, and this is the first report of this NPS in the EU. The samples contained 4F-PBP cut with inositol in a relative mass fraction around 40:60, based on a multitarget qNMR method for simultaneous identification and quantification of mixtures. This methodology allowed the direct and unequivocal identification of both compounds in the mixture, as well as the precise determination of the corresponding amounts, with a very significant time-gain in comparison to conventional HPLC methods, for instance. It should be stressed that using NMR is possible to detect non-volatile compounds, like *myo*-inositol, that do not appear in GC-MS unless derivatized. To the authors knowledge, this is the first time that inositol is reported as a cutting agent in a seizure of synthetic cathinones and, more importantly, being the major compound. This fact can be a hint that in Portugal, such as in other countries, after these types of substances become illegal, there is a downtrend in the purity of the products. This decrease in purity may lead to serious health consequences, with unpredictable dangerous effects, since consumers used to products with low purity can easily overdose when subsequently using a pure sample.

Acknowledgments

The authors acknowledge Fundação para a Ciência e a Tecnologia (FCT) for financial support through projects UID/MULTI/00612/2013, UID/QUI/00100/2013 and Rede Nacional de Espectrometria de Massa REDE/1501/REM/2005. This work was done within the scope of the protocol established between Faculdade de Ciências da Universidade de Lisboa (FCUL), Laboratório de Polícia Cientifica da Polícia Judiciária (LPC/PJ) and Faculdade de Fármacia da Universidade do Porto (FFUP). The authors thank the staff from the Toxicology Sector of LPC/PJ.

References

[1] C.L. German, A.E. Fleckenstein, G.R. Hanson, Bath salts and synthetic cathinones: an emerging designer drug phenomenon, Life Sci. 97 (2014) 2–8.
[2] Council Decision 2005/387/JHA of 10 May 2005 on the information exchange, risk-assessment and control of new psychoactive substances. Official Journal L 127, 20/05/2005 P. 0032–0037. Available from: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CELEX:32005D0387:EN:HTML [Last accessed February 2, 2014].
[3] UN, Single convention on narcotic drugs, 1961 (1961). Available from: https://www.unodc.org/pdf/convention_1961_en.pdf [Last accessed February 2, 2014].
[4] UN, Convention on Psychotropic Substances of 1971 (1971). Available from: https://www.unodc.org/pdf/convention_1971_en.pdf [Last accessed February 2, 2014].
[5] EMCDDA, European Drug Report 2014: Trends and developments (2014). Available from: http://www.emcdda.europa.eu/attachements.cfm/att_228272_EN_TDAT14001ENN.pdf [Last accessed February 2, 2014].
[6] K. Zaitsu, M. Katagi, H. Tsuchihashi, A. Ishii, Recently abused synthetic cathinones, alpha-pyrrolidinophenone derivatives: a review of their pharmacology, acute toxicity, and metabolism, Forensic Toxicol. 32 (2014) 1–8.
[7] D.P. Katz, D. Bhattacharya, S. Bhattacharya, J. Deruiter, C.R. Clark, V. Suppiramaniam, M. Dhanasekaran, Synthetic cathinones: a khat and mouse game, Toxicol. Lett. 229 (2014) 349–356.
[8] P.C. Meltzer, D. Butler, J.R. Deschamps, B.K. Madras, 1-(4-methylphenyl)-2-pyrrolidin-1-yl-pentan-1-one (pyrovalerone) analogues: A promising class of monoamine uptake inhibitors, J. Med. Chem. 49 (2006) 1420–1432.
[9] UNODC, Global SMART Programme report 2013: The challenge of New Psychoactive substances. Available from: http://www.unodc.org/documents/scientific/NPS_2013_SMART.pdf [Last accessed February 2, 2014].
[10] J.P. Kelly, Cathinone derivatives: a review of their chemistry, pharmacology and toxicology, Drug Test Anal. 3 (2011) 439–453.
[11] R.B. Rothman, M.H. Baumann, Monoamine transporters and psychostimulant drugs, Eur. J. Pharmacol. 479 (2003) 23–40.
[12] A.J. Eshleman, K.M. Wolfrum, M.G. Hatfield, R.A. Johnson, K.V. Murphy, A. Janowsky, Substituted methcathinones differ in transporter and receptor interactions, Biochem. Pharmacol. 85 (2013) 1803–1815.
[13] L.D. Simmler, T.A. Buser, M. Donzelli, Y. Schramm, L.H. Dieu, J. Huwyler, S. Chaboz, M.C. Hoener, M.E. Liechti, Pharmacological characterization of designer cathinones in vitro, Brit. J. Pharmacol. 168 (2013) 458–470.
[14] J.B. Zawilska, J. Wojcieszak, Designer cathinones–an emerging class of novel recreational drugs, Forensic Sci. Int. 231 (2013) 42–53.
[15] M.H. Baumann, J.S. Partilla, K.R. Lehner, E.B. Thorndike, A.F. Hoffman, M. Holy, R.B. Rothman, S.R. Goldberg, C.R. Lupica, H.H. Sitte, S.D. Brandt, S.R. Tella, N.V. Cozzi, C.W. Schindler, Powerful cocaine-like actions of 3,4-methylenedioxypyrovalerone (MDPV), a principal constituent of psychoactive 'Bath Salts' products, Neuropsychopharmacology 38 (2013) 552–562.
[16] A. Rickli, M.C. Hoener, M.E. Liechti, Monoamine transporter and receptor interaction profiles of novel psychoactive substances: para-halogenated amphetamines and pyrovalerone cathinones, Euro. Neuropsychopharmacol. (2015).
[17] Decreto-Lei no54/2013 Portaria 154/2013, 2254 3, 2013, pp. 2254-2257. Available from: https://dre.pt/pdf1sdip/2013/04/07500/0225402257.pdf [Last accessed February 2, 2014].
[18] F. Westphal, T. Junge, B. Klein, G. Fritschi, U. Girreser, Spectroscopic characterization of 3,4-methylenedioxypyrrolidinobutyrophenone: A new designer drug with alpha-pyrrolidinophenone structure, Forensic Sci. Int. 209 (2011) 126–132.
[19] S.D. Brandt, H.R. Sumnall, F. Measham, J. Cole, Analyses of second-generation 'legal highs' in the UK: Initial findings, Drug Test Anal. 2 (2010) 377–382.
[20] D. Zuba, Identification of cathinones and other active components of 'legal highs' by mass spectrometric methods, Trac-Trend Anal. Chem. 32 (2012) 15–30.
[21] R.P. Archer, Fluoromethcathinone, a new substance of abuse, Forensic Sci. Int. 185 (2009) 10–20.
[22] M.R. Alotaibi, S.M. Husbands, I.S. Blagbrough, ^{1}H, ^{13}C, ^{15}N HMBC, and ^{19}F NMR spectroscopic characterisationof seized flephedrone, cut with benzocaine, J. Pharm. Biomed. Anal. (2015).
[23] 4-Methylethcathinone (4-MEC): Critical review report, WHO Expert Committee on Drug Dependence, thirty-sixth meeting, Geneva 2014.
[24] G.F. Pauli, S.N. Chen, C. Simmler, D.C. Lankin, T. Godecke, B.U. Jaki, J.B. Friesen, J.B. McAlpine, J.G. Napoitano, Importance of purity evaluation and the potential of quantitative H-1 NMR as a purity assay, J. Med. Chem. 57 (2014) 9220–9231.
[25] P.A. Hays, Proton nuclear magnetic resonance spectroscopy (NMR) methods for determining the purity of reference drug standards and illicit forensic drug seizures, J. Forensic Sci. 50 (2005) 1342–1360.
[26] R. Lopes, M.J.V. Brito, H. Gaspar, R.B. Silva, Validation of the quantification of cathinones in plant feeders by ^{1}H NMR, in: Eurachem 2014: Quality analytical measurements, Lisbon, Portugal May 2014.
[27] R.L.T.F. Lopes, Validação da quantificação de catinonas por qRMN ^{1}H em drogas com efeitos psicoativos. Master Thesis. Faculdade de Ciências, Universidade de Lisboa, Lisboa, Portugal, (2014).
[28] D. Zuba, B. Byrska, Prevalence and co-existence of active components of legal highs', Drug Test Anal. 5 (2013) 420–429.
[29] B. Miserez, O. Ayrton, J. Ramsey, Analysis of purity and cutting agents in street mephedrone samples from South Wales, Forensic Toxicol. 32 (2014) 305–310.
[30] A.M. Araújo, M.J. Valente, M. Carvalho, D.D. Silva, H. Gaspar, F. Carvalho, M.L. Bastos, P.G. Pinho, Raising awareness of new psychoactive substances: chemical analysis and in vitro toxicity screening of 'legal high' packages containing synthetic cathinones, Arch. Toxicol. (2014).
[31] V.M.R. Zancajo, J. Brito, M.P. Carrasco, M.R. Bronze, R. Moreira, A. Lopes, Analytical profiles of legal highs containing cathinones available in the area of Lisbon, Portugal, Forensic Sci. Int. 244 (2014) 102–110.