Title: Analytical profiles of "legal highs" containing cathinones available in the area of Lisbon, Portugal

Authors:

Victor M.R. Zancajo [a,*], J. Brito [b], Marta P. Carrasco [c], M.R. Bronze [c,d], Rui Moreira [c], Alvaro Lopes [a,e]

[a] Instituto Superior de Ciências da Saúde Egas Moniz, Quinta da Granja, Monte de Caparica, 2829-511 Caparica, Portugal
[b] WDXRFLab - Instituto Superior de Ciências da Saúde Egas Moniz, Quinta da Granja, Monte de Caparica, 2829-511 Caparica, Portugal
[c] Instituto de Investigação do Medicamento (iMed.ULisboa), Faculdade de Farmácia, Universidade de Lisboa, Av. Prof. Gama Pinto, 1649-003 Lisboa, Portugal
[d] ITQB, Instituto de Tecnologia Química e Biológica, Universidade Nova de Lisboa, Avenida da República, Estação Agronómica Nacional, 2780-157 Oeiras, Portugal
[e] Faculdade de Farmácia, Universidade de Lisboa, Av. Prof. Gama Pinto, 1649-003 Lisboa, Portugal

*Corresponding author

Email: rodriguez.zancajo@gmail.com

Phone: +34 622125467

Co-author: Alvaro Lopes

Email: alvaro.lopes@campus.ul.pt

Abstract:

Thirteen "legal highs" were purchased in different "smart shops" in the area of Lisbon, Portugal, during the month of February 2013. The samples were analysed by a battery of analytical methods including Fourier transform infra-red (FTIR), gas chromatography coupled with mass spectrometry (GC-MS), proton nuclear magnetic resonance (^{1}H-NMR) and wavelength dispersive X-ray fluorescence (WD-XRF). Active ingredients were found either as single component or in mixtures in the different products. The cathinone derivative methedrone was present in three products; it is suspected to have a particular high toxicity and narrow therapeutic window linked with the methoxy group. A total of seven compounds were identified: 4-Fluoromethcathinone, ethcathinone, buphedrone, methedrone, pentedrone, 3,4-

Dimethylmethcathinone and 4-Methylethcathinone. Analytical profiles of all the samples were obtained and compared.

Elemental composition of the products was obtained by XRF analysis. The inorganic profiles obtained contain useful information and can be used to distinguish and classify samples according to their origin.

Keywords: "Legal highs"; Cathinones; Methedrone; Elemental composition; X-Ray fluorescence.

Acknowledgements

The authors would like to thank the European Commission for funding of this project through the Erasmus Mundus Program, Rosário Bronze for performing GC-MS, Marta Carrasco for NMR spectroscopy and José Brito for obtaining the XRF data and for his time and support.

Introduction

"Legal highs" have been banned in many countries during the last years. The government of Portugal announced the intention of banning these substances on November 2012, based on the Council Decision 2005/387/JHA on information exchange, risk-assessment and control of new psychoactive substances submitted by the European Commission with the support of EMCDDA, its Scientific Committee, Europol and Member States. Final version of the new law (Decreto-Lei nº54/2013 Portaria 154/2013) was published on the April 17th 2013 in "*Diário da República, 1.ª série - N.º 75 - 17 de abril de 2013"* together with a list of 159 substances that became illegal to possess, sell or distribute. This list includes 34 cathinone derivatives [1].

The new Portuguese legislation was based on the "precautionary principle" of risk assessment with a wide scope on new psychoactive substances (NPS) generally considered as "…substances not specifically framed and controlled under ongoing specific legislation that, in pure form or in a preparation, can pose a threat to public health comparable to the substances contained in that legislation, with danger to life or to health and physical integrity due to effects on the central nervous system and that may induce significant changes in motor function as well as mental functions, namely, critical judgment and reasoning behaviour, often with states of delirium, hallucinations or extreme euphoria and that can cause addiction and, in certain cases, produce lasting or even permanent damage on the health of consumers."

Consumption of these substances not only implies the risk due to their psychoactive and toxic properties, but the hazard of containing additional toxic compounds, such as leaching metals from the reaction vessels, residues of catalysts, dyes and contamination in adulterants or diluents. Manganese poisoning is a possibility in users if permanganate was used in their synthesis and they are not correctly purified [2] . Methoxylated derivatives present a special case of toxicity. Methedrone (4-Methoxymethcathinone) has been given its name trivially, too similar to mephedrone which implies the risk of misidentification by consumers. Methedrone is the β-keto analogue of paramethoxymethamphetamine (PMMA) and it has been responsible for at least two fatalities [3], where it was found in concentration similar to concentration levels quantified in livings. Methedrone has shown to release norepinephrine and dopamine

similar to PMMA and paramethoxyamphetamine (PMA) [4]. These drugs have same effects as 3,4-methylenedioxy-*N*-methylamphetamine (MDMA), but with a lower onset, leading the users to consume more. The results are a quicker increase in body temperature and blood pressure as well as in blood concentration and more hazardous than MDMA. They have a particular high toxicity and narrow therapeutic window linked with the methoxy group. Accordingly, PMA and PMMA have been responsible of at least 6 fatalities [5]. Hence, it seems that methedrone recreational use entails higher risks than consumption of other related compounds.

This study presents the results of a wide range of analysis on 13 "legal highs" products that were purchased in three different "smart shops" in the area of Lisbon. Synthetic cathinone derivatives were found in all the products (Figure 1).

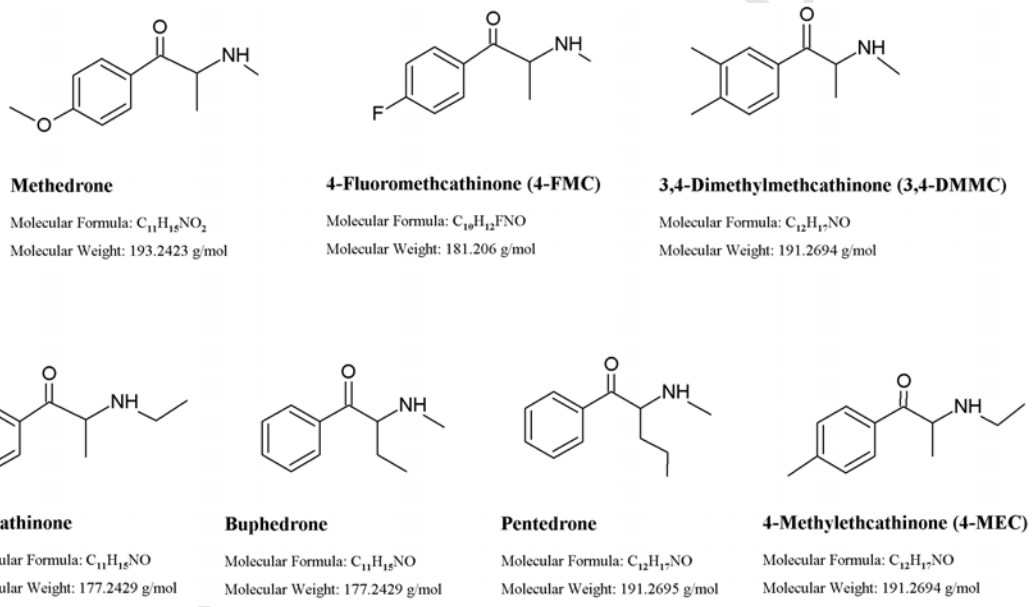


Figure 1 (one column and half): Chemical structures, molecular formula and molecular weight of cathinone derivatives identified in this study.

Materials and methods

Methanol used as solvent for the GC-MS analysis and methanol-d4 used for NMR were purchased from Sigma-Aldrich (Germany) and were analytical grade.

Gas Chromatography Mass Spectrometry

A gas chromatograph mass detector GC-MS Shimadzu GC-2010 (Kyoto, Japan) with a column Varian Factor Four VF-5ms (30 m x 0.25 mm x 0.25 µm) was used. 2 µl of

sample at a concentration of 0.5 mg/ml was injected in split mode 1:50. Helium was the carrier gas. The initial temperature was 80 ºC, held for 1 min, then increased till 150 ºC at 15 ºC/min, maintained for 5 min and finally increased to 295 ºC at 25 ºC/min, and held for 3 minutes more. The injector temperature was 250 ºC and the GC-MS interface transfer line temperature was 280 ºC. The mass spectrometer was operated in Electron Ionization mode (EI) with electron beam energy of 70 eV. The range 29-450 m/z was collected. Mass spectra were compared with NIST MS libraries for "legal highs" [6,7].

Fourier Transform Infrared Spectroscopy

Samples were screened by Attenuated Total Reflectance (ATR)-FTIR using a Perking Elmer Spectrometer 65 (Massachusetts, USA) equipped with the software "Perkin Elmer Spectra". 20 mg of each product was placed directly in the instrument. The IR spectra were obtained in the range of 4000 to 600 cm^{-1}, with 35 scans at 4 cm^{-1} resolution. Spekwin32 software for optical spectroscopy, Version 1.71.6.1, was used for processing and visualizing the spectra. FTIR spectra of products number 1-8 were also obtained after being irradiated with XRF.

X-Ray Fluorescence

Products number 1-8 were analysed by WD-XRF. The whole remaining amount of each product, approximately 0.9 g, was placed in a Bruker plastic-film vial specific for XRF analysis. Products were analysed with a Bruker S4 Pioneer, in the range of 0-30 kV at an intensity of 134 mA and energy 4.02 kW in helium atmosphere. Software used for spectra analysis was "Spectra plus (Bruker)".

Ultraviolet-Visible Spectrometry

A Perkin Elmer Lambda 25 UV/Vis Spectrometer was used to obtain the UV-visible spectra of the products to evaluate the unexpected change in colour occurred due to X-Rays irradiation. Products were analysed with a slit width of 1 nm at a scan speed of 480 nm/min. 5 mg of products number 1-8 were dissolved in 1 ml of distilled water and placed in a quartz micro cuvette.

Proton Nuclear Magnetic Resonance

^{1}H NMR spectra of products number 1, 4, 10 and 13 were obtained at a temperature of 293 K. The measurements were performed on a Bruker ARX-400 (Bruker Bio Spin GmbH, Karlsruhe, Germany) operating at 400 MHz. 5 mg of each sample were dissolved in 500 µl of methanol d-4 (CD_3OD), which was also used as a reference.

Results and discussion

Products evaluation

All the products were presented as white powders in small plastic bags with striking designs and names labels. The information provided with the products was in general poor, written in English, Portuguese or both languages. Some of the products were labelled as "plant feeders" and "not for human consumption". Modes of use were described per square meter or per plant, with a recommended dose of 0.2 grams. General information was inconsistent for products 1 and 6. Ingredients of these products were described per capsule, although they were presented in powder. Products labelled with the same name but with different lot number, advertised different ingredients, such as in the case of Blast (products nº 1, 5 and 9) and Bloom (nº 2 and 6). Previous studies has also found inconsistent information provided regarding the ingredients of "legal highs" [8]. According to the average price of the products (38 Euros per gram compared with 8-10 euros per gram reported in 2010 [9]), it seems that "legal highs" containing cathinone derivatives increased their price in the last years or the price in Portugal was higher than the average in the European Union.

Table 1 (one column): Number assigned to each product, and summary information including name, weight, price, origin and active ingredients identified.

PRODUCT NUMBER	LEGAL HIGH NAME	SMART SHOP	WEIGHT ADVERTISED	PRICE	ACTIVE INGREDIENTS DETECTED
1	Blast	Magic Mushroom Lisbon	1 g	36 €	4-Fluoromethcathinone Caffeine
2	Bloom	Magic Mushroom Lisbon	1 g	36 €	Ethcathinone Methedrone Pentedrone Caffeine

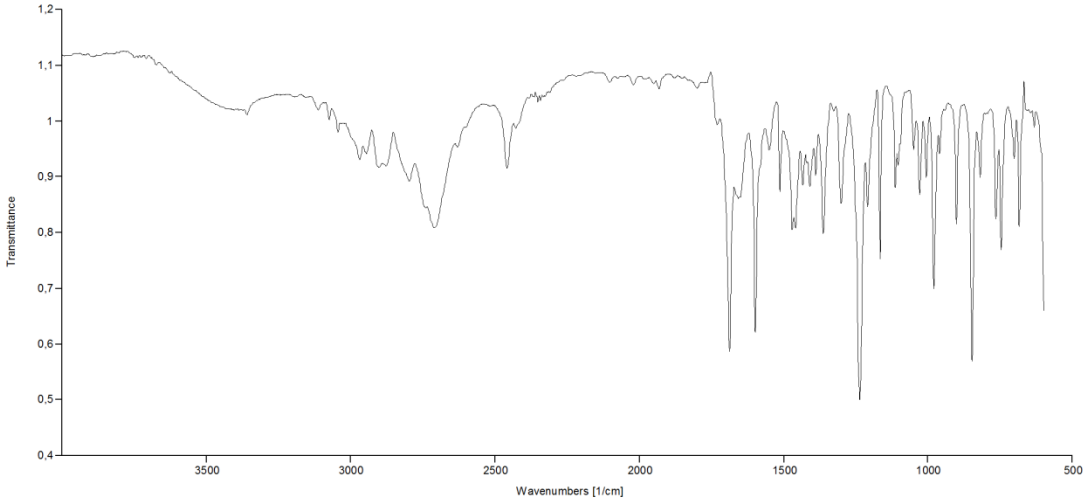
3	Blow	Magic Mushroom Lisbon	1 g	38 €	4-Methylethcathinone Caffeine
4	Charlie	Magic Mushroom Lisbon	1 g	37 €	Ethcathinone Buphedrone
5	Blast	Free Mind Lisbon	1 g	36 €	4-Fluoromethcathinone Caffeine
6	Bloom	Free Mind Lisbon	1 g	36 €	Ethcathinone Methedrone Pentedrone Caffeine
7	Blow	Free Mind Lisbon	1 g	38 €	4-Methylethcathinone Caffeine
8	Charlie	Free Mind Lisbon	1 g	37 €	Ethcathinone Buphedrone
9	Blast	Magic Mushroom Almada	0.5 g	19.5 €	4-Fluoromethcathinone Caffeine
10	Bliss	Magic Mushroom Almada	0.5 g	16.5 €	Methedrone
11	Blow	Magic Mushroom Almada	0.5 g	20.5 €	4-Methylethcathinone Caffeine
12	Charlie	Magic Mushroom Almada	0.5 g	20 €	Ethcathinone Buphedrone
13	Invader Space Ship	Magic Mushroom Almada	0.250 g	10.5 €	3,4-DMMC

Chemical analysis

Active ingredients were found in all thirteen "legal highs" products purchased for this study (Table 1). The following synthetic cathinones were identified 4-Fluoromethcathinone (4-FMC), ethcathinone (EC), buphedrone, methedrone, pentedrone, 3,4-Dimethylmethcathinone (3,4-DMMC) and 4-Methylethcathinone (4-MEC).

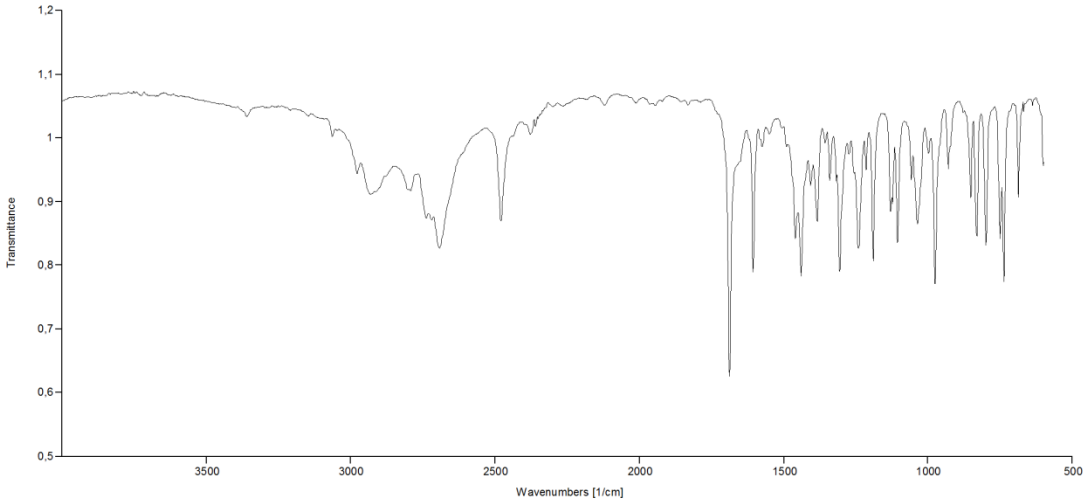
ATR-FTIR spectra of products were consistent with the molecular structure of synthetic cathinones (figures 2-5). All spectra presented a very intense peak at 1680-1690 cm^{-1} (stretch C=O), another intense peak at 1600 cm^{-1} (stretch C=C) due to the presence of an aromatic ring and bands with relative low intensity at frequencies near to 3200 cm^{-1} and 2450-2490 cm^{-1} corresponding to an amine. Noticeable differences were found in the fingerprint region between 600 cm^{-1} and 1200 cm^{-1} for each product that helped to identify the synthetic cathinone present in the sample for products containing only one cathinone derivative as main component (products 1, 3, 5, 7, 9, 10, 11 and 13). This suggests relatively pure samples. Products labelled with the same name showed undistinguishable IR spectra. IR spectra of 4-FMC, 4-MEC, methedrone and 3,4-

187 DMMC have been previously published [10–13].



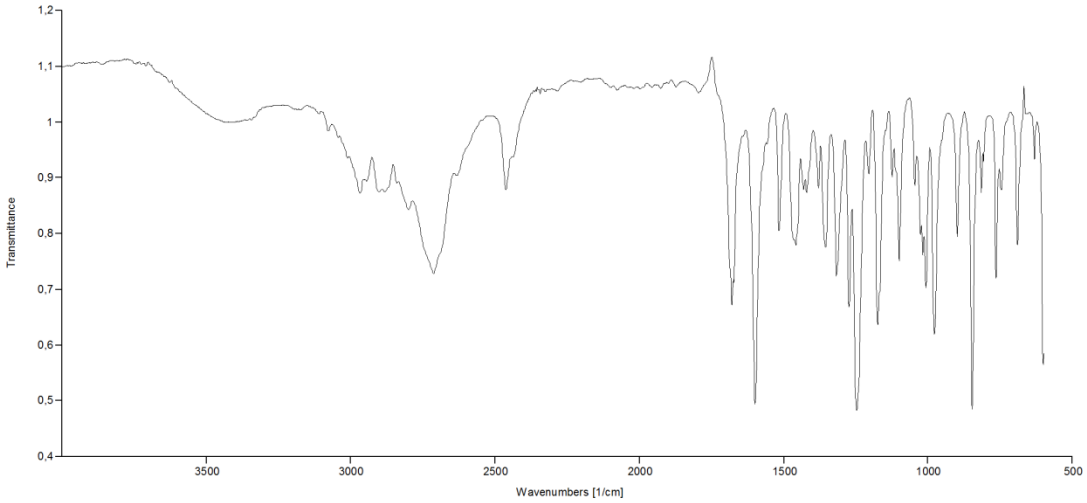
188

189 **Figure 2 (one column and half):** FTIR spectra of product Blast (4-FMC).



190

191 **Figure 3 (one column and half):** FTIR spectra of product Blow (4-MEC).



192

193 **Figure 4 (one column and half):** FTIR spectra of product Bliss (methedrone).

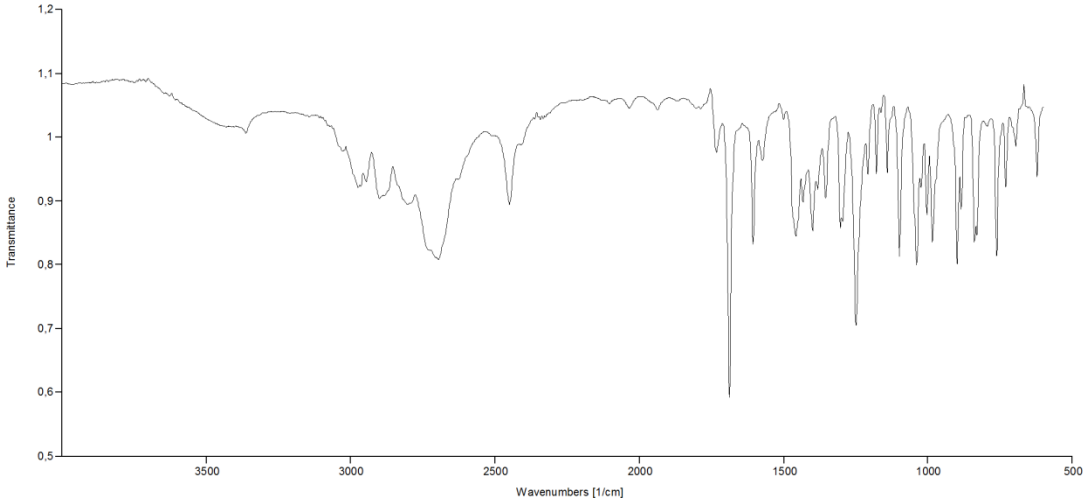
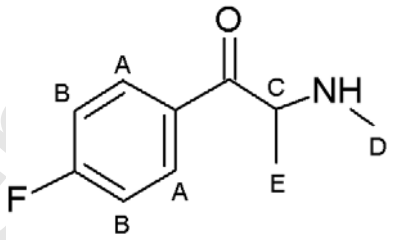


Figure 5 (one column and half): FTIR spectra of product Invader Space Ship (3,4-DMMC).

It was confirmed by GC-MS analysis that products nº 1, 5 and 9 (labelled as "Blast") contain 4-FMC as a major ingredient. ^{1}H NMR data supported the previous findings of MS and confirmed the presence of 4-FMC. See Table 2. Chemical characterization of 4-FMC is available in the literature[10].

Table 2 (one column): ^{1}H NMR data for 4-FMC.



Position	*δ (ppm)*	*Integration*	*Multiplicity*	*J(Hz)*
E	1.58	3.06	d	7.2
D	2.77	3.04	s	-
C	5.10	1.06	m	7.2
A	7.35	2.00	t	8.7
B	8.15	2.03	m	-

4-MEC was identified as main ingredient of products 7 and 11 ("Blow"). Analytical data of 4-MEC has been published [11]. EC was found in products number 2 and 6 and

together with buphedrone in products 4, 8 and 12. Retention times of cathinone derivatives under GC conditions have been reported [14] and helped to confirm the identity of chemicals present in the samples. Fragmentation patterns of cathinones have been previously proposed and observed under EI conditions [15,16]. Fragmentation patterns observed on the mass spectra of products containing buphedrone and ethcathinone were very similar; both compounds produced the same characteristic ions. The ^{1}H NMR spectrum confirmed the presence of the both compounds together in a sample (figure 6). Both compounds showed the signal corresponding to the protons from chiral carbon atoms of buphedrone and ethcathinone overlapped, and appeared as a multiplet. However, the number of remaining signals, corresponding to the alkyl substituent chain was the twice that it would be expected in the presence of only one compound. The intensity of the signals was approximately half of the expected value compared with the intensity of signals assigned to aromatic protons, indicating the presence of both, buphedrone and EC in similar amounts. Methanol (CD_3OD), used as solvent, was set at 3.31 ppm on the NMR spectra. Water also appeared in all the spectra at 4.8 ppm, presumably due to the hygroscopic character of methanol.

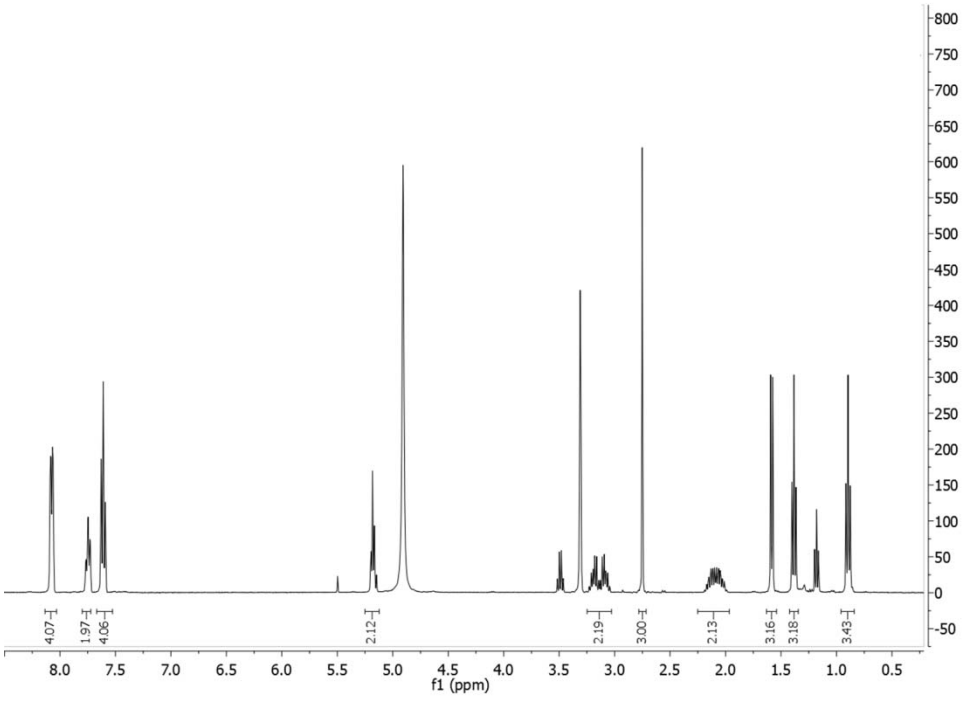


Figure 6 (one column and half): ^{1}H NMR spectra of product 4 (containing ethcathinone and buphedrone). Methanol was set at 3.31 ppm (CD_3OD). Diethyl ether was also identified, 1.18 ppm (t) and 3.55 ppm (q), water (H_2O) appeared at 4.8 ppm.

Methedrone was identified as the main component in product number 10 and together with other cathinones (ethcathinone, and pentedrone) in products number 2 and 6. Base peak in mass spectra of methedrone, as it is expected for all cathinones, was the iminium ion, in this case, m/z 58 ($C_3H_8N^+$). Peaks at m/z 135 and m/z 107 with low intensity correspond to acylium ion ($C_8H_7O_2^+$) and the ion resultant of the loss of carbon monoxide ($C_7H_7O^+$), characteristic ions for methedrone [15], and consistent with a methoxy group substituent in the aromatic ring (figure 7). Molecular ion was absent in the mass spectra of methedrone. No molecular ions could be detected for any of the cathinones identified.

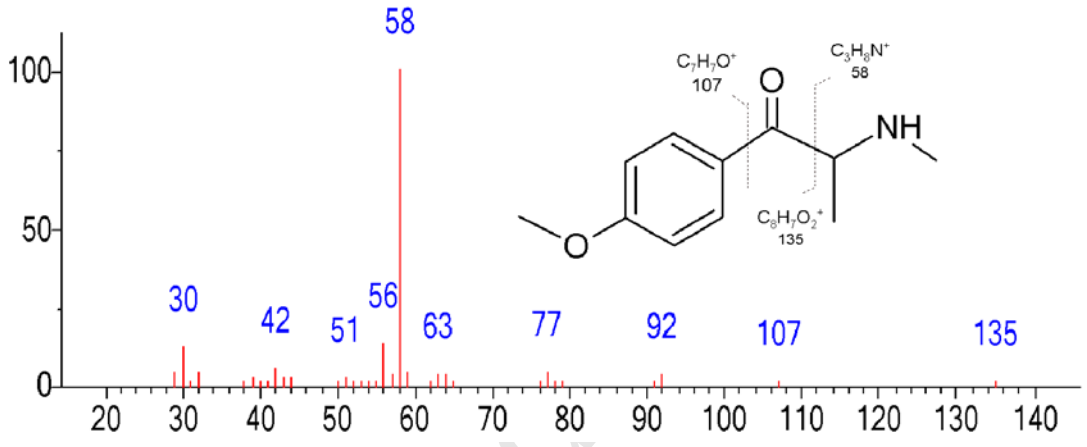
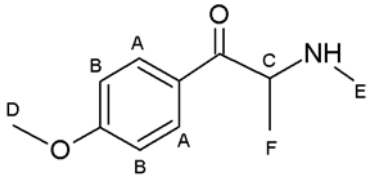


Figure 7 (one column and half): Mass spectrum of methedrone.

Presence of methedrone was also confirmed by NMR (Table 3, Figure 8). No NMR data of methedrone has been previously reported. Proton attached to chiral carbon was identified as a quartet at δ 5.04 ppm. Two doublets in the aromatic region 8.04 ppm and 7.11 ppm, with coupling constants of 8.9 Hz, integrating both by approximately two, supported substitution in *para*. Hydrogens belonging to the methoxy group were identified as a singlet, at δ 3.92 ppm.

Figure 8 (one column and half): ^{1}H NMR spectrum of methedrone (product 10), methedrone chemical structure (upper-right insert) and its expansion of the aromatic region (upper-left insert). Methanol was set at 3.31 ppm (CD_3OD). Diethyl ether was also identified, 1.18 ppm (t) and 3.55 ppm (q), water (H_2O) appeared at 4.8 ppm.

Table 3 (one column): ^{1}H NMR data for methedrone.



Position	*δ (ppm)*	*Integration*	*Multiplicity*	*J(Hz)*
F	1.57	3.03	d	7.2
E	2.75	3.02	s	-
D	3.92	3.00	s	-
C	5.04	1.12	q	7.2
B	7.11	2.16	d	8.9
A	8.04	2.00	d	8.9

NMR revealed the presence of diethyl ether in all the samples analysed, δ 1.18 ppm (t) and δ 3.55 ppm (q). Diethyl ether is used as a solvent for the base compounds in a final step of the most syntheses proposed for cathinones, and to obtain the hydrochloric or hydrobromic salts of cathinones and amphetamines by dissolving or bubbling HCl gas on the diethyl ether [17–19], which are the most probable sources of diethyl ether contamination found in the products.

X-Ray Fluorescence

Wavelength dispersive X-Ray Fluorescence (WD-XRF) was used to obtain an inorganic chemical profile of products 1-8. Concentrations of light elements (C, H, O and N) are expressed as approximations referred to concentration of cellulose for universal calibration. Chlorine, bromine, sodium, sulphur, calcium, iron, copper, silicon, aluminium, ruthenium and molybdenum were identified in different quantities on the products.

Table 4 (two columns): Elemental composition of products 1-8, concentration is expressed in mg of element by grams of sample and error in %.

Product nº	*1*		*2*		*3*		*4*		*5*		*6*		*7*		*8*	
Weight	*0.833 g*		*0.892 g*		*0.92 g*		*0.83 g*		*0.852 g*		*0.875 g*		*0.94 g*		*0.883 g*	
	Conc (mg/g)	*Error %*	*Conc (mg/g)*	*Error %*	*Conc (mg/g)*	*Error %*	*Conc mg/g*	*Error %*	*Conc (mg/g)*	*Error %*	*Conc (mg/g)*	*Error %*	*Conc (mg/g)*	*Error %*	*Conc (mg/g)*	*Error %*
CHO	850	-	834	-	830	-	810	-	862	-	835	-	826	-	801	-
Cl	147.5	0.21	154.7	0.21	169	0.20	185.8	0.20	133.7	0.22	152.7	0.21	173.1	0.20	194.5	0.19
Br	2.618	0.24	10.36	0.12	0.62	0.60	3.24	0.22	3.783	0.19	11.24	0.11	0.565	0.64	3.195	0.23
Na	-	-	0.58	16.7	-	-	1.1	10.0	-	-	0.63	15.9	-	-	1.1	10.0
S	-	-	0.27	3.90	0.178	5.31	0.242	4.27	-	-	0.29	3.72	0.08	9.40	0.26	4.11
Ca	-	-	0.06	10.20	0.167	5.02	0.07	10.0	-	-	0.06	10.60	0.07	9.43	0.08	8.51
Fe	0.03	8.63	0.05	6.35	0.04	7.18	0.06	5.49	0.03	7.80	0.04	7.23	0.05	6.75	0.06	5.95
Cu	0.03	8.51	-	-	-	-	-	-	0.02	8.92	0.03	7.86	0.03	9.40	0.03	8.71
Si	-	-	-	-	0.18	9.37	-	.	-	-	-	-	0.19	8.66	-	-
Ru	-	-	-	-	0.03	18.1	-	.	-	-	-	-	-	-	-	-
Al	-	-	0.11	11.1	-	-	-	.	-	-	0.05	7.86	-	-	-	-
Mo	-	-	-	-	-	-	-	.	-	-	0.02	28.30	-	-	-	-

The high amounts of chlorine detected in the samples (13.4 % to 19.4 %) together with

the absence of any sing of covalent chlorine in the GC-MS and H^1 NMR analysis indicated that cathinones were in hydrochloric salt form. This chlorine was stoichiometrically in agreement with the quantity of cathinones estimated by the amount of carbon, hydrogen and oxygen detected, confirming high purity of cathinones present on the samples. Bromine was the second more abundant element detected in all the samples, but in a much lower level than chlorine, discarding the possibility of synthetic cathinones presented as hydrobromic salt. No manganese or chromium was detected in any of the samples analysed. Magnesium was advertised as ingredient in products number 1 and 6 in the form of magnesium stearate, however no traces of magnesium were found in any of the products.

In order to see if elemental composition obtained by XRF can be used to separate and classify samples containing synthetic cathinones, the data was treated with Tanagra multivariate analysis first in an unsupervised learning process to produce hierarchical clusters and later to produce Principal Component Analysis (Figure 9). Four PCs were created containing relevant information. When combinations of PC1, PC2 and PC3 were plotted, effective separation of the samples consistent with their name and active ingredients was achieved, suggesting the feasibility of creating a prediction model with PCA able to classify samples containing synthetic cathinones according to their origin. Data was also treated with The Unscrambler X version 10.3, which produced comparable results.

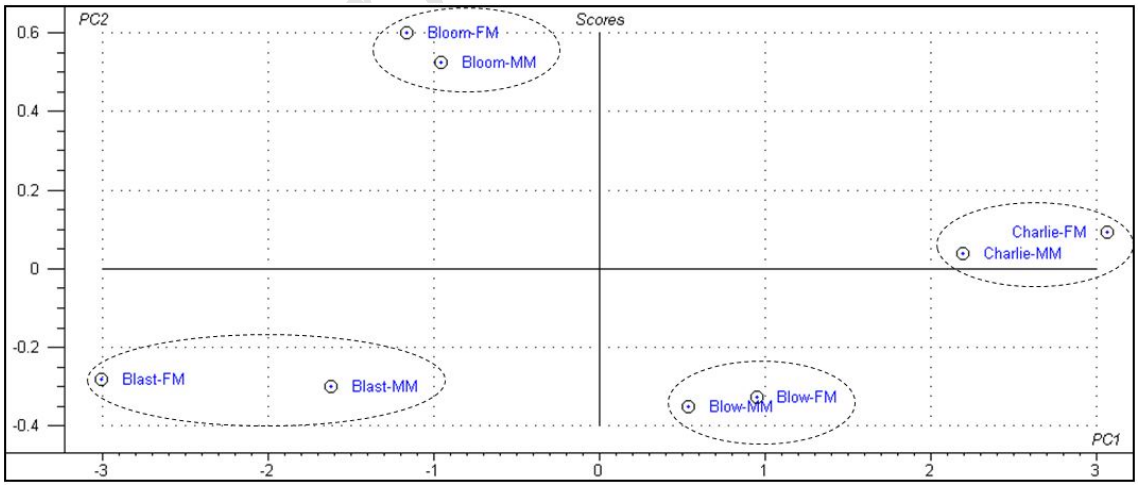


Figure 9 (one column): PCA scores plot for XRF data. Products with same name and same psychoactive ingredient purchased in different shops clustered together.

Unexpected change in colour was observed in different extent on the products after X-Ray irradiation. Products number 1 and 5 turned pink, whilst products 4 and 8 turned orangeish. The rest of the products lost their pure white colour. UV-Vis spectra of products 1-8 after X-Ray irradiation were compared with UV-Vis spectra from the original products, however no differences were found in the spectra taken before and after X-ray irradiation. No absorption bands were found in the visible region for products that change colour. This suggested a reversion in the colour when diluting the salt in distilled water.

FTIR and NMR did not reveal any additional information; the spectra were identical to the ones obtained for all the products in their previous states independently of their acquired colours, supporting the absence of chemical changes in products predicted by the non-destructive character of XRF as analytical technique. The change in colour observed remitted gradually in one week, until being just barely appreciable. However, the difference in colour between irradiated and non-irradiated products could still be noticed several weeks after exposure.

Conclusions

The products studied in this project contained active ingredients either as single component or in mixtures. All cathinones derivatives identified (4-FMC, EC, buphedrone, methedrone, pentedrone, 3,4-DMMC, 4-MEC) belong to the "classic cathinones group", analogues of amphetamines, being considered as the first generation of "legal highs" [20].

IR spectrometry can be used to identify the cathinone derivative present in "legal highs" samples which are highly pure and only contain one cathinone derivative.

As a result of the change in legislation it was not possible to purchase more products and evaluate their consistency on time. Although the changes on Portuguese legislation seem far from being able to solve the problem that "legal highs" represent to society. The list of banned substances by Portuguese law comprises 159 substances, however, as the EMCDDA has reported 199 new substances classified as "legal highs" a minimum of 40 substances remain yet in a "legal void", actually only few of the possible cathinone derivatives or isomers are listed, and new substances are continually appearing. Hence, new modifications in the Portuguese legislation will need to be taken

in future. Still a completed list of cathinone derivatives that can be synthetized seems unrealistic. Instead, more generic controls (whole family of cathinones) seem better approach.

The available data from New Zealand, United Kingdom (UK) or Germany shows that by banning these substances a decrease in their availability is reached, however, these actions have not been able to make them disappear, and although banned, they are often seized and appear to be summed up to the current illegal market of drugs [21,22]. On the basis of what has happened in other countries these products may remain in the Portuguese market even after being forbidden.

Identification of novel psychoactive substances is an arduous task for forensic laboratories due to the great number of substances present in the market in last years, very high prices or absence of standards, and subtle differences among these substances. None or few studies have been published evaluating human toxicity, potential for addiction, acute overdose or long-term effects. Thus, toxicological evaluation needs to be performed in order to elucidate relative potency among different derivatives, possible interactions with other drugs as well as neurotoxicity and behaviour toxicity of synthetic cathinones. In order to guarantee public health, these products should be monitored regularly, the new substances identified and the knowledge of the scientific community increased by producing reliable analytical data of these compounds.

Trace metals found in the products might be present in raw materials, reagents or solvents used, or they can be contamination from the equipment used during the synthesis, instead of being intendedly introduced. These trace metals can function as important chemical markers and may serve to distinguish between samples [23]. To the best of author knowledge this is the first time than inorganic profiles of samples containing synthetic cathinones are presented. Products showed different elemental compositions between them, but very similar for the products labelled with the same name (Table 3). Products nº 1 and 5 (Blast) and nº 2 and 6 (Bloom) purchased in different smart shops and also with different ingredients advertised clustered together suggesting a common origin. These chemicals are most probably synthetized in "underground" laboratories, following simple and inexpensive synthetic routes using easily obtainable starting materials, the chemicals synthetized will depend on the demand as well as the availability of precursor materials. Several synthetic pathways have been proposed for substituted cathinones [22–24], with the use of the corresponding propiophenone and bromine being the easiest and most probable. The

inorganic composition may provide information about synthetic pathways. It is difficult to know if bromide present comes from a synthesis using reactives containing bromo or from any other source of contamination. But chemicals obtained by different synthetic routes will show different inorganic profiles. If forensic laboratories have a large enough number of seize samples, the inorganic composition could help to link several seizures to the same producer and to classify seized samples.

References

[1] Decreto-Lei n°54/2013 Portaria 154/2013, 2254 3 -, https://dre.pt/pdf1sdip/2013/04/07500/0225402257.pdf. (2013) 2254–2257.

[2] EMCDDA, Synthetic cathinones, www.emcdda.europa.eu/publications/drug-profiles/synthetic-cathinones, (2012).

[3] M. Wikström, G. Thelander, I. Nyström, R. Kronstrand, Two Fatal Intoxications with the New Designer Drug Methedrone (4-Methoxymethcathinone) Autopsy cases, J. Anal. Toxicol. 34 (2010) 594–598.

[4] L.D. Simmler, a Rickli, M.C. Hoener, M.E. Liechti, Monoamine transporter and receptor interaction profiles of a new series of designer cathinones., Neuropharmacology. 79C (2013) 152–160. doi:10.1016/j.neuropharm.2013.11.008.

[5] S.S. Johansen, a C. Hansen, I.B. Müller, J.B. Lundemose, M.-B. Franzmann, Three fatal cases of PMA and PMMA poisoning in Denmark., J. Anal. Toxicol. 27 (2003) 253–256. doi:NO_DOI.

[6] SWGDRUG. Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG) SWGDRUG MS Library Version 1.8 (Visited 09.04. 2013). Available at: http://www.swgdrug.org/ms.htm, (n.d.).

[7] Cayman Chemical. Cayman Spectral Library V 05162013. (Visited 16.05.2013) at https://www.caymanchem.com/app/template/SpectralLibrary.vm, (2013).

[8] M. Baron, M. Elie, L. Elie, An analysis of legal highs: do they contain what it says on the tin?, Drug Test. Anal. 3 (2011) 576–81. doi:10.1002/dta.274.

[9] J. Hillebrand, D. Olszewski, R. Sedefov, Legal highs on the Internet., Subst. Use Misuse. 45 (2010) 330–40. doi:10.3109/10826080903443628.

[10] R.P. Archer, Fluoromethcathinone, a new substance of abuse., Forensic Sci. Int. 185 (2009) 10–20. doi:10.1016/j.forsciint.2008.11.013.

[11] P. Jankovics, A. Váradi, L. Tölgyesi, S. Lohner, J. Németh-Palotás, H. Koszegi-Szalai, Identification and characterization of the new designer drug 4'-

methylethcathinone (4-MEC) and elaboration of a novel liquid chromatography-tandem mass spectrometry (LC-MS/MS) screening method for seven different methcathinone analogs., Forensic Sci. Int. 210 (2011) 213–20. doi:10.1016/j.forsciint.2011.03.019.

[12] Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG) Monographs., Http://www.swgdrug.org/monographs.htm. (n.d.).

[13] J.F. Casale, P.A. Hays, Differentiation of 3,4-Dimethylmethcathinone (3,4-DMMC) from its Dimethyl Aryl-Positional Isomers, Microgram J. 9 (n.d.) 75–83.

[14] W.G. and R.U. Viorica Lopez-Avila*, A. Technologies, S. Clara, Mass spectral fragmentation of cathinones by high-resolution TOFMS using a soft ionization source, J. Pharm. Sci. Innov. Re Search Artic. 1 (2012) 44–53.

[15] D. Zuba, Identification of cathinones and other active components of "legal highs" by mass spectrometric methods, TrAC Trends Anal. Chem. 32 (2012) 15–30. doi:10.1016/j.trac.2011.09.009.

[16] C.R. Maheux, C.R. Copeland, Chemical analysis of two new designer drugs: buphedrone and pentedrone., Drug Test. Anal. 4 (2012) 17–23. doi:10.1002/dta.385.

[17] L. Aalberg, K. Andersson, C. Bertler, M.D. Cole, Y. Finnon, H. Huizer, et al., Development of a harmonized method for the profiling of amphetamines. II. Stability of impurities in organic solvents., Forensic Sci. Int. 149 (2005) 231–241. doi:10.1016/j.forsciint.2004.06.019.

[18] O.I.G. Khreit, M.H. Grant, T. Zhang, C. Henderson, D.G. Watson, O.B. Sutcliffe, Elucidation of the Phase I and Phase II metabolic pathways of (±)-4'-methylmethcathinone (4-MMC) and (±)-4'-(trifluoromethyl)methcathinone (4-TFMMC) in rat liver hepatocytes using LC-MS and LC-MS^2., J. Pharm. Biomed. Anal. 72 (2013) 177–85. doi:10.1016/j.jpba.2012.08.015.

[19] J.D. Power, P. McGlynn, K. Clarke, S.D. McDermott, P. Kavanagh, J. O'Brien, The analysis of substituted cathinones. Part 1: chemical analysis of 2-, 3- and 4-methylmethcathinone., Forensic Sci. Int. 212 (2011) 6–12. doi:10.1016/j.forsciint.2011.04.020.

[20] D. Zuba, B. Byrska, Prevalence and co-existence of active components of "legal highs," Drug Test. Anal. DOI 10.1002/dta.1365. (2012) available online at: wileyonlinelibrary.com/journa. doi:10.1002/dta.1365.

[21] 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. doi:10.1002/dta.155.

[22] G.S. Winder, N. Stern, A. Hosanagar, Are “Bath Salts” the next generation of stimulant abuse?, J. Subst. Abuse Treat. (2012) doi:10.1016/j.jsat.2012.02.003. doi:10.1016/j.jsat.2012.02.003.

[23] R.S. Ortiz, K.C. Mariotti, N. V Schwab, G.P. Sabin, W.F.C. Rocha, E.V.R. de Castro, et al., Fingerprinting of sildenafil citrate and tadalafil tablets in pharmaceutical formulations via X-ray fluorescence (XRF) spectrometry., J. Pharm. Biomed. Anal. 58 (2012) 7–11. doi:10.1016/j.jpba.2011.09.005.

[24] M. Osorio-olivares, C. Rezende, S. Sepu, B.K. Cassels, R.F. Baggio, J.C. Mun, A two-step method for the preparation of homochiral cathinones, 14 (2003) 1473–1477.

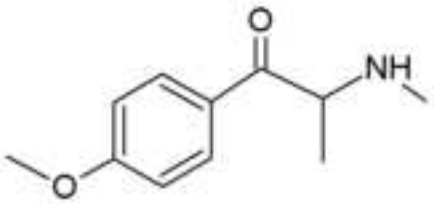
[25] H. Belhadj-Tahar, N. Sadeg, Methcathinone: a new postindustrial drug., Forensic Sci. Int. 153 (2005) 99–101. doi:10.1016/j.forsciint.2005.04.023.

[26] R.P. Archer, K. Williams, J. Wiley, Reference materials for new psychoactive substances, (2011) 505–514. doi:10.1002/dta.317.

Highlights:

- We produced a snapshot of the availability of "legal highs" in Portugal.
- Analytical data of the novel psychoactive substances identified are presented.
- Methedrone, a special case of toxicity of cathinones, was found in three samples.
- Elemental composition of samples was obtained by XRF.
- Inorganic profiles can be used to identify common origin of samples.

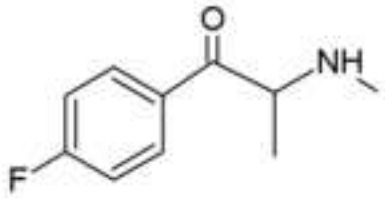
Figure 1



Methedrone

Molecular Formula: $C_{11}H_{15}NO_2$

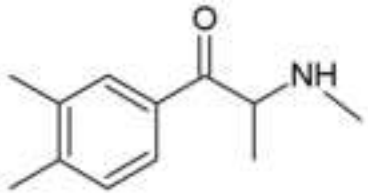
Molecular Weight: 193.2423 g/mol



4-Fluoromethcathinone (4-FMC)

Molecular Formula: $C_{10}H_{12}FNO$

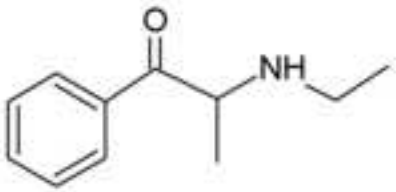
Molecular Weight: 181.206 g/mol



3,4-Dimethylmethcathinone (3,4-DMMC)

Molecular Formula: $C_{12}H_{17}NO$

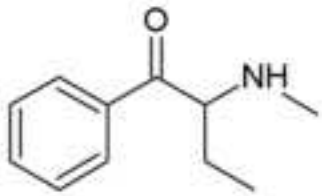
Molecular Weight: 191.2694 g/mol



Ethcathinone

Molecular Formula: $C_{11}H_{15}NO$

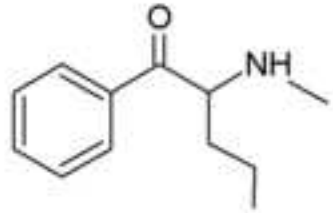
Molecular Weight: 177.2429 g/mol



Buphedrone

Molecular Formula: $C_{11}H_{15}NO$

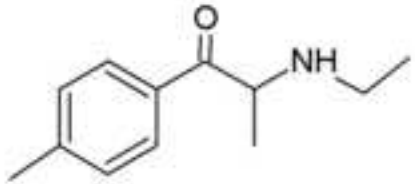
Molecular Weight: 177.2429 g/mol



Pentedrone

Molecular Formula: $C_{12}H_{17}NO$

Molecular Weight: 191.2695 g/mol



4-Methylethcathinone (4-MEC)

Molecular Formula: $C_{12}H_{17}NO$

Molecular Weight: 191.2694 g/mol

Figure 2

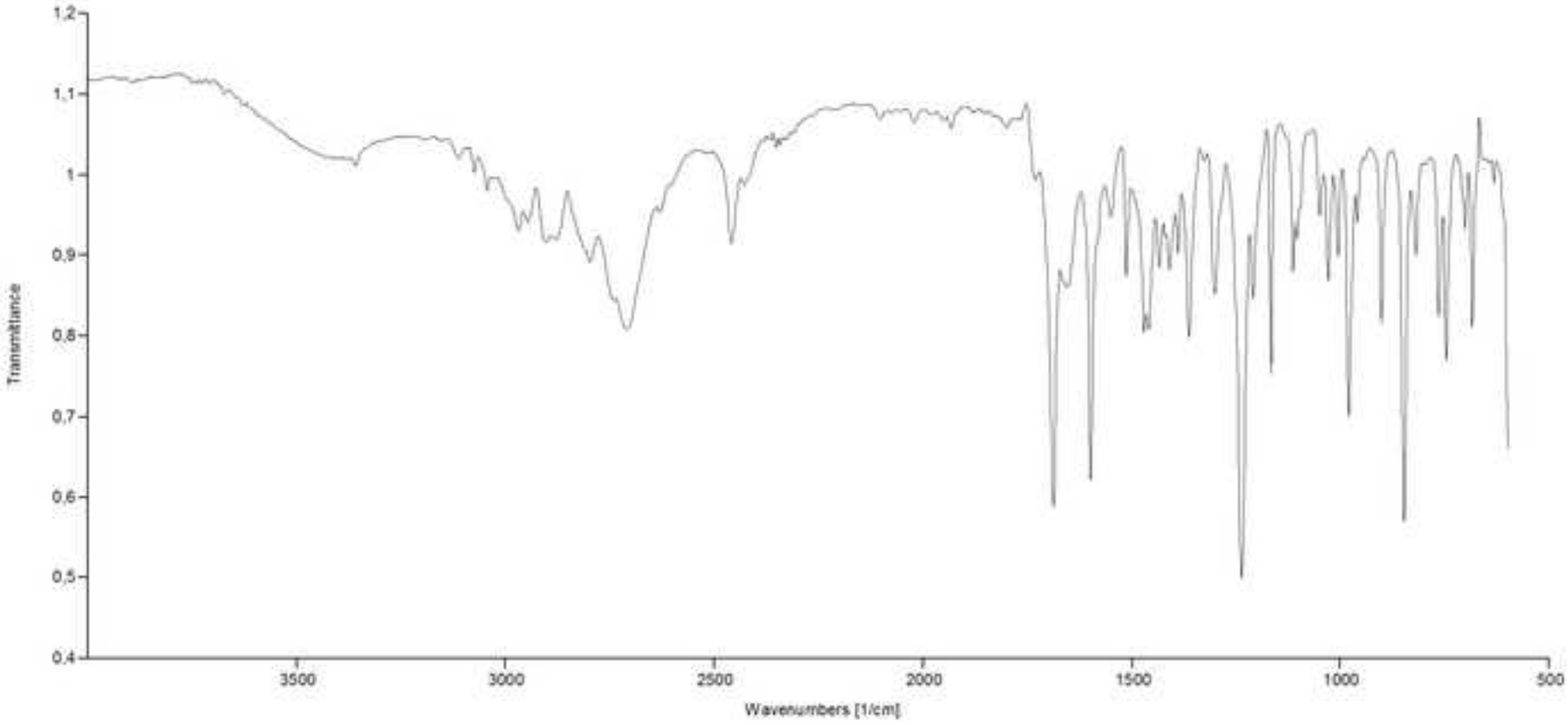

1,2
1,1
1
0,9
0,8
0,7
0,6
0,5
0,4
Transmittance
3500
3000
2500
2000
1500
1000
500
Wavenumbers [1/cm]

Figure 3

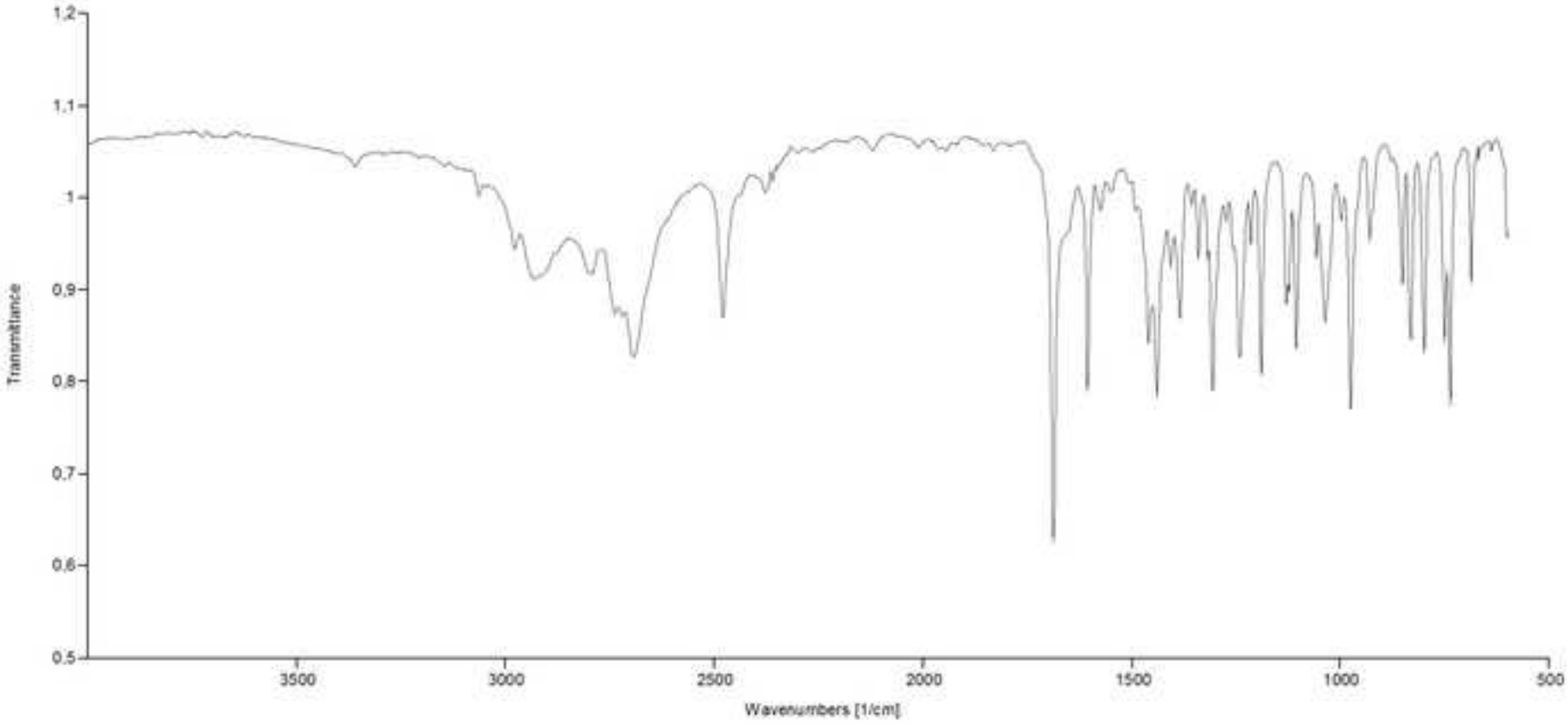

1,2
1,1
1
0,9
0,8
0,7
0,6
0,5
Transmittance
3500
3000
2500
2000
1500
1000
500
Wavenumbers [1/cm]

Figure 4

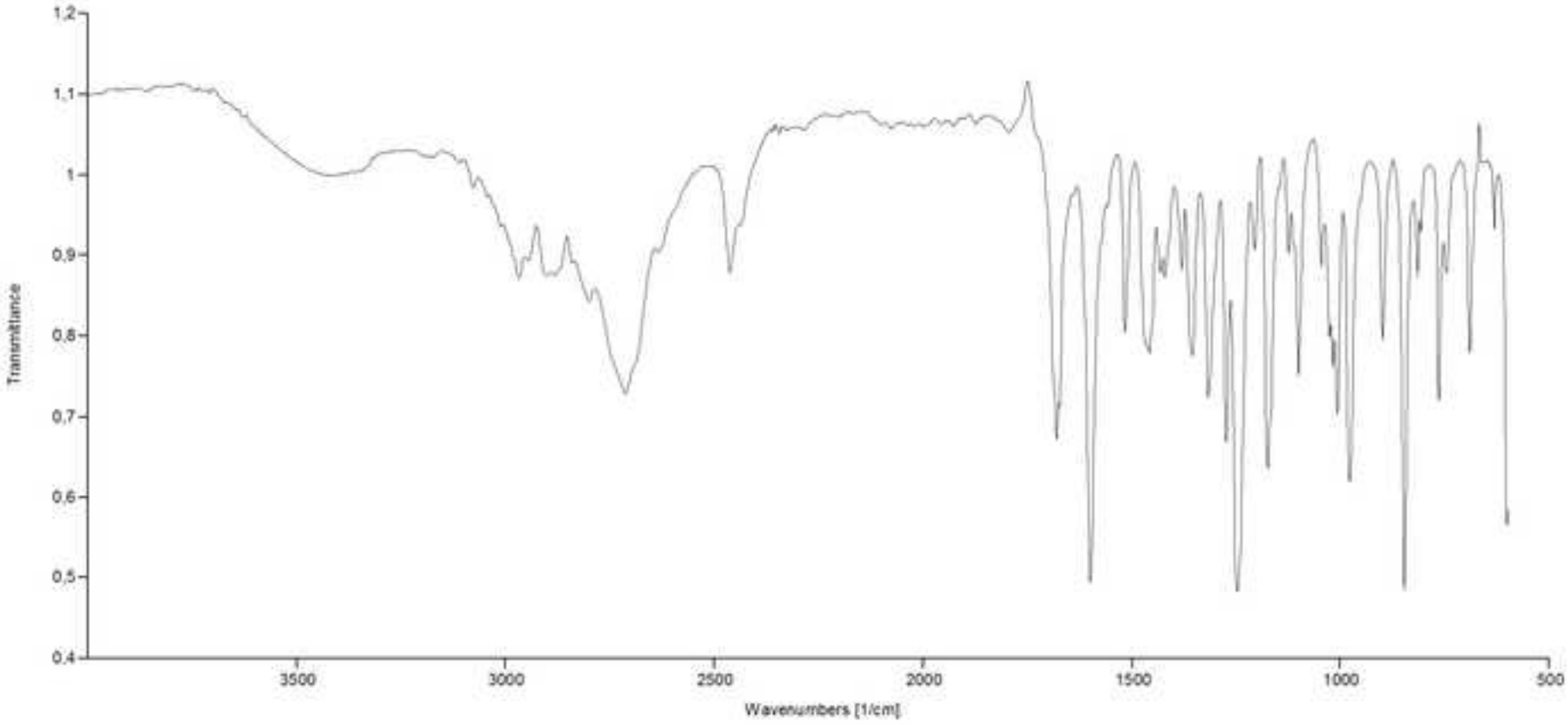

1,2
1,1
1
0,9
0,8
0,7
0,6
0,5
0,4
Transmittance
3500
3000
2500
2000
1500
1000
500
Wavenumbers [1/cm]

Figure 5

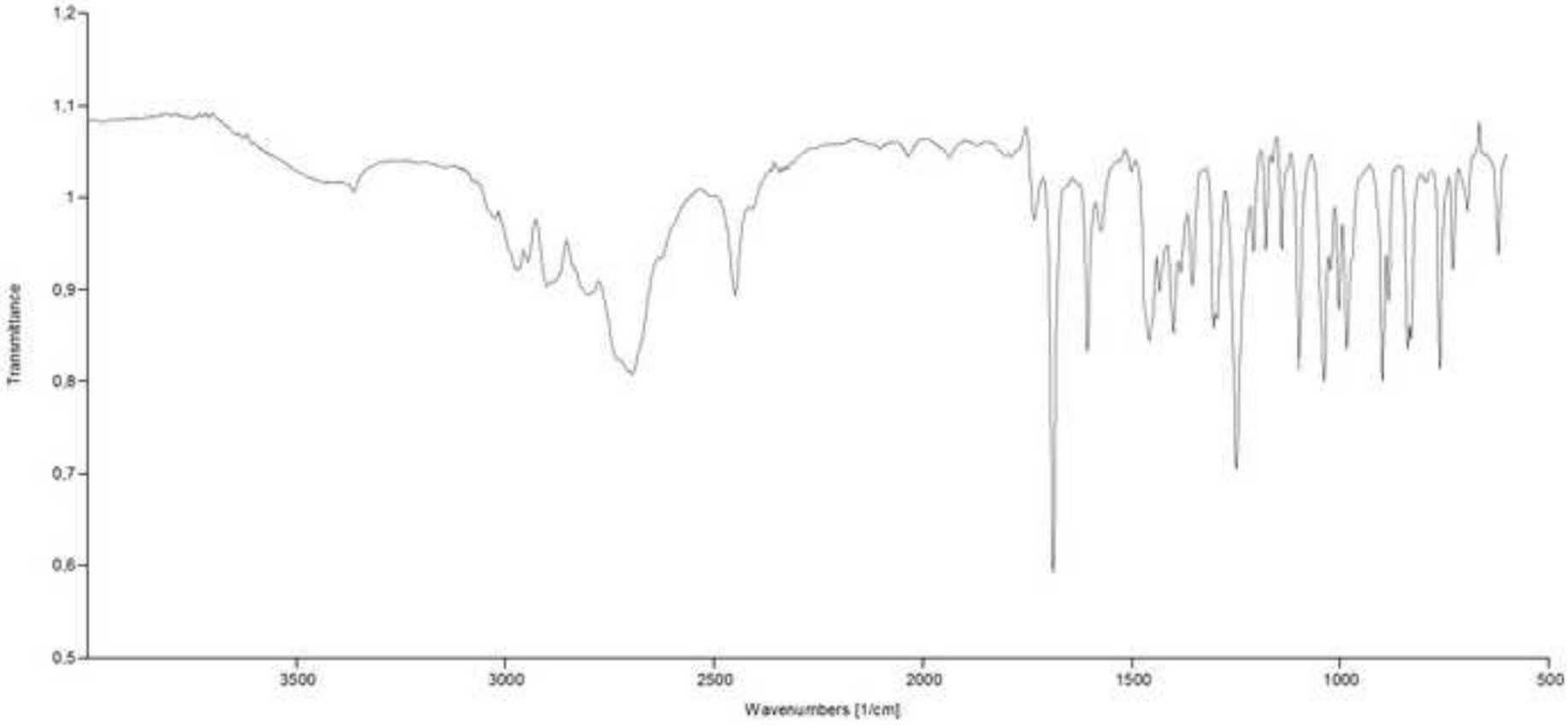

1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
Transmittance
3500
3000
2500
2000
1500
1000
500
Wavenumbers [1/cm]

Figure 6

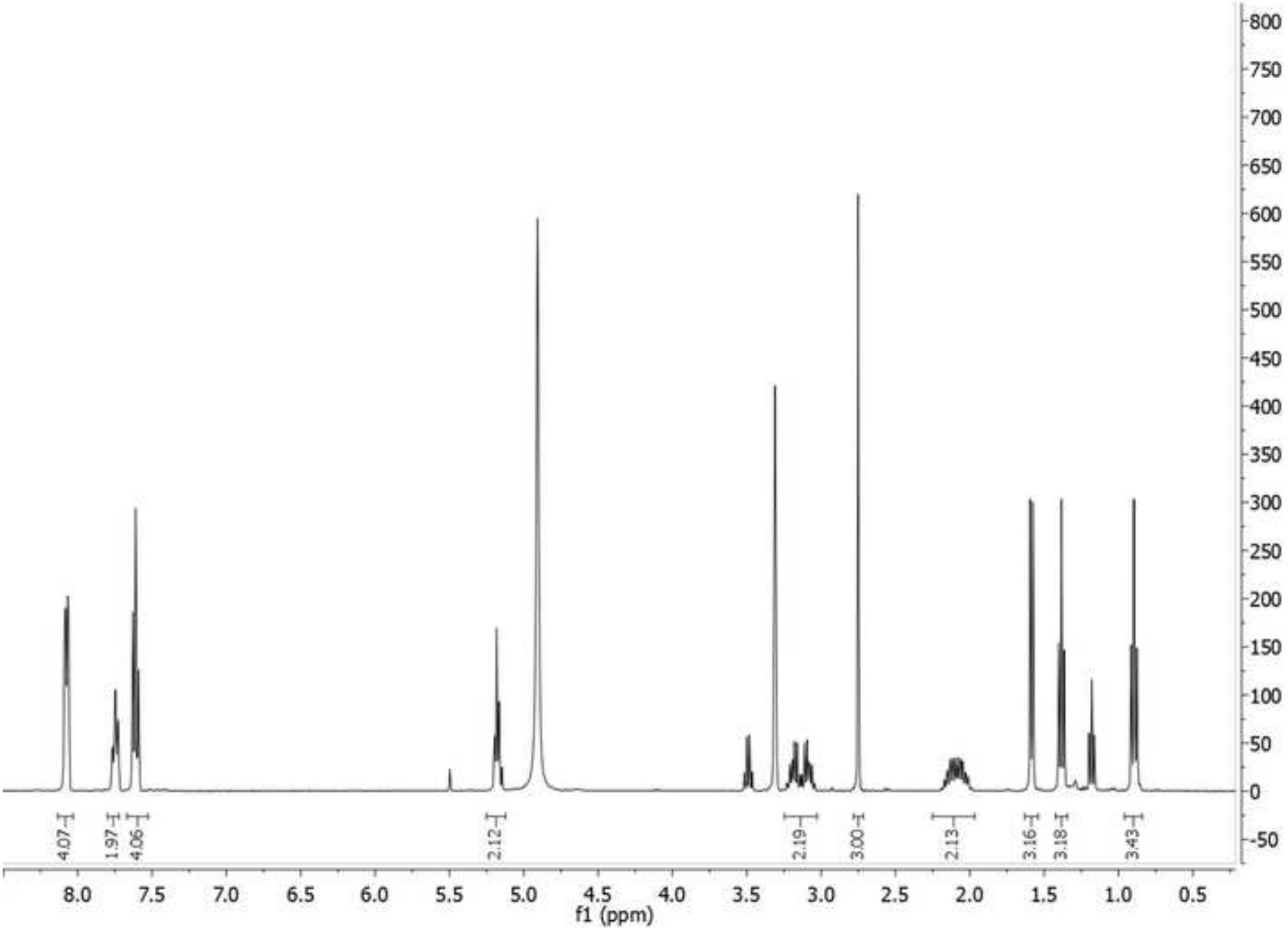

4.07
1.97
4.06
2.12
2.19
3.00
2.13
3.16
3.18
3.43
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f1 (ppm)
800
750
700
650
600
550
500
450
400
350
300
250
200
150
100
50
0
-50

Figure 7

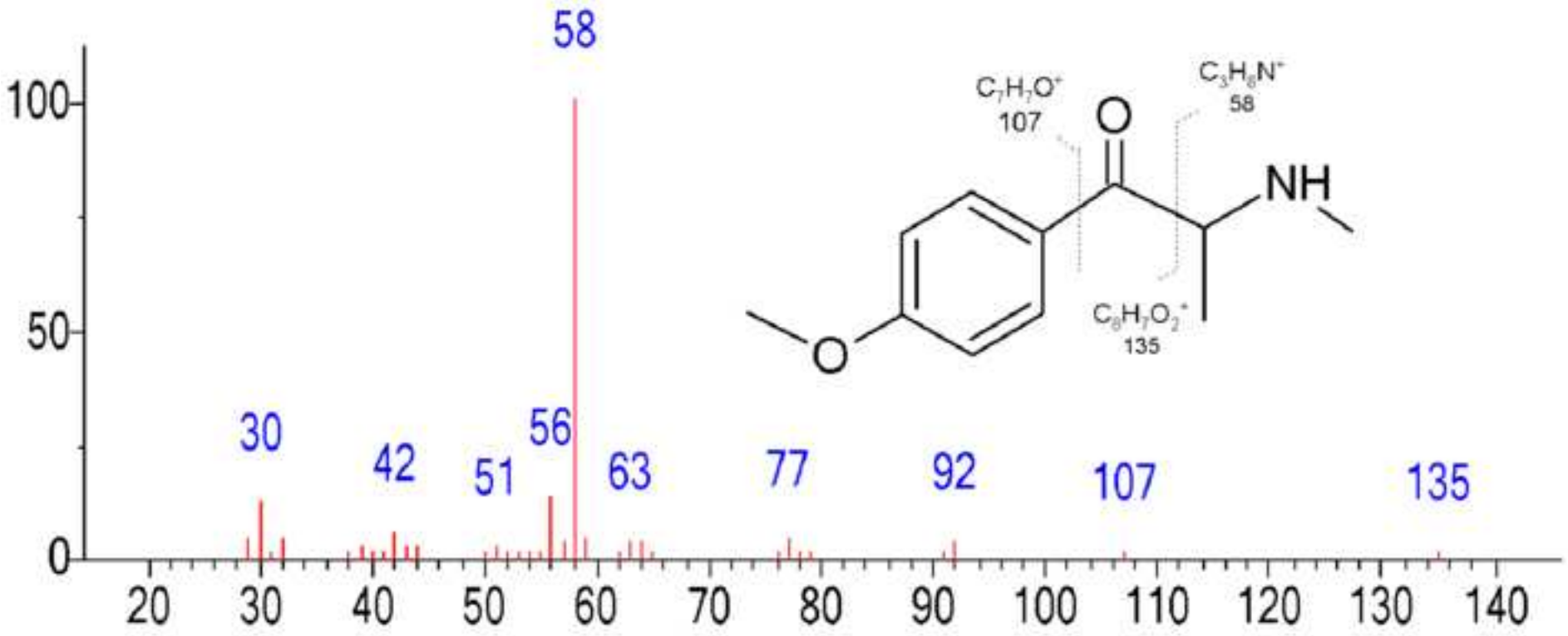

58
100
50
0
30
42
51
56
63
77
92
107
135
20
30
40
50
60
70
80
90
100
110
120
130
140
C7H7O+
107
C3H8N+
58
C8H7O2+
135
O
NH
O

Figure 8

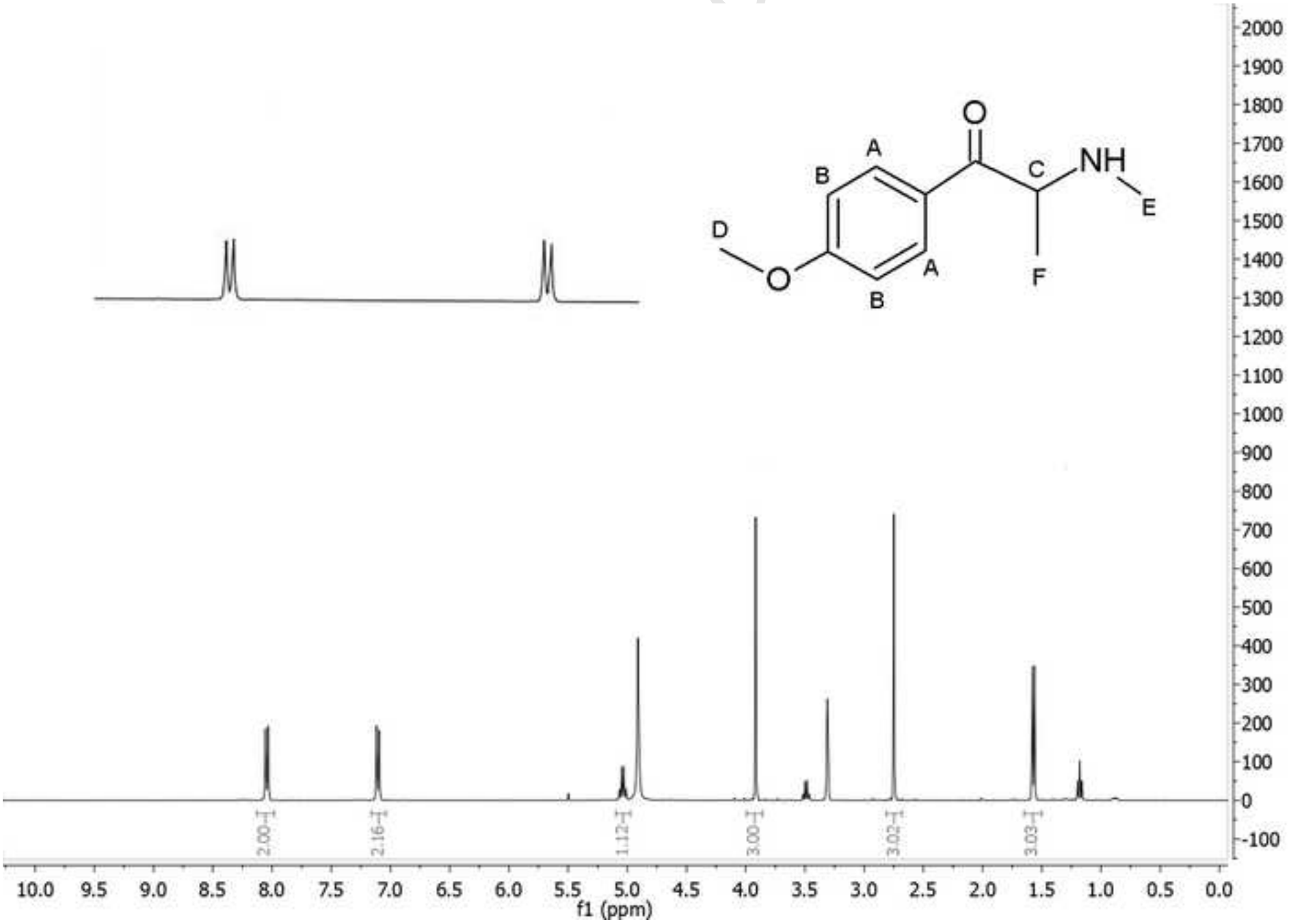
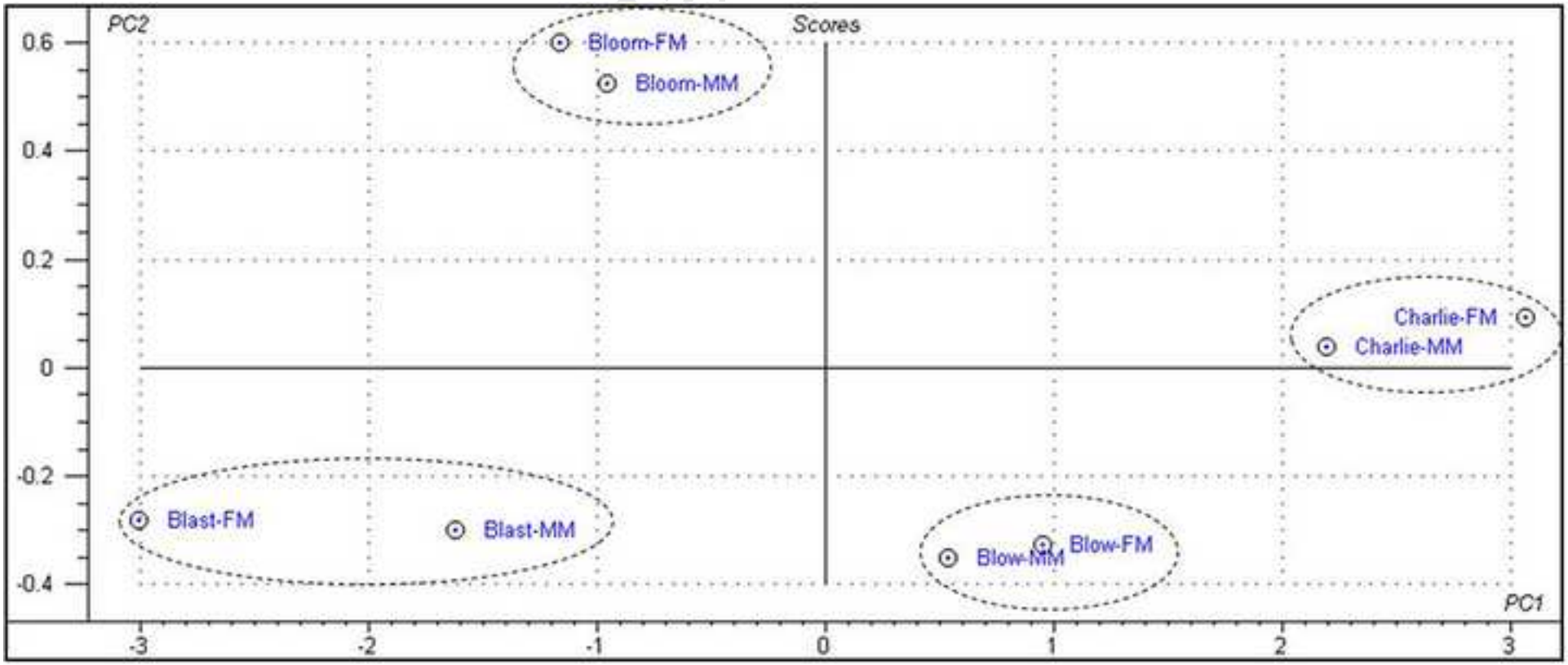

O
A
B
C
NH
E
D
O
A
B
F
2000
1900
1800
1700
1600
1500
1400
1300
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
-100
2.00
2.16
1.12
3.00
3.02
3.03
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

Figure 9


PC2
Scores
Bloom-FM
Bloom-MM
Charlie-FM
Charlie-MM
Blast-FM
Blast-MM
Blow-MM
Blow-FM
PC1
0.6
0.4
0.2
0
-0.2
-0.4
-3
-2
-1
0
1
2
3