# Analysis Using Gas Chromatography - Mass Spectrometry (GC-MS) of Some Ethnobotanical Products

**MIHAELA CORLADE-ANDREI[1], CARMEN DIANA CIMPOESU[1], DAN DRAGOS[2]*, MIRELA NICOLOV,[2] ELENA BUTNARU[1]**
[1] "Grigore T. Popa" University of Medicine and Pharmacy, 16 Universitatii Str., 700115, Iasi, Romania
[2] "Victor Babes" University of Medicine and Pharmacy,Faculty of Farmacy, 2 Eftimie Murgu Sq., Timisoara, Romania

*Romania ranks fourth in the world for the consumption of ethnobotanical products, behind England, Germany and the Netherlands, although in 2008, our country was not in the ranking. The study intends to identify the substances contained in ethnobotanical products. This was possible using gas chromatography-mass spectrometry. The results revealed the presence of psychoactive substances in some ethno botanical products. The presence of these substances explains the symptoms of the ethnobotanical product poisoned patients who come to the emergency department.*

*Keywords: drugs, ethnobotanical products, gas chromatography, psychoactive substances*

In Romania, the first cases of ethnobotanical product poisoning appeared in 2009.

According to the reports of the Emergency Departments in the whole country, the consumption of products with psychoactive effect had an ascending trend. If in 2009 the number of reported emergencies was of 999, in 2012 their number increased up to 5373 [12].

Romania ranks fourth in the world for the consumption of ethnobotanical products (figure 1), behind England, Germany and the Netherlands although in 2008, our country was not in the ranking.

Along with Bulgaria, Estonia and Latvia, Romania is one of the countries where people have very little knowledge on and are the least informed about the effects determined by drug consumption. This fact determines that curiosity and 'team spirit' come first among the young people.

The patients' treatment diagnosed with acute poisoning induced by ethnobotanical product consumption represents a challenge for the doctors working in the Emergency Department, their attention being focused on the problems which may endanger the vital functions.

The lack of information about the chemical composition, the source of the possibly dangerous compounds and substances used to produce the drugs make it difficult to assess the risks for health and the toxicity level. Some ethnobotanical products are consumed combined with alcohol and other illegal drugs, which cause even more dangerous side effects.

The uncertainty regarding the identity and purity of the drug used in ethnobotanical products represents their main danger, the diagnosis and treatment of these patients being extremely difficult.

## Experimental part

*Material and method*

Reagents and samples: ethnobotanical products (Flower Magic, Pure by Magic, Grass supreme potpourri, Elixir Moonwalk, Caramelo de Coca, Hash lollipop), spectral library, bi-distilled water, methyl alcohol.

The samples were identified by means of a population study conducted in Iasi City, on 25 June 2011, for the International Day against Drug Abuse and Illicit Trafficking [3].

There were examined six ethnobotanical products: Flower Magic and Pure by Magic (white powder sold as plant fertilizer or bath salts, consumed by inhalation), Grass supreme potpourri (vegetable fragments used to make cigarettes), Elixir Moonwalk (an umber-brownish fluid and strong alcohol smell, consumed as such or combined with juice and/or alcohol), Caramelo de Coca and Hash lollipop (green or brown candy, containing, as it is written on the packaging, coca extract and hashish).

They are considered to be the most known and implicitly the most consumed ethnobotanical products [3].

To analyse the substances contained in the ethnobotanical products identified by the population study we chose to use the gas chromatography-mass spectrometry.

The methanol extracts of the samples were analysed using this technique by means of the FOCUS GC equipment (Thermo Electron Corporation), having a 30 m long capillary column TR-5MS and with the inner diameter of 0.25 mm. As carrier gas we used helium and the flow rate was of 1 mL/min.

The working conditions to examine the fluids: column temperature = 100°C (2 min)- 300°C (10 min); growth rate of the air oven temperature = 30°C/min; injector temperature = 290°C; transfer temperature = 300°C; scanning field = 40 – 400 u (unified atomic mass unit), scanning time: 0.74 s;

Conditions for powder analysis (Pure by Magic, Magic Power): temperature programme of the air oven = 50°C lasts 1 min; 15°C/min. up to 280°C, lasts 6.33 min; 10° C/ min at 300°C, lasts 10.34 min ; injector temperature = 250°C ; transfer temperature = 280°C; scanning field = 40 – 500 u, scanning time: 0.94 s.

## Results and discussions

The signal generated in the detector is visualized as a chromatogram peak and depending on the calibration curve and the area of the peak obtained from the analysis of the sample we determined the substance of the respective sample.

Thus, of all analysed products, only two were identified as substances with psychoactive properties.

In the composition of the product Pure by magic there were identified two pyrovalerones (MDPV and MDPBP) (fig. 2).

* *email: dan.dragos@umft.ro*

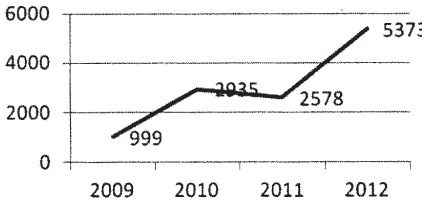


Fig 1. Medical emergencies due to the consumption of psychoactive substances 2009-2012

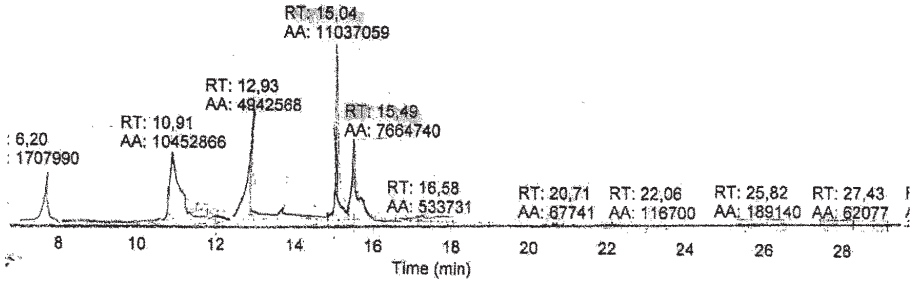


Fig. 2. Chromatogram of the product Pure by Magic

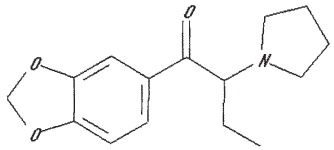

Fig.3. Chemical structure of methylenedioxy-pyrovalerone (MDPV)

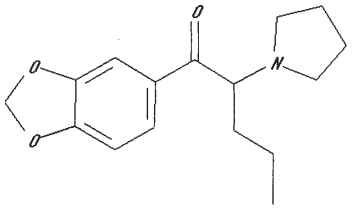

Fig.4. Chemical structure of 3',4'- methylenedioxy-αpyrrolidinebutyro-phenone (MDPBP)

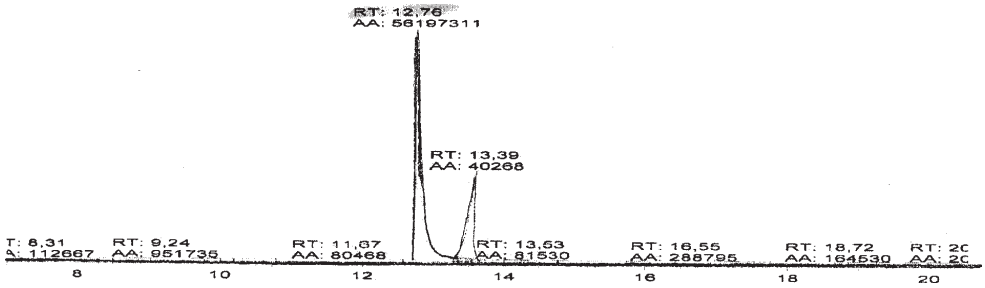


Fig. 5. Chromatogram of the product Grass supreme potpourri

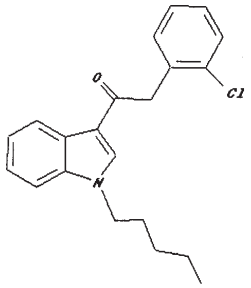

Fig. 6. Chemical structure of 1-penthyl-3-(2-chlorophenyl) indol (JWH-203)

Methylenedioxypyrovalerone (MDPV) and 1-(3,4-methylenedioxyphenyl)-2-(pyrrolidine-1-butyrophenone (MDPBP) are psychoactive drugs with stimulating properties, which act as norepinephrine- dopamine reuptake inhibitors (NDRI ) (fig. 3,4).

The consumption of these pyrovalerones affects CYP450, CYP2D6, 2C19 and also determines the COMT 1 phase of the hepatic metabolism, turning into methylcatechol and pyrrolidine, which also turn into uridine 5'-diphosho-glucuronosyltransferase, which may allow them to be excreted by the kidneys. Only a small part of the metabolites is excreted in the faeces. At the molecule level, this can be seen as the demethylation of the methylenedioxypyrovalerone (CYP2D6), followed by the methylation of the aromatic ring, by means of the catechol-O-methyltransferase. Then the aromatic ring methylation and the side chain take place, followed by the oxidization of the pyrimidine ring at the proper lactam. Then the aromatic ring detaches and opens at the adequate carboxylic acid [6,8].

The effects due to the consumption of MDPV are similar to the ones of cocaine, amphetamine and methyl-phenidate. The ways of consumption include oral consumption, inhaler, smoking, intrarectal and intravenous administration. This is supposed to be active at 3-5 mg, with typical doses ranging from 5 to 20 mg.

The effects expected by the consumers are: euphoria, increased sociability, sexual stimulation /aphrodisiac effects, diminished perception of the need to eat and sleep.

The acute effects may include: extreme anxiety/ agitation, sometimes it progresses to violent behaviour, insomnia, psychotic illusions, confusion, tachycardia, arterial hypertension, precordial pain, breathing difficulty, tachypnea, coughing, hyperthermia, shivers, sweat, bruxism, nausea, abdominal pain and transit disorders.

The psychic symptoms may persist for a long period of time. The physical symptoms may progress to rhabdomyolysis, renal and hepatic failure. The primary psychological effects last for 3 to 4 h approximately.

The presence of 1-penthyl-3-(2-chlorophenyl) indol (JWH-203) was identified in the product Grass supreme potpourri (fig. 5).

JWH-203 is one of the most complex types of synthetic Cannabis, agonist both for CB1 and for CB2, having an almost equal affinity, its effects on the body and brain being more intense than the ones of THC, which is just a partial agonist of Cannabis. (fig. 6)

Although synthetic Cannabis was first used for therapeutic purposes, JWH018 alters the mentality of an addict and may cause extreme anxiety, panic attacks, and convulsions. This is due to the fact it affects the GABA neurotransmitters. [5]

The effects caused by JWH018 consumption include: anxiety, panic attacks, hyperreactivity alternating with lethargy, excessive thirst, serious memory loss and dementia, severe disorientation, tachycardia, palpitations, loss of appetite, weight loss, injected eyes and eye lid drooping.

In the other analysed products there were not identified any psychoactive substances, although, in some cases, they were mentioned as ingredients (cocaine in the composition of the product Caramelo de Coca and hashish in the composition of the product Hash Lollipop).

## Conclusions

The consumption of ethnobotanical products represents a complex issue and it is not one of the problems indulges simple conclusions. However, we may draw a clear conclusion: ethnobotanical products considered as not being 'risky', contain dangerous substances, with noxious effects, which may even lead to death [13].

The ethnobotanical products are produced in clandestine laboratories and its strength may vary a lot.

The products composition is not exact, accurate, being changed in order to remove the product from the list of forbidden drugs.

The identification of these products in the composition of the ethnobotanical products, using gas chromatography-mass spectrometry, explains the clinical and paraclinical manifestations of the patients who need medical assistance in the Emergency Department of 'Sfântul Spiridon' Emergency Clinical Hospital of Iasi.

The phenomenon of expansion to the national level of the ethnobotanical substance consumption makes it necessary to know the composition of these products in order to be able to administer a targeted treatment to the patient, not just a symptomatic one, based on the anamnesis and on the clinical examination.

## References


1. BACONI D., BĂLĂLĂU D., ABRAHAM P. Abuzul şi toxicodependenţa: mecanisme, manifestări, tratament, legislaţie, Editura Medicală, Bucureşti, 2008.
2. BACONI D., BĂLĂLĂU C. Toxicologia substanţelor de abuz, Editura Universitară „Carol Davila", Bucureşti, 2013.
3. CORLADE-ANDREI M., CIMPOEŞU D., BUTNARU E. Aspecte privind consumul de produse etnobotanice - Rev. Medico-Chirurgicală, Iaşi-2011; 115(4): p1069-1072
4. COSMAN D., COMAN H. Management şi tratament în toxicomanii, Editura Medicală Universitară "Iuliu Haţieganu", Cluj-Napoca, 2005.
5. HUFFMAN J.W., SZKLENNIK P.V., ALMOND A., et all, 1-Pentyl-3-phenylacetylindoles, a new class of cannabimimetic indoles, Bioorg. Med. Chem. Lett. 15, 2005.
6. MEYER M., PENG D., SCHUSTER F., MAURER H., Studies on the metabolism of the α-pyrrolidinophenone designer drug methylenedioxy-pyrovalerone (MDPV) in rat and human urine and human liver microsomes using GC–MS and LC–high-resolution MS and its detectability in urine by GC–MS, Journal of Mass Spectrometry, 2010.
7. OISTEANU A. Narcotice in cultura romană. Istorie, religie şi literatura, Editura Polirom, Bucureşti, 2011
8. STRANO-ROSSI S., CADWALLADER A.B., TORRE X., BOTRE F., Toxicological determination and in vitro metabolism of the designer drug methylenedioxypyrovalerone (MDPV) by gas chromatography/mass spectrometry and liquid chromatography/quadrupole time-of-flight mass spectrometry. Rapid Commun Mass Spectrom., 2010.
9. WELLS D., Dead men had used bath salts, Warren Times, 2011.
10. WHITE SR, EITZEN EM JR, KLEIN KR. Toxicology of hazardous chemicals ed. New York, NY, 2004.
11. *** http://www.chromatography-online.org.
12. *** http://www.ana.gov.ro.
13. WYMAN JF, LAVINS ES, ENGELHART D, ARMSTRONG EJ, SNELL KD, BOGGS PD, TAYLOR SM, NORRIS RN, MILLER FP. Postmortem tissue distribution of MDPV following lethal intoxication by „bath salts" J Anal Toxicol. 2013 Apr; 37(3):182-5


Manuscript received: 28.07.2014