UNIVERSITY OF MEDICINE AND PHARMACY
„CAROL DAVILA", BUCHAREST
DOCTORAL SCHOOL

PHARMACY FIELD

Research on the characterization of drug users and analytical diagnosis of consumption

PH.D. THESIS SUMMARY

Ph.D. supervisor:
UNIV. PROF. DR. BACONI DANIELA LUIZA

Ph.D. Student:
CIUCĂ (ANGHEL) DANIELA-MĂDĂLINA

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Introduction

1. The motivation for choosing the theme

The new psychoactive substances (NPS), with the most well-known classes being synthetic cannabinoids and synthetic cathinone, are widely known by the general public as "legal highs" and "ethnobotanical drugs". It has been observed that synthetic cannabinoids (molecules that mimic the effects of cannabis) and synthetic cathinone (molecules that mimic the effects of amphetamines) develop polymorphic symptomatology among drug users, making it difficult to define a toxidrome.

Therefore, this doctoral thesis aims to respond to the need for an easy diagnosis of new psychoactive substance use, given its expanding nature caused by the emergence of new molecules belonging to the aforementioned classes.

The research results have highlighted a concerning general population exposure to drug use. The most frequent adverse reactions have been found to be in the psychological sphere, with the study emphasizing possible correlations between various manifestations (hallucinations, impaired thinking, etc.) and specific categories of abused substances. The extraction and analysis method using gas-chromatography coupled with mass spectrometry (GC-MS) technique allowed the detection of substances of interest as well as methadone and its metabolite EDDP (2-ethylidene-1,5-3,3-diphenylpyrrolidine).

2. Hypotheses

One of the hypotheses addressed in the research is the detection and possible quantitative determination of drugs and their metabolites in biological samples, to provide specialists (doctors, health authorities, and law enforcement representatives) with an objective means of diagnosing drug use. In addition to analytical methods, the research in this thesis aims to highlight specific characteristics of NPS consumption that can also contribute to rapid diagnosis.

To achieve these objectives, several research methods have been employed. Initially, an analysis of the current drug use situation and the general population's exposure to drugs was conducted through an international survey-based research, followed by targeted investigation into defining aspects for diagnosing NPS consumption. These aspects include identifying the most frequent adverse reactions and their correlation with specific classes of abused substances (based on survey research and interviews), identifying hematological, biochemical, cardiological,

psychiatric, and psychological parameters that change in the context of different drug use (based on a retrospective study conducted on drug users enrolled in treatment programs), as well as analyzing individual personality traits that predispose individuals to drug use, acting as triggers for addictive behavior (based on interviews).

In order to detect and quantitatively determine drugs and their metabolites in biological samples, the development of an optimal extraction method for NPS from biological samples was considered. Additionally, a successfully validated GC-MS method was applied to determine methadone and its main metabolite, EDDP, in biological samples to assess compliance with methadone treatment among the study participants. Furthermore, in the analytical study, the selection of the optimal type of biological sample for methadone dosage during substitution treatment was pursued.

I. Current state of knowledge

1. General considerations regarding the use of substances of abuse

Drug consumption remains high across all member countries of the European Union, each having its own consumption characteristics (EMCDDA 2022, p. 8), with the same upward trend in drug consumption also observed in Romania (ANA 2021, p. 23). "The most commonly used drug at the European level" is cannabis (EMCDDA 2022, p. 8), while at the national level, the first place is occupied by new psychoactive substances (ANA 2021, p. 50). It is also concerning that reports of consumption of cannabis adulterated with synthetic cannabinoids have increased. These substances mimic the effects of tetrahydrocannabinol (THC), and although their toxicity is not fully understood, it should be taken into consideration (EMCDDA 2022, p. 12). New psychoactive substances have a dynamic evolution, with the European Monitoring Centre for Drugs and Drug Addiction monitoring 880 new molecules with psychoactive properties by the end of 2021, of which 52 were reported for the first time in Europe (EMCDDA 2022, p. 38).

2. Main substances of abuse

In the attempt to combat drug abuse and addiction, research in recent decades has focused on understanding the mechanism of action of substances with high abuse potential. Taking into account the action of the most common types of drugs on systems and receptors, common points can be identified between drug classes as follows:

- within the ***opioidergic*** system act heroin (on μ, κ, and δ receptors), alcohol (μ receptors).

- within the ***GABAergic*** system act alcohol ($GABA_A$ and $GABA_C$ receptors), benzodiazepines ($GABA_{2\alpha}$ and $GABA_{1\alpha}$ receptors), ɤ-hydroxybutyric acid ($GABA_B$ receptors), and barbiturate derivatives ($GABA_A$ receptors).

- within the ***serotonergic*** system act synthetic cathinone (serotonin transporters (5-HT)), LSD ($5\text{-}HT_{2A}$ receptors), amphetamines ($5\text{-}HT_{1A}$, $5\text{-}HT_{2A}$, and $5\text{-}HT_{2C}$ receptors), alcohol ($5\text{-}HT_3$ receptors), MDMA, khat, and mescaline ($5\text{-}HT_{1A}$ and $5\text{-}HT_{2A}$ receptors).

- within the ***dopaminergic*** system act synthetic cathinone (dopamine transporters (DAT)), LSD (D_2 and D_3 receptors), amphetamines, MDMA, khat, alcohol, cocaine, and phencyclidine (D_2 receptors).

- within the ***adrenergic*** system act synthetic cathinone (norepinephrine transporters (NET)), LSD (α2 receptors), amphetamines, cocaine, khat, and mescaline (α2 receptors).

- within the ***cannabinoid*** system act synthetic cannabinoids (CB_1 and CB_2 receptors), THC (CB_1 and CB_2 receptors), and alcohol.

- within the ***glutamatergic*** system act (on NMDA receptors) ketamine, dextromethorphan, phencyclidine, and alcohol.

Regarding symptomatology, *opioids* are characterized by respiratory depression and miosis (Trecot et al., 2008, p. S134-S135), as well as the development of psychological and physical dependence. The latter is manifested through withdrawal syndrome, which includes symptoms such as rhinorrhea, mydriasis, myalgia, piloerection, tachycardia, and anxiety (Baconi & Bălălău, 2013, p. 43; Wang, 2019, pp. 234-235). The acute effects of *amphetamine* use are mostly psychiatric in nature and can even lead to psychosis resembling schizophrenia (Harro, 2015, p. 179). *Cocaine* consumption leads to a subjective increase in performance and physical strength, but various hallucinations (visual, auditory, tactile - associated with illusions of parasitosis (Brewer et al., 2008, p. 483)), as well as illusions and paranoid ideas, can occur (Brady et al., 1991, p. 509). The effects of *MDMA* consumption include symptoms such as tachycardia, chest pain, nausea, sweating, hypertension, mydriasis, bruxism, tachypnea, cardiac arrest, and hepatotoxicity (Luethi & Liechti, 2020, p. 1085). *Khat* consumption induces mild euphoria, increased energy, decreased appetite, increased vigilance, and increased self-confidence (Omar et al., 2015, p. 1; Kelly, 2011, p. 1). The use of *synthetic cathinone* is primarily associated with sympathomimetic toxicity (Luethi & Liechti, 2020, p. 1092), as well as skeletal muscle injuries (Zhou et al., 2019, p. 2). The syndrome

characterized by paranoia, severe agitation, and violent behavior has been described as "excited delirium" (Weinstein et al., 2017, p. 4-5). *Mescaline* consumption has been associated with "mystical experiences" (Uthaug et al., 2022, p. 312). *LSD* consumption does not produce dependence or withdrawal symptoms, but it leads to profound alterations of the psyche. The characteristics of LSD consumption include sensory disturbances ("flashbacks") and perceptual changes. Acute *ketamine* intoxication has been associated with effects such as nausea, vomiting, dizziness, dysphoria, confusion, and hallucinations (rarely). *Dextromethorphan* leads to dissociative effects, visual hallucinations, and potentiation of long-term memory. Acute intoxication with *gamma-hydroxybutyric acid* (GHB) begins with symptoms such as nausea, sedation, dizziness, myoclonus, deep coma, bradycardia, respiratory depression, hypothermia, and even death. Chronic *cannabis* use leads to dependence and the emergence of withdrawal syndrome, manifested by tremors, nystagmus, sweating, and irritability (Patel & Marwaha, 2022, p. 6). *Synthetic cannabinoids* induce effects similar to cannabis but stronger and more prolonged. Recent studies also confirm dissociative and psychedelic effects (Theunissen et al., 2022, p. 1256). The risk of psychosis and psychiatric complications is higher with synthetic cannabinoids compared to natural cannabis (Hervas 2017, p. 45). Acute intoxication with *barbiturate derivatives* manifests as central nervous system depression up to coma (Suddock & Cain, 2021, p. 3). Manifestations of acute *benzodiazepine* intoxication include lethargy, slurred speech, ataxia, coma, respiratory arrest, and hyporeflexia (Andrade et al., 2021). *Alcohol* consumption leads to the development of dependence and the manifestation of withdrawal syndrome, which includes hallucinations, tremors, and seizures (Costardi et al., 2015, p. 383).

3. Critical evaluation of analytical methods published in the literature for the analysis of synthetic cannabinoids and cathinone in biological samples

Studies have shown that the vacutainer used for blood sample collection can influence the stability of the analyte/metabolite. Synthetic cathinone are more stable when biological samples are collected in vacutainers with NaF preservative compared to EDTA (ethylenediaminetetraacetic acid) or whole blood samples (Aldubayyan et al., 2021, p. 54; Toennes & Kauert 2001, p. 343), while for synthetic cannabinoids, vacutainers without preservatives are preferably used (Toennes & Kauert, 2001, p. 341). The most suitable organic solvent (with the best recoveries and minimal influence on the type of biological sample) for cathinone extraction using the liquid-liquid method is ethyl acetate, while for synthetic cannabinoids, organic solvents such as ethyl acetate or a mixture

of hexane and ethyl acetate have been used with good results. The pH also plays a crucial role in analyte extraction. For cathinone, most derivatives have a pKa higher than 9, so alkalization of the sample is necessary before extraction, while for synthetic cannabinoids, the pH varies depending on the chemical structure of the compound being analyzed. Although liquid chromatographic methods successfully identify analytes, the GC-MS method has the advantage of being more accessible, with a shorter analysis time, a simple interface, and the possibility of analysis in FULL scan and SIM (selected ion monitoring) modes.

4. Critical evaluation of analytical methods published in the literature for the determination of methadone and the EDDP metabolite in biological samples

Blood samples are collected in vacutainers with EDTA (for obtaining plasma). Satisfactory results regarding extraction from biological samples have been obtained using liquid-liquid extraction with organic solvents (either in a mixture or separately) at alkaline pH (considering the pKa of methadone, which has a value of 8.3). Both methadone and its main metabolite, 2-ethylidene-1,5-3,3-diphenyl pyrrolidine (EDDP), can be easily determined by analyzing urine and plasma using a liquid-liquid extraction method with an organic solvent at alkaline pH, followed by analysis using GC-MS technique in both FULL scan and SIM modes. Previous studies have demonstrated that EDDP, although inactive, can be used as a biomarker in urine samples for monitoring methadone treatment (Baconi et al., 2016, p. 521).

II. Personal contributions

5. Assessment of drug knowledge and use based on a study in the general population

To gather more information regarding the population's exposure to drug use, specifically to collect information on the adverse effects of these NSPs, we conducted an international survey-based research. The questionnaire was administered in three languages (Romanian, English, and Spanish) and consisted of 45 questions.

The objectives of the questions included in the first part of the questionnaire were: characterizing the study participants' groups based on socio-demographic parameters; determining the level of exposure to drug use among the participating population; assessing aspects related to consumers' perception of drugs, drug consumption, and awareness of the consequences associated with drug use; assessing the level of knowledge about substances of abuse among the population;

defining the prevalence of drug consumption and characterizing it (methods of consumption, frequency of use, potential addictive nature).

The main objectives of the questions included in the second part of the questionnaire were: identifying patterns of use (single drug, combined with similar substances or alcohol); identifying the desired effects experienced by users when consuming these substances; and identifying the adverse effects experienced after consumption.

The research included a total of 840 participants, with 760 participants from Romania. It was observed that all social classes were affected. The most well-known substances of abuse were cannabis, cocaine, and heroin. The term "cathinone" was less familiar, but there is a considerable popularity with terms such as ethnobotanical drugs and "bath salts". The most commonly used substances of abuse were cannabis and alcohol. The most commonly experienced adverse effects included palpitations (cardiovascular), dry mouth (ENT), nausea (gastrointestinal), increased libido (genitourinary), tingling sensations (musculoskeletal), dizziness (neurological), mydriasis (ophthalmologic), tachycardia (sympathomimetic toxidrome), drowsiness, and increased energy (psychological).

6. **Outlining trends in drug use by analyzing the Romanian Anti-SIDA Association (ARAS) database**

The main objective of this study was to assess the dynamics of new psychoactive substances by accessing the ARAS database.

Following the analysis of data collected between 2017 and 2020 among drug users enrolled in the national anti-AIDS program through ARAS, a total of 71,248 requests were recorded, with 72.40% being male individuals. There is a constant upward trend in heroin consumption (from 93.09% in 2017 to 96.03% in 2020). "Legal highs" showed a decreasing trend in consumption during the period 2017-2019 (progressive decline from 1.94% to 0.12%), with a resurgence of interest starting in 2020 (increase from 0.12% to 0.38%). The majority of registered users associate heroin with various other types of drugs, and the preferred mode of consumption is through injection, which is why they visit needle exchange centers (within the "Syringe" program).

7. **Retrospective study on the hematological, biochemical, and cardiological characterization of drug users included in the treatment program at the C.E.T.T.T. "St. Stelian"**

In order to determine the statistically significant parameters correlated with the abuse of certain classes of substances, we conducted a retrospective study in collaboration with the Evaluation and Treatment Center for Youth Drug Addiction - C.E.T.T.T "St. Stelian," over a retrospective period of 6 years (January 2015 - January 2021). The main objectives of this study were: to determine the altered hematological, biochemical, and urinary parameters in the context of psychoactive substance use and obtain correlations regarding cardiac changes and substance abuse, using the Chi-square test for independence (expressed by the formula X^2 (DF = degrees of freedom, N = sample size) = chi-square statistical value, p = p-value) (Mihalaș & Lungeanu, 2011, pp. 101-104).

Out of a total of 2572 patients, the majority were male.

The result of the Chi-square test (X^2 (3, 1820) = 312.9504, p-value <0.00001) indicates statistical differences between drug categories. Hallucinations appear to be predominantly correlated with the consumption of cannabis and "legal highs" (Żukiewicz-Sobczak et al., 2012, p. 310).

Although opioids are known to have a dependency character, the statistical analysis highlights a higher correlation between withdrawal symptoms and "legal highs" (X^2 (4, 2433) = 161.54, p-value <0.00001).

Among the hematological and biochemical parameters, the relationship between these variables was found to be insignificant for most parameters, except for GGT (gamma-glutamyl transferase) (X^2 (3, 452) = 12.5153, p-value 0.005816). Among the analyzed drug categories, alcohol is the substance that showed the highest frequency of increased GGT values. Studies have demonstrated that GGT is a well-known biomarker for assessing heavy alcohol consumption (Peterson, 2004, pp. 32-33) and that there is a causal relationship between increased GGT values and heavy alcohol intake (Whitfield, 2001, p. 263). A homogeneous decrease in the erythrocyte series and an increase in lymphocytes were observed in all analyzed groups (homogeneity coefficient, CV < 20) (CV_{MCH} = 13.01, CV_{MCHC} = 4.55, CV_{LYM} = 13.22).

A total of 584 cardiac examinations were recorded, of which 54.62% showed at least one parameter modification. There are statistically significant differences between the consumer groups (X^2 (4, 393) = 31.2219, p-value < 0.00001), with alcohol consumers being more prone to cardiac impairment compared to other consumer groups. In the group of opioid consumers, high frequencies of bundle branch block (12.81%), axis deviation (10.31%), myocardial infarction

(10%), and abnormal ECG (34.68%) were recorded, results that are supported by findings from other studies (Wallner et al., 2008, pp. 1988-1992). In the cannabis consumer group, 11.11% experienced a myocardial infarction, while 48.14% had an abnormal ECG. Among "legal highs" consumers, bundle branch block was the most frequent cardiac modification (18.33%). Increases in the QT interval, axis deviation, ST-segment elevation, and P wave were also noted within this group. Similar results to ours have also been highlighted in various case reports during the investigation of electrocardiographic parameters in patients consuming synthetic cannabinoids: increased P wave (Ozturk et al., 2018, pp. 296-300; Aydin Sunbul et al., 2016, p. 486), prolonged QT interval (Ozturk et al., 2018, pp. 296-300; McKeever et al., 2015, p. 129), and fibrillation (Efe et al., 2017, p. 362).

8. Retrospective study on the psychological and psychiatric characterization of drug users included in the treatment program at the C.E.T.T.T. "St. Stelian"

As part of the retrospective study "Consumption of new psychoactive substances", the research aimed to determine the psychiatric and psychological parameters modified in the context of psychoactive substance use and evaluate possible correlations between these parameter changes and substance abuse.

The study group included 604 patients, of which 183 underwent psychological examination and 421 were examined from a psychiatric perspective. The majority of patients included in the study were male. There were no significant differences in terms of age and consumption tendencies between male and female participants.

Taking into account the admission diagnosis, statistical differences were obtained between drug user categories, and it was found that psychotic disorders are predominantly triggered by cannabis use (X^2 (2, 514) = 10.9104, p-value = 0.004274), while personality and behavioral disorders are correlated with opioid use (X^2 (3, 593) = 8.1516, p-value = 0.04298). Users of "legal highs" are more likely to develop schizophrenia (X2 (2, 484) = 17.7272, p-value = 0.000141) compared to other consumer groups. Depressive disorders are common among all consumer groups.

The psychiatric examination demonstrated the following:

Cannabis users are more susceptible to attention deficit (X^2 (3, 414) = 9.5446, p-value = 0.022861), "legal highs" users exhibit alterations in illness awareness (X^2 (2, 358) = 7.1637, p-value = 0.027824), and opioid users have a predisposition to decreased thinking (X^2 (3, 414) =

8.278, p-value = 0.040602) and self-preservation instincts (X^2 (3, 414) = 8.9039, p-value = 0.030596). These results are supported by data from the specialized literature (Urits et al., 2021, p. 7; Schuster et al., 2018, p. 9). Consistent with the findings of other researchers, our results indicate that perceptual changes (primarily manifested by the occurrence of hallucinations) occur primarily in the case of "legal highs" use (X^2 (3, 414) = 17.2647, p-value = 0.000623) (Żukiewicz-Sobczak et al., 2012, p. 310). Without notable differences between consumer groups, disturbances in circadian rhythm (manifested by either insomnia or decreased sleep quality due to interruptions or nightmares) occur regardless of the abused substance, a finding supported by the results of other studies in the field (Flemmen, Unhjem & Wang, 2014, p. 12).

The psychological examination indicated that "legal highs" users are more likely to exhibit alterations in thinking, such as delusional ideation or suicidal thoughts, compared to other consumers (X^2 (1, 130) = 13.4625, p-value = 0.000243), a result supported by other studies in the field (Urban et al., 2011, p. 431).

9. Interviews with drug addicts

In collaboration with C.E.T.T.T. "Sf. Stelian," we conducted a study based on the interview technique. The study took into account Adlerian (primarily) and Freudian principles and consisted in conducting the interview titled "Drug Use. The Cause-Effect Relationship: Trauma-Addiction" with 20 patients from the hospital. The patients received for completion the questionnaire "Assessment of Drug Use - Effects Experienced by the Patient due to Drug Consumption". Simultaneously, the questionnaire "Drug Use. The Cause-Effect Relationship: Trauma-Addiction" was administered to 20 non-consumer volunteers (comparison group).

The objectives of the study were: to determine possible triggering factors of dependent behavior, highlight the main adverse effects experienced in the past by drug users, and identify possible correlations between the consumption of new psychoactive substances and specific adverse reactions experienced. To perform the statistical analysis, the responses to the questions were replaced with numbers (according to the methodology), and the Pearson correlation test was applied, where r (degrees of freedom) = r statistic, p = p-value. The result of the Pearson test is significant at values of $p < .05$ (Mihalaș and Lungeanu, 2011, pp. 101-106).

Statistically significant correlations were obtained between drug use and male sex (R(38) = -0.603 with p-value = .000038), education (R(38) = -0.928 with p-value < .00001), unhappy

childhood (R(38) = -0.405; p = .009532), violence (R(38) = 0.436; p = .004875), and risk awareness (R(38) = -0.616; p = .000024).

Regarding specific adverse reactions related to NSP consumption, correlations with paranoia (R(18) = 0.798; p = 0.000025), visual hallucinations (R(18) = 0.704; p = .00054), auditory hallucinations (R(18) = 0.707; p =.00049), and the specific smell of the skin caused by these substances (R(18) = 0.905; p <.00001) were confirmed. Similar results were obtained in an international study on population exposure to drugs (Ciucă Anghel et al., 2022b, p. 430).

The qualitative analysis highlighted the fact that dependent individuals become vulnerable to drug use due to various weaknesses in their personality, a finding supported by the results obtained by other researchers (Hokm et al., 2018, p. 537). Measured through the discrepancy between self and ideal self, low self-esteem is strongly correlated with drug use. This result is supported and complemented by the strong positive correlation between drug use and feelings of anxiety (Taylor & Del Pilar, 1992, p. 896) and feelings of inferiority (Matthews, Dwyer & Snoek in 2017 (p. 281) from other studies.

10. Identification of substances of abuse from biological samples from drug-using patients using the GC-MS method

The study "Intoxication with new psychoactive substances" was conducted in collaboration with C.E.T.T.T. "Sf. Stelian" and its main objective was a rapid diagnosis of the consumption of new psychoactive substances, particularly cannabinoids and synthetic cathinone.

The inclusion criteria for the study were: patients who were consumers of psychoactive substances, specifically drugs known by street names such as "legal highs," "ethnobotanicals," synthetic cannabinoids, cathinone, "Spice," "Pure Magic," "bath salts"; age over 18 years; positive urine test for drug use; recommendation from the attending physician for analysis, following suspicion of altered behavior related to the use of the implicated substances; voluntary declaration of "legal highs" consumption by the patient.

Sample collection was performed using vacuum tubes with anticoagulant (EDTA tripotassium/dipotassium/disodium), vacuum tubes without coagulation activators, and sterile urine containers. The blood samples were centrifuged and separated. Both the obtained serum and plasma, as well as the urine samples, were frozen at -20°C.

For the extraction of the target analytes from the biological samples, a liquid-liquid extraction method was applied using a mixture of organic solvents (n-hexane: ethyl acetate, 9:1, v/v) at alkaline pH (pH 10). The sample-to-extraction solvent ratio was 1:2.5. The samples underwent extraction by vortex agitation for 15 minutes, followed by centrifugation for 15 minutes at 3500 rpm at 4°C. The upper organic layer was separated and evaporated under a nitrogen stream at 40°C. The residues were reconstituted with methanol. The injected volume in GC-MS was 1 µL. The samples were analyzed using the GC-MS method, with the following operating parameters:

- Column temperature of 100°C for 2 minutes, followed by an increase of 15°C/minute up to 160°C, held for 1 minute, then further increased at a rate of 15°C/minute up to 280°C, held for 5 minutes; total analysis time of 20 minutes.
- Carrier gas: helium, flow rate of 1 mL/minute.
- MS operating parameters: solvent delay time of 2 minutes, mass range: 20 - 500 m/z, positive ions, injector temperature of 200°C; working in split mode, transfer line temperature of 250°C, ion source temperature of 200°C.
- The acquisition of chromatograms in FULL scan mode.

The presence of characteristic ions at m/z 149 and 121 at t_R 10.76 in the case of patient 3 led to suspicion of the presence of a compound with a methylenedioxyphenyl ring, such as methylone, butylone, pentylone, MDPPP, MDPBP, or MDPV, in the sample.

The results suggest a possible presence of flephedrone (t_R 13.20) in patients 10 (Fig. 10.1) and 11, but without a standard for this substance, a definite identification cannot be supported.

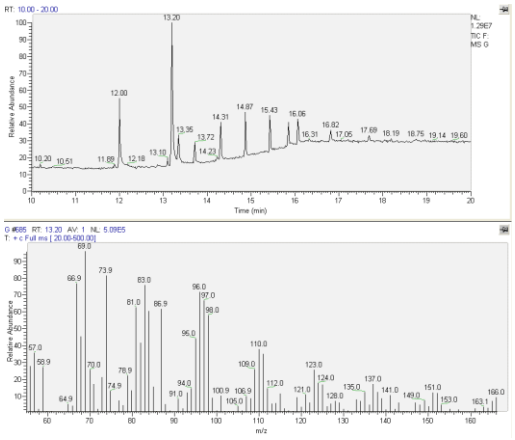

Fig. 10.1. Chromatogram obtained from the analysis of serum sample in patient no.10, t_R 13.20; present ions m/z 55, 69, 74, 83, 97, 110, 123, and 151.

Since the spectra at specific retention times are identical in the case of patient 3 and patient 9, patient 10 and patient 11, and patient 11 and patient 2, it was suspected that these similarities could be due to a common medication in the treatment regimen. By analyzing the patients' records, although common medications were identified in the treatment regimens (patients 3 and 9: diazepam and valproic acid; patients 10 and 11: valproic acid; patients 2 and 11: paracetamol), comparison with spectra of suspected substances (according to the National Institute of Standards and Technology, NIST database) disproved this hypothesis. According to the medical records of the patients included in the study, participants with serial numbers 9, 11, and 13 were highlighted as suspected consumers of new psychoactive substances. However, this consumption could not be confirmed through the analysis of available samples.

The presence of characteristic ions at m/z 72 for methadone (at t_R 13.80), and m/z 277 for EDDP (at t_R 13.12) in the urine spectra of patients indicates the successful application of the analysis method for detecting these analytes (Table X.1). The fact that these ions were not detected in the serum samples indicates urine as the preferred biological sample for the detection and determination of methadone and EDDP (which will be confirmed in Chapter 11 as well).

Table X.I. Detection of methadone and EDDP in urine samples from patients suspected of "legal highs" use

Patient Code Number	**EDDP (m/z 277, t_R 13,12)**	**MTD (m/z 72, t_R 13,80)**
1	-	-
2	+	+
3	-	-
4	-	+
5	-	+
7	-	+
8	+	-
10	-	-

„+” : positive result ; „-”: negative result

11. Identification and quantification of methadone and the EDDP metabolite in plasma and urine from patients undergoing substitution treatment, by the GC-MS method

As part of the study "Intoxication with new psychoactive substances," the research aimed at collecting medical data from the patients included in the study and determining the substance of

interest (methadone) and its metabolite (EDDP) from biological samples using high-performance chromatographic methods.

The objectives were: to measure the levels of methadone in the biological fluids of opioid-dependent patients undergoing methadone substitution treatment to assess treatment compliance; to detect the metabolite EDDP in the biological fluids; to compare urine and plasma samples to identify the more suitable biological sample for rapid detection of the analyzed substances; to confirm a relationship between plasma concentrations of methadone and the administered dose.

Sample collection was performed according to the procedure described in *Chapter 10*.

In order to create the calibration curves, an initial stock standard solution of methadone was prepared with a concentration of 1 mg/mL, followed by dilution in methanol at a ratio of 1:100 to obtain a working solution with a concentration of 10 µg/mL. By diluting the working solution in control urine and control plasma, successive concentration samples were obtained to create the calibration curve. The concentration range for the urine samples in the calibration curve was 0.025 - 3 µg/mL, while for the plasma samples it was 0.1 - 1 µg/mL. Diphenylamine 0.01% in methanol was used as the internal standard.

For the extraction of methadone and EDDP from the biological samples, a liquid-liquid extraction method was employed using a mixture of organic solvents (n-hexane and 2-propanol, 97:3, v/v) at alkaline pH (pH 10), as follows:

For the extraction of methadone and EDDP from biological samples, a liquid-liquid extraction method was applied using a mixture of organic solvents (n-hexane:2-propanol, 97:3, v/v) at alkaline pH (pH 10, achieved with 2M KOH). The sample-to-extraction solvent ratio was 1:4 v/v. A 0.01% diphenylamine in methanol was used as an internal standard. The tubes were vortexed for 15 minutes and then centrifuged for 10 minutes at 3400 rpm, at 15°C. The upper organic layer was separated and transferred to a new (dry and clean) tube and then evaporated under a nitrogen stream at 40°C. The residue was reconstituted in 100 µL of methanol, and 1 µL was injected into GC-MS.

The samples were analyzed using the GC-MS method, with the following operating parameters:

- Column temperature: 150 ºC for 1 minute, then increased at a rate of 10 ºC/min up to 220 ºC, followed by a further increase at a rate of 30 ºC/min up to 280 ºC, with a hold time of 1 minute; total analysis time of 12 minutes.

- Carrier gas: helium, flow rate of 1 mL/min.
- MS operating parameters: solvent delay time of 6 minutes, mass range of 50 - 650 m/z, positive ions mode, injector temperature of 220 ºC; working in split mode, transfer line temperature of 260 ºC, ionization source temperature of 200 ºC.
- Full scan mode was used for data acquisition, as well as selected ion monitoring (SIM) mode, using the ion with m/z = 72 for methadone and the ion with m/z = 277 for EDDP.

The majority of study participants were males aged between 20 and 30 years, which can be explained by the fact that drug initiation typically occurs in early life, during adolescence, and early adulthood. Most of the patients were opiate users and 50% have a history of methadone substitution treatment, an aspect that is explained by the specifics of the center.

Regarding the doses of methadone administered in the treatment regimen, although the literature suggests optimal methadone doses ranging from 80 to 150 mg/day (Durrani & Bansal 2022, p. 4), our study results align with findings from other studies in the field, which highlight a preference for moderate methadone doses (ranging from 90 to 100 mg/day) to avoid withdrawal symptoms (Anderson & Kearney, 2000, p. 43; Baconi et al., 2018; Loimer & Schmid, 1992, p. 241). However, administering higher doses of methadone significantly reduces the chances of relapse and illicit drug use (Strain et al., 1999, p. 1000).

Most patients, in addition to methadone treatment, also take other medications from the anticonvulsant class (37.5%), anxiolytics (18.75%), antipsychotics (18.75%), and antidepressants (25%), indicating, as supported by the findings of other studies in the field, a higher rate of psychiatric comorbidities among opioid users (Zhu et al., 2021).

The GC-MS procedure for determining methadone using liquid-liquid extraction with an alkaline organic solvent mixture was applied and discussed comparatively for urine and plasma samples.

Qualitative analysis, conducted by scanning the samples in FULL mode, resulted in positive findings for the presence of methadone in both urine and plasma samples from almost all patients receiving methadone treatment. Positive results in patients without methadone treatment at the time of sample collection indicate clear evidence of extra consumption. Quantitative analysis, performed by scanning the samples in SIM mode, also yielded conclusive results (Table XI.1).

The proportional relationship between the daily dose of methadone, urine levels of methadone, and the presence of EDDP in urine samples has been established by various studies in

the field (Baconi et al., 2018; George & Braithwaite, 1999). These studies have demonstrated the presence of increased urine levels of the metabolite EDDP in patients with continuous methadone administration due to the induction of methadone metabolism (Wolf et al., 2014, p. 137). The varying urine concentrations of methadone in the case of the same daily dose administered to different patients can be explained either by extra consumption in one patient or by individual variations related to methadone metabolism, as shown in previous studies (Kharasch, 2017).

The results indicated higher levels of methadone in urine compared to plasma, highlighting a broader distribution of the analyte of interest in urine. The lower plasma levels can be attributed to plasma fluctuations (due to tolerance) and lower extraction efficiency from plasma, supported by the findings of other studies in the field (Moffat, Osselton & Widdop, 2011, p. 1649; Vasile et al., 2014, p. 1209; Baconi et al., 2016, p. 526).

Table XI.1. Determination of plasma and urine levels of methadone (Ciucă Anghel et al., 2022a, p. 7).

No.	**MTD** (mg)	**U**	**P**	**Urine**		**Plasma**		**Q_{MTD} Peak area**	**Concentration MTD** (µg/mL)
				q_{MTD}	**q_{EDDP}**	**q_{MTD}**	**q_{EDDP}**		
1	-	Da	-	+	+ slab	NA	NA	32982_U	0,41
2	100	-	Da	NA	NA	+	+	77755_P	0,072
3	-	Da	-	+ slab	-	NA	NA	9158_U	0,29
4	100	Da	-	+	+ slab	NA	NA	167055_U	1,06
5	100	Da	-	+	+	NA	NA	1490729_U	> 3 (*4,366)
6	110	Da	-	+	+	NA	NA	2787530_U	> 3 (*3,012)
7	110	Da	-	+	+	NA	NA	3523925_U	> 3 (*6,697)
8	-	Da	-	+	+	NA	NA	53850_U	0,51
9	-	Da	Da	+	+ slab	+ slab	+	10513_U 2506_P	0,30 0,165
10	-	-	Da	NA	NA	-	-	-	-
11	-	-	Da	NA	NA	+ slab	-	4624_P	0,93
12	50	-	Da	NA	NA	+	+	32958_P	>1
13	-	-	Da	NA	NA	-	-	-	-
14	100	-	Da	NA	NA	+	-	4779_P	0,013

No. (patient order number); MTD (oral methadone dose), U (available urine sample), P (available plasma sample), EDDP (2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine), "-" (negative result of the analysis), "+" (positive result of the analysis); qMTD (qualitative methadone analysis), qEDDP (qualitative EDDP

analysis); Q (quantitative analysis); NA (unavailable sample); Peak Area: AU – urine area, AP – plasma area; *concentration obtained through dilution adjustment.

III. Conclusions and personal contributions

The research combined aspects from clinical laboratory, cardiology, psychology, and psychiatry, highlighting their interdisciplinary nature. The results can serve as support for future research on new psychoactive substances in any of the mentioned fields.

Among the conclusions drawn from the conducted research, the following ones stand out as significant.

No class of substances of abuse has exclusivity for a particular receptor system.

Although "legal highs" are highly popular on the illicit drug market, they remain unknown in terms of composition.

The majority of adverse symptoms experienced by users of new psychoactive substances are of a psychological nature, followed by effects on the ENT (ear, nose, throat) area, cardiac, neurological, and ophthalmological effects.

Regarding consumption trends, the data emphasize **a resurgence of "legal highs" in the attention of consumers**, justifying the research topic.

Hallucinations seem to be primarily correlated with the consumption of Cannabis and "legal highs".

Although opioids are known to be addictive, **the statistical analysis highlights an increased correlation between withdrawal symptoms and "legal highs"**. In the case of cannabis consumption, the occurrence of withdrawal syndrome has not been consistently observed.

The presence of bilirubin pigments or urobilinogen in urine does not correlate with a specific type of substance abuse.

The presence of various comorbidities (positive test for HIV, HBV, HCV), can be correlated with both cannabis use and intravenous drug administration (in the case of opioid users).

There are significant statistical differences in the elevation of GGT levels between consumer groups, which is associated with alcohol consumption. The increase in the GGT parameter value could be explained as a result of liver diseases, as well as due to the combination of alcohol with

heroin, which, through the injectable route of administration, significantly increases the risk of contracting HIV, HBV, or HCV.

Homogeneity is observed (coefficient of variation CV < 20) regarding hematological parameters: increased LYM (lymphocytes), decreased MCH (mean corpuscular hemoglobin), and MCHC (mean corpuscular hemoglobin concentration).

Electrocardiogram (ECG) abnormalities are common in the context of drug use. Cardiac impairment can also be induced by high doses of administered methadone.

Cardiac impairment is primarily determined by alcohol consumption, with significant statistical differences between consumer groups. The advanced age of alcohol consumers also contributes to cardiac impairment within this group of consumers.

The EKG changes recorded in 48.14% of the cannabis users included in the study indicate the **harmful effects of cannabis on the myocardium**.

Bundle branch block is the most common cardiac modification among users of "legal highs". Increases in QT interval, axis deviation, ST-segment elevation, and P-wave abnormalities were also observed.

Psychotic disorders are predominantly triggered by cannabis consumption, while personality and behavioral disorders are correlated with opioid use. Depressive disorders are common among all groups of consumers.

Attention deficit is correlated with cannabis use, altered consciousness is associated with "legal highs", diminished thinking and self-preservation instincts are observed in opioid use, and **perceptual changes (hallucinations) are prevalent in the case of "legal highs" consumption**.

"Legal highs" users are more likely to develop schizophrenia and psychotic disorders compared to other groups of consumers.

Regarding circadian rhythm changes, there are no notable differences between groups of consumers.

In terms of psychological profile, compared to other consumers, **"legal highs" users are more likely to exhibit changes in thinking, such as delusional ideation or suicidal thoughts**.

Men are more prone to drug use.

Various childhood traumas such as feelings of unhappiness, experiencing nightmares or unpleasant dreams in early childhood, as well as exposure to violence (both towards oneself and as an observer), can induce a tendency towards addictive behavior.

The main triggering factor for addictive behavior remains the lack of access to information (lack of education) correlated with a lack of risk awareness. Various weaknesses in an individual's personality and low self-esteem contribute to this.

Regarding specific adverse reactions to NPS consumption, correlations have been confirmed with paranoia, hallucinations (both visual and auditory), as well as the specific skin odor imprinted by these substances.

Acid hydrolysis of urine samples does not provide additional benefits in analysis for detecting new psychoactive substances (NPS).

The long duration of time from the moment of consumption to the moment of biological sample collection, as well as from the moment of sample collection to the moment of analysis, can negatively influence the results of the analyses.

The proposed extraction and analysis method allows for the detection of NPS as well as methadone and EDDP, requiring further validation studies initially on forensic samples and later on biological samples from drug users. Urine is the preferred biological sample for analysis.

Moderate doses of methadone (ranging from 90 to 100 mg/day) are preferred for methadone maintenance treatment to avoid withdrawal symptoms.

There is a high rate of psychiatric comorbidities among opioid users.

Different urine concentrations of methadone in the situation of administering the same daily dose of methadone to different patients either indicate excessive consumption or indicate individual characteristics related to methadone metabolism.

Urine contains higher levels of methadone than plasma, indicating a broader distribution of the analyte of interest in urine, making it more suitable for the chosen method than plasma.

The proposed analysis procedure provides satisfactory results and can be used in clinical laboratories for the rapid determination of methadone levels, particularly in verifying treatment compliance (by detecting extra consumption).

Methadone concentrations in biological fluids have been shown to be related to the administered doses, and urine is more suitable, compared to plasma, for the chosen method.

The originality of the research

First and foremost, the originality of this work is given by the fact that, **for the first time in Romania, survey-based research investigates both the population's exposure to drugs and the adverse effects experienced by consumers**.

In the thesis, we conducted a **comprehensive characterization of various categories of drug users in terms of hematological, biochemical, cardiological, psychological, and psychiatric aspects**, which has not been done before. **The obtained results could have a significant impact on the approach to drug-consuming patients, particularly in terms of easier diagnosis**.

The conducted research highlights certain significant aspects associated with the use of "legal highs": the occurrence of withdrawal symptoms, susceptibility to experiencing alterations in consciousness and thinking (delusional or suicidal ideation), as well as perceptual changes (hallucinations). Additionally, "legal highs" users are more prone to developing psychiatric pathologies, schizophrenia, and psychotic disorders compared to other groups of users. Through these findings **the thesis makes an important contribution to the characterization of the toxicological profile of these substances**, as currently, specialists face a lack of scientific data regarding new psychoactive substances.

By correlating aspects of childhood and individual personality with drug use, this study demonstrates the importance of conducting anonymous surveys or interviews on a larger scale regarding drug consumption in detoxification centers or schools. Such surveys could be useful in the early detection of potential addictive behaviors among adolescents. Additionally, the study results confirm the necessity of informative campaigns on drug consumption as a preventive measure in schools.

The results obtained from the analysis of biological samples provide a basis for the development of a new method for the detection and determination of NPS, as well as methadone and EDDP.

This study opens up possibilities for future research, such as validating the proposed analytical method for the detection and determination of NPS in biological samples, determining the reversible/irreversible nature of psychological changes in these consumers, and conducting comparative studies on cardiac impairment among synthetic cannabinoid users *vs*. cathinone users.

By intersecting with the field of psychiatry, this work opens up new research directions regarding activation patterns and neuropsychological processing, including the association of alexithymia (defined as the inability to recognize and verbalize emotions) with drug consumption.

❖ **Limitations**

People's reluctance to respond to questionnaires, in general, is exacerbated when it comes to addressing an extremely sensitive topic, both socially and legally, such as disclosing the illegal use of various substances.

A comparison of hematological, biochemical, urinary, cardiologic, psychological, and psychiatric parameters was made with respect to normal values (of a healthy individual), as it was not possible to evaluate them prior to drug use.

Regarding the analysis of adverse reactions reported by the interview participants, these are confessions based on the subjective perception of the participant and were not medically validated at the time they were experienced.

In the analysis of biological samples, in most cases, it is not known with certainty whether the consumed substance of abuse belongs to the class of interest (i.e., whether it is a synthetic cathinone or cannabinoid). Additionally, the lack of reference substances makes it difficult to identify them solely through comparison with the spectral library and available data from the specialized literature. The biological samples were quantitatively limited, as they were needed for both the detection of synthetic cannabinoids and cathinone (Chapter 10) and the identification and quantification of methadone and EDDP (Chapter 11). Moreover, a major disadvantage of the analysis of plasma samples is the significantly smaller available volume of samples.

Selective Bibliography

1. Agenția Națională Antidrog (ANA) (2021) *Raport Național Privind Situația Drogurilor*, disponibil la http://ana.gov.ro/wp-content/uploads/2022/03/RN_2021.pdf (accesat 01 februarie 2023).
2. Aldubayyan, A.A., Castrignanò, E., Elliott, S. & Abbate, V. (2021) „Stability of synthetic cathinones in clinical and forensic toxicological analysis-Where are we now?" *Drug testing and analysis*, 13(1), pp. 44–68.
3. Anderson, I.B. & Kearney, T.E. (2000) „Use of methadone", *The Western Journal of Emergency Medicine*, 172(1), pp. 43–46.
4. Andrade, A.L.M., De Micheli, D., Silva, E.A. da, Lopes, F.M., Pinheiro, B. de O. & Reichert, R.A. (Eds.) (2021) „Psychology of Substance Abuse: Psychotherapy, Clinical Management and Social Intervention", *Springer International Publishing*.
5. Aydin Sunbul, E., Sunbul, M., Terzi, A., Calli, S., Koca, E., Bilici, R., & Citak, S. (2016) „The Effect of Synthetic Cannabinoids on P-Wave Dispersion: An Observational Study", *Medical principles and practice: international journal of the Kuwait University, Health Science Centre*, 25(5), pp. 483–487.
6. Baconi D.L. & Bălălău C. (2013) „*Toxicologia substanțelor de abuz*", Editura Universitară Carol Davila, București, p. 7.
7. Baconi, D.L., Ciobanu, A.-M., Margină, D., Negrei, C., Bârcă, M., Ilie, M. & Bălălău, D. (2008) „Dynamics of the biochemical parameters in heroin addict patients during the methadone substitution therapy", *Toxicology Letters*, 180.
8. Baconi, D.L., Ciobanu, A.-M., Vasile, R.D., Vlăsceanu, A.M., Nedelescu, M. & Stan, M. (2018) „Methadone Treatment for Heroin Dependence" in Drug Addiction Zhao, F. and Li M., (Eds.), *IntechOpen*, London, United Kingdon, pp.117-133.
9. Baconi, D.L., Popescu, G., Ciobanu A.-M., Stan, M., Vlăsceanu, A.M., & Bălălău, C. (2016) „EDDP metabolite as biomarker for monitoring of methadone substitution treatment", *Farmacia*, 64, pp. 521-527.
10. Brady, K.T., Lydiard, R.B., Malcolm, R., & Ballenger, J.C. (1991) „Cocaine-induced psychosis", *The Journal of clinical psychiatry*, 52(12), pp. 509–512.

11. Brewer, J.D., Meves, A., Bostwick, J.M., Hamacher, K.L. & Pittelkow, M.R. (2008) „Cocaine abuse: dermatologic manifestations and therapeutic approaches", *Journal of the American Academy of Dermatology*, 59, pp. 483–487.
12. **Ciucă Anghel, D.-M.**, Stan, M., Bălălău, C., Tudor, G. & Baconi, D. L. (2022). „Toxicology of new psychoactive substances: an exposure to drug use", *Farmacia*, 70(3), pp. 425-433.
13. **Ciucă-Anghel, D.-M.**, Stan, M., Bălălău, C., Anghel., E.E., Bălălău, D., Paunica, I., Dimitriu, A.S., Paunica, S. & Baconi, D.L. (2022) „Adverse effects of new psychoactive drug use. Psychological insights of addiction", *Mediterranean Journal of Clinical Psychology* 10(3).
14. Costardi, J.V., Nampo, R.A., Silva, G.L., Ribeiro, M.A., Stella, H.J., Stella, M.B. & Malheiros, S.V. (2015) „A review on alcohol: from the central action mechanism to chemical dependency", *Revista da Associacao Medica Brasileira (1992)*, 61(4), pp. 381–387.
15. Durrani, M. & Bansal, K. (2022) Methadone. *In StatPearls* [Internet]. Publisher: Treasure Island (FL): StatPearls Publishing.
16. Efe, T.H., Felekoglu, M.A., Çimen, T. & Doğan, M. (2017) „Atrial fibrillation following synthetic cannabinoid abuse. Turk Kardiyoloji Dernegi arsivi", *Turk Kardiyoloji Derneginin yayin organidir*, 45(4), pp. 362–364.
17. Flemmen, G., Unhjem, R. & Wang, E. (2014) „High-intensity interval training in patients with substance use disorder", *BioMed research international*, 616935.
18. George, S. & Braithwaite, R.A. (1999) „A pilot study to determine the usefulness of the urinary excretion of methadone and its primary metabolite (EDDP) as potential markers of compliance in methadone detoxification programs", *Journal of Analytical Toxicology*, 23(2), pp. 81–85.
19. Harro J. (2015) „Neuropsychiatric Adverse Effects of Amphetamine and Methamphetamine", *International review of neurobiology*, 120, pp. 179–204.
20. Heal, D.J., Smith, S.L., Gosden, J. & Nutt, D.J. (2013) „Amphetamine, past and present--a pharmacological and clinical perspective", *Journal of psychopharmacology (Oxford, England)*, 27(6), pp. 479–496.
21. Hokm Abadi M.E., Bakhti M., Nazemi M., Sedighi S. & Mirzadeh Toroghi E. (2018) „The relationship between personality traits and drug type among substance abuse", *Journal of Research & Health*, 8(6), pp. 531–540.
22. Kelly J.P. (2011) „Cathinone derivatives: a review of their chemistry, pharmacology and toxicology", *Drug testing and analysis*, 3(7-8), pp. 439–453.

23. Kharasch E.D. (2017) „Current Concepts in Methadone Metabolism and Transport", *Clinical Pharmacology in Drug Development*, 6(2), pp. 125–134.
24. Liechti M.E. (2017) „Modern Clinical Research on LSD", *Neuropsychopharmacology: official publication of the American College of Neuropsychopharmacology*, 42(11), pp. 2114–2127.
25. Loimer, N., Schmid, R. (1992) „The use of plasma levels to optimize methadone maintenance treatment", *Drug Alcohol Dependence*, 30(3), pp. 241–246.
26. Luethi D, Liechti M.E. (2020) „Designer drugs: mechanism of action and adverse effects", *Archives of Toxicology*, 94(4), pp. 1085-1133.
27. Matthews, S., Dwyer, R., & Snoek, A. (2017) „Stigma and Self-Stigma in Addiction", *Journal of bioethical inquiry*, 14(2), pp. 275–286.
28. McKeever, R.G., Vearrier, D., Jacobs, D., LaSala, G., Okaneku, J., & Greenberg, M.I. (2015) „K2-not the spice of life; synthetic cannabinoids and ST elevation myocardial infarction: a case report", *Journal of medical toxicology : official journal of the American College of Medical Toxicology*, 11(1), pp. 129–131.
29. Mihalaș G.I., & Lungeanu D. (2011) *Biostatistică*. Timișoara: Editura Victor Babeș; pp. 101-104.
30. Moffat A.C., Osselton M.D., Widdop B. (eds) (2011) Clarke's analysis of drugs and poisons, 4th ed., Publisher: Pharmaceutical Press: London, Chicago, pp. 1649.
31. Observatorul European pentru Droguri și Toxicomanie (OEDT) (2022), *Raportul european privind drogurile 2022: Tendințe și evoluții, Oficiul pentru Publicații al Uniunii Europene, Luxemburg.* Disponibil la: https://www.emcdda.europa.eu/system/files/publications/14644/2022.2419_RO_02_wm.pdf (accesat 14 iulie 2022).
32. Omar, Y.S., Jenkins, A., Altena, M. v., Tuck, H., Hynan, C., Tohow, A., Chopra, P., & Castle, D. (2015) „Khat Use: What Is the Problem and What Can Be Done?", *BioMed research international*, 472302.
33. Ozturk, H.M., Erdogan, M., Alsancak, Y., Yarlioglues, M., Duran, M., Boztas, M.H., Murat, S.N., & Ozturk, S. (2018) „Electrocardiographic alterations in patients consuming synthetic cannabinoids", *Journal of psychopharmacology (Oxford, England)*, 32(3), pp. 296–301.
34. Patel, J., & Marwaha, R. (2022) Cannabis Use Disorder. *In StatPearls.* StatPearls Publishing.

35. Peterson K. (2004) „Biomarkers for alcohol use and abuse--a summary", *Alcohol research & health*, 28(1), pp. 30–37.
36. Sanchez Hervas, E. (2017) „Synthetic cannabinoids: characteristics, use and clinical implications", *Archives of Psychiatry and Psychotherapy*, 19(2), pp. 42-48.
37. Schuster, R.M., Gilman, J., Schoenfeld, D., Evenden, J., Hareli, M., Ulysse, C., Nip, E., Hanly, A., Zhang, H., & Evins, A.E. (2018) „One Month of Cannabis Abstinence in Adolescents and Young Adults Is Associated With Improved Memory", *The Journal of clinical psychiatry*, 79(6), 17m11977.
38. Strain E.C., Bigelow G.E., Liebson I.A., Stitzer M.L. (1999) „Moderate- vs high-dose methadone in the treatment of opioid dependence: a randomized trial", *JAMA*, 281, pp. 1000-1005.
39. Suddock, J.T., & Cain, M.D. (2021). Barbiturate Toxicity. *In StatPearls*. StatPearls Publishing.
40. Taylor, D.N., & Del Pilar, J. (1992) „Self-Esteem, Anxiety, and Drug Use", *Psychological Reports*, 71(3), pp. 896–898.
41. Theunissen, E.L., Reckweg, J.T., Hutten, N., Kuypers, K., Toennes, S.W., Neukamm, M.A., Halter, S., & Ramaekers, J.G. (2022) „Psychotomimetic symptoms after a moderate dose of a synthetic cannabinoid (JWH-018): implications for psychosis", *Psychopharmacology*, 239(5), pp. 1251–1261.
42. Toennes, S.W., & Kauert, G.F. (2001) „Importance of vacutainer selection in forensic toxicological analysis of drugs of abuse", *Journal of analytical toxicology*, 25(5), pp. 339–343.
43. Trescot, A.M., Datta, S., Lee, M., & Hansen, H. (2008) „Opioid pharmacology", *Pain physician*, 11(2 Suppl), pp. S133–S153.
44. Urban, M., Rudecki, T., Wróblewski, D., & Rabe-Jabłońska, J. (2011) „Zaburzenia psychotyczne zwiazane z przewlekłym przyjmowaniem mefedronu. Opis przypadku [Psychotic disorders related with chronic use of mephedrone. Case report]", *Psychiatria polska*, 45(3), pp. 431–437.
45. Urits, I., Charipova, K., Gress, K., Li, N., Berger, A.A., Cornett, E.M., Kassem, H., Ngo, A.L., Kaye, A.D., & Viswanath, O. (2021) „Adverse Effects of Recreational and Medical Cannabis", *Psychopharmacology bulletin*, 51(1), pp. 94–109.

46. Uthaug, M.V., Davis, A.K., Haas, T.F., Davis, D., Dolan, S.B., Lancelotta, R., Timmermann, C., & Ramaekers, J.G. (2022) „The epidemiology of mescaline use: Pattern of use, motivations for consumption, and perceived consequences, benefits, and acute and enduring subjective effects", *Journal of psychopharmacology (Oxford, England)*, 36(3), pp. 309–320.
47. Vasile, R.D., Baconi, D.L., Hudiţă, C., Bârcă, M., Bălălău, C., Ciobanu, A.-M. (2014) „Methadone plasma levels in heroin addict patients during substitution therapy", *Farmacia*, 62(6), pp. 1202-1213.
48. Wallner, C., Stöllberger, C., Hlavin, A., Finsterer, J., Hager, I., & Hermann, P. (2008) „Electrocardiographic abnormalities in opiate addicts", *Addiction (Abingdon, England)*, 103(12), pp. 1987–1993.
49. Wang S. (2019) „Historical Review: Opiate Addiction and Opioid Receptors", *Cell transplantation*, 28(3), pp. 233–238.
50. Weinstein, A.M., Rosca, P., Fattore, L., & London, E.D. (2017) „Synthetic Cathinone and Cannabinoid Designer Drugs Pose a Major Risk for Public Health", *Frontiers in psychiatry*, 8, 156.
51. Whitfield J.B. (2001) „Gamma glutamyl transferase", *Critical reviews in clinical laboratory sciences*, 38(4), pp. 263–355.
52. Wolf, C.E., Goldstein, A., Poklis, J.L., Poklis, A. (2014) „Evaluation of an enzyme immunoassay for the detection of methadone metabolite EDDP [2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine] in urine", *Journal of Clinical Laboratory Analysis*, 28(2), pp. 136–140.
53. Zhou X., Luethi D., Sanvee G.M., Bouitbir J., Liechti M.E., Krähenbühl S. (2019) „Molecular Toxicological Mechanisms of Synthetic Cathinones on C2C12 Myoblasts", *International Journal of Molecular Sciences*, 20(7), 1561.
54. Zhu, Y., Mooney, L.J., Yoo, C., Evans, E.A., Kelleghan, A., Saxon, A.J., Curtis, M.E., Hser, Y.I. (2021) „Psychiatric comorbidity and treatment outcomes in patients with opioid use disorder: Results from a multisite trial of buprenorphine-naloxone and methadone", *Drug Alcohol Dependence*, 228:108996, pp. 1-17.
55. Żukiewicz-Sobczak, W., Zwoliński, J., Chmielewska-Badora, J., Krasowska, E., Piątek, J., Sobczak, P., Wojtyła, A., Fornal, E., Kuczumow, A., & Biliński, P. (2012) „Analysis of psychoactive and intoxicating substances in legal highs", *Annals of agricultural and environmental medicine*, 19(2), pp. 309–314.

List of published scientific papers

1. Ciucă Anghel, D. M., Stan, M., Bălălău, C., Tudor, G., Baconi, D. L. (2022) „Toxicology of new psychoactive substances: an exposure to drug use", Farmacia, 70(3), pp. 425-433. Link către publicație: https://doi.org/10.31925/farmacia.2022.3.7

2. Ciucă Anghel, D. M; Anghel, E. E.; Stan, M.; Tudor, G.; Dumitriu, A. S.; Paunica, S.; and Baconi, D. L. (2022) „Psychological and psychiatric characterization of various groups of drugs users", Journal of Mind and Medical Sciences: Vol. 9: Iss. 2, Article 8, p. 255-265; Link către publicație: https://scholar.valpo.edu/jmms/vol9/iss2/8

3. Ciucă Anghel, D.-M., Ciobanu A.-M., Guțu C. M., Stan M., Tudor G., and Baconi D. L. (2022) „GC-MS Analysis of Methadone and EDDP in Addicted Patients under Methadone Substitution Treatment: Comparison of Urine and Plasma as Biological Samples", Molecules 27(23): 8360. Link către publicație: https://doi.org/10.3390/molecules27238360

4. Ciucă-Anghel, M.D., Stan, M., Bălălău, C., Anghel., E.E., Bălălău, D., Paunica, I., Dimitriu, A.S., Paunica, S., Baconi, D.L. (2022) „Adverse effects of new psychoactive drug use. Psychological insights of addiction", Mediterranean Journal of Clinical Psychology 10(3). Link către publicație: https://doi.org/10.13129/2282-1619/mjcp-3572

5. Ciucă Anghel D.-M., Vlăsceanu A.M., Tudor G., Ciobanu A.-M., Stan M., Nițescu G.V., Florou D., Tsatsakis A.M., Baconi D.L. (2023) „Characterization of various groups of drugs users. Highlights on legal highs users", Farmacia 71(1):18-28. Link către publicație: https://doi.org/10.31925/farmacia.2023.1.3

6. Gheorghiu, O. R. C., Ciobanu, A. M., Guțu, C. M., Chițescu, C. L., Costea, G. V., Anghel, D. -M., Vlasceanu, A. M., et al. (2023) „Determination of Phosphodiesterase Type-5 Inhibitors (PDE-5) in Dietary Supplements", Molecules, 28(10), 4116. MDPI AG. Link către publicație: http://dx.doi.org/10.3390/molecules28104116

7. Ciucă Anghel, D.-M., Nițescu, G. V., Tiron, A., Guțu, C. M., Baconi, D. L. (2023) „Understanding the Mechanisms of Action and Effects of Drugs of Abuse" Molecules, 28(13), 4969. Link către publicație: https://doi.org/10.3390/molecules28134969