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8-9 APRIL 2019, MAASTRICHT, NETHERLANDS

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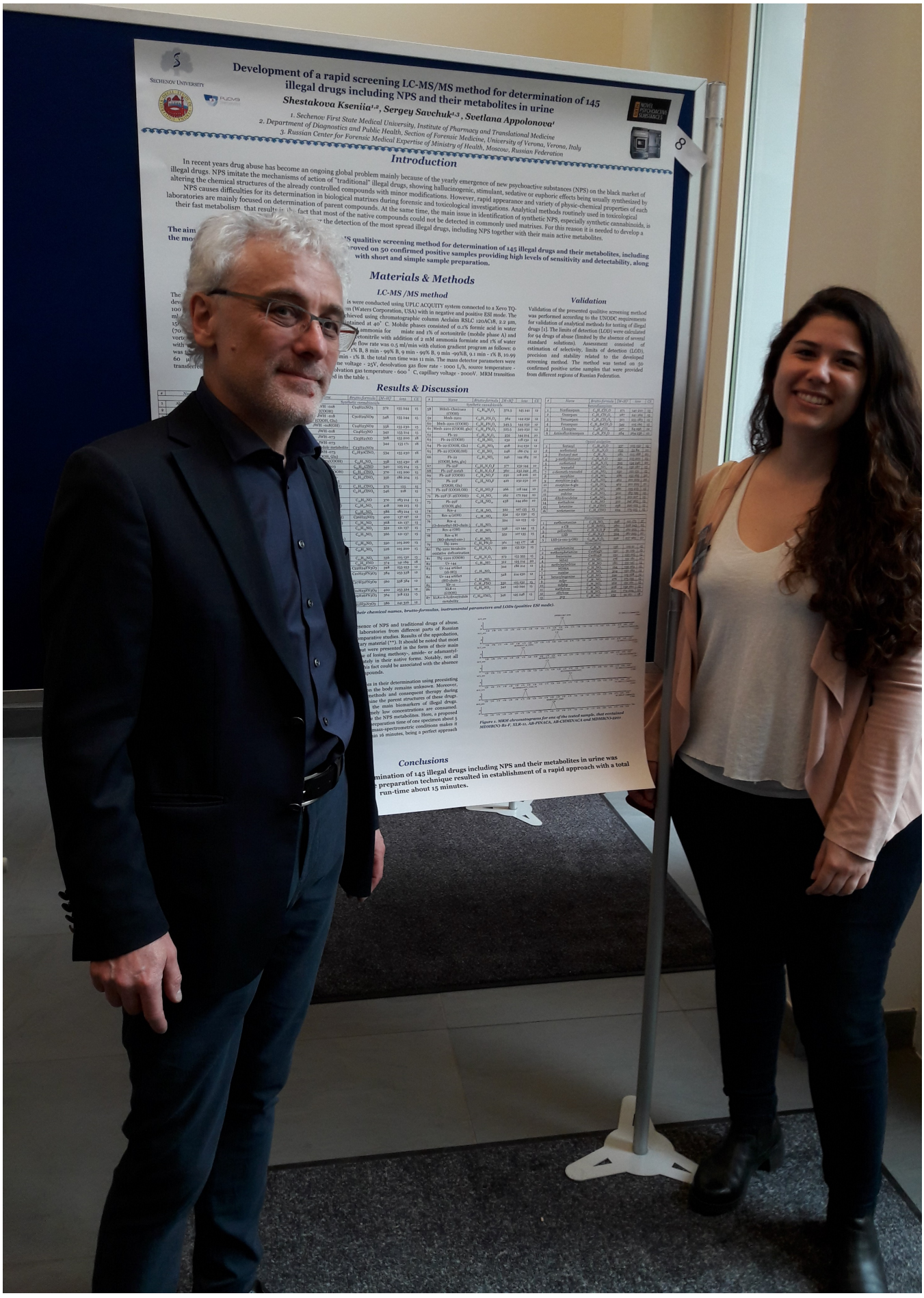

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VI International Conference on Novel Psychoactive Substances (NPS), Maastricht, The Netherlands

NEW GC-MS APPROACH FOR ANAESTHETICS DETECTION IN COMPLICATED BIOLOGICAL MATRICES. DETECTION OF CARFENTANYL IN PUTRID TISSUE, A CASE STUDY

INTRODUCTION

- Last year in Russia, approx. 0.03% from total seized drugs were fentanyl and/or its analogues[1]
- According to results (2012-2018) from the Russian forensic and toxicology laboratories, 1-5% of drugs found in biological samples (mostly urine) were fentanyls. This happened in cities which have common borders with European countries, such as Pskov, Saint-Petersburg and Kaliningrad. In Yekaterinburg, the capital of Ural region, this percentage is 2. In other regions this ratio was close to zero[1]
- In the European Union, over 70% of the 1.600 or so seizures of new synthetic opioids reported in 2016 were fentanyl derivatives as reported by the EMCDDA. They accounted for the 2.3% of the total number of seizures[2]

OBJECTIVE: Creation of the a new GC-MS approach for carfentanyl and related compounds detection in complicated biological matrices

MATERIALS AND METHODS

LLE

To 5mL of biological samples (blood, urine and lysis products) 10mL of distilled water and 0.5mL of a saturated solution of NaOH were added and extracted with 10mL of hexane. The extract was evaporated and reconstructed in 150μL of acetonitrile

SPE

Biological fluids were extracted on Bond Elute Certify cartridges. Protein fragments of homogenate were precipitated with TCA and then underwent the same treatment as biological fluids

RESULTS & DISCUSSION

GC-MS

- For sensitive FULL SCAN Confirmation step of SIM fentanyls detection→ creation of SIM methods including minor and isotopic ions for each target substances
- Opportunity to get full spectra from SIM results for individual target substances (SIM-SPECTRA) suitable for AMDIS MS lib identification
- SIM-SPECTRA confirmation step after standard GC-MS-SIM screening

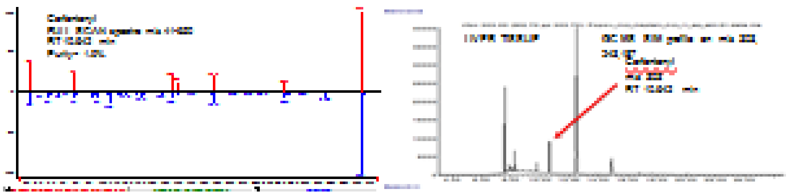

Figure 1. Full Scan and SIM spectra of carfentanyl in the liver tissue. Sample treated with LLE after heptane extraction with acid cleanup re-extraction step. The cleanness of the obtained spectra is shown in this figure.

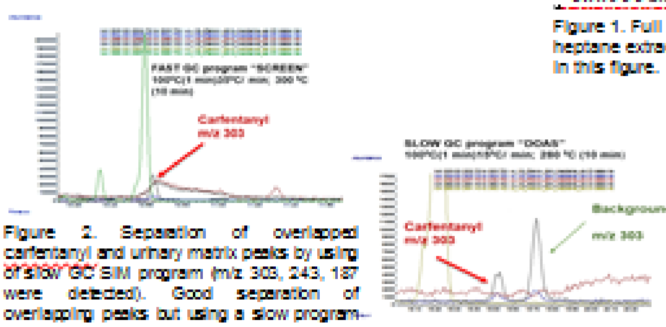

Figure 2. Separation of overlapped carfentanyl and urinary matrix peaks by using of slow GC SIM program (m/z 303, 243, 187 were detected). Good separation of overlapping peaks but using a slow program has decreased carfentanyl intensity (peaks are not so sharp)

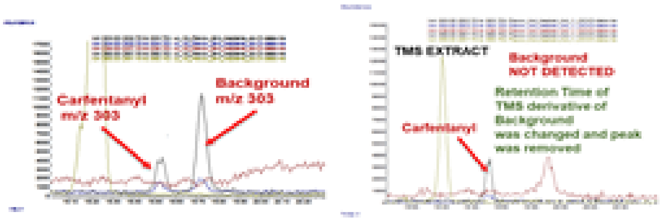

Figure 3. Removal of the urinary matrix peak (closed to carfentanyl) containing m/z 303 ion by derivatization of heptane extract with BSTFA (Carfentanyl does not form the TMS derivative)

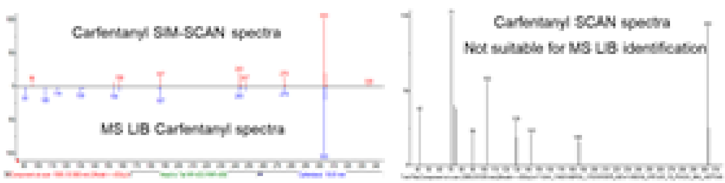

Figure 4. GC-MS confirmation of fentanyls by SIM identification in putrid liver tissue. For sensitive FULL SCAN confirmation step of SIM fentanyls detection SIM methods were created including minor and isotopic ions for each target substance. It was an opportunity to get full spectra from SIM results for individual target substances (SIM-SPECTRA) suitable for AMDIS MS lib identification. SIM-SPECTRA confirmation step was used after standard GC-MS-SIM screening

One segment SIM-SPECTRUM fentanyls method for Shimadzu GC-MSQP 2010 Ultra and Agilent 5975-77

SIM	303 M+	304	305	335	275	247	243	187	154	158	276	244	105	0.00	0.00
SIM	259 M+	260	261	230	216	203	215	202	172	159	160	145	146	110	257
SIM	245 M+	189	188	132	131	130	118	96	91	93	77	79	0.00	0.00	0.00

m/z 303 M+ Carfentanyl, RT=10.64 min. * - locked on diphenylamine (5.54 min)
m/z 257 3-MF; Isotope 1 RT= 9.69 min; Isotope 2 RT= 9.98 min
m/z 245 M+ fentanyl, RT=9.58 min
LOD: 170 ng/ml SCAN mode; 35 ng/ml for SIM and SIM SPECTRA mode
No losses of sensitivity of GC-SIM detection using SIM-SPECTRUM method were observed

CONCLUSION

- ✓ The best carfentanyl S/N was at the heptane extraction of "liquid around tissue" (LLE with basic hydrolysis). This has proved a better sample treatment than SPE for putrid biological samples
- ✓ Addition of matrix ions in SIM screening method lead to an increase in specificity of carfentanil CG-MS detection
- ✓ The use of fentanyls GC-MS SIM SPECTRA allows to confirm the presence of target substances with 10 times high sensitivity

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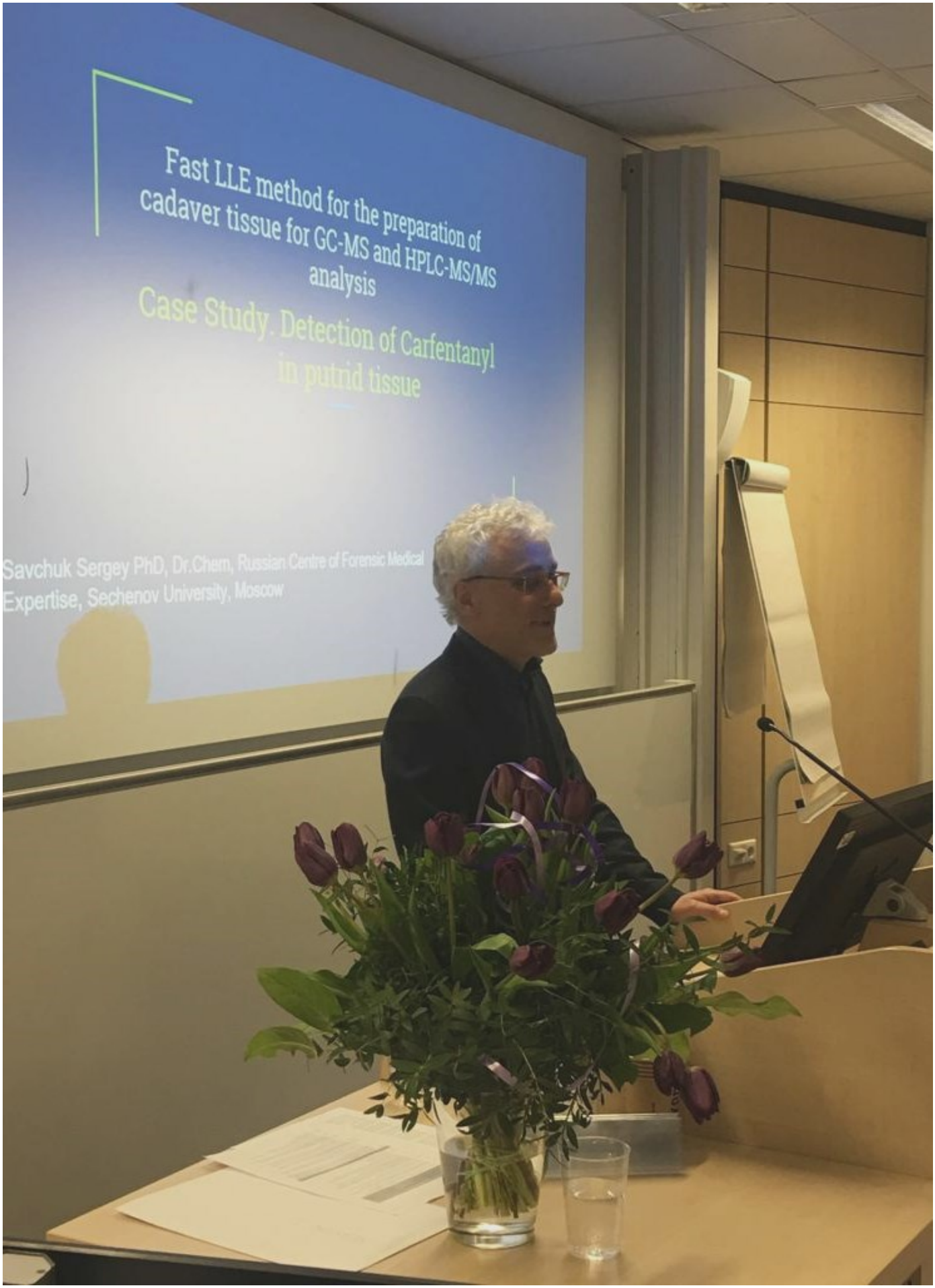

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Development of a rapid screening LC-MS/MS method for determination of 145 illegal drugs including NPS and their metabolites in urine

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2. Department of Diagnostics and Public Health, Section of Forensic Medicine, University of Verona, Verona, Italy
3. Russian Center for Forensic Medical Expertise of Ministry of Health, Moscow, Russian Federation

Introduction

In recent years drug abuse has become an ongoing global problem mainly because of the yearly emergence of new psychoactive substances (NPS) on the black market of illegal drugs. NPS imitate the mechanisms of action of "traditional" illegal drugs, showing hallucinogenic, stimulant, sedative or euphoric effects being usually synthesized by altering the chemical structures of the already controlled compounds with minor modifications. However, rapid appearance and variety of physicochemical properties of each NPS causes difficulties for its determination in biological matrices during forensic and toxicological investigations. Analytical methods routinely used in toxicological laboratories are mainly focused on determination of parent compounds. At the same time, the main issue in identification of synthetic NPS, especially synthetic cannabinoids, is their fast metabolism, that results in the fact that most of the native compounds could not be detected in commonly used matrices. For this reason it is needed to develop a screening method that will cover the detection of the most spread illegal drugs, including NPS together with their main active metabolites.

The aim of this study was to develop a fast LC-MS/MS qualitive screening method for determination of 145 illegal drugs and their metabolites, including the most popular NPS, in urine. The method was approved on 50 confirmed positive samples providing high levels of sensitivity and detectability, along with short and simple sample preparation.

Materials & Methods

Sample preparation

The dilute-and-shoot method for the developed screening procedure was as follows: 100 µl of urine was transferred to a plastic 1.5 ml eppendorf vial with following addition of 150 µl of mixture containing acetonitrile:water (70:30). The resulted solution was after vortexed for 5 seconds and centrifuged 5 min with 15000 rpm. After 40 µl of supernatant was transferred to a new vial with addition of 60 µl of distilled water, vortexed and transferred to a vial for LC-MS analysis.

LC-MS /MS method

The LC-MS analysis were conducted using UPLC ACQUITY system connected to a Xevo TQ-S micro IVD System (Waters Corporation, USA) with ion negative and positive ESI mode. The separation was achieved using chromatographic column Acclaim RSLC 120 AQ (C), 2.2 µm, 100 x 2.1 mm maintained at 40° C. Mobile phases consisted of 0.1% formic acid in water with addition of 2 mM ammonium formiate and 0.1% of acetonitrile (mobile phase A) and 0.1% formic acid in acetonitrile with addition of 2 mM ammonium formiate and 0.1% of water (mobile phase B). The flow rate was 0.5 ml/min with elution gradient program as follows: 0 min - 1% B, 1 min - 1% B, 8 min - 99% B, 9 min - 99% B, 9 min - 99% B, 9.1 min - 1% B, 10.99 min - 1% B, 11 min - 1% B. the total run time was 11 min. The mass detector parameters were as follows: cone voltage - 25V, desolvation gas flow rate - 1000 L/h, source temperature - 148 ° C, desolvation gas temperature - 600 ° C, capillary voltage - 3000 V. MRM transition are presented in the table 1.

Validation

Validation of the presented qualitive screening method was performed according to the UNODC requirements for validation of analytical methods for testing of illegal drugs [3]. The limits of detection (LOD) were calculated for 94 drugs of abuse (limited by the absence of several standard solutions). Assessment consisted of estimation of selectivity, limits of detection (LOD), precision and stability related to the developed screening method. The method was tested on 50 confirmed positive urine samples that were provided from different regions of Russian Federation.

Results & Discussion

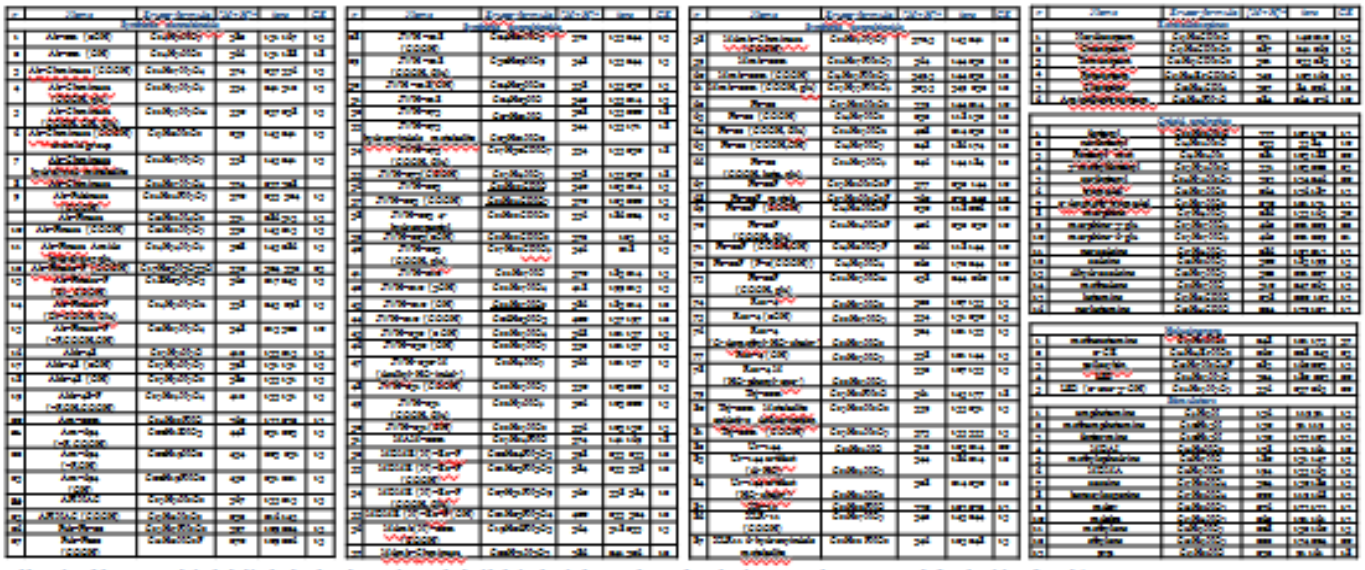

Table 1. List of the compounds included in the developed screening method with their chemical names, brutto-formulae, instrumental parameters and LODs (positive/negative mode).

The method was tested on 50 proved positive cases, associated with the presence of NPS and traditional drugs of abuse. (figure 1). For this purpose, urine samples were provided by toxicological laboratories from different parts of Russian Federation, being before confirmed to be positive during routine multicentral comparative studies. Results of the approbation, including chromatograms for all analyzed samples are presented in the supplementary material (**). It should be noted that most of the synthetic cannabinoids, were not identified in their native structures, but were presented in the form of their main derivatives, mostly in the form of carboxylic moieties, produced by the cleavage of linking methoxy-, amido- or adamantyl-groups. Alternately, most of the synthetic cathinones were detected predominately in their native forms. Notably, for all compounds, determined by GC-MS were identified by the developed approach. This fact could be associated with the absence of the preliminary extraction and extremely low concentration of the analyzed compounds.

The constant rise of new "designer drugs" poses a critical issue due to the difficulties in their determination using preexisting analytical methods. As a result, the full extent of the effects of these new drugs on the body remains unknown. Moreover, such limitations are critical in the identification of appropriate detoxification methods and consequent therapy during overdoses. Most NPS quickly metabolize and so, it is usually impossible to determine the parent structures of these drugs. Therefore, investigations of NPS metabolism should be conducted to evaluate the main biomarkers of illegal drugs. Compared to "traditional narcotics", NPS have a higher potency, and so, extremely low concentrations are consumed. Consequently, highly sensitive analytical methods should be developed to determine the NPS metabolites. Here, a proposed 'dilute-and-shoot' shoot method is rapid, showing high sensitivity with total sample preparation time of one specimen about 5 minutes. The developed LC-MS/MS method using provided chromatographic and mass-spectrometric conditions makes it possible to determine main drugs of abuse including NPS and their metabolites within 16 minutes, being a perfect approach for preliminary drug screening analysis.

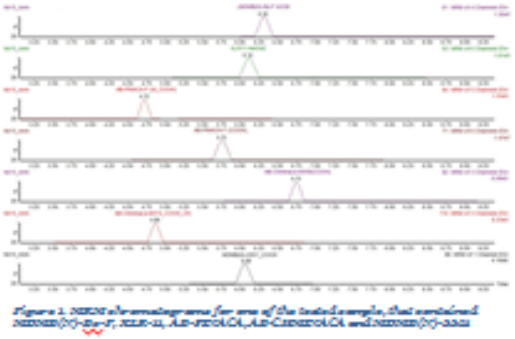

Figure 1. XIC chromatograms for one of the tested samples, that contained MDMB(N)-Bz-F, XLR-11, AB-FUBICA, AB-CHMFUBICA and MDMB(N)-2201

Conclusions

A sensitive and fast LC-MS/MS method for determination of 145 illegal drugs including NPS and their metabolites in urine was developed. The utilization of a diluting-shoot sample preparation technique resulted in establishment of a rapid approach with a total run-time about 15 minutes.

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FAST LLE METHOD FOR THE PREPARATION OF CADAVER TISSUE FOR GC-MS AND HPLC-MS/MS ANALYSIS. *CASE STUDY. DETECTION OF CARFENTANYL IN PUTRID TISSUE*

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2. Sechenov First Moscow State Medical University, Moscow, Russian Federation
3. Russian Centre for Forensic Medical Expertise of Ministry of Health, Moscow, Russian Federation

INTRODUCTION

- Last year in Russia, approx. 0.03% from total seized drugs were fentanyl and/or its analogues[1]
- According to results (2012-2018) from the Russian forensic and toxicology laboratories, 1-5% of drugs found in biological samples (mostly urine) were fentanyls. This happened in cities which have common borders with European countries, such as Pskov, Saint-Petersburg and Kaliningrad. In Yekaterinburg, the capital of Ural region, this percentage is 2. In other regions this ratio was close to zero[1]
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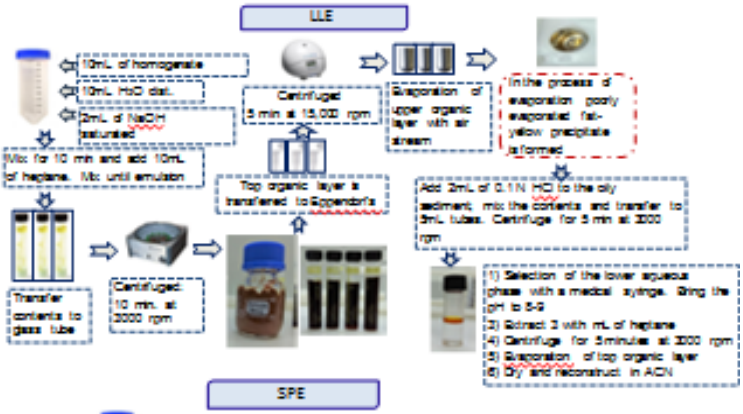
OBJECTIVE: Creation of the a new GC-MS approach for carfentanyl and related compounds detection in complicated biological matrices

MATERIALS AND METHODS



RESULTS & DISCUSSION

GC-MS

- For sensitive FULL SCAN Confirmation step of SIM fentanyls detection→ creation of SIM methods including minor and isotopic ions for each target substances
- Opportunity to get full spectra from SIM results for individual target substances (SIM-SPECTRA) suitable for AMDIS MS lib identification
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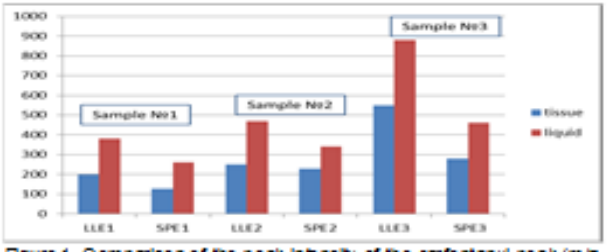

Figure 1. Comparison of the peak intensity of the carfentanyl peak (m/z 303) in the tissues and in the liquids around the tissues (liquid) in SIM mode. The intensity from the "liquids" was higher than the tissues. In addition, for all cases LLE was better than SPE as for tissues and as for "liquids".

QTOF

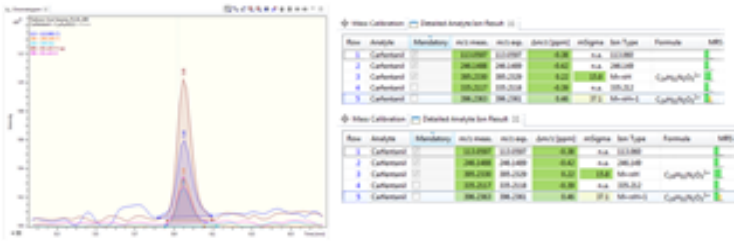

Figure 2. QTOF confirmation of Carfentanyl in tissue

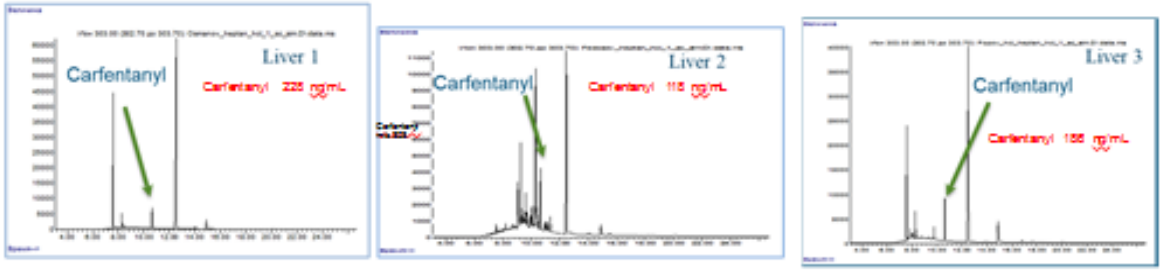

Figure 3. QTOF confirmation: Results of Carfentanyl SIM determination in three liver samples using the "liquid around tissue" m/z187, 243,303.

CONCLUSION

- ✓ The best carfentanyl S/N was at the heptane extraction of "liquid around tissue" (LLE with basic hydrolysis). This has proved a better sample treatment than SPE for putrid biological samples
- ✓ Addition of matrix ions in SIM screening method lead to an increase in specificity of carfentanil CG-MS detection
- ✓ The use of fentanyls GC-MS SIM SPECTRA allows to confirm the presence of target substances with 10 times high sensitivity

1. Zaitsina O.L., V.V.Shilov, I.N.Lodygin, I.N.Grigoriev. Features of the detection of fentanyl derivatives in the urine by gas and liquid chromatography coupled mass spectrometry. Toxicological Bulletin - 2016 N3, P.46-47 (Russian language)
2. European Monitoring Centre for Drugs and Drug Addiction. European Drug Report 2018: Trends and Developments

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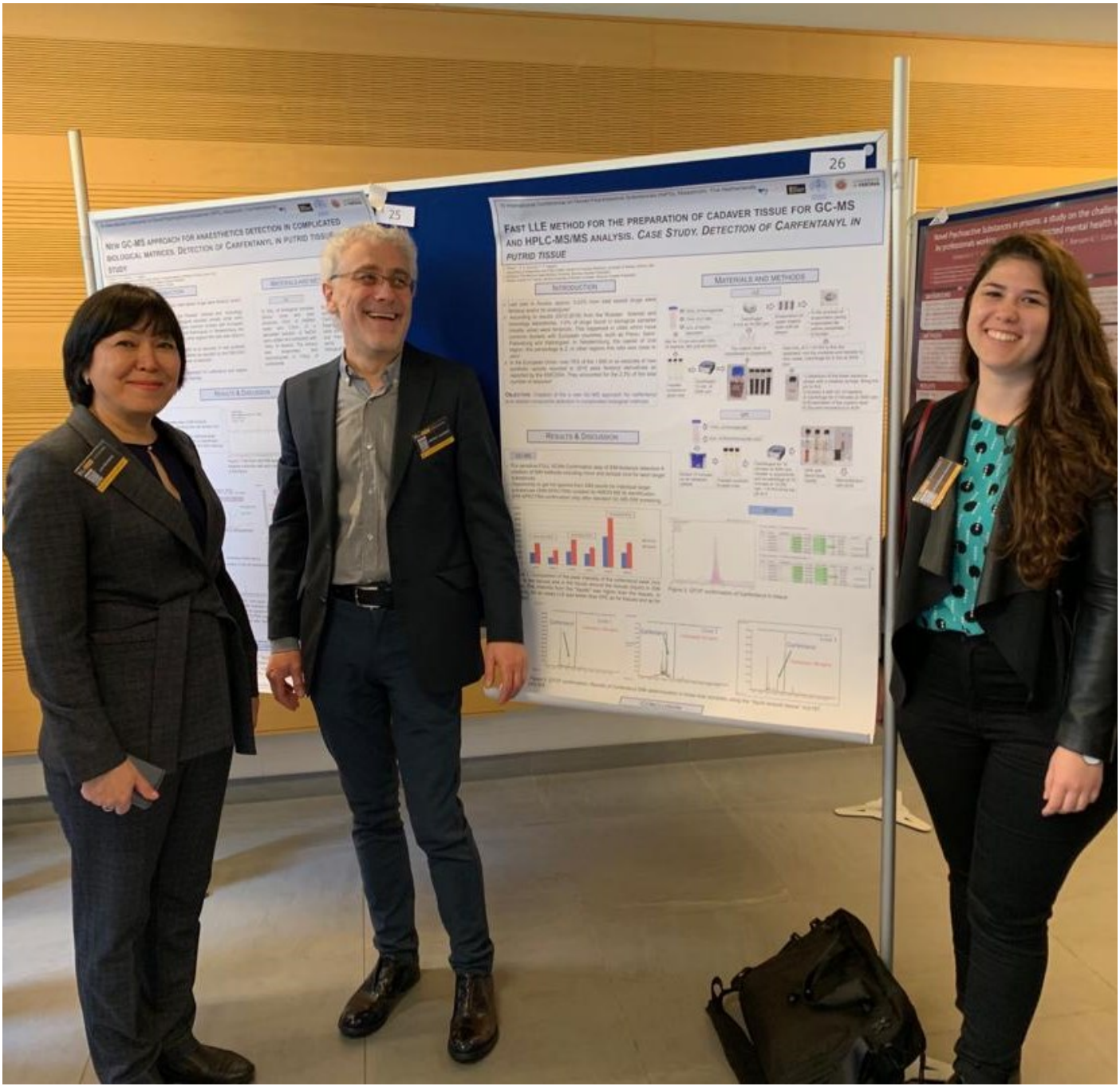

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Laboratory diagnostics of acute poisoning with 3,4-methylenedioxy-α-pyrrolidinohexanophenone (3,4-MDPHP) in the Sverdlovsk region

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Introduction

In recent years, synthetic cathinones in Russia occupy a leading position in the etiology of acute drug poisoning. In 2018, cases of acute poisoning of 3,4-methylenedioxy-α-pyrrolidinohexanophenone (**3,4-MDPHP**) were detected in the Sverdlovsk region. 3,4-MDPHP is a structural analogue of MDPV and has a psychostimulant effect. Upon admission to the intensive care unit and intensive care, patients using MDPHP experienced psychosis, vomiting, hyperthermia.

The aim of this study is the identification of metabolites of 3,4-MDPHP in the biomaterial of patients with acute poisoning.

Methods

The study was conducted on clinical blood and urine specimens, collected from patients admitted to the intensive care unit of Sverdlovsk Regional Poison Treatment Centre, from January to October 2018.

Sample preparation

Urine and blood samples were collected at the time of presentation to the hospital.

Urine samples. For the cleavage of glucuronides 0.5 ml phosphate buffer (pH 4,8) and 25 µL β-glucuronidase were added to 1.0 ml of urine and the sample was incubated at 55 °C for one hour. The mixture was shaken, centrifuged, the supernatant was transferred to a pre-prepared mixed phase solid phase extraction cartridge SampleQ Evidex (200 mg / 3 ml) (Agilent Technologies). To urine samples without hydrolysis and after hydrolysis, 2 ml of 1/15 M phosphate buffer (pH 4.8) was added. The contents of the vials were centrifuged at 3000 rpm for 10 minutes, the centrifugate was separated from the sediment and added to an SPE cartridge.

The column was preconditioned with 2 ml of methanol and 2 ml of 1 / 15M phosphate buffer (pH 4.8). The column was washed with 1 ml of 1 / 15M phosphate buffer (pH 4.8) and 1 ml of 10% methanol. The base fractions were eluted using 2 ml of methylene chloride / isopropanol / 25% ammonium hydroxide (2 / 1 / 0,1 v/v). The second fractions were eluted using 2 ml of n-hexane / ethyl acetate (2/1 v/v). The eluate was evaporated to dryness and reconstructed in 100 µl of the mobile phase for LCMS or 100 µl of ethyl acetate for GCMS.

Blood samples. Acetonitrile (500 µL) and zinc sulfate (1.0 g) were added to 500 µl of plasma, obtained by centrifuging whole blood at 2500 rpm. After extraction and centrifugation, supernatant was blown to dryness with air at room temperature and reconstructed.

Assay

A comprehensive screen for synthetic cathinone and its metabolites was carried out using gas chromatography/mass spectrometry and liquid chromatography/high-resolution tandem mass spectrometry.

GC–MS analysis of the extracts dissolved in ethyl acetate was performed with gas chromatograph equipped with single quadrupole mass selective detector Shimadzu GCMS-QP2010 Ultra (Shimadzu Corporation, Japan) with use DB-17 ms column (30m×0.25mm×0.33um).

Biotransformation products were analyzed using **HPLC/ HRMS** of the Agilent 1290 Infinity II chromatographic system coupled with the Agilent 6545 Q-TOF LC/MS tandem quadrupole time-of-flight mass spectrometry detector (Agilent Technologies, USA).

Results and discussion

Most of the ingested dose of 3,4-MDPHP is excreted unchanged in the urine. A large peak of the unchanged substance was detected on the chromatograms when screening plasma and urine using the GC-MS method (Fig. 1, 2).

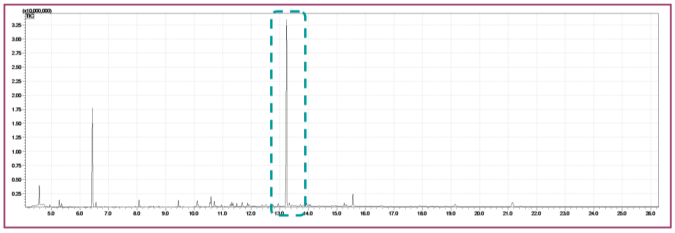

Fig.1 Total ion chromatogram of the urine of a patient with acute poisoning with 3,4-MDPHP

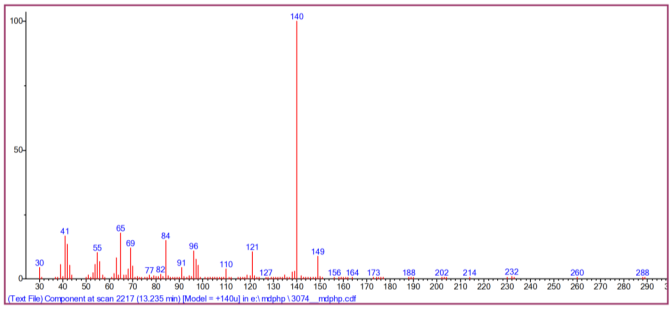

Fig.2 Mass spectrum of 3,4-MDPHP

Search of metabolites was carried out on the assumption that the metabolism of 3,4-MDPHP, as well as the fragmentation of molecules, would be similar to 3,4-MDPV and other cathinones. The main metabolite is the product of oxidation of the unchanged compound along the pyrrolidine ring at position 2 with the formation of a lactam cycle (Fig. 3). HRMS analysis indicated the protonated molecule $[M+H]^+$ with *m/z* 304.1539, which corresponds to the compound with the formula $C_{17}H_{21}NO_4$ (exact value 304.1543, accident error -0.4 ppm for sample). Pathways of fragmentation of the main metabolite of 3,4-MDPHP correspond to the directions of fragmentation of the oxo-metabolite 3,4-MDPV.

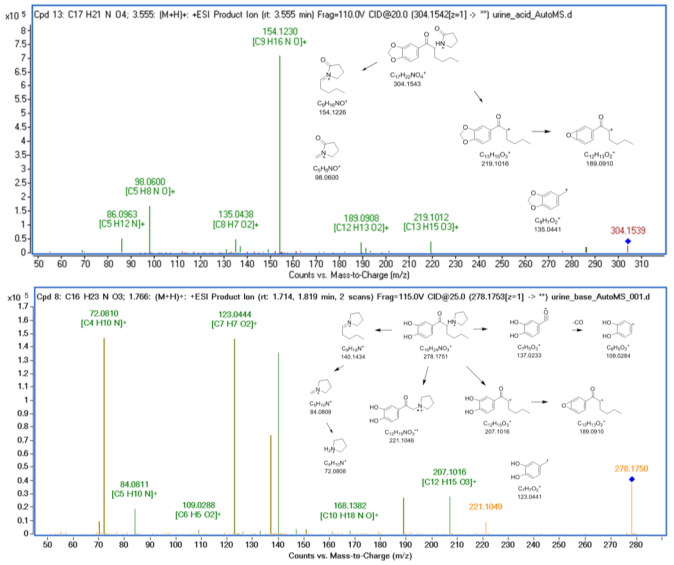

Fig 3. CID spectra of the main metabolites of 3,4-MDPHP

The second direction of biotransformation is associated with the dealkylation of the methylenedioxy substituent on the benzene ring, followed by methylation of the hydroxy group in position 3.

One of the obtained spectra showed a $[M+H]^+$ with *m/z* 278.1750, which corresponds to the compound $C_{16}H_{23}NO_3$ (Fig.3). The presence of the ions with *m/z* 140.1434, *m/z* 84.0808 and *m/z* 72.0808 indicated an unaltered pyrrolidine ring. The ions with *m/z* 123.0441, 137.0233 and 109.0284 indicates the loss of a carbon atom occurred in the methylenedioxy group.

Identified a range of metabolites obtained by opening the pyrrolidine ring with the formation of aliphatic aldehyde and its subsequent oxidation to carboxylic acid. In such compounds, ions with *m/z* 304.1543, 274.1438 and 228.1383 while maintaining signal with *m/z* 149.0233 confirm that all structural changes are focused on the pyrrolidine ring.

In addition, metabolites have been identified with simultaneous opening of the pyrrolidine ring and dealkylation of the methylenedioxy group. Another metabolic pathway of 3,4-MDPHP is associated with the reduction of the ketone group.

The main pathways of reactions I phase biotransformation are presented in Fig. 4.

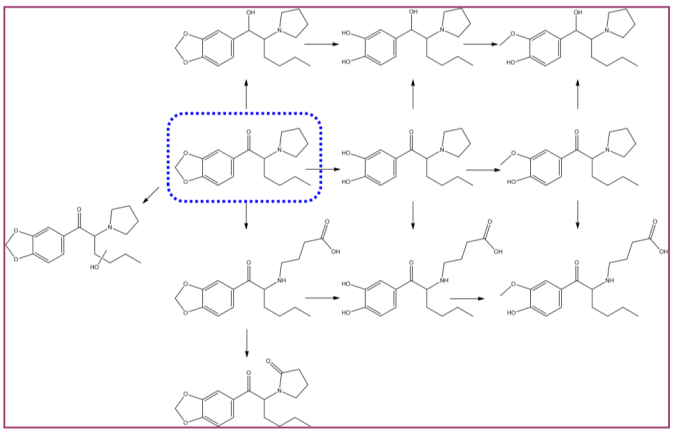

Fig 4. Metabolic pathway I phase proposed for 3,4-MDPHP in humans

Conclusions:

The main pathways of metabolism of 3,4-MDPHP were studied. Structural elucidation of the 3,4-MDPHP and its metabolites was carried out by identifying the characteristic production structures. Over 10 phase I and II metabolites of 3,4-MDPHP have been identified. The main pathways of metabolism are associated with O-demethylation, hydroxylation, reduction of the ketone group and conjugation with glucuronic acid. The obtained data can be used to detect and identify new psychoactive substances in the biomaterial of patients with acute poisoning.

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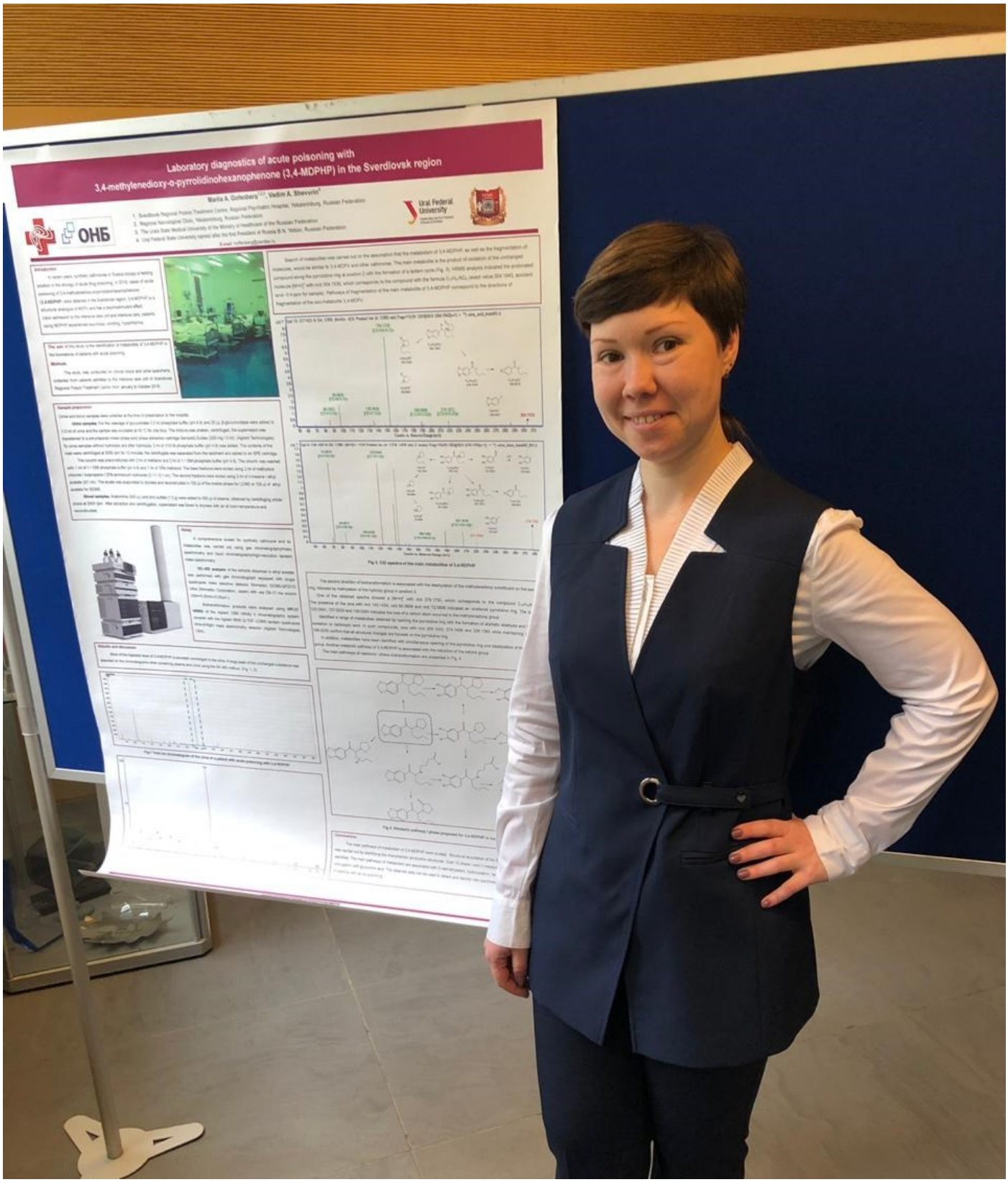

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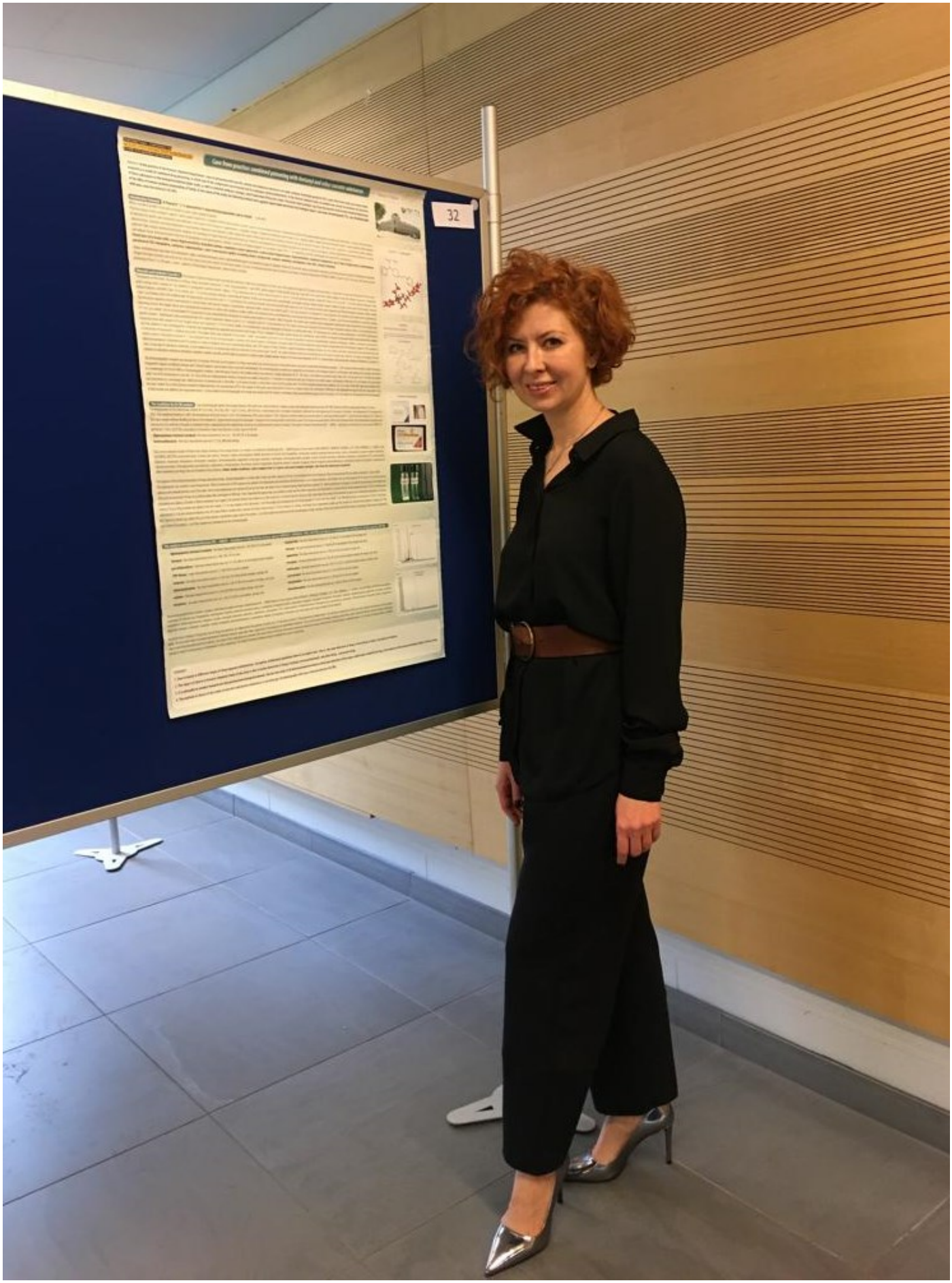

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Identification of psychoactive substances and their metabolites in human urine, blood, hair and nails in case of combined use of ADB-FUBINACA and alpha-PVP

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Introduction

Two men (D., 29-years-old and G., 32-years-old) were hospitalized in January 2018 in a state of acute intoxication after smoking a small amount of unknown to them "spice" and intranasal use of alpha-PVP. Because both of them suddenly felt badly, their behavior became inadequate, vomiting occurred, a girlfriend of one of them called an ambulance.

In addition to mental and behavioral disorders, the following intoxication symptoms were observed: nausea, vomiting, impaired coordination, tremor, mydriasis, tachycardia, tachypnea, hypertension, hyperthermia. Diuresis was reduced in both patients. Laboratory studies indicated leukocytosis, hyperglycemia, elevated aminotransferases. Venous blood gas analysis showed respiratory acidosis with elevated anion interval. The coagulation parameters were normal. An alcohol blood test was negative.

Patient D. by the time of hospitalization in a state of coma, he regained consciousness on the second day of treatment. On the third day of treatment, the following symptoms were observed in both patients: retardation, severe muscle weakness, drowsiness, slow thinking and dysarthria. A decrease in cognitive function was observed. Behavior, mental and somatic conditions of both men returned to normal after 7 days in the hospital after drug consumption.

The aim of the study was to identify markers of new psychoactive substances (NPS) that caused changes in the mental and somatic conditions of patients in biological samples (urine, blood, hair, nails).

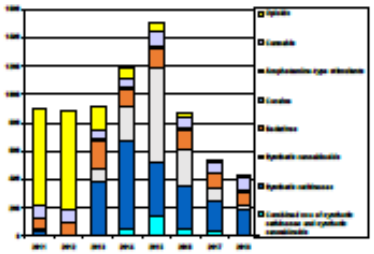

Fig.1 Trends in drug use among the examined persons in 2011-2018

Sample preparation

Samples of urine, blood, fingernails, hair on the head and armpits were taken on the first day of hospitalization.

- 3.0 ml of urine
- 1.5 ml of blood

Samples were hydrolyzed and extracted twice after adjustment to pH 8-9, then to pH 2-3 with heptane/ethyl acetate (7:1;v/v). Evaporated extracts were silylated with a mixture of BSTFA+1% TMCS / ethyl acetate (1:1;v/v) and analyzed by GC-MS or dissolved in acetonitrile and analyzed by LC-MS-MS.

- 100 mg of head hair
- 100 mg of armpit hair
- 30 mg of fingernails

Samples were rinsed 5 times with 1.5 ml of methanol for 15 min. Each of the washings was evaporated, reconstituted in 100 µl of acetonitrile and analyzed. After the methanol washings, the samples were cut in small fragments and sonicated for 4 hours with 3 ml of methanol. Finally, the extracts were evaporated, in 100 µl of acetonitrile and analyzed by GC-MS and LC-MS-MS.

GC–MS conditions

GC–MS analysis of prepared samples was performed with an Agilent 7890A gas chromatograph connected to a 5975C mono-quadrupole mass-selective detector (Agilent Technologies). The GC conditions were [4,5]: Rxi-5ms capillary column (30 m x 0.25 mm internal diameter, 0.25 lm film thickness, Restek); 280 °C interface temperature, 280 °C injector temperature; injection mode, splitless; injection volume, 1 µl; carrier gas (He), flow rate 1.2 ml/min; oven temperature program, initial temperature at 100 °C (1-min hold) followed by ramp at 25 °C/min up to 300 °C (7-min hold). The MS conditions were: ion source temperature, 230 °C; ionization mode, electron ionization (EI) at 70 eV; detection gain, 1,476 V; identification, scan mode; scan range, m/z 41–650; solvent delay, 3 min.

Fig 2. Laboratory for forensic chemistry, analytical equipment

LC-MS-MS conditions

LC-MS-MS analysis of prepared samples was performed using a Dionex UltiMate 3000 HPLC system coupled to an AmaZon speed Bruker mass spectrometer. Chromatographic separation was performed using a column Acclaim® RSLC 120 C18 2.2 µm, 120Å 2.1 x 100 mm (Dionex). For gradient elution, the mobile phases 2mM ammonium formate, 0.1% formic acid, 1% acetonitrile in deionized water (mobile phase A) and 2mM ammonium formate, 0.1% formic acid, 1% deionized water in acetonitrile (mobile phase B) were used with the time program: 0-1 min 1% eluent B, 1-8 min gradient up to 95% eluent B, 8-9 min 95% eluent B. Final conditioning for 2 min 1% eluent B. The flow rate was 0.5 ml/min. The column oven was set to 40 °C, the autosampler was set to 12 °C. The MS conditions were: capillary voltage, 4500V; nitrogen temperature (drying gas), 320 °C; nebulizing gas pressure, 29.3 psi. The detection was performed in the MS1, MS2, MS3 (full scan) mode; mass range, 70–800 m/z; using the Toxtuper_Custom.M method in a mode of simultaneous registration of positive and negative ions in the search windows of target substances [5].

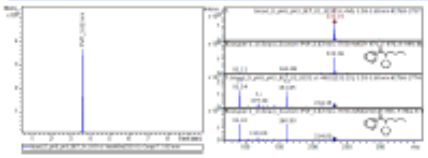

Fig.3 Alpha-PVP, LC-MS-MS extracted ion chromatogram and mass-spectra.

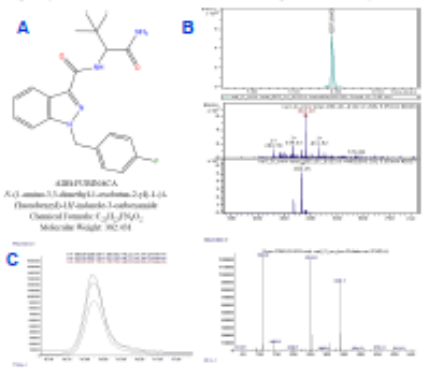

Fig.4 ADB-FUBINACA chemical structure (A), LC-MS-MS extracted ion chromatogram and mass-spectra (B), GC-MS extracted ion chromatogram and mass-spectra (C).

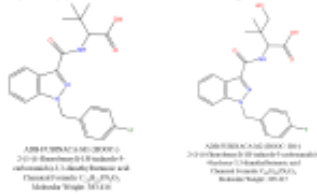

Fig.5 Biotransformation products of ADB-FUBINACA:
M1 - hydrolytic deamination; M2 - hydrolytic deamination + hydroxylation.
These metabolites are the same as the MDMB-FUBINACA metabolites.

Results

Alpha-PVP was found in all urine, blood, nails and hair samples.

ADB-FUBINACA was found in washings of fingernails and head hair and a little amount in sonicated nail extract. Parent substance was not found in blood and urine.

ADB-FUBINACA marker (-COOH) was found in blood and urine.

ADB-FUBINACA marker(-COOH -OH) was found only in urine.

Compounds which were found in urine, blood, nails and hair (patient D., 29-years-old)

Identified substances/metabolites	Intensity, mV				
	Nails	Head hair	Armpit hair	Blood	Urine
a-PVP	3.5 e7	2.2 e7	4.7 e6	3.0 e6	1.3 e8
ADB-FUBINACA	2.3 e8	3.4 e7	n/d	n/d	n/d
ADB-FUBINACA marker (-COOH)	n/d	n/d	n/d	6.8 e4	4.0 e7
ADB-FUBINACA marker(-COOH -OH)	n/d	n/d	n/d	n/d	4.7 e6

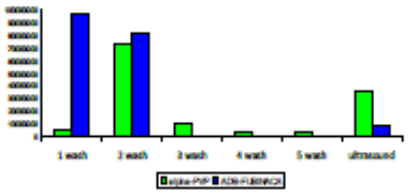

Fig. 6 Profile of compounds which were found in washings and in ultrasonic extract of the fingernails of D., 29-years-old . On the Y-axis the intensity of the chromatographic peaks are shown at the base ion.

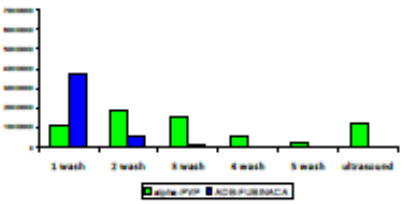

Fig. 7 Profile of compounds which were found in washings and in ultrasonic extract of the head hair of D., 29-years-old . On the Y-axis the intensity of the chromatographic peaks are shown at the base ion.

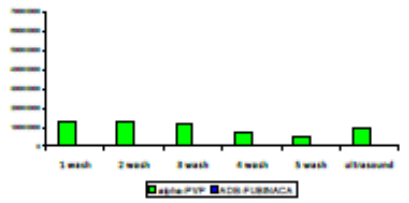

Fig. 8 Profile of compounds which were found in washings and in ultrasonic extract of the armpit hair of D., 29-years-old . On the Y-axis the intensity of the chromatographic peaks are shown at the base ion.

Conclusions

Consumption markers of ADB-FUBINACA and alpha-PVP were found in biological samples of patients.

ADB-FUBINACA was detected only on surface of head hair and fingernails due to passive exposure while smoking, but not detected in cortex, as well as in armpit hair because it was not a systematic "herbal blend" consumption. Only metabolites of ADB-FUBINACA were found in urine and blood due to almost complete biotransformation in the human body.

alpha-PVP was detected both on surface and in cortex of hair and nails, because it was a systematic consumption. Alpha-PVP and its metabolites were found in blood and in urine.

Each of these new psychoactive substances in itself has powerful hallucinogenic properties. Probably the cause of severe neuropsychiatric and somatic disorders was the combined effect of ADB-FUBINACA and alpha-PVP.

References

1. Drummer O.H. Chromatographic screening techniques in systematic toxicological analysis. Review. //Journal of Chromatography B. 1999. V. 733. pp. 27 – 45
2. Mass Spectral Library SUDMED_2235_AMDISLIB/NISTLIB/AGSLIB_20190217. Editor and supervisor Pechnikov A.L. Available at: http://www.sudmed.ru/index.php?showtopic=6924&st=555 (Accessed Feb 2019).
3. Maurer H.H., Pfleger K., Weber A.A. (2011) Mass Spectral and GC Data of Drugs, Poisons, Pesticides, Pollutants and Their Metabolites. 4th, reserved and enlarged edition. Weinheim: WILLEY-VCH Verlag,V.1, pp. 4 - 21.
4. Rizvanova L.N., Savchuk S.A., Appolonova S.A. Study on optimization of chemical-toxicological analysis procedures in view of Federal Law of 13 July 2015 № 230-FZ entry into force. Narkologiya [Narcology], 2015, no. 10, pp. 72-86. (In Russian)
5. Savchuk S.A. Detection of synthetic cannabimimetics, illegal drugs, psychoactive substances and their metabolites in urine, hair and nails using liquid chromatography with mass spectrometric detection. Information letter. Narkologiya [Narcology], 2014, no. 8, pp. 42-52. (In Russian)
6. Savchuk S.A., Grigoryev A.M. Khromato-massspektrometricheskiy analiz v narkologicheskoi i toksikologicheskoi praktike [Chromatography Coupled with MassSpectrometry Analysis in Drug Control and Toxicology Practice]. Moscow: Lenand Publ., 2013. 224 p. (In Russian)
7. Uchiyama N., Matsuda S., Kawamura M., Kikura-Hanajiri R., Goda Y. Two new-type cannabimimetic quinolinyl carboxylates, QUPIC and QUCHIC, two new cannabimimetic carboxamide derivatives, ADB-FUBINACA and ADBICA, and five synthetic cannabinoids detected with a thiophene derivate a-PVT and an opioid receptor agonist AH-7921 identified in illegal products. //Forensic Toxicol. 2013. - DOI 10.1007/s11419-013-0182-9.

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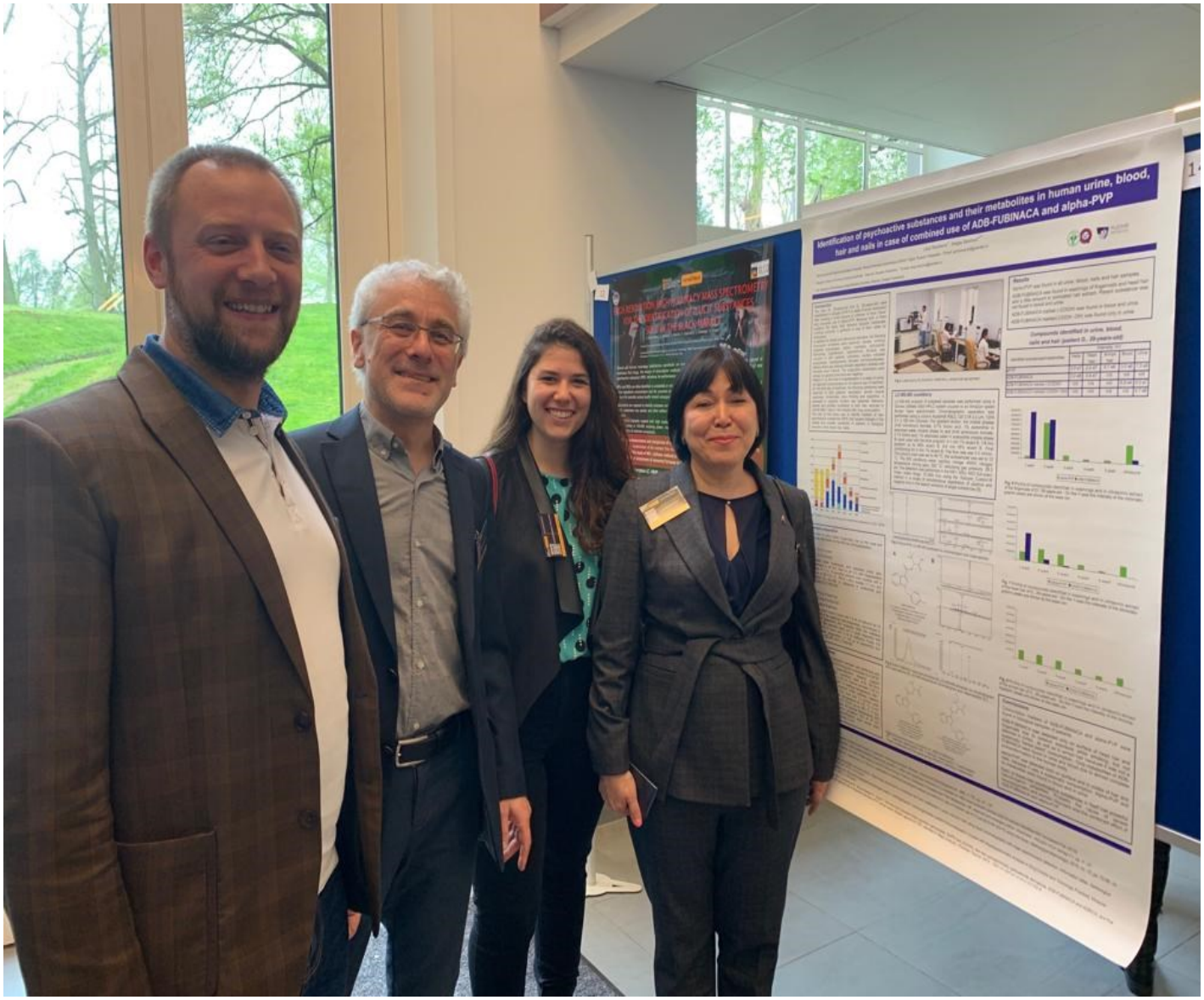

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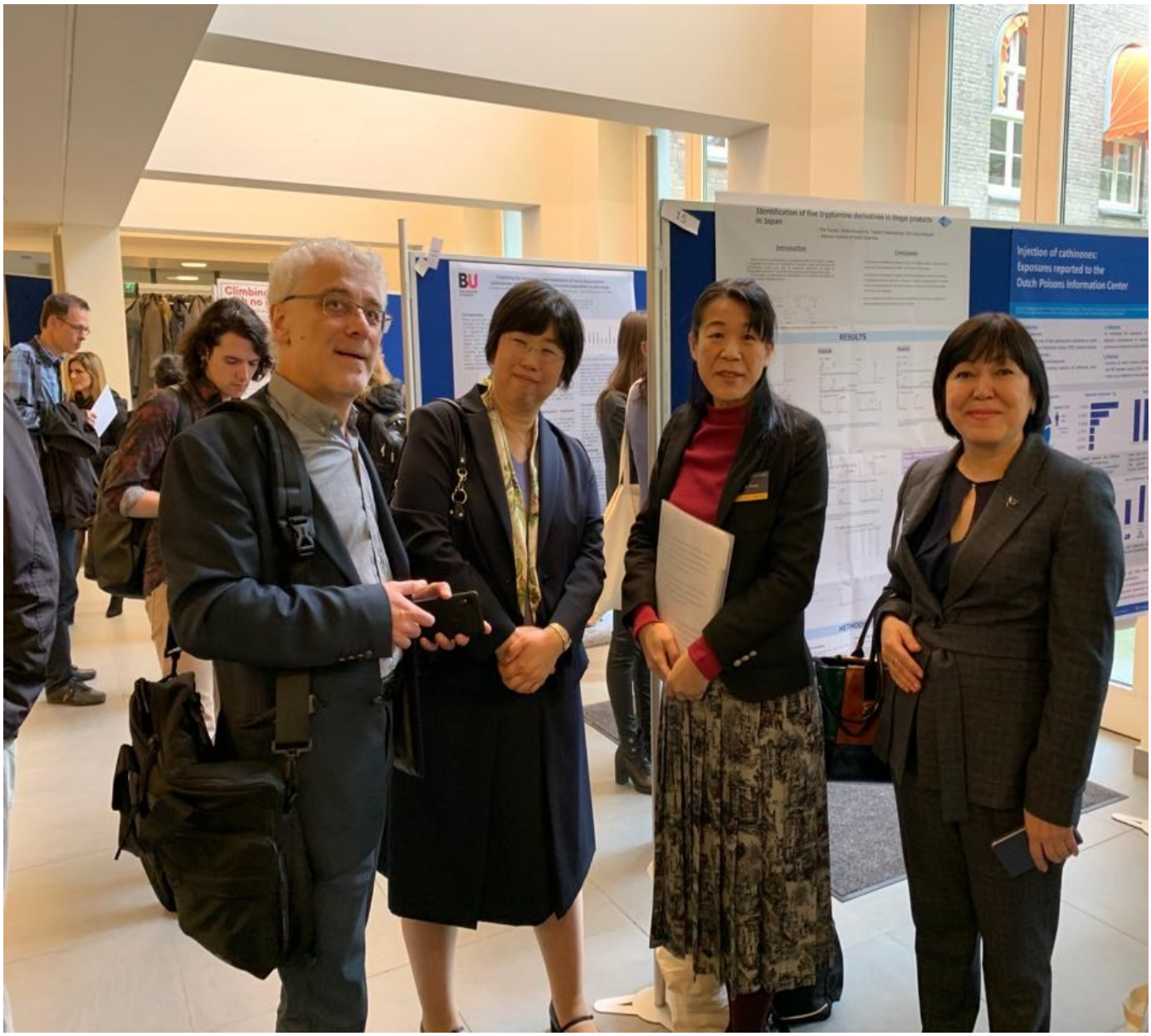

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