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V International Conference on
NOVEL PSYCHOACTIVE SUBSTANCES
23-24 OCTOBER 2017 IN VIENNA, AUSTRIA

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A rational toxicology-oriented approach to survey NPS

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Introduction

The worldwide spread of the so-called New Psychoactive Substances (NPS) imposes a drastic re-evaluation of the standard approaches of analytical toxicology, typically oriented to the determination of the most traditional drugs of abuse (i.e. cocaine, opiates, barbiturates, amphetamines, THC, etc.) In fact, the traditional strategy based on a preliminary screening using immunoassays followed by a "confirmation" of the presumptively "positive" samples using chromatographic techniques with mass spectrometric detection is clearly inadequate to face the great structural variability of NPS and, in many cases, their rapid metabolic conversion and short lifetimes in the biological fluids. The rapid change of the structures of NPS also hinders the study of their pharmaco-toxicological activity and metabolism, which are needed for their scheduling as Controlled Substances. Here, three alternative approaches to study NPS from different analytical and pharmacological points of view are presented.

APPROACH #1

Application of hair analysis to identify the use of synthetic cannabinoids

Experimental design

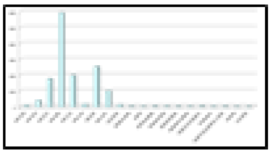
- Hair samples from 800 subjects with histories of cannabis use undergoing a toxicological screening (driving license)
- All subjects had tested negative for THC and THC-COOH in urine and hair in serial tests
- Hair samples were re-tested for synthetic cannabinoids: see results

Analytical methods and results

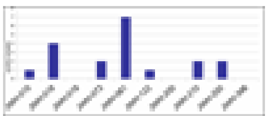
Urine samples were collected in the first 2 hours of hospitalization. The double liquid-liquid extraction (LLE) at pH 8-9 and at pH of 2-3 after alkaline hydrolysis [5, 6] was used for isolation of MDMB(N)-2201 metabolites from urine.

Sample ID	Age	Year	Results
1	21	2009	JWH-250: 3 pg/mg, JWH-073: 17 pg/mg, JWH-018: 6 pg/mg
2	40	2010	JWH-081: 16 pg/mg
3	33	2010	JWH-122: 125 pg/mg
4	29	2010	JWH-081: 1200 pg/mg
5	30	2010	JWH-018: 10 pg/mg
6	39	2011	JWH-081: 34 pg/mg
7	27	2011	JWH-018: 11 pg/mg
8	19	2011	JWH-210: 1.2 pg/mg
9	45	2011	JWH-081: 11 pg/mg
10	39	2011	JWH-018: 10 pg/mg, JWH-073: 13 pg/mg
11	21	2011	JWH-081: 27 pg/mg
12	30	2012	JWH-081: 14 pg/mg
13	33	2012	JWH-210: 1 pg/mg
14	23	2013	JWH-073: 4 pg/mg, JWH-081: 132 pg/mg, JWH-018: 4 pg/mg

Comparison between NPS in hair and in the market



NPS in hair



NPS in the market n=999

Discussion and Conclusions

- Compulsory toxicological testing may induce drug users to shift to NPS to hide their addiction.
- Toxicological data on NPS use do not fit well with the information from materials seized in the market

  E.g.: AM-2201 and AM-694 present in the market, not in the hair; JWH-018, frequently detected in the hair, rare in the market.
- Explanation (tentative): toxicological data do not reflect only the availability of the compounds in the market, but also the preference of the customers.

APPROACH #2

Evaluation of NPS incorporation pattern in hair and nails

Purpose: to check the embedding of NPS in hair and nails and the patterns of binding to the solid matrix (proof of concept)

Experimental design: analysis of NPS in hair and nail samples from a multi-drug user (case report, Ulan-Ude, Buryatia, Russia) using a sequential six stage elution with methanol and determination of NPS by GC-MS in each individual eluate.

Analytical methods

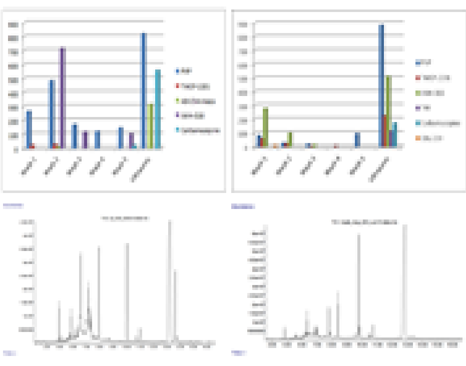
[see also poster: S. Savchuk, S. Appolonova, V. Salomatin, A. Gerasso, F. Tagliaro. Hair and nail analysis for NPS. Sequential rinsing for differentiation between surface drug contamination and drug content in the cortex]

Sample preparation: 70-100 mg of finely cut samples (hair or nails) washed 5 times with 2 ml of methanol, and finally incubated with further 3 ml of methanol and sonicated on an ultrasonic bath for 6 h. All extracts were evaporated to dryness, added 150 µl of ACN and analyzed by GC-MS (and LC-MS/MS).

Analyses

- GC-MS: Agilent 5975, 5977, full scan
- Column: HP-5MS 30m, 0.25 mm, 0.25 mm. Const Pressure 55 kpa
- Method 1: 100°C (1 min), 25°C /min, 300°C (10 min)
- Method 2: 100°C (1 min), 15°C /min, 280°C (30 min)

Results



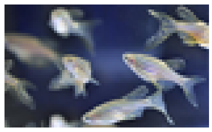
Discussion and Conclusions

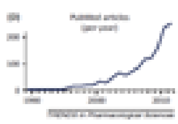
- Keratinized tissues (hair and nails) are ideal matrices to study the "use" of drugs in the population because of:
  - presence and stability of the parent drugs (no metabolism)
  - wide window of detectability (several months)
  - only chronic or repeated use gives positive results (occasional use is of less clinical concern).
- In particular, the present study confirms that many NPS are embedded in the hair and, for the first time, in nails.
- Nail analysis, tentatively, looks a good alternative to hair testing, since loosely bond compounds, reasonably reflecting external contamination of the samples, can be almost completely removed, differently from hair, by serial washings.

APPROACH #3

Zebrafish as a model to study NPS

Zebrafish (Danio rerio) is a new popular model organism in biomedical research. The utility of both adult and larval zebrafish in neuroscience has grown in the past decades because of its high physiological and genetic homology and behavioural similarity to humans.





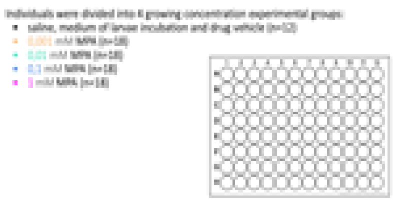
Purpose: to check the suitability of the Zebrafish model for a preliminary rapid screening of the NPS appearing in the market, before a formal pharmaco-toxicological testing in mammals.

Methods

Wild type zebrafish larvae (aged 5 days) were placed into a 96 multiwell plate in the DanioVision Observation Chamber and locomotory activity was recorded and analyzed by EthoVision XT software.

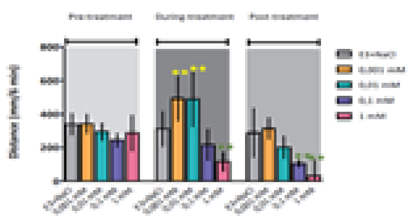
After a day of locomotory activity record, animals were treated with Methiopropamine (MPA), a thiophene ring-based structural analog of methamphetamine.

Locomotory activity was monitored for the 2 days.



Results

Analysis of the overall diurnal activity of larvae (AUC)



Discussion and Conclusions

The AUC analysis reveals an increase of total activity: 37%, 58%, 42% for the 0.001, 0.01 and 0.1 mM MPA, respectively. 1 mM MPA is associated with a reduction of locomotor activity of about 25%.

The effect persists the day after treatment, with an increased activity of 17% (0.001 mM) and of 12% (0.01 mM) in the lower doses and a reduction of 24% (0.1 mM) and of 54% (1mM) in the higer ones.

In conclusion, the larval Zebrafish model looks promising as a rapid, low cost and simple screening test for NPS (only 2 mg of drug needed, easy ethical approval).

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Hair and nail analysis for NPS. Sequential rinsing for differentiation between surface drug contamination and drug content in the cortex

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Introduction

Traditionally, the sample preparation of hair and nail analyses include two stages: washing followed by hydrolysis of the matrix or sonication and by quantitative analysis. In the present work, we have tested a selective sequential procedure including six steps in order to prevent "false positive" results from external contamination of the sample and hence to achieve a better reliability of the analysis for forensic toxicological applications.

Aim 1: differentiation between external contamination and content of NPS in the sample matrix (hair and nails) by comparing the presence of the target substances in 5 consecutive methanol washings from cut hair and nails, and in 6- hour ultrasonic methanol extracts following the washings.

Aim 2: study of the reproducibility of NPS distribution by comparing the results obtained from hair and nails collected from hands, legs and feet of the same person.

Sample Collection

20-100 mg of hair and 10-40 mg of nails were taken from the patients of narcology clinics in Northern region of Russia.

The concentrations of the studied NPS were in the range 1.0 -3.4 ng/mg.

Sample preparation

Samples were cut in small fragments and rinsed on vortex 5 times with 0.5 ml of methanol for 30 s. All wash fluids were evaporated to dryness, reconstituted in 150 µl of acetonitrile and analyzed. After the methanol washings, further aliquots of 3 ml of methanol were added to the samples and sonicated for 6 hours. Finally, the extracts were evaporated, reconstituted and analysed as described above.

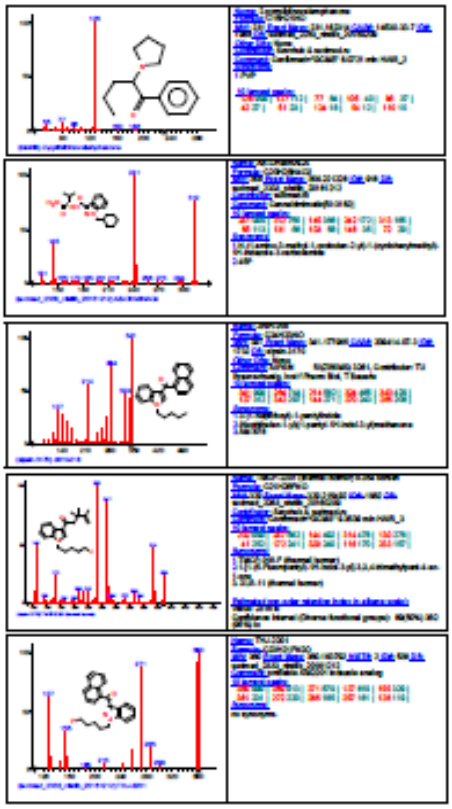

Fig. 1 Mass-spectra of new psychoactive substances which were found in washings and in ultrasonic extracts [2]

Methods

- The GC-MS analyses were performed in full-scan mode (41-650 amu) on a model 7890B 5977 MSD fitted with a HP-5MS column (30 m × 0.25 mm × 0.25 µm, Agilent Technologies). Method 1 "SCREEN": 100ºC (1 min),35 ºC /sec, 300ºC(10 min). Method 2 "DOAS": 100ºC (1 min),15 ºC /sec, 280ºC(30 min)
- The LC-MS/MS analyses were performed on a Toxtyper instrument (Bruker Daltonics, Bremen) consisting of a Ultimate 3000 HPLC (Dionex) with an Acclaim® RSLC 120 C18 column (2.2 µm, 120A 2.1 × 100 mm, Dionex) coupled to a Bruker ion trap mass spectrometer .

1. Hair results

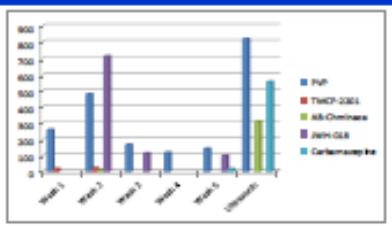

Fig. 2 The profile of compounds which were found in washings and in ultrasonic extract of the hair of the patient B-VA. On the Y-axis the intensity of the chromatographic peaks are shown at the base ion. CBL-2201 was not confirmed because the spectrum was not clear.

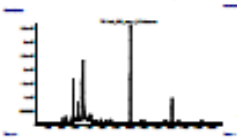

Fig. 3 The GC-MS TIC profile of methanolic extract of "HAIR wash 2".

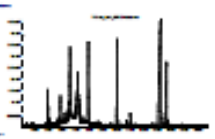

Fig. 4 The GC-MS TIC profile of ultrasonic extract of hair.

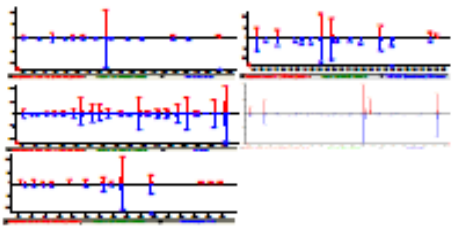

Fig. 5 Mass-spectra of psychoactive substances which were found in washings and in ultrasonic extract of hair. The old cannabimimetic JWH-018 was detected along with AB-CHMINACA, TMCP-2201, THJ-2201 and PVP.

2. Results of finger nails

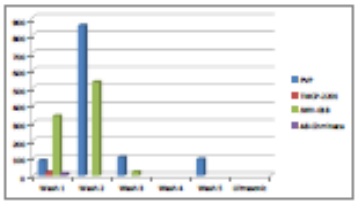

Fig. 6 The profile of compounds which were found in washings and in ultrasonic extract of the finger nails of the patient B-VA. On the Y-axis the intensity of the chromatographic peaks are shown at the base ion.Target substances were not found in ultrasonic extract. (Perhaps due to problem with sample preparation)

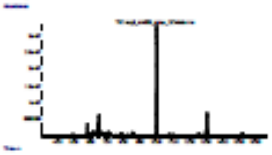

Fig. 7 The GC-MS TIC profile of methanolic "wash 2" of finger nails.

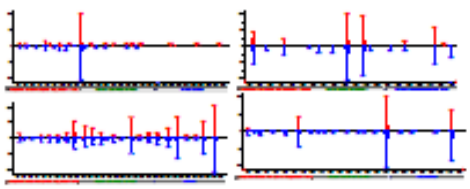

Fig. 8 Mass-spectra of psychoactive substances which were found in washings of the finger nails of the patient B-VA. The old cannabimimetic JWH-018 was detected along with TMCP-2201, THJ-2201 and PVP.

3. Results of toes nails

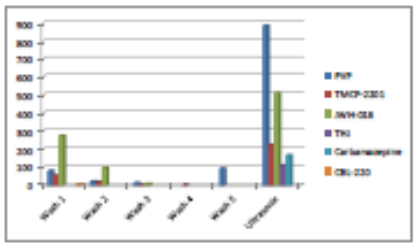

Fig. 9 The profile of compounds which were found in washings and in ultrasonic extract of the toes nails of the patient B-VA. On the Y-axis the intensity of the chromatographic peaks are shown at the base ion. AB-CHMINACA was not found, CBL-2201 was not confirmed, spectrum is not clear.

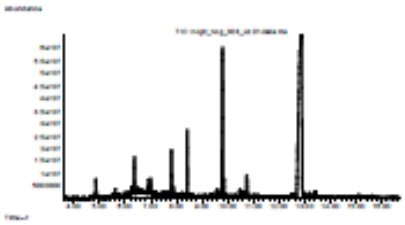

Fig. 10 The GC-MS TIC ultrasonic extract of toe nails.

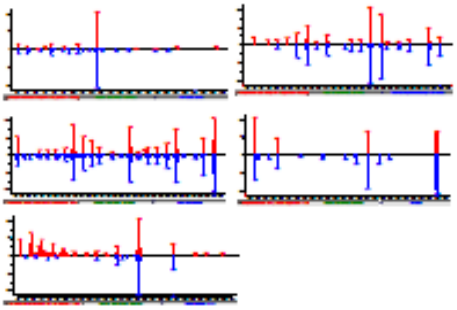

Fig. 11 Mass-spectra of psychoactive substances which were found in washings and in ultrasonic extract of the toes nails of the patient B-VA. The old cannabimimetic JWH-018 was detected along with carbamazepine, TMCP-2201, THJ-2201 and PVP.

Results

AB-CMINACA was found only in washings of finger nails, it is not confirmed.

JWH-018, TMCP-2201, THJ-2201 and PVP were found in ultrasonic extracts of hair and toe nails in concentration higher than in washes, confirmed.

Carbamazepine was found in ultrasonic extracts of hair and toe nails, not found in washes, confirmed.

CBL-2201 is not confirmed, spectra is not clear, was found only in washes.

Conclusion

The GC-MS-SCAN results of NPS detected in 5 methanol rinses and ultrasonic extract of toenail cuttings of a drug user. Similar profiles of NPS were obtained from the hair of the same drug user. In cases with high concentrations of drugs, the typical profiles showed significant traces of the target compounds in rinse 1 and 2. In rinse 3, 4 and 5 the concentrations decreased to zero and sharply increased in the last extract obtained after sonication.

On the side of interpretation, the presence of the target compounds in rinses 1 and 2 could reasonably be the result of surface contamination and extraction of the substances from the cuticle of the biological structures (the cuticle in nails is negligible). However, in the case of nails, because of a much smaller ratio between surface area and volume, the possibility of significant interferences from the presence of target impurities is reasonably smaller than in hair. The high recovery of the analytes after intensive sonication can be interpreted as a result from their extraction from the cortex of the samples. Since similar patterns of elution were obtained from the different samples analysed, the tested approach, although preliminarily, looks suitable to face the problems of the analysis of NPS in keratinized tissues, such as hair and nails.

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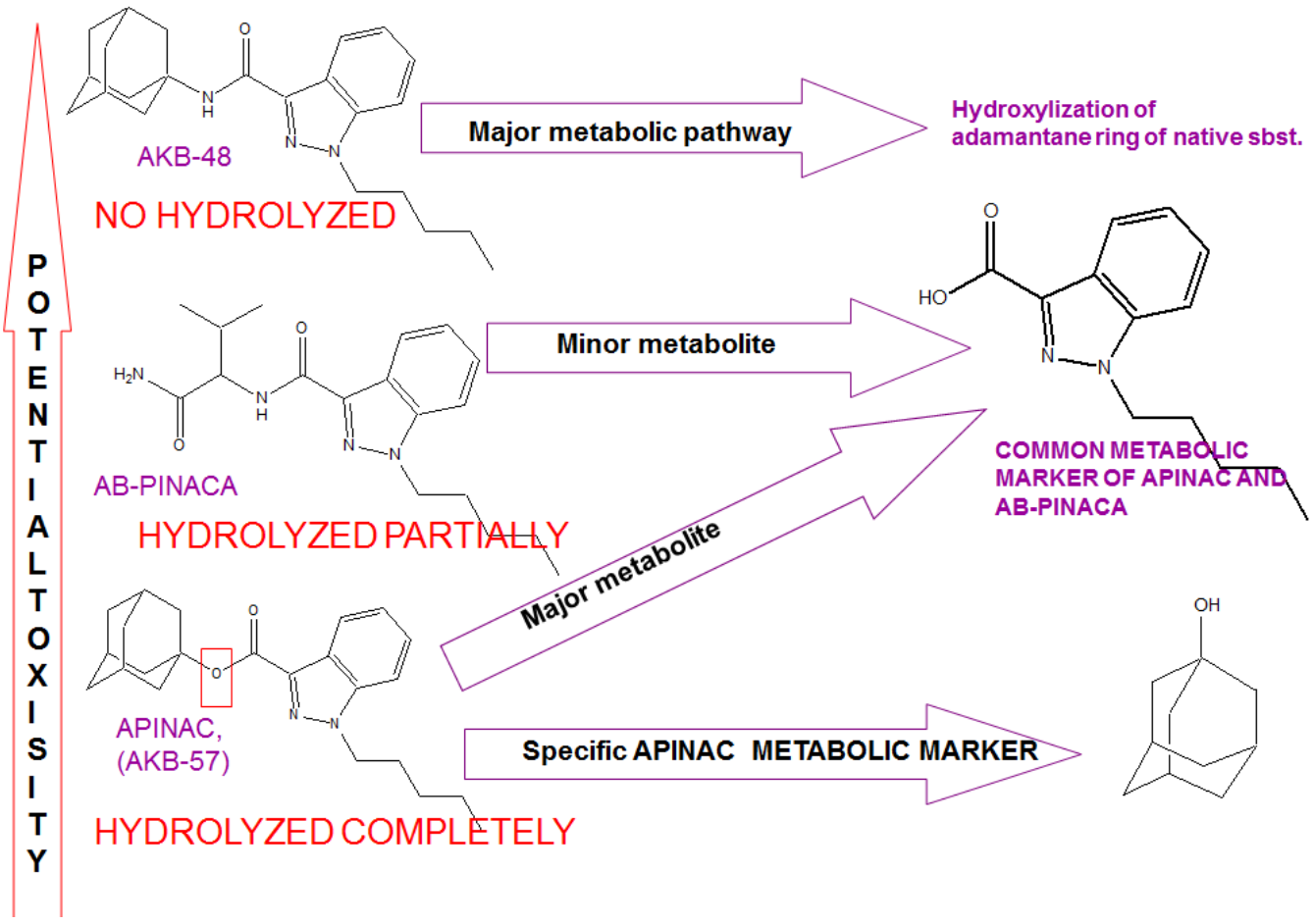
Research Institute of Pharmacy

In vivo metabolic studies of synthetic cannabinoid APINAC

Sergey Savchuk, Ph.D, D.Chem.Sci.

Comparison of APINAC metabolic pathways and cannabimimetics with related structures



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State budgetary institution of health committee

VOLGOGRAD REGIONAL CLINICAL NARCOLOGICAL DISPANCERY CHEMICAL-TOXICOLIGICAL LABORATORY

Head of laboratory Danil Kuznetsov

EXPERIENCE OF THE VOLGOGRAD CHEMICAL-TOXICOLOGICAL LABORATORY IN SCREENING SMOKING MIXTURES AND PSYCHOSTIMULANTS IN URINE

V INTERNATIONAL CONFERENCE ON NOVEL PSYCHOACTIVE SUBSTANCE 2017 VIENNA

Volgograd region

CONSISTS OF 36 AREAS.
6 CITIES OF REGIONAL VALUE
POPULATION about 2,500,000
Red dots indicate cities where the greatest number of analyzes come from

700 crimes related to drugs in the Volgograd region for the first 6 months of 2017

Arrows mark the main paths of drugs in the region

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Detection carfentanil, other derivatives of fentanyl, and their metabolites in biological fluids by GC-MS

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[2] Russian Center of Forensic Medical Expertise, Moscow, Russian Federation

Introduction

At the end of 2012 in the Pskov region was a mass death from an overdose of an unknown surfactant. Analysis of biological fluids from corpses and living persons by gas chromatography / mass spectrometry (GC-MS) after liquid-liquid extraction have been not identified narcotic substances. Only related compounds, such as diphenhydramine and its metabolites, suggests the fact that the use of heroin or similar to the substance. Using the operational information, it was revealed the allegedly new use is not included at the time the Lists, substance fentanyl series – carfentanil, which is a means for euthanasia of large animals.

According to the international classification of fentanyl and other compounds fentanyl series are synthetic opioids – substances that have a morphine-like effect on people, but having a chemical structure that differs from morphine.

To date, synthesized hundreds of analogs of fentanyl, which differ only in strength and duration, more than 12 circulate on the illicit drug market in various countries. In our area common carfentanil and 3-methylfentanyl.

Carfentanil is one of the most active substances from the group of the studied derivatives of fentanyl, which has a narrow range of safe doses. Due to the high lipid solubility, it manifests its activity in various ways it enters the body, including inhalation of the vapor or aerosol. The result is a one-minute inhalation of vapors carfentanil in dogs occurs immobilization with loss of consciousness.

High efficiency, low dosages needed to create the effect, produced by the multiplicity of effects make detection of derivatives of fentanyl, which is in illegal sales is very difficult.

Methods

The liquid-liquid extraction (LLE) at pH 8-9 and solid-phase extraction (SPE) was used for isolation of derivatives of fentanyl from urine.

In the use of high doses of drugs carfentanil and 3-methylfentanyl it is possible to detect and when used to isolate normal LLE. But most often we have to concentrate.

A screening analysis was performed on gas chromatograph Agilent 6890 and MAESTRO 7820 with mass-selective detector Agilent 5975 (Agilent Technologies)(fig.3).. Confirmation was performed by HPLC-MS/MS using Agilent 6460 and Toxtyper Bruker system with mass spectrometer AmaZon Speed (Bruker)

Urine Samples Preparation

LLE

6.0 ml of urine was extracted with sodium chloride, carbonate buffer and 3 ml of a mixture of dichloromethane/ heptane/ isopropanol (6:3:1;v/v). After phase separation by centrifugation, the organic extracts were transferred to vials. After phase separation by centrifugation, the organic extracts were evaporated at 30°C. Evaporated extracts were reconstituted into 100 µl of ethyl acetate and analyzed by GC-MS

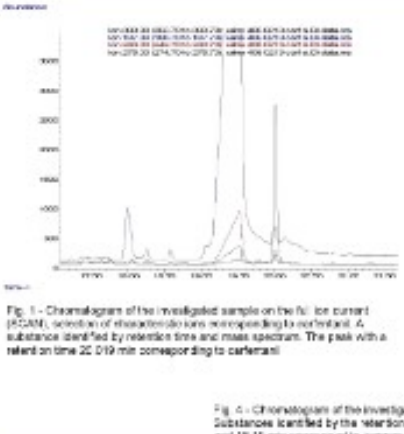
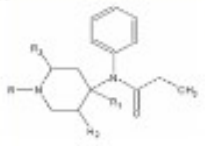


Fig. 1 - Chromatogram of the investigated sample on the full ion current (SCAN), selection of characteristic ions corresponding to carfentanil. A substance identified by retention time and mass spectrum. The peak with a retent on time 20.019 min corresponding to carfentanil

A



Fentanyl

R = CH_2CH_2Ph, R_1 = R_2 = R_3 = H

Carfentanil

R = CH_2CH_2Ph, R_1 = $COOCH_3$, R_2 = R_3 = H

3-methylfentanyl

R = CH_2CH_2Ph, R_2 = CH_3, R_1 = R_3 = H

B, C

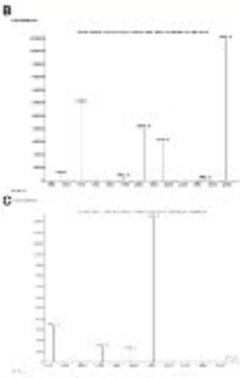


Fig.3 Chemical structure of the compounds fentanyl series (A), mass spectra of 3-methylfentanyl (B), mass spectra of carfentanil (C).

GC-MS conditions

GC–MS analysis of prepared samples was performed with an Agilent 6890N gas chromatograph and MAESTRO 7820 connected to a 5975C mono-quadrupole mass-selective detector (Agilent Technologies). The GC conditions were [5, 6]: HP-5ms capillary column (30 m x 0.25 mm internal diameter, 0.25 µm film thickness); 280 °C interface temperature, 280 °C injector temperature; injection mode, splitless; injection volume, 1 µl; carrier gas (He), flow rate 2 ml/min; oven temperature program, initial temperature at 70 °C (1-min hold) followed by ramp at 15 °C/min up to 280 °C (7-min hold). The MS conditions were: ion source temperature, 230 °C; ionization mode, electron ionization (EI) at 70 eV; detection gain, 1.800 V; solvent delay, 8 min; identification, scan mode and sim mode; scan range, m/z 41–650;

Characteristic ions: carfentanil—303, 187, 275, 243, 3-methylfentanil—259,190, 203, 216

Fig.2 Laboratory analytical equipment

Mass Spectra Libraries

Well-known MS libraries such as NIST, MPW, WILEY, updated not so quickly as new psychoactive substances appear. This makes it difficult to diagnose cases of drug or NPS intoxication.

Authors used the MS libraries of NPS and their metabolites, created by leading experts of the Russian Federation in the field of analytical toxicology:

- the GC-MS NPS library «Sudmed_2362» created by Russian professional community under supervising of Alex Pechnikov (contain 2362 spectra 29/05/2017) [3];

That MS librarie constantly updated as new psychoactive substances appear.

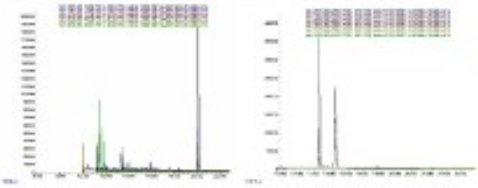


Fig. 4 - Chromatogram of the investigated sample (SIM) of characteristic ions corresponding to the 3-methylfentanyl and carfentanil. Substances identified by the retention times and mass spectra. On the left the chromatogram peaks with retention times of 17.7 min and 18.15 min correspond to isomers 3-methylfentanyl, 19.5 min – carfentanil. On the right of the chromatogram peak with a retention

Urine Samples Preparation

SPE

Samples was prepared using cartridges for solid phase extraction (SPE) Agilent SampliQ Evidex or Bond Elute Certify 3 ml, 200 mg with a mixed phase. For small quantities of sample we were used Bond Elut Plexa.

Conditioning of the column

Was passed through the cartridge one by one: 3 ml of methanol, 3 ml of phosphate buffer (pH = 6,0).

5-10 ml of the urine sample

Rinsing the column

Was passed through the cartridge one by one: 3 ml 0.1M HCl, 1.5 ml of methanol

Elution of analyte: 3 ml of the mixture dichloromethane / isopropanol / ammonia (78/20/2)

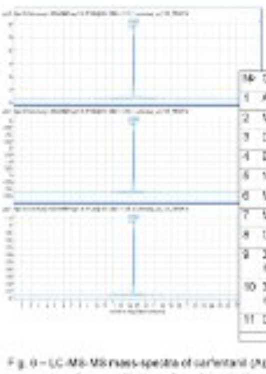
The organic extracts were evaporated at 30°C. Evaporated extracts were reconstituted into 100 µl of ethyl acetate and analyzed by GC-MS

Keeping biomaterial through time

We also have conducted studies on the persistence of these substances in the biomaterial in time. Biological material, which was analyzed and stored in a freezer at -25°C, showed good persistence of analytes after 4 years, which is evident when comparing the chromatograms in Fig. 5.

Fig. 5 – Chromatogram of the same test samples (SIM) of characteristic ions corresponding to the 3-methylfentanyl and carfentanil, different on-time performance. Substances identified by the retention times and mass spectra. The chromatogram on the left 2012 on the right chromatogram of 2016. Peaks with retention times of 17.7 (18.3) and 18.19 min (18.8) min correspond to isomers 3-methylfentanyl, 19.5 (20.20) min – carfentanil

A



B

№	Components	purity	ret.	RT
1	Amfetamine	75	93	4.55
2	Methamfetamine	97	99	5.11
3	Diphenhydramine	99	96	11.35
4	EDDP	3.0	93	12.49
5	Naphazoline	100	96	12.64
6	Methadon	80	84	13.39
7	Methorphan	100	98	13.50
8	Cocaine	73	97	15.25
9	3-methylfentanil isomer 1	75	96	18.20
10	3-methylfentanil isomer 2	81	97	18.77
11	Carfentanil	24	96	20.18

Fig. 6 – LC-MS-MS mass-spectra of carfentanil (A), retention times and other parameters for quantitation of analytes in the spiked urine

Results

As practice shows, the solution of such problems possible in the application at the stage of sample preparation method, solid phase extraction, allowing the simultaneous separation, isolation and concentration of target components.

To date, the bladder a mixture containing the main injecting drug (fig.6), which is used as a control in the territory of the Russian Federation. Identification data of drugs, including derivatives fentanyl series, can serve as an indicator of the sensitivity of the equipment.

Conclusions

The efficiency of gas chromatography / mass spectrometry analysis of biological samples for the detection and identification of fentanyl derivatives in routine practice of the laboratories, provided only GC-MS.

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Professional testing of forensic laboratories, round 2/2016
Number of participated laboratories :
Urinary samples were analyzed in 23 lab (23 GC-MS, 4 LC-MS/MS)
Hair samples were analyzed in 12 lab (12 GC-MS, 3 LC-MS/MS)
Urine samples PT 8,9,11 (2/2016) :
Urine_PT _16: lab staff urine after eating of baking with poppy seeds. Sample contained MORPHINE.
Urine_PT _17: MIX of drug users urine was previously analyzed by GC-MS, LC-MS/MS, LC-TOF. Sample contained: metabolites of PB-22F, AB-CHMINACA, MDMB (N) -Bz-F, ADB-FUBINACA, AB PINACA, α-PVP,
Urine_PT _18(blank): caffeine, theobromine.
The main problem: 5 lab showed false positive results in Urine_PT _18(blank)).
Substances were identified in Hair PT 5V (1/2015): MDPV, XLR11
Fifth international conference on novel psychoactive substances

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Drug analysis quality assurance program for Russian forensic toxicology laboratories. One step forward to assure and improve quality

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Introduction

It is known that forensic toxicology laboratories participates in quality assurance programs in order to monitor and demonstrate the quality and reproducibility of their analyses. Participation in proficiency tests can report many advantages to forensic toxicology laboratories. It represents an important tool to assess and monitor the quality performance of the laboratory for a specific test. It provides a mean to compare laboratory work with other laboratories of the same field [1, 2, 3]. All the organizations that carry out proficiency testing of laboratories for drug analysis is mainly used spike method to biological samples.

However, there is a problem of acquiring (in the absence of production) of certified analytical comparative samples for metabolites / markers of new psychoactive substances. There is also a problem of legislative restrictions on the use of chemical compounds whose turnover is prohibited.

We have developed the program of external quality assurance using urine and hair samples obtained from drug consumers.

The coordinators of the Russian comparative exercises in the field of analytical toxicology are: Institute of Pharmacy and Translational Medicine, I.M. Sechenov First Moscow State Medical University (Moscow, Russian Federation) and the Internet portal "MachToxlab" (www.toxlab.ru). Comparative exercises are conducted at a frequency of 2 times a year. More than 150 forensic and toxicology laboratories in Russia working in hospitals and in bureau of forensic expertise. Number of laboratories which took part in proficiency testing: 23 laboratories participating in 2015, 49 in 2016 [4].

Objectives

The aim of present study was to create Russian program of external quality assurance using real urine and hair samples obtained from drug consumers. The main objectives were:

- Development of methods with minimum false positive and negative results;
- Harmonization of GC-MS and HPLC-MS/MS methods;
- Rapid identification and confirmation of structure NPS and their metabolites;
- Generation of MS libraries with permanent updates;
- Development methods for determination of NPS in hair and nails;
- Proficiency testing of Russian laboratories permanent updates.

Fig.1 Test samples, the Russian program of external quality assurance

Test Samples

For proficiency testing of laboratories we use the quality control samples (fig.1):

1) Real multicomponent urine samples from common drugs and NPS consumers, were prepared by combining the same type of so-called "positive" urine samples;

2) Real hair samples from common drugs and NPS consumers;

3) Ethanol spiked QC samples.

In each round, laboratories are invited to analyze three urine test samples, one hair test sample and three ethanol spiked test samples. Using normal laboratory screening and confirmatory tests, laboratories are required to analyze the samples for the controlled drugs and related compounds, including their metabolites.

The samples for Russian program of external quality assurance before starting and after finishing of round test were analyzed by GC-MS, UPLC-MS/MS, UPLC-IT-TOF to confirm the composition and quality of long storage.

Some laboratories participating in program used these samples for optimization and testing of analytical techniques (table 1, 2).

Table 1. Comparison of sample preparation methods for GC-MS, UPLC-MS/MS[5]

Identified substances/ metabolites in PT-9-urine sample	Intensity, mV					
	GC-MS		UPLC-MS/MS			
	LLE	SPE	LLE	SPE	DAS (4:1)	DAS (1:1)
MDPV	1.1 e6	1.0 e5	2.6 e7	3.2 e7	1.5 e7	1.1 e7
aPVP	8.0 e6	2.0 e5	1.4 e8	1.2 e8	1.0 e8	8.1 e7
PB-22F marker (-COOH)	3.5 e5	1.0 e5	1.0 e6	1.1 e6	-	-
5F-AB-PINACA marker (-COOH)	2.6 e6	1.0 e5	3.6 e6	2.1 e6	1.1 e6	-
AB-CHMINACA marker (-COOH)	7.0 e6	2.0 e5	7.0 e6	9.3 e6	8.1 e5	4.9 e5
XLR11 marker (-COOH) degradant	3.5 e6	2.0 e4	2.2 e7	2.5 e7	5.3 e6	2.6 e6
MDMB(N)-6z-F marker (-COOH)	8.0 e4	2.0 e4	1.4 e6	8.1 e5	-	-

Fig.2 Agilent 7890 or 7820 gas chromatograph connected to an Agilent 5975 or 5977 mass-selective detector, the main analytical equipment in forensic and toxicology laboratories of Russian Federation

Analytical Equipment

The main analytical equipment in forensic and toxicology laboratories of Russian Federation are Agilent 7890 (7820) gas chromatograph connected to an Agilent 5975 (5977) monoquadrupol mass-selective detector (Agilent Technologies) (fig.2).

In some laboratories, HPLC-MS / MS and HPLC-TOF systems (Bruker, Agilent Technologies, Shimadzu) are also used to identify compounds (fig. 3,4).

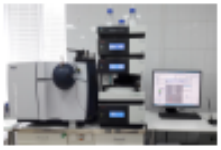


Fig.3 HPLC-MS/MS (ion trap) Bruker Toxtyper for screening identification of NPS metabolites

Fig.4 HPLC-MS/MS Shimadzu IT-TOF for confirmation identification of NPS metabolites

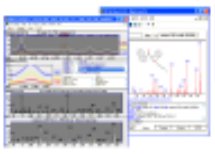


Fig.5 The GC-MS NPS library «Sudmed_2362» created by Russian professional community under supervising of Alex Pechnikov (contain 2362 spectra 29/05/2017)

Fig.6 MS libraries for LC-MS (QQQ, ION TRAP, Q-TOF) created by Andrey Grigoryev and Sergey Savchuk, contain170 NPS abst/methabolites MS data

Mass Spectral Data

Well-known MS libraries such as NIST, MPW, WILEY, updated not so quickly as new psychoactive substances appear. This makes it difficult to diagnose cases of drug or NPS intoxication. Participants in the Russian program of external quality assurance use the MS libraries of NPS and their metabolites, created by leading experts of the Russian Federation in the field of analytical toxicology [6, 7] (fig. 5,6). These MS libraries constantly updated as new psychoactive substances appear.

Table 2. Comparison of GC-MS programs: in order to determine the degree of interference of matrix connections on the quality of AMDIS identification with three different temperature programs: sharp (35 deg / min), smooth (15 deg / min) and medium (25 deg / min), Agilent 7890/5975 [8]

Identified substances/metabolites in PT-4-urine sample	SCREEN (sharp) RT, min	DOAS (smooth) RT, min	NvPND (medium) RT, min
Quinoline-6-ol, TMC	5.12	8.42	6.56
MDPV	7.33	13.22	9.86
PB-22_M HY TMS	7.45	13.52	9.92
PB-FUB22_M1_TMS	7.58	13.74	10.12
PB-22-M HY (ooc-) TMS	8.05	14.59	10.94
PB-22-M HY (HO-chain-) 2TMS	8.24	14.99	11.32
PB-FUB22_M2_TMS	8.49	15.41	11.69
AB-PINACA-M1 (COOH); TMS-	-	16.61	12.66
5F-AB –PINACA-M (HOOC-) TMS	9.84	17.62	13.32
PB-FUB22_M3_TMS	9.86	17.47	13.37
AB-FUBINACA-M (HOOC-) TMS	10.86	19.94	14.75

Results

The number of laboratories which participated in Russian program of external quality assurance: 23 laboratories participating in 2015 and 49 laboratories in 2016. For example, substances and their metabolites that have been identified in urine and hair samples.

PT-4-urine (2015/1): MDPV, PB-22, PB-FUB22, AB-PINACA, 5F-AB-PINACA, AB-FUBINACA.

PT-6-urine (2015/1): aPVP, amphetamine, cocaine, methadone, codeine, morphine, diphenhydramine, doxylamine, phenobarbital, naproxen, ketoprofen, diclofenac, ibuprofen, metamizole, chloroquine, tropicamide, phenyramine, THC-COOH.

PT-6-urine (2015/1): caffeine, theobromine (the so-called "negative" sample from a person who does not consume drugs, NPS and nicotine).

PT-6-urine (2015/2): α-PVP, amphetamine, desomorphine, codeine, morphine, PB-22F, AB-CHMINACA, XLR11, THC-COOH, acetaminophen, tropicamide, naproxene, carbamazepine.

PT-6-urine (2015/2): α-PVP, MDPV, PB-22F, AB-CHMINACA, XLR11, MDMB(N)-6z-F, ADB-FUBINACA, AB-PINACA, acetaminophen.

PT-11-urine (2015/2): caffeine, theobromine.

PT_6v_hair (2015/2): MDPV, XLR11 (thermal isomer).

PT-12-urine (2016/1): α-PVP, 5F-AB-PINACA, AB-CHMINACA, MDMB(N)-2201, MDMB(N)-6z-F, THC-COOH.

PT-13-urine (2016/1): α-PVP, amphetamine, MDA, codeine, morphine, thebaine, AB-CHMINACA, phenobarbital.

PT-14-urine (2016/1): caffeine, theobromine.

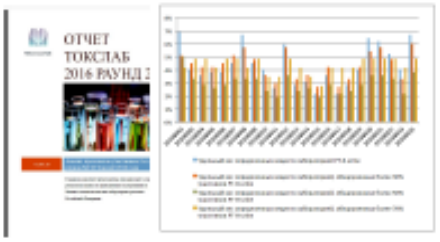
PT-16-urine (2016/2): morphine, gamma-amino-beta-phenylbutyric acid.

PT-17-urine (2016/2): α-PVP, AB-CHMINACA, MDMB(N)-2201, ADB-FUBINACA, AB-PINACA, 5F-AB-PINACA, diphenhydramine.

PT-18-urine (2016/2): caffeine, theobromine.

The summary report is formed according to the results of each round of the Russian program of external quality assurance (fig. 7). It is published on the Internet portal "MachToxlab" (www.toxlab.ru).

In addition, coordinators of comparative exercises offer and provide consulting assistance to laboratories participating in the Russian program of external quality assurance. One of the main tasks of individual expert consultations is to prevent false positive and false negative results in forensic chemical analysis.



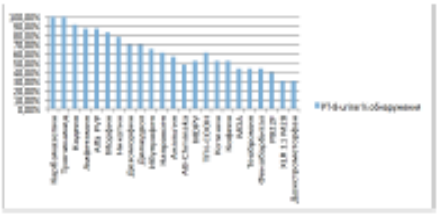


Fig.7 The summary report is formed according to the results of each round of the Russian program of external quality assurance

Conclusion

The first Russian program of external quality assurance of drug analysis in urine and hair using authentic samples was organized. Qualitative performance is good in the majority of laboratories, with a low incidence of false results. The main problem: false positive results in BLANK PT sample. The false positive results were obtained from the nine out of the 49 participating laboratories and the 23 out of the 49 participating laboratories had a problem with a correct identification of target analytes and only the 17 laboratories achieved satisfactory results for all compounds. As a results the 40 out of the 49 participating laboratories founded not less than 70-80% of target substances.

The results demonstrated that the performance was fair and further improvement is needed. Nevertheless, the programm has provided a good external mechanism for participants to evaluate their analytical capability and to identify any testing inadequacy on urine and hair analysis for drug analysis.

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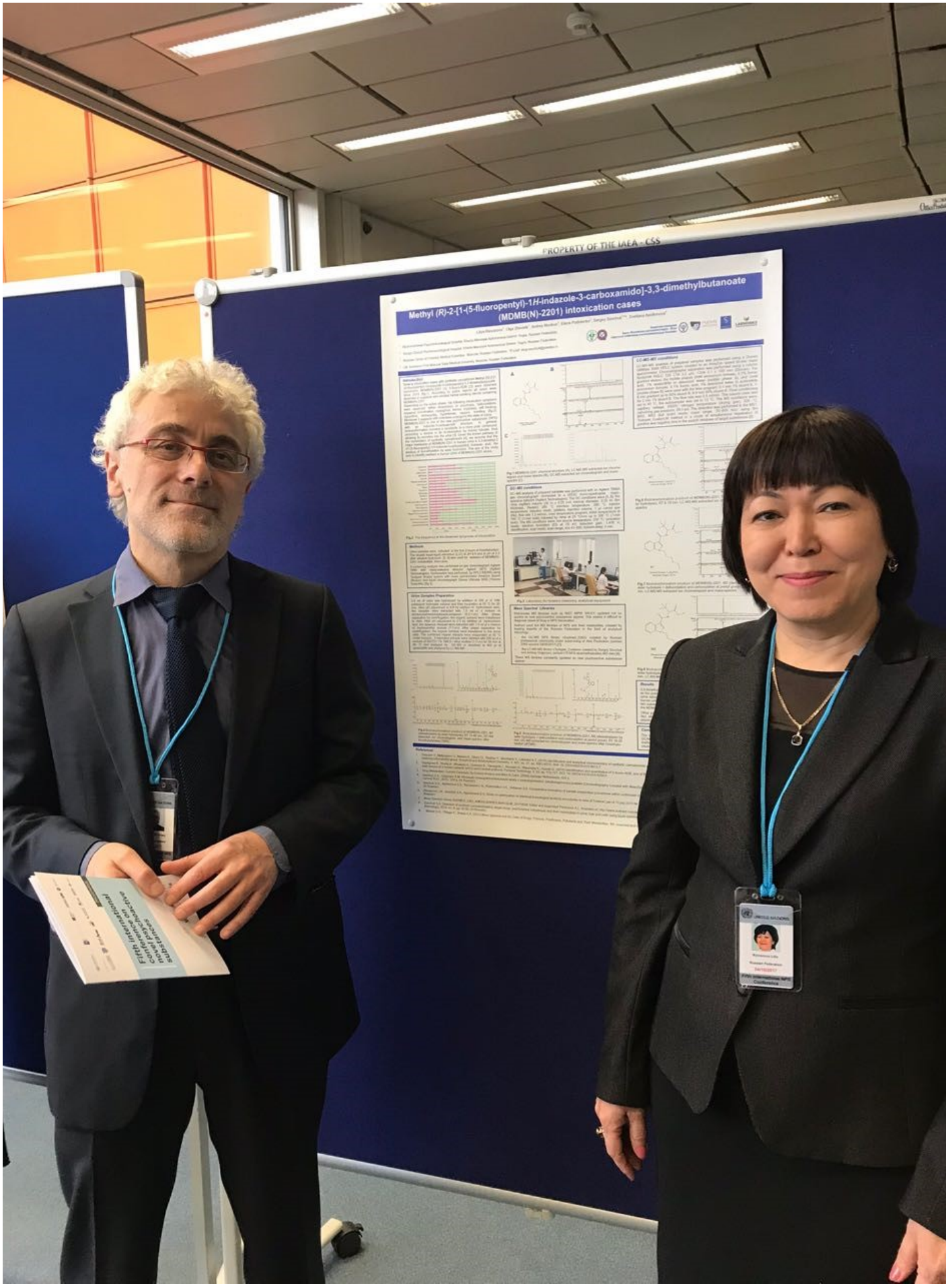

EXAMINATION OF INTERCELLULAR FLUID IN THE PRACTICE OF FORENSIC CHEMICAL RESEARCH

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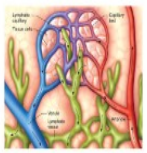
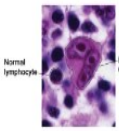
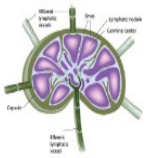






Medicolegal diagnosis of poisonings

Source of the data used in case of medicolegal diagnosis of poisonings are: investigation materials, medical documents of the victim, data of a medicolegal research of a corpse, results of judicial chemical analysis and other additional researches.

The outside and internal research of a corpse in a morgue is faced by mutually adding tasks. In case of an outside research aim to set the signs specifying:

1) on the way of arrival of poison to an organism;

2) on a chemical entity of poison;

3) rate of approach of death.

The purpose of an internal research of a corpse is establishment:

1) ways of introduction of poison;

2) the organs and fabrics struck most;

3) character contact (chemical burns) idistro-ficheskikh of changes of internals;

4) existence and character of the developed complications;

5) signs characteristic of effect of separate poisons;

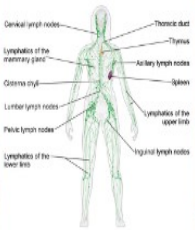
6) immediate cause and rate of approach of death;

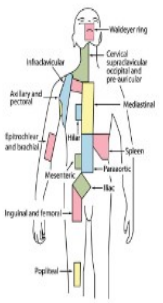
7) an intake of materials for additional laboratory researches.

The major among additional methods is the judicial and chemical research of internals, fabrics and the liquid environments of an organism. Its purpose is poison detection, determination of its quantitative contents and distribution in an organism.

The negative result of a judicial and chemical research not always excludes poisoning. In case of undoubted poisoning it can be caused by the following reasons: lifetime transformations of poison in an organism, release of poison from an organism, application of antidotal therapy, the wrong intake of biological material for judicial chemical analysis, the wrong storage of the excepted biological material, the wrong choice of a technique of chemical analysis, small sensitivity of the applied technique of a chemical research, technical errors.

The positive result of a judicial and chemical research not always testifies to poisoning. Can be the reasons of the positive result of such analysis (in the absence of poisoning): endogenous formation of poison in case of different diseases, the long reception of medicines, the long professional contact with poison, posthumous formation of some poisons when rotting a corpse, posthumous penetration of poison into corpse fabrics from the soil or clothes, deliberate posthumous introduction of poison, accidental hit of poison in case of the wrong sanitarian processing of a corpse, error in the organization and technique of a sou-debno-chemical research. Therefore, the medicolegal proof of poisoning shall be result of assessment of all collected data.





Pipina E. B. , Savchuk S. A.

Because of the difficulty of sampling of biological material for sample preparation in forensic chemical studies, MUCH of the Bureau of judicial medical examination of the city of Tomsk was used the method of isolation in the freezing of interstitial(tissue) fluid from the internal organs proposed by Pechnikova A. L.

This method was applied because of the availability of biological material, as the sampling of biological fluids from a corpse is not always possible for various reasons. as the samples were investigated deaths from various injuries, namely a death in the ambulance after providing the necessary medical manipulations.

After a series of comparative studies where similar methods of isolation (sample preparation) were used intercellular fluid, blood, bile, urine, from the same corpse was made a conclusion about informative result which can be obtained in the study of intercellular (tissue) fluid. The study was performed on a gas chromatograph with mass selective detector the Agilent 7820 "маэстро"

Sample preparation for forensic chemical study: of 50 g of biological material (the internal organs : brain, liver, kidney, adipose tissue) were frozen at -18° C, after which the biological material is thawed at room temperature.

The intercellular liquid is obtained when the food is thawed tissues were collected in 800 µl, were placed in Eppendorf with 1 g of sodium chloride was added to 700 µl of acetonitrile. Within 1 minutes turn on the vortex. It then spun in a centrifuge at 15 thousand rpm. The supernatant layer was evaporated in a current of air, pererastala in 150 µl ethyl acetate and 1 µl was injected into the chromatograph evaporator.

Gas chromatograph Agilent Technologies 7820 Maestrowith mass selective detector. Column with methylphenylsiloxane capillary phase HP-5MS UI(30m x0.250mm; thickness of the phase layer 0.25 µm). The temperature of the column thermostat 50C (0.5 min), 99s/min 100C 1 min,15C/min,280S (30 min). The delay time of the solvent 3.6 minutes. The inclusion of the cathodes from the beginning of the analysis of 36 minutes.

Evaporator temperature is 270C, the analytical interface 280S, source Temperature of ions 230C, the temperature of the mass analyzer 150C. Analysis of constant pressure carrier gas. As carrier gas helium grade"A"-1 µl/min the mass Range m/z 41-650a.e.m. The input sample without dividing the flow amount 1мкл (by means of the autosampler.) Analysis in the scanning mode on the full ion current.

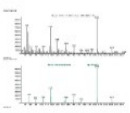
The analysis was carried out in the mode of DOAS. The retention times of determined compounds were obtained according to the method of fixing the retention times; the setup for this method was carried out on diphenylamine (internal standard). (identification RTL – AMDIS libraries of mass spectra SAV46;48 MPW2011; MPW2007; DESDRUG_2011; TOX3; EKBDRUGS_11; AIPSIN_2240; NISTDRUG; NISTTOX).

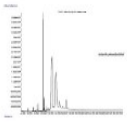
Thus in the analyzed samples of blood, bile, urine not found : drugs, narcotic, potent substances. In the extracellular fluid from the brain to the liver, kidneys detected : phenobarbital, methylphenobarbital, metoprolol.

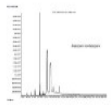
In intercellular fluid obtained from the adipose tissue discovered: phenobarbital, methylphenobarbital, metoprolol, ketamine, tramadol, diazepam.

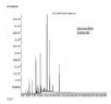
Studies have been conducted on the biological material was obtained from 30 cases of death in trauma.

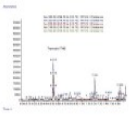
The result that can be concluded about the simple, informative study of the intercellular fluid obtained from adipose tissue by the method of freezing.











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Laboratory diagnostics of acute poisoning with Synthetic Cannabinoid Receptors Agonists in the Sverdlovsk region

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Introduction

In recent years, abuse of new psychoactive substances ("designer drugs"), including Synthetic Cannabinoid Receptors Agonists (SCRA) has become widespread in the Russian Federation, which has led to a sharp increase in the number of acute poisoning by these compounds.

The aim of this study is to assess the possibility of laboratory diagnosis of SCRA poisoning for the study of blood and urine by gas chromatography-mass spectrometry (GCMS)

Methods

The study was conducted on 1652 clinical urine specimens, collected from patients admitted to the intensive care unit of Sverdlovsk Regional Poison Treatment Centre, from January 2013 to December 2016. In 112 cases also examined whole blood taken when patients entered the intensive care unit. A comprehensive screen for SCRA was carried out using GCMS

Sample preparation

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

Urine samples. Sodium hydroxide (2 ml, 25%) and internal standard (50 µl, 100 µg/ml ketorolac in water) were added to urine (10 ml) and the mixture was heated at 60°C for 60 min. After cooling, the pH was adjusted to 2–3 with hydrochloric acid solution (6M). The mixture was extracted with hexane – ethyl acetate 7:1 (2 ml), centrifuged and the organic phase was blown to dryness with air at room temperature.

Blood samples. Acetonitrile (3ml), deionized water (3 ml), internal standard and sodium chloride (1.0 g) were added to blood (1ml). After extraction and centrifugation, supernatant was blown to dryness with air at room temperature.

Derivatization of the residues for the gas chromatography-mass spectrometry (GC-MS) analysis was performed by methylation. Methylation of the samples was carried out using a mixture of acetone and methyl iodide (70 µl of each) and anhydrous potassium carbonate (2 -5 mg) at 60°C for 40 min. The organic phase was blown to dryness with air at room temperature. The samples obtained were extracted with hexane twice, organic phase was blown to dryness with air.

GC–MS conditions

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) under the following conditions: DB-17 ms column (30m×0.25mm×0.33m) with helium as carrier gas (6.6 ml/min); oven temperature program was 90°C (0.5 min), 20°C/min (285°C, 9.1 min), and 30°C/min (295°C, 10.32 min). Injector temperature was 300°C. The injection volume was 1ll with a split ratio of 1:2. The GC/MS interface temperature was 300°C. The temperature of the ion source was 200°C. Mass spectra were recorded in electron ionization (EI) mode (70 eV), scan range m/z 40–550 for assay metabolites of SCRA. Processing of the data was performed using the software «AMDIS / NIST MS Search»

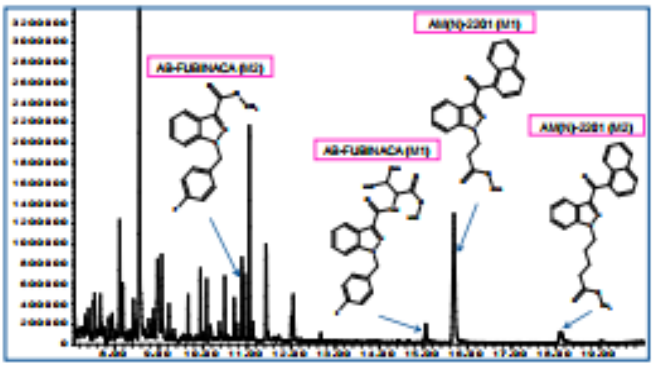

Fig.1. Total ion current chromatogram of the urine of a patient with acute poisoning with AB-FUBINACA (N-(1-Carbamoyl-2-methylpropyl)-1-(4-fluorobenzyl)-1H-indazole-3-carboxamide) and AM(N)-2201 [1-(5-Fluoropentyl)-1H-indazol-3-yl](naphthalen-1-yl) methanone

Results and discussion

In 932 cases (56.4%) in the urine, metabolites and markers of various SCRA were detected: derivatives of cycloalkanecarbonylindole, indole-3-carboxamide, indole-3-carboxylic acid, indazole-3-carboxamide, indazole-3-carboxylic acid, naphthoylindazole (Fig. 1, 2).

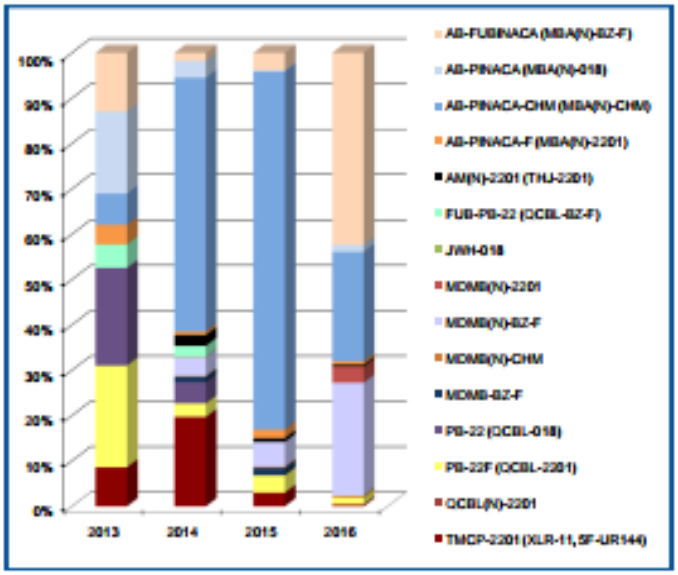

Fig.2. Types of SCRA identified in the urine of patients with acute poisoning

Blood samples were studied in patients whose urine contained metabolites of the SCRA, but study of whole blood on the content of SCRA gave a positive result in only 26 of 112 examined (23.2%) (Fig.3). As a result of the study, we found no correlation between the exposure time of the toxicant and its detectability in the blood (Table 1).

For the analysis of blood poisoning with SCRA, the sensitivity of the GC-MS method is not sufficient and it is necessary to use highly sensitive analytical methods such as HPLC-MS/MS.

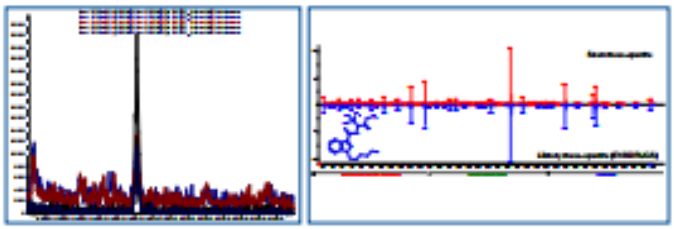

Fig.3. Extract ion chromatogram of the blood of a patient with acute poisoning with MDMB(N)-2201 (Methyl 2-[1-(5-fluoropentyl)-1H-indazole-3-carboxamido]-3,3-dimethylbutanoate) and mass-spectra of MDMB(N)-2201

Table 1. The number of cases of detection of SCRA in the blood by GC-MS

Number of patients with acute SCRA poisoning	Exposure time of the SCRA	Number of cases of detection of SCRA in whole blood
2	30-40 minutes	1 (50.0%)
13	1-1.5 hours	4 (30.8%)
22	2-3 hours	5 (22.7%)
25	3-5 hours	2 (8.0%)
10	6 hours and more	4 (40.0%)
40	unknown	10 (25.0%)

Conclusions:

SCRA acute poisoning is widespread in the Russian Federation. The study of blood for the content of SCRA and their metabolites by GCMS in the diagnosis of acute poisoning is inexpedient.

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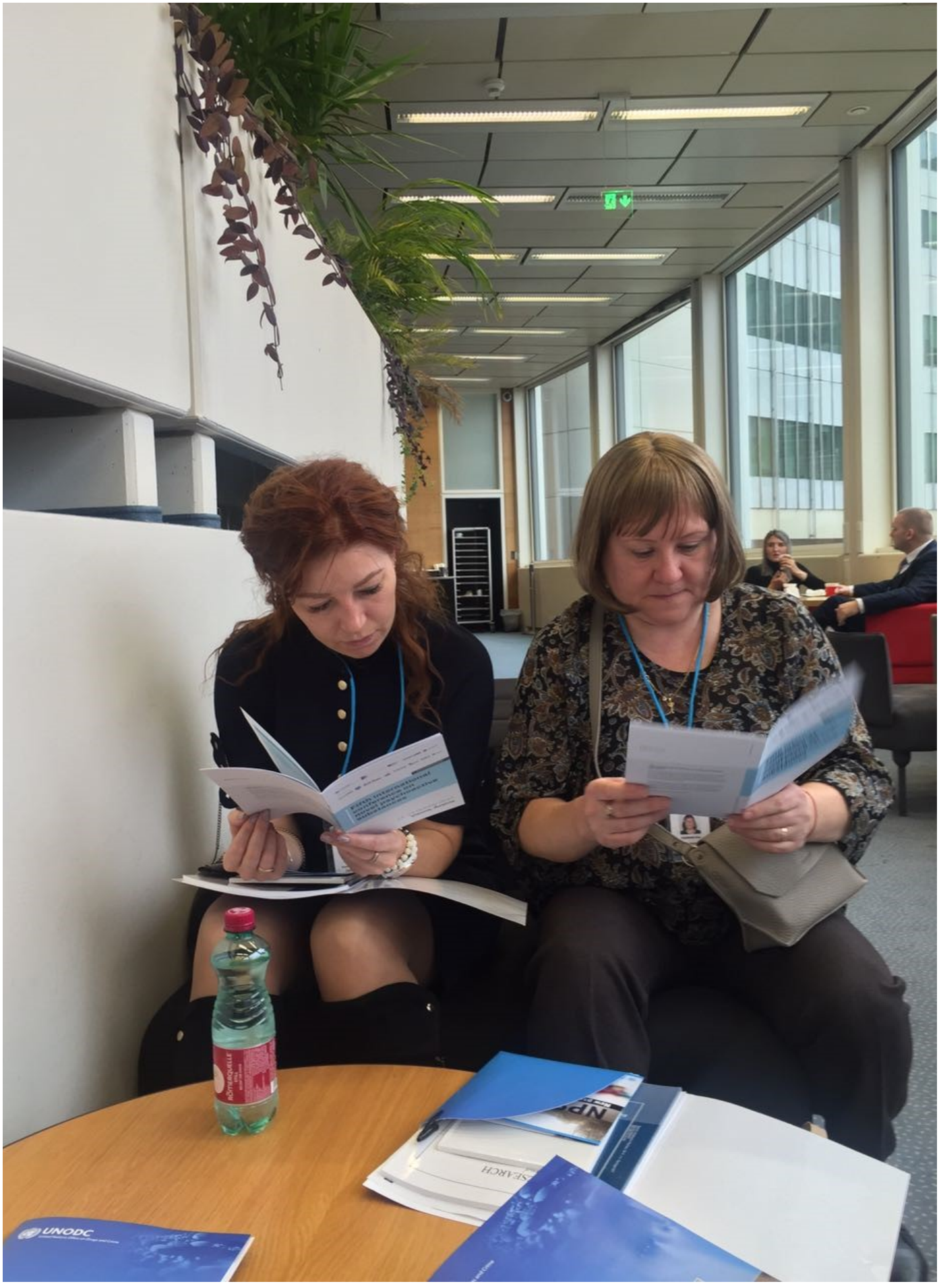

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Quick routine definition of (±)-11-nor-9-carboxy-delta-9-THC TMS in plasma using ISOLUTE SLE +Supported Liquid Extraction and GC/MS

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Introduction:

In 2016 year, Russia changed the legislation (procedure for conducting a medical examination for intoxication).

To establish the fact of consumption of cannabis, it was indicated to take blood or urine sampling at the choice of an individual. Previous sample preparation did not give fast, reproducible results. Introduction of technology the ISOLUTE SLE +Supported Liquid Extraction solved the problem.

Fig.1.: The logo of the clinic and photo.

Fig.2.: Cartridge

Methods:

Plasma blood is used for analysis. The analysis was done by GC/MS (SCAN mode).
Blood should be collected in a vacuum tube with EDTA
Sample preparation
HYDROLYSIS
Hydrolyze plasma with heat at 70°C for 1 hours. (Base hydrolysis: 2ml plasma add 100µl 10N KOH)
Cool and adjust pH to 3.5– 4(by adding 200µL glacial acetic acid)
ISOLUTE SLE +Supported Liquid Extraction :
SAMPLE LOAD:
Place the ISOLUTE SIE + columns or plate on a suitable processing manifold. Ensure collection vessel is in position.
Load hydrolyzed plasma (1ml) onto the column. Sample may percolate through the frit at this stage.
Apply a short pulse of vacuum (~10" Hg/~0.2 bar) or positive pressure.
Sample should be fully loaded within 1 minute, depending on sample viscosity.
SAMPLE SOAK:
Once loaded wait 5 minutes for sample to completely absorb and form extraction layer.
ANALYTE ELUTION:
Check the collection vessel is in position.
Apply 2,0 ml extraction solvent (Dichloromethane)(ROTS) and allow to flow for 5 minutes under gravity.
Apply 2,0 ml extraction solvent (tert.– Butylmethylether) (ROTS) and allow for 5 minutes under gravity.
POST EXTRACTION:
The eluate evaporated in the heat flow 40°C.
TMS DERIVATIZATION:
Derivatize samples with addition of Ethyl acetate (100µl)(ROTH), (MSTFA) N– Methyl—N—trimethylsilyl trifluor
(o) acetamid(e) 50(µl) (MACHEREY-NAGEL), recap and heat at 70°C for 40 min.

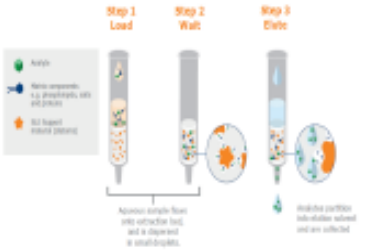


Fig.3: Fundamental Steps for ISOLUTE SLE+

GC/MS Analysis Conditions:
Column: HP-5MS UI (order number: 190918-433UI)
Dimensions: 30meterX0,25mmX0,25µm
Injection:0,5 µl splitless for 0,7 min at 280°C
Carrier: Constant pressure flow Helium
Oven: 50°C(0,5min), 99°C/min 100°C(1min), 15°C/min, 280°C(25min).
Detector: MSD SCAN MODE
Transfer line=280°C.

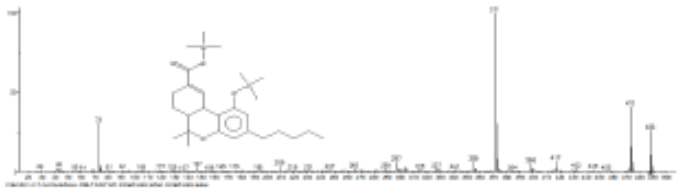


Fig.4: Mass spectral library//NIST/EPA/NIH,2014

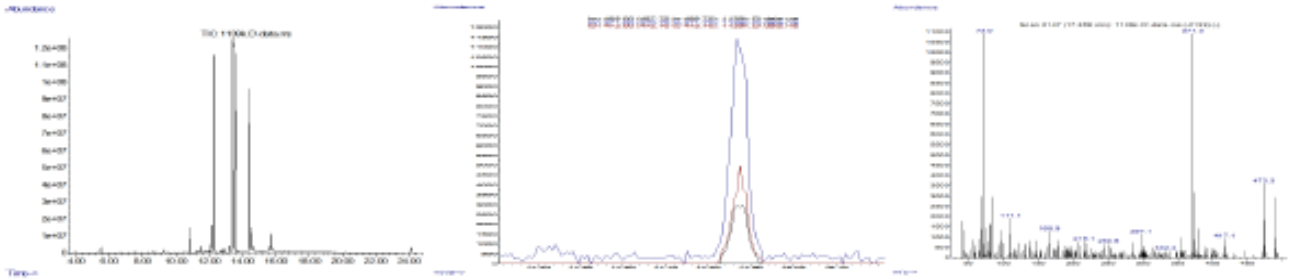
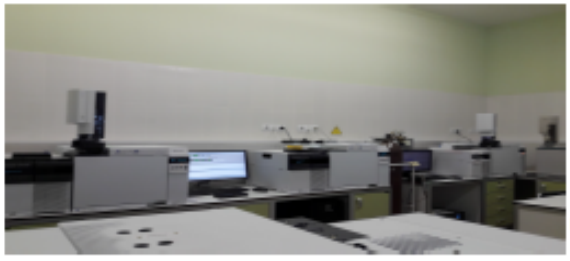


Fig.5. :GC-MS chromatogram and mass-spectra, metabolite of (±)-11-nor-9-carboxy-delta-9-THC after derivatization, TMS



Globally, cannabis is one of the most widely used illicit drugs. The naturally occurring cannabinoids found in hemp species bind to receptors in the brain and cause sensations of relaxation and calm.
Clinical symptoms:

- anxiety
- difficulty with speech
- violation of motor function

Results:

For a complete manifold with samples, the extraction time takes no more than 15 minutes.
Extract of hydrolyzate does not require additional purification.
This sample preparation improves the identification of the analyte.
Detection limit ≤ 100ng/ml.

Fig.8.:Instrumentation: Мастро Agilent 7820A GC– mass selective detector Agilent 5977E

Conclusions:

The described method can be confidently used for routine analysis of (±)-11-nor-9-carboxy-delta-9-THC TMS in plasma. The application of this technique increases the efficiency of work laboratories.

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Methyl *(R)*-2-[1-(5-fluoropentyl)-1*H*-indazole-3-carboxamido]-3,3-dimethylbutanoate (MDMB(N)-2201) intoxication cases

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Introduction

Several intoxication cases with synthetic cannabinoid Methyl (R)-2-[1-(5-fluoropentyl)-1H-indazole-3-carboxamido]-3,3-dimethylbutanoate (synonyms: MDMB(N)-2201 [1], 5-fluoro-ADB [2]) were observed since 2015 (fig.1). According to police reports all cases were observed in suspects who inhaled herbal smoking blends containing MDMB(N)-2201.

Depending on the action phase, the following intoxication symptoms were observed: either drowsiness or psychosis, hallucinations, impaired coordination, nystagmus, tremor, mydriasis, stiff thinking, dysarthria, tachycardia, hyperemia, nausea, vomiting (fig.2). Moreover 3 suspects with overdose underwent the state of coma.

MDMB(N)-2201 is one of the new psychoactive substances (NPS) with an indazole-3-carboxamide structure. In general, biotransformation converts a xenobiotic to a more polar compound promoting a decline in its re-absorption by kidney tubules, thus allowing its excretion into the urine [3]. Given the known pathway of the metabolism of synthetic cannabinoids [4], we assume that the major metabolite of MDMB(N)-2201 in human urine is 3,3-dimethyl-2-[1-(5-fluoropentyl)-1H-indazole-3-carboxamido] butanoic acid, the product of demethylation by ester hydrolysis. The aim of the study was to identify markers in human urine of MDMB(N)-2201 abuse.

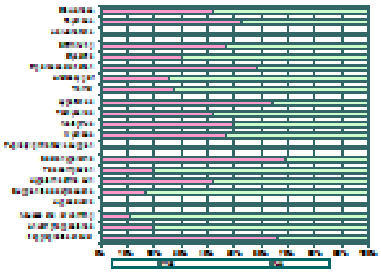

Fig.2 The frequency of the observed symptoms of intoxication

Methods

Urine samples were collected in the first 3 hours of hospitalization. The double liquid-liquid extraction (LLE) at pH 9 and at pH 3 after alkaline hydrolysis [5, 6] was used for isolation of MDMB(N)-2201 metabolites from urine.

A screening analysis was performed on gas chromatograph Agilent 7890 with mass-selective detector Agilent 5975 (Agilent Technologies). Confirmation was performed by HPLC-MS/MS using Toxtyper library system with mass spectrometer AmaZon Speed (Bruker) and liquid chromatograph Dionex Ultimate 3000 (Thermo Scientific) (fig.3).

Urine Samples Preparation

2.0 ml of urine was hydrolyzed by addition of 200 µl of 10M potassium hydroxide solution and then incubation at 60 °C for 30 min. After pH adjustment to 9 by addition of hydrochloric acid, the samples were extracted with 1.5 ml of a mixture of dichloromethane/heptane/isopropanol (6:3:1 v/v). After phase separation by centrifugation, the organic extracts were transferred to vials. After pH adjustment to 3 by addition of hydrochloric acid, the aqueous residues were extracted with 1.5 ml of a mixture of heptane/ethyl acetate (7:1 v/v). After phase separation by centrifugation, the organic extracts were transferred to the same vials. The combined organic extracts were evaporated at 40 °C under vacuum. Evaporated extracts were silylated with 20 µl of a mixture of BSTFA+1% TMCS / ethyl acetate (1:1 v/v) for 30 min at 80 °C and analyzed by GC-MS or dissolved in 100 µl of acetonitrile and analyzed by LC-MS/MS.

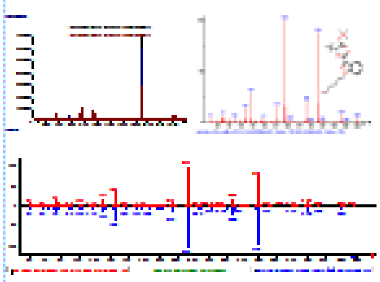

Fig.4 Biotransformation product of MDMB(N)-2201, M1 (demethylation by ester hydrolysis), RT 15.48 min. GC-MS extracted ion chromatogram and mass-spectra after trimethylsilylation (TMS).

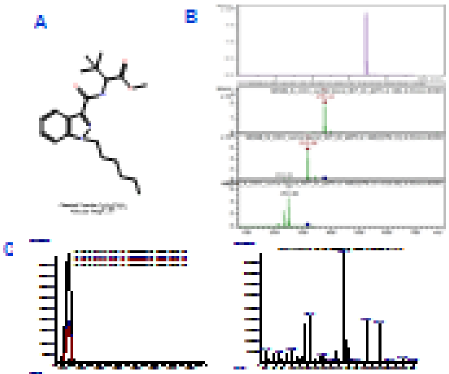

Fig.1 MDMB(N)-2201 chemical structure (A), LC-MS/MS extracted ion chromatogram and mass-spectra (B), GC-MS extracted ion chromatogram and mass-spectra (C).

GC–MS conditions

GC–MS analysis of prepared samples was performed with an Agilent 7890A gas chromatograph connected to a 5975C monoquadrupole mass-selective detector (Agilent Technologies). The GC conditions were [5, 6]: Rxi-5ms capillary column (30 m × 0.25 mm internal diameter, 0.25 µm film thickness, Restek); 280 °C injection 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, 1.076 V; identification, scan mode; scan range, m/z 41–650; solvent delay, 3 min.

Fig.3 Laboratory for forensic chemistry, analytical equipment

Mass Spectral Libraries

Well-known MS libraries such as NIST, MPW, WILEY, updated not so quickly as new psychoactive substances appear. This makes it difficult to diagnose cases of drug of NPS intoxication.

Authors used the MS libraries of NPS and their metabolites, created by leading experts of the Russian Federation in the field of analytical toxicology:

- the GC-MS NPS library «Sudmed_2980» created by Russian professional community under supervision of Alex Pechnikov (contain 2980 spectra 2008-2017) [7];
- the LC-MS/MS library «Toxtyper_Custom» created by Sergey Savchuk and Andrey Grigoryev, contain 70 NPS's and metabolites MS data [8].

These MS libraries constantly updated as new psychoactive substances appear.

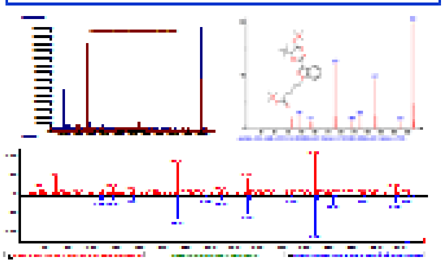

Fig.5 Biotransformation product of MDMB(N)-2201, M2 (demethylation by ester hydrolysis + defluorination and carboxylation at pentyl group), RT 16.02 min. GC-MS extracted ion chromatogram and mass-spectra after trimethylsilylation (diTMS).

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 × 100 mm (Dionex). For gradient elution, the mobile phases: 2 mM ammonium formate, 0.1% formic acid, 1% acetonitrile in deionized water (mobile phase A) and 2 mM 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, 1–9 min gradiently to 95% eluent B, 9–9 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 10 °C. The MS conditions were: capillary voltage, 4500 V; nitrogen temperature (drying gas), 200 °C; nebulizing gas pressure, 29.0 psi. The detection was performed in the MS, MS2, MS3 (full scan) mode; mass range, 70–800 m/z; using the Toxtyper_Custom method in a mode of simultaneous registration of positive and negative ions in the search windows of target substances [8].

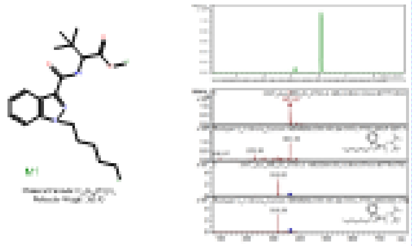

Fig.6 Biotransformation product of MDMB(N)-2201, M1 (demethylation by ester hydrolysis), RT 6.19 min. LC-MS-MS extracted ion chromatogram and mass-spectra.

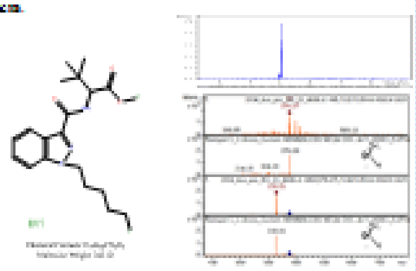

Fig.7 Biotransformation product of MDMB(N)-2201, M2 (demethylation by ester hydrolysis + defluorination and carboxylation at pentyl group), RT 3.07 min. LC-MS-MS extracted ion chromatogram and mass-spectra.

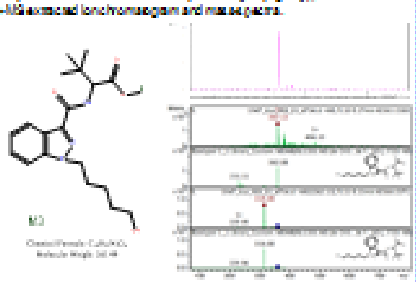

Fig.8 Biotransformation product of MDMB(N)-2201, M3 (demethylation by ester hydrolysis + defluorination and hydroxylation at pentyl group), RT 3.17 min. LC-MS-MS extracted ion chromatogram and mass-spectra.

Results

3,3-dimethyl-2-[1-(5-fluoropentyl)-1H-indazole-3-carboxamido] butanoic acid as the predominant metabolite of MDMB(N)-2201 (fig. 4, 6) was found in all urine samples. Minor metabolites of MDMB(N)-2201 were also detected in human urine samples (fig. 5, 7, 8). All identified metabolites are the same for two substances: MDMB(N)-2201 and ADB-F-PINACA. Native substance of the MDMB(N)-2201 was not detected in any of the analyzed urine samples. Other psychoactive substances were not identified in urine samples. This fact allows us to conclude that smoking blends were free from other psychoactive ingredients.

Conclusions

The ability to identify MDMB(N)-2201 and ADB-F-PINACA metabolites by GC-MS and HPLC-MS/MS as the biomarker of NPS is shown. However, our method does not allow to identify which of these two substances was the cause of intoxication.

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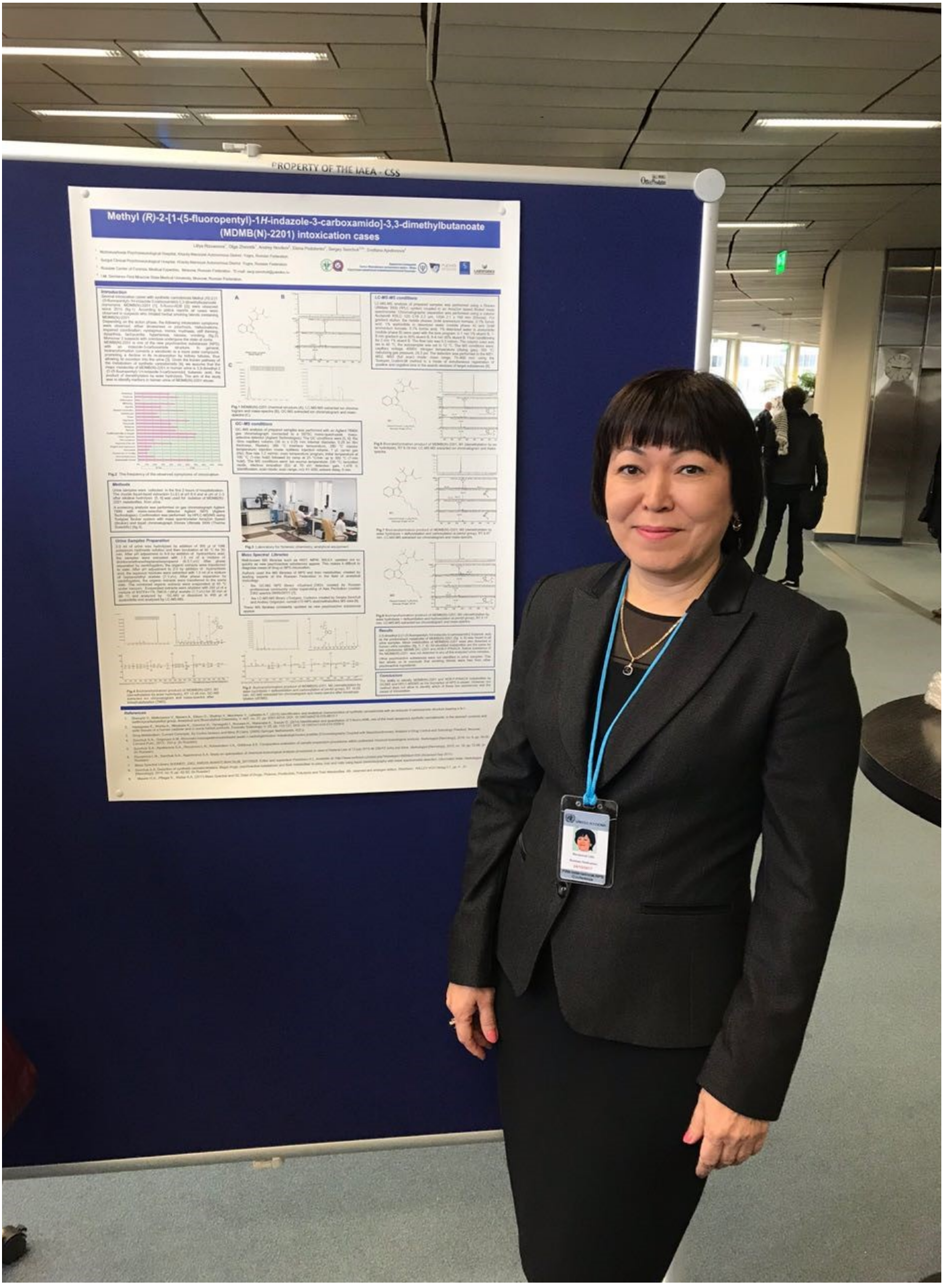

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