DESENVOLVIMENTO E APLICAÇÃO DE UM MÉTODO DE TRIAGEM RÁPIDA PARA DETERMINAÇÃO DE NOVAS SUBSTÂNCIAS PSICOATIVAS E SEUS METABOLITOS EM URINA

DEVELOPMENT AND APPLICATION OF A RAPID SCREENING METHOD FOR DETERMINATION OF NEW PSYCHOACTIVE SUBSTANCES AND THEIR METABOLITES IN URINE

РАЗРАБОТКА И ПРИМЕНЕНИЕ БЫСТРОГО СКРИНИНГОВОГО МЕТОДА ДЛЯ ОПРЕДЕЛЕНИЯ НОВЫХ ПСИХОАКТИВНЫХ СОЕДИНЕНИЙ И ИХ МЕТАБОЛИТОВ В МОЧЕ

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RESUMO

O surgimento contínuo de novas substâncias psicoativas no mercado negro de drogas ilegais, bem como a falta de informações sobre sua influência no corpo humano, enfrenta vários desafios em sua determinação por técnicas analíticas padrão. Além disso, o rápido metabolismo de novas substâncias psicoativas se revela na ausência de possibilidade na identificação de suas estruturas nativas em fluidos biológicos. Este estudo apresenta um novo método de triagem para determinação de 137 substâncias psicoativas, incluindo seus metabólitos. O método 'diluir e disparar' foi escolhido como a técnica preferida de preparação de amostras. e consistiu na diluição 1:5 das amostras de urina com a solução de acetonitrila e água (30:70), seguida de ionização por *eletrospray* - cromatografia líquida - análise em espectrometria de massa em tandem. O método qualitativo desenvolvido foi validado de acordo com os requisitos do Escritório das Nações Unidas sobre Drogas e Crime, que incluíam avaliação de seletividade, limites de detecção, precisão e estabilidade. Além disso, o método apresentado foi testado em 50 amostras de urina positivas certificadas contendo diferentes drogas de abuso. A análise confirmatória foi realizada usando uma abordagem de espectrometria de massa de alta resolução. O método de triagem apresentado oferece a possibilidade de determinação simultânea de canabinóides sintéticos (96), analgésicos opiáceos (16), estimuladores (13), alucinógenos (5), benzodiazepínicos (5) e medicamentos não classificados (10) durante uma corrida. As avaliações de validação do novo método mostraram altas taxas de especificidade, seletividade, precisão e estabilidade intra e inter-dias, com o limite de detecção variando de 1 a 5 ng·mL⁻¹. Ao mesmo tempo, testes de 50 amostras positivas mostraram excelente aplicabilidade do método de triagem desenvolvido para análises preliminares de rotina em laboratórios toxicológicos.

Palavras-chave: *Espectrometria de massa por cromatografia, métodos de triagem, novas substâncias psicoativas.*

ABSTRACT

The ongoing appearance of new psychoactive substances on the black market of illegal drugs, as well as the lack of information on their influence on the human body, faces several challenges in their determination by standard analytical techniques. Moreover, the rapid metabolism of new psychoactive substances reveals in the absence of possibility in the identification of their native structures in biological fluids. This study presents a new screening method for determination 137 psychoactive substances including their metabolites. 'Dilute-and-shoot' method was chosen as the preferable sample preparation technique, and consisted of 1:5 dilution of urine specimens with the solution of acetonitrile and water (30:70) followed by electrospray ionization – liquid

chromatography-tandem mass spectrometry analysis. The developed qualitative method was validated according to United Nations Office on Drugs and Crime requirements that included assessment of selectivity, limits of detection, precision, and stability. In addition, the presented method was tested on 50 certified positive urine specimens containing different drugs of abuse. The confirmatory analysis was performed using a high-resolution mass-spectrometry approach. The presented screening method provides the possibility of simultaneous determination of synthetic cannabinoids (96), opioid analgesics (16), stimulants (13), hallucinogens (5), benzodiazepines (5) and non-classified drugs (10) during one run. The validation assessments of the novel method have shown high rates of its specificity, selectivity, intra- and inter-day precision and stability with the limit of detection ranged from 1 to 5 ng·mL⁻¹. At the same time, tests of 50 positive samples showed excellent applicability of the developed screening method for routine preliminary screening analysis in toxicological laboratories.

Keywords: *Chromatography-mass spectrometry, screening methods, new psychoactive substances.*

АННОТАЦИЯ

Активное появление новых психоактивных препаратов на черном рынке наркотических средств, а также отсутствие достаточной информации об их влиянии на организм формирует ряд трудностей в их обнаружении рутинно используемыми аналитическими методами. Более того, за счет активного метаболизма психоактивных препаратов определение их нативных структур в биологических жидкостях становится практически невозможным. Таким образом, в данной работе представлен новый скрининговый метод, направленный на определение 137 психоактивных препаратов и их метаболитов. Метод "Dilute-and-shoot" был выбран как наиболее предпочтительный метод пробоподготовки. Он заключался в разведении образца в отношении 1:5 смесью ацетонитрил: вода (30:70) с последующим анализом методом жидкостной хроматографии - tandemной масс-спектрометрии. Разработанный метод был валидирован в соответствии с требованиями Управления ООН по наркотикам и преступности, включающим оценку селективности, предела обнаружения, точности и стабильности. Подтверждающий анализ проводился с использованием масс-спектрометрии высокого разрешения. Представленный метод дает возможность одновременного определения синтетических каннабиноидов (96), опиоидных анальгетиков (16), стимуляторов (13), галлюциногенов (5), бензодиазепинов (5) и неклассифицированные соединения (10) во время одного инструментального анализа. Валидация нового метода продемонстрировала высокие уровни специфичности, селективности, точности и стабильности, с диапазоном предела обнаружения 1-5 нг/мл. Помимо этого, апробация на 50 положительных образцах показала отличную практическую значимость разработанного скринингового метода для рутинного предварительного скринингового анализа в токсикологических лабораториях.

Ключевые слова: *Хромато-масс-спектрометрия, скрининговая методика, новые психоактивные вещества.*

1. 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 (Simolka *et al.*, 2012). NPS also referred to as "designer drugs" has become a worldwide phenomenon that attracts special attention to scientific and toxicological societies (Grabenauer *et al.*, 2016). Following the United Nations Office on Drugs and Crime (UNODC, 2013), the amount of seized NPS and its recreational use are remarkably growing since 2010th. Currently, more than 800 NPS are under control by the European Monitoring Centre for Drugs and Drug Addiction

(EMCDDA), and thus, it has become a common obstacle (UNODC, 2018; Savchuk *et al.*, 2017).

NPS imitate the mechanisms of action of "traditional" illegal drugs showing hallucinogenic (e.g., 1p-LSD, 2C-I), stimulant (e.g., methylon, PVP), sedative (e.g., etizolam, clonazolam) or euphoric (e.g., carfentanyl, 3-methylfentanyl) effects and are usually synthesized by altering chemical structures of the already controlled compounds with minor modifications (Gómez-Ruiz *et al.*, 2007; Savchuk *et al.*, 2017). For this reason, until these substances are not included in the list of prohibited and controlled illegal drugs, they can unpunishably be dispersed all over the world. Usually, they are sold with the trade names "research chemicals", "bath salts" or "exotic

incenses".

Generally, the use of blood or saliva as a biological matrix for screening methods of illegal drugs may provide indications of recent exposure and link intoxication to the causative agents (Dresen *et al.*, 2011; Castaneto *et al.*, 2015). Nevertheless, urine is still the most common and applicable matrix in toxicological laboratories due to its non-invasive collection, longer detection time windows, and higher metabolite concentrations (Abdulaziz *et al.*, 2016).

Due to the lack of the appropriate information concerning interactions of NPS with other drugs, as well as their possible impurities and unknown consumed amount of the drug, it is usually challenging to choose the adequate dosage during its illegal consumption (Gurney *et al.*, 2014). Moreover, the higher binding affinity of NPS to receptors (mainly cannabinoid and opioid receptors) compared with one the of traditional drugs, results in a higher severity of the side effects (Keller *et al.*, 2009; Yeakel *et al.*, 2013). As a result, the number of severe intoxications increases that is mostly associated with the need for emergency medical service and occasionally–deaths (Buser *et al.*, 2014; Mir *et al.*, 2011, Centers for Disease Control and Prevention, 2013). At the same time, in several cases, the utilization of NPSs for medical purposes is legal, while its non-medical usage is under specific control almost all over the world.

The still increasing appearance of NPS on the black market and number of overdose cases urges scientists to the creation of fast, accurate, and sensible methodologies for the precise determination of the NPS along with traditional drugs of abuse. Moreover, rapid appearance and variety of physic-chemical properties of each NPS cause several difficulties in their determination in biological matrixes during forensic and toxicological investigations. Therefore, it is needed to develop fast, selective, and sensitive approaches.

Analytical methods routinely used in toxicological laboratories are mainly focused on the determination of parent compounds. At the same time, the main issue in the identification of synthetic NPS, especially synthetic cannabinoids, is their fast metabolism resulting in the fact that most of the native compounds could not be detected in commonly used matrixes, even after first minutes of its consumption (Tyrkko *et al.*, 2013).

For this reason, it is needed to develop a screening method that will cover the preliminary

determination of the most spread illegal drugs, including NPS, together with their primary active metabolites.

Chromatographic separation methods, coupled with mass-spectrometric determination techniques, are commonly applied in different toxicological studies providing high rates of sensitivity, selectivity, and specificity (Ibáñez *et al.*, 2013). Until recently, the most preferred mass-spectrometric approaches in terms of routine toxicological analysis were GC-MS methods (Tsujikawa *et al.*, 2014). However, GC-MS is usually time consuming and in most cases, requires steps of extraction and derivatization. For this reason, fast dilute-and-shoot LC-MS/MS screening methods have become the most prevalent techniques usually used for preliminary screening analysis (Xu *et al.*, 2007; Aszyk *et al.*, 2016; Wu *et al.*, 2012). During the last several years, many methods for illegal drug screening have been published. Table 1 represents comparison between last released screening methods with various sample preparation and instrumental approaches.

Thus, this study aimed to develop a fast LC-MS/MS qualitative screening method for the determination of 137 psychoactive substances and their metabolites, including the most popular NPS, in the urine. The method was approved on 50 confirmed positive samples providing high levels of sensitivity and detectability, along with short and simple sample preparation.

2. MATERIALS AND METHODS

2.1. Chemicals

Chemicals were purchased from LGC standards, Cayman Chemical and Sigma-Aldrich. All utilized solvents were of HPLC grade. Acetonitrile, formic acid, and methanol were purchased from Merk (USA).

2.2. Biological material

Urine samples from 50 positive drug users were obtained from cases investigated in the certified toxicological laboratories and were verified by gas chromatography with electron impact mass spectrometry (GC-MS-EI) and UPLC-Q-TOF (information not presented). Drug-free blank urine samples for the development and validation of the method were collected from region specimen banks and were confirmed not to

have any drugs of abuse. The certified urine with the quantified amount of the screened drugs was purchased from the WHO UNODC professional blind tests.

2.3. Specimen procedure

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 the following addition of 150 µl of a mixture containing acetonitrile:water (70:30). The resulted solution was after vortexed for 15 seconds and centrifuged 5 min at 15000 rpm. After 40 µl of supernatant was transferred to a new vial with the addition of 60 µl of *Milli-Q® ultrapure water*, vortexed, and transferred to a vial for LC-MS analysis.

2.4. Chromatographic and mass-spectrometric conditions

The LC-MS analysis was conducted using the UPLC ACQUITY system connected to a Xevo TQ-S micro IVD System (Waters Corporation, USA) with an electrospray source operated in positive and negative ion ionization mode. The separation was achieved using chromatographic column Acclaim RSLC 120 AC18, 2.2 µm, 100×2.1mm equipped with guarding column: Acquity UPLC® BEH C18, 5.0×2.1 mm (Waters Inc. USA) maintained at 40°C. Mobile phases consisted of 0.1% formic acid in water with the addition of 2 mM ammonia formate and 1% of acetonitrile (mobile phase A) and 0.1% formic acid in acetonitrile with the addition of 2 mM ammonia formate and 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 minutes. Ions were registered in both positive and negative ionization in the Selected Reaction Monitoring (SRM) mode by monitoring precursor ions and its two fragment-ions. 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 - 2000V. The equipment maintenance and data analysis were performed using Mass Lynx V4.2.

2.5. Validation

Validation of the presented qualitative screening method was performed according to the UNODC requirements for validation of analytical methods for testing of illegal drugs (UNODC,

2009).

The full qualitative validation procedure was undertaken for 12 drugs of abuse related to different classes of controlled drugs (synthetic cannabinoids, opioids, phenylamines, benzodiazepines). The validation assessment consisted of estimation of selectivity, limits of detection (LOD), precision, and stability of the developed screening method. For the selectivity study, the drug-free blank urine samples collected from health individuals were analyzed in three replicates and were compared to the analyzed three replicates of QC samples, which contained compounds being under validation. Further, there were calculated relative standard deviations of analyzed compounds that did not exceed 15% and 20% in the extreme points, respectively. The precision of the developed method was conducted by analyzing the reproducibility of the received results based on 5 repeated analyses of spiked samples at three concentration levels: lowest (5 ng/ml), medium (50 ng/ml) and highest (150 ng/ml). The stability study was undertaken to assess the stability of the analytes during short and long-term storage. For this purpose, there were conducted comparison analyses of samples right after its sample preparation, in 72 hours of its storage at +4°C.

2.6. Testing on real positive samples

The method was tested on 50 confirmed positive urine samples. For this purpose, urine samples were provided by toxicological laboratories from different regions of the Russian Federation for round multicentral comparative studies. All provided samples were confirmed and reported before to be positive by means of employing traditional gas-chromatography mass-spectrometry methods.

2.7. Confirmatory high resolution LC-MS method

The confirmatory analysis was performed using UHPLC-QTOF-MS, contained Bruker Elute liquid chromatography coupled to high-resolution quadrupole time-of-flight mass-spectrometer. Chromatographic separation was performed using ACQUITY UPLC BEH C18 (100 x 2.1 mm) (BRU-18C182-100 mm). Temperatures of the column and autosampler were maintained at 40 °C and 4 °C, respectively. Mobile phase A contained 1% of Methanol in water with the addition of 5 mM ammonia formate in 0.1% formic acid, while Mobile phase B consisted of methanol with five mM ammonia formate in 0.1% formic acid. The flow rate was 0.2 ml/min. The gradient elution was

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. Ionization was performed in a positive electrospray mode with m/z range 30-1000 m/z. ESI source parameters were: capillary voltage - 4500V; N₂ temperature 220 °C with flow rate 8 l/min and collision energy - 7eV.

3. RESULTS AND DISCUSSION

The presented screening LC-MS/MS method was developed for the determination of 137 illegal drugs, including "traditional illegal drugs" (such as amphetamines or 'marihuana'), NPSs, and their main active metabolites. In many cases, NPS is abused together with traditional drugs of abuse. Therefore, the goal of this study was to establish a method that covers different classes of the most spread drugs of abuse. As a result, the developed screening method allows the opportunity for simultaneous determination of synthetic cannabinoids (88), opioid analgesics (16), stimulants (13), hallucinogens (5), benzodiazepines (5) and non-classified drugs (10) during one LC-MS run (Table 2).

3.1. Optimization of the instrumental methods

Optimization of the separation conditions is one of the critical steps in the development of screening methods. It serves for the identification of all analyzed analytes by separation of their isomers, elimination interfering signals, and certifying elution of all compounds of interest within the selected gradient. Moreover, chromatographic optimization may serve for the establishment of the retention time for all screened compounds that result in narrow and symmetrical peaks of each analyte. Due to the big difference in the chemical nature of the screened compounds, in the presented study, it was considered to use the C18 phase column, which, to our knowledge, is one of the most optimal stationary phases commonly used for the routine toxicological analysis. To provide appropriate conditions for protonation of basic groups in several NPS it is more common to use buffers with acidic pH. Optimization of the mobile phase composition for HPLC systems plays a key role in the quality of chromatographic separation. Thus, in this study there were tested several components of mobile phase B, such as: pure acetonitrile; acetonitrile + 0.1% formic acid; acetonitrile + 2mM ammonium formate + 0.1% formic acid; acetonitrile + 2mM ammonium formate + 1% water + 0.1% formic acid; pure methanol. The utilization

of pure acetonitrile or methanol by itself, as well as with the addition of 1% water, gave good and intensive peaks for only neutral or weakly acidic analytes. At the same time, the addition of organic modifiers (such as 2mM ammonia formate) increased the sensitivity for mostly all of the screened compounds.

Therefore, according to the received results it was found out that the best separation of all analytes presented in the method (at the low signal-to-noise ratio and high rates of sensitivity and resolution) could be achieved using following mobile phases: phase A – deionized water + 0.1% formic acid + 2mM ammonia formate + 1% acetonitrile; phase B – acetonitrile + 0.1% formic acid + 2mM ammonia formate + 1% distilled water.

Besides that, to increase sensitivity in the determination of NPS, the maintenance of mass-spectrometry optic and fragmentation conditions of parent ions was conducted. For this reason, there were analyzed certified urine samples containing morphine in the concentration of 0.1 µg/ml. Cone voltages and collision energies were optimized.

For the following optimization step of the study, there were determined main MRM transitions of each compound. Using Masslynx software, at least two MRM transitions were selected for each of the analytes. Ionization was performed using ESI, maintained in both positive and negative mode. Negative ESI provided better detectability for the acid derivatives. Optimized MRM transitions and collision energies for positive and negative ESI are presented in Tables 3 and 4, respectively.

3.2. Optimization of the sample preparation step

The assessment of linearity and sensitivity of the optimized LC-MS conditions demonstrated good results that allowed performing the following optimization of the sample preparation method. 'Dilute-and-shoot' was chosen to be the method of choice for our preliminary screening method as it is the most rapid technique, as well as provides fewer chances of losing target compounds (as could be present while using solid-phase or liquid-liquid extractions). To find out the appropriate conditions for the 'Dilute-and-shoot' method, to 100 µl of the certified urine, containing Morphine (in concentration 100 ng/ml), there were added different solvent solutions: Pure acetonitrile, Acetonitrile (AcN):water (H₂O) (7:1); 150 µl of the mixture AcN : H₂O with consequent addition of 60 µl of H₂O to the 40 µl of the initial mixture.

It was found out that the third approach was the most optimal for the NPS screening, since the addition of water obstructs the elution of the substance in the form of two peaks, while the presence of the organic phase contributes to the sensitivity of the target analytes signals.

3.3. Validation

Validation was performed for 12 selected compounds related to the most spread classes of prohibited substances and was aimed to confirm that the method was satisfied for the determination of leading groups of illegal drugs. The method proved to be selective and specific for the tested compounds by the analysis of blank urine samples that showed the absence of any interfering signals. At the same time, analysis of the quality control solutions at the concentration equal to 5 ng/ml provided purity of all received signals, regarded to analyzed compounds. The precision assessment was performed by analyzing the reproducibility of signals received from analysis at three concentration levels: 5 ng/ml, 50 ng/ml, and 150 ng/ml.

After three repeats of the analysis at each concentration level, the relative standard deviation of the received signals was not more than 15% at high concentrations and 20% – at low concentrations. The received LODs of all analyzed compounds were between the limit of 0.5-10 ng/ml. Furthermore, the already prepared samples were stable during long-term storage for 72 hours at +4°C, by calculating relative standard deviations (RSDs) of the signals immediately after preparation of the samples, in 72 hours. The signals at each time-point remained approximately unchanged (RSD < 15%) that proved the stability of the analyzed substances (Table 5).

The selected gradient program was established in the way that it could separate the main components of the method. The presented method includes 350 MRM transitions with the respected cycle of 0.015 seconds for each compound.

3.4. Application of the method on real positive samples

Real urine samples that contained markers of drug abuse were obtained from different authorized toxicological laboratories. All samples were before examined to be positive using complex routine methods, usually utilized in forensic laboratories. Thus, the method was tested on 50 proved positive cases, associated with the presence of NPS and traditional drugs of abuse

(Table 6).

It should be noted that most of the synthetic cannabinoids, were not identified in their native structures, but were presented in the form of its leading derivatives, mostly carboxylic moieties, produced by the expense of losing methoxy-, amide- or adamantyl- groups. Alternately, most of the synthetic cathinones were detected predominately in their native forms. Notably, not all compounds, determined by GC-MS, were identified by the developed approach. This fact could be associated with the absence of preliminary extraction and extremely low concentration of the analyzed compounds.

An example of one of the positive samples is presented in Figure 1. It includes chromatograms received using the developed preliminary screening method as well as the results of a confirmatory analysis performed using high resolution mass-spectrometry (as described in 2.8).

The results of the application of the method showed that the developed approach might find practical applications for clinical purposes. 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 body is 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 drug consumptions. Compared to "traditional narcotics", NPSs have a higher potency, and 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 a total sample preparation time of one specimen about 5 minutes. The developed approach makes it possible to determine the main drugs of abuse including NPS and their metabolites within 15 minutes, being a perfect approach for preliminary drug analysis.

4. CONCLUSIONS:

A novel methodology was developed, a sensitive and fast LC-MS/MS method for the determination of 137 illegal drugs, including NPS

and their metabolites in urine. The presented method gives the possibility for the determination of parent substances, as phase I and phase II metabolites. The developed approach provided excellent performance in terms of turnaround, selectivity, and sensitivity. The utilization of a diluting-shoot sample preparation technique resulted in the establishment of a rapid approach with a total run-time of about 15 minutes. Moreover, the developed method could potentially be utilized for preliminary screening purposes in chemical toxicological laboratories being easily transformed between different LC-MS/MS instruments.

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Table 1. Description of screening methods with various sample preparation and instrumental approach

Analyzed compounds	Sample preparation	Instrumental analysis	Ref.
Screening of 64 NPS in Dry Blood spots (DPS) phenethylamines cathinones piperazines tryptamines	1. 10 µl of blood pipetted onto the DBS, diffused and dried for 3 hours; 2. DBS were punched and collected in the Eppendorf tube; 3. Extraction: +500 µl MeOH + 10 µl IS; vortex (15 min); 4. Supernatant + 10 µl 0.25% HCl MeOH and reconstitution in 100 µl of H ₂ O	RP-LC-MS/MS Run time – 10 min Scheduled multiple reaction monitoring (sMRM)	(Ambach <i>et al.</i> , 2013)
Quantitative analysis of 56 NPS in whole blood and urine Stimulant and hallucinogenic NPS (piperazines, tryptamines, amphetamines, cathinones)	Blood samples: SPE at PH 6, elution by Rapid and simple LC-MS/MS screening of 64 novel psychoactive substances using dried blood spots. Urine samples: LLE in 0.1M NaOH with ethyl acetate	LC-MS/MS ESI(+) sMRM	(Ambach <i>et al.</i> , 2015)
Determination of 40 NPS and 4 their metabolites Piperazines (8) Amphetamines (4) Synthetic cathinones (28) and related metabolites (4)	Urine SPE on cation exchange extraction (SOLA SCX); Elution with 2% NH ₄ OH in dichloromethane/2-propanol (95:5, v/v).	LC-HRMS Screening and confirmation - in full scan and data dependent MS ² .	(Beck <i>et al.</i> , 2014)
93 drugs of abuse and their metabolites 47 conventional metabolites (28 parents and 19 metabolites) 46 NPS (44 parent drugs and 2 metabolites) 35 drugs of abuse and metabolites:	Urine SPE pH 6. SPE cartridges (Waters Oasis MCX, 3 cc, 60 mg)	UHPLC-MS/MS ESI (+/-) MRM	(Tang <i>et al.</i> , 2014)
Amphetamines (7); THC-COOH; Opiates (6); Cocainics (3); Benzodiazepines (16); others (2)	SPE (pH 5) using the Bond ElutPlexa PCX column Elution with chloroform–isopropyl alcohol (80:20) and 3 ml chloroform–isopropyl alcohol–ammonium hydroxide (80:20:3)	LC/MS/MS ESI (+/-)	(Shin <i>et al.</i> , 2014)
414 compounds of illegal drugs	Protein precipitation with acetonitrile and 5-fold dilution	LC-MS/MS sMRM-IDA-EPI	(Verplaetse <i>et al.</i> , 2013)
	Urine and plasma SPE 9 Thermo Scientific Hypersep Verify CX 200 mg mixed mode cartridges	LC-MS/MS	(Kozak <i>et al.</i> , 2018)
50 NPS in postmortem samples (region, blood, and urine) 26 “legal highs”	whole blood or urine LLE at Ph>9 with 70:30 <i>n</i> -butyl chloride:ethyl acetate Urine five-fold dilution	LC-MS/MS LC-MS/MS ESI (+)	(Fagiola <i>et al.</i> , 2018) (Al-Saffar <i>et al.</i> , 2013)

Table 2. List of the compounds included in the developed screening method with their chemical names, brutto-formulas collision energies (positive ESI mode)

Synthetic cannabinoids
<p>AB-001 M1 (diHydroxylation)</p> <p>$C_{24}H_{31}NO_3$ 381.5078</p>	<p>AB-001 M2 (Hydroxylation)</p> <p>$C_{24}H_{31}NO_2$ 365.5084</p>	<p>AB-CHMINACA M1 (Carboxylation, hydroxylation of deaminated moiety)</p> <p>$C_{20}H_{27}N_3O_4$ 373.4461</p>	<p>AB-CHMINACA M2 (Carboxylation, Glucuronidation)</p> <p>$C_{26}H_{35}N_3O_9$ 533.5708</p>	<p>AB-CHMINACA M3 (Carboxylation, Hydroxylation, Glucuronidation)</p> <p>$C_{26}H_{35}N_3O_{10}$ 549.5702</p>
<p>AB-CHMINACA M4 (Carboxylation, Hydrolysis of amide)</p> <p>$C_{15}H_{18}N_2O_2$ 258.1368</p>	<p>AB-CHMINACA M5 (Carboxylation)</p> <p>$C_{20}H_{27}N_3O_3$ 357.4467</p>	<p>AB-CHMINACA</p> <p>$C_{20}H_{28}N_4O_2$ 356.4692</p>	<p>AB-FUBINACA M1 (Carboxylation)</p> <p>$C_{20}H_{20}FN_3O_3$ 369.3895</p>	<p>AB-PINACA</p> <p>$C_{18}H_{26}N_4O_2$ 330.4246</p>
<p>AB-PINACA M1 (Carboxylation)</p> <p>$C_{18}H_{26}N_3O_3$ 331.4094</p>	<p>AB-PINACA M2 (Amide hydrolysis, Glucuronidation)</p> <p>$C_{24}H_{34}N_3O_9$ 507.5335</p>	<p>AB-PINACA-F M1 (Carboxylation)</p> <p>$C_{18}H_{24}FN_3O_3$ 349.3998</p>	<p>AB-PINACA-F M2 (Oxidative defluorination, amide hydrolysis)</p> <p>$C_{18}H_{23}N_3O_5$ 361.3923</p>	<p>AB-PINACA-F M3 (dicarboxylation, Glucuronidation)</p> <p>$C_{24}H_{31}N_3O_{11}$ 537.5164</p>
<p>AB-PINACA-F M4 (Defluorination, amide hydrolysis)</p> <p>$C_{18}H_{25}N_3O_4$ 347.4088</p>	<p>AKB-48</p> <p>$C_{23}H_{31}N_3O$ 365.5117</p>	<p>AKB-48 M1 (Dihydroxylation)</p> <p>$C_{23}H_{31}N_3O_3$ 397.5105</p>	<p>AKB-48 M2 (Hydroxylation)</p> <p>$C_{23}H_{31}N_3O_2$ 381.5111</p>	<p>AKB-48 M3 (Hydroxylation, Carboxylation)</p> <p>$C_{23}H_{29}N_3O_4$ 411.4941</p>
<p>AM-2201</p>	<p>AM-694 M1 (Defluorination Carboxylation)</p>	<p>AM-694 M2 (Defluorination Hydroxylation)</p>	<p>AM-694 M3 (Hydroxylation)</p>	<p>APINAC</p>

$C_{24}H_{22}FNO$ 359.4360 APINAC M1 (Carboxylation)	$C_{20}H_{18}INO_3$ 447.2663 FUB-PB22	$C_{20}H_{20}INO_2$ 433.2828 FUB-PB22 M1 (Carboxylation)	$C_{20}H_{19}IFNO_2$ 451.2732 FUB-PB22 (Carboxylation, Glu)	$C_{23}H_{30}N_2O_2$ 366.4965 JWH-018
$C_{13}H_{16}N_2O_2$ 232.2783 JWH-018 M1 (Carboxylation, Glu)	$C_{25}H_{17}FN_2O_2$ 396.4131 JWH-018 M2 (Hydroxylation)	$C_{16}H_{12}NO_2F$ 269.2704 JWH-018 M3 (Carboxylation)	$C_{22}H_{20}FNO_8$ 445.3945 JWH-073	$C_{24}H_{23}NO$ 341.4455 JWH-073 M1 (Hydroxylation at indazole)
$C_{30}H_{29}NO_9$ 547.5526 JWH-073 M2 (Carboxylation, Glu)	$C_{24}H_{23}NO_2$ 357.4450 JWH-073 M3 (Carboxylation)	$C_{24}H_{21}NO_3$ 371.4284 JWH-203	$C_{23}H_{21}NO$ 327.4189 JWH-203 M1 (Carboxylation)	$C_{23}H_{21}NO_2$ 343.4183 JWH-203 M2(4-pentyl hydroxylation)
$C_{29}H_{27}NO_9$ 533.5260 JWH-203 M3 Dihydroxylation	$C_{23}H_{19}NO_3$ 357.4019 JWH-203 M4 (Carboxylation, glu)	$C_{21}H_{22}ClNO$ 339.8585 JWH-210	$C_{21}H_{20}ClNO_3$ 369.8414 JWH-210 M1 (Trihydroxylation)	$C_{21}H_{22}ClNO_2$ 355.8579 JWH-210 M2 (Hydroxylation)
$C_{21}H_{22}ClNO_2$ 371.8673 JWH-210 M3 (Carboxylation)	$C_{27}H_{28}ClNO_9$ 545.9655 JWH-250 M1 Dihydroxylation	$C_{26}H_{27}NO$ 369.4987 JWH-250 M2 (Hydroxylation)	$C_{26}H_{27}NO_4$ 417.4969 JWH-250 M3 (Carboxylation)	$C_{26}H_{27}NO_2$ 385.4981 JWH-251 M1 (Carboxylation)
$C_{26}H_{25}NO_3$ 399.4816 JWH-251 M2 (Carboxylation, Glu)	$C_{22}H_{25}NO_4$ 367.4382 JWH-251 M3 (Hydroxylation)	$C_{22}H_{25}NO_3$ 351.4388 MAM-2201	$C_{22}H_{23}NO_4$ 365.4223 MDMB(N)-Bz-F	$C_{22}H_{23}NO_3$ 349.4223 MDMB(N)-Bz-F M1 (Carboxylation)

$C_{28}H_{31}NO_9$ 525.5470 MDMB (N)-Bz-F M2 (Carboxylation, Glu) $C_{27}H_{31}FN_3O_9$ 559.5402 MMB-2201	$C_{22}H_{25}NO_2$ 335.4394 MDMB (N)-Bz-F M3 (indazole Hydroxylation) $C_{21}H_{23}FN_3O_4$ 399.4155 MMB-2201 M1 (Carboxylation)	$C_{25}H_{24}FNO$ 373.4626 MDMB(N)-2201 M1 (Carboxylation) $C_{19}H_{26}FN_3O_3$ 363.4264 MMB-2201 M2 (Carboxylation, glu)	$C_{22}H_{24}FN_3O_3$ 397.4427 MDMB-CHMINACA $C_{22}H_{31}N_3O_3$ 385.4998 PB-22	$C_{21}H_{23}FN_3O_3$ 383.4161 MDMB-CHMINACA M1 (Carboxylation) $C_{21}H_{29}N_3O_3$ 371.4732 PB-22 M1 (Carboxylation, Ester hydrolysis)
$C_{20}H_{27}FN_2O_3$ 362.4384 PB-22 M2 (Carboxylation, Glu) $C_{20}H_{25}NO_8$ 407.4144 PB-22F M3 (Carboxylation, Glu) $C_{20}H_{24}NO_8F$ 425.4049 RCS-4M1 (Dihydroxylation)	$C_{19}H_{25}FN_2O_3$ 348.4118 PB-22 M3 (Carboxylation, hydroxylation) $C_{14}H_{17}NO_3$ 247.2897 PB-22F M4 (Carboxylation, Hydroxylation) $C_{14}H_{16}NO_3F$ 265.2801 RCS-4 M2 (O-demethyl-OH)	$C_{25}H_{33}FN_2O_9$ 524.5359 PB-22 M4 (Carboxylation, ketone formation, glu) $C_{20}H_{23}NO_9$ 421.3979 PB-22F M5 (Carboxylation, defluorination) $C_{14}H_{15}NO_4$ 261.2732 RCS-4 M3 (Hydroxylation)	$C_{23}H_{22}N_2O_2$ 358.4330 PB-22F $C_{23}H_{21}N_2O_2F$ 376.4334 PB-22F M6 (Carboxylation, defluorination, glu) $C_{20}H_{23}NO_{10}$ 437.3973 RCS-4 M4 (HO-phenyl-oxo-)	$C_{21}H_{23}FN_3O_3$ 383.4161 THJ-2201 M1 (Carboxylation) $C_{21}H_{29}N_3O_3$ 371.4732 PB-22 M1 (Carboxylation, Ester hydrolysis) $C_{14}H_{17}NO_2$ 231.2903 PB-22F M2 (Carboxylation, Ester hydrolysis) $C_{14}H_{16}NO_2F$ 249.2807 RCS-4 $C_{21}H_{23}NO_2$ 321.4128 THJ-2201
$C_{21}H_{23}NO_4$ 353.4116 THJ-2201 M1 (defluorination)	$C_{20}H_{21}NO_3$ 323.3856 THJ-2201 M2 (Carboxylation,	$C_{21}H_{23}NO_3$ 337.3692 UR-144	$C_{22}H_{25}NO_3$ 351.3957 UR-144 M1 (Dihydroxylation)	$C_{23}H_{21}FN_2O$ 360.4240 UR-144 M (Hydroxylation)

<chem>C23H22N2O2</chem> 358.4330 XLR-11 <chem>C21H28FNO</chem> 329.4515	defluorination) <chem>C23H20N2O3</chem> 372.4165 XLR-11 M1 (Carboxylation, defluorination) <chem>C21H27NO3</chem> 341.4440	<chem>C21H29NO</chem> 311.4611 XLR11 M2 (Hydroxylation at indazole) <chem>C21H28FNO2</chem> 345.4509	<chem>C21H29NO3</chem> 343.4599	<chem>C21H29NO2</chem> 327.4605
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Synthetic opioids

Fentanyl <chem>C22H28N2O</chem> 336.4705	Norfentanyl <chem>C14H20N2O</chem> 232.3214	Fentanyl M <chem>C19H24N2</chem> 280.4072	3-methylfentanyl <chem>C23H30N2O</chem> 350.4971	Carfentanyl <chem>C24H30N2O3</chem> 394.5066
Tramadol <chem>C16H25NO2</chem> 263.3752	O-desmethyl-tramadol <chem>C15H23NO2</chem> 249.3486	Morphine <chem>C17H19NO3</chem> 285.3377	Morphine-glucuronide <chem>C23H27NO9</chem> 461.4618	Norcodeine <chem>C17H19NO3</chem> 285.3377
Codeine <chem>C18H21NO3</chem> 299.3643	Dihydrocodeine <chem>C18H23NO3</chem> 301.3801	Methadone <chem>C21H27NO</chem> 309.4452	Ketamine <chem>C13H16ClNO</chem> 237.7252	Norketamine <chem>C12H14ClNO</chem> 223.6987

Halucinogens

Methoxetamine <chem>C15H21NO2</chem> 247.3327	2-CB <chem>C10H14BrNO2</chem> 260.1277	Psilocybin <chem>C12H17N2O4P</chem> 284.2481	LSD <chem>C20H25N3O</chem> 323.432	LSD (2-oxo-3-OH) <chem>C20H25N3O3</chem> 355.4308
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Stimulators

Amphetamine <chem>C9H13N</chem> 135.2062	Methamphetamine <chem>C10H15N</chem> 149.2328	Fentermine <chem>C10H15N</chem> 149.2328	MDAI <chem>C10H11NO2</chem> 177.1998	Methylephedrine <chem>C11H17NO</chem> 179.263
MDMA <chem>C11H15NO2</chem> 193.2423	Cocaine <chem>C17H21NO4</chem> 303.3529	Benzoylcgonine <chem>C17H21NO4</chem> 289.3264	MDPV <chem>C16H21NO3</chem> 275.3428	MDPBP <chem>C15H19NO3</chem> 261.3163

 Methylole $C_{11}H_{13}NO_3$ 207.2258	 Ethylole $C_{12}H_{15}NO_3$ 221.2524	 PVP $C_{15}H_{21}NO$ 231.3333		
Benzodiazepines				
 Nordiazepam $C_{15}H_{11}ClN_2O$ 270.7136	 Oxazepam $C_{15}H_{11}ClN_2O_2$ 286.7130	 Temazepam $C_{16}H_{13}ClN_2O_2$ 300.7396	 Fenazepam $C_{15}H_{10}BrClN_2O$ 349.6097	 Clozapine $C_{18}H_{19}ClN_4$ 326.8233
 7-Amino flunitrazepam $C_{16}H_{14}FN_3O$ 283.3002				
Other substances				
 Pyracetam $C_6H_{10}N_2O_2$ 142.1558	 Caffeine $C_8H_{10}N_4O_2$ 194.1906	 Hydroxyquinoline C_9H_7NO 145.1579	 Nicotine $C_{10}H_{14}N_2$ 162.2316	 Dimedrol $C_{17}H_{21}NO$ 255.3547
 Dicycloverine $C_{19}H_{35}NO_2$ 309.4867	 Carbamazepine $C_{15}H_{12}N_2O$ 236.2686	 hydroxycarbamazepine $C_{15}H_{12}N_2O_2$ 252.2679	 Chlorprotixen $C_{18}H_{18}ClNS$ 315.8602	

Table 3. List of the compounds included in the developed screening method with their chemical names, brutto-formulas collision energies(negative ESI mode)

#	Name	Brutto-formula	[M+H] ⁺	Fragment ions	CE (eV)
Synthetic cannabinoids					
1	AB-001 M1 (dihydroxylation)	$C_{24}H_{31}NO_3$	382	151, 167	15
2	AB-001 M2 (Hydroxylation)	$C_{24}H_{31}NO_2$	366	151,188	18
3	AB-CHMINACA M1 (Carboxylation, hydroxylation of deaminated moiety)	$C_{20}H_{27}N_3O_4$	374	257,356	13
4	AB-CHMINACA M2 (Carboxylation, Glucuronidation)	$C_{26}H_{35}N_3O_9$	534	241,312	13
5	AB-CHMINACA M3 (Carboxylation, Hydroxylation, Glucuronidation)	$C_{26}H_{35}N_3O_{10}$	550	257, 238	13
6	AB-CHMINACA M4 (Carboxylation, Hydrolysis of amide group)	$C_{15}H_{18}N_2O_2$	259	145, 241	13
7	AB-CHMINACA M5 (Carboxylation)	$C_{20}H_{28}N_4O_3$	358	145,241	13
8	AB-CHMINACA	$C_{20}H_{28}N_4O_2$	357	257, 328	13
9	AB-FUBINACA M1 (Carboxylation)	$C_{20}H_{20}FN_3O_3$	370	253,324	15
10	AB-PINACA	$C_{18}H_{26}N_4O_2$	331	286, 313	15

11	AB-PINACA M1 (Carboxylation)	$C_{18}H_{25}N_3O_3$	332	145, 215	15
12	AB-PINACA M2 (Amide hydrolysis, Glucuronidation)	$C_{24}H_{34}N_3O_9$	508	145, 215, 286	15
13	Ab-Pinaca-F M1 (Carboxylation)	$C_{18}H_{24}FN_3O_3$	350	177, 145, 213, 233, 304, 332	25
14	AB-PINACA-F M2 (Oxidative defluorination, amide hydrolysis)	$C_{18}H_{23}N_3O_5$	362	217, 245	15
15	AB-PINACA-F M3 (dicarboxylation, Glucuronidation)	$C_{24}H_{31}N_3O_{11}$	538	245, 298	15
16	AB-PINACA-F M4 (Oxidative defluorination, amide hydrolysis)	$C_{18}H_{25}N_3O_4$	348	213, 302	10
17	AKB-48	$C_{23}H_{31}N_3O$	412	133, 151, 215	15
18	AKB-48 M1 (Dihydroxylation)	$C_{23}H_{31}N_3O_3$	398	131, 133, 151	15
19	AKB-48 M2 (Hydroxylation)	$C_{23}H_{31}N_3O_2$	382	133, 151	15
20	AKB-48 M3 (Hydroxylation, Carboxylation)	$C_{23}H_{29}N_3O_4$	412	133, 151	15
21	AM-2201	$C_{24}H_{22}FNO$	360	155, 232	15
22	AM-694 M1 (Defluorinization, Carboxylation)	$C_{20}H_{18}INO_3$	448	231, 203	15
23	AM-694 M2 (Defluorinization, Hydroxylation)	$C_{20}H_{20}INO_2$	434	203, 231	15
24	AM-694 M3 (Hydroxylation)	$C_{20}H_{19}IFNO_2$	452	231, 221	15
25	APINAC	$C_{23}H_{30}N_2O_2$	367	135, 215	15
26	APINAC M1 (Carboxylation)	$C_{13}H_{16}N_2O_2$	232	216, 145	14
27	FUB-PB22	$C_{25}H_{17}FN_2O_2$	397	109, 224	15
28	FUB-PB22 M1 (Carboxylation)	$C_{16}H_{12}NO_2F$	270	109, 226	15
29	FUB-PB22 (Carboxylation, Glucuronidation)	$C_{22}H_{20}FNO_8$	446	109, 270	15
30	JWH-018	$C_{24}H_{23}NO$	342	155, 214	15
31	JWH-018 M3 (Carboxylation)	$C_{24}H_{21}NO_3$	372	155, 244	15
32	JWH-018 M1 (Carboxylation, Glucuronidation)	$C_{30}H_{29}NO_9$	548	155, 244	15
33	JWH-018 M2 (Hydroxylation)	$C_{24}H_{23}NO_2$	358	155, 230	15
34	JWH-073	$C_{23}H_{21}NO$	328	155, 200	18
35	JWH-073 M1 (Hydroxylation at indazole)	$C_{23}H_{21}NO_2$	344	155, 171	18
36	JWH-073 M2 (Carboxylation, Glucuronidation)	$C_{29}H_{27}NO_9$	534	155, 230	18
37	JWH-073 M3 (Carboxylation)	$C_{23}H_{19}NO_3$	358	155, 230	18
38	JWH-203	$C_{21}H_{22}ClNO$	340	125, 214	15
39	JWH-203 M1 (Carboxylation)	$C_{21}H_{20}ClNO_3$	370	125, 200	15
40	JWH-203 M2 (4-pentyl hydroxylation)	$C_{21}H_{22}ClNO_2$	356	186, 204	15
41	JWH-203 M3 (Dihydroxylation)	$C_{21}H_{22}ClNO_2$	372	125	15
42	JWH-203 M4 (Carboxylation, Glucuronidation)	$C_{27}H_{28}ClNO_9$	546	218, 200	15
43	JWH-210	$C_{26}H_{27}NO$	370	183, 214	15
44	JWH-210 M1 (Trihydroxylation)	$C_{26}H_{27}NO_4$	418	199, 215	15
45	JWH-210 M2 (Hydroxylation)	$C_{26}H_{27}NO_2$	386	183, 214	12
46	JWH-210 M3 (Carboxylation)	$C_{26}H_{25}NO_3$	400	137, 197	12
47	JWH-250 M1 (Dihydroxylation)	$C_{22}H_{25}NO_4$	368	121, 137	15
48	JWH-250 M2 (Hydroxylation)	$C_{22}H_{25}NO_3$	352	121, 137	15
49	JWH-250 M3 (Carboxylation)	$C_{22}H_{23}NO_4$	366	121, 137	15
50	JWH-251 M1 (Carboxylation)	$C_{22}H_{23}NO_3$	350	105, 200	15
51	JWH-251 M2 (Carboxylation, Glucuronidation)	$C_{28}H_{31}NO_9$	526	105, 200	15
52	JWH-251 M3 (Hydroxylation)	$C_{22}H_{25}NO_2$	336	105, 130	15
53	MAM-2201	$C_{25}H_{24}FNO$	374	141, 169	18
54	MDMB(N)-Bz-F	$C_{22}H_{24}FN_3O_3$	398	253, 253	12

55	MDMB (N)-Bz-F M1 (Carboxylation)	C ₂₁ H ₂₃ FN ₃ O ₃	384	253, 338	12
56	MDMB (N)-Bz-F M2 (Carboxylation, Glucuronidation)	C ₂₇ H ₃₁ FN ₃ O ₉	560	338, 384	12
57	MDMB (N)-Bz-F M3 (Hydroxylation at indazole)	C ₂₁ H ₂₃ FN ₃ O ₄	400	253, 324	12
58	MDMB(N)-2201 M1 (Carboxylation)	C ₁₉ H ₂₆ FN ₃ O ₃	364	318, 233	15
59	MDMB-CHMINACA	C ₂₂ H ₃₁ N ₃ O ₃	386	241, 326	12
60	MDMB-CHMINACAM1 (Carboxylation)	C ₂₁ H ₂₉ N ₃ O ₃	372,5	145, 241	12
61	MMB-2201	C ₂₀ H ₂₇ FN ₂ O ₃	364	144, 232	12
62	MMB-2201 M1 (Carboxylation)	C ₁₉ H ₂₅ FN ₂ O ₃	349.5	144, 232	12
63	MMB-2201 M2 (Carboxylation, glucuronidation)	C ₂₅ H ₃₃ FN ₂ O ₉	525.5	349.4, 232	12
64	PB-22	C ₂₃ H ₂₂ N ₂ O ₂	359	144, 214	10
65	PB-22 M1 (Carboxylation, Ester hydrolysis)	C ₁₄ H ₁₇ NO ₂	232	118, 132	12
66	PB-22 M2 (Carboxylation, glucuronidation)	C ₂₀ H ₂₅ NO ₈	408	214, 232	12
67	PB-22 M3 (Carboxylation, hydroxylation)	C ₁₄ H ₁₇ NO ₃	248	186, 174	12
68	PB-22 M4 (Carboxylation, ketone formation, glucuronidation)	C ₂₀ H ₂₃ NO ₉	246	144, 184	12
69	PB-22F	C ₂₃ H ₂₁ N ₂ O ₂ F	377	232, 144	10
70	PB-22F M2 (Carboxylation, Ester hydrolysis)	C ₁₄ H ₁₆ NO ₂ F	250	118, 132, 206	10
71	PB-22F M3 (Carboxylation, Glucuronidation)	C ₂₀ H ₂₄ NO ₈ F	426	232, 250	10
72	PB-22F M4 (Carboxylation, Hydroxylation)	C ₁₄ H ₁₆ NO ₃ F	266	118, 144	10
73	PB-22F M5 (Carboxylation, oxidative defluorination)	C ₁₄ H ₁₅ NO ₄	262	118, 144, 172, 244	10
74	PB-22F M6 (Carboxylation, oxidative defluorination, glucuronidation)	C ₂₀ H ₂₃ NO ₁₀	438	244, 260	10
75	RCS-4	C ₂₁ H ₂₃ NO ₂	322	107, 135	15
76	RCS-4 M1 (Dihydroxylation)	C ₂₁ H ₂₃ NO ₄	354	151, 230	15
77	RCS-4 M2 (O-demethyl-HO-chain-)	C ₂₀ H ₂₁ NO ₃	324	121, 153	15
78	RCS-4 M3 (Hydroxylation)	C ₂₁ H ₂₃ NO ₃	338	121, 144	15
79	RCS-4 M4 (HO-phenyl-oxo-)	C ₂₂ H ₂₅ NO ₃	352	107, 135	15
80	THJ-2201	C ₂₃ H ₂₁ FN ₂ O	361	145, 177	18
81	THJ-2201 M1 (Oxidative defluorination)	C ₂₃ H ₂₂ N ₂ O ₂	359	155, 231	15
82	THJ-2201 M2 (Carboxylation, Oxidative defluorination)	C ₂₃ H ₂₀ N ₂ O ₃	373	155, 355	15
83	UR-144	C ₂₁ H ₂₉ NO	312	125, 214	20
84	UR-144 M1 (Dihydroxylation)	C ₂₁ H ₂₉ NO ₃	344	186, 214	12
85	UR-144 M2 (Hydroxylation)	C ₂₁ H ₂₉ NO ₂	328	214, 230	12
86	XLR -11	C ₂₁ H ₂₈ FNO	330	125, 232	15
87	XLR-11 M1 (Carboxylation, Oxidative defluorination)	C ₂₁ H ₂₇ NO ₃	342	101, 125, 171, 244	15
88	XLR11 M2 (Hydroxylation at indazole)	C ₂₁ H ₂₈ FNO ₂	346	125, 248	15

Opioid analgetics

1	Fentanyl	C ₂₂ H ₂₈ N ₂ O	337	105,132	15
2	Norfentanyl	C ₁₄ H ₂₀ N ₂ O	233	55,84	10
3	Fentanylmet	C ₁₉ H ₂₄ N ₂	281	105,188	20
4	3-methylfentanyl	C ₂₃ H ₃₀ N ₂ O	351	105,202	25
5	Carfentanyl	C ₂₄ H ₃₀ N ₂ O ₃	395	134,246	20
6	Tramadol	C ₁₆ H ₂₅ NO ₂	264	136,187	15
7	O-desmethyl-tramadol	C ₁₅ H ₂₃ NO ₂	250	121,131	15
8	Morphine	C ₁₇ H ₁₉ NO ₃	286	153,165	30
9	Morphine-3-glucuronide	C ₂₃ H ₂₇ NO ₉	462	201,229	22
10	Morphine-6-glucuronide	C ₂₃ H ₂₇ NO ₉	462	201,229	21
11	Norcodeine	C ₁₇ H ₁₉ NO ₃	286	193,215	15
12	Codeine	C ₁₈ H ₂₁ NO ₃	300	183,199	15
13	Dihydrocodeine	C ₁₈ H ₂₃ NO ₃	302	201,227	15
14	Methadone	C ₂₁ H ₂₇ NO	310	247,265	15

15	Ketamine	C ₁₃ H ₁₆ CINO	238	220,125	15
16	Norketamine	C ₁₂ H ₁₄ CINO	224	179,125	15
Halucinogens					
1	Methoxetamine	C ₁₅ H ₂₁ NO ₂	248	121,175	37
2	2-CB	C ₁₀ H ₁₄ BrNO ₂	260	228,243	25
3	Psilocybin	C ₁₂ H ₁₇ N ₂ O ₄ P	285	160,205	15
4	LSD	C ₂₀ H ₂₅ N ₃ O	324	180, 223, 281	20
5	LSD M1 (2-oxo-3-OH)	C ₂₀ H ₂₅ N ₃ O ₃	356.5	237, 265	20
Stimulators					
1	Amphetamine	C ₉ H ₁₃ N	136	119,91	15
2	Methamphetamine	C ₁₀ H ₁₅ N	150	91,119	15
3	Fentermine	C ₁₀ H ₁₅ N	150	133,105	15
4	MDAI	C ₁₀ H ₁₁ NO ₂	178	131,161	10
5	Methylephedrine	C ₁₁ H ₁₇ NO	180	131,147	15
6	MDMA	C ₁₁ H ₁₅ NO ₂	194	135,163	15
7	Cocaine	C ₁₇ H ₂₁ NO ₄	304	150,182	15
8	Benzoylecgonine	C ₁₇ H ₂₁ NO ₄	290	119,168	15
9	MDPV	C ₁₆ H ₂₁ NO ₃	276	175, 135	15
10	MDPBP	C ₁₅ H ₁₉ NO ₃	262	191,161	15
11	Methylone	C ₁₁ H ₁₃ NO ₃	208	132,160	15
12	Ethylone	C ₁₂ H ₁₅ NO ₃	222	174,204	20
13	PVP	C ₁₅ H ₂₁ NO	232	91,161	18
Benzodiazepines					
1	Nordiazepam	C ₁₅ H ₁₁ CIN ₂ O	271	140, 210	15
2	Oxazepam	C ₁₅ H ₁₁ CIN ₂ O ₂	287	241,269	15
3	Temazepam	C ₁₆ H ₁₃ CIN ₂ O ₂	301	255, 283	15
4	Fenazepam	C ₁₅ H ₁₀ BrCIN ₂ O	349	105, 169	15
5	Clozapine	C ₁₈ H ₁₉ CIN ₄	327	84,296	12
6	7-aminoflunitrazepam	C ₁₆ H ₁₄ FN ₃ O	284	264, 236	10
Others					
1	Pyracetam	C ₆ H ₁₀ N ₂ O ₂	143	126, 98	10
2	Caffeine	C ₈ H ₁₀ N ₄ O ₂	195	110, 138	15
3	Hydroxyquinoline	C ₉ H ₇ NO	146	118, 128	5
4	Nicotine	C ₁₀ H ₁₄ N ₂	163	106, 131	5
5	Dimedrol	C ₁₇ H ₂₁ NO	256	152, 167	15
6	Dicycloverine	C ₁₉ H ₃₅ NO ₂	310	155, 165	10
7	Carbamazepine	C ₁₅ H ₁₂ N ₂ O	237	194, 175	15
8	2-hydroxycarbamazepine	C ₁₅ H ₁₂ N ₂ O ₂	253	210, 180	15
9	Chlorprotixen	C ₁₈ H ₁₈ CINS	316	231, 271	15

Table 4. List of the compounds included in the developed screening method with their chemical names, brutto-formulas collision energies(negative ESI mode)

#	Name	Brutto-formula	[M+H] ⁺	Fragment ions	CE
1	Phenobarbital	C ₁₂ H ₁₂ N ₂ O ₃	231	144,188	15
2	THC-COOH	C ₂₁ H ₂₈ O ₄	343	191,245	15
3	Nordiazepam	C ₁₅ H ₁₁ ClN ₂ O	269	241,223	15
4	Oxazepam	C ₁₅ H ₁₁ ClN ₂ O ₂	285	257,205	15
5	Morphine	C ₁₇ H ₁₉ NO ₃	284	248,108	15
6	AB-FUBINACA M1(Carboxylation)	C ₂₀ H ₂₀ FN ₃ O ₃	368	322,215	15
7	MDMB(N)-2201 M1(Carboxylation)	C ₁₉ H ₂₆ FN ₃ O ₃	362	318,185	15
8	MMB-2201 M1(Carboxylation)	C ₁₉ H ₂₅ FN ₂ O ₃	347.5	303,184	15
9	MDMB-CHMINACAM1(Carboxylation)	C ₂₁ H ₂₉ N ₃ O ₃	370.5	343,214	15
10	AB-CHMINACA M5 (Carboxylation)	C ₂₀ H ₂₇ N ₃ O ₃	356	213,312	15
11	AB-CHMINACA M5 (Hydroxylation)	C ₂₀ H ₂₇ N ₃ O ₄	371	229,238	15
12	AB-PINACA-F M1 (Carboxylation)	C ₁₈ H ₂₄ FN ₃ O ₃	348	185,205	15
13	AB-PINACA-F M2(Oxidative defluorination to COOH, amide hydrolysis)	C ₁₈ H ₂₃ N ₃ O ₅	360	260,216	15
14	AB-PINACA-F M4 (Oxidative defluorination, amide hydrolysis)	C ₁₈ H ₂₅ N ₃ O ₄	346	113,169	15
15	PVP	C ₁₅ H ₂₁ NO	230	114,184	15
16	Benzoylecgonine	C ₁₇ H ₂₁ NO ₄	288	208,125	15
17	MDMB-CHMINACAM1(Carboxylation)	C ₂₁ H ₂₉ N ₃ O ₃	370	326,243	15
18	MDMB (N)-Bz-F M1 (Carboxylation)	C ₂₁ H ₂₃ FN ₃ O ₃	382	336,294	15
19	PB-22 M1 (Carboxylation, Ester hydrolysis)	C ₁₄ H ₁₇ NO ₂	230	127,155	15
21	PB-22F M2(Carboxylation, Ester hydrolysis)	C ₁₄ H ₁₆ NO ₂ F	248	204,136	15
22	XLR-11 M1 (Carboxylation, Oxidative defluorination)	C ₂₁ H ₂₇ NO ₃	340	171,240	15

Table 5. Validation parameters of the method

Name of compounds	LOD ng/ml	Reproducibility RSD,%			RT RSD,%	Stability 72 hr RSD,%
		5 ng/ml	50 ng/ml	150 ng/ml		
Amphetamine	1	3.3	3.3	0.77	3.05	2.93
Buprenorphine	0.5	1.17	1.17	2.51	2.01	2.24
7-amino flunitrazepam	1	8.79	8.79	2.89	0.52	2.42
Nordiazepam	5	0.71	0.71	0.71	2.00	0.18
Morphine	0.5	6.32	6.32	2.61	2.84	3.29
Oxazepam	5	1.4	1.4	1.33	0.55	1.54
Temazepam	5	2.41	2.41	2.59	0.96	2.19
APINAC	5	4.98	4.98	12.65	0.24	10.7
Cocaine	5	4.98	4.98	10.30	1.17	7.75
THC-acid	5	8.88	8.88	1.77	0.59	1.11
Methylecgonine	5	8.61	8.61	19.08	1.84	2.88
Benzoylecgonine	5	7.69	5.12	4.38	1.45	3.18

Table 6. List of the tested samples

Nº			Detected compounds
1	Tramadol	26	PVP
2	Cocaine, amphetamine, methamphetamine	27	AB-Chminaca
3	AB-chminaca, THC	28	AB-Chminaca, AB-fubinacaMDMB(N)-2201
4	a-PVP, AB-chminaca	29	AB-Fubinaca
5	Morphine	30	AB-chminaca
6	a-PVP, dimedrol, codeine, Morphine	31	Ab-fubinaca
7	AB-chminaca	32	AB-fubinaca
8	PB-22, AB-chminaca, MDMB(N)- 2201	33	pvp
9	AB-fubinaca, 5F-ab-pinaca, MDMB(N)-2201	34	AB-PINACA-F MDMB-Bz-F MDMB-2201
10	5F-AB-PINACA, AB-fubinaca, PVP	35	AB-fubinaca
11	MDMB(N)-2201	36	PVP, AB-Chminaca
12	MDMB(N)-2201, 5F-ab-pinaca	37	AB-Chminaca
13	AB-chminaca, 5F-AB-PINACA, MDMB(N)-Bz-F	38	MDMB(N)-2201
14	AB-chminaca, 5F-AB-PINACA, MDMB(N)-2201, PVP	39	Morphine
15	aPVP, ethylon, methoxetamine	40	Morphine, ketamine
16	AB-chminaca, 5F-AB-PINACA, MDMB(N)-2201	41	MDPV, phenobarbital
17	AB-chminaca, 5F-AB-PINACA, XLR-11, AB-PINACA	42	PVP, AB-Chminaca
18	PB-22F	43	Methadone, MMB-2201 MDMB-2201
19	PB-22	44	PVP, MDPV
20	MDPBP	45	AB-Chminaca, AB-fubinaca, MDMB- chminaca
21	MDPBP, PVP, AB-Chminaca	46	PB-22F, AB-PINACA-F
22	MDPBP	47	Mephedrone, PVP
23	PVP, MDMB(N)-2201 , carbamazepine	48	PVP
24	PVP	49	Amphetamine, methamphetamine, carfentanyl, 3-methylfentanyl, methadone
25	MDMB(N)-2201	50	MDMB-Chminaca, AB-fubinaca THJ-2201

Figure 1. Results for the tested sample №13 showing chromatograms and confirmatory Q-TOF spectra of **A**–5F-AB-PINACA M1; **B** - MDMB(N)-Bz-F M1 and **C** -AB-CHMINACA M4.