Pharmacy

The development of a liquid chromatography/tandem mass spectrometry screening method for the identification of various designer drugs and other substances of abuse

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Abstract

Background: Manufacturers of new synthetic drugs have used the opportunities posed by the principles of the free market and the lack of appropriate legislation and successfully entered the European market, posing a serious health threat, especially for young people. Therefore, analytical methods capable of detecting and identifying designer drugs are continually needed. Apart from new synthetic narcotics, also legal over-the-counter pharmaceuticals, which taken in higher doses may produce stimulation or hallucinations, gain popularity among teenagers.
Material and methods: In this work a fast and reliable liquid chromatography coupled to tandem mass spectrometry with time of flight analyzer (LC-MS/MS-TOF) screening method was developed.
Results: The developed method allows simultaneous detection and identification of 18 compounds – various types of stimulants and hallucinogens with particular emphasis on pharmaceutical substances used for these purposes. The chromatographic separation was optimized and the unequivocal identification of each substances was enhanced by MS and MS/MS spectra evaluation.
Conclusions: The usefulness of the elaborated method as a screening method was confirmed by the analysis of 18 smart drugs in powdery, tablet or capsule forms and 5 bulk powders. Impurities from the syntheses – DBZP from BZP synthesis, 3-isoFMC residual from 3FMC synthesis – were found in numerous samples, which proves their poor quality.

Key words: Screening method, tandem mass spectrometry, time of flight, designer drugs, synthetic cathinones, piperazines, drugs of abuse.

Background

The will of experience the psychotropic and narcotic effects causes people to continually develop new methods to induce a stimulation of central nervous system (CNS). Even after revising and updating the lists of scheduled drugs, new compounds with slightly modified structures, which fall outside the law, are designed and introduced on the market. Thus, they may not only be sold legally and uncontrolled but also avoid detection [1]. Legal status of novel designer drugs combined with their psychotropic effects, encourage people,

especially teenagers and young adults, with the desire to use stimulants, but with the fear of legal consequences at the same time. Products containing designer drugs were marketed under the umbrella terms like "legal highs", "club drugs", "smart drugs" and sold at clubs, festivals or special shops called "smart shops" or "head shops".

Recreational drugs become more prevalent at the end of the 20th century. Originally, club drugs containing varied stimulants were used on all-night dance parties to maintain physical activity or to enhanced the alter state of consciousness [2]. One of the most recognized substance was methamphetamine (MA), a derivative of amphetamine, developed as a compound for treating asthma, congestion of nasal mucosa, ADHD and obesity. However, due to its properties (promoting wakefulness, improving mood and attention, appetite suppressing), it gained popularity among students or truck drivers and became one of the most used illicit drug [3,4]. MA products mostly consist of powders or crystalline form. Because of a high abuse potential and severe side effects, it was listed in Schedule II of the United Nations 1971 Convention on Psychotropic Substances [5]. Then, the 3,4-methylenedioxymethamphethamine (MDMA) known as "ecstasy" become widely abused [2]. It has a structure close related to MA and it was also included to the UN Convention on Psychotropic Substances. The Report "Synthetic drug production in Europe" prepared by EMCDDA [6] suggests that, although the availability of MDMA was the lowest in 2009, it is increasing again from 2010.

Due to decreased availability and purity of MA, MDMA and other typical drugs of abuse, synthetic cathinones became one of the most developing group of stimulants. They are derivatives of cathinone – an alkaloid naturally found in *Catha edulis* (khat). They belong to beta-ketoamphetamines and therefore possess amphetamine-like properties. The users reported euphoria, increased energy, empathy, openness and sexual stimulation but also tachycardia, hypertension and other cardiovascular symptoms. A lot of them were marketed in powder, capsule or tablet form as bath salts, plant food or soil conditioners. The most frequently used substances are 4'-methylmethcathinone (mephedrone), 3 and 4-fluoromethcathinone (3-FMC, 4-FMC), dimethylcathinone, ethcathinone, methedrone, methylenedioxypyrovalerone (MDPV), naphtylpyrovalerone (naphyrone), methylone, ethylone and butylone [1,7,8,9,10].

Besides synthetic cathinones, various part-pills contained 1-benzylpiperazine (BZP) – a recreational stimulant substance which belongs to a group of aryl-substituted piperazines. This group includes, amongst others, m-trifluorophenyl piperazine (TFMPP), 1-(3-chlorphenyl)piperazine (mCPP), p-fluorophenylpiperazine (pFPP), 1-(4-methoxyphenyl)-piperazine (pMeOPP), 1-methyl-4-benzylpiperazine (MBZP) and dibenzylpiperazine (DBZP) which is possibly a synthetic impurity of BZP [11,2]. Combinations of BZP and TFMPP were often detected. The study carried out on rats showed that administration of the mixture of BZP and TFMPP mimics the molecular mechanism of MDMA and, consequently, its psychoactive effects [13]. BZP was originally synthesized in 1944 as a potential antihelminthic but the trials were terminated because of adverse effects of BZP [10].

The presence of another class of synthetic stimulants, known as pipradol derivatives, on recreational drug market was discussed by Coppola and Mondola [14]. The main representatives of this group are pipradol (developed for obesity, depression and narcolepsy treatment), desoxypipradol (2-DPMP; developed in 1953 for treating ADHD, depression and narcolepsy or to wake patients following anaesthesia), diphenylprolinol (D2PM) and diphenylmethylpyrrolidine (desoxy D2PM). They produce amphetamine-like effects, but with prolonged neuropsychiatric symptoms [15].

Ketamine is a pharmaceutical agent used in human and veterinary surgeries, because of its analgetic, anaesthetic and sedative properties. However, due to the same reasons, it was used for non-medical purposes and distributed as a club drug. Ketamine uptake may produce hallucinations and dissociative state [2,16].

In addition to above substances, many of which are already controlled and whose purity is uncertain, users are also interested in widespread pharmaceuticals, which may produce stimulation or hallucinations when taken in higher doses. Legal over-the-counter cough medicine

dextromethorphan (DXM) represents one of the most commonly abused pharmaceutical substance particularly among teenagers. Its popularity is partially due to the belief that non-medical use of DXM is a safe and easily accessible alternative to other "illicit" recreational drugs such as MA, MDMA or ketamine. DXM is sold as an antitissive pharmaceutical product in a dose ranges from 10 to 30 mg, in capsule, tablet, lozenge or syrup forms. At doses higher than medically used, it produces euphoria, distorted perception, hallucinations and dissociative anaesthesia typical to NMDA agonists such as ketamine [3]. As DXM, apart from paracetamol, pseudoephedrine, guaifenesin, saccharin, propylene glycol, alcohol, is very often added to combination cough medicines, abusers have developed a simple home acid-base extraction technique to produce "free-base" DXM from an OTC cold preparations (guaifenesin and excipients). In this way, they avoid adverse reactions caused by the ingestion of increased doses of all unwanted concurrent substances, when they reach target DXM dose [17]. Another easily available pharmaceutical drug used for recreational purposes is diphenhydramine. It is an OTC antihistamine and sedative compound, which is sometimes used because of its dystonic reactions and muscle rigidity, however, its abuse liability profile is rather low [18,19]. The presence of glaucine in party pills was also reported [20]. Glaucine is an alkaloid with bronchodilating and anti-inflammatory properties which is still therapeutically used as an antitissive agent in several European countries. However, glaucine may produce sedation, fatigue, lethargy, hallucinations and dissociative-type symptoms and this is the reason of its non-medical usage [21]. Local anesthetics such as benzocaine, procaine, lidocaine, and dimethocaine were frequently added to the liquid or powdery type products to simulate the effect of cocaine [22,23,24]. Lidocaine and benzocaine were even used as common adulterants of cocaine sold as a "street drug" [25].

Detection and identification of such diverse groups of compounds at ever-changing drug scene remains a great challenge for control laboratories. There are several techniques employed for screening or confirmation of drugs identity including nuclear magnetic resonance spectroscopy (NMR) [26] invaluable for structure elucidation of unknown substances, non-destructive techniques such as infrared spectroscopy (IR) [24], Raman spectroscopy [27], X-ray diffractometry [28] often used for rapid confirmation of identified compound and combined techniques which are more suitable for complex mixture analysis: gas chromatography-mass spectrometry (GC-MS) [29,30], liquid chromatography-mass spectrometry (LC-MS) [31,32,7] or capillary electrophoresis–laser induced fluorescence (CE-LIF) [33]. However, most of these methods concern a separation and identification of one chemical group e.g. substituted cathinones [29], methylenedioxy derivatives [7] or ring-substituted amphetamines [30].

The aim of this work was to develop a fast and reliable screening LC-MS/MS-TOF method that allows simultaneous detection and identification of various types of stimulants and hallucinogens with particular emphasis on pharmaceutical substances used for these purposes.

Material and Methods

Chemicals

Methanol (MeOH) and acetonitrile (ACN) from POCH s.a. both suitable for LC-MS in terms of purity, formic acid (FA) from Park Scientific Limited, acetic acid from APPLICHEM both suitable for HPLC, and doubly distilled water additionally purified in the Nanopure Diamond UV Deionization System from Barnstead were used throughout. Reference standards: lidocaine hydrochloride was supplied by Astra Zeneca, caffeine and diphenhydramine hydrochloride were purchased from Sigma-Aldrich. Dextrometorphan hydrobromide CRS and procaine hydrochloride CRS were obtained from EDQM. As a glaucine reference material a national reference standard in a form of hydrochloride salt was used. Due to unavailability of other reference standards, BZP, DBZP, TFMPP, D2PM, MA,MDMA, 3-FMC, mephedrone, butylone, MDPV were extracted from samples purchased from smart shops or provided for testing. Their identity was confirmed by LC-MS/MS. Ketamine was extracted from a veterinary drug – Ketamina 10% injection aqueous solution 10ml (ketamine hydrochloride – 115.33 mg/ml) Biowet Puławy Sp. z o.o.

Samples

Most samples were submitted to Polish National Medicines Institute for examination. Several samples were collected from smart shops. Analyzed samples were in various dosage forms such as tablets, capsules and powders packed in sachets or in Ziploc bags.

Equipment and chromatographic conditions

Unequivocal identification of analytes was performed by a tandem mass spectrometer with time-of-flight analyzer (MS/MS-TOF) which is known as an instrument of high mass resolution. It enables a very accurate mass detection, resulting in the assignment to a molecular formula. The identification is further enhanced by the analysis of the isotopic pattern of the compounds. Additionally, obtained MS/MS spectra allow for structure determination.

All experiments were performed on a LC Ultimate 3000 system (Dionex, a part of Thermo Fisher Scientific) equipped with a pump, a degasser, an autosampler and a column heater coupled to a tandem mass spectrometer MicrOTOF-Q II (Bruker Daltonik) with electrospray ionization (ESI) and time of flight (TOF) analyzer. Nitrogen generator Parker dh FNS provided high purity nitrogen gas to the mass spectrometer. The MS settings were: electrospray ionization (ESI) in the positive ion mode, dry gas (nitrogen) flow rate 8.0 L min^{-1}, nebulizer 0.8 Ba, the dry heater 170°C, the capillary voltage 4500 V and end plate offset -500 V. MS data were recorded in a full scan mode (from 50 to 800 m/z).

Data was acquired and processed using Chromeleon v. 6.8 software (Dionex, a part of Thermo Fisher Scientific), Hystar 3.2. and ESI Compass 1.3 for micrOTOF/maXis software (Bruker Daltonik). Separations were performed on a C18 analytical column (Hypersil GOLD, 100 mm× 2.1 mm; 3 µm particle size; Thermo Fisher Scientific) under gradient elution. Unless otherwise stated, a solvent A consisted of water-acetonitrile-formic acid (90:10:0.1, v/v/v) and a solvent B was a mixture of methanol-acetonitrile-formic acid (90:10:0.1, v/v/v). An applied linear gradient was as follows: initially 0% B from 0 to 5 min, then linear gradient to 16% B at 9 min, subsequently, linear to 40% B at 15 min, constant 40% B to 17 min, finally return to 0 % B and equilibration for 7 min. The flow rate was 0.15 ml min^{-1}. Analyses were carried out at 25°C and the injection volume was 1.0 µl.

Standard solutions

Stock solutions of reference standards were prepared at concentrations of approximately 1 mg ml^{-1}. Ketamine solution was diluted to the concentration of 1 mg ml^{-1}. Caffeine standard solution was prepared at a concentration of 500 µg ml^{-1}. The mixture of methanol-water (50:50, v/v) was used throughout to dissolve each standard. All solutions were stored in refrigerated conditions.

Sample solutions

Samples in powder form and powders obtained from crushed tablets were homogenized. Then, approximately 1 mg of each sample was dissolved with methanol-water (50:50, v/v) and sonicated for 10 min. Finally, extracts were filtered through 0.22 µm filter before use.

Test solution

The test solution was used for the LC-MS method development. It was obtained by mixing of each standard solution – equivalent to 100 µg of each substance, 50 µl of BZP, TFMPP, MA and MDMA solutions into 10 ml volumetric flask.

Results

Method optimization

Optimization of mobile phase components was performed based on chromatographic parameters such as good peak shapes and the resolution. As all target compounds possess more or less polar structures, reverse phase (RP) chromatography with C18 stationary phase was chosen.

Because of a wide chemical diversity of investigated substances, an isocratic elution was not suitable to achieve separation in reasonable time of analysis. Hence, a gradient elution was employed. Preliminary experiments were performed using several mixtures of ACN and H2O in different proportions with 0.1% (v/v) FA. In a wide gradient from 10 to 80% of ACN a rapid

elution of all compounds was achieved with good separation of most compounds except for MA with MDMA and lidocaine with ketamine, which coeluted. Gradient modifications allowed for a partial separation of MA and MDMA but there was no improvement in lidocaine and ketamine separation (Fig.1). An organic component was changed to methanol. The application of the same wide gradient 10-80% of organic solvent resulted in a very good separation of lidocaine and ketamine but other compounds coeluted: MA with MDMA, DXM with diphenhydramine and caffeine with lidocaine. Caffeine was retained stronger when MeOH was used and its peak was recorded after MDMA. Different gradient programs were tested. Using a gradient from 15 to 50% of MeOH partial separation of two critical pairs (MA with MDMA and DXM with diphenhydramine) was achieved. Caffeine with lidocaine still coeluted. In order to enhance separation, the addition of ACN to the mobile phase was considered. With 1% of ACN, no resolution improvement was obtained. Further increase of ACN concentration resulted in a faster elution of caffeine until it was well separated (at 10% of ACN).

Also the resolution between DXM and diphenhydramine was better. However, increased elution power of the mobile phase during the isocratic step – water-MeOH-ACN-FA (80:10:10:0.1, v/v/v/v) – caused too fast BZP elution, without any retention and, additionally, the loss of separation of MA and MDMA. For this reason, initial composition of the mobile phase was changed. Instead of adding ACN, the entire content of MeOH was replaced by ACN leading to the final composition of solvent A – water-ACN-FA (90:10:0.1, v/v/v). Solvent B consisted of MeOH-ACN-FA (90:10:0.1, v/v/v). The addition of ACN to the solvent B prevented the decrease of the ACN concentration in the course of gradient elution. With a gradient program described in Subsection "Equipment and chromatographic conditions", all peaks are well separated with the exception of DXM and diphenhydramine for which a baseline separation was not obtained (Fig.2, p. 6).

Apart from formic acid, acetic acid as pH modifiers was also tested. Although peaks resolution was not worse, BZP was eluted almost without retention and the noise was significantly higher, which resulted in decreased method sensitivity.

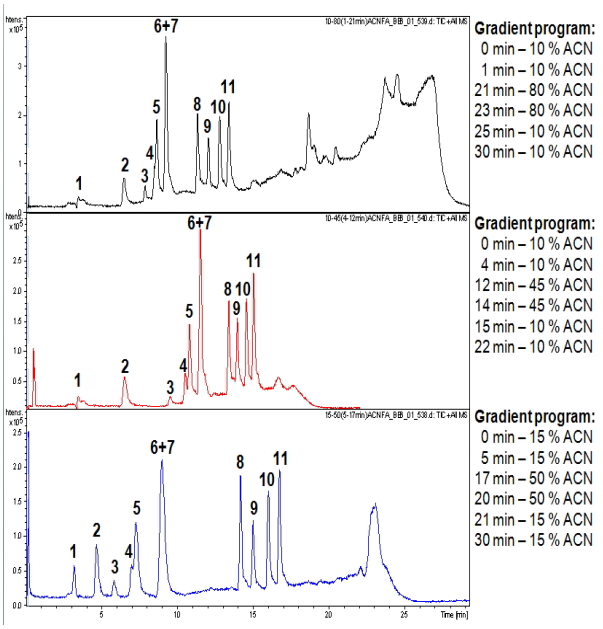

Figure 1: Total Ion Chromatograms (TIC) recorded for different gradient programs with ACN as an organic modifier of the mobile phase; Identified peaks: 1 – BZP, 2 – procaine, 3 – caffeine, 4 – MA, 5 – MDMA, 6 – lidocaine, 7 – ketamine, 8 – glaucine, 9 – TFMPP, 10 – DXM, 11 – diphenhydramine.

In the next stage of the study, other compounds such as mephedrone, 3-FMC, 3-isoFMC, butylone, DBZP, MDPV and D2PM were analysed in the selected chromatographic conditions (Fig.3 p. 8). Obtained data indicated that all of them were eluted within the time of analysis. Butylone, DBZP, 3-FMC, its isomer and MDPV would be separated from the rest of tested compounds. Mephedrone peak had the same retention time as lidocaine and D2PM very close to glaucine. However, this is not a limitation as MS data provides detailed information to confirm the target analytes.

MS/MS data

For unambiguous identification of the analysed compounds, the chromatographic system was coupled with electrospray tandem mass spectrometer with time of flight analyser. Obtained MS and MS/MS data was summarized in Table 1 (p. 7). Isomers 3-FMC and 3-isoFMC possess the same molecular formulas, resulting in identical MS spectra. Although, the elaborated method allowed the chromatographic separation of these isomers, their unequivocal distinction was possible when tandem MS experiments were

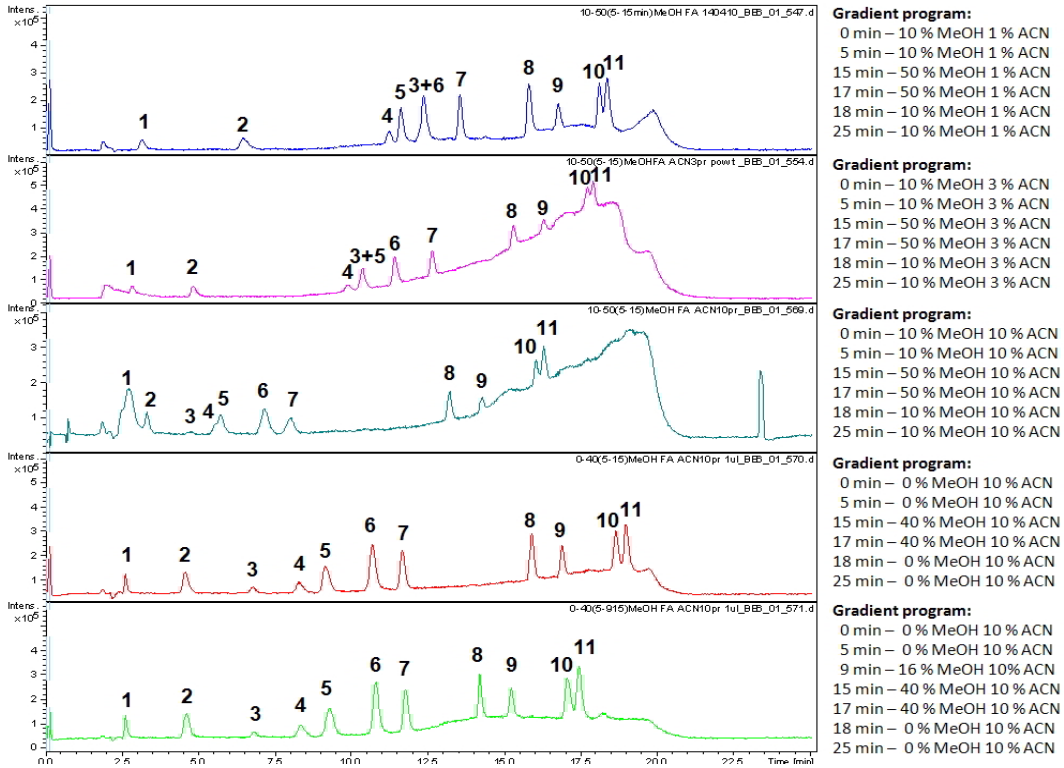

Figure 2: Total Ion Chromatograms (TIC) recorded for different gradient programs with ACN/MeOH mixture as an organic modifier of the mobile phase; Identified peaks: 1 – BZP, 2 – procaine, 3 – caffeine, 4 – MA, 5 – MDMA, 6 – lidocaine, 7 – ketamine, 8 – glaucine, 9 – TFMPP, 10 – DXM, 11 – diphenhydramine

performed. MS/MS spectra of 3-FMC and its isomer are presented in Fig.4. Despite the same precursor ions at m/z 182.09, slight but significant differences of their fragmentation patterns were observed. In 3-FMC MS/MS spectrum product ion at m/z 149 was detected whereas 3-isoFMC gives the fragment at m/z 151.

As it was mentioned in Subsection "Method optimization", the baseline separation of DXM and diphenhydramine was not achieved. It is shown, in Fig.3 (p. 8), that peaks of lidocaine and mephedrone had the same retention times. Also glaucine and D2PM were eluted from the column at similar time. However, all these critical pairs could be easily distinguished by the evaluating the corresponding mass spectra. The MS and MS/MS spectra are depicted in Fig.5 and 6 (p. 9 and 10) and they are completely different. Molecular formulas and masses of each compound are different, hence different precursor ions define them. Moreover, they represent different classes of substances and their structures differ significantly, therefore, their MS/MS spectra had no product ion in common and they are characterized by completely different fragmentation patterns.

Sample analysis

The developed LC-MS/MS method was applied to the analysis of 23 samples. Most of them were smart drugs in original attractive packaging advertised as collector's products or food fertilizers. Analyzed tablets had different intense colours and some of them had stamped symbols. Samples in powder forms were closed in silver foil bags. Five samples were delivered as bulk powders. The extraction procedure was described in Subsection "Sample solutions". As shown in Table 2 (p. 8) eight tablet samples (1-8) contained BZP and always they were contaminated with DBZP. In six of them, additionally, TFMPP was detected (Samples 3-8). These results confirmed the earlier works [11,34] in which mixtures of BZP and TFMPP were reported. In one sample (9) only caffeine was found but in sample 11 caffeine was a one of several active substances. Substituted cathinones were found in all tested bulk powders as well as in six smart

Table 1: MS and MS/MS data of all identified compounds.

Compound name	Molecular formula	[M+H]+ theoretical	[M+H]+ measured	Error [ppm]	mSigma	Collision energy [eV]	MS/MS
BZP (benzylpiperazine)	$C11H_{16}N_2$	117.1386	117.1391	-2.9	6.7	20	85, 91
Procaine	$C_{13}H_{20}N_2O_2$	237.1598	237.1598	-0.3	2.7	21	100, 120, 164
3-isoFMC (3-isofluoromethcathinone)	$C_{10}H_{12}FNO$	182.0976	182.0973	1.5	6.6	20	103, 123, 151, 164
3-FMC (3-fluoromethcathinone)	$C_{10}H_{12}FNO$	182.0976	182.0979	-1.8	0.9	20	103, 123, 149, 164
Caffeine	$C_8H10N_4O_2$	195.0877	195.0878	-0.7	4.9	20	110, 138
MA (methamphetamine)	$C_{10}H_{16}N$	150.1277	150.1280	-2.1	6.1	20	91, 119
MDMA (methylenedioxymethamphetamine)	$C_{11}H_{16}NO_2$	194.1176	194.1177	-1.0	8.6	20	105, 133, 135, 163
Butylone	$C_{12}H_{16}NO_3$	222.1125	222.1131	-2.9	5.3	21	174, 204
Mephedrone	$C_{11}H_{15}NO$	178.1226	178.1232	-3.1	5.9	20	91, 119, 145, 160
Lidocaine	$C_{14}H_{22}N_2O$	235.1805	235.1809	-1.5	8.0	21	86
Ketamine	$C_{13}H_{16}ClNO$	238.0993	238.1002	-3.5	8.4	21	125, 163, 179, 207, 220
DBZP (dibenzylpiperazine)	$C_{18}H_{22}N_2$	267.1856	267.1844	4.2	3.6	22	91, 120, 134, 175
MDPV	$C_{16}H_{22}NO_3$	276.1594	276.1602	-2.7	7.0	22	126, 135, 175, 205
Glaucine	C21H25NO4	356.1856	356.1864	-2.2	3.9	24	279, 294, 310, 325
D2PM (diphenylprolinol)	$C_{17}H_{20}NO$	254.1539	254.155.	-1.2	7.2	21	130, 158, 208
TFMPP	$C_{11}H_{14}F_3N_2$	231.1104	231.1109	-2.5	4.0	21	188
DXM (dextromethorphan)	$C_{18}H_{25}NO$	272.2009	272.2005	1.3	5.5	22	147, 215
Diphenhydramine	$C_{17}H_{21}NO$	256.1696	256.1698	-0.8	13.1	21	152, 167

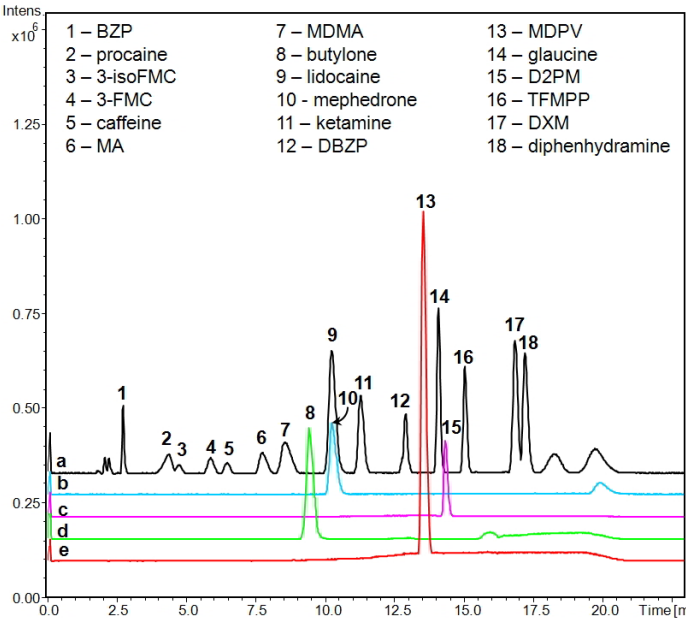

Figure 3: Total Ion Chromatograms (TIC) recorded for the mixture of 13 compounds (a), mephedrone solution (b), D2PM solution (c), butylone solution (d), MDPV solution (e).

Table 2: Results of a qualitative analysis of the investigated samples.

Sample No.	Sample name	Dosage form	Identified substance
1	Fast and Furious	Tablet (pink)	BZP, DBZP
2	Fast and Furious	Tablet (pink)	BZP, DBZP
3	Bolts	Tablet (blue)	BZP, DBZP, TFMPP
4	Bolts	Tablet (blue)	BZP, DBZP, TFMPP
5	Devils	Tablet (pink)	BZP, DBZP, TFMPP
6	Smiley's	Tablet (yellow)	BZP, DBZP, TFMPP
7	XXX	Tablet (pink)	BZP, DBZP, TFMPP
8	Diablo XXX	Tablet (cream)	BZP, DBZP, TFMPP
9	Move	Tablet (blue)	caffeine
10	Super fly	Powder	3-FMC, 3-isoFMC
11	Charge +	Powder	3-FMC, caffeine, lidocaine
12	Boom	Powder	mephedrone
13	Doves	Tablet (white)	butylone
14	Summer daze	Tablet (yellow)	butylone
15	Ivory wave	Powder	MDPV, lidocaine
16	Groove	Tablet (pink)	Glaucine
17	Neuroblast	Tablet (light pink)	D2PM
18	Chemistry	Capsule (green)	D2PM
19	—	Bulk powder	mephedrone
20	—	Bulk powder	mephedrone
21	—	Bulk powder	butylone
22	—	Bulk powder	butylone
23	—	Bulk powder	MDPV

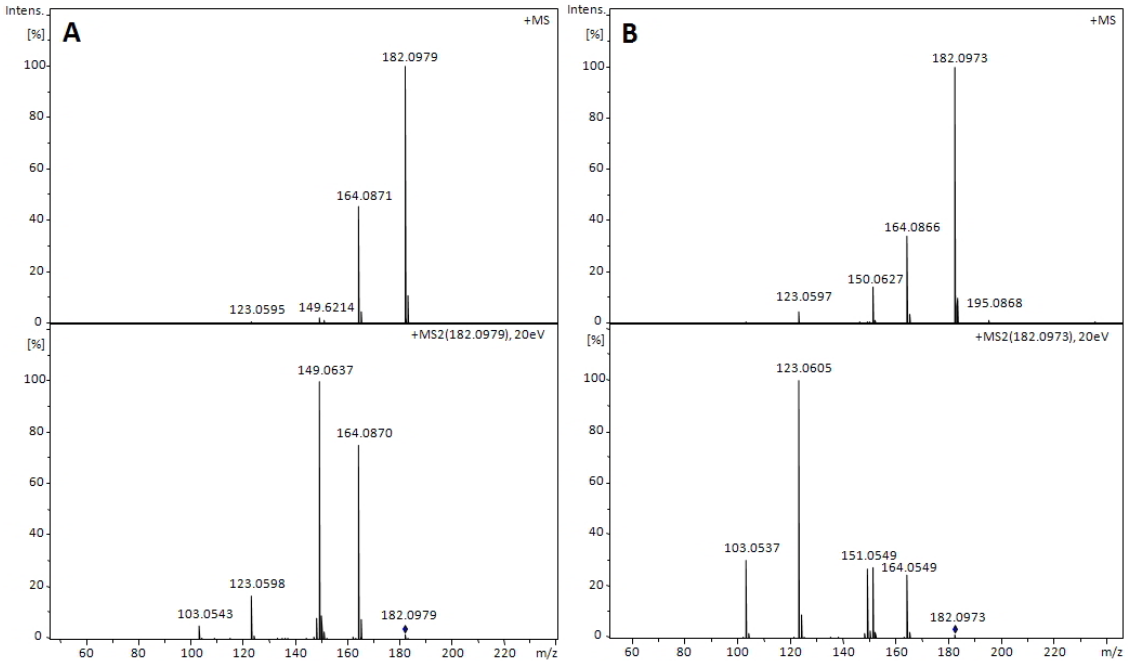

Figure 4: MS and MS/MS (+MS2) spectra obtained for 3-FMC (A) and 3-isoFMC (B).

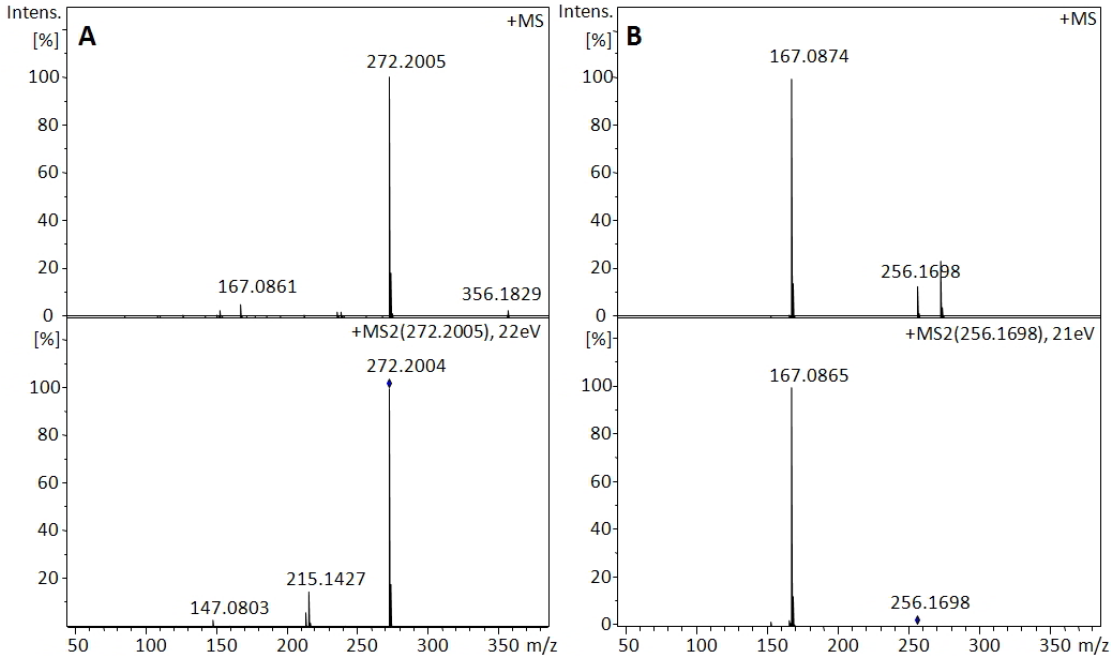

Figure 5: MS and MS/MS (+MS2) spectra obtained for DXM (A) and diphenhydramine (B)

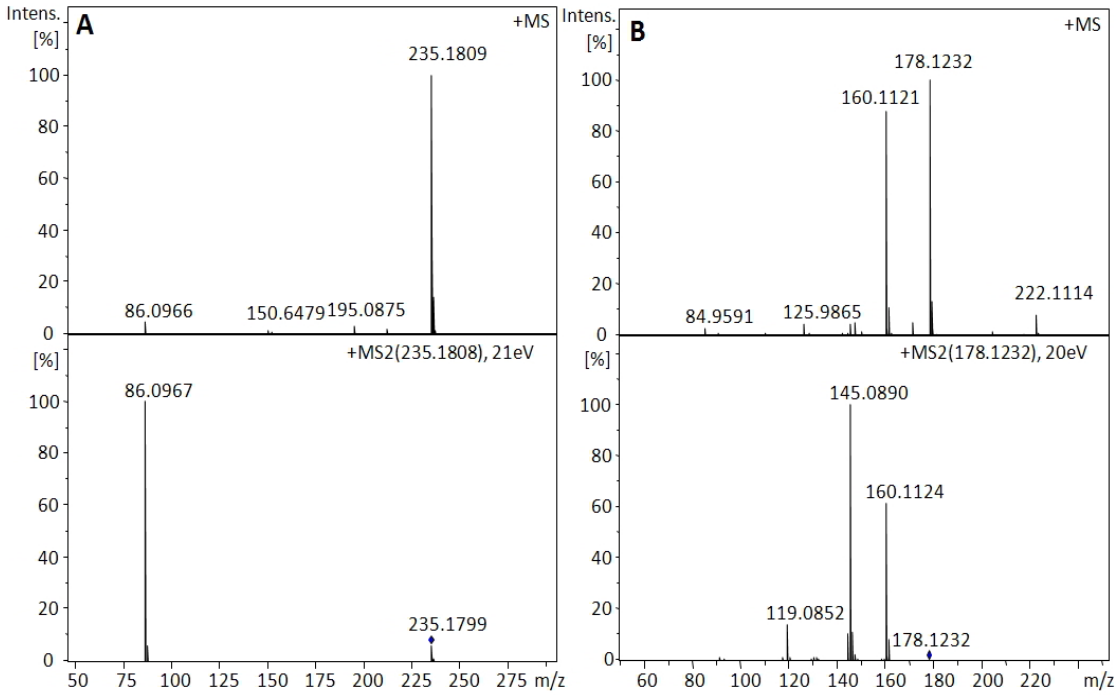

Figure 6: MS and MS/MS (+MS2) spectra obtained for lidocaine (A) and mephedrone (B).

drugs. Mephedrone was detected in samples 12, 19 and 20, its fluoro analogue 3-FMC in samples 10 and 11, two methylenedioxy derivatives of cathinone – butylone and MDPV – were determined in samples 13, 14, 21, 22 and 15, 23 respectively. The presence of diphenylprolinol (D2PM) was found in samples 17 and 18. Moreover, two powdery products contained a pharmaceutical ingredient – lidocaine – as an addition to cathinones.

Discussion

The LC MS/MS-TOF method was developed for 11 substances used because of their stimulating or hallucinogenic properties. All of them eluted within 18 min. Peaks of subsequent seven compounds were also recorded within the time of analysis. These results indicate that the elaborated method has a potential of detecting and indentifying other, even novel analytes, particularly from cathinones and piperazines groups. The method probably would not be effective for the analysis of synthetic cannabinoids – another increasing class of designer drugs, which are usually found in herbal blends for smoking. A lot of them possess structures with a long hydrocarbon chain which could delay their elution. If such problem existed, the gradient program should be modified to allow a faster elution of synthetic cannabinoids.

MS/MS-TOF was found to be a powerful tool which enables unambiguous identification of variety of compounds. A baseline separation of analytes which possess different molecular masses is not necessary for their identification. On the other hand, compounds with the same molecular formulas can be distinguished by the analysis of fragmentation data from MS/MS experiments. The discrimination of two skeletal isomers (3-FMC and 3-isoFMC) was confirmed. However, MS/MS spectra may not be sufficient to differentiate positional isomers. A position of the substituent is only changed e.g. position on the benzene ring for aromatic isomers. As a result they usually are characterized by the identical fragmentation patterns and chromatographic separation of such isomers is required. If it cannot be achieved, further analytical techniques such as NMR and IR spectroscopy need to be involved.

The elaborated method was employed for the qualitative analysis of 23 samples. The majority of investigated smart drugs had the forms characteristic for pharmaceutical dosage forms – tablets or capsules. They contained either

single substances which possess stimulating or hallucinogenic properties or mixtures of different compounds whose cumulative impact on the body imitate the action of narcotic and psychotropic drugs. The combination of BZP with TFMPP mimics the effects of MDMA. Powders composed of 3-FMC or MDPV with the addition of local anaesthetic – lidocaine were designed probably to simulate the usage of cocaine. Its dosage form is suitable for sniffing, which is the most common method of cocaine use [35]. Moreover, such mixtures produce both CNS stimulation and anaesthesia as cocaine. Several pharmaceutical substances were detected, besides lidocaine, also caffeine and glaucine. Bulk powders consisted of single substances, most likely to serve as active ingredients for the preparation of smart drugs. The production process of these products is not controlled, therefore, the samples of low quality, containing impurities or by-products were detected. The presence of DBZP in samples with BZP and isomer 3-isoFMC in samples with 3-FMC evidenced of a failure in the synthesis of active compound and the lack of any purification steps.

Conclusions

The developed LC-MS/MS-TOF method allows for detection and identification of 18 active compounds from different chemical groups: piperazines (BZP, DBZP, TFMPP), cathinones (3-FMC, 3-isoFMC, mephedrone, butylone, MDPV), amphetamines (MA, MDMA), pipradol derivative (D2PM) and various pharmaceutical ingredients used for non-medical purposes due to their stimulating, hallucinogenic or anaesthetic properties (ketamine, caffeine, DXM, diphenhydramine, lidocaine and procaine). Data obtained from both chromatographic separation and a mass spectrometer (MS and MS/MS spectra) enable an unambiguous identification of targeted analytes.

The usefulness of the elaborated method as a screening method was confirmed by the analysis of 18 smart drugs in powdery or tablet forms and 5 bulk powders. Impurities from the syntheses – DBZP from BZP synthesis, 3-isoFMC residual from 3FMC synthesis – were found in numerous samples, which proves their poor quality.

References:

1. Johnson LA, Johnson RL, Portier RB: Current "legal highs". J Emerg Med, 2013; 44(6):1108-15.
2. Koesters SC, Rogers PD, Rajasingham CR: MDMA ('ecstasy') and other 'club drugs'. The new epidemic. Pediatr Clin North Am. 2002; 49(2):415-33.
3. Chomchai C, Manaboriboon B: Stimulant methamphetamine and dextromethorphan use among Thai adolescents: implications for health of women and children, J Med Toxicol, 2012;8(3):291-4.
4. Vearrier D, Greenberg MI, Miller SN et al: Methamphetamine: history, pathophysiology, adverse health effects, current trends, and hazards associated with the clandestine manufacture of methamphetamine. Dis Mon. 2012; 58(2):38-89.
5. EMCDDA–Europol joint publications. Methamphetamine: a European Union perspective in the global context, EMCDDA, Europol, Lisbon, 2009.
6. EMCDDA Report, Perspective on drugs: Synthetic drug production in Europe. 28.05.2013.
7. Archer RP: Fluoromethcathinone, a new substance of abuse. Forensic Sci Int. 2009; 185(1-3):10-20.
8. Fornal E: Identification of substituted cathinones: 3,4-Methylenedioxy derivatives by high performance liquid chromatography–quadrupole time of flight mass spectrometry. J Pharm Biomed Anal, 2013; 81-82:13-9.
9. Zuba D: Identification of cathinones and other active components of legal highs by mass spectrometric methods. Trends Anal. Chem, 2012; 32:15-30.
10. 1Prosser JM, Nelson LS: The toxicology of bath salts: a review of synthetic cathinones. J Med Toxicol, 2012; 8(1):33-42.
11. Europol-EMCDDA Joint Report on a new psychoactive substance: 1-benzylpiperazine (BZP), EMCDDA, Lisbon, 2007.
12. Cohen BM, Butler R: BZP-party pills: a review of research on benzylpiperazine as a recreational drug. Int J Drug Policy, 2011; 22(2):95-101.
13. Baumann MH, Clark RD, Budzynski AG et al: N-substituted piperazines abused by humans mimic the molecular mechanism of 3,4-methylen edioxymethamphetamine (MDMA, or 'Ecstasy'). Neuropsychopharmacology, 2005; 30(3):550-60.
14. Coppola M, Mondola R: Research chemicals marketed as legal highs: the case of pipradrol derivatives. Toxicol Lett, 2012; 212(1):57-60.
15. Corkery JM, Elliott S, Schifano F et al: 2-DPMP (desoxypipradrol, 2-benzhydrylpiperidine, 2-phenylmethylpiperidine) and D2PM (diphenyl-2-pyrrolidin-2-yl-methanol, diphenylprolinol): A preliminary review. Prog Neuropsychopharmacol Biol Psychiatry, 2012; 39(2):253-8.

16. Dillon P, Copeland J, Jansen K: Patterns of use and harms associated with non-medical ketamine use. Drug Alcohol Depend, 2003; 69(1):23-8.
17. Hendrickson RG, Cloutier RL: "Crystal dex:" free-base dextromethorphan. J Emerg Med, 2007; 32(4):393-6.
18. Lindsay L, White ML: Herbal Marijuana Alternatives and Bath Salts – "Barely Legal" Toxic Highs. Clin Pediatr Emerg Med, 2012; 13(4):283-91.
19. Jaffe JH, Bloor R, Crome I et al: A postmarketing study of relative abuse liability of hypnotic sedative drugs. Addiction, 2004; 99(2):165-73.
20. Dargan PI, Button J, Hawkins L et al: Detection of the pharmaceutical agent glaucine as a recreational drug. Eur J Clin Pharmacol, 2008; 64(5):553-4.
21. Geppert B, Wachowiak R, Zaba C: Glaucine as a non-declared active component of "legal highs". Z Zagadnien Nauk Sadowych, 2010; 84:401-8.
22. Kikura-Hanajiri R, Uchiyama N, Kawamura M et al: Changes in the prevalence of synthetic cannabinoids and cathinone derivatives in Japan until early 2012. Forensic Toxicol, 2013; 31:44–53.
23. Sägmüller B, Schwarze B, Brehm G et al.: Identification of illicit drugs by a combination of liquid chromatography and surface-enhanced Raman scattering spectroscopy. J Mol Struct, 2003; 661-662: 279–290.
24. Choe S, Heo S, Choi H et al: Analysis of pharmaceutical impurities in the methamphetamine crystals seized for drug trafficking in Korea. Forensic Sci Int, 2013; 227(1-3):48-51.
25. 25Magalhães EJ, Nascentes CC, Pereira LS et al: Evaluation of the composition of street cocaine seized in two regions of Brazil. Sci Justice, 2013; 53(4):425-32.
26. Westphal F, Junge T, Girreser U: Mass, NMR and IR spectroscopic characterization of pentedrone and pentylone and identification of their isocathinone by-products. Forensic Sci Int, 2012; 217(1-3):157-67.
27. Stewart SP, Bell SE, Fletcher NC et al: Raman spectroscopy for forensic examination of – ketophenethylamine "legal highs": Reference and seized samples of cathinone derivatives. Anal Chim Acta, 2012; 711:1-6.
28. Trzybiński D, Niedziałkowski P, Ossowski T: Single-crystal X-ray diffraction analysis of designer drugs: hydrochlorides of metaphedrone and pentedrone. Forensic Sci Int, 2013; 232(1-3):e28-32.
29. Elie MP, Elie LE, Baron MG: Keeping pace with NPS releases: fast GC-MS screening of legal high products. Drug Test Anal, 2013; 5(5):281-90.
30. Daeid NN, Savage KA, Ramsay D et al: Development of gas chromatography–mass spectrometry (GC–MS) and other rapid screening methods for the analysis of 16 'legal high' cathinone derivatives. Sci Justice, 2013; article in press.
31. Jankovics P, Váradi A, Tölgyesi L et al: Identification and characterization of the new designer drug 4'-methylethcathinone (4-MEC) and elaboration of a novel liquid chromatography-tandem mass spectrometry (LC-MS/MS) screening method for seven different methcathinone analogs. Forensic Sci Int, 2011; 210(1-3):213-20.
32. Al-Saffar Y, Stephanson NN, Beck O: Multicomponent LC-MS/MS screening method for detection of new psychoactive drugs, legal highs, in urine-experience from the Swedish population. J Chromatogr B Analyt Technol Biomed Life Sci, 2013; 930:112-20.
33. Chen KF, Lee H, Liu JT et al: A microwave-assisted fluorescent labeling method for the separation and detection of amphetamine-like designer drugs by capillary electrophoresis. Forensic Sci Int, 2013; 228(1-3):95-9.
34. Young SA, Thrimawithana TR, Antia U et al: Pharmaceutical quality of "party pills" raises additional safety concerns in the use of illicit recreational drugs. N Z Med J, 2013; 126(1376):61-70.
35. Cocaine: a European Union perspective in the global context, EMCDDA; Europol, Lisbon, 2010.