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Clinical value of analytical testing in patients presenting with New Psychoactive Substances intoxication

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Abstract

New psychoactive substances (NPS) have emerged worldwide in recent years, posing a threat to public health and a challenge to drug policy. NPS are usually derivatives or analogues of ”classical” recreational drugs designed to imitate their effects while circumventing regulations. This article provides an overview of benefits and limitations of analytical screening in managing patients presenting with acute NPS toxicity. NPS typically cannot be analytically identified with the usual immunoassay tests. In order to detect NPS using an immunoassay, antibodies specifically binding to the new structures would have to be developed, which is complicated by the rapid change of the NPS market. Activity-based assays could circumvent this problem since no prior knowledge on the substance structure is necessary. However, ”classical” recreational drugs activating the same receptors could lead to false positive results. Liquid or gas chromatography coupled with mass spectrometry is a valuable NPS analysis tool, but its costs (e.g. equipment), run time (results usually within hours vs. minutes in case of immunoasssays) and the need for specialized personnel hinder its use in clinical setting, while factors such as lack of reference standards can pose further limitations. Although supportive measures are sufficient in most cases for adequate patient management, the detection and identification of NPS can contribute significantly to public health and safety in cases of e.g. cluster intoxications and outbreaks, and to the investigation of these novel compounds' properties. However, this requires not only availability of the necessary equipment and personnel, but also collaboration between clinicians, authorities and laboratories.

Introduction

The recreational use of psychoactive substances is common; in the European Union it is estimated that more than 92 million or just over a quarter of 15- to 64-year-olds have used illicit drugs at least once in their lives [1]. In recent years, in addition to "classical" recreational drugs, new substances have emerged worldwide, leading to a wider range of available compounds than in the past [1-3]. According to the United Nations Office on Drugs and Crime (UNODC), NPS are defined as "new narcotic or psychotropic drugs, in pure form or in preparation, not controlled by either the 1961 United Nations Single Convention on Narcotic Drugs nor the 1971 United Nations Convention on Psychotropic Substances, but which may pose a public health threat comparable to that presented by substances listed in these conventions" [4].

Based on their chemical structure and/or pharmacology NPS can be categorized into different classes, such as synthetic cannabinoids, also known as synthetic cannabinoid receptor agonists (SCRAs), synthetic opioids, phenethylamines (e.g. amphetamines including cathinones, N-2-methoxybenzyl-phenethlylamines (NBOMes) etc.), piperazines, tryptamines, piperidines, and benzodiazepines. Although NPS are usually derivatives or analogues of "classical" recreational drugs (Fig. 1) produced to imitate their effects while circumventing regulations, the alterations of their structure can have a great influence on their pharmacokinetic and pharmacodynamic properties even within the same class [2], thus making it difficult to predict their pharmacokinetics (e.g. bioavailability, elimination half-life), psychoactive effects, potency, and toxicity.

The NPS phenomenon started in 2004 with the "Spice" products being sold in Western Europe and Japan in head-shops and via the Internet as herbal blends [5,6]. These products were advertised as legal alternatives to cannabis (therefore also named "legal highs") [7]. Due to the availability and limited legislative control, and because they could not be detected with the

usual commercial drug screening methods, “Spice” became quickly popular especially among young people [5,7,8]. However, in 2009 Auwärter et al. could show that SCRAs (JWH-018 and CP47,497) which had been sprayed on the leaves and not the herbal blends themselves accounted for the psychoactive effects of these products [5].

Meanwhile, other NPS (over 700 substances) have also been identified. Products are often provided as powders and are labelled (among others) as “laboratory reagents” or “dietary supplements”, and with warnings such as “not for human consumption” [8]. In 2005, the European Union established an Early Warning System (EWS) monitored by the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) and Europol. Until 2015, a rise of the number of newly identified substances was observed (41 NPS identified for the first time in 2010, 49 in 2011, 73 in 2012, 81 in 2013, 101 in 2014, 98 in 2015), with a decline seen since 2016, (66, 51 and 55 NPS identified for the first time in 2016, 2017, and 2018, respectively) [1,8,9]. Nevertheless, this means that approximately one new chemical product is entering the illicit drug market each week.

Some of these compounds were initially developed to study different types of receptors and are therefore potent agonists, exhibiting high receptor binding affinities and strong psychoactive effects at low doses [10,11]. For example, the phytocannabinoid Δ^9-tetrahydrocannabinol (Δ^9-THC), the main psychoactive constituent of cannabis, acts as a partial agonist at the cannabinoid receptors 1 and 2 (CB_1 and CB_2), while most SCRAs are full agonists and generally have higher affinity for the CB_1 and CB_2 receptors [12,13]. Since psychoactive and behavioral effects are produced via CB_1 agonism, this can result in greater potential for serious neuropsychiatric toxicity compared with cannabis. Additionally, lower doses of SCRAs are needed to achieve psychoactive effects, posing further difficulties associated with their detection.

As a further example, activation of the serotonin (5-HT) receptor 5-HT_{2A} has been shown to mediate effects of hallucinogens with a strong correlation between hallucinogenic potencies in humans and receptor affinity *in vitro* [10,14-16]. The NBOMes (2,5-dimethoxy-*N*-benzylphenethylamines) are serotonin 5-HT_{2A} receptor agonists similar to other hallucinogenic compounds, but present a higher receptor affinity compared to phenethylamine analogues without an N-benzyl moiety [10,11], thus leading to stronger effects and responses, including fatal intoxications [17-20]. The route of consumption also varies depending on the type of NPS. For example, herbal blends such as "Spice" are often smoked, while tryptamines are often sniffed or ingested dissolved in a drink [21], and highly potent substances such as NBOMes are consumed sublingually on blotter paper similar to LSD [22].

Next to the dynamic NPS market (i.e. rapid introduction of a variety of new substances on the drug market in order to evade legislations) and the broad toxicological differences among the substances that cannot be predicted only based on the chemical structure, factors that make the identification and investigation of NPS difficult are related to their analytical detection in human samples. The immunoassays that are most commonly used at the emergency department (ED) of hospitals to rapidly screen for psychoactive drugs typically cannot detect NPS. Moreover, even more specific methods such as liquid-chromatography coupled to tandem mass spectrometry (LC-MS/MS) or gas-chromatography coupled to mass spectrometry (GC-MS) may not include the newest NPS, which can also lead to underdetection [23]. This poses problems for various scientists and working groups such as analytical chemists, clinical pharmacologists, emergency physicians, and forensic scientists trying to detect and investigate NPS and potential problems related to their consumption. These aspects will be discussed in more detail in the present article.

Analytics

The approach for detection and identification of psychoactive drugs generally consists of two analytical steps, a preliminary screening and a confirmation test [24,25]. Since it is not feasible to directly test all samples with the more limited available confirmation methods, preliminary screening methods are used to filter presumptive positive samples and thus help to decide which type of subsequent confirmation method should be used for further identification [25].

Samples and sample treatment

The analysis for NPS can be performed in matrices such as blood, urine, hair, nails, oral fluid and tissue samples [26,27]. Urine and blood are the most commonly used samples in clinical setting, while the rest of the listed options are usually regarded as alternatives. Less conventional matrices include dried blood spots with capillary blood, which is also used for neonatal metabolic disease screening [28] and has recently gained more attention in clinical and forensic toxicology also for other applications [29-31]. Advantages of these alternative techniques are their longer detection time window and the less invasive sample collection and easier storage and shipping [29]. Usually, urine is the matrix collected for drug testing in clinical settings, because sampling is non-invasive, it is readily available, and has a longer detection window compared to blood. However, in some emergency departments sampling of blood might be more common because it is needed also for other analyses and patients may not be able/willing to provide urine. Although urine has a longer detection window and typically higher analyte and metabolite concentrations than blood [32], the amount of liquids consumed might influence the urine concentration of an analyte and due to metabolism it is possible that only biotransformation products (metabolites) and not the parent compound are present. For this reason metabolites are often included in confirmation methods. Furthermore, especially for substances with a long detection window a positive result does not necessarily reflect the

impairment at the time of the sampling. Blood has the advantage that samples cannot be easily manipulated and the analytical results better reflect the compounds currently present in the body.

In order to protect the instruments and have better results, sample preparation is often necessary prior to sample analysis. The type of sample preparation technique depends on the matrix, on the physical and chemical properties of the investigated analytes and the level of sensitivity and specificity required for a certain analysis [24]. Blood samples can be extracted using methods such as protein precipitation, liquid-liquid extraction (LLE), salting-out assisted liquid-liquid extraction (SALLE), solid phase extraction (SPE) or microwave assisted extraction (MAE) [24,33-35]. The extraction of urine samples can be performed by SPE or LLE [24] but also simple dilution of the samples (dilute and shoot methods) have been reported [35,36].

Preliminary screening tests

Immunoassays

Immunoassay-based psychoactive drug screening tests are biochemical tests that use selective antigen-antibody binding to mostly qualitatively determine the presence of a compound [25]. Immunoassays can be classified into non-competitive and competitive. An example of a non-competitive assay is a "sandwich immunoassay", in which two different antibodies create a "sandwich" around the antigen. The second antibody is labelled and its amount is directly proportional to the amount of antigen in the sample. In a competitive assay a known amount of labelled antigen is allowed to compete for a limited number of binding sites, therefore, the amount of bound labelled antigen is inversely proportional to the amount of antigen present in the sample [37].

Qualitative immunoassays have a certain cut-off as limit of detection (which can vary depending on the investigated question and possible consequences, e.g. clinical vs. forensic setting) leading to false negative results in case of low concentrations present in the sample (e.g. after use of high potency drugs such as some NPS), and give in most cases either a positive or a negative result. However, semi-quantitative tests have also been reported [38]. For most substances typical immunoassay tests detect the chemical class (e.g. benzodiazepines, opiates, amphetamines etc.) and not a specific substance, while for some substances the specific substance is detected (e.g. methadone, cocaine, benzoylecgonine (cocaine metabolite), 6-monoacetylmorphine (heroin metabolite), phencyclidine) (Table 1).

Furthermore, immunoassays are not very specific, meaning that cross reactivity with other compounds with a similar structure is possible, resulting in false positive results.

Since NPS have a high structural diversity, immunoassays typically cannot detect them or are too unspecific [5,7]. Thus, although SCRAs are cannabinoid receptor agonists like THC they are not detected by most immunoassays. Furthermore, although cathinones are amphetamines and differ from them only by the addition of a keto-group at the beta-carbon (Fig. 1), they are generally not detected by immunoassays. In contrast, some novel benzodiazepines may produce a positive benzodiazepine class screening test result. In order to be able to detect or exclude NPS using an immunoassay, further antibodies specifically binding to the new structures would have to be developed, which is time consuming and results in delays (thus not keeping up with the dynamic NPS market) and higher costs [42]. Although some actions have been undertaken in this direction during the last years with some immunoassays being developed for the detection of commonly used NPS such as some of the SCRAs [25], NPS immunoassays are currently clearly not part of the clinical routine. Kronstrand et al. [43] concluded in their evaluation of the Immunalysis Spice K2 homogenous enzyme immunoassay (HEIA) (Pomona, CA, USA) that an LC-MS/MS screening approach is a superior strategy to

immunoassays, because of the rapid change of synthetic cannabinoid structures. Similarly, Franz et al. [42] evaluated the diagnostic efficiency of immunoassays for screening urine for SCRAs and their results showed an insufficient cross-reactivity for the SCRAs available on the market at the time of the study and their metabolites. Furthermore, the investigated immunoassays had a high cut-off, which also contributed to a high proportion of false-negative results. Therefore, Franz et al. advised against the use of immunoassays for screening urine for SCRAs in clinical and forensic settings [42].

Activity-based assays

Recently, Canneart et al. [44] reported upon an activity-based assay for screening of SCRAs in biological samples. This assay is based on the mechanism of receptor activation combined with the principle of functional complementation of a split Nanoluc® luciferase [45]. Once either cannabinoid receptor (CB_1/CB_2), fused to one of the luciferase, is activated, an engineered β-arrestin 2, fused to the other part, is recruited. The resulting restoration of luciferase activity leads to a measurable bioluminescence [44,45]. Therefore, SCRAs could be detected pharmacologically based on their cannabinoid activity. Hence, no prior knowledge on their structure is necessary [44,45]. However, since phytocannabinoids, although less potent, may also produce a positive result, conventional assays might be necessary to differentiate between synthetic and natural compounds (e.g. Δ^9-THC) in case of positive samples [46,47]. The same mechanism has been used when targeting for opioid activity by activation of the μ-opioid receptor, and this assay has been applied successfully to 107 authentic post-mortem blood samples for the detection of opiates and (synthetic) opioids [48].

Mass Spectrometry

Meyer and Maurer [49] concluded in their review, that only mass spectrometry (MS), after a variety of separation methods, provides the high level of flexibility, sensitivity and selectivity needed for a robust and reliable detection of NPS [26]. Chromatography coupled with MS is a powerful tool of analysis when it comes to whole blood, serum and urine samples. Depending on the molecular properties and the desired strength of separation, different methods can be chosen. Nowadays, liquid chromatography (LC) or gas chromatography (GC) are the most commonly used separation techniques in clinical settings. Using a stationary and a mobile phase, the chromatographic techniques separate the compounds, which can then be identified and quantified with the MS based on their mass-to-charge ratio.

Untargeted Screening

In recent years, untargeted screening using high-resolution mass spectrometry (HR-MS/MS) of urine samples has gained attention for the screening of not only "classical" but also novel substances, such as NPS [47]. Even though the lack of certified reference material and mass spectral libraries still poses a problem, the ability of HR-MS/MS to determine a compound's or a fragment's mass with sufficiently high accuracy makes it a valuable tool for the identification of NPS [24]. Nonetheless, this technique is specialized, time-consuming, and expensive and therefore not routinely available in most clinical settings. Additionally, the target analytes need to be present in a sufficiently high concentration in order to trigger an acquisition [47]. HR-MS/MS can be operated in data-independent acquisition (DIA) modes, which provide comprehensive full scan MS and MS/MS and qualitatively analyses of samples [24,50]. These techniques have the additional advantage that data can be retrospectively analysed for new analytes and therefore re-extraction or re-analysis of samples is not necessary [24].

Additionally, it is not necessary to have a library with certified reference standards (for the comparison of retention times or mass spectra), thus overcoming the problem of the availability of NPS reference standards.

Targeted Screening

Using multi-analyte LC-MS/MS approaches such as selected reaction monitoring (SRM) enables the detection and (if desired) quantification of several hundred analytes in one chromatographic run [51]. The selectivity and number of monitored transitions defines here the identification power [50]. Targeted screenings are traditionally performed using low-resolution MS/MS devices [26] such as Qtrap instruments, which are usually routinely present in clinical laboratories. However, SRM methods only target analytes implemented in the method and are therefore not capable to detect unexpected or unknown compounds [26]. Furthermore, certified reference standards are needed for method development, which can pose a problem in the context of the dynamic NPS market. Additionally, the newest NPS may not be available or they may have to be obtained from other countries, which can lead to delays and problems with federal customs [24]. In the literature, a vast amount of LC-MS and GC-MS methods for the detection and quantification of NPS have been reported [52-56], including an in-depth review of all possible HR-MS methods for the analysis of NPS by Pasin et al. [24].

Value of NPS analytics in clinical setting

As with intoxication-related presentations in general, in cases presenting with NPS toxicity, decisions regarding patient management are usually based on the patient's (or witnesses') report regarding symptoms and substance(s) used and the clinical presentation (e.g. signs and

symptoms in accordance with a specific toxidrome). In most cases those are the first, and in some cases the only information available since analytical screening is not available at all medical facilities, and even when available, it is not necessarily performed for all intoxication cases or the results are not immediately available. Furthermore, other factors such as patients not willing to cooperate or not being able to provide a urine sample at presentation might also further complicate sample acquisition and can lead to delays. In line with this, in an analysis within the European Drug Emergencies Network (Euro-DEN) Plus project which has been collecting data on ED presentations due to acute recreational drug/NPS toxicity since 2013, a toxicological screening was routinely performed only in the minority (15%) of the cases, which probably reflects normal practice in most European hospitals [57]. In this study reflecting the real world conditions, NPS were detected only when MS methods were available and used and in none of the cases when only an immunoassay was used, despite reported NPS use in some cases [57]. Analytically detected NPS included: the phenethylamine 2,5-dimethoxy-4-chlorphenethylamine (2C-C), the synthetic cathinones 3,4-methylenedioxypyrovalerone (MDPV), a-pyrrolidinopentiophenone (a-PVP), pentylone, and mephedrone, and the SCRAs 5F-PB-22 and 5F-AKB48. Self-reported NPS included phenethylamines of the 2C- and 2D-series compounds (2C-B, 2C-C, DOC) and NBMOes (25B-NBOMe), synthetic cathinones (mephedrone, methedrone, 3-methylmethcathinone (3-MMC), 4-methylethcathinone (4-MEC)), paramethoxymethamphetamine (PMMA), benzodifurans (Bromo-DragonFLY), tryptamines (dimethyltryptamine (DMT)), and also products named "teenage mutant ninja turtle", "devil bandit", "charge white", "blue ghost". In contrast to NPS, a relatively high agreement between the immunoassay and the MS results was found for "classical" recreational substances such as methadone (100% agreement), cocaine (96% agreement), heroin (92% agreement), and cannabis (84% agreement) in cases for which both analytical methods were performed (n=213). Although these findings demonstrate the importance of additional

analytical methods such as MS for the detection of NPS, these methods are rarely routinely available, mainly due to their high costs (e.g. equipment, solvents, standards), long run time (results usually available within hours vs. minutes in case of immunoasssays) and the need for specialized personnel [57]. Less complex screening tools such as high-resolution accurate-mass (HRAM) spectrometry using libraries that can be regularly updated from forensic networks can facilitate some of these aspects, but the financial barrier remains the main limitation regarding their use. Furthermore, as mentioned above, even specific and reliable MS methods cannot or may not be set-up to detect all NPS. Factors that can affect the window of detection (e.g. elimination half-life of the compound and its metabolites) can also lead to false negative results. For example, γ-hydroxybutyrate (GHB) has a short plasma elimination half-life (20-50 minutes) that results in a short detection window (≤ 4-5 hours in blood and ≤ 12 hours in urine) [58]. On the other hand, substances such as benzodiazepines (parent compounds and/or metabolites with long elimination half-lives) and cannabis can be detectable in samples days or even weeks after use. Therefore, management of recreational drug/NPS toxicity is usually based on the substances used being reported and supportive based on the clinical presentation (e.g. administration of benzodiazepines in case of agitation) and the detection of specific NPS compounds often more of scientific and epidemiologic than of immediate clinical practical interest.

However, although symptomatic measures are in most NPS intoxication cases sufficient for adequate patient management, there are some instances where timely identification of the exact compound can be of public health value. For example, new NPS on the market can often lead to local cluster intoxications, as was the case on July 12, 2016 in New York, where 33 persons were exposed to an unknown drug with consequent behavioural abnormalities, described by bystanders as "zombielike" [59]. Using liquid chromatography-quadrupole time-of-flight mass spectrometry (LC-QTO/FMS), the potent synthetic cannabinoid AMB-FUBINACA (in vitro

85 times more potent than Δ^9-THC in reference to CB_1 receptor activation) [60] was identified in the product used and its metabolite in the serum of patients transported to local medical centres, thus enabling timely information of medical professionals and health authorities [59]. However, in line with the limitations mentioned above, the identification of the compound took several days, required a sophisticated MS analysis, and only the metabolite could be detected in patients' samples, since due to rapid biotranformation of the parent compound, it is often not detectable/only at low levels present [59]. Other recent outbreaks with increased number of cases with severe effects following exposure to SCRAs include cases of acute kidney injury after use of XLR-11, agitated delirium linked to ADB-PINACA and severe illness and deaths associated with MAB-CHMINACA [61], and case series with other NPS compounds (e.g. seven analytically confirmed cases with 25I-NBOMe (clinical features included tachycardia, hypertension, agitation/aggression, hallucinations, seizures, hyperpyrexia, clonus, and acute kidney injury), identified by LC-MS/MS analysis [18]). Analytical identification of the specific compound in such cases, although time consuming and challenging, can significantly contribute to the timely detection of trends and high risk substances which has important implications for public health and public safety. However, this requires not only availability of the necessary equipment and personnel, but also collaboration between clinicians, authorities and laboratories, e.g. in order to timely provide adequate samples for analysis.

Besides enabling prompt identification of trends and related health risks, analytical confirmation of NPS can also contribute to improving our knowledge regarding these less investigated substances and our understanding of the several, often unpredicted differences and similarities between compounds. For example, the pharmacokinetic and pharmacodynamic properties of SCRAs can differ considerably from the differently structured natural compound Δ^9-THC and intoxications with these compounds are considered to be associated with a higher risk of adverse effects (including fatalities) and driving impairment compared to cannabis [62-

65]. These findings are important especially in the light of the increasing interest in medical use of cannabis in the recent years since they contribute to the risk assessment and can be used to optimize patient safety and regulations (e.g. implementation of precaution measures but also avoidance of unnecessary restrictions) and inform about risks related to specific agents.

In theory, the patient's self-report could be used instead of a challenging analytical method to gain information about the consumed substance(s). This information in combination with a thorough clinical examination could facilitate linking specific effects to specific substances even in cases with no analytical results available. However, self-reports have several limitations, since NPS names are often used incorrectly (e.g. 2C-P instead of 2C-B [66]), patients might not know which substances they have used, and NPS use might not be reported, e.g. in cases of uncooperative or comatose patients, or because of fear of the legal consequences. Furthermore, even if reported correctly to the patient's knowledge, the product information of NPS is often unreliable with products not containing what they claim and/or more than one compounds, and lack of consistency even among products with the same name, as it has been shown in studies analysing NPS products purchased online [67,68]. Therefore, self-reports cannot reliably replace analytical methods in order to identify use of specific NPS. In clinical practice, NPS will remain undetected if not reported and MS or similar analysis will be required to correctly identify the compound.

Conclusions

In clinical setting urine or blood samples are usually used for qualitative analysis in cases presenting with recreational drug/NPS toxicity, but the currently commercially available rapid immunoassay screening tests typically cannot identify NPS. Although in most cases, management is based on the patient's self-report and clinical presentation and supportive

measures are sufficient, identification of specific compounds can be of public health and public safety value in cases of outbreaks and cluster intoxications with NPS. However, next to the availability of the necessary equipment and personnel, collaborations between clinicians, authorities and laboratories are also essential for optimizing identification of currently used NPS. Due to numerous limitations of self-reports more elaborated chromatographic and mass spectrometric methods are needed for identification and quantification of specific substances. However, these expensive and time-consuming methods have also limitations. Development of immunoassays using antibodies specifically binding to specific NPS or/and activity-based assays could facilitate rapid detection of some NPS in the future. Until then, chromatography coupled with mass spectrometry remains the most powerful and reliable tool for NPS detection and therefore, although of limited use in acute clinical settings, indispensable when it comes to forensic investigations and cases with potential legal consequences.

Competing Interests

There are no competing interests to declare.

Nomenclature of Targets and Ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY.

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Table 1. Cut-off values for specific substances and substance classes detected with some commercially available immunoassays

			Triage TOX Drug Screen (Alere) [39]	**CEDIA, DRI (Thermo Fischer) [40]**	**Rapi Test (MD Doctors) [41]**
Substance / substance class	**Target Analyte**	**Comment**	**Cut-off (ng/mL)**	**Cut-off (ng/mL)**	**Cut-off (ng/mL)**
Amphetamine	D-amphetamine	Parent	1000	50	300/500/1000
	D-methamphetamine	Parent	1000	50	300/500/1000
	MDMA	Parent		50	500
	MDEA	Parent		50	
Barbiturates	Pentobarbital	Parent	300		
	Secobarbital	Parent			300
Benzodiazepine	Estazolam	Parent	300		
	Nordiazepam	Metabolite		50	
	Oxazepam	Parent		50	200/300
	Hydroxy-alprazolam	Metabolite		50	
	Hydroxy-bromazepam	Metabolite		50	
	7-amino-flunitrazepam	Metabolite		50	
	Lorazepam	Parent		50	
Cocaine	Benzoylecgonine	Metabolite	300	30	150/300
Nicotine	Cotinine	Metabolite			100
Opiates	Morphine	Parent	300	25	300/1000
Opioids	Tramadol	Parent			100
	Buprenorphine	Parent			10
	Methadone	Parent	3000		300
	EDDP	Metabolite		50	100/300
	Norfentanyl	Metabolite			20
	Oxycodone	Parent			100
	Propoxyphene	Parent			300
Phencyclidine	Phencyclidine	Parent	25		25
Tetrahydrocannabinol	11-nor-9 carboxy-Δ-9-THC	Metabolite	50	10	20/50/150
Ethanol	Ethyl glucuronide	Metabolite		100	

Figure 1. Structural formulas of phenethylamine (basic structure) and representative substituted phenethylamines: the classical recreational drugs amphetamine and methamphetamine, and some new psychoactive substances, i.e. the synthetic cathinone mephedrone, the 2C-series compound 2,5-dimethoxy-4-bromophenethylamine (2C-B), the 2D-series compounds 2,5-dimethoxy-4-iodoamphetamine (DOI) and 2,5-dimethoxy-4-chloroamphetamine (DOC), and the NBMOe 4-iodo-2,5-dimethoxy-N-(2-methoxybenzyl)phenethylamine (25I-NBOMe)