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Brief Communication

Evaluation of commercial multi-drug oral fluid devices to identify 39 new amphetamine-designer drugs

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ABSTRACT

Recently, the diffusion on the black market of new psychoactive substances not controlled and often sold as 'legal highs', is exponentially increasing in Europe. Generally, the first analysis for these drugs involves an immunoassay screening in urine or plasma. Actually, there is growing interest in the use of oral fluid (OF) as alternative specimen over conventional biological fluids for drug testing, because of the significant advantages, as a non-invasive collection under direct observation without undue embarrassment or invasion of privacy, and a good correlation with plasma analytical data. Few assays have been developed for detection of new psychoactive compounds in biological samples, so it is important to investigate how they may or may not react in pre-existing commercial immunoassays. In this paper, two different multi-drugs oral fluid screen devices (OFDs) (Screen® Multi-Drug OFD and GIMA One Step Multi-Line Screen Test OFD) were evaluated to determine the cross-reactivity of thirty-nine new amphetamine designer drugs, including twelve substances officially recognized as illicit by italian legislation. Cross-reactivity towards most drugs analyzed was <1 in assays targeting amphetamine (AMP) or methamphetamine (MET). Only two (p-methoxyamphetamine and p-methoxymethamphetamine) of all tested amphetamines gave a positive result.

1. Introduction

Recently, the diffusion on the drug market of new psychoactive substances not controlled and often sold as 'legal highs' is exponentially increasing in Europe. 'Legal highs' are substances of synthetic or natural origin having psychotropic properties. More than 280 legal highs and other new psychoactive synthetic drugs are actually monitored by EU drug agency [1]. Generally, the first analysis for these drugs involves an immunoassay screening in urine or plasma. If the screening test is positive, additional tests by gas chromatography–mass spectrometry (GC–MS) or liquid chromatography–mass spectrometry (LC–MS) must be performed.

Actually, there is growing interest in the use of oral fluid (OF) as alternative specimen over conventional biological fluids (urine, blood) for drug testing. The great advantages of these matrix are a non-invasive collection under direct observation without undue embarrassment or invasion of privacy, and a good correlation with plasma analytical data [2–4]. Drug concentrations in OF reflect the free, unbound parent drug and lipophilic metabolites circulating in the blood. Since these are the forms of the drug which cross the blood–brain barrier and effect performance and behavior, OF is a good specimen for detecting drug involvement in driving behavior or impairment of performance. Drug and lipophilic metabolite concentrations in OF are a function of the drug's pKa, plasma, and OF pH and the fraction of drug bound to OF and plasma protein [2].

Italian legislation on traffic safety has recently accepted oral fluid as a possible alternative biological sample for toxicological analysis [5]. The national guidelines establishing how to perform the analysis and the practical issues regarding which device and cut-off values should be used are however still undetermined.

There have been numerous studies regarding the performance characteristics of commonly used OFDs for known abuse drugs [6–9]. Most of these studies reported good agreement between screening and confirmatory results.

Differently, few assays have been developed for detection of new psychoactive compounds; it is important to investigate how new 'legal highs' may or may not react in pre-existing and broad diffused commercial immunoassay kits.

The main objective of this study is to evaluate whether two commercial OFDs (Screen® Multi-Drug OFD and GIMA One Step Multi-Line Screen Test OFD) can be an effective alternative for screening thirty-nine new amphetamine designer drugs. The ability and degree of reliability of these devices in spiked samples are examined. Between the thirty-nine amphetamine compounds analyzed, twelve (PMA, PMMA, 2,5-DMA, DOB, DOM, DOET, 2C-B, 2C-I, 2C-T-2, 2C-T-7, TMA, TMA-2) are already classified as

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psychotropic substances by Italian legislation [10,11], so their consume and diffusion are punishable by law. A low cross-reactivity towards these compounds may result in these abused substances not being detected in forensic case samples.

2. Materials and methods

2.1. Chemicals and reagents

Hydrochloride salt forms of p-methoxyamphetamine (PMA), p-methoxymethamphetamine (PMMA), 2,5-dimethoxy-amphetamine (2,5-DMA), 2,5-dimethoxy-4-bromoamphetamine (DOB), 2,5-dimethoxy-4-chloroamphetamine (DOC), 2,5-dimethoxy-4-ethylamphetamine (DOET), 2,5-dimethoxy-4-iodoamphetamine (DOI), 2,5-dimethoxy-4-methylamphetamine (DOM), 2,5-dimethoxy-4-nitroamphetamine (DON), 2,5-dimethoxy-4-propylamphetamine (DOPR), 2,5-dimethoxy-4-bromophenethylamine (2C-B), 2,5-dimethoxy-4-iodophenethylamine (2C-I), 2,5-dimethoxy-4-nitrophenethylamine (2C-N), 2,5-dimethoxy-4-methylphenethylamine (2C-M), 2,5-dimethoxy-4-methylthiophenethylamine (2C-T), 2,5-dimethoxy-4-ethylthiophenethylamine (2C-T-2), 2,5-dimethoxy-4-isopropylthiophenethylamine (2C-T-4), 2,5-dimethoxy-4-cyclo hexylthiophenethylamine (2C-T-5), 2,5-dimethoxy-4-propylthiophenethylamine (2C-T-7), 2,5-dimethoxy-4-(2-methoxyethyl) thiophenethylamine (2C-T-13), 2,5-dimethoxy-4-isobutylthiophenethylamine (2C-T-17), 2,5-dimethoxy-4-methylthioamphetamine (ALEPH), 2,5-dimethoxy-4-ethylthioamphetamine (ALEPH-2), 2,5-dimethoxy-4-cyclohexylthioamphetamine (ALEPH-5), 2,5-dimethoxy-4-propylthioamphetamine (ALEPH-7), 2,5-dimethoxy-4-cyclopropylmethylthioamphetamine (ALEPH-8), 2,5-dimethoxy-4-(2-methoxyethyl) thioamphetamine (ALEPH-13), 2,5-dimethoxy-4-isobutylthioamphetamine (ALEPH-17), 3,4,5-trimethoxyamphetamine (TMA), 2,4,5-trimethoxyamphetamine (TMA-2), 2,3,4-trimethoxyamphetamine (TMA-3), 2,4,6-trimethoxyamphetamine (TMA-6), 3,4-methylenedioxy-N-isopropylamphetamine (MDIP), 3,4-methylenedioxy-N-benzylamphetamine(MDBZ), 3,4-methylenedioxy-N-cyclopropylmethylamphetamine (MDCPM), 2-(3, 4-methylenedioxyphenyl)-2-methoxyethylamine (BOH), 4-methyl-2, 5-dimethoxy-β-hydroxyphenethylamine (BOHD), 2-(4-bromo-2, 5-dimethylenedioxyphenyl)-2-methoxyethylamine (BOB), 2-(2,5-dimethoxy-4methylphenyl)-2-methoxyethylamine (BOD) were synthesized in our laboratory according to the methods of Shulgin and Shulgin [12]. Aqueous stock solutions of all the mentioned amphetamines were prepared at 10 μg/ml, and they were diluted to appropriate concentrations (10; 20; 50; 100; 200; 1000; 2000; 5000 ng/ml) with a drug-free pooled oral fluid sample collected by 10 volunteers.

2.2. Immunoassay OF tests

Oral fluid screening tests were performed using Screen® Multi-Drug OFD (SCREEN ITALIA Srl, Perugia, Italy), and GIMA One Step Multi-Line Screen Test OFD (GIMA Spa, Gessate, Milan, Italy), according to the manufacturer's instructions [13,14]. These multi-drug tests are made to detect amphetamine (AMP), cocaine

Table 1
Cross-reactivities of thirty-nine amphetamine designer drugs on Screen® test.

Analyte	% Cross-reactivity	
	AMP	MET
p-Methoxyamphetamine (PMA)	25	<1
p-Methoxymethamphetamine (PMMA)	<1	250
2,5-Dimethoxyamphetamine (2,5-DMA)	<1	<5
2,5-Dimethoxy-4-bromoamphetamine (DOB)	<1	<1
2,5-Dimethoxy-4-chloroamphetamine (DOC)	<1	<1
2,5-Dimethoxy-4-ethylamphetamine (DOET)	<1	<1
2,5-Dimethoxy-4-iodoamphetamine (DOI)	<1	<1
2,5-Dimethoxy-4-methylamphetamine (DOM)	<1	<1
2,5-Dimethoxy-4-nitroamphetamine (DON)	<1	<1
2,5-Dimethoxy-4-propylamphetamine (DOPR)	<1	<1
2,5-Dimethoxy-4-bromophenethylamine (2C-B)	<1	<1
2,5-Dimethoxy-4-iodophenethylamine (2C-I)	<1	<1
2,5-Dimethoxy-4-nitrophenethylamine (2C-N)	<1	<1
2,5-Dimethoxy-4-methylphenethylamine (2C-M)	<1	<1
2,5-Dimethoxy-4-methylthiophenethylamine (2C-T)	<1	<1
2,5-Dimethoxy-4-ethylthiophenethylamine (2C-T-2)	<1	<1
2,5-Dimethoxy-4-isopropylthiophenethylamine (2C-T-4)	<1	<1
2,5-Dimethoxy-4-cyclohexylthiophenethylamine (2C-T-5)	<1	<1
2,5-Dimethoxy-4-*n*-propylthiophenethylamine (2C-T-7)	<1	<1
2,5-Dimethoxy-4-(2-methoxyethyl)thiophenethylamine (2C-T-13)	<1	<1
2,5-Dimethoxy-4-i-butylthiophenethylamine (2C-T-17)	<1	<1
2,5-Dimethoxy-4-methylthioamphetamine (ALEPH)	<1	<1
2,5-Dimethoxy-4-ethylthioamphetamine (ALEPH-2)	<1	<1
2,5-Dimethoxy-4-cyclohexylthioamphetamine (ALEPH-5)	<1	<1
2,5-Dimethoxy-4-*n*-propylthioamphetamine (ALEPH-7)	<1	<1
2,5-Dimethoxy-4-cyclopropylmethylthioamphetamine (ALEPH-8)	<1	<1
2,5-Dimethoxy-4-(2-methoxyethyl) thioamphetamine (ALEPH-13)	<1	<1
2,5-Dimethoxy-4-isobutylthioamphetamine (ALEPH-17)	<1	<1
3,4,5-Trimethoxyamphetamine (TMA)	<1	<1
2,4,5-Trimethoxyamphetamine (TMA-2)	<1	<1
2,3,4-Trimethoxyamphetamine (TMA-3)	<1	<1
2,4,6-Trimethoxyamphetamine (TMA-6)	<1	<1
3,4-Methylenedioxy-N-isopropylamphetamine (MDIP)	<1	<1
3,4-Methylenedioxy-N-benzylamphetamine(MDBZ)	<1	<1
3,4-Methylenedioxy-N-cyclopropylmethylamphetamine (MDCPM)	<1	<1
2-(3,4-Methylenedioxyphenyl)-2-methoxyethylamine (BOH)	<1	<1
2-(4-Bromo-2,5-dimethylenedioxyphenyl)-2-methoxyethylamine (BOB)	<1	<1
2-(2,5-Dimethoxy-4methylphenyl)-2-methoxyethylamine (BOD)	<1	<1
2-(2,5-Dimethoxy-4methylphenyl)-2-idroxyethylamine (BOHD)	<1	<1