Journal of Analytical Toxicology 2015;39:144–151
doi:10.1093/jat/bku133 Advance Access publication December 9, 2014

Technical Note

Determination of Designer Drug Cross-Reactivity on Five Commercial Immunoassay Screening Kits

Laura E. Regester*, Jeffrey D. Chmiel, Justin M. Holler, Shawn P. Vorce, Barry Levine and Thomas Z. Bosy

Division of Forensic Toxicology, Armed Forces Medical Examiner System, Dover AFB, DE, USA

*Author to whom correspondence should be addressed. Email: laura.e.regester.ctr@mail.mil

The detection of new designer drugs is often a difficult issue in forensic urine drug testing as immunoassays are the primary screening methodology for drugs of abuse in many of these laboratories. Cross-reactivity of compounds with immunoassay kits can either aid or complicate the detection of a variety of drug and drug metabolites. For instance, emerging designer drugs that share structural similarities to amphetamines and phencyclidine (PCP) have the potential to cross-react with assays designed to detect these compounds. This study evaluates the cross-reactivity of five commercially available immunoassay reagent kits for 94 designer drugs on a Roche/Hitachi Modular P automated screening instrument. The compounds used in this study are grouped by structural class as follows: 2,5-dimethoxyamphetamines, 2C (2,5-dimethoxyphenethylamines), β-keto amphetamines, substituted amphetamines, piperazines, α-pyrrolidinopropiophenones, tryptamines and PCP analogs. A drug concentration of 100 μg/mL was used to determine cross-reactivity for each assay and resulted in the following positive rates: Microgenics DRI® Ecstasy enzyme assay (19%), Microgenics DRI® Phencyclidine enzyme assay (20%), Lin-Zhi Methamphetamine enzyme immunoassay (39%), Siemens/Syva® EMIT®II Plus Amphetamines assay (43%) and CEDIA® DAU Amphetamine/Ecstasy assay (57%). Of the 94 designer drugs tested, 14% produced a negative response for all five kits. No designer drug used in this study generated a positive result for all five immunoassay kits.

Introduction

Commercially available immunoassays remain the most common screening method utilized by forensic urine drug testing laboratories. There are a variety of proprietary biochemical and enzymatic reporting methodologies employed by manufacturers to achieve acceptable screening results, these include kinetic interaction of microparticles in solution (KIMS), cloned enzyme donor immunoassay (CEDIA) and enzyme multiplied immunoassay technique (EMIT). These tests are a quick, accurate and relatively cheap approach to eliminate negative samples from further, more costly, analysis. Cross-reactivity with unintended non-targeted compounds is a significant limitation that exists when using immunoassays. The cross-reactivity of an immunoassay can be loosely defined as the propensity of its antibody to bind to compounds the assay was not specifically designed to detect. A positive immunoassay result from these 'non-targeted' compounds still requires confirmation testing, adding time and expense to the overall analytical process.

Compounds structurally related to amphetamine (AMP), methamphetamine (METH), 3,4-methylenedioxy-*N*-methylamphetamine (MDMA) and phencyclidine (PCP) are becoming increasingly popular as new drugs of abuse. These designer drugs are often synthesized through structural modifications of existing drugs to circumvent laboratory and law enforcement efforts to identify and control abuse of these compounds. They commonly have hallucinogenic and stimulant properties which can often result in toxicity to the user. For example, there has been a report of acute psychosis with methylenedioxypyrovalerone (MDPV) (1). Reports of death have been reported from recreational use of designer drugs alone or in combination with 5-IT (2, 3), methylone (4, 5), *para*-methoxyamphetamine (PMA) (6–8), mephedrone (9, 10), *para*-methoxymethamphetamine (PMMA) (6), AMT (11), 5-MeO-DMT (12), mCPP (13), MDPV (4, 14, 15), 1-benzylpiperazine (BZP) (16), methedrone (17), 2C-I (18) and TFMPP (16). A number of these compounds have been added to the Schedule 1 listing of compounds by the Federal Controlled Substances Act or are regulated by the Federal Analogue Act, indicating a high potential for abuse without an acceptable medical use (19).

An added complication to the positive screening results produced by non-targeted compounds is the difficulty in performing specific high volume screening for these compounds. As one compound in the structural series is identified, many others are being synthesized and marketed. This poses serious challenges to the manufactures of immunoassays and the screening sections of laboratories electing to use them. Randox (Crumlin, UK) currently manufactures Biochip array technology for the detection of designer-type drugs which is capable of screening for 10 synthetic cathinones and 12 phenylpiperazine and benzylpiperazine compounds (20). While this technology is available, it is limited to the detection of a relatively small number of designer drugs. Due to the lack of specific immunoassays capable of detecting an increasing number of designer-type drugs, these compounds may go undetected, even when present at high urine concentrations.

It can be especially difficult to detect designer drugs in cases where they are combined with structurally similar compounds (e.g., MDMA). Many of these drugs are frequently sold as counterfeit ecstasy tablets (6, 13). New designer drugs may go undetected if a specimen containing a combination of a new designer drug and MDMA is screened solely by immunoassay. If MDMA is confirmed and quantitated, but no further testing is applied to detect the designer drug then it may not be identified. There have been numerous reports of designer drugs not being detected by primary immunoassay screening methods, and requiring a more elaborate alkaline drug extraction and analysis using gas chromatography–mass spectrometry (GC-MS) or liquid chromatography tandem mass spectrometry (LC-MS-MS) (14, 18, 21–23). Alternatively, some studies have reported cases that screened positive for AMPs or PCP (24) and confirmed negative for these drug targets (25–29), but are reported positive for designer drugs. The potential for false-positives on screening tests increases with the presence of structurally similar compounds to those of interest.

Table I
Cross-Reactivity Results for Assays in μg/mL

Drug	Acronym	CEDIA® Amphetamine/ Ecstasy	Syva® EMIT® II Plus Amphetamine	LIN-ZHI Methamphetamine	Microgenics DRI® MDMA	Microgenics DRI® PCP
2,5-Dimethoxyamphetamines						
2,5-Dimethoxy-4-ethylamphetamine	DOEt	30.00	4.50	N	N	N
2,5-Dimethoxy-4-methylamphetamine	DOM	40.00	5.50	N	N	N
2,5-Dimethoxyamphetamine	DMA	30.00	10.00	N	N	N
4-Bromo-2,5-dimethoxyamphetamine	DOB	20.00	3.00	N	N	N
4-Chloro-2,5-dimethoxyamphetamine	DOC	5.00	1.00	N	N	N
4-Iodo-2,5-dimethoxyamphetamine	DOI	30.00	4.00	N	N	N
2C Series						
25-I-NBOMe		N	N	N	N	N
2C-C-NBOMe	25C-NBOMe	N	N	N	N	N
4-Bromo-2,5-dimethoxy-*N*-[(2-methoxyphenyl)methyl]-benzeneethanamine	25B-NBOMe	N	N	N	N	N
4-Bromo-2,5-dimethoxy-β-phenethylamine	2C-B	50.00	3.00	N	N	N
4-Chloro-2,5-dimethoxy-β-phenethylamine	2C-C	80.00	5.00	N	N	N
4-Ethyl-2,5-dimethoxy-β-phenethylamine	2C-E	N	45.00	N	N	N
4-Ethylthio-2,5-dimethoxy-β-phenethylamine	2C-T-2	N	35.00	N	N	N
4-Iodo-2,5-dimethoxy-β-phenethylamine	2C-I	50.00	2.00	N	N	N
4-Isopropylthio-2,5-dimethoxy-β-phenethylamine	2C-T-4	N	45.00	N	N	N
4-Methylthio-2,5-dimethoxy-β-phenethylamine	2C-T	N	13.00	N	N	N
4-*n*-Propylthio-2,5-dimethoxy-β-phenethylamine	2C-T-7	100.00	9.00	N	N	N
Tryptamines						
α-Methyltryptamine	AMT	20.00	2.50	N	N	N
4-Hydroxy-diethyl-tryptamine	4-OH-DET	N	N	N	N	N
5-Methoxy-*N*-methyl-*N*-isopropyltryptamine	5-Meo-MiPT	N	40.00	N	N	N
N,N-Diallyl-5-methoxytryptamine	5-methoxy-DALT	N	N	N	N	78.00
N,N-Diisopropyl-5-methoxytryptamine	5-MeO-DiPT	N	N	N	N	N
N,N-Diisopropyltryptamine	DiPT	N	N	N	N	N
N,N-Dimethyl-5-methoxytryptamine	5-MeO-DMT	N	N	N	N	N
N,N-Dimethyltryptamine	DMT	40.00	N	30.00	N	N
N,N-Dipropyltryptamine	DPT	30.00	N	N	N	N
α-Methyl-5-methoxytryptamine	5-MeO-AMT	40.00	6.00	N	N	N
α-pyrrolidinopropiophenone						
2-Naphthylpyrovalerone (Naphyrone)		70.00	N	N	N	11.00
3,4-Methylendioxy-α-pyrrolidinobutiophenone	MDPBP	40.00	N	80.00	N	24.00
3,4-Methylenedioxypyrovalerone	MDPV	100.00	N	N	N	3.50
3,4-Methylenedioxy-α-pyrrolidinopropiophenone	MDPPP	30.00	N	N	95.00	60.00
4-Methoxy-α-pyrrolidinohexanophenone	MPHP	N	N	N	N	1.70
4-Methoxy-α-pyrrolidinopropiophenone	MOPPP	40.00	N	N	N	26.00
4-Methyl-α-pyrrolidinobutiophenone	4-MPBP	N	N	55.00	N	9.00
4-Methyl-α-pyrrolidinopropiophenone	MPPP	N	N	80.00	N	18.50
Pyrovalerone		N	N	N	N	2.00
α,α-Diphenyl-2*R*-pyrrolidinemethanol	D2PM	N	N	N	N	65.00
α-Pyrrolidinobutiophenone	α-PBP	N	N	90.00	N	4.60
α-Pyrrolidinopentiophenone	α-PVP	N	N	N	N	1.45
α-Pyrrolidinopentiothiophenone	α-PVT	N	N	N	N	0.60
α-Pyrrolidinopropiophenone	α-PPP	N	N	N	N	7.00
Beta-keto amphetamines						
1-(-1,3-benzodioxol-5-yl)-2-(methylamino)-1-pentanone (pentylone)		10.00	N	N	N	N
2-(Benzylamino)-1-(*p*-tolyl)propan-1-one (benzedrone)	4-MBC	N	75.00	N	N	N
3,4-Methylenedioxy-*N*-ethylcathinone (Ethylone)	β-keto-MDEA	30.00	100.00	55.00	96.00	N
3,4-Methylenedioxy-*N*-methylcathinone (Methylone)	β-keto-MDMA	40.00	N	25.00	80.00	N
3,4-Dimethylmethcathinone	3,4-DMMC	N	N	60.00	N	N
3,4-Methylenedioxybuphedrone (Butylone)	β-keto-MBDB	15.00	N	16.00	60.00	N
4-Ethylethcathinone	4-EEC	60.00	N	50.00	N	N
4-Fluoromethcathinone (Flephedrone)	4-FMC	N	N	N	N	N
4-Methoxymethcathinone (Methedrone)		40.00	N	50.00	100.00	N
4-Methylethcathinone	4-MEC	80.00	37.00	50.00	N	N
4-methylmethcathinone (Mephedrone)	4-MMC	80.00	N	25.00	N	N
Cathinone	khat	N	N	N	N	N
Diethylcathinone		N	N	N	N	N
Dimethylone	β-keto-MDDMA	100.00	N	30.00	N	N
Methcathinone (ephedrone)	Cat	N	N	35.00	N	N
N,N-Dimethylcathinone		N	N	50.00	N	N
n-Ethylcathinone		100.00	100.00	65.00	N	N
α-Methylamino-butyrophenone (buphedrone)	MABP	30.00	N	11.00	N	N
α-Methylamino-valerophenone (pentedrone)		30.00	N	40.00	N	N
β-Keto-ethylbenzodioxolylbutanamine (eutylone)	β-keto-EBDB	10.00	N	60.00	55.00	N
Piperazines						
1-Cyclohexyl-4-(1,2-diphenylethyl)-piperazine, dihydrochloride	MT-45	N	N	N	N	N
1-((3-Trifluoromethyl)phenyl)piperazine	1-(3-TFMPP)	7.00	N	0.70	N	N
1-((4-Trifluoromethyl)phenyl)piperazine	1-(4-TFMPP)	90.00	N	N	N	N
1,4-Dibenzylpiperazine	DBZP	N	80.00	N	N	N
1-Benzylpiperazine	BZP	25.00	N	30.00	N	N
Chlorophenylpiperazine	mCPP	7.00	N	1.50	N	N

(*continued*)

Table I Continued

Drug	Acronym	CEDIA® Amphetamine/ Ecstasy	Syva® EMIT® II Plus Amphetamine	LIN-ZHI Methamphetamine	Microgenics DRI® MDMA	Microgenics DRI® PCP
Substituted amines						
1-(8-Bromobenzodifuran-4-yl)-2-aminopropane (bromodragonfly)	BDF	N	0.80	N	N	N
2-Aminoindane	2-AI	100.00	N	N	N	N
3,4,5-Trimethoxyamphetamine	TMA	N	40.00	N	N	N
4-Chloro-α-ethylphenethylamine (AEPCA)	4-CAB	5.00	9.00	90.00	20.00	N
4-Fluoromethamphetamine	4-FMA	1.50	2.50	1.50	11.00	N
4-Methylamphetamine	4-MA	9.00	7.00	15.00	100.00	N
4-Methylthioamphetamine	4-MTA	2.00	5.00	20.00	2.00	N
5-(2-Aminopropyl)-2,3-dihydro-1*H*-indene (IAP)	5-APDI	3.00	3.00	4.00	20.00	N
5-(2-Aminopropyl)-2,3-dihydrobenzofuran (3-desoxy-MDA)	5-APDB	1.00	0.20	25.00	0.20	N
5-(2-Aminopropyl)benzofuran (benzo fury)	5-APB	2.00	2.00	10.00	2.00	N
5-(2-Aminopropyl)indole(5-API)	5-IT	10.00	30.00	12.50	N	N
5,6-Methylenedioxy-2-aminoindane	MDAI	75.00	40.00	N	N	N
5-Iodo-2-aminoindan	5-IAI	40.00	4.00	N	N	N
6-(2-aminopropyl)benzofuran	6-APB	5.00	8.50	8.00	16.00	N
Escaline		N	N	N	N	N
Mescaline		N	N	N	N	N
Methiopropamine	MPA	4.00	0.90	1.00	N	N
Methylenedioxyethylamphetamine	MDEA	0.25	5.00	13.00	0.40	N
Methylhexanamine (dimethylamylamine)	DMAA	50.00	5.00	N	N	N
p-Fluoroamphetamine	4-FA	15.00	2.95	50.00	37.50	N
p-Methoxyamphetamine	PMA	4.00	7.00	35.00	5.00	N
p-Methoxymethamphetamine	PMMA	0.90	10.00	1.00	2.00	N
PCP analogs						
2-Diphenylmethylpiperidine	2-DPMP	N	N	N	N	21.00
4-Methoxyphencyclidine	4-MeO-PCP	N	N	N	N	1.50
Benzothiophenylcyclohexylpiperidine (benocyclidine)	BCP	N	N	N	N	0.40
Methoxetamine	MXE	N	N	N	N	90.00
Other						
(−)-2-B-carbomethoxy-3-B-14-fluorophenyl tropane (WIN 35428)	CFT	N	N	N	N	N

N, negative at 100 μg/mL.
Concentrations where a positive response was found in μg/mL for each assay.

Designer drugs can be categorized into subgroups: 2,5-dimethoxyamphetamines, 2C (2,5-dimethoxyphenethylamines), β-keto amphetamines, substituted amphetamines, piperazines, α-pyrrolidinopropiophenones, tryptamines and PCP analogs. Previous studies have evaluated the cross-reactivity potential of many designer drugs using radioimmunoassay (RIA) (30), CEDIA (31), EMIT (25, 27, 29, 31), enzyme-linked immunosorbent assay (ELISA) (32–33), KIMS (25, 26, 29, 34), FPIA (35) and on-site drugs of abuse urinary screening tests (36). It has been reported that some AMP-like drugs have limited or no cross-reactivity with commercial immunoassays (8, 10, 29, 32). Even the most popular bath salts, mephedrone and MDPV, showed little to no cross-reactivity in one particular study (31). There are a number of factors that could affect the degree of cross-reactivity for a particular immunoassay kit. One study demonstrated higher sensitivity toward AMP analogs when calibration was accomplished with d-METH rather than d-AMP (34). Structure modifications, to include substitution on the amine nitrogen, stereochemistry, groups on the aromatic ring and size and number of substituents can affect cross-reactivity presumably by altering the antigenic profile and therefore the antibody's binding affinity (30, 35).

This study reports the cross-reactivities of 94 designer drugs with five commercially available immunoassay reagent kits. All kits evaluated in this study use monoclonal antibodies that are intended to increase specificity to their target analytes. Additionally, the results of this study may help determine those concentrations for which the designer drugs analyzed might be expected to cause a positive result for each of the assays evaluated.

Materials and methods

Immunoassay and sample preparation

A Roche/Hitachi Modular P automated screening instrument (Indianapolis, IN) was used to screen urine samples. The kits used were Siemens/Syva® EMIT®II Plus Amphetamines assay (Newark, DE), Microgenics DRI® Ecstasy and Phencyclidine enzyme assays (Fremont, CA), Lin-Zhi Methamphetamine enzyme immunoassay (Sunnyvale, CA) and CEDIA® DAU Amphetamine/Ecstasy Assay (Fremont, CA). The kits were calibrated on the Modular P analyzer using d-METH spiked at 0.50 μg/mL (for Syva® and CEDIA® AMP kits), d-METH spiked at 0.25 μg/mL (for Lin-Zhi METH kit), MDMA spiked at 0.50 μg/mL (for Microgenics DRI® Ecstasy kit) and PCP spiked at 0.025 μg/mL (for Microgenics DRI® PCP kit) with certified reference standards purchased from Cerilliant (Round Rock, TX) in certified drug-free urine. Negative (75% cutoff concentration) and positive (125% cutoff concentration) controls were included in the initial calibration. All immunoassays were performed in accordance with the manufacturers' instructions, except for the Lin-Zhi METH assay which was calibrated with a 0.25-μg/mL cutoff rather than 0.50 μg/mL. The 94 drugs chosen for this study were purchased from either Cayman Chemical (Ann Arbor, MI) or Cerilliant. Samples were prepared at 100 μg/mL in certified drug-free urine and tested using each assay. Those that were negative required no further testing. Samples that produced a positive result with one or more of the assays were subject to additional testing at decreasing concentrations until the spiked sample produced a negative response.

Drug	Acronym	R1	R2	R3	R4	R5	R6	R7
2,5- Dimethoxyamphetamines								
2,5-dimethoxy-4-ethylamphetamine	DOEt	CH_2CH_3						
2,5-dimethoxy-4-methylamphetamine	DOM	CH_3						
2,5-dimethoxyamphetamine	DMA	H						
4-bromo-2,5-dimethoxyamphetamine	DOB	Br						
4-chloro-2,5-dimethoxyamphetamine	DOC	Cl						
4-iodo-2,5-dimethoxyamphetamine	DOI	I						
2C Series								
25-I-NBOMe	25-I-NBOMe	I	CH_2-C_6H_4-O-CH_3					
2C-C-NBOMe	25C-NBOMe	Cl	CH_2-C_6H_4-O-CH_3					
4-bromo-2,5-dimethoxy-*N*-[(2-methoxyphenyl)methyl]-benzeneethanamine	25B-NBOMe	Br	CH_2-C_6H_4-O-CH_3					
4-bromo-2,5-dimethoxy-β-phenethyamine	2C-B	Br	H					
4-chloro-2,5-dimethoxy-β-phenethylamine	2C-C	Cl	H					
4-ethyl-2,5-dimethoxy-β-phenethylamine	2C-E	CH_2CH_3	H					
4-ethylthio-2,5-dimethoxy-β-phenethylamine	2C-T-2	SCH_2CH_3	H					
4-iodo-2,5-dimethoxy-β-phenethylamine	2C-I	I	H					
4-isopropylthio-2,5-dimethoxy-β-phenethylamine	2C-T-4	$SCH(CH_3)_2$	H					
4-methylthio-2,5-dimethoxy-β-phenethylamine	2C-T	SCH_3	H					
4-*n*-propylthio-2,5-dimethoxy-β-phenethylamine	2C-T-7	$SCH_2CH_2CH_3$	H					
Tryptamines								
α-methyltryptamine	AMT	H	CH_3	H	H	H		
4-hydroxy-diethyl-tryptamine	4-OH-DET	H	H	CH_2CH_3	CH_2CH_3	OH		
5-methoxy-*N*-methyl-*N*-isopropyltryptamine	5-Meo-MiPT	$O-CH_3$	H	CH_3	$CH(CH_3)_2$	H		
N,N-diallyl-5-methoxytryptamine	5-methoxy-DALT	$O-CH_3$	H	$CH_2CH=CH_2$	$CH_2CH=CH_2$	H		
N,N-diisopropyl-5-methoxytryptamine	5-MeO-DiPT	$O-CH_3$	H	$CH(CH_3)_2$	$CH(CH_3)_2$	H		
N,N-diisopropyltryptamine	DiPT	H	H	$CH(CH_3)_2$	$CH(CH_3)_2$	H		
N,N-dimethyl-5-methoxytryptamine	5-MeO-DMT	$O-CH_3$	H	CH_3	CH_3	H		
N,N-dimethyltryptamine	DMT	H	H	CH_3	CH_3	H		
N,N-dipropyltryptamine	DPT	H	H	$CH_2CH_2CH_3$	$CH_2CH_2CH_3$	H		
α-methyl-5-methoxytryptamine	5-MeO-AMT	$O-CH_3$	CH_3	H	H	H		
α-pyrrolidinopropiophenone								
2- naphthylpyrovalerone (Naphyrone)			-C_4H_4-	$CH_2CH_2CH_3$				
3,4-methylendioxy-α-pyrrolidinobutiophenone	MDPBP		-O-CH_2-O-	CH_2CH_3				
3,4-methylenedioxypyrovalerone	MDPV		-O-CH_2-O-	$CH_2CH_2CH_3$				
3,4-methylenedioxy-α-pyrrolidinopropiophenone	MDPPP		-O-CH_2-O-	CH_3				
4-methyl-α-pyrrolidinohexanophenone	MPHP	H	CH_3	$CH_2CH_2CH_2CH_3$				
4-methoxy-α-pyrrolidinopropiophenone	MOPPP	H	$O-CH_3$	CH_3				
4-methyl-α-pyrrolidinobutiophenone	4-MPBP	H	CH_3	CH_2CH_3				
pyrovalerone		H	CH_3	$CH_2CH_2CH_3$				
α-pyrrolidinobutiophenone	α-PBP	H	H	CH_2CH_3				
α-pyrrolidinopentiophenone	α-PVP	H	H	$CH_2CH_2CH_3$				
α-pyrrolidinopropiophenone	α-PPP	H	H	CH_3				
Beta-keto amphetamines								
1-(–,3-benzodioxol-5-yl)-2-(methylamino)-1-pentanone (Pentylone)			-O-CH_2-O-	$CH_2CH_2CH_3$	CH_3	H		
2-(benzylamino)-1-(p-tolyl)propan-1-one (Benzedrone)	4-MBC	H	CH_3	CH_3	CH_2-C_6H_5	H		
3,4-methylenedioxy-*N*-ethylcathinone (Ethylone)	β-keto-MDEA		-O-CH_2-O-	CH_3	CH_2CH_3	H		
3,4-methylenedioxy-*N*-methylcathinone (Methylone)	β-keto-MDMA		-O-CH_2-O-	CH_3	CH_3	H		
3,4-dimethylmethcathinone	3,4-DMMC	CH_3	CH_3	CH_3	CH_3	H		
3,4-methylenedioxybuphedrone (Butylone)	β-keto-MBDB		-O-CH_2-O-	CH_2CH_3	CH_3	H		
4-ethylethcathinone	4-EEC	H	CH_2CH_3	CH_3	CH_2CH_3	H		
4-fluoromethcathinone (Flephedrone)	4-FMC	H	F	CH_3	CH_3	H		
4-methoxymethcathinone (Methedrone)		H	$O-CH_3$	CH_3	CH_3	H		
4-methylethcathinone	4-MEC	H	CH_3	CH_3	CH_2CH_3	H		
4-methylmethcathinone (Mephedrone)	4-MMC	H	CH_3	CH_3	CH_3	H		
Cathinone	khat	H	H	CH_3	H	H		
Diethylcathinone		H	H	CH_3	CH_2CH_3	CH_2CH_3		
dimethylone	β-keto-MDDMA		-O-CH_2-O-	CH_3	CH_3	CH_3		
Methcathinone (ephedrone)	Cat	H	H	CH_3	CH_3	H		
N,N-Dimethylcathinone		H	H	CH_3	CH_3	CH_3		
n-Ethylcathinone		H	H	CH_3	CH_2CH_3	H		
α-methylamino-butyrophenone (buphedrone)	MABP	H	H	CH_2CH_3	CH_3	H		
α-methylamino-valerophenone (pentedrone)		H	H	$CH_2CH_2CH_3$	CH_3	H		
β-keto-ethylbenzodioxolylbutanamine (eutylone)	β-keto-EBDB		-O-CH_2-O-	CH_2CH_3	CH_2CH_3	H		
Piperazines								
1-cyclohexyl-4-(1,2-diphenylethyl)-piperazine, dihydrochloride	MT-45	C_6H_{11}	CH-C_6H_5,-CH_2-C_6H_5					
1-((3-trifluoromethyl)phenyl)piperazine	1-(3-TFMPP)	H	C_6H_4-CF_3					
1-((4-trifluoromethyl)phenyl)piperazine	1-(4-TFMPP)	H	C_6H_4-CF_3					
1,4-dibenzylpiperazine	DBZP	CH_2-C_6H_5	CH_2-C_6H_5					
1-benzylpiperazine	BZP	H	CH_2-C_6H_5					
chlorophenylpiperazine	mCPP	H	C_6H_4-Cl					
Substituted Amines								
1-(8-bromobenzodifuran-4-yl)-2-aminopropane (bromodragonfly)	BDF		-O-CH=CH-	Br	-O-CH=CH-		CH_3	H
2-aminoindane	2-AI	H	H	H		-CH_2-		H
3,4,5-trimethoxyamphetamine	TMA	H	$O-CH_3$	$O-CH_3$	$O-CH_3$	H	CH_3	H
4-chloro-α-ethylphenethylamine (AEPCA)	4-CAB	H	H	Cl	H	H	CH_2CH_3	H
4-Fluoromethamphetamine	4-FMA	H	H	F	H	H	CH_3	CH_3
4-Methylamphetamine	4-MA	H	H	$O-CH_3$	H	H	CH_3	H
4-methylthioamphetamine	4-MTA	H	H	$S-CH_3$	H	H	CH_3	H
5-(2-aminopropyl)-2,3-dihydro-1H-indene (IAP)	5-APDI	H	-CH_2-CH_2-CH_2-		H	H	CH_3	H
5-(2-aminopropyl)-2,3-dihydrobenzofuran (3-desoxy-MDA)	5-APDB	H	-CH_2-CH_2-O-		H	H	CH_3	H
5-(2-aminopropyl)benzofuran (benzo fury)	5-APB	H	-CH=CH-O-		H	H	CH_3	H
5-(2-aminopropyl)indole(5-API)	5-IT	H	-CH=CH-NH-		H	H	CH_3	H
5,6-methylenedioxy-2-aminoindane	MDAI	H	-O-CH_2-O-		H		-CH_2-	H
5-iodo-2-aminoindan	5-IAI	H	H	I	H		-CH_2-	H
6-(2-aminopropyl)benzofuran	6-APB	H	-O-CH=CH-		H	H	CH_3	H
escaline		H	$O-CH_3$	$O-CH_2CH_3$	-$O-CH_3$	H	H	H
mescaline		H	$O-CH_3$	$O-CH_3$	-$O-CH_3$	H	H	H
methylenedioxyethylamphetamine	MDEA	H	-O-CH_2-O-		H	H	CH_3	CH_2CH_3
p-fluoroamphetamine	4-FA	H	H	F	H	H	CH_3	H
p-methoxyamphetamine	PMA	H	H	$O-CH_3$	H	H	CH_3	H
p-methoxymethamphetamine	PMMA	H	H	$O-CH_3$	H	H	CH_3	CH_3

Figure 1. Base structures and substituents for designer drug classes.

Table II
Screened Negative at 100 μg/mL on All Five Assays

Structural class	Drug	Acronym
2C	25-I-NBOMe	
	2C-C-NBOMe	25C-NBOMe
	4-Bromo-2,5-dimethoxy-*N*-[(2-methoxyphenyl)methyl]-benzeneethanamine	25B-NBOMe
Beta-keto amphetamines	4-Fluoromethcathinone (Flephedrone)	4-FMC
	Cathinone	khat
	Diethylcathinone	
Piperazines	1-Cyclohexyl-4-(1,2-diphenylethyl)-piperazine, dihydrochloride	MT-45
Other	(−)-2-B-carbomethoxy-3-B-14-fluorophenyl tropane (WIN 35428)	CFT
Substituted amines	Escaline	
	Mescaline	
Tryptamines	4-Hydroxy-diethyl-tryptamine	4-OH-DET
	N,N-Diisopropyl-5-methoxytryptamine	5-MeO-DiPT
	N,N-Diisopropyltryptamine	DiPT
	N,N-Dimethyl-5-methoxytryptamine	5-MeO-DMT

Results and discussion

This study reports the cross-reactivity profiles for the five immunoassays tested. Table I groups each designer drug by structural class and presents the lowest concentrations in μg/mL where a 'positive' response was obtained. The concentrations reported in Table I were identified by screening samples spiked at various concentrations until a negative response was produced. The results for each assay are discussed below to include the percentage of positive results produced as well as the class of designer drugs that produced the result. Observed structural trends are reported with proposals of possible explanations for cross-reactivity.

CEDIA® DAU Amphetamine/Ecstasy assay

A positive response was observed for 57% of the 94 compounds when using the CEDIA® DAU Amphetamine/Ecstasy assay. The CEDIA® Amphetamine/Ecstasy kit was the most non-specific as it generated the highest number of positive responses for the immunoassay kits tested. Positive rates for this assay based on structural class are as follows: 2,5-dimethoxyamphetamines (100%), substituted amines (81%), piperazines (66%), β-keto amphetamines (65%), tryptamines (40%), 2C (36%) and α-pyrrolidinopropiophenones (35%) (see Table I).

All samples containing 2,5-dimethoxyamphetamines screened positive using the CEDIA® kit. Only 2C drugs with smaller substituents produced positive results with this assay. The 2C drugs with larger substituents (see Figure 1); for example, 2C-E, 2C-T-2 and 2C-T-4 did not elicit a positive response when using the CEDIA® kit and 2C-T-7 only gave a positive response at the highest concentration tested, 100 μg/mL. It is suspected that the bulky substitutions at the 4-position of the phenethylamine base structure may interfere with antibody binding. Compounds of the α-pyrrolidinopropiophenones class that were more structurally similar to MDMA also produced positive results for the CEDIA® assay. Substituted amines produced positive results using this assay, the exception were those compounds with substitutions on R2 of the aromatic ring (see Figure 1).

Petrie *et al.* tested 42 designer AMP compounds, including 2C, piperazines, 4-substituted amphetamines, 2,5-methoxyamphetamines and β-keto amphetamines by CEDIA® and found that compounds cross-reacted at the concentration of 5.0 μg/mL for all of the compounds that are also presented here (31). However, in this study, the same compounds generally cross-reacted at concentrations >5.0 μg/mL. Additionally, all of the 2C-T drugs tested produced a negative response at 5.0 μg/mL and those tested by Petrie *et al.* did cross-react. There is not enough information in the article to deduce why these differences occurred.

Siemens/Syva® EMIT® II Plus Amphetamines assay

Of the 94 designer drugs tested, 43% produced a positive response on the Syva® EMIT II Plus Amphetamine Assay. All of the 2,5-dimethoxyamphetamines, 86% of substituted amines, 72% of 2C drugs, 30% of tryptamines, 20% of β-keto amphetamines and 16% of piperazines screened positive on this assay (see Table I).

Substituted amines produced more positive results on the Syva® AMP assay than β-keto amphetamines. The keto-group present in β-keto amphetamine compounds appears to prohibit successful competition for antibody binding sites on the Syva® assay possibly due to delocalization of the nitrogen electrons to the carbonyl group (see Figure 1).

It is important to note that in this study calibration of AMP kits was completed with d-METH; in the published literature, d-AMP is often used as a calibrator. Vorce *et al.* found that DMAA gave a positive response at 3.1 μg/mL when calibration was completed with 0.50 μg/mL d-AMP, whereas the presented study gave a positive response at 5.0 μg/mL when calibrated at the same concentration with d-METH on the Syva® EMIT® II Plus assay (25). In another study, Dickson *et al.* gave comparable negative results for BZP, mCPP and TFMPP when calibrated with d-AMP rather than d-METH for Syva® EMIT® II Plus (29). When comparing cross-reactivity results, differences may be present if the calibration drug is different depending on the calibrant's degree of reactivity. Lekskulchai and Mokkhavesa have also shown that there are differences in the sensitivity depending on the drug used for calibration (34).

Lin-Zhi Methamphetamine enzyme immunoassay

The manufacturer's instructions were modified for the Lin-Zhi Methamphetamine immunoassay for a cutoff concentration of 0.25 μg/mL from the instructed 0.50 μg/mL. Positive responses were produced for 39% of the compounds on the Lin-Zhi

Drug	Acronym
α-pyrrolidinopropiophenone	
4-methyl-α-pyrrolidinopropiophenone	MPPP
α,α-diphenyl-2R-pyrrolidinemethanol	D2PM
α-pyrrolidinopentiothiophenone	α-PVT
Substituted amines	
Methiopropamine	MPA
methylhexanamine (dimethylamylamine)	DMAA
PCP analogs	
2-Diphenylmethylpiperidine	2-DPMP
4-methoxyphencyclidine	4-MeO-PCP
benzothiophenylcyclohexylpiperidine (benocyclidine)	BCP
Methoxetamine	MXE
Other	
(-)-2-B-carbomethoxy-3-B-14-fluorophenyl tropane (WIN 35428)	CFT

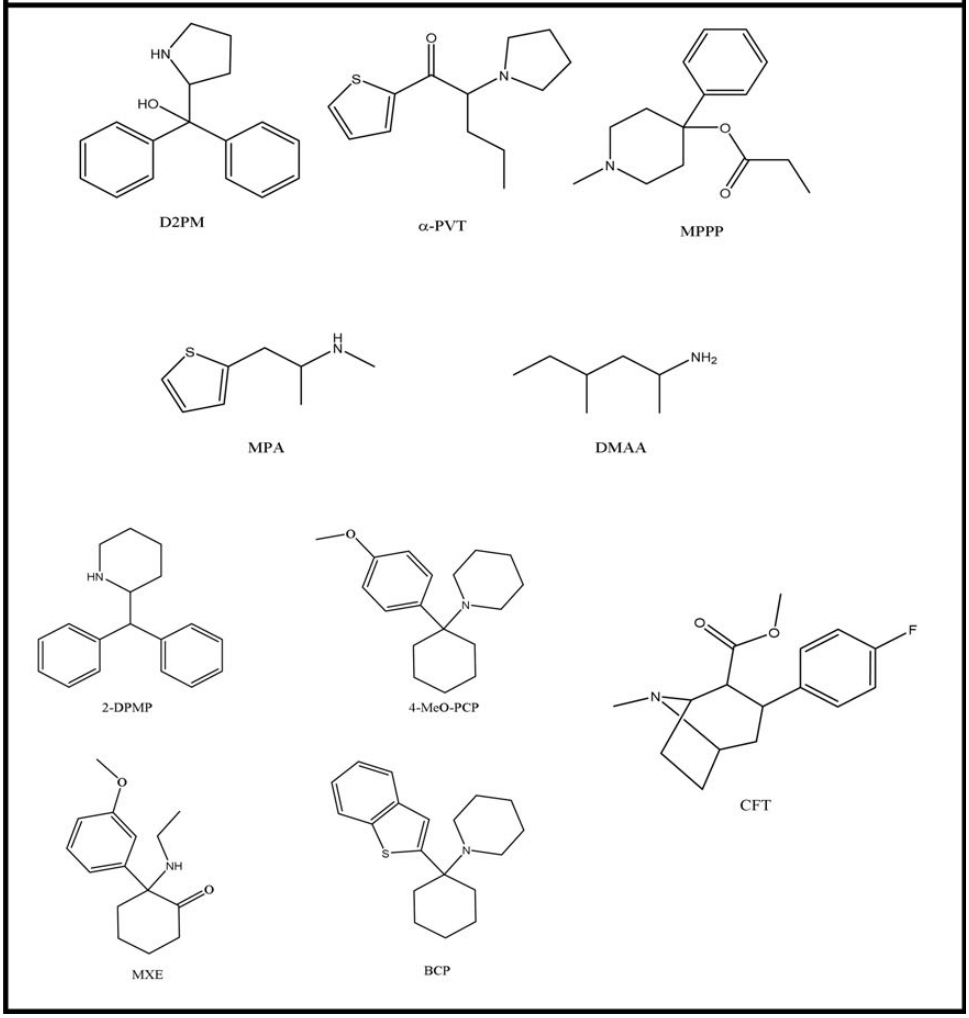

Figure 2. Structures of miscellaneous designer drugs.

Methamphetamine enzyme immunoassay. This assay gave the fewest positive responses of the three AMP immunoassay kits tested. β-keto amphetamines (75%), substituted amines (63%), piperazines (50%), α-pyrrolidinopropiophenones (28%) and tryptamines (10%) gave positive responses with this assay (see Table I).

Only one tryptamine, DMT, gave a positive response for this assay. DMT has a very simple tryptamine structure with only

dimethyl substitutions to the amine, therefore allowing it to cross-react with the assay targeted for methamphetamine (see Figure 1). Of the α-pyrrolidinopropiophenones tested, 28% produced positive responses for the Lin-Zhi METH assay.

Microgenics DRI® Ecstasy enzyme assay

Of the 94 designer drugs tested, 19% gave positive responses on the Microgenics DRI® MDMA kit. Those that did give positive responses consisted of 54% of substituted amines, 25% of β-keto amphetamines and 7.1% of α -pyrrolidinopropiophenones tested (see Table I).

The β-keto amphetamines that produced a positive response had the following: either a methoxy or methylenedioxy group attached to the aromatic ring, a mono-substituted amine and either a methyl or ethyl group on the α-carbon (see Figure 1). Similarly, substituted amines that produced a positive result required an aromatic ring with one to two substituents and a methyl or ethyl group on the α-carbon and no additional nitrogens other than the one in the amine group to produce a positive result. 3,4-Methylenedioxy-α-pyrrolidinopropiophenone (MDPPP), the one α-pyrrolidinopropiophenone that gave a positive response, has a methylenedioxy group attached to the aromatic ring and a methyl group attached to the α-carbon, making it most structurally similar to MDMA.

Microgenics DRI® Phencyclidine enzyme assay

The Microgenics DRI® PCP immunoassay produced positive results for 20% of the designer compounds tested at 100 μg/mL. The positive rate for this assay was 100% for PCP analogs and α-pyrrolidinopropiophenones and 10% for tryptamines.

This was expected since the compounds tested had more structural similarities to AMP. It is important to note that, in most of the published studies for designer drug immunoassay cross-reactivity, the papers are targeted to AMP and MDMA immunoassays. In this study, a PCP immunoassay was also included. Experience from casework has indicated that some of these drugs have produced false-positive screening results with PCP immunoassays. This observation was validated as all of the α-pyrrolidinopropiophenones and PCP analogs as well as one of the 10 tryptamines tested gave a positive screening result for the PCP assay (see Table I). Additionally, all of the PCP analogs, 42% of the α-pyrrolidinopropiophenones and the sole tryptamine, 5-methoxy-DALT, screened negative for all assays tested except for this PCP enzyme assay. Generally, the less substituted α-pyrrolidinopropiophenones (see Figure 1), such as α-PVP, α-PVT and α-PPP, gave positive responses at lower concentrations than those that were more substituted.

Summary

Five commercially available immunoassay screening kits were tested to determine the concentrations at which designer drugs would cross-react for each kit. Of the 94 designer drugs tested in this study, 80 gave a positive result on at least one of the five commercial immunoassays evaluated. The following positive rates were obtained: Microgenics DRI® Ecstasy enzyme assay (19%), Microgenics DRI® Phencyclidine enzyme assay (20%), Lin-Zhi Methamphetamine enzyme immunoassay (39%), Siemens/Syva® EMIT®II Plus Amphetamines assay (43%) and CEDIA® DAU Amphetamine/Ecstasy assay (57%).

Fourteen designer drugs generated a negative result on all five assays (see Table II). Of the compounds that screened negative on all kits evaluated, most had either large structural substituents connected to the amine nitrogen or groups added to the aromatic ring (see Figures 1 and 2). For example, the 2C drugs that screened negative for all assays had methyl-methoxyphenyl groups connected to the amine nitrogen. These large additions may sterically hinder the binding antibody thereby causing a negative response.

Alternatively, none of the designer drugs tested produced a positive result on all five assays. Only 17 designer drugs, primarily substituted amines (82%) and β-keto amphetamines (17%), produced a positive result for all three AMP screening kits. The results from this study will be useful for laboratories to determine if they can detect and presumably classify designer drugs. Further work would be beneficial in determining the reasoning for designer drug cross-reactivity and the degree of reactivity based on structural modifications.

Acknowledgments

The authors would like to acknowledge the help of Jillian Neifeld in this project.

Funding

This work was funded in part by the American Registry of Pathology.

Conflict of Interest statement

The opinions or assertions presented hereafter are the private views of the authors and should not be construed as official or as reflecting the views of the Department of Defense, its branches, the US Army Medical Research and Materiel Command or the Armed Forces Medical Examiner System.

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