

Colorimetric detection and chromatographic analyses of designer drugs in biological materials: a comprehensive review

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Abstract A number of analogues of phenethylamine and tryptamine, which are prepared by modification of the chemical structures, are being developed for circulation on the black market. Often called “designer drugs,” they are abused in many countries, and cause serious social problems in many parts of the world. Acute deaths have been reported after overdoses of designer drugs. Various methods are required for screening and routine analysis of designer drugs in biological materials for forensic and clinical purposes. Many sample preparation and chromatographic methods for analysis of these drugs in biological materials and seized items have been published. This review presents various colorimetric detections, gas chromatographic (GC)–mass spectrometric, and liquid chromatographic (LC)–mass spectrometric methods proposed for designer drug analyses. Basic information on extractions, derivatizations, GC columns, LC columns, detection limits, and linear ranges is also summarized.

Keywords Designer drug · GC–MS · LC–MS(–MS) · Phenethylamines · Tryptamines · Piperazines

Abbreviations

AA	Acetic anhydride
ACN	Acetonitrile
<i>N</i> -Ac-2'-FMC	<i>N</i> -Acetyl-2'-fluoromethacathinone
<i>N</i> -Ac-3'-FMC	<i>N</i> -Acetyl-3'-fluoromethacathinone
<i>N</i> -Ac-4'-FMC	<i>N</i> -Acetyl-4'-fluoromethacathinone
4-AcO-DIPT	4-Acetoxy- <i>N,N</i> -diisopropyltryptamine
ALEPH	2,5-Dimethoxy-4-methylthioamphetamine
ALEPH-2	2,5-Dimethoxy-4-ethylthioamphetamine
ALEPH-5	2,5-Dimethoxy-4-cyclohexylthioamphetamine
ALEPH-7	2,5-Dimethoxy-4-(<i>n</i> -propylthioamphetamine
AMT	α -Methyltryptamine
AP	Amphetamine
BDB	2-Amino-1-(3,4-methylenedioxyphenyl)butane
4-Brom-2,5-dimethoxy-BZP	4-Bromo-2,5-dimethoxybenzylpiperazine
2-Brom-4,5-dimethoxy-BZP	2-Bromo-4,5-dimethoxybenzylpiperazine
5-Brom-2,4-dimethoxy-BZP	5-Bromo-2,4-dimethoxybenzylpiperazine
BZP	<i>N</i> -Benzylpiperazine
2C-B	2,5-Dimethoxy-4-bromophenethylamine
2C-B-DragonFLY	1-(8-Bromobenzo[1,2- <i>b</i> ;4,5- <i>b'</i>]difuran-4-yl)-2-aminoethane

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2C-B-FLY	1-(8-Bromo-2,3,6,7-tetrahydrobenzo[1,2-b;4,5-b']difuran-4-yl)-2-aminoethane	DOM	2,5-Dimethoxy-4-methyl-amphetamine
2C-C	2,5-Dimethoxy-4-chlorophenethylamine	DOPR	2,5-Dimethoxy-4-propylamphetamine
2C-D	2,5-Dimethoxy-4-methylphenethylamine	DPT	<i>N,N</i> -Dipropyltryptamine
CE	Capillary electrophoresis	EAMP	Ethylamphetamine
2C-E	2,5-Dimethoxy-4-ethylphenethylamine	ECF	Ethyl chloroformate
2C-H	2,5-Dimethoxyphenethylamine	EP	Ephedrine
2C-I	2,5-Dimethoxy-4-iodophenethylamine	2'-FMC	2'-Fluoromethacathinone
2C-N	2,5-Dimethoxy-4-nitrophenethylamine	3'-FMC	3'-Fluoromethacathinone
2-CPP	1-(2-Chlorophenyl)piperazine	4'-FMC	4'-Fluoromethacathinone
3-CPP	1-(3-Chlorophenyl)piperazine, mCPP	4-FMP	4-Fluoro- α -methylphenethylamine
4-CPP	1-(4-Chlorophenyl)piperazine	GC	Gas chromatography
2C series	4-Substituted 2,5-dimethoxyphenethylamines	HFBA	Heptafluorobutyric anhydride
2C-T	2,5-Dimethoxy-4-methylthiophenethylamine	HMMA	4-Hydroxy-3-methoxymethamphetamine
2C-T-2	2,5-Dimethoxy-4-ethylthiophenethylamine	HMMA-Glu	4-Hydroxy-3-methoxymethamphetamine-glucuronide
2C-T-4	2,5-Dimethoxy-4-isopropylthiophenethylamine	HMMA-Sul	4-Hydroxy-3-methoxymethamphetamine-sulfate
2C-T-5	2,5-Dimethoxy-4-cyclohexylthiophenethylamine	5-HT	5-Hydroxytryptamine, serotonin
2C-T-7	2,5-Dimethoxy-4-(<i>n</i>)-propylthiophenethylamine	IS	Internal standard
DIPT	<i>N,N</i> -Diisopropyltryptamine	JWH-018	1-Naphthalenyl(1-pentyl-1 <i>H</i> -indol-3-yl)methanone
2,5-DMA	2,5-Dimethoxyamphetamine	LC	Liquid chromatography
DMT	<i>N,N</i> -Dimethyltryptamine	LLE	Liquid–liquid extraction
DOB	2,5-Dimethoxy-4-bromoamphetamine	LOD	Limit of detection
DOB-DragonFLY	1-(8-Bromobenzo[1,2-b;4,5-b']difuran-4-yl)-2-aminopropane	LOQ	Limit of quantitation
DOB-FLY	1-(8-Bromo-2,3,6,7-tetrahydrobenzo[1,2-b;4,5-b']difuran-4-yl)-2-aminopropane	LSD	Lysergic acid diethylamide
DOC	2,5-Dimethoxy-4-chloroamphetamine	MA	Methamphetamine
DOET	2,5-Dimethoxy-4-ethylamphetamine	MBDB	<i>N</i> -Methylbenzodioxolylbutanamine
DOI	2,5-Dimethoxy-4-iodoamphetamine	bk-MBDB	2-Methylamino-1-(3,4-methylenedioxyphenyl)-butan-1-one, butylone
		MBTFA	<i>N</i> -Methylbis-(trifluoroacetamide)
		MBZP	1-Benzyl-4-methyl-piperazine
		MDA	3,4-Methylenedioxyamphetamine
		MDBZP	1-(3,4-Methylenedioxybenzyl)-piperazine
		bk-MDDMA	2-Dimethylamino-1-(3,4-methylenedioxyphenyl)-propan-1-one
		MDEA	3,4-Methylenedioxy- <i>N</i> -ethylamphetamine

bk-MDEA	2-Ethylamino-1-(3,4-methylenedioxyphenyl)-propan-1-one, ethylone	MPBP	4'-Methyl- α -pyrrolidinobutyrophenone
MDMA	3,4-Methylenedioxymethamphetamine	MPHP	4'-Methyl- α -pyrrolidinohexanophenone
bk-MDMA	2-Methylamino-1-(3,4-methylenedioxyphenyl)-propan-1-one, methylone	2-MPP	1-(2-Methoxyphenyl)piperazine
MDPPP	<i>R,S</i> -3',4'-Methylenedioxy- α -pyrrolidinopropiophenone	3-MPP	1-(3-Methoxyphenyl)piperazine
MDPV	3,4-Methylenedioxy-pyrovalerone	4-MPP	1-(4-Methoxyphenyl)piperazine
ME	Methylephedrine	MPPP	<i>R,S</i> -4'-Methyl- α -pyrrolidinopropiophenone
<i>N</i> -Me-DOB-DragtonFLY	1-(8-Bromobenzo[1,2- <i>b</i> ;4,5- <i>b'</i>]difuran-4-yl)-2-methylaminopropane	MRM	Multiple reaction monitoring
<i>N</i> -Me-DOB-FLY	1-(8-Bromo-2,3,6,7-tetrahydrobenzo[1,2- <i>b</i> ;4,5- <i>b'</i>]difuran-4-yl)-2-methylaminopropane	MS	Mass spectrometry
<i>N</i> -Me-4-FMP	1-(4-Fluorophenyl)- <i>N</i> -methylpropan-2-amine	MSTFA	<i>N</i> -Methyl- <i>N</i> -trimethylsilyltrifluoroacetamide
5-MeO-AMT	5-Methoxy- α -methyltryptamine	4-MTA	4-Methylthioamphetamine
5-MeO-DALT	<i>N,N</i> -Diallyl-5-methoxytryptamine	4-OH-DIPT	4-Hydroxy- <i>N,N</i> -diisopropyltryptamine
5-MeO-DET	5-Methoxy- <i>N,N</i> -diethyltryptamine	4-OH-DMT	4-Hydroxy- <i>N,N</i> -dimethyltryptamine
5-MeO-DIPT	5-Methoxy- <i>N,N</i> -diisopropyltryptamine	<i>p</i> -OH-MA	4-Hydroxymethamphetamine
5-MeO-DMT	5-Methoxy- <i>N,N</i> -dimethyltryptamine	<i>p</i> -OH-MA-Glu	4-Hydroxymethamphetamine-glucuronide
5-MeO-DPT	5-Methoxy- <i>N,N</i> -di- <i>n</i> -propyltryptamine	<i>p</i> -OH-MA-Sul	4-Hydroxymethamphetamine-sulfate
5-MeO-EIPT	5-Methoxy- <i>N</i> -ethyl- <i>N</i> -isopropyltryptamine	<i>N</i> -OH-MDA	<i>N</i> -Hydroxy-3,4-methylenedioxyamphetamine
5-MeO-EPT	<i>N</i> -Ethyl-5-methoxy- <i>N</i> -propyltryptamine	<i>N</i> -OH-MDMA	<i>N</i> -Hydroxy-3,4-methylenedioxy-methamphetamine
MeOH	Methanol	3-OH-4-MeO-MA	3-Hydroxy-4-methoxymethamphetamine
5-MeO-MIPT	<i>N</i> -Isopropyl-5-methoxy- <i>N</i> -methyltryptamine	PCEEA	<i>N</i> -(1-Phenylcyclohexyl)-2-ethoxyethanamine
MeOPP	1-(4-Methoxyphenyl)piperazine	PCMEA	<i>N</i> -(1-Phenylcyclohexyl)-2-methoxyethanamine
MIPT	<i>N</i> -Methyl- <i>N</i> -isopropyltryptamine	PCMPA	<i>N</i> -(1-Phenylcyclohexyl)-3-methoxypropanamine
MMDA	3-Methoxy-4,5-methylenedioxyamphetamine	PCPR	<i>N</i> -(1-Phenylcyclohexyl)-propanamine
MOPPP	<i>R,S</i> -4'-Methoxy- α -pyrrolidinopropiophenone	PDMS	Polydimethylsiloxane
		PEA	β -Phenethylamine
		PFPA	Pentafluoropropionic anhydride
		PMA	<i>p</i> -Methoxyamphetamine
		PMEA	<i>p</i> -Methoxyethylamphetamine
		PMMA	<i>p</i> -Methoxymethamphetamine
		PPA	Phenylpropanolamine

PPP	<i>R,S</i> - α -Pyrrolidinopropiophenone
PVP	1-Phenyl-2-pyrrolidin-1-ylphentan-1-one, α -pyrrolidinovalophenone
SERT	Serotonin transporter
SIM	Selected ion monitoring
SPDE	Solid-phase dynamic extraction
SPE	Solid-phase extraction
SPME	Solid-phase microextraction
TFA	Trifluoroacetic acid
TFAA	Trifluoroacetic anhydride
TFMPP	1-(3-Trifluoromethyl)-piperazine
TFAP	<i>S</i> -(–)- <i>N</i> -(Trifluoroacetyl)propyl chloride
TLC	Thin-layer chromatography
TMA-1	3,4,5-Trimethoxyamphetamine
TMA-2	2,4,5-Trimethoxyamphetamine
TMA-3	2,3,4-Trimethoxyamphetamine
TMA-4	2,3,5-Trimethoxyamphetamine
TMA-5	2,3,6-Trimethoxyamphetamine
TMA-6	2,4,6-Trimethoxyamphetamine
TMF	3-Methylfentanyl
TMSCI	Trimethylsilylchloride
TOFMS	Time-of-flight mass spectrometry
TPC	Trifluoroacetyl-L-propylchloride
UPLC	Ultra performance liquid chromatography
UV	Ultraviolet

Introduction

All over the world, hallucinogens and/or stimulants have long been abused to experience their psychotic effects. Each country has its own laws controlling the sale, synthesis, supply, and use of these drugs, with the ultimate goal of exterminating drug abuse. However, the amount of confiscated drugs increases every year. In the 1980s, many fentanyl derivatives with molecular structures similar to that of heroin were available on the black market. More recently, chemical variants of phenethylamines and tryptamines have become available on the black market. These chemicals, obtained by modifying a side chain or a functional group of existing drugs, are not controlled by the law and have become known as “designer drugs.” The use of these drugs

has been increasing among young people, with “rave parties” in particular being characterized by their use. It is difficult to monitor such drugs by preexisting laws; their chemical structures are similar to but different from those of illegal drugs. Because their chemical structures are quite similar to those of the drugs being controlled, these drugs show psychoactive effects that are equal to or stronger than those of the original drugs. Therefore, the use of these drugs is very dangerous to human health.

The identification of these drugs by scientific methods is the first step to decrease and exterminate abuse of these emerging drugs. In this review, we present various types of detection techniques that have been published, including colorimetric, immunochemical, and chromatographic methods, although some reviews on similar subjects are available [1–5].

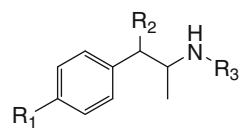
Drug groups and their chemical structures

Throughout history and up to the present day, various plants and mushrooms have been used in religious or cultural ceremonies. Over the years, scientists have suspected that these plants contained some bioactive compounds that could be used to treat some types of diseases. After extraction and purification, scientists unexpectedly found stimulant(s) or hallucinogen(s) (such as morphine, cocaine, or LSD), instead of medicinal compounds. Later, some researchers focused their attention on finding and synthesizing more active drugs.

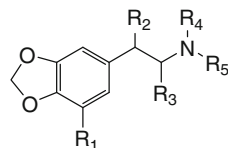
Since the introduction of fentanyl derivatives into the black market, many types of designer drugs have become available. The drug groups and their structures are shown in Figs. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11. The PEA derivatives usually imitate amphetamines in their effects. Many psychoactive PEA derivatives, which act as psychedelics and/or hallucinogens were listed in a book entitled *Pihkal: a chemical love story* [6]. Some hallucinogenic compounds, such as LSD, ibogaine, and yohimbine, contain the tryptamine skeleton in their structures. Thorough investigation of dozens of tryptamine compounds was also published by Shulgin and Shulgin in *Tihkal: the continuation* [7]. PEA, tryptamine, piperidine, and piperazine derivatives can be synthesized by modifying MDA, lysergic acid, and phenethylamine. The majority of the drugs have hallucinogenic effects. Recently, steroid designer drugs have been detected in urine samples obtained from athletes [8–13].

Bioactive mechanisms

MA causes the norepinephrine, dopamine, and 5-HT transporters to reverse their direction of flows [14–16].

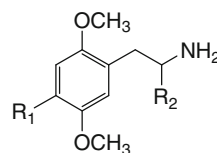
Fig. 1 Structures of phenethylamine designer drugs

Name	R ₁	R ₂	R ₃
AP	H	H	H
MA	H	H	CH ₃
PMA	OCH ₃	H	H
4-MTA	SCH ₃	H	H
PMMA	OCH ₃	H	CH ₃
PMEA	OCH ₃	H	C ₂ H ₅
4-FMP	F	H	H
<i>N</i> -Me-4-FMP	F	H	CH ₃
<i>p</i> -OH-MA	OH	H	CH ₃
Mephedrone	CH ₃	=O	CH ₃

Fig. 2 Structures of methylenedioxyphenethylamine designer drugs

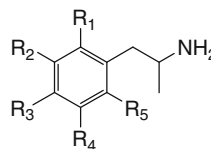
Name	R ₁	R ₂	R ₃	R ₄	R ₅
MDA	H	H	CH ₃	H	H
MDMA	H	H	CH ₃	CH ₃	H
MDEA	H	H	CH ₃	C ₂ H ₅	H
MBDB	H	H	C ₂ H ₅	CH ₃	H
MMDA	OCH ₃	H	CH ₃	H	H
BDB	H	H	C ₂ H ₅	H	H
<i>N</i> -OH-MDA	H	H	CH ₃	OH	H
<i>N</i> -OH-MDMA	H	H	CH ₃	OH	CH ₃
bk-MDMA	H	=O	CH ₃	CH ₃	H
bk-MBDB	H	=O	C ₂ H ₅	CH ₃	H
bk-MDEA	H	=O	CH ₃	C ₂ H ₅	H
bk-MDDMA	H	=O	CH ₃	CH ₃	CH ₃

Fig. 3 Structures of 1,4-dimethoxyphenethylamine designer drugs



Name	R ₁	R ₂	Name	R ₁	R ₂
2C-B	Br	H	2,5-DMA	H	CH ₃
2C-C	Cl	H	DOM	CH ₃	CH ₃
2C-D	CH ₃	H	DOET	C ₂ H ₅	CH ₃
2C-E	C ₂ H ₅	H	DOPR	C ₃ H ₇	CH ₃
2C-H	H	H	DOB	Br	CH ₃
2C-I	I	H	DOC	Cl	CH ₃
2C-N	NO ₂	H	DOI	I	CH ₃
2C-T	SCH ₃	H	ALEPH	SCH ₃	CH ₃
2C-T-2	SC ₂ H ₅	H	ALEPH-2	SC ₂ H ₅	CH ₃
2C-T-4	<i>S-iso</i> -propyl	H	ALEPH-5	<i>S-cyclohexyl</i>	CH ₃
2C-T-5	<i>S-cyclohexyl</i>	H	ALEPH-7	<i>S-iso</i> -propyl	CH ₃
2C-T-7	<i>S-n</i> -propyl	H			

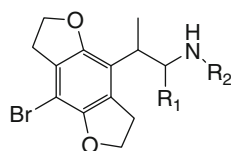
Fig. 4 Structures of ring substituted phenethylamine designer drugs



Name	R ₁	R ₂	R ₃	R ₄	R ₅
TMA-1	H	CH ₃ O	CH ₃ O	CH ₃ O	H
TMA-2	CH ₃ O	H	CH ₃ O	CH ₃ O	H
TMA-3	CH ₃ O	CH ₃ O	CH ₃ O	H	H
TMA-4	CH ₃ O	CH ₃ O	H	CH ₃ O	H
TMA-5	CH ₃ O	CH ₃ O	H	H	CH ₃ O
TMA-6	CH ₃ O	H	CH ₃ O	H	CH ₃ O

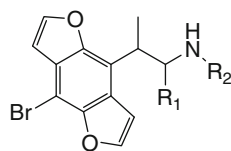
This inversion leads to release of the neurotransmitters from the vesicles to the cytoplasm and from the cytoplasm to the synapse, causing stimulation of the postsynaptic receptors. MA also indirectly prevents the reuptake of these neurotransmitters, causing them to remain in the synaptic cleft for a prolonged period. In addition, MA is a potent neurotoxin that causes dopaminergic-neuron degeneration. High doses of MA produce declines in several markers of brain dopamine and 5-HT neurons; dopamine and 5-HT

concentrations, their uptake sites, and activities of tyrosine hydroxylase and tryptophan hydroxylase are all reduced after the administration of MA. Dopamine has been proposed as playing a role in MA-induced neurotoxicity, because experiments that reduce dopamine production or block the release of dopamine led to a decrease in the toxic effects of MA administration. Ring-substituted phenethylamines, such as MDMA (Fig. 2) and 4-substituted 2,5-dimethoxyphenethylamines (2C series; Fig. 3) act as



Name	R ₁	R ₂
2C-B-FLY	H	H
DOB-FLY	CH ₃	H
N-Me-DOB-FLY	CH ₃	CH ₃

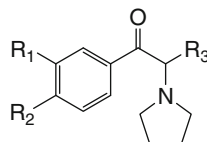
Fig. 5 Structures of (tetrahydrobenzodifuranyl)aminoalkane designer drugs



Name	R ₁	R ₂
2C-B-DragonFLY	H	H
DOB-DragonFLY	CH ₃	H
N-Me-DOB-DragonFLY	CH ₃	CH ₃

Fig. 6 Structures of (benzodifuranyl)aminoalkane designer drugs

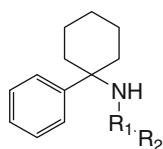
Fig. 7 Structures of pyrrolidinopropiophenone designer drugs



Name	R ₁	R ₂	R ₃
PPP	H	H	CH ₃
MPPP	H	CH ₃	CH ₃
MOPPP	H	OCH ₃	CH ₃
Pyrovalerone	H	CH ₃	C ₃ H ₇
MPBP	H	CH ₃	C ₂ H ₅
MPHP	H	CH ₃	C ₄ H ₉
MDPV	-O-CH ₂ -O-		C ₃ H ₇
MDPPP	-O-CH ₂ -O-		CH ₃

releasing agents of 5-HT, norepinephrine, and dopamine [17–20]. After entering neurons via the monoamine transporters, MDMA inhibits the vesicular monoamine transporter, which results in increased concentrations of 5-HT, norepinephrine, and dopamine in the cytoplasm and induces their release by reversing the direction of flows for their respective transporters through a process of phosphorylation. MDMA also acts as a weak 5-HT₁ and 5-HT₂ receptor agonist, and its metabolite MDA augments this activity. The word “entactogen” was first used by Nichols [21], and the unusual entactogenic effects of MDMA have been hypothesized to be, at least partly, the result of indirect oxytocin secretion via activation of the serotonergic system [21].

Neuropharmacological and electrophysiological data strongly suggest the existence of postsynaptic receptors for tryptamine distinct from those for 5-HT [22–25]. There may be a rostrally projecting neuronal tryptamine-containing system arising from cell bodies in or near the median raphe nucleus. The demonstration of specific receptors for tryptamine in the central nervous system strongly indicates a neurotransmitter role for tryptamine, although a role of tryptamine as a modifier of the central 5-HT systems cannot be ruled out. It is also possible that 5-HT and tryptamine may be mediators of functionally opposite neuronal pathways. On the other hand, tryptamine designer drugs, such as 5-MeO-DIPT (Fig. 9), have been shown to act as blocker-type inhibitors of SERT-mediated uptake and to have no effect on 5-HT release. Chronic treatments with 5-MeO-DIPT produced serotonergic neurotoxicity [22–25].



Name	R ₁	R ₂
PCEEA	C ₂ H ₄	OC ₂ H ₅
PCMEA	C ₂ H ₄	OCH ₃
PCMPA	C ₃ H ₆	OCH ₃

Fig. 8 Structures of *N*-(1-phenylcyclohexyl)-2-alkoxyethanamine designer drugs

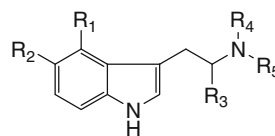
Metabolisms

The metabolisms of designer drugs have been keenly studied by Maurer's group, and some reviews on this subject have been published by them [26–29]. PEA-type designer

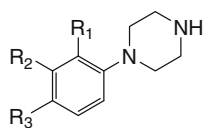
drugs (Figs. 1, 3, 4) are mainly metabolized through two routes: *N*-dealkylation or ring hydroxylation followed by methylation. Each parent drug is useful as a marker, because all metabolites cannot be detected in every case; in *N*-alkylated-type drugs, the parent drug and the *N*-dealkylated metabolite are primarily excreted into urine. However, hydroxylation is a major metabolic pathway for nonalkylated drugs such as AP. The hydroxylated drugs are usually excreted into urine following conjugation with glucuronic acid and/or sulfate. When the metabolites are extracted from the biological materials, it should be noted that the characteristics of metabolites are quite different from those of the parent compounds, because they have different functional groups such as amines, hydroxides, or conjugates.

The methylenedioxy and/or ring-substituted PEA-type designer drugs (Fig. 2) are metabolized via two overlapping routes; one route is methylenedioxy ring cleavage and/or *O*-demethylation to dihydroxy derivatives followed by the methylation of one of the hydroxyl groups, while the other route is *N*-dealkylation.

Fig. 9 Structures of tryptamine designer drugs



Name	R ₁	R ₂	R ₃	R ₄	R ₅
AMT	H	H	CH ₃	H	H
DMT	H	H	H	CH ₃	CH ₃
DIPT	H	H	H	<i>iso</i> -propyl	<i>iso</i> -propyl
DPT	H	H	H	<i>n</i> -propyl	<i>n</i> -propyl
MIPT	H	H	H	H	<i>iso</i> -propyl
4-AcO-DIPT	AcO	H	H	<i>iso</i> -propyl	<i>iso</i> -propyl
4-OH-DIPT	OH	H	H	<i>iso</i> -propyl	<i>iso</i> -propyl
4-OH-DMT	OH	H	H	CH ₃	CH ₃
5-MeO-AMT	H	CH ₃ O	CH ₃	H	H
5-MeO-DALT	H	CH ₃ O	H		
5-MeO-DET	H	CH ₃ O	H	C ₂ H ₅	C ₂ H ₅
5-MeO-DIPT	H	CH ₃ O	H	<i>iso</i> -propyl	<i>iso</i> -propyl
5-MeO-DPT	H	CH ₃ O	H	<i>n</i> -propyl	<i>n</i> -propyl
5-MeO-DMT	H	CH ₃ O	H	CH ₃	CH ₃
5-MeO-EIPT	H	CH ₃ O	H	C ₂ H ₅	<i>iso</i> -propyl
5-MeO-EPT	H	CH ₃ O	H	C ₂ H ₅	<i>n</i> -propyl
5-MeO-MIPT	H	CH ₃ O	H	CH ₃	<i>iso</i> -propyl



Name	R ₁	R ₂	R ₃
2-CPP	Cl	H	H
3-CPP	H	Cl	H
4-CPP	H	H	Cl
2-MPP	CH ₃ O	H	H
3-MPP	H	CH ₃ O	H
4-MPP	H	H	CH ₃ O
TFMPP	H	CF ₃	H

Fig. 10 Structures of ring substituted piperazine designer drugs

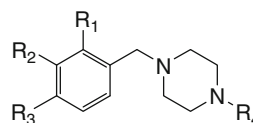
The tryptamine-type designer drugs, such as 5-MeO-DIPT (Fig. 9), are mainly metabolized via *N*-dealkylation, *O*-dealkylation, and hydroxylation at the 6 position of an indole ring, followed by conjugation with sulfate or glucuronic acid [30–34].

Analytical methods

Colorimetric detection

Colorimetric reactions, such as spot tests and TLC with spray to produce a color, are used for screening or preliminary identification of seized materials and residues extracted from biological materials, because it is easy to observe color changes without use of special instruments [35–38].

Fig. 11 Structures of ring substituted benzylpiperazine designer drugs



Name	R ₁	R ₂	R ₃	R ₄
BZP	H	H	H	H
MBZP	H	H	H	CH ₃
MDBZP	-O-CH ₂ -O-		H	H
2-Brom-4,5-dimethoxy-BZP	CH ₃ O	CH ₃ O	Br	H
4-Brom-2,5-dimethoxy-BZP	CH ₃ O	Br	CH ₃ O	H
5-Brom-2,4-dimethoxy-BZP	Br	CH ₃ O	CH ₃ O	H

Almost all designer drugs have nitrogen atom(s) in their molecular structures. Thus, colorimetric reagents that react with nitrogen are usually used for detection tests; these include the Marquis reagent, Simon's reagent, Dragendorff reagent, and others. Fluorometric and chemiluminescent assays are more sensitive than colorimetric ones, but require a chemical probe that excites the drug. With the Marquis reagent, all drugs containing nitrogen in the molecules produce a yellow to brown color [35]. With Simon's reagent, drugs with secondary amines, such as bk-MDMA, produce a blue to purple color. Ehrlich's reagent produces a blue to purple color by reaction with tryptamines, such as 5-MeO-DIPT. The detection limit of a colorimetric method for MDMA using chromotropic acid as reagent was 0.1 mM [36]. The detection limits for tryptamines were 10–50 µg/ml with Ehrlich's reagent, 50 µg/ml with the Marquis reagent, and 0.5–2.0 µg/ml with tetrabromophenolphthalein ethyl ether [37].

A preliminary systematic method for the screening of designer drugs was constructed by combining these colorimetric reagents [38]. However, the sensitivity was much lower than that of other chromatographic methods and specificity for each drug is lacking; it needs purification and condensation of analytes from biological materials.

Immunochemical detection

Recently, immunoassay kits, such as Acro Rapid MDMA Urine Test, DrugSmart Cassette, and RapiCard InstaTest, have been developed and these are now widely used for the detection of illegal drugs in urine or saliva. These kits are more useful than colorimetric methods, because they do not require reagents or special tools and results are obtained quickly and easily. Kits for MDMA or MDA detection are

available, but those for other designer drugs are not on the commercial market.

Chromatographic analyses according to drug categories and detection methods

Chromatographic methods such as TLC, GC, LC, and CE are commonly used to identify and quantitate designer drugs in biological materials. In addition, various chromatographic methods are coupled with MS for routine analysis of illegal drugs. The published methods for analysis of designer drugs in biological materials and seized items are summarized in Tables 1, 2, 3, 4, 5, 6 [39–115].

Extraction

Although many sample preparation procedures have been reported, the following four methods are most commonly used for extraction of analytes from biological materials [116]: (1) deproteinization, (2) headspace extraction, (3) LLE, and (4) SPE. The methods are chosen according to the purposes and the instruments to be used, and they have both advantages and disadvantages.

Deproteinization with MeOH and/or ACN is sometimes performed before LC–MS(–MS) analysis. This procedure is very simple, but nonvolatiles such as peptides and lipids are included in the supernatant fraction and affect column separation and contaminate ionization chambers. Therefore, this is usually not suitable for GC–MS analysis.

LLE is still widely used for sample preparation because of its simplicity and low cost. In this technique, analytes can be systematically extracted and purified according to their physicochemical properties. For designer drug extraction, dichloromethane, chloroform, chlorobutane, ethyl acetate, and mixtures of some of these are used as extraction solvents. However, the solvents need to be evaporated carefully to avoid losing the highly volatile targets. Relatively large amounts of extraction solvent are required, and the extraction process is sometimes complicated. Moreover, the organic solvents used in this technique are toxic to both humans and the environment. The procedure is also time consuming, and the number of samples that can be analyzed simultaneously is limited. An automated system of LLE for biological samples has been constructed [117], but such a robotic system is expensive and is not widely used. The formation of an emulsion during extraction is also a critical problem.

To overcome these problems, SPE has been developed. In this technique, silica gel or a polymer resin is embedded in a cartridge as a solid adsorbent material [118]. The analytes are adsorbed onto the surface of the sorbent when the sample solution flows through the cartridge. Endogenous impurities can be removed by passing an aqueous

Table 1 GC–MS methods for simultaneous determination of designer drugs in biological and seized materials

Targets	Sample(s)	Purification	Derivatization	Column	LOD(s) (ng/ml)	Linear range (ng/ml)	Reference
51 Phenethylamines, 32 tryptamines, 21 piperazines	Purchased products, synthesized standards	LLE	–	DB-5MS (Agilent)	–	–	[5]
TMA-1, TMA-2, 2C-B, 2C-I, 2C-T-2, 2C-T-7, 5-MeO-DMT, AMT, 5-MeO-MIPT, 5-MeO-DIPT	Purchased products, synthesized standards	–	–	DB-5MS (Agilent)	0.1 ppm	10–200 ppm	[39]
Phenethylamines, tryptamines, piperazines, and other abused drugs	Blood, urine	SPE (FOCUS)	Acylation (AA)	HP-5MS (Agilent)	–	10–5000	[40]
Phenethylamines, tryptamines, piperazines, and other abused drugs	Urine	SPE (FOCUS)	Acylation (AA)	HP-1MS (Agilent)	–	100–5000 (in most drugs)	[41]
AMT, DMT, 2C-B, DPT, 2C-T-7, 5-MeO-DIPT	Blood, urine	SPE (ZCDAU020)	Acylation (PFPA)	ZB-1 (Phenomenex)	5 and 10	50 and 1000	[42]
AP, MA, MDA, MDMA, MDEA, BDB, MBDB, MeOPP, 2-CPP, TFMP, PMA, PMMA, 4-MTA, BZP, MDBZP	Plasma	SPE (Isolute Confirm HCX)	Acylation (HFBA)	HP-5MS (Agilent)	1	5–1000	[43]

Table 2 GC–MS methods for determination of phenethylamine designer drugs in biological and seized materials

Target(s)	Sample(s)	Purification(s)	Derivatization(s)	Column(s)	LOD(s) (ng/ml)	Linear range(s) (ng/ml)	Reference
2C-B-FLY, DOB-FLY, <i>N</i> -Me-DOB-FLY, 2C-B-DragonFLY, DOB-DragonFLY, <i>N</i> -Me-DOB-DragonFLY	Synthesized standards	–	Acylation (TFAA)	DB-1MS, DB-5MS, DB-17MS (Agilent)	20 (2C-B-FLY, 2C-B-DragonFLY), 10 (other compounds)	–	[44]
<i>N</i> -OH-MDA, <i>N</i> -OH-MDMA	Synthesized standards	–	–	DB-5MS (Agilent)	–	–	[45]
bk-MBDB, bk-MDEA, bk-MDMA, mephedrone	Rat and human urine	SPE (Isolute Confirm HCX), LLE	Methylation (diazomethane), acylation (AA), combined methylation/acylation	HP-1 (Agilent)	1	–	[46]
Fluorophenethylamines	Seized compounds, synthesized standards	–	–	DB-1 (Agilent)	1 µg/ml	–	[47]
4-Methylmethcathinone, 2-fluoromethamphetamine, α -phthalimidopropiophenone, <i>N</i> -ethylcathinone	Seized compounds, synthesized standards	–	Acylation (PFPA)	HP-1 (Agilent)	–	–	[48]
PVP	Rat urine	SPE (Bond Elut Certify)	Methylation, acylation, combined methylation/acylation, silylation	HP-1 (Agilent)	–	–	[49]
AP, MA, MDA, MDMA, MBDB, EAMP, 4-MTA, 2C-B	Urine	LLE (diatomaceous earth)	Chiral derivatization (TPC)	DB-5 (Agilent)	0.5 (AP), 3 (MA)	20–1000	[50]
PMMA and its metabolites	Rat urine, plasma and tissues	SPE (SPEC PLUS DAU, plasma and tissues), LLE (urine)	Acylation (AA)	DB-5MS (Agilent)	10 (plasma), 10 ng/g (tissues)	10–2000 (plasma), 20–5000 ng/g (tissues)	[51]
DOB-DragonFLY	Seized liquid, liver, blood, urine, and vitreous humor	LLE	–	HP-5MS (Agilent)	–	–	[52]
2'-FMC, 3'-FMC, 4'-FMC, <i>N</i> -Ac-2'-FMC, <i>N</i> -Ac-3'-FMC, <i>N</i> -Ac-4'-FMC	Purchased capsule, synthesized standards	LLE	Acylation (AA)	HP-5MS (Agilent)	–	–	[53]
bk-MBDB, bk-MDEA, and their metabolites	Urine	LLE	Acylation (TFAA)	DB-5MS (Agilent)	–	–	[54]
MDPV	Seized compounds	LLE	–	DB-1 (Agilent)	–	–	[55]

Table 2 continued

Target(s)	Sample(s)	Purification(s)	Derivatization(s)	Column(s)	LOD(s) (ng/ml)	Linear range(s) (ng/ml)	Reference
AP, MA, MDA, MDMA, MDEA	Hair	LLE	Chiral derivatization (TFAP), acylation (PFPA)	5% Phenyl-methylsilicone (Agilent)	0.1–0.5 ng/mg	0.2–20 ng/mg (AP, MA, MDA), 0.5–20 ng/mg (MDMA, MDEA)	[56]
bk-MBDB, bk-MDEA, bk-MDDMA	Synthesized standards	LLE	Acylation (TFAA)	DB-5MS (Agilent)	–	–	[57]
2C-H, 2,5-DMA, 2C-B, DOB, 2C-I, DOI	Synthesized standards	–	Acylation (TFAA)	DB-5MS (Agilent)	2 ng/injection	3–100 µg/ml	[58]
TMA-1, TMA-2, TMA-3, TMA-4, TMA-5, TMA-6	Synthesized standards	–	Acylation (TFAA)	DB-1MS, DB-5MS, DB-17MS (Agilent)	–	–	[59]
DOC and its metabolites	Rat urine	LLE	Acylation (AA)	HP-1 (Agilent)	–	–	[60]
DOM and its metabolites	Rat urine	LLE	Acylation (AA)	HP-1 (Agilent)	–	–	[61]
PMEA and its metabolites	Blood, urine	LLE	Acylation (TFAA)	DB-5MS (Agilent)	–	–	[62]
MDA, ME, PEA, AP, MA, PPA, PMA, EP, PMMA, MDA, 4-MTA, MDMA, MBDB	Human whole blood	SPE (FOCUS)	Acylation (AA)	HP-5MS (Agilent)	5–50	50–5000	[63]
MPBP, MPHP	Seized compounds, synthesized standards	–	–	DB-1 (Agilent)	–	–	[64]
DOB	Seized compounds	–	–	Dimethylsiloxane	–	–	[65]
bk-MDMA, MBDB, methcathinone	Rat plasma and hair	SPE (Bond Elut Certify)	Acylation (PFPA)	DB-35MS (Agilent)	–	10–2000 (except for MBDB; 50–2000) for rat plasma, 5.0–200 ng/mg (except for methcathinone; 1.0–50.0 ng/mg) for rat hair	[66]
2C-B and its metabolites	Rat urine	LLE	Acylation (AA)	HP-1 (Agilent)	–	–	[67]
DOI and its metabolites	Rat urine	LLE	Acylation (AA)	HP-1 (Agilent)	–	–	[68]
AP, MA, MDMA, MDA	Hair	SPME (fiber coated with polydimethylsiloxane)	Acylation (ECF)	PTE-5 (Supelco)	0.01–0.5 ng/mg	0.02–5.0 ng/mg (AP), 0.01–10 ng/mg (MA), 0.50–10 ng/mg (MDA, MDMA)	[69]
2C-D and its metabolites	Rat urine	LLE, SPE	Methylation (diazomethane), acylation (AA, MBTFA), combined methylation/acylation	HP-1 (Agilent)	20	–	[70]

Table 2 continued

Target(s)	Sample(s)	Purification(s)	Derivatization(s)	Column(s)	LOD(s) (ng/ml)	Linear range(s) (ng/ml)	Reference
2C-I and its metabolites	Rat urine	SPE (Isolute Confirm HX), LLE	Methylation (diazomethane), acylation (AA, MBTFA), combined methylation/acylation	HP-1 (Agilent)	50	–	[71]
2C-E and its metabolites	Rat urine	LLE	Acylation (AA)	HP-1 (Agilent)	10	–	[72]
Fluoro-methoxy-phenylalkylamines	Seized compounds	–	Acylation	DB-1 (Agilent)	–	–	[73]
AP, MA, MDA, MDMA, MDEA	Urine	SPE (Cerex CLIN II)	Acylation (4-carboxyhexafluorobutyl chloride)	DB-5MS (Agilent)	31.3 (AP, MA, MDMA, MDEA), 62.5 (MDA)	–10000 (MA), –12000 (AP, MDA, MDMA, MDEA)	[74]
MDMA, MDA	Hair, urine	Liquid extraction (hair)	Acylation (TFAA)	HP-5MS (Agilent)	0.125 ng/mg (hair)	0.5–250 ng/mg (hair)	[75]
MPBP and its metabolites	Rat urine	SPE (Isolute Confirm HX)	Methylation, acylation, combined methylation/acylation, silylation(MSTFA)	HP-1 (Agilent)	100	–	[76]
4-MTA and its metabolites	Urine	SPE (Isolute Confirm HX), LLE	Acylation (AA), methylation (diazomethane)	HP-1 (Agilent)	30	–	[77]
2C-B and its metabolites	Mice urine	LLE	<i>N</i> -TFA- <i>O</i> -TMS derivatization (MSTFA, MBTFA), <i>N</i> , <i>O</i> -TFA-derivatization (TFAA), <i>N</i> , <i>O</i> -TMS derivatization (MSTFA, TMSCl)	HP-1 (Agilent)	–	–	[78]
PMMA and its metabolites	Rat urine	LLE	Acylation (AA, HFBA), methylation (diazomethane), methylation plus acylation	HP-1 (Agilent)	10–50	–	[79]
MPHP and its metabolites	Rat urine	SPE (Isolute Confirm HX)	Silylation (MSTFA) acylation (AA)	HP-1 (Agilent)	5	–	[80]
MOPPP and its metabolites	Rat urine	SPE (Isolute Confirm HX)	Silylation (MSTFA), ethylation (diazomethane), acylation (AA)	HP-1 (Agilent)	50	–	[81]
MDPPP and its metabolites	Rat urine	SPE (Isolute Confirm HX)	Silylation (MSTFA), ethylation (diazomethane), acylation (AA)	HP-1 (Agilent)	50	–	[82]
PPP, MPPP and their metabolites	Rat urine	SPE (Isolute Confirm HX)	Silylation (MSTFA), ethylation (diazomethane), acylation (AA)	HP-1 (Agilent)	50	–	[83]
AP, MA, MDA, MDMA, MDEA, BDB, MBDB	Hair	SPDE (a hollow needle with an internal coating of PDMS)	Acylation (MBTFA)	HP-5MS (Agilent)	0.07–0.19 ng/mg	0.05–20 ng/mg (AP, MA, MDA), 0.1–20 ng/mg (MDMA, BDB), 0.2–20 ng/mg (MDEA, MBDB)	[84]
MPPP and its metabolites	Rat urine	SPE (Isolute Confirm HX)	Ethylation (diazomethane)	HP capillary, cross linked methylsilicone	100	–	[85]

Table 2 continued

Target(s)	Sample(s)	Purification(s)	Derivatization(s)	Column(s)	LOD(s) (ng/ml)	Linear range(s) (ng/ml)	Reference
MDA, MDMA, MDEA, BDB, MBDB	Urine and liver microsomes of humans and rats	SPE (Bond Elut C18, Isolute Confirm HCLX)	Acetylation and/or methylation	–	3	10–500	[86]
MDEA and its metabolites	Urine	LLE	Acylation (AA)	HP capillary, cross linked methylsilicone	5	–	[87]

solution through the cartridge. Sorbent materials can be chosen according to the physicochemical properties of the analytes. A cation-exchanging sorbent and/or a hydrophobic sorbent (C_{18} or C_8) is normally used for extraction of designer drugs, because the pK_a value of the targets is largely over 9.

SPME is very suitable for automation of drug extraction from biological samples [69], but is not highly popular for designer drug analyses at this time, probably because of its low efficiency.

Headspace extraction is very simple and thus suitable for automation. However, designer drugs are not volatile and need derivatization for GC–MS analysis. We have used headspace extraction in combination with SPME after in-matrix derivatization for GC–MS analysis of MDMA and MDA in human hair [69].

Hydrolysis

Target drugs themselves can be detected from urine samples in clinical or forensic laboratories, when their concentrations in urine are relatively high. However, it is preferable that all metabolites are identified and quantitated for biological samples. As described in the metabolism section, the drugs absorbed into a body are metabolized through demethylation or hydroxylation followed by conjugation with glucuronic acid and/or sulfate. A urine sample is usually hydrolyzed with a concentrated acid or enzyme(s), because the conjugated standards are usually not commercially available, although the glucuronide metabolites were directly detected in a few studies [119]. Hydrochloric acid is commonly used for hydrolysis, and a mixture of β -glucuronidase and sulfatase is also used for this purpose. The decomposition of psilocin by acid hydrolysis was noted in the previous report [120], and, subsequently, the hydrolysis of tryptamine-type drugs was achieved by enzymatic reaction. In addition, selection of a β -glucuronidase from a different origin is important for complete hydrolysis of tryptamine-type drugs [121].

Derivatization

As shown in Figs. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, all designer drugs contain an amino or imino group. The analysis of free amines such as AP, MA, MDA, and MDMA by GC or GC–MS is associated with difficulties in sensitivity and reproducibility, because of adsorption on and interaction with glass GC inserts and analytical columns causing poor peak resolution and tailing. To overcome these problems, derivatization is commonly used. Although many kinds of derivatization reagents have been examined, a common option is the acylation of amines by AA/pyridine, TFAA, PFPA, HFBA, or MBTFA. AA and

Table 3 GC-MS methods for determination of tryptamine and other designer drugs in biological and seized materials

Type	Target(s)	Sample(s)	Purification(s)	Derivatization(s)	Column	LOD(s) (ng/ml)	Linear range (ng/ml)	Reference
Tryptamine	5-MeO-DPT	Urine	LLE (Extrelut)	Acylation (TFAA)	HP-5MS (Agilent)	5	10–2000	[88]
	5-MeO-DIPT, 5-MeO-MIPT	Urine	LLE	–	HP-5MS (Agilent)	–	–	[89]
	2-Brom-4,5-dimethoxy-BZP, 4-brom-2,5-dimethoxy-BZP	Seized compounds, synthesized standards	LLE	–	DB-1 (Agilent)	–	–	[90]
	BZP, TEMPP and their metabolites	Urine	SPE (OASIS HLB)	Acylation (TFAA)	DB-5MS (Agilent)	50–1000 (scan), 20–500 (SIM)	–	[91]
Piperazine	3-CPP and its metabolites	Rat urine	LLE	Acylation (AA, TFAA), methylation (diazomethane)	HP-1 (Agilent)	50	–	[92]
	4-MPP and its metabolites	Rat urine	LLE	Acylation (AA)	HP-1 (Agilent)	50–100	–	[93]
	BZP and its metabolites	Rat and human urine	LLE	Acylation (AA, TFAA, HFBA), methylation (diazomethane)	HP capillary, cross linked methylsilicone	100	–	[94]
	BZP, 4-MPP, TEMPP	Synthesized standards, purchased products	LLE	Acylation (AA, TFAA)	HP-5MS (Agilent)	–	–	[95]
Fentanyl	α -Methylfentanyl and its metabolites	Rat urine	LLE	Acylation (TFAA)	OV-17	200 ng/injection (α -fentanyl), 400 ng/injection (nor-fentanyl and ω , ω -1 nor-fentanyl)	–	[96]
	Phencyclidine	PCEA, PCMEA, and their metabolites	SPE (Bond Elut Certify), LLE	Acylation (MBTFA), silylation (MSTFA)	HP-1 (Agilent)	–	–	[97]
Phencyclidine	PCMPA and its metabolites	Rat urine	SPE (Bond Elut Certify), LLE	Acylation (MBTFA), silylation (MSTFA)	HP-1 (Agilent)	–	–	[98]
	PCPR and its metabolites	Rat urine	SPE (Isolute Confirm HXC), LLE	Acylation (AA, MBTFA)	HP-5, HP-1 (Agilent)	–	–	[99]
Cannabimimetic indole	Cannabicyclohexanol, JWH-018	Seized compounds, synthesized standards	–	–	HP-IMS (Agilent)	–	–	[100]
	JWH-018	Seized compounds	Liquid extraction	–	HP1-MS (Agilent)	–	–	[101]

Table 4 LC–MS(–MS) methods for simultaneous determination of various typed designer drugs in biological and seized materials

Targets	Sample	Purification	Detection	Column	Mobile phase	LOD(s) (ng/ml)	Linear range (ng/ml)	Reference
Phenethylamines, tryptamines, phenylpiperazines	Serum	SPE (Chromabond Drug)	LC–MS–MS (MRM)	Synergi Polar RP (Phenomenex)	Ammonium formate/(MeOH/formic acid)	1.0–5.0	–	[102]
MDMA, 2C-D, 2C-B, 2C- B-Fly, 2C-T-2, 2C-I, 2C- E, 2-CPP, 4-OH-DIPT, 4-AcO-DIPT	Urine	SPE (Bond Elut Certify), LLE	LC–MS (SIM)	Hypersil Gold (Thermo Electron)	Ammonium bicarbonate/ACN	8–16	Up to 2000	[103]
AP, MA, MDMA, MDMA, MBDB, morphine, 6-monoacetylmorphine, benzoylcegonine, cocaine	Plasma	SPE (OASIS HLB)	LC–MS (scan, SIM)	Atlantis dC18 (Waters)	ACN/ammonium formate	0.5 and 1.0	2–250	[104]

TFAA are widely used because of their cost effectiveness and ease of handling. However, these reagents are sensitive to moisture, and such treatments require the complete removal of moisture. Some research groups have investigated the stability of acyl derivatives of amphetamines in the solid state and in solution. TFA derivatives in ethyl acetate or *n*-hexane are stable at 5°C for only a few hours [122, 123]. Although pentafluoropropionic and heptafluorobutyric derivatives of amphetamines are more stable than the corresponding trifluoroacetic derivatives, a relatively long time is required to remove excess reagent, such that the recovery of the derivatives is decreased by vaporization. Alkylchloroformates quickly react with amines in aqueous solution, and the derivatives can be extracted with organic solvents [124, 125]. This reagent has significant potential for use in simple derivatization procedures and decreases recovery problems because of the low volatility of the derivatives.

Recently, chiral separation has become necessary, because some designer drugs contain a chiral center. For example, selegiline is used for treatment of Parkinson disease and is metabolized to *l*-MA in the human body [126]. To discriminate an abuse of MA from the medical use of selegiline, it is necessary to discriminate between the *l*-type and the *d*-type [127] by GC–MS. TPC was used for chiral derivatization for GC–MS analysis, and the product diastereomers were separated for amphetamines and some designer drugs [50]. TPC is now commercially available.

TLC detection

TLC is widely used for detection of designer drugs in seized products, because of its simplicity and low cost. In one described procedure [128], the seized products were dissolved in MeOH, and aliquots were developed on a silica gel plate (normal-phase chromatography). The developing solvent used was a mixture of chloroform/dioxane/ethyl acetate/28% ammonia solution (25:60:10:5, v/v/v/v) or MeOH/28% ammonia solution (100:1.5, v/v). Spots were detected under UV radiation at 254 nm. In addition, fluorescamine reagent was sprayed on the plates, and fluorescent spots were visualized under UV radiation at 365 nm. MA, a secondary amine, can be detected with Simon's reagent. In another report [38], hexane/acetone/triethylamine (10:10:1, v/v/v) or MeOH/25% ammonia solution (200:3, v/v) was used as a developing solvent. All of the 29 drugs tested resulted in a color change in the presence of the iodoplatinate reagent, but some drugs showed no color change with Dragendorff reagent. Some drugs overlapped with other drugs, and not every drug was completely separated by TLC. The detection limit of the drugs was about 1 µg/spot.

Table 5 LC–MS(–MS) methods for determination of phenethylamine designer drugs in biological and seized materials

Target(s)	Sample(s)	Purification	Detection(s)	Column(s)	Mobile phase	LOD(s) (ng/ml)	Linear range(s) (ng/ml)	Reference
2C-B-FLY, DOB-FLY, <i>N</i> -Me-DOB-FLY, 2C-B-DragonFLY, DOB-DragonFLY, <i>N</i> -Me-DOB-DragonFLY	Synthesized standards	–	LC–MS (SIM)	L-column ODS2 (CER), CAPCELL PAK phenyl (Shiseido)	MeOH/ammonium formate	5 (<i>N</i> -Me-DragonFLY, DOB-DragonFLY), 10 (other compounds)	–	[44]
2C-B-FLY, DOB-FLY, <i>N</i> -Me-DOB-FLY, 2C-B-DragonFLY, DOB-DragonFLY, <i>N</i> -Me-DOB-DragonFLY	Synthesized standards	–	LC–MS–MS (product ion scan, SRM)	CAPCELL PAK phenyl (Shiseido)	MeOH/ammonium formate	0.5 (2C-B-DragonFLY, DOB-FLY, DOB-FLY), 0.1 (<i>N</i> -Me-DOB-FLY, <i>N</i> -Me-DOB-DragonFLY)	–	[44]
<i>N</i> -OH-MDMA and its metabolites	Rat plasma, urine, hair	SPE (Bond Elut Plexa)	UPLC–MS–MS (MRM)	ACQUITY HSS T3 (Waters)	Formic acid/formic acid/ACN	0.1 (each drug in rat plasma and urine), 5 pg/mg (each drug in rat hair)	0.5–500 (each drug in rat plasma and urine), 0.01–0.75 ng/mg (<i>N</i> -OH-MDMA, <i>N</i> -OH-MDA in rat hair), 1–50 ng/mg (MDMA, MDA in rat hair)	[105]
ALEPH, ALEPH-2, ALEPH-5, ALEPH-7, 2C-T, 2C-T-2, 2C-T-5, 2C-T-7	Urine	SPE (Bond Elut C ₁₈)	LC–MS–MS (SRM)	Polaris C ₁₈ (Varian)	Acetic acid/ACN	1.2–4.9	1–100	[106]
AP, MA, MDA, MDMA, ketamine, norketamine	Hair	Formic acid under ultrasonication	LC–MS–MS (SRM)	Synargi Polar (Phenomenex)	Acetic acid/MeOH	0.1	0.5–25	[107]
DOB-DragonFLY	Seized liquid, liver, blood, urine, vitreous humor	LLE	LC–MS–MS (MRM), UPLC–TOFMS, HPLC–DAD	C18 Mercury MS Synergi (Phenomenex, LC–MS–MS)	(Ammonium acetate/formic acid)/(MeOH/ACN) (LC–MS–MS)	0.2 µg/kg (blood, LC–MS–MS)	0.5–50 µg/kg (blood, LC–MS–MS)	[52]
bk-MBDB, bk-MDEA, and their metabolites	Urine	Deproteinization	LC–MS (SIM), LC–MS–MS (product ion scan)	L-column ODS2 (CER)	Ammonium acetate/MeOH	10–25 (LC–MS)	0.1–10 µg/ml (LC–MS)	[54]
AP, MA, MDA, MDMA, <i>p</i> -OH-MA, <i>p</i> -OH-MA-Sul, <i>p</i> -OH-MA-Sul, HMMA, HMMA-Glu, HMMA-Sul	Blood	Protein precipitation	LC–MS–MS (product ion scan, MRM)	L-column ODS (CER)	Ammonium formate/MeOH	–	10–5000 (MA, MDMA), 10–500 (AP, MDA), 1–500 (<i>p</i> -OHMA, HMMA), 2–500 (<i>p</i> -OHMA-Glu, <i>p</i> -OHMA-Sul, HMMA-Glu, HMMA-Sul)	[108]

Table 5 continued

Target(s)	Sample(s)	Purification	Detection(s)	Column(s)	Mobile phase	LOD(s) (ng/ml)	Linear range(s) (ng/ml)	Reference
2,5-DMA, 2C-B, 2C-H, 2C-I, DOB, DOI	Seized compounds	–	LC-MS (scan)	ACQUITY UPLC BEH C18 (Waters)	Ammonium acetate/ACN	2.5 ng/injection (2C-H, 2,5-DMA, 2C-I, DOI), 5 ng/injection (2C-B, DOB)	1–100 µg/ml (2C-H, 2,5-DMA, 2C-I, DOI), 2–100 µg/ml (2C-B, DOB)	[58]
TMA-1, TMA-2, TMA-3 (IS), TMA-6	Urine	SPE (Bond Elut C ₁₈)	LC-MS (SIM)	–	Acetic acid/ACN	4.26–9.12	10–200	[109]
PMEA and its metabolites	Blood, urine	On-line extraction (MS-pak PK-2A)	LC-MS (SIM)	L-column ODS (CER)	(Formic acid/ACN) (formic acid/ACN)	50–600 pg (blood), 20–250 pg (urine)	0.1–20 µg/ml	[62]
PMEA and its metabolites	Blood, urine	Deproteinization	LC-MS-MS (product ion scan)	L-column ODS (CER)	Ammonium formate buffer/MeOH	–	–	[62]
MDA, MDMA, HMMA, HMMA-Glu, HMMA-Sul, 3-OH-4-MeO-MA	Urine	Dilution with MeOH and centrifugation	LC-MS (scan, SIM), LC-MS-MS (product ion scan)	L-column ODS (CER)	Ammonium formate/MeOH	5 (MDMA, HMMA, 3-OH-4-MeO-MA), 10 (HMMA-Glu, HMMA-Sul), 50 (MDA)	0.03–20 (HMMA, 3-OH-4-MeO-MA), 0.1–50 (HMMA-Glu, HMMA-Sul, MDA), 10–1000 (MDMA)	[110]
AP, MDMA, MDA	Blood, hair	LLE	LC-MS-MS (APCI, MRM)	LiChroCART (Merck)	Formic acid/ACN/forimic acid	5 (blood), 0.1 ng/mg (hair)	10–1000 (blood), 0.2–20 ng/mg (hair)	[111]
MDA, MDMA, MDEA, BDB, MBDB	Urine and liver microsomes of humans and rats	SPE (Bond Elut C ₁₈ , Isolute Confirm HXC)	LC-MS	HP Hypersil RP-18 (Agilent)	ACN/ triethylammonium acetate	–	–	[86]
AP, MA, MDA, MDEA, MDMA, MBDB, 2C-B, and related phenethylamines	Serum	SPE (Bond Elut C ₁₈)	LC-MS (APCI, scan, SIM)	Superspher 100 RP-18 (Merck)	Ammonium formate/ACN	1–5	5–500	[112]

Table 6 LC–MS(–MS) methods for determination of piperazine and other designer drugs in biological and seized materials

Type	Target(s)	Sample(s)	Purification(s)	Detection	Column	Mobile phase	LOD(s) (ng/ml)	Linear range(s) (ng/ml)	Reference
Piperazine	2-Brom-4,5-dimethoxy-BZP, 4-brom-2,5-dimethoxy-BZP	Seized compounds, synthesized standards	–	LC–MS–MS (scan, product ion scan)	AQUA C18 (Phenomenex)	Ammonium acetate/MeOH	–	–	[90]
	BZP, TFMP	Urine	LLE, SPE (ZCDAU020)	LC–MS (SIM)	Synergi Polar RP (Phenomenex)	Formic acid/ACN	100	100–5000 (BZP), 100–10000 (TFMP)	[113]
Fentanyl	BZP, TFMP, and their metabolites	Urine	SPE (OASIS HLB)	LC–MS (scan, SIM)	SCX (Shiseido)	Ammonium acetate/ACN	5–40 (scan), 0.2–1 (SIM)	10–10000 (SIM)	[91]
	Fentanyl, <i>cis</i> -TMF, <i>trans</i> -TMF	Blood, urine	LLE	LC–MS–MS	Gemini C18 (Phenomenex)	–	–	–	[114]
Cannabimimetic indole	Cannabicyclohexanol, JWH-018	Seized compounds, synthesized standards	–	UPLC–MS (scan)	ACQUITY UPLC HSS T3 (Waters)	Formic acid/ (formic acid/ACN)	–	10–500 µg/ml	[100]
Phosphodiesterase 5 inhibitor (ED drug)	JWH-018	Seized compounds	MeOH under ultrasonication	LC–MS (scan)	ACQUITY UPLC HSS T3 (Waters)	Formic acid/ (formic acid/ACN)	–	–	[101]
	Piperidenafil	Purchased products	–	LC–MS (scan)	Zorbax SB-C ₁₈ (Agilent)	Formic acid/ (formic acid/ACN)	0.1 mg/l (UV)	0.81–162 mg/l (UV)	[115]

GC–MS detection

To improve the selectivity and sensitivity, GC coupled with MS has been used to detect drugs in biological materials and seized items. A nonpolar or slightly polar column (such as HP-1 or HP-5) is commonly used for GC separation.

In a typical pretreatment procedure before GC–MS analysis of designer drugs [43], a mixed-mode SPE cartridge was used for extraction, and the analytes were eluted with 1 ml of MeOH/aqueous ammonia (98:2, v/v) into 1.5-ml polypropylene reaction vials. Each eluate was evaporated to dryness under a stream of nitrogen at 56°C. After addition of 20 µl of HFBA, the reaction vials were sealed and put on a rotary shaker for 15 s. Derivatization was carried out under microwave irradiation.

In the FocusTM screening method [40], 1 ml of urine sample was mixed with 0.1 ml of 1 M acetate buffer (pH 5) and was hydrolyzed by 50 µl of β-glucuronidase (type HP-2, Sigma, St. Louis, MO, USA) at 37°C for 2 h. To this solution, 1 ml of distilled water was added, and the mixture was vortex-mixed for 30 s, sonicated for 5 min, and centrifuged at 850g for 15 min. The supernatant fraction was applied to a FocusTM column that had been conditioned with 1 ml of MeOH and 1 ml of distilled water. The column was rinsed with 1 ml of distilled water and 1 ml of 10% ACN. The analytes were eluted with 0.75 ml of 0.1% TFA in ACN. The eluate was then evaporated to dryness under a stream of nitrogen at 60°C. The residue was dissolved in 50 µl of pyridine, and 50 µl of AA was added to the solution to carry out acetylation. The mixture was incubated at 60°C for 30 min, and then the solvent was evaporated to dryness at room temperature. The residue was dissolved in 100 µl of ethyl acetate containing 1 µl of diazepam-*d*₅ (IS), and a 2-µl aliquot of the solution was injected into a GC–MS instrument.

LC–MS(–MS) detection

In a typical screening method [13], urine hydrolyzed with β-glucuronidase from *Escherichia coli* was adjusted to pH 9–10 with a mixture of sodium hydrogen carbonate and sodium carbonate (10:1, w/w). LLE with diethyl ether was performed using sodium sulfate as a salting-out agent. After centrifugation, the organic layer was separated, acidified with acetic acid in ethyl acetate, and evaporated to dryness under nitrogen at 50°C. The remaining residue was reconstituted with 100 µl of reconstitution solvent [A:B = 80:20 (v/v); A: 5 mM ammonium formate in 0.01% formic acid, B: a mixture of ACN/water (90:10 v/v) containing 5 mM ammonium formate and 0.01% formic acid]. In this procedure, 241 small molecules were said to be screened by LC–TOFMS.

For analysis of thioamphetamine designer drugs [106], urine samples (1 ml) were spiked with 50 ng of 2C-T- d_4 (IS) and mixed with 1 ml of 100 mM hydrogen carbonate buffer (pH 10). The mixture was applied to a Bond Elut C₁₈ SPE column, which had been activated and conditioned with 1 ml of MeOH and 1 ml of 100 mM hydrogen carbonate buffer (pH 10). After application of the sample, the column was washed with 2 ml of MilliQ water and dried by passing through a stream of air for 5 min. The analytes were then eluted with 2 ml of MeOH and analyzed by LC–MS–MS. Linear calibration curves were obtained for all analytes (1–100 ng/ml) with correlation coefficients not smaller than 0.99. Although the range of linearity is relatively narrow, it includes the critical values for regulatory cut-off levels for routine analysis of abused drugs in urine. The limits of detection and quantitation ranged from 1.2 to 4.9 and from 3.2 to 9.6 ng/ml, respectively.

In addition, for analyses of hallucinogenic designer drugs [103], urine samples with 1 ml of 0.1 M phosphate buffer (pH 6.0) underwent a SPE procedure using Bond Elut Certify columns. The columns were preconditioned with 2 ml of MeOH and 2 ml of 0.1 M phosphate buffer (pH 6.0), then washed with 1 ml of 1.0 M acetic acid and 4 ml of MeOH. Analytes were eluted with 2 ml of ethyl acetate with 2% ammonium hydroxide and analyzed by LC–MS. Absolute analytical recoveries obtained after extraction for three different samples were always higher than 70%. The ranges of the limits of detection and quantitation were 8–16 and 27–53 ng/ml, respectively.

As another example [102], plasma samples (1 ml) were spiked with 20 μ l of IS solution (10 μ g/ml each of fenfluramine- d_{10} and phencyclidine- d_5 , and 5 μ g/ml each of AP- d_5 , MDMA- d_5 , MDEA- d_5 , ketamine- d_4 , cocaine- d_3 , and dimethylphenylpiperazine). The samples were then diluted with phosphate buffer (pH 6), mixed on a rotary shaker, and loaded onto SPE cartridges (Chromabond Drug) that had been conditioned with MeOH and adjusted with phosphate buffer to pH 6. After three washing steps (water 1 ml, flow rate 2 ml/min; acetic acid 1 ml, flow rate 2 ml/min; MeOH 1 ml, flow rate 2 ml/min), the analytes were eluted with 1.5 ml of dichloromethane/isopropanol/25% ammonia (80:20:2; v/v/v). The eluate was evaporated under a gentle stream of nitrogen at 30°C. During this evaporation step, a mixture of concentrated hydrochloric acid and 2-propanol (3:1, v/v) was added to prevent the loss of volatile amphetamines. The limits of detection were between 1.0 and 5.0 ng/ml.

Conclusions

The number of abusers of designer drugs has increased remarkably worldwide. In particular, the number of

confiscation cases of MDMA and its related compounds has increased dramatically, and the urgent need for tightened control over them is apparent. Simple screening methods are required for detection of designer drugs in biological materials and seized items. Although commercial kits for routine screening of MDMA and MDA are available, none is available for other designer drugs. Colorimetric and chromatographic methods have been presented in this review; a suitable method should be chosen for each laboratory. Although various human sample materials can be used for analysis, urine and blood are the first choices. However, designer drugs exist in urine and blood for only a short period. Samples such as hair and nails, which can prove long-term use of designer drugs, are likely to receive much attention in the future.

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