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Enantiomeric composition of abused amine drugs: chromatographic methods of analysis and data interpretation

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1. Introduction

1.1. Enantiomers

Enantiomers are pairs of nonsuperimposable mirror-image compounds, typically include one or more asymmetric (chiral) carbons (carbon bonded to four different groups). The two individual components of an enantiomer-pair have identical chemical and physical properties, differing only in the way they react with other chiral compounds and the direction in which they rotate plane-polarized light, and therefore, cannot be resolved by conventional chromatographic methodologies.

The *configuration* of the groups bonded to the chiral atom is designated as *S* or *R*, while D/+ and L/− are used to describe the *direction* in which a polarized light is rotated by the molecule. A left-handed or levorotatory (L/−) compound rotates polarized light to the left while a right-handed or dextrorotatory (D/+) compound rotates polarized light to the right. Configuration alone does not predict the light-rotating direction, a full description of a compound often includes designations for the configuration (*S*/*R*) and the light-rotating characteristic (D/L or +/−). When the quantities of two enantiomers in a solution are exactly equal, it becomes optically inactive and is called a racemate.

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Biological systems are typically chiral; thus, drugs derived from natural sources commonly exhibit optical activity while synthetic products—unless specifically synthesized for optical purity—exist as racemates. For the same reason, biological systems often react differently toward each component of an enantiomer-pair. For example, the L-isomers of some barbiturates act as depressants while the D-isomers act as convulsants.

1.2. Significance of enantiomeric analysis in the pharmaceutical industry

"Chirality" is now a topic at the forefront of academic research and drug development. For example, the 2001 Noble Prize in Chemistry has been awarded to "three scientists who devised techniques for catalytic asymmetric synthesis—the use of chiral catalysts to accelerate the production of single-enantiomer compounds for pharmaceutical use and a wide range of other applications." [1] On the other hand, US Food and Drug Administration's regulations place major emphases on the characterizations of enantiomeric compositions of pharmaceutical products [2]. Drug firms are also actively involved in developing new chiral drugs as single enantiomers and in carrying out "racemic switches"—redeveloping racemic mixture drugs as single enantiomers [3]. As a result, approximately 40% of all dosage-form drug sales in 2000 was of single enantiomers, in contrast to approximately one-third in 1999 [3].

1.3. Significance of enantiomeric analysis in forensic science laboratories

Enantiomeric characterization and composition determination are also important issues in forensic laboratories involving in the analysis of abused drugs. Enantiomeric analyses are often carried out to: (a) provide information for sentencing guidance for certain drug-related offenses; (b) assist in drug-related investigations; and (c) determine whether the drug of concern derives from a controlled substance.

Although enantiomeric specifications have long been an important factor in the scheduling of commonly abused drugs, current US Federal drug codes are so worded that both optical isomers are included. For example, both optical isomers of amphetamine, methamphetamine, and cocaine and both natural and synthetic cocaine are Schedule II drugs [2] as shown below:

- US Code 1308.12 (b) (4): "Coca leaves (9040) and any salt, compound, derivative or preparation of coca leaves (including cocaine (9041) and ecgonine (9180) and their salts, isomers, derivatives and salts of isomers and derivatives), and any salt, compound, derivative, or preparation thereof which is chemically equivalent or identical with any of these substances, . . ."
- US Code 1308.12 (d) (1): "Amphetamine, its salts, optical isomers, and salts of its optical isomers"
- US Code 1308.12 (d) (2): "Methamphetamine, its salts, isomers, and salts of its isomers"

However, the United States Sentencing Commission guidelines manual distinguishes between the enantiomers of methamphetamine for the purpose of sentencing. One gram of

L-methamphetamine is considered to be the equivalent of 40 g of marijuana, while 1 g of D-methamphetamine or racemic methamphetamine is equivalent to 10 kg of marijuana [4].

Enantiomeric analysis can also assist the investigation process. For example, the source of cocaine samples—a question often asked in the investigation process or in the court—may be elucidated by enantiomeric analysis [5,6]. Cocaine samples derived from the extraction of coca leaves contain only L-cocaine, while completely synthetic cocaine samples usually contain both D- and L-cocaine and diastereomers. Enantiomeric analysis can also provide valuable information concerning clandestine conversions of norephedrine and norpseudoephedrine to amphetamine and ephedrine and pseudoephedrine to methamphetamine [7–11]. The single enantiomer starting materials tend to produce single enantiomer products—single enantiomer *S*(D)-methamphetamine (D-methamphetamine) from L-ephedrine or D-pseudoephedrine and *R*(L)-methamphetamine (L-methamphetamine) from D-ephedrine or L-pseudoephedrine. It should be cautioned, however, L-methamphetamine has also been extracted from the Vicks Inhaler® [12] and D-methamphetamine-based prescription drugs [13].

Enantiomeric composition determinations of amphetamine and methamphetamine in biological specimens serve well for confirming or contradicting the stated amphetamine and methamphetamine sources of these compounds [14,15]. This is possible because of the following enantiomeric characteristics of certain amphetamine/methamphetamine-containing substances: (a) L-methamphetamine is used in Vicks Inhaler® [12]; (b) prescription methamphetamine is in D-form; and (c) amphetamine and methamphetamine resulting from the use of licit medicine, such as deprenyl (selegiline), an antiparkinsonian, are L-isomers [13].

Enantiomeric composition alone, however, does not always lead to a definite conclusion. For example, the detection of a racemic mixture of methamphetamine may indicate illicit use; the same observation may also derive from (a) prescription use of a racemic precursor molecule such as furfenorex [16]; or (b) legitimate use of D-methamphetamine along with the concurrent use of L-methamphetamine (Vicks Inhaler®) [12] or deprenyl [13]. Detection of single enantiomer D-methamphetamine, however, does not necessarily prove use of licit prescription either. As mentioned above, single enantiomer D-methamphetamine can be manufactured from L-ephedrine or D-pseudoephedrine [10].

1.4. Approaches in chromatographic separation of enantiomers and coverage of this review

A substantial number of studies have been conducted on the use of capillary electrophoresis (CE) for enantiomeric composition determinations. CE may even become the method of choice when an effective and robust interface (between the CE and the mass spectrometric detecting device) is developed and become conveniently available. Since CE approaches have been covered by an earlier issue of this journal, they will be excluded in this review. Only chromatographic methods will be addressed.

This review will be limited to various chromatographic methods for enantiomeric determination of amine drugs, with emphases on amphetamine and methamphetamine, their precursor drugs, and structurally closely related compounds; and significance pertaining to the observed enantiomeric composition data.

There have been a number of reviews on topics hereby addressed. For examples "Chiral stationary phases for gas–liquid chromatographic separation of enantiomers" [17], "Determination of methamphetamine enantiomer ratios in urine by GC–MS" [16], "Amphetamine and methamphetamine determinations in biological samples by high performance liquid chromatography" [18], "Liquid chromatographic analysis of enantiomeric composition of abused drugs" [19], "Determination of amphetamine, methamphetamine and amphetamine-derived designer drugs or medicaments in blood and urine" [21], "Methamphetamine—properties and analytical methods of enantiomer determination" [20], and "Illegal and legitimate use? Precursor compounds to amphetamine and methamphetamine" [22] have been published in the 1983–2000 period. Relevant information in these reviews will be summarized along with newer information.

2. Chromatographic approaches in the determination of enantiomeric compositions

Enantiomeric analysis of amphetamine and related drugs can be accomplished by a variety of analytical techniques, including nuclear magnetic resonance (NMR) approaches [23]. Among methods available, gas chromatography (GC) and high-pressure liquid chromatography (HPLC), in combination with various detecting devices, are most commonly used. Interest in enantiomeric purity, metabolic studies, and pharmaceutical applications has lead to the development and use of a variety of *chiral derivatizing reagents* and *chiral stationary phases* greatly facilitating GC and HPLC applications.

2.1. Chiral derivatization and gas chromatographic analysis

Chiral derivatization, followed by GC separation of the resulting diastereomeric pairs, is the most common approach adapted for enantiomeric analysis of amphetamine and related drugs and metabolites [16]. Thanks to its commercial availability and effectiveness, trifluoroacetyl-L-prolyl chloride (L-TPC, Structure **1** in Fig. 1) is widely used and has been thoroughly studied. Many of these studies are summarized in the front section of Table 1.

In addition to trifluoroacetyl, other fluorinated groups, such as heptafluorobutyryl [46–49] have also been adapted. For example, Srinivas et al. [49] used heptafluorobutyryl-L-prolyl chloride (L-HPC, Structure **2** in Fig. 1) for the derivatization of several racemic amphetamines under aqueous-alkaline condition (pH 9.5). The resulting diastereomeric pairs can be satisfactorily resolved under properly selected isothermal conditions. This reagent has also been used for enantiomeric analysis of methylenedioxymethamphetamine (MDMA) and its metabolites, methylenedioxyamphetamine (MDA), 4-hydroxy-3-methoxymethamphetamine (HMMA), and 4-hydroxy-3-methoxyamphetamine (HMA), in rat [50] and human [51] specimens. In this latter study, selected derivatization was achieved through a reaction with L-HPC, followed by a reaction with *N*-methyl-*N*-trimethylsilyltrifluoroacetamide, resulting *N*-L-HPC-*O*-TMS (trimethylsilyl) derivatives. Preliminary results of the analysis of urine samples indicated an enantioselective metabolism in the *N*-demethylation pathway for MDMA in humans [51].

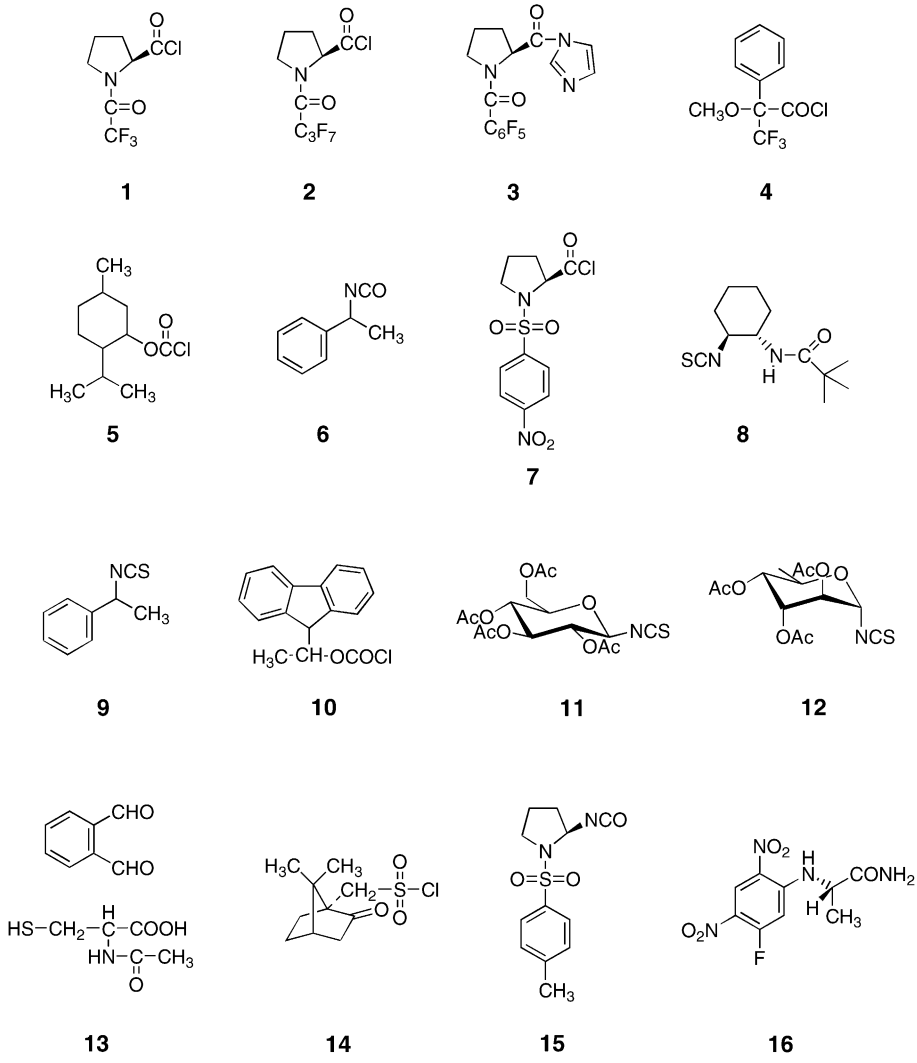


Fig. 1. Structures of commonamphetamines chiral derivatizing reagents: *N*-trifluoroacetyl-L-prolyl chloride (**1**), *N*-heptafluorobutyryl-L-prolyl chloride (**2**), *N*-pentafluorobenzoyl-L-prolyl imidazolide (**3**), α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (**4**), menthyl chloroformate (**5**), 1-phenylethyl isocyanate (**6**), 4-nitrophenylsulfonyl-L-prolyl chloride (**7**), (1*S*, 2*S*) *N*-[(2-isothiocyanato)-cyclohexyl]-pivalinoyl amide (**8**), 1-phenylethyl isothiocyanate (**9**), 9-fluorenylethyl chloroformate (**10**), 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl isothiocyanate (**11**), 2,3,4-tri-*O*-acetyl-α-D-arabinopyranosyl isothiocyanate (**12**), *o*-phthaldialdehyde/*N*-acetyl-L-cysteine (**13**), D-camphor-10-sulfonyl chloride (**14**), *N*-(*p*-toulenesulfonyl) prolyl isocyanate (**15**), 1-fluoro-2,4-dinitro-phenyl-5-L-alaninamide (**16**).

Table 1
Chiral derivatization and GC procedures for enantiomeric analysis of amphetamine and related drugs and metabolites

Reagent[a]	Compound analyzed[b]	Specimen	Stationary phase	Detection	Reference
1	AM	Std	SP-2100 (Hewlett-Packard, Avondale, PA)	EI-MS	[24]
1	AM, MAM	Forensic sample	SP-2100	EI-MS	[25]
1	AM, MAM	Human urine	Nonpolar (95% dimethyl–5% phenyl methyl-polysiloxane) fused silica	EI-MS	[26]
1	AM, MAM	Human urine	HP-5MS (Hewlett-Packard)	EI-MS	[27]
1	AM, α-phenylethylamine	Forensic sample	Ultra-1; Ultra-2 (Hewlett-Packard, Palo Alto, CA)	FID, FTIR	[28]
1	AM	Rat liver microsome	HP-1 (Hewlett-Packard)	FID	[29]
1	AM, MAM	Human urine	HP-1; DB-17 (J&W Scientific, Rancho Cordova, CA)	EI-MS	[30–33]
1	AM, MAM	Human urine	Cross-linked methylsilicone (Hewlett-Packard)	EI-MS	[34]
1	AM, MAM	Standard	Ultra-1	EI-MS	[35]
1	AM, MAM	Human urine, serum	HP-5MS	EI-MS	[36]
1	AM, MAM	Human urine	DB-5 (J&W Scientific)	FID, EI-MS	[37]
1	AM, MAM	Human urine, blood	Crosslinked 5% phenyl methyl silicone	EI-MS	[38]
1	Methcathinone	Standard	HP-1	EI-MS	[39]
1	AM, MAM, MDA, MDMA, MDEA	Human urine	DB-17, HP-1; ZB-50 (Phenomenex)	EI-MS	[40]
1	MDA, MDMA	Blood	Methylsilicone column (Hewlett-Packard)	EI-MS	[41,42]
1	MDMA, MDA	Rat blood	Methylsilicone column	EI-MS	[43]
1, **2**	MDEA, MDA	Rat brain	5% phenyl methyl silicone (Hewlett-Packard, Palo Alto, CA)	EI-MS	[44]
1	MDMA, MDA	Blood, tissue	HP-1	EI-MS	[45]
2	Methylphendiate	Blood, urine	1.5% OV-7/1.5% OV210 on Chromosorb-AW DMCS (Supelco, Cokville, Ont., Canada)	NPD, FID	[46]
2	Methoxyphenamine, 2-OH-MAM, 2-*O*Me-AM, 2-*O*Me-5-OH-AM,	Human urine	Cross-linked dimethyl silicone fused-silica (Hewlett-Packard: NJ)	NPD	[47]
2	Norfenfluramine, fenfluramine	Human plasma	Capillary OV-225 (Terochem Labs, Edmonton, Alberta, Canada)	ECD	[48]

Table 1 (*continued*)

Reagent[a]	Compound analyzed[b]	Specimen	Stationary phase	Detection	Reference
2	Norfenfluramine, fenfluramine amphetamine, methamphetamine, 2-OMe-AM, *p*-*O*Me-AM, 3,4-dimethoxyamphetamine, methylphendiate, norephedrine, ephedrine	Standard	Capillary OV-225	ECD	[49]
2	MDMA, MDA, HMMA, HMA	Rat urine, brain	DB-5 coupled to DB 1301 (J&W Scientific, Rancho Cordova, CA)	NCI-MS	[50]
2	MDMA, MDA, HMMA, HMA	Urine	DB-5MS (J&W Scientific, Folsom, CA)	PCI-MS	[51]
3	AM	Human plasma, saliva	5% OV-275 coated on Chromosorb W-AW (Supelco, Bellefonte, PA)	CI-MS	[52]
(−)-**4**	AM, *p*-OH-AM, *p*-OMe-AM, 2,5-dimethoxy-4-methylamphetamine	Rat urine	3% phenyl methyl silicone on dimethylchloro-silane-treated diatomite support	MS	[53]
(−)-**4**	AM	Rat urine	3% OV-17 coated on Gas Chrom Q support	MS	[54]
(+)-**4**	AM, MAM	Human blood, gastric	Shimadzu CBP-5 (Shimadzu)	FID, MS	[55]
(+)-**4**	AM, MAM, *p*-OH-AM, norephedrine, norpseudoephedrine, ephedrine, pseudoephedrine,	Human urine	Cross-linked 5% phenylmethylsilicone (SE-54)(Hewlett-Packard)	NPD, MS	[56–58]
(+)-**4**	MAM, ephedrine, pseudoephedrine, methcathinone	Standard	DB-5	MS	[59]
(−)-**4**	MDMA, MDA	Human urine, plasma	DB-17, HP Ultra 1	MS	[60]
(−)-**5**	AM, MAM	Human urine	DB-5, DB-17	MS	[61]

[a] Only structure designations are listed. See Fig. 1 for the structures and names of these chiral derivatization reagents.

[b] AM: amphetamine; MAM: methamphetamine; MDMA: methylenedioxymethamphetamine; MDA: methylenedioxyamphetamine; MDEA: methylenedioxyethylamphetamine; HMMA: 4-hydroxy-3-methoxymethamphetamine; HMA: 4-hydroxy-3-methoxyamphetamine.

Various combinations of fluorinated groups and amino acids have been explored to create series of chiral derivatizing reagents. For example, Souter [62] evaluated the utilizations of a variety of amino acids, including L-proline, L-leucine, L-valine and L-alanine, coupled with trifluoroacetyl, pentafluoropropionyl, and heptafluorobutyryl groups.

The use of *N*-pentafluorobenzoyl-*S*-L-prolyl-1-imidazolide (PFBPI, Structure **3** in Fig. 1) by Matin et al. [52] exemplified further deviation from this basic structural framework. This reagent has also been studied by others as summarized in the middle section of Table 1.

L- and D-α-Methoxy-α-(trifluoromethyl)phenylacetyl chloride (L- and D-MTPA, Structure **4** in Fig. 1) is a chiral pair of derivatization reagent that has also been extensively studied. For example, L-MTPA was used to determine the enantiomeric compositions of amphetamine and methamphetamine [53,54]; however, poor derivatization yields for methamphetamine was reported. (This difficulty was overcome by overnight incubation in a study using D-MTPA [55].) Fallon et al. [60] used L-MTPA to derivatize MDMA and MDA in human plasma and urine, while Shin and Donike [56] used D-MTPA to separate the enantiomers of amphetamines, phenol alkylamines, and hydroxyamines. In this latter study, prior to *N*-acylation, amine salts were converted to free bases and the hydroxy group was protected by TMS, TES (triethylsilyl), or the *t*-BDMS (*t*-butyldimethylsilyl) groups in forming *O*-silyl ether through the reaction to *N*-methyl-*N*-silylamide, *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide, *N*-methyl-*N*-(triethylsilyl)trifluoroacetamide, or *N*-methyl-*N*-(*t*-butyldimethylsilyl)trifluoroacetamide, respectively. All resulting *N*-MTPA and *O*-silylated diastereomeric pairs were well separated. This same approach has also been applied to investigate the stereoselective metabolism of famprofazone [56,57] and selegiline [58] in humans. A slightly different version of this reagent, D-α-methoxy-α-(trifluoromethyl)phenyl acetic acid, was used by LeBelle et al. [59] to analyze ephedrine, pseudoephedrine, methamphetamine, and methcathinone.

Other chiral derivatizing reagents with different structural features are also shown in Fig. 1, while their major applicational characteristics are summarized in the later section in Table 1. For example, Hughes et al. [61] analyzed amphetamine and methamphetamine in urine as carbamate derivatives following reaction with L-menthyl chloroformate (Structure **5** in Fig. 1). With this approach, L-methamphetamine was well resolved from illicit D-methamphetamine using a commonly used achiral stationary phase (DB-5).

2.2. Chiral derivatization and high-pressure liquid chromatographic analysis

Although not as convenient as GC, LC-based methodologies allow for (a) the selection of larger and hopefully more effective derivatizing groups and (b) the use of an "active" mobile phase (see Section 2.5), which introduces an additional parameter not available to GC-based approaches. Table 2 summarizes the use of various chiral derivatization reagents to form diastereomers which were typically resolved by reverse-phase C18 columns from various manufacturers.

In an early study, Miller et al. [63] compared four chiral reagents, L-MTPA, 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl isothiocyanate (GITC, Structure **11** in Fig. 1), (*R*)-D-1-phenylethyl isocyanate (PEIC, Structure **6** in Fig. 1), and 2,3,4-tri-*O*-acetyl-α-D-arabinopyranosyl isothiocyanate (AITC, Structure **12** in Fig. 1), for their effectiveness in resolving amphetamine enantiomers. Derivatization reactions were accomplished under mild conditions (25–75 °C) and were complete for all substrates within 60 min. The diastereomeric derivatives were separated by a C18 column with

Table 2
Chiral derivatization and HPLC procedures for enantiomeric analysis of amphetamine and related drugs and metabolites

Reagent[a]	Compound analyzed[b]	Specimen	Stationary phase	Detection mode	Reference
(−)-**4**, (+)-**6**, **11**, **12**	AM, *p*-Cl-AM, 2,5-dimethoxy-4-methyl-AM, 2,5-dimethoxy-4-thiomethyl-AM, 2,4-dimethoxy-5-methyl-AM	Standard	C18 (IBM Instruments, Danbury, CT)	UV (220 or 254 nm)	[63]
(+)-**9**	DOM, ephedrine	Standard	ODS (Beckmann, Berkeley, CA)	UV (254 nm)	[64]
8	AM, MAM	Standard	ODS (Hypersil)	UV (254 nm)	[65]
(−)-**10**	AM, MAM	Rat serum	C18 (Alltech Associated, Deerfield, IL)	Fluorescence, FAB-MS	[66]
(−)-**10**	AM, *p*-OH-AM	Rat serum, urine	C18 (Alltech Associated)	Fluorescence	[67]
(−)-**10**	MAM, *p*-OH-MAM, AM, *p*-OH-AM	Human urine	C18 (Alltech Associated)	Fluorescence	[68]
(−)-**10**	AM, *p*-OH-AM	Rat liver	C18	Fluorescence	[69]
(+)-**10**	Ephedrine, MAM, pseudoephedrine	Forensic sample	$5C_{18}$-AR (Waters Associates, Nacalai, Tesque)	Fluorescence	[70]
Polymeric FMOC-L-proline	AM	Spiked urine	C18 (EM Science, Cherry Hill, NJ)	Fluorescence, UV–VIS, polarimeter	[71]
		Spiked plasma	C18-DB (Supelco, Bellefonte, PA)	Fluorescence	[72]
11	AM, MAM, ephedrine, pseudoephedrine, norephedrine, pseudonorephedrine,	Forensic sample	C18 (Waters Associates)	UV (254 and 280 nm)	[8,10,11]
7	AM, MAM	Standard	Zorbax-Sil (Du Pont, Wilmington, DE) Supelcosil LC-Si (Supelco)	UV (254 nm)	[73,74]
15	AM	Standard	Silica gel (Waters Associates, Tianjing, China)	UV (254 nm)	[75]
14	AM, ephedrine, pseudoephedrine	Standard	C18; C8 (Perkin-Elmer)	UV (260 nm)	[76]
OPA/ homochiral thiol	AM, *p*-OH-AM, *p*-Cl-AM	Standard	C18 (Waters Associates)	Fluorescence	[77]
13	AM	Human urine	100 RP18 (Merck)	Fluorescence	[78]
16	AM, MAM	Human urine	C18 (Alltech Associates)	UV (340 nm)	[79]

[a] Only structure designations are listed. See Fig. 1 for the structures and names of these chiral derivatization reagents.
[b] AM: amphetamine; MAM: methamphetamine; DOM: 2,5-dimethoxy-4-methylamphetamine.

methanol–water mobile phase. In general, diastereomeric pairs resulting from the reaction with GITC, AITC, or MTPA were better resolved than those derived from PEIC.

Noggle et al. [8,10,11] separated the enantiomers of ephedrine, pseudoephedrine, amphetamine, and methamphetamine using GITC as the chiral derivatization agent. The resulting amphetamine diastereomeric pair was not well resolved. This difficulty was also reported when 4-nitrophenylsulfonyl-L-prolyl chloride (NPSP, Structure **7** in Fig. 1) was used [73,74]. Kleidernigg and Lindner [65] synthesized chiral derivatizing reagent (1*S*, 2*S*) *N*-[(2-isothiocyanato)-cyclohexyl]-pivalinoyl amide ((*S*,*S*)-PDITC, Structure **8** in Fig. 1) for the resolution of amphetamine and methamphetamine. The resulting diastereomeric thioureas was reportedly better separated than the corresponding GITC diastereomeric derivatives.

Gal and Sedman [64] evaluated D-1-phenylethyl isothiocyanate (PEITC, Structure **9** in Fig. 1) as a chiral derivatizing agent for the derivatization of enantiomeric 2,5-dimethoxy-4-methylamphetamine (DOM) and ephedrine, followed by the separation by a C18 column.

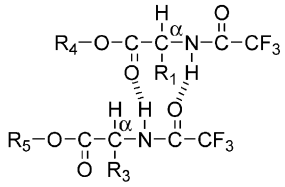
Hutchaleelaha et al. [66,67] and Sukbuntherng et al. [68] from the same group used L-(9-fluorenyl)ethyl chloroformate (L-FLEC, Structure **10** in Fig. 1) to react with amphetamine, methamphetamine, and their hydroxy metabolites in urine (after β-glucuronidase enzymatic cleavage of conjugates) for the formation of fluorescent diastereomers to facilitate fluorescence detection. This same reagent was used by Gunaratna and Kissinger [69] to investigate the stereoselective metabolism of amphetamine in rat liver microsomes by the cytochrome *P*-450 enzymes. D-FLEC was used by Chen et al. [70] for the analysis of methamphetamine enantiomers in forensic samples.

Gao and Krull [71] developed an on-line solid-phase derivatization approach using UV-fluorescence detection for the determination of amphetamine enantiomers in urine. Polymeric 9-fluorenylmethyl chloroformate-L-proline (FMOC-L-proline) was used in this study which was applied to the analysis of D,L-amphetamine in human plasma [72].

Desai and Gal [77] described an enantiomeric analytical method for amphetamine based on the reaction with *o*-phthaldialdehyde (OPA) and homochiral thiols. The resulting highly fluorescent isoindole diastereomeric derivatives were resolved using a C18 column. Pastor-Navarro et al. [78] developed a two-dimensional column-switching method for on-line quantitation of amphetamine enantiomers in pharmaceuticals and urine. The method used a C18 material for purification and a mixture of OPA and *N*-acetyl-L-cysteine (Structure **13** in Fig. 1) as the derivatization reagent.

2.3. Chiral gas chromatographic methods

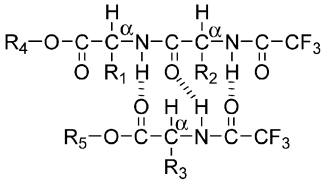
Chiral stationary phases (CSP) in GC columns allow for the analysis of enantiomeric compositions without using prior formation of diastereomeric derivatives. (However, achiral derivatizations are often used to improve the analytes' chromatographic behavior and mass spectrometric characteristics [80].) Peptide, diamide, and ureide are used for the preparation of chiral stationary phases. Representative structures of these phases are shown in Fig. 2. Further description of these phases and their performance characteristics is available in a review by Liu and Ku [17],



Monopeptide ester phase (Solvent)

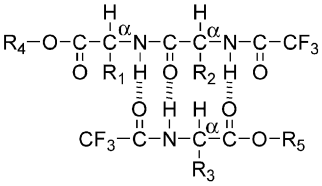
Amino acid derivative (Solute)

17



L-Dipeptide ester phase

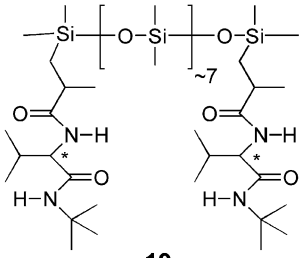
D-Configuration of solute



L-Dipeptide ester phase

L-Configuration of solute

18



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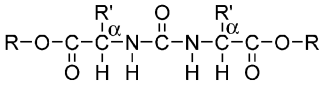

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Fig. 2. Structures of monopeptide solvent–solute interaction (**17**), interactions of an *N*-TFA dipeptide ester (solvent) with D- and L-*N*-TFA amino acid esters (solute) (**18**), structure of the chiral stationary phase Chirasil-Val (**19**), general formula of ureide phases (**20**).

Table 3
Chiral GC procedures for enantiomeric analysis of amphetamine and related drugs and metabolites

Chiral stationary phase	Compound analyzed[a]	Specimen	Derivatizing reagent[b]	Detection	Reference
Heptakis(3-*O*-acetyl-2,6-di-*O*-pentyl)-β-cyclodextrin	AM, *p*-OH-AM	Standard	TFA	FID	[81]
2,6-di-*O*-pentyl-3-*O*-trifluoroacetyl-γ-cyclodextrin; 2,6-di-*O*-pentyl-3-*O*-trifluoroacetyl-γ-cyclodextrin; 2,6-di-*O*-pentyl-3-*O*-propionyl-γ-cyclodextrin	AM, MAM	Standard	TFA, AC	FID	[82]
Chiraldex G-PN; Chiraldex β-DM (Advanced Separation Technologies)	AM, MAM, ephedrine, pseudoephedrin, deprenyl	Standard	TFA, AC	FID	[83]
2,6-di-*O*-pentyl-3-*O*-trifluoroacetylated-β-cyclodextrin/OV 17	AM	Standard		FID	[84]
Chirasil-Val (Applied Science)	AM	Standard	L-TPC	EI-MS	[24]
Chirasil-Val	AM, MAM	Forensic sample	L-TPC	EI-MS	[25]
Chirasil Dex-CB (Chrompack)	Demethylselegiline, AM, MAM	Urine, plasma	PFP	FID, EI-MS	[85]

[a] AM: amphetamine; MAM: methamphetamine.
[b] TFA: trifluoroacetyl; AC: acetyl; L-TPC: trifluoroacetyl-L-prolyl; PFP: pentafluoropropionyl.

while their applications to the analysis of amphetamine and related drugs are summarized in Table 3. Representative applications are further reviewed below.

In 1988, König et al. [81] resolved enantiomers of *N*-trifluoroacetylamphetamine and *N*,*O*-di-(trifluoroacetyl)-4-hydroxyamphetamine on a capillary GC column which was coated with 2,6-di-*O*-pentyl-3-*O*-acetyl-β-cyclodextrin stationary phase.

Jin and Beesley [82] compared the effectiveness and the elution orders of capillary GC columns coated with three different chiral phases, 2,6-di-*O*-pentyl-3-*O*-trifluoroacetyl-β-cyclodextrin (B-TA), 2,6-di-*O*-pentyl-3-*O*-trifluoroacetyl-γ-cyclodextrin (G-TA) and 2,6-di-*O*-pentyl-3-*O*-propionyl-γ-cyclodextrin (G-PN) for the resolution of amphetamine and methamphetamine enantiomers. Following the derivatization by acetic anhydride or trifluoroacetic anhydride, separation was carried out isothermically. All enantiomers of amphetamine and methamphetamine were separated within 30 min with separation factor ranging between 1.02 and 1.06.

Armstrong et al. [83] used Chiraldex G-PN column to resolve trifluoroacetylated derivatives of amphetamine, methamphetamine, ephedrine, and pseudoephedrine enantiomers. Unexpectedly, trifluoroacetylated amphetamine has the opposite enantiomeric elution order as acetylated amphetamine.

Wan et al. [84] used a mixed G-TA and OV-7 mixed phases for the analysis of underivatized amphetamine and claimed improvement in thermal stability and shorter analysis time.

Hasegawa et al. [85] adapted Chirasil Dex-CB column to differentiate the metabolites derived from the use of selegiline from the abuse of methamphetamine. Urine and plasma

specimens collected from selegiline users were derivatized with pentafluoropropionic anhydride (PFPA) and the observed demethylseligiline, methamphetamine, and amphetamine were characterized as L-isomers.

2.4. Chiral high-pressure liquid chromatographic methods

Chiral stationary phases used for HPLC applications are grouped into five categories [86] as outlined in Table 4. Representative structures of these phases are illustrated in Fig. 3. Further description of these phases and their performance characteristics are available in a review by Sellers et al. [19], while their applications to the analysis of amphetamine and related drugs are summarized in Table 5. Representative applications are further reviewed below.

S-L-Naphthylurea column combined with UV detection was used for the analysis of 3,5-dinitrobenzoyl chloride (3,5-DNB) derivatized amphetamine enantiomers [87,88]. Bourque and Krull [89] developed an analytical approach for on-line solid-phase derivatization using a polymeric 3,5-DNB derivatizing reagent for the determination of D,L-amphetamine in urine.

The enantiomers of primary, secondary, and some tertiary amines were derivatized as carbamate derivatives formed by reaction with β-naphthyl chloroformate. The enantiomeric carbamates are resolved on an available Pirkle-type HPLC chiral stationary phase consisting of (*R*)-*N*-(3,5-dinitrobenzoyl)-phenylglycine covalently bonded to silica, by using a mobil phase consisting a mixture of isopropanol in hexane [90].

Nagai et al. [91] assayed the enantiomeric compositions of amphetamine and methamphetamine in human hair using a chiral cellulose-base column. In this application, the analysts were derivatized as acetamide. Methamphetamine isomers and its metabolites excreted in rat urine [92] were derivatized as benzoyl derivatives and analyzed by the combined Chiralcel OB-H and OJ columns, which offered good peak resolution and L/D ratio data. Mixtures of amphetamine and methamphetamine could be separated simultaneously within 25 min. Faster metabolism of L-isomers occurred after the administration of racemic methamphetamine and amphetamine, which confirms the metabolic stereospecificity. This approach also reported for simultaneous determination of optical isomers of methamphetamine, amphetamine, *p*-hydroxymethamphetamine, and *p*-hydroxyamphetamine in rats [93] and humans urine [94]. Nagai et al. [95–97] investigated the time-lapse changes of racemic ethylamphetamine in rats and humans urine base on the similar procedure. In 1998, Matsushima et al. [98] using Chiralcel OB-H and Finepack SIL

Table 4
Category of chiral stationary phase for HPLC application

Type	Force for stereoisomeric retention
Type I	Hydrogen bonding, pi–pi and dipole stacking
Type II	Primarily attractive interaction; inclusion complexes also play an important role
Type III	Formation of inclusion complexes in chiral cavities
Type IV	Formation of diastereoisomeric metal complexes with a selector ligand bound to the stationary phase
Type V	Hydrophobic and polar interaction with protein bound to the silica support

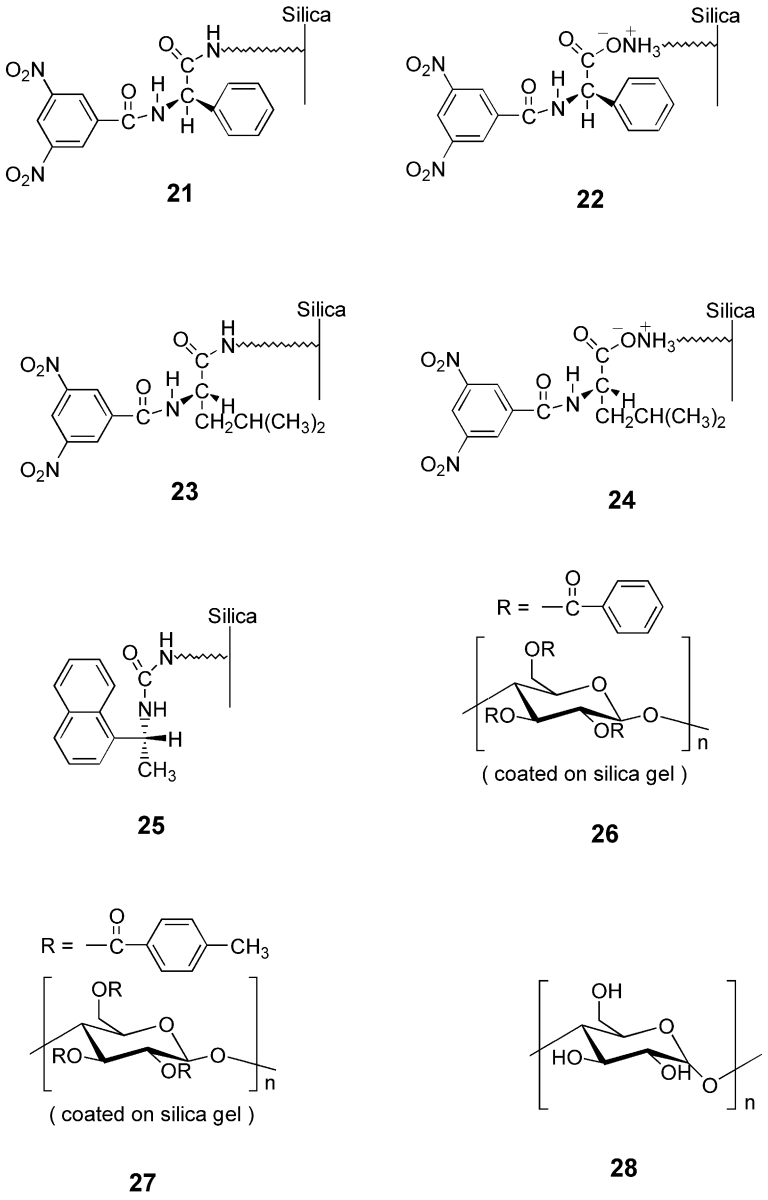

Fig. 3. Structures of HPLC chiral stationary phases. Type I: (*R*)-*N*-(3,5-dinitro-benzoyl)phenylglycine (covalent) (**21**), (*R*)-*N*-(3,5-dinitrobenzoyl)phenylglycine (ionic) (**22**), (*S*)-*N*-(3,5-dinitrobenzoyl)leucine (covalent) (**23**), (*S*)-*N*-(3,5-dinitrobenzoyl) leucine (ionic) (**24**), Supelcosil-LC-(*S*)-naphthyl urea (**25**); Type II: Chiracel OB (**26**), Chiracel OJ (**27**); Type III: β-Cyclodextrin (**28**).

columns for identification of the optical activity and simultaneous analysis of racemates MDA, MDMA and MDEA, and the urinary excretion of their optical isomers in rats was investigated.

Katagi et al. [99] determined the optical isomers methamphetamine and metabolites of amphetamine and *p*-hydroxymethamphetamine in human urine directly using HPLC thermospray–mass spectrometry with chiral β-CD phenylcarbamate-bonded silica column (ULTRON ES-PhCD by Shinwa Chemical Ind., Kyoto, Japan).

Al-Dirbashi el al. derivatized methamphetamine and its metabolites in human urine and hair (100–103) by fluorescent reagent, 4-(4,5-diphenyl-1*H*-imidazol-2-yl)-benzoyl chloride (DIB). The resulting enantiomeric pair was separated using a semi-micro Chiracel OD-RH column (Daicel Chemical Ind., Tokyo, Japan).

β-Cyclodexdrin chiral stationary phase has been used in several studies involving amphetamine or related drugs. For example, Rizzi et al. [104] adapted this chiral phase, ChiraDex (Merck, Darmstadt, Germany), to compare the efficiency in resolving the enantiomers of amphetamine, methamphetamine, and various ring-substituted amphetamines using two different approaches: (a) without derivatization and (b) derivatization with phenyl isothiocyanate (PITC), naphthyl isothiocyanate (NITC), and 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate (AQC). This approach is further compared with chiral derivatization approach (Marfey's reagent) using a C18 column. Lemr et al. [105] used a β-cyclodexdrin stationary phase (ChiraDex) to study the influence of various parameters (mobil phase composition, pH, organic solvent, salt nature and concentration, flow rate, injection amount, and temperature) on enantiomeric separation of ephedrine, methamphetamine, and selegiline. Furthermore, Sadeghipour and Veuthey [106] separated the enantiomers of MDA, MDMA, MDEA, and MBDB on β-cyclodexdrin packed columns (Astec Cyclobond I 2000 and Astec Cyclobond I 2000 RSP by Advanced Separation Technology, Whippany, NJ, USA) coupled to a fluorimetric detector.

Makino et al. [107] developed a direct assay system for urine specimens. This approach included two separation processes. A strong cation-exchange precolumn was used to remove neutral anionic substances in urine; methamphetamine enantiomers trapped in the column are then transferred to and separated by a phenyl-β-cyclodexdrin-bonded semi-microcolumn (Chiral Drug™ by Shiseido: Tokyo, Japan).

Chiral stationary phases derived from crown ethers have also been applied to the analysis of amphetamine and related drugs. For example, DAICEL CROWNPACK CR (+) (Daicel Chemicals, Japan) was used by Makino et al. [108] for the determination of methamphetamine and amphetamine enantiomers. As little as 0.1% D-amphetamine in bulk methamphetamine could be determined.

2.5. Enantiomeric separation by active mobile phases in high-pressure liquid chromatographic methods

Transient diastereomeric complex formation is the fundamental mechanism underlying chiral separation of enantiomers. Although not a widely adapted approach; nevertheless, transient complexes can be formed between the analyte and a chiral component in the mobile phase. The variance in elution times is a result of the rate of complex formation as well as the difference in the affinities of the resulting complexes for the mobile phase or for the stationary phase. Wainer [86] summarized enantiomer resolutions involving the addition of chiral additives to the mobile-phase through the formation of three types of transient

Table 5
Chiral stationary phase for HPLC procedures for enantiomeric analysis of amphetamine and related drugs and metabolites

Chiral stationary phase	Compound analyzed[a]	Specimen	Derivatizing reagent	Detection[b]	Reference
Supelcosil LC-(*S*)-naphthylurea (Supelco UK, Poole, UK)	AM	Human urine	3,5-DNB	LCD-6AV	[87]
Supelcosil LC-(*S*)-naphthylurea (Supelco, Bellefonte, PA)	AM, MAM, ephedrine, pseudopehedrine, norephedrine	Human urine	3,5-DNB	DAD-UV	[88]
Supelcosil LC-(*S*)-naphthylurea	AM, norephedrine	Human urine	3,5-DNB	UV–VIS	[89]
(*R*)-*N*-(3,5-dinitrobenzoyl)-phenylglycine covalently bonded (Regis)	AM, MAM, ephedrine, pseudopehedrine, norephedrine, norpseudopehedrine, *p*-OH-AM, benzphetamine	Standard	β-naphthyl chloroformate	UV–VIS	[90]
Chiralcel OB, OJ column (Diacel Ind., Tokyo, Japan)	AM, MAM	Human hair	Acetyl	UV (220 nm)	[91]
Chiralcel OB, OJ column	AM, MAM	Rat urine	Benzoyl	UV (220 nm)	[92]
Chiralcel OB, OJ column	AM, MAM, *p*-OH-MAM, *p*-OH-AM	Rat urine	Benzoyl	UV (220 nm)	[93]
Chiralcel OB-H column	AM, MAM	Human urine	Benzoyl	UV (220 nm), OR-2 polarimeter (450 nm)	[94]
Chiralcel OB-H column	EAM, AM	Rat urine	Benzoyl	UV (220 nm)	[95]
Chiralcel OB-H, OJ column	EAM, AM, *p*-OH-EAM, *p*-OH-AM	Rat urine	Benzoyl	UV (220 nm)	[96]
Chiralcel OB-H column	EAM, AM	Human urine	Benzoyl	UV (220 nm)	[97]
Chiralcel OB-H, Finepack SIL (Jasco, Tokyo, Japan)	MDA, MDMA, MDEA	Rat urine	None	UV (220 nm), polarimeter (450 nm)	[98]
Ultron ES-PhCD, ES-CD (Shinwa Chemical Ind.)	MAM, AM, *p*-OH- MAM	Human urine	None	TSP-MS, UV (220 nm)	[99]
Chiralcel OD-RH (Diacel Ind.)	AM, MAM, *p*-OH- MAM	Human urine, hair	DIB	Fluorescence	[100–103]
ChiraDex (Merck)	AM, MAM, *p*-OH-AM, *p*-OMe-AM, MDMA, MDEA, DOB, DOET, 2,5-dimethoxy-MAM DMA,	Forensic sample	PITC, NITC, AQC	UV	[104]
ChiraDex	Ephedrine, MAM, selegiline	Standard	None	UV (206 nm)	[105]

Table 5 (*continued*)

Chiral stationary phase	Compound analyzed[a]	Specimen	Derivatizing reagent	Detection[b]	Reference
Astec Cyclobond I 2000, I 2000 RSP (Advanced Separation Technologies)	MDA, MDMA, MDEA, MBDB	Standard	None	Fluorescence	[106]
Chiral Drug™ (Shiseido)	AM, MAM	Human urine	None	DAD (210 nm)	[107]
DAICEL CROWNPACK CR (+) (Diacel)	MAM, AM	Human urine	None	DAD	[108]
ChiraDex; Chiral CHB (Chrom Tech: Hängersten, Sweden)	MDEA, MDA, HME	Human plasma	None	Fluorescence, electrochemical	[109]
(+)-(18-Crown-6)-2,3,11, 12-tetracarboxylic acid bonded to silica gel	AM	Standard	None	Absorbance	[110]
Regis Pirkle covalent Phenylglycine; Regis Pirkle Ionic Phenylglycine	AM, MAM	Standard	L-TPC	UV (254 nm), MS	[111]

[a] AM: amphetamine; MAM: methamphetamine; MDMA: methylenedioxymethamphetamine; MDA: methylenedioxyamphetamine; MDEA: methylenedioxyethylamphetamine; DOB: 4-bromo-2,5-dimethoxyamphetamine; DOET: 2,5-dimethoxy-4-ethylamphetamine; DMA: 2,5-dimethoxyamphetamine; MBDB: *N*-methyl-1-(3,4-methylenedioxyphenyl)-2-butanamine; HME: *N*-ethyl-4-hydroxy-3-methoxyamphetamine.

[b] 3,5-DNB: 3,5-dinitrobenzoyl chloride; DIB: 4-(4,5-diphenyl-1*H*-imidazol-2-yl)-benzoyl chloride; PITC: phenyl isothiocyanate; NITC: naphthyl isothiocyanate; AQC: 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate; L-TPC: trifluoroacetyl-L-prolyl.

diastereomeric complexes: inclusion complexes, ion pairs, and transition metal ion complexes.

This approach has been applied to the analysis of amphetamine-related drugs. For example, Brunnenberg and Kovar [109] incorporated β-cyclodexdrin in the mobile phase for the analysis of MDEA and MDA and a chiral protein phase (chiral-CBH) for *N*-ethyl-4-hydroxy-3methoxyamphetamine (HME) to investigate the enantioselective metabolism of MDEA and its metabolites (MDA and HME) in human plasma.

2.6. Combined application of chiral derivatization reagent and chiral stationary phase

Approaches involving combined use of chiral derivatization and chiral stationary phase GC and HPLC analysis have also been explored. For example, Liu and Ku [24] and Liu et al. [25] combined L-TPC derivatization and Chirasil-Val (Applied Sciences, State College, PA) chiral stationary phase GC for the analysis of amphetamine enantiomers. With this approach, the two minor diastereomeric pair derived from D-TPC impurity were also resolved, resulting in the observation of four chromatographic peaks; the optical purity of the chiral derivatization reagent L-TPC can thus be determined.

In a parallel HPLC/MS study using an ionic *N*-3,5-(dinitrobenzoyl)phenylglycine chiral phase (Regis, Morton Grove, IL) [111], enantiomers of amphetamine and methamphetamine were resolved. Another study [112] conducted by the same group involved the use of

L-TPC and GITC chiral derivatization reagents and achiral (C18) and chiral stationary (SUPELCOSIL® LC-(*S*) Naphthyl Urea phases (Supelco, Bellefonte, PA)) for the analysis of ephedrine and pseudoephedrine. The following observations were reported:

- Differences in the elution orders of the resulting L-TPC and GITC derivatives. The order for the resulting L-TPC derivatives is: L-ephedrine, D-ephedrine, D-pseudoephedrine, and L-pseudoephedrine, while the order for the GITC derivatives is: L-pseudoephedrine, D-pseudoephedrine, L-ephedrine, and D-ephedrine.
- With the HPLC parameters investigated, the C18 column does not provide adequate base-line resolution for the four components of ephedrine and pseudoephedrine. With optimal HPLC parameters, the chiral column achieved base-line separation for L-TPC derivatives (with the exception of D-ephedrine and D-pseudoephedrine) and similar base-line separation for GITC derivatives (with the exception of L-ephedrine and D-pseudoephedrine). Sequential analysis of L-TPC and GITC derivatives, with the naphthyl urea chiral column using THF/water compositions of 20:80 and 30:70, respectively, will allow for base-line separation and quantitation of four enantiomeric compositions—D-/L-ephedrine and D-/L-pseudoephedrine.
- The detector's (254 nm) responses toward the GITC derivatives were approximately 60 times greater than that for the corresponding L-TPC derivatives.

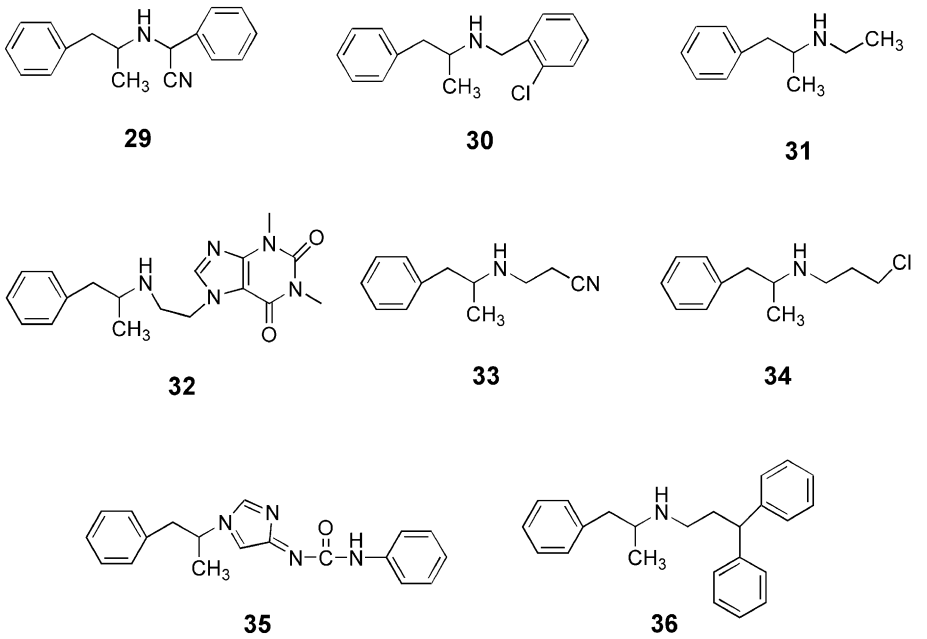

Fig. 4. Structures of amphetamine-generating precursor compounds: amphetaminil (**29**), clobenzorex (**30**), ethylamphetamine (**31**), fenethylline (**32**), fenproporex (**33**), mefenorex (**34**), mesocarb (**35**), prenylamine (**36**).

3. Stereospecific metabolic processes and the effect of urine pH condition

Metabolic fates of many amphetamine-related drugs have been thoroughly studied. The most important chemical processes are: aromatic hydroxylation at the 4-position, aliphatic hydroxylation at the β-carbon position, *N*-dealkylation, oxidative deamination, *N*-oxidation, and conjugation of the nitrogen.

N-demethylation reaction of methamphetamine was reportedly stereospecific with D-enantiomer being more rapidly proceeded. Thus, 16 h after the administration of racemic methamphetamine, *S*-D-amphetamine was shown to be predominant [113]. On the other hand, D-amphetamine was known to proceed with the β-hydroxylation at a faster rate [114,115]. Since the percentage of D-amphetamine was reportedly higher than the relative percentage of D-methamphetamine [14], the preferential conversion of D-methamphetamine to D-amphetamine is apparently more significant than the preferential β-hydroxylation of D-amphetamine.

Urine pH conditions can significantly affect the excretion patterns of amphetamines. Since amphetamines are basic drugs (with pK_a approximately 9.9), reabsorption at the kidneys is insignificant under acidic urine conditions, resulting in the excretion of more parent drugs. Under alkaline urine conditions, more significant reabsorption occurs, with a net effect of increased drug half-lives. Increased metabolic degradation will be observed.

Although urine pH conditions do not appear to cause differential excretion of D- and L-enantiomers [116], its effect on the reabsorption rate will have a secondary effect on the observed enantiomeric composition.

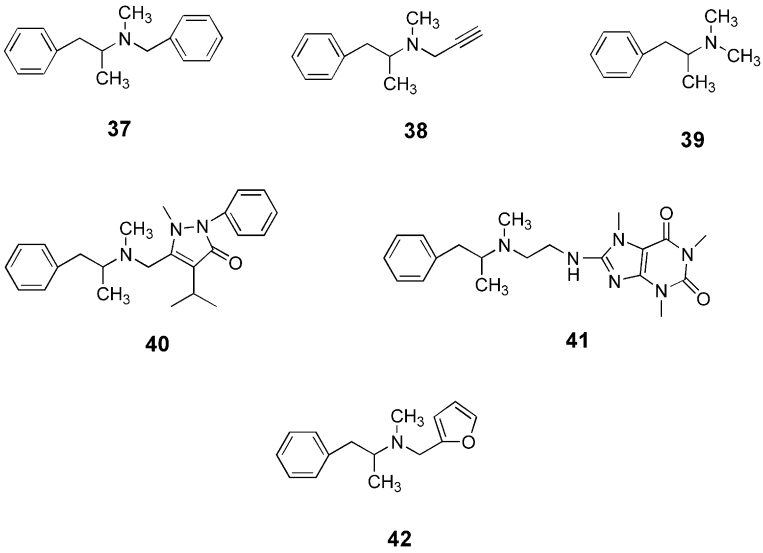

Fig. 5. Structures of amphetamine and methamphetamine-generating precursor compounds: benzphetamine (**37**), deprenyl (**38**), dimethylamphetamine (**39**), famprofazone (**40**), fencamine (**41**), furfenorex (**42**).

4. Amphetamine and methamphetamine metabolic precursor drugs

It is now well known that the use of a number of drugs can result in metabolic production of amphetamine (alone) or methamphetamine and amphetamine [16,21,22,117]. Many of these *precursor compounds* belong to the category of anorectics but are often used for treating obesity; they are used because of their own therapeutic activities and are not administered for the purpose of producing amphetamine or methamphetamine. Drugs that have been studied and reportedly producing amphetamine are: amphetaminil, clobenzorex, ethylamphetamine, fenethylline, fenproporex, mesocarb, prenylamine, and mefenorex (Fig. 4). Those are known to produce methamphetamine and amphetamine are: benzphetamine, deprenyl (selegiline), dimethylamphetamine, famprofazone, fencamine, and furfenorex (Fig. 5).

The review of these compounds in this article will be limited to (a) methods used to determine the enantiomeric compositions of these compounds and their metabolites; (b) enantiomeric composition characteristics of these drugs and their metabolites, especially amphetamine and methamphetamine; and (c) interpretative value of the observed enantiomeric composition. Thus, the focus of the review is placed in analytical methodology and data interpretation that may be helpful to differentiating amphetamine or methamphetamine produced by these precursor drugs from those derived from the abuse of amphetamine or methamphetamine. Most significant information in these aspects is summarized in Tables 6 and 7.

Table 6
Precursors for amphetamine

Name	Structure[a]	Medical use	Urinary specific metabolites	Chirality	Reference
Amphetaminil	**29**	Psychostimulant; anorectic	–	–[b]	[118–120]
Clobenzorex	**30**	Anorectic; psychostimulant; sympathominetic	4-Hydroxyclobenzorex; clobenzorex	(+)	[121–127]
Ethylamphetamine	**31**	Psychostimulant; anorectic; sympathominetic	Ethylamphetamine; *p*-OH-ethylamphetamine	Racemic	[95–97, 128,129]
Fenethylline	**32**	Psychostimulant	Theophylline metabolites	–[b]	[130–134]
Fenproporex	**33**	Anorectic; psychostimulant	Fenproporex	Racemic	[31,130, 135–137]
Mefenorex	**34**	Psychostimulant; anorectic	Mefenorex; 4-hydroxymefenorex	–[b]	[138,139]
Mesocarb	**35**	Psychostimulant	Mono- and di-hydroxymesocarb	–[b]	[140]
Prenylamine	**36**	Coronary vasodilator	Diphenylpropylamine	Racemic	[141–149]

[a] Only structure designations are listed. See Fig. 4 for the structures and names of these drugs.
[b] Not reported.

Table 7
Precursors for methamphetamine and amphetamine

Name	Structure[a]	Medical use	Urinary specific metabolites	Chirality	Reference
Benzphetamine	**37**	Anorectic	1-(4-Hydroxyphenyl)-2-(*N*-benzylamino)-propane	(+)	[32,150–156]
Deprenyl (selegiline)	**38**	Antiparkinsons; MAO-B inhibitor	Desmethyldeprenyl; deprenyl-*N*-oxide	(−)	[34,85, 157–161]
Dimethylamphetamine	**39**	Illicit drug	Dimethylamphetamine-*N*-oxide; dimethylamphetamine	–[b]	[162–165]
Famprofazone	**40**	Antipyretic; analgesic	Famprofazone; *p*-hydroxydemethyl-famprofazone	Racemic	[33,36, 166–168]
Fencamine	**41**	Psychostimulant	Fencamine	Racemic	[153]
Furfenorex	**42**	Anorectic	1-Phenyl-2-(*N*-methyl-*N*-γ-valerolactonyl-amino) propane	–[b]	[170,171]

[a] Only structure designations are listed. See Fig. 5 for the structures and names of these drugs.
[b] Not reported.

4.1. Amphetamine-generating drugs

Metabolic products of *Amphetaminil* include amphetamine, hydrocyanic acid, and benzaldehyde. Following administration to humans or rats, the unchanged parent drug was not detectable in the urine sample [118–120]. The enantiomeric composition of this drug has not been reported in the open literature.

Clobenzorex was first reported as a racemic drug [121], but was later identified as *S*-D-enantiomer [122]. This drug has been used as an adulterant in traditional Chinese medicine [123–125]. A series of recent studies conducted by Valtier and Cody also confirmed the chirality of this drug [126]. In these studies, chiral derivatization reagent, L-TPC, and HP-1 column were used for enantiomeric determination.

Although the use of this drug can result in the observation of amphetamine, it is rather easy to differentiate the source of the observed amphetamine, thanks to the presence of 4-hydroxyclobenzorex (a metabolite of clobenzorex) which is detectable for at least as long as amphetamine [127].

The chirality of *Ethylamphetamine* has long been established as racemic mixture [128]. Time-lapse changes of D- and L-enantiomers observed in human [97] and rat [95,96] urine have also been studied. HPLC methods using Chiralcel OB-H and Chiral OJ columns (Daicel Chemical Ind.) columns were found effective for enantiomeric determination [99].

Under controlled conditions, the *S*-D-enantiomer was metabolized more rapidly than the L-enantiomer [128]. The time-lapse changes of D- and L-enantiomers of racemic ethylamphetamine in the urine of rats and humans has been studied by Nagai et al. [95–97]. In humans, the excreted dose of parent *R*-L-ethylamphetamine was found in higher percentage than that of *S*-D-ethylamphetamine, while the percentage of the excreted D-amphetamine metabolite was found larger than that of L-amphetamine. The L/D ratios ranged between 1.22 and 1.29. Rat studies produced different results, in where higher levels of

D-enantiomers of ethylamphetamine and amphetamine were excreted than the L-enantiomers. The ratio of L/D was 0.51.

Presence of the parent drug and the hydroxylated metabolites, which can be detected at a higher concentration and longer period after use, can be used to confirm the use of this drug [129].

Fenethylline is used medically as a psychostimulant for the treatment of children with attention deficient disorders and has been used for the treatment of narcolepsy [130] or as an antidepressant [131]. Enantiomeric composition of this drug has not been reported in the open literature.

In a study using radiolabeled fenethylline, a relatively small amount (3.6%) of the parent drug was found to excrete intact, while the percentages of the amphetamine, hippuric acid, and 4-hydroxyamphetamine metabolites were found to be 24.5, 27.2, and 6.6%, respectively [132]. Theophylline, the *N*-dealkyl metabolite, was also found in urine. During the first 24 h, the measured amount was approximately 13.7% of the dose given [132].

Human studies of three volunteers (30-mg oral administration) conducted by Yoshimura et al. [133] showed that carboxymethyl-theophylline and amphetamine were major metabolites in the urine. Carboxymethyl-theophylline was found to disappear faster than amphetamine, with only trace levels at 24–48 h. Amphetamine was found in higher concentration in the same time interval.

Kikura and Nakahara [134] investigated the detection of fenethylline and its metabolites in human hair, following single-dose (50 mg/day, 1 day, $n = 1$) and multiple-dose (50 mg/day, 3 days, $n = 5$) oral administration. In the proximal 1-cm segments, the concentrations of fenethylline and amphetamine detected from subjects with multiple- and single-dose were 0.51 ± 0.23 and 0.35 ± 0.12 and 0.25 and 0.11 ng/mg, respectively. With the exception of one sample, the concentration of fenethylline in human hair was found to range from 1.2 to 2.7 times greater than that of amphetamine. This is in contrast to rapid disappearing of fenethylline in urine. Thus, hair samples may be valuable for confirming the use of fenethylline.

Fenproporex is classified as a nonstimulant anorectic drug used for short-term treatment of moderate to severe obesity [121,130]. Tognoni et al. [135] demonstrated that administration of fenproporex leads to formation of considerable amounts of amphetamine in the body via cleavage of the nitrogen-cyanoethyl bond. A small amount of the parent drug is excreted for a period of approximately 3-h postdose, whereas the metabolite amphetamine is detectable for days [136].

Amphetamine was detected in urine specimens collected from all five subjects studied. The peak concentration appeared at approximately 6–20-h postdose and ranged from approximately 1200 to 2100 ng/ml. It was detected (>5 ng/ml) in the urine for up to 119 h [31]. Metabolic amphetamine was a mixture of D- and L-enantiomers.

In a later study [137], fenproporex was detected for longer periods than previously reported. All urine samples with amphetamine higher than 500 ng/ml were also found to contain detectable amounts of the parent drug for longer than 20-h postdose. The presence of the parent drug and its relative concentration provides conclusive evidence for the involvement of this drug.

Early study has shown the excretion of minimal (1%) intact *mefenorex* in rat and human [138]. More recent study by Kraemer et al. [139] identified mefenorex and 13 metabolites,

including 4-hydroxymefenorex, 4-hydroxyamphetamine, and 4-hydroxynorephedrine, in urine. The metabolite 4-hydroxymefenorex is a useful indicator and is found in much higher concentrations than the parent drug. After a single dose of 80 mg mefenorex, the parent drug could only be found for 16–20 h and 4-hydroxymefenorex for about 32 h. Amphetamine could be detected from 32 to 68 h after ingestion. No study on the enantiomeric composition of this drug was found in the open literature.

Since the parent drug and specific metabolites are not detectable for as long as the metabolite amphetamine, misinterpretation of positive amphetamine tests is possible.

Rat study [140] on the metabolism of *Mesocarb* showed minimal excretion (1%) of the parent drug, while the dihydroxy, hydroxy, and amphetamine metabolites showed 60%, 22%, and 4%, respectively. No study on the enantiomeric composition of this drug was found in the open literature.

Prenylamine is marketed as a racemic mixture. The metabolism of this drug has been well studied [141–149]. *R*-L-Naphthylethyl isocyanate was used as the chiral derivatization reagent to study the enantioselective characteristic on the metabolism of this drug [144]. Metabolism of *S*-D-prenylamine was faster than *R*-L-prenylamine, with *S*-D-metabolites excreted more rapidly. In plasma, the amount of *R*-L-prenylamine and *S*-D-prenylamine was found in 4:1 ratio [146].

In addition to amphetamine, 4-hydroxyamphetamine, norephedrine, 4-hydroxynorephedrine, and diphenylpropylamine were identified as metabolites [147–149].

4.2. Methamphetamine and amphetamine-generating drugs

Benzphetamine is synthesized from *S*-D-methamphetamine as a pure *S*-D-enantiomer. It is prescribed as an anorectic in the treatment of obesity as adjunct to a reduced caloric diet [150,151]. The metabolism of benzphetamine is nearly complete with little, if any, excreted as unchanged drug [152,153]. 1-(4-Hydroxyphenyl)-2-(*N*-benzylamino)propane, an aromatic hydroxylation and *N*-demethylation product, has been identified as the major metabolite in urine [154,155]. Typically, samples tested for amphetamine and methamphetamine are not hydrolyzed. Hydrolysis is needed for full recovery of 1-(4-hydroxyphenyl)-2-(*N*-benzylamino)propane. Recently, Sato et al. [156] developed a β-glucuronidase hydrolysis, solid-phase extraction, and LC–MS procedure for the analysis of benzphetamine and its five metabolites in rat urine.

With respect to the excretion of metabolites in the urine, Cody and Valtier [32] have reported two distinctly different results following a single 50-mg oral dose of benzphetamine–HCl. It was shown that the metabolic production of amphetamine derived from methamphetamine and, to a substantial degree, also from demethylbenzphetamine. Thus, several subjects studied excreted substantially higher level of methamphetamine, while others excreted more amphetamine than methamphetamine.

The following analytical findings resulting from the use of benzphetamine may help identify the source of amphetamine and methamphetamine: (a) potential identification of 1-(4-hydroxyphenyl)-2-(*N*-benzylamino)propane; (b) methamphetamine and amphetamine exist in *S*-D-enantiomeric forms; and (c) amphetamine/methamphetamine >1 for those individuals with metabolic characteristic in generating higher amphetamine level than methamphetamine.

Deprenyl (selegiline), in combination with levodopa, is used primarily for treating Parkinson's disease [157,158]. Deprenyl was shown to be extensively metabolized to form desmethyldeprenyl, methamphetamine, amphetamine, and their conjugated *p*-hydroxy derivatives [159,160]. Desmethyldeprenyl can be detected in urine for less than 12 h after a single oral dose of 2.5–10 mg deprenyl [34,85]. Recently, Katagi et al. [161] identified deprenyl-*N*-oxide in two deprenyl users' urine, with abundance higher than three times of desmethyldeprenyl.

Deprenyl serves as a good example for using enantiomer data to differentiate the sources of amphetamine and methamphetamine derived from the use of licit drugs from abused substances. Deprenyl is manufactured as *R*-L-enantiomer; thus generating L-amphetamine and L-methamphetamine metabolites, while those derived from abused substances exists as racemates or D-enantiomers. In cases in which L-methamphetamine is administered, differentiation can be made based on the identification of unique deprenyl metabolites, such as deprenyl-*N*-oxide or desmethyldeprenyl.

Dimethylamphetamine is not known as a prescription drug. Dimethylamphetamine showed similar metabolic pattern in rats and humans, with the exception of more pronounced aromatic hydroxylation level in rats [162–164]. Major metabolic products in humans include the parent drug, dimethylamphetamine-*N*-oxide, methamphetamine, and amphetamine. Recently, Katagi et al. [165] developed a simple and sensitive LC–MS procedure for the simultaneous determination of dimethylamphetamine and its metabolites dimethylamphetamine-*N*-oxide, methamphetamine, and amphetamine in urine.

Since dimethylamphetamine-*N*-oxide in urine can be satisfactorily detected in urine even 3–5 days after the intake of dimethylamphetamine, it can serve as an effective indicator for identifying dimethylamphetamine use.

Famprofazone is a component of the multi-ingredient medication Gewodin used as an antipyretic and analgesic (with some slight sympathomimetic properties) [130]. Each tablet contains 25 mg famprofazone, 250 mg paracetamol (acetaminophen), 75 mg isopropylphenazone and 30 mg caffeine. This drug has been demonstrated to metabolically produce methamphetamine and amphetamine and has been shown to be the cause of positive urine drug testing [166].

Cody [33] investigated the enantiomeric composition of metabolic amphetamine and methamphetamine following a single 50-mg dose of famprofazone and reported higher concentration of L-methamphetamine than D-methamphetamine in each sample. L-Methamphetamine in the first sample was 67% which increases to 100% in the last few sample. The composition of amphetamine enantiomers were much closer, with L-amphetamine beginning at approximately 50% and rising only to approximately 55% in the later samples. These enantiomeric composition characteristics are of reference value for determining the source of amphetamine and methamphetamine.

The identification of 3-hydroxymethylpyrazolone metabolite provides better proof of famprofazone administration. However, it should be noted that 3-hydroxymethylpyrazolone is also a metabolite of propylphenazone. Intake of famprofazone can be proved through the identification of the unchanged parent drug or the longer-lasting metabolite, *p*-hydroxydemethylfamprofazone in urine [167–169].

Mallol et al. [169] studied the metabolism of *Fencamine* in rats and humans and reported the detection of intact fencamine in humans for 48 h following a 50-mg oral dose

of the drug. Approximately 32% of the dose was excreted as the intact drug in 48 h and 26.6% in the first 24 h in humans.

Fencamine is manufactured as a racemic mixture. Enantiomeric composition of the resulting metabolites can be of assistance in confirming whether fencamine is ingested.

Administration of *Furfenorex* to human resulted in nearly complete conversion with very little of the parent drug excreted in urine. Of various metabolites identified, 1-phenyl-2-(*N*-methyl-*N*-γ-valerolactonylamino)propane was found to be unique and characteristic of the use of furfenorex [170]. This metabolite was also excreted at higher levels than amphetamine, methamphetamine, and their hydroxylated compounds, the sum of which represents only about 6% of the dose [153,171].

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