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Analysis of enantiomers of chiral phenethylamine drugs by capillary gas chromatography/mass spectrometry/flame–ionization detection and pre-column chiral derivatization

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Abstract

Several important chiral phenethylamine agents such as mexiletine, fenfluramine, amphetamine, methamphetamine and *N*-*n*-propylamphetamine show stereoselective disposition in humans and large differences in therapeutic relevance and toxicity. To analyze the enantiomers of chiral amine drugs, stereoselective methods were developed to separate those enantiomers on an achiral capillary gas chromatography by pre-column chiral derivatization with *S*-(−)-*N*-(fluoroacyl)-prolyl chloride. The stereoselectivity and sensitivity can be improved by chiral derivatization. The methods established offer enantioselective, simple, flexible and economic approaches for the analysis of chiral amine drug enantiomers in biological fluids. The methods have been used to determine *S*-(+)-methamphetamine in human forensic samples and to analyze enantiomers of amphetamine and fenfluramine in rat liver microsomes.

Keywords: Chiral derivatization; Gas chromatography; Enantiomers; Chiral amine drug

1. Introduction

A drug containing an asymmetric carbon atom is termed a chiral drug. Most synthetic chiral drugs were marketed as racemates consisting of an equimolar mixture of two enantiomers. In general, only one enantiomer is pharmacologically active and the other is inactive or without toxicological relevance. The separation of enantiomers is very important

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in the pharmaceutical industry. Consistent with the current focus on issues of stereoselectivity, regulatory agencies have placed emphasis on safety and efficacy of stereoisomers in drug research and development [1]. Therefore, there is a growing need for methods to separate and determine drug enantiomers. This has been one of the most difficult and challenging issues in separation science, because two enantiomers have identical chemical and physical properties, except for the opposing directions in which they rotate plane-polarized light. Development of the chiral chromatographic method suitable for the resolution and determination of optically active drugs is more than an academic exercise or useful only for the antipode impurity; it satisfies apparent bioanalytical needs.

Several important chiral phenethylamine agents such as mexiletine, fenfluramine, amphetamine, methamphetamine and *n*-propylamphetamine demonstrate stereoselective disposition in humans and large differences in therapeutic relevance and toxicity.

Two approaches to the separation of chiral drug enantiomers by using gas chromatography have been developed. One is separation of the enantiomer directly on a chiral stationary phase. The other is conversion of the enantiomer into its related diastereomers, following pre-column derivatization with chiral derivatization agents on an achiral stationary phase.

Our laboratory has developed stereospecific chromatography methods [2–7] for the determination of the enantiomers of ofloxacin, propafenone, propranolol glucuronides, atenolol, metoprolol, *p*-hydroxyphenylphenylhydantion, mephenytoin, etc. The purpose of this study is to explore the use of capillary column gas chromatography with hydrogen flame ionization detector (FID) or mass spectrometer detector (MSD) to determine of several chiral phenethylamine compounds. Specifically, achiral stationary phase was used to separate *S*-(−)-*N*-fluoroacetyl prolyl derivatives, such as mexiletine, fenfluramine, amphetamine, methamphetamine and *N*-*n*-propylamphetamine.

2. Materials and methods

2.1. Materials

Amphetamine sulfate, methamphetamine hydrochloride, mexiletine hydrochloride and fenfluramine hydrochloride were obtained from the National Institute for Drug Control of China (Beijing, China). *N*-*n*-Propylamphetamine, *R*-(−)-amphetamine, *S*-(+)-amphetamine and *S*-(+)-methamphetamine were obtained from the Sigma (St. Louis, MO, USA). *S*-(−)-*N*-(trifluoroacetyl)-prolyl chloride (*S*-(−)-TFAPC) was purchased from the Aldrich Chemical (St. Louis, MO, USA). Sodium hydroxide, triethylamine, chloroform, ethyl acetate, anhydrous sodium sulfate and all other materials were analytical and chromatographic grade.

2.2. Chromatography

2.2.1. GC/MSD method

HP 5790 gas chromatograph/5790 mass spectrometer detector system (Palo Alto, CA, USA) was used to this study. The gas chromatograph was equipped with a HP-1 fused-

silica capillary column (12 m long, 0.25 mm inside diameter and 0.25-μm film thickness); He was used as the carrier gas. The injector temperature was maintained at 250 °C. After injection, the oven temperature was held at 120 °C for 1 min and then linearly programmed to 275 °C at a rate of 10 °C/min. The mass spectrometer was operated in the electron impact model at 70 eV; the source temperature was maintained at 200 °C. Mass unit and relative abundance were calibrated with perfluorotributylamine, and spectra were collected in the range *m*/*z* from 45 to 450. The full scan and selective ion monitor (SIM) model was used to analyze drug enantiomers.

2.2.2. GC/FID method

Shimadzu GC-15A gas chromatography system (Shimadzu, Japan) was equipped with a HP-1 fused-silica capillary column (12 m long, 0.25 mm inside diameter and 0.25-μm film thickness), a hydrogen flame ionization detector and a Shimadzu C-R4A chromatopac data system. High-purity nitrogen was used as the carrier gas at a head pressure of 1 kg/cm^2 and makeup gas at flow rate of 15 ml/min. The detector temperature was set at 275 °C and the injector was maintained at 250 °C. After injection, the oven temperature was held at 100 °C for 4 min, then linearly programmed to 280 °C at a rate of 8 °C/min and was maintained at 280 °C for 2 min.

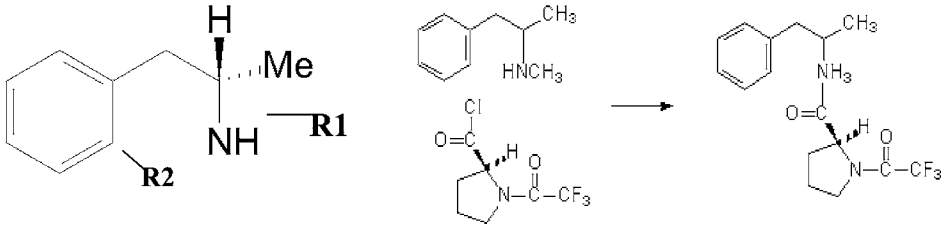
2.3. Analytical procedures

2.3.1. Aqueous phase derivatization method

All test tubes was vapor-phase silanized, rinsed with methanol and oven dried before use. Urine or liver homogenate was added to a 10-ml Teflon-lined, screw-capped test tube. The pH of the sample was adjusted to 9 by 10 M NaOH, then 0.5 ml of chilled 1 M sodium bicarbonate/sodium carbonate buffer (pH 9.0). After sample was mixed well, 40 μl of *S*-(−)-TFAPC was added, and the tube was vortexed for 30 min at room temperature. The resulting mixture was saturated with NaCl and adjusted to pH 9, then 0.5 ml of chilled 1 M sodium bicarbonate/sodium carbonate buffer (pH 9.0) and extracted with 4 ml of ethyl acetate (EtOAc) by gently rocking for 15 min. After phases separate, the organic layer was washed with 2 ml of deionized water. The organic layer was evaporated to dryness under a gentle stream of air at 40 °C. The residue was cooled to room temperature and reconstituted with EtOAc. An aliquot of 1 μl of analyte was analyzed by GC/MSD.

2.3.2. Organic phase derivatization method

All test tubes was silanized, rinsed with methanol and oven dried before use. A 1.0-ml sample of microsomal mixture spiked with chiral amine was piped into a 15-ml screw-capped test tube. The pH of the sample was adjusted to 12–13 with 40% NaOH. The mixtures were extracted with 2 ml of chloroform by rotatory shaking for 1 min. After centrifugation at 4000 rpm for 10 min, the aqueous phase was removed by aspiration. The remaining organic phase was dried by anhydrous sodium sulfate, then transferred to another clean screw-capped test tube. A 10-μl sample of triethylamine as a catalytic agent and 40 μl *S*-(−)-TFAPC were added into the test tube and mixed. The tube was capped and allowed to react for 15 min at room temperature by gentle rocking. The chloroform



Drug	R1	R2
amphetamine	H	H
methamphetamine	CH_3	H
N-n-propylamphetamine	n-C_3H_7	H
fenfluramine	C_2H_5	CF_3

Fig. 1. The structures of chiral amine drugs and their *S*-(−)-TFAPC derivatives.

layer was washed with 2 ml distilled water and evaporated to dryness at 50 °C water bath under a gentle stream of air. The residues were allowed to cool to room temperature and reconstituted with 40 μl EtoAc prior to injection. A 1-μl sample was injected into the GC/FID system.

3. Results and discussion

Amphetamine, methamphetamine, *N*-*n*-propylamphetamine, mexiletine and fenfluramine are derivatives of phenethylamine. All compounds contain one chiral carbon and exist in enantiomeric pairs. The chiral amine enantiomers were reacted with *S*-(−)-TFAPC and converted to diastereomers of amide derivatives (Fig. 1).

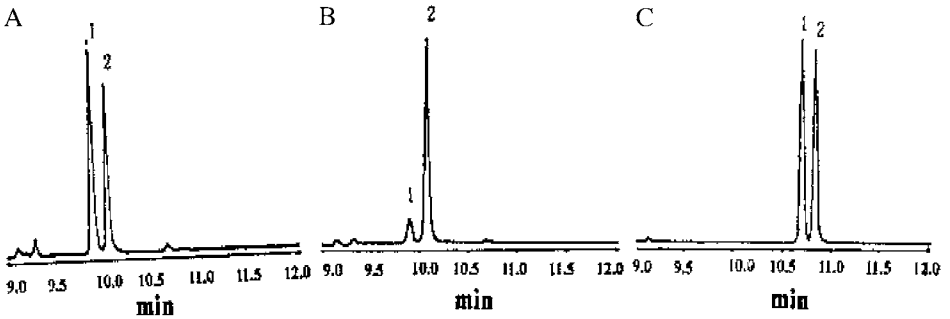


Fig. 2. GC/MS/EI of *S*-(−)-TFAPC diastereomers of methamphetamine and *N*-*n*-propylamphetamine. (A) The chromatogram of racemic methamphetamine *S*-(−)-TFAPC diastereomers. (B) The chromatogram of *S*-(+)-methamphetamine *S*-(−)-TFAPC diastereomer. (C) The chromatogram of racemic *N*-*n*-propylamphetamine *S*-(−)-TFAPC diastereomers. (1 and 2): *R*-(−)- and *S*-(+)-enantiomer.

3.1. Determination of methamphetamine enantiomers by GC/MSD

The chromatograms of separation of *S*-(−)-TFAPC derivatives of methamphetamine and *N*-*n*-propylamphetamine diastereomers are shown in Fig. 2. The diastereomers have

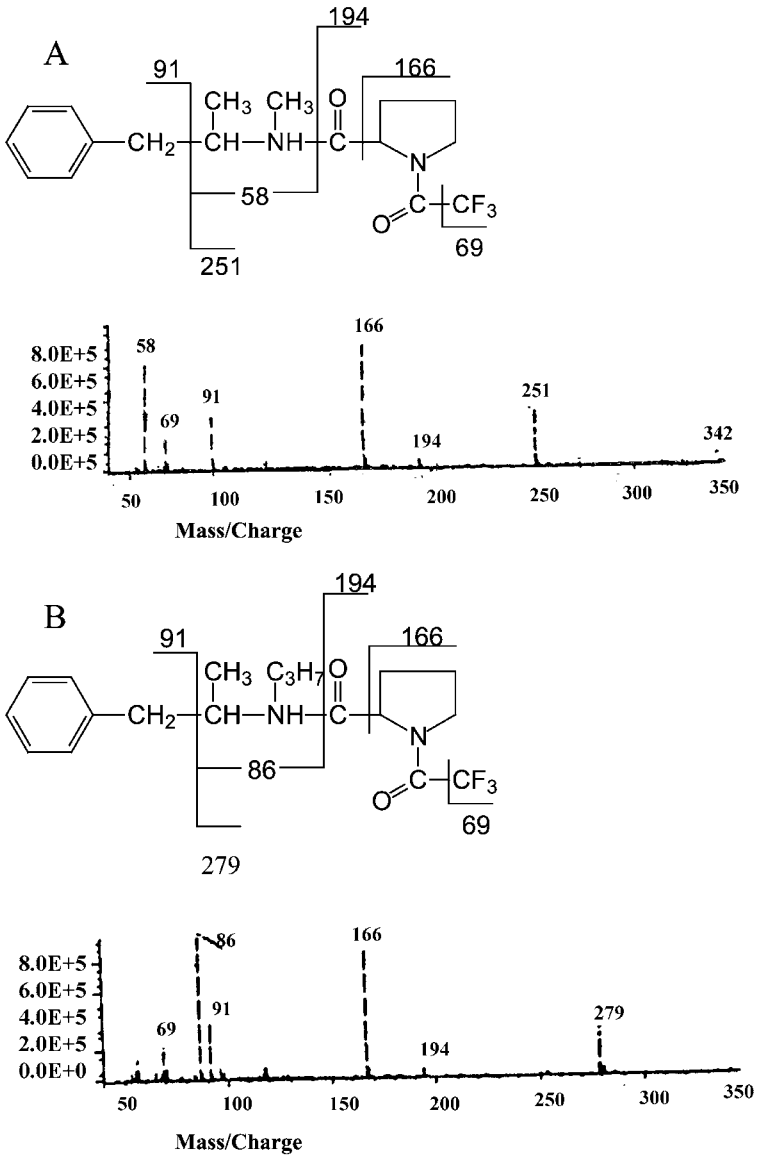


Fig. 3. Mass spectra of methamphetamine–*S*-(−)-TFAPC derivatives (A) and mass spectra of *N*-propylamphetamine–*S*-(−)-TFAPC derivatives (B).

similar mass spectra, which are shown in Fig. 3; these mass spectra correspond to published mass spectra of the *S*-(−)-TFAPC derivative of chiral amine drugs. The elution order of methamphetamine diastereomers was determined by injecting references of *R*-(−)- and *S*-(+)-methamphetamine derivatized with *S*-(−)-TFAPC and their respective retention times were determined.

For assay of methamphetamine enantiomers, the calibration curves were obtained by analyzing urine samples spiked with 10–2000 ng/ml *R*-(−)- and *S*-(+)-methamphetamine. The *S*-(−)-TFAPC derivatives were analyzed by GC/MSD in SIM mode. Monitoring ions were set at *m*/*z* 251 for methamphetamine and *m*/*z* 279 for internal standards, *N*-*n*-propylamphetamine. The method is linear from 10.0 to 2000.0 ng/ml for each enantiomer, and the regression equations based on enantiomer concentration versus the peak-area ratios based on enantiomer and internal standard were $R = 3.542 \times 10^{-4} + 3.607 \times 10^{3}$ C ($r = 0.9999$) for *S*-(+)-methamphetamine and $R = 1.087 \times 10^{-4} + 4.366 \times 10^{3}$ C ($r = 0.9991$) for *R*-(−)-methamphetamine. To determine the precision of the derivatization and extraction procedures, the urine sample spiked with methamphetamine and *N*-*n*-propylamphetamine were treated according to the analytical and chromatographic procedure. The ratio of peak area for methamphetamine and *N*-*n*-propylamphetamine is 0.94 ± 0.09 ($n = 6$) and 1.01 ± 0.04 ($n = 6$), respectively. The sensitivity (LOQ) of the method was 10 ng/ml(RSD < 20%). The derivatives of methamphetamine and *N*-*n*-propylamphetamine stored in −20 °C were stable for at least 3 days.

A urine and a liver sample from the forensic source were determined and the results showed the concentration of *R*-(−)-methamphetamine and *S*-(+)-methamphetamine to be 340.3 and 3816 ng/ml in urine, respectively, and 109.4 and 1073 ng/ml in liver, respectively. Determining whether the *S*-(+)-, *R*-(−)-, or *RS*-(±)-isomers of methamphetamine are present in urine and liver samples will help determine its source. Methamphetamine is presently available in legitimate pharmaceutical products only as the *S*-(+)-enantiomer. Fig. 4 shows the chromatograms of methamphetamine in urine (A) and liver (B).

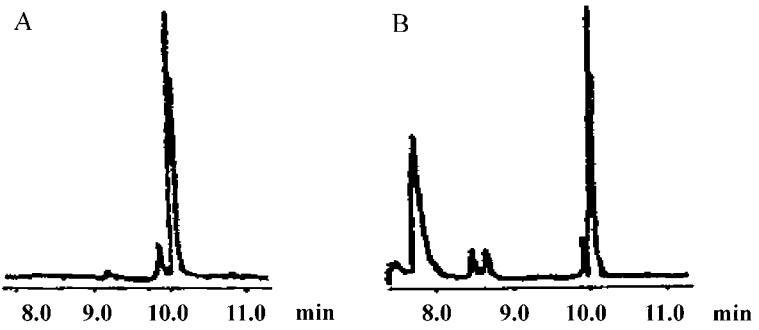


Fig. 4. Chromatograms of *S*-(+)-methamphetamine–*S*-(−)-TFAPC derivative in urine (A) and liver (B) sample from forensic source.

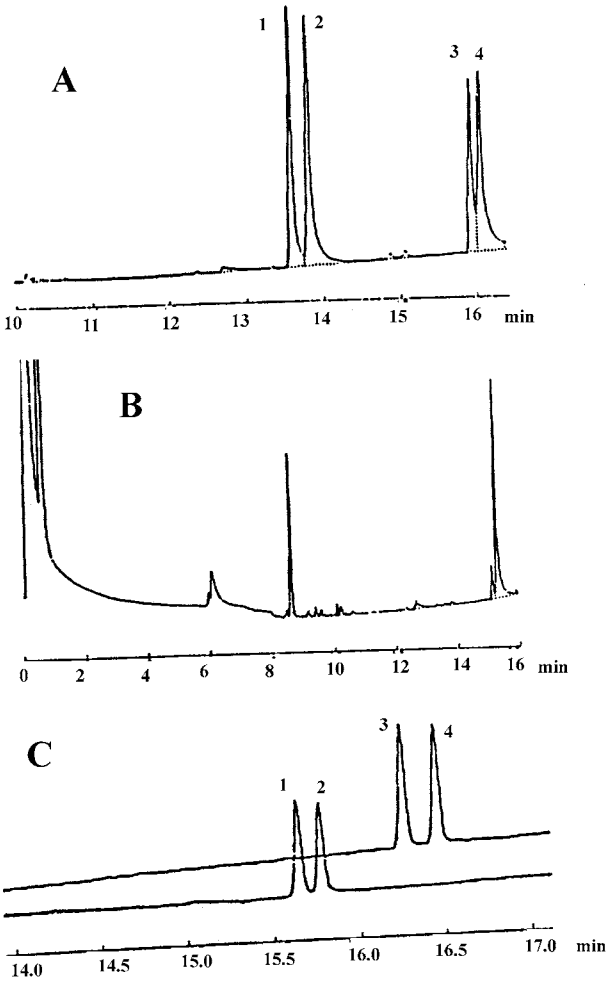


Fig. 5. Chromatograms of chiral amine enantiomers *S*-(−)-TFAPC diastereomers in rat microsomes (A) 1 and 2: *R*-(−)- and *S*-(+)-amphetamine; 3 and 4: *R*-(−)- and *S*-(+)-mexiletine, (B) *S*-(+)-methamphetamine; (C) 1 and 2: *R*-(−)- and *S*-(+)-fenfuramine, 3 and 4: *R*-(−)- and *S*-(+)-*n*-propylamphetamine.

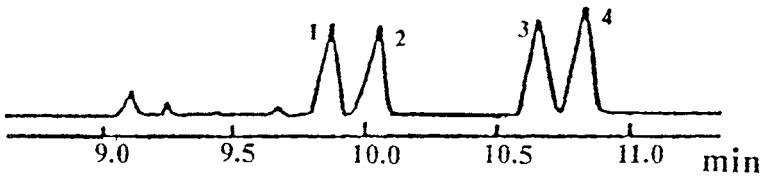


Fig. 6. Chromatogram of methamphetamine and *N*-*n*-propylamphetamine *S*-(−)-HFTPC diastereomers. 1 and 2: *R*-(−)- and *S*-(+)-methamphetamine, 3 and 4: *R*-(−)- and *S*-(+)-*N*-*n*-propylamphetamine.

3.2. Separation of enantiomers of chiral amine drugs by GC/FID

Fig. 5 showed the chromatograms of separation of derivatives amphetamine, fenfluramine, mexiletine, *N*-*n*-propylamphetamine and *S*-(+)-methamphetamine enantiomers *S*-(−)-TFAPC diastereomers by GC/FID.

The method developed allowed study of the metabolic depletion of amphetamine and fenfluramine enantiomers in rat hepatic microsome. The results showed that the metab-

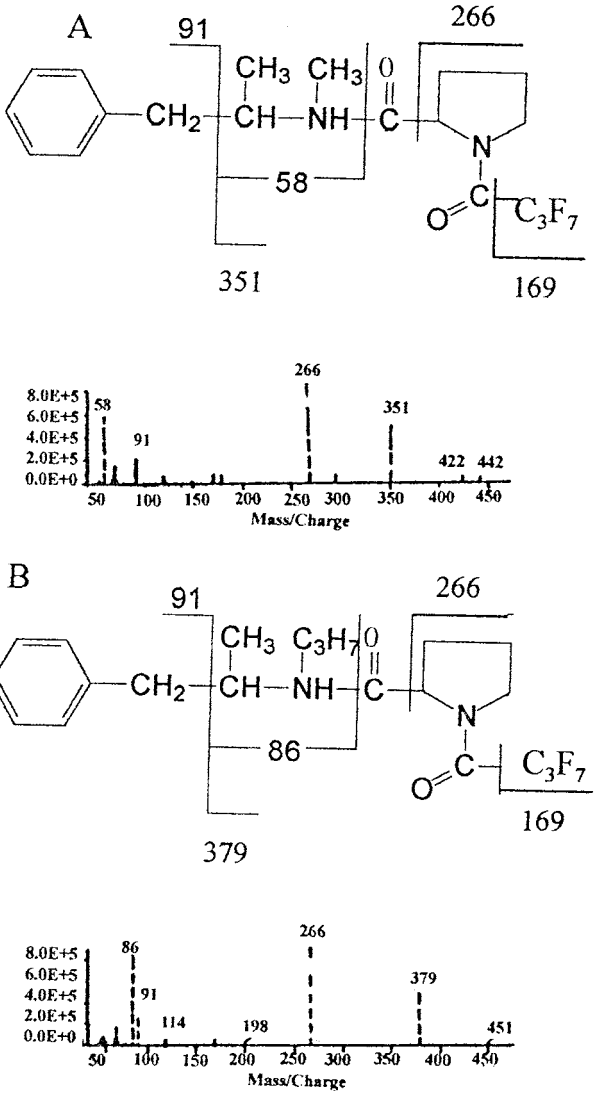


Fig. 7. Mass spectra of methamphetamine–*S*-(−)-HFTPC derivatives (A) and mass spectra of *N*-*n*-propylamphetamine–*S*-(−)-HFTPC derivatives (B).

olism of *R*-(−)-amphetamine was more rapid than that of *S*-(+)-antipode, and the difference of the stereoselectivity was increased with incubation time. The concentration ratio of *R*/*S* enantiomer was decreased from 0.95 to 0.71 when incubation time was from 5 to 40 min. Similarly, the metabolism of *R*-fenfluramine was more rapid than that of the *S*-enantiomer, but the concentration ratio of *R*/*S*-fenfluramine was about 0.66 and almost not changed.

3.3. Discussion

S-(−)-TFAPC is a useful chiral derivatization reagent for separation of chiral amine drugs enantiomers on achiral capillary column. The prolyl-peptide bone is thought to enhance the difference in physical properties of its diastereomer derivatives and to cause consequent enhancement of chromatographic separation. *S*-(−)-TFAPC is able to derivatize amine enantiomers either in aqueous bio-samples or organic solvent (phase) after enantiomers were extracted. The aqueous phase derivatization is simpler than the organic phase derivatization. The final sample solution from aqueous phase derivatization is cleaner than that from organic phase derivatization.

Mass spectrometry was used to confirm the identity of the diastereomeric derivatives. The mass spectra of the derivatized diastereomers were essentially identical (Fig. 3). The fragmentation pattern coincided with that of the derivative of TFAPC.

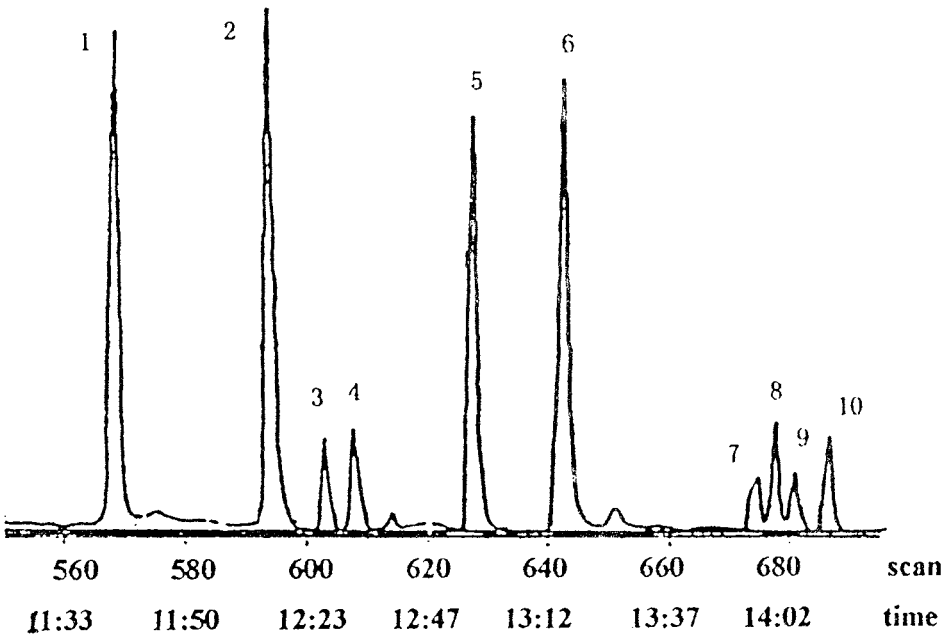


Fig. 8. Chromatogram of MDPA, MDMA and its three metabolites MDA, HMM and HMA diastereomers of derivatized with *S*-(−)-HFTPC. 1 and 2: *R*-(−)- and *S*-(+)-HMA, 3 and 4: *S*-(+)- and *R*-(−)-HMM, 5 and 6: *R*-(−)- and *S*-(+)-MDA, 7 and 9: *R*-(−)- and *S*-(+)-MDMA, 8 and 10: *R*-(−)- and *S*-(+)-MDPA.

Another useful CDR, *S*-(−)-heptafluorobutyl propyl chloride (*S*-(−)-HFBPC), was also used to separate chiral amine enantiomers successfully. Fig. 6 show the separation of methamphetamine and *N*-*n*-propylamphetamine diastereomers derivatized with *S*-(−)-HFBPC on a HP-1 fused-silica capillary column (12 m × 0.25 mm i.d., 0.25-μm film thickness) by GC/MSD. Fig. 7 shows the mass spectra of methamphetamine and *N*-*n*-propylamphetamine *S*-(−)-HFTPC diastereomers. Fig. 8 shows the separation of 3,4-(methylenedioxy)propylamphetamine (MDPA), 3,4-(methylenedioxy)methamphetamine (MDMA) and its three metabolites 3,4-(methylenedioxy)amphetamine(MDA), 4-hyroxy-3-methoxy methamphetamine (HMM) and 4-hyroxy-3-methoxy amphetamine(HMA) diastereomers of derivatized with S-(−)-(−)-HFBPC on an capillary column (15 m × 0.25 mm i.d., 0.25-μm film thickness, DB-5 fused-silica capillary column coupled to 15 m × 0.25 mm i.d., 0.25 μm film thickness, DB1301 fused-silica capillary column) by GC/electron capture negative ion chemical ionization mass spectrometry. However, those compound enantiomers were not separated on an capillary chiral column(ChiralVal).

4. Summary

In conclusion, several chiral amine drug enantiomers have been derivatized with *S*-(−)-*N*-(trifluoroacetyl)-prolyl chloride and separated as diastereomers by capillary GC with MSD or FID detection. Stereoselectivity and sensitivity can be improved through chiral derivatization. The methods established in this paper offer enantioselective, simple and economic approaches for the analysis of chiral amine drug enantiomers in biological fluids.

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