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Enantioseparation of methamphetamine by supercritical fluid chromatography with cellulose-based packed column

Hiroki Segawa*, Yuko T. Iwata, Tadashi Yamamuro, Kenji Kuwayama, Kenji Tsujikawa, Tatsuyuki Kanamori, Hiroyuki Inoue

National Research Institute of Police Science, 6-3-1, Kashiwanoha, Kashiwa, Chiba 277-0882, Japan

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ABSTRACT

The enantiomers of methamphetamine were differentiated by supercritical fluid chromatography (SFC) with an enantioselective cellulose-based packed column. The optimization of the chromatographic conditions was achieved by changing column temperature, co-solvent proportion, additive concentration, flow rate and back pressure. In particular, the additive concentration crucially changed the resolution between the enantiomers. After determining the optimized conditions, the enantiomers of methamphetamine were successfully separated. The analytical precision, accuracy and limit of detection were checked by using the authentic standard and seized real samples. We believe that chiral SFC is a promising method for enantioseparation of forensic samples.

1. Introduction

Chirality of the seized drugs of abuse can provide the valuable information for criminal investigation. For example, the proportion of enantiomers of methamphetamine (MA) is deeply related to its synthetic route and raw materials used in the clandestine laboratory. The representative starting materials of the illicitly manufactured MA are 1-phenyl-2-propanone (P2P), ephedrine (EP) and pseudoephedrine (PEP). In the case of P2P, MA can be synthesized through the reductive amination or Leuckart reaction. The synthesized MA is racemic, namely the 1:1 mixture of (*S*)-(+)-MA (*S*-MA) and (*R*)-(−)-MA (*R*-MA). In the case of EP and PEP, MA can be synthesized through the direct reductive reactions such as Nagai method, Moscow method, Rosenmund method, Birch reduction and Hypo method. Emde method using the chlorinated EP and PEP is also known. The synthesized MA from (1*R*, 2*S*)-(−)-EP and (1*S*, 2*S*)-(+)-PEP is normally pure *S*-MA. Thus the chiral analysis of the seized MA provides the information on the synthetic method. In combination with the impurity profiling, we can have a clue as to the distribution pathway of the illicitly manufactured MA [1–7].

Presently, chiral chromatography technique is a choice to perform the enantioseparation. The combination of chiral derivatization and achiral stationary phase have been reported for enantioseparation by gas chromatography (GC) [8–12]. In liquid chromatography (LC), chiral stationary phase was applied [13–17]. Capillary electrophoresis (CE) using the chiral selective additive such as cyclodextrin has also been used for this purpose [18–22]. Recently, supercritical fluid chromatography (SFC) is attracting enormous attention in the field of separation science including chiral separation [23–29]. Compared with GC, SFC does not require any derivatization. SFC is more cost-effective than LC because the consumption of organic solvents used as the component of the mobile phase is decreased. In addition, the reproducibility of the retention time in SFC, which is frequently cited as a disadvantage of CE, is satisfactory due to the strict control of the temperature and the back pressure. It is thus expected that SFC becomes a choice of the chiral analysis of the seized drugs of abuse.

In this study, we applied SFC with the cellulose-based chiral stationary phase for enantioseparation of MA. The chromatographic conditions were optimized through investigating the behavior of resolution between the peaks of *S*-MA and *R*-MA against type of column, column temperature, co-solvent proportion, additive concentration, flow rate and back pressure. After optimization, racemic MA was successfully separated. We checked the repeatability of the retention and the intensity, and the ability of estimating the ratio of *S*-MA and *R*-MA. At last, the established method was applied to the seized real samples with crystalline form and compared the result with that of the chiral GC analysis. Our strategy of method development is considered to be helpful to apply chiral SFC analysis to other drugs of abuse.

* Corresponding author.
E-mail address: segawa@nrips.go.jp (H. Segawa).

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2. Experimental

2.1. Chemicals

Methanol (MeOH), 2-propanol (2PrOH), ethyl acetate (EtOAc), formic acid (FA), sodium dihydrogen phosphate, sodium hydrogen phosphate, sodium carbonate, 28% aqueous ammonia (NH_3) were purchased from Wako Pure Chemical Industries (Osaka, Japan). Trifluoroacetic anhydride (TFAA) was purchased from GL Science (Tokyo, Japan). *N*-benzylisopropylamine was purchased from Tokyo Chemical Industry (Tokyo, Japan). Ultrapure water was laboratory produced by Milli-Q (Merck Millipore, Billerica, MA, USA).

Authentic standard of MA (*S*-MA and racemic) was stocked as hydrochloride salt solution in MeOH. *S*-MA was obtained from Dainippon Pharmaceutical (Osaka, Japan), and racemic MA was synthesized through reductive amination from racemic EP (Fuji Chemical Industries, Toyama, Japan). In order to make the solution with particular *S*-MA/*R*-MA ratio, the standard of *S*-MA solution and that of racemic MA solution were mixed. 2PrOH was used for diluting the MA solution.

2.2. Extraction of MA in the real sample with crystalline form

The extraction of MA from the real sample followed the method reported by Inoue et al. [1]. Briefly, the real samples after homogenization of 50 mg were weighed and dissolved in the 1 mL of cleaning solvent which was a mixture of 0.1 M phosphate buffer (pH 7.0) and 10% sodium carbonate solution with the ratio of 4:1. As an extraction solvent, 0.5 mL of EtOAc was added, and the sample tube was shaken. After centrifugation, the upper organic layer was transferred into a screw vial. This sample solution was stocked as the starting solution of the following analysis.

For SFC analysis, the sample solution was diluted to 100 fold by 2PrOH and was supplied as a sample (final concentration was about 1 mg/mL). For GC analysis, trifluoroacetylation was performed as follows; 10 μL of the starting solution, 0.2 mL of EtOAc and 0.2 mL of TFAA were mixed and reacted for 20 min at 50 °C. After evaporation of the solvent under nitrogen gas flow, 1 mL of EtOAc was added to dissolve the trifluoroacetylated (TFA) sample (final concentration was about 1 mg/mL). This TFA sample was supplied for GC analysis.

2.3. Chromatographic conditions of SFC

SFC was performed on an ultra performance convergence chromatography system (Acquity UPC^2, Waters Milford, MA, USA) equipped with Trefoil CEL2 column (Waters). The dimension of the column is 3.0 mm inner diameter and 2.5 μm particle size. Two different column length of 50 mm and 150 mm were used. The CHIRALPAK IC-3/SFC and CHIRALCEL OJ-3/SFC (Daicel, Osaka, Japan) columns were also used in the screening step. MeOH with NH_3 of various concentrations was used as the co-solvent for SFC. In the screening step, other co-solvent (2PrOH) and additives such as diethylamine (DEA), cyclohexylamine (CHA) and methanolamine (MEA) (Wako Pure Chemical Industries) were examined. The co-solvent with additives was prepared in-house and filtered. The flow of the mobile phase was performed with isocratic mode. A photo diode array (PDA) attached to the UPC^2 system was used as a detector. The scan range was from 210 nm to 400 nm with 20 Hz. The wavelength resolution was 1.2 nm. When the chromatograms by PDA were shown in the following, absorption coefficients at the 210 nm of wavelength was extracted.

2.4. Mass spectrometry

Mass spectrometry (MS) was performed with a Q-TOF mass spectrometer, Synapt high-definition MS system (Waters). The SFC and MS systems were directly interfaced by a PEEKsil tube of 50 μm in diameter with the makeup flow (MeOH with 0.1% FA) of 0.1 mL/min. MS was performed with positive mode electrospray ionization, capillary voltage of 4 kV, source temperature of 120 °C, dry gas flow rate of 800 L/h at 400 °C, cone voltage of 20 V, trap cell collision energy of 8 eV and transfer cell collision energy 4 eV. In this study, product ion scan was used for evaluating the limit of detection (LOD) with the precursor ion of *m/z* 150 (protonated molecule).

2.5. Chromatographic conditions of GC

GC was performed on a 6890N gas chromatograph (Agilent Technologies, Santa Clara, CA, USA) equipped with a flame ionization detector (FID). The column was BETA DEX 225, Supelco (Bellefonte, PA, USA) with 0.25 mm of inner diameter, 30 m of column length and 0.25 μm of film thickness. The column oven was kept 100 °C for 0.1 min, and raised to 140 °C at a rate of 2 °C/min. The injection port temperature was 210 °C and that at FID was 220 °C. The carrier gas was helium at a rate of 1.2 mL/min. The sample injection volume was 0.2 μL with splitless injection.

2.6. Data analysis

The peak area of the chromatograms was calculated by the software equipped with the SFC and GC (MassLynx for SFC and Chemstation for GC). To estimate the resolution and the LOD, chromatographic peaks were fitted by Gaussian function and the peak height, width, retention time and the amplitude of noise were calculated by Solver add-in in Microsoft Excel 2013.

3. Results and discussions

3.1. Screening of the optimized chromatographic conditions

The screening of the chromatographic conditions of SFC was commenced with choosing an appropriate chiral stationary phase. We examined Trefoil CEL2 (50 mm), CHIRALPAK IC-3/SFC and CHIRALCEL OJ-3/SFC. All three columns possess cellulose-based chiral selector, but the position and the types of the functional groups at the terminal benzene ring are different. We found that only Trefoil CEL2 column showed a slight separation and the other two did not. Therefore, we tried to optimize the chromatographic conditions with using Trefoil CEL2 column in the following experiment.

Subsequently the effect of the types of co-solvent and additive was examined. When MeOH and 2PrOH were used as co-solvent, peak separation was found only by MeOH. 2PrOH provided a single and very broad peak. When NH_3, DEA, CHA and MEA were used as additive, NH_3 showed the good resolution. Since the co-solvent with NH_3 is easy to prepare, we used MeOH with NH_3 as a co-solvent in the following experiment.

Next, the behavior of the resolution between *S*-MA and *R*-MA against co-solvent proportion, additive concentration, flow rate, column temperature and back pressure was investigated. Fig. 1 shows the behavior of the resolution against the above mentioned parameters. For co-solvent proportion, the lower the proportion was, the better the resolution was (Fig. 1a). However, the retention time significantly got slow when the co-solvent proportion was decreased (about 9 min for 5% proportion, and about 4.2 min for 7.5%) and the analysis time increased. It was found that the additive concentration in the co-solvent was deeply related to the enantioseparation. The plot Fig. 1a clearly shows that the resolution was dependent on the concentration of the additive in the co-solvent (comparing the different symbols in Fig. 1a). Basically the resolution got better as the additive concentration