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Rapid enantiomeric separation and simultaneous determination of phenethylamines by ultra high performance liquid chromatography with fluorescence and mass spectrometric detection: application to the analysis of illicit drugs distributed in the Japanese market and biological samples

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A rapid enantiomeric separation and simultaneous determination method based on ultra high performance liquid chromatography (UHPLC) was developed for phenethylamine-type abused drugs using (*R*)-(−)-4-(*N*,*N*-dimethylaminosulfonyl)-7-(3-isothiocyanatopyrrolidin-1-yl)-2,1,3-benzoxadiazole ((*R*)-(−)-DBD-Py-NCS) as the chiral fluorescent derivatization reagent. The derivatives were rapidly enantiomerically separated by reversed-phase UHPLC using a column of 2.3-μm octadecylsilica (ODS) particles by isocratic elution with water–methanol or water–acetonitrile systems as the mobile phase. The proposed method was applied to the analysis of products containing illicit drugs distributed in the Japanese market. Among the products, 1-(3,4-methylenedioxyphenyl)butan-2-amine (BDB) and 1-(2-methoxy4,5-methylenedioxyphenyl)propan-2-amine (MMDA-2) were detected in racemic form. Furthermore, the method was successfully applied to the analysis of hair specimens from rats that were continuously dosed with diphenyl(pyrrolidin-2-yl)methanol (D2PM). Using UHPLC-fluorescence (FL) detection, (*R*)- and (*S*)-D2PM from hair specimens were enantiomerically separated and detected with high sensitivity. The detection limits of (*R*)- and (*S*)-D2PM were 0.12 and 0.21 ng/mg hair, respectively (signal-to-noise ratio (S/N) = 3). Copyright © 2012 John Wiley & Sons, Ltd.

Keywords: phenethylamines; diphenyl(pyrrolidin-2-yl)methanol (D2PM); (*R*)-(−)-DBD-Py-NCS; chiral derivatization method; ultra high performance liquid chromatography (UHPLC)

Introduction

Health hazards caused by the abuse of illicit drugs occur frequently among young people and have become a serious concern. Such drugs are easily obtainable via the Internet, adult shops, street markets, and so on. The use of illicit drugs is also the gateway to narcotic and psychostimulant drugs abuse. In Japan, the Pharmaceutical Affairs Law was revised, and the regulation was tightened by introducing a system of controlled substances, designated as Shitei-Yakubutsu, in April 2007 (31 compounds and 1 plant).[1,2] Under this Act, compounds that have potential harmful health effects are designated Shitei-Yakubutsu, and rapid response to such compounds is facilitated. This system temporarily decreased the distribution of designated substances in Japan. However, due to synthetic modification, structural analogs of designated compounds may slip past regulations. As of August 2011, 60 substances (classified as 26 phenethylamines, 12 tryptamines, 6 alkyl nitrites, 4 piperazines, 10 cannabinoids, 1 diterpene, and 1 plant) are listed as designated substances; the list is continually revised and improved as necessary. Diphenyl(pyrrolidin-2-yl)methanol (D2PM), and 1-(2-fluorophenyl)-*N*-methylpropan-2-amine (*N*-methyl-2FMP), were recently added to the designated substances list; there is concern that analogs of these substances may be distributed as new illicit drugs. In particular, D2PM and its analogs are organocatalysts used for various asymmetric syntheses; however, such chemical reagents are

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likely abused.[3–7] To prevent distribution of these substances, it is crucial to establish an analytical method of detection before they enter the Japanese market. Therefore, development of simple and rapid screening methods of illicit drugs and their structurally related compounds is required.

Most phenethylamine compounds are chiral, and their enantiomers can possess different pharmacological activities and pharmacokinetic/pharmacodynamic properties. For example, it is well known that the enantiomers of methamphetamine and amphetamine differ in their biological and metabolic activities. The *d*-isomer has the greatest biological activity, whereas the *l*-isomer is far less active.[8,9] Therefore, it is important to ensure enantiomeric purity by chiral separation. Furthermore, relevant information can be gathered by indentifying the manufacturing method, the producer countries, and their sources by analyzing impurities and determining of the ratio of optical isomers in the distributed illicit drugs.

Numerous strategies for enantiomeric separation of chiral compounds are available using various separation techniques such as gas chromatography (GC), high performance liquid chromatography (HPLC), supercritical fluid chromatography (SFC), capillary electrophoresis (CE), and capillary electrochromatography (CEC). [10–18] Among these methods, HPLC is one of the most effective tools for chiral separation. The methods are divided into three broad classes: chiral stationary phase (CSP) methods, chiral mobile phase methods, and chiral derivatization methods. [15–17] CSP methods use packing materials combined with chiral molecules at the carrier surface as the stationary phase. A number of CSP methods have already been developed and are widely used. However, to perform chiral separations of target enantiomers by HPLC, the CSP must be selected through a trial-and-error process based solely on prior experience. Chiral mobile phase methods form a diastereomer complex by passing the sample through a column using a mobile phase containing chiral molecules. This method does not require the column to be packed with chiral molecules or complicated handling; however, the kinds of enantiomers that can be separated using this method are limited. Indeed, direct chromatographic separation using hydroxypropyl-β-cyclodextrin (HP-β-CD) as a chiral mobile phase additive has been investigated for the chiral separation of amphetamine and its derivatives; baseline separations could not be achieved because of the peak broadening.[19] These basic compounds tend to broaden peaks in consequence of residual silanol in the column. On the other hand, the chiral derivatization method does not require the comparatively expensive analytical column containing a CSP, and analysis can be performed using a conventional HPLC column such as an ODS column. Guillame *et al*. reported that *N*-α-(2,4-dinitro-5-fluorophenyl)-L-alaninamide (Marfey's reagent) and 2,3,4-tri-O-acetyl-α-D-arabinopyranosyl isothiocyanate (AITC) would be effective for enantiomeric separation of amphetamine, its derivatives, and several β-blockers (atenorol, propranorol, and so on) using HPLC–UV.[19] Furthermore, using a fluorescence derivatization method such as HPLC-fluorescence (HPLC-FL) detection has the advantage of highly sensitive detection.

In our previous study, we reported an HPLC-FL method for the enantiomeric separation of D2PM and psychotropic methylphenidate (MPH) using a chiral fluorescent derivatization reagent, (*R*)-(−)-4-(*N*,*N*-dimethylaminosulfonyl)-7-(3-isothiocyanatopyrrolidin-1-yl)-2,1,3-benzoxadiazole ((*R*)-(−)-DBD-Py-NCS).[20] However, to the best of our knowledge, the method and the reagent have not been applied for the analysis of other drugs.

In this study, we conducted to develop a method for rapid enantiomeric separation and simultaneous determination of phenethylamine-type abused drugs and established a detection method using UHPLC-FL and electrospray ionization time-of-flight mass spectrometry (ESI-TOF-MS). UHPLC is an analytical technique developed in the last half decade that is an extension of conventional HPLC techniques; it uses small particles in the separation column and pumping of the mobile phase under ultra-high pressure conditions.[21–23] Application to the analysis of phenethylamine drugs distributed on the Japanese market and the analysis of rat-hair specimens after oral dose of racemic, (*R*)-, and (*S*)-D2PM are also discussed.

Experimental

Materials and reagents

The hydrochloric acid salts of racemic phenethylamines, i.e. 1-(3,4-methylenedioxyphenyl)butan-2-amine (BDB), 1-(4-iodo-2,5-dimethoxyphenyl)propan-2-amine (DOI), 2-ethylamino-1-phenylpropan-1-one (*N*-ethylcatinone), 1-(4-fluorophenyl)propan-2-amine (4-FMP), 2-methylamino-1-(3,4-methylenedioxyphenyl)butan-1-one (bk-MBDB), 2-ethylamino-1-(3,4-methylenedioxyphenyl)propan-1-one (bk-MDEA), 1-(2-fluorophenyl)-*N*-methylpropan-2-amine (*N*-methyl-2FMP), 2-(methylamino)-1-(4-methylphenyl)propan-1-one (4-methylmethocatinone), 1-(2-methoxy4, 5-methylenedioxyphenyl)propan-2-amine (MMDA-2), and 1-(4-methoxyphenyl)-*N*-methylpropan-2-amine (PMMA) were obtained from the National Institutes of Health Sciences (NIHS, Tokyo, Japan). Products sold in the past as legal substances on the Japanese market were used for the determination of the phenethylamine-type abused drugs. Racemic methylphenidate hydrochloride (MPH), (2R,2′R)-(+)-*threo*-methyl α-phenyl-α-(2-piperidyl)acetate hydrochloride (D-MPH), (2S,2′S)-(−)-*threo*-methyl α-phenyl-α-(2-piperidyl)acetate hydrochloride (L-MPH), and leucine enkephalin were purchased from Sigma-Aldrich Chemical Co. (St Louis, MO, USA). (*R*)-Diphenyl(pyrrolidin-2-yl)methanol ((*R*)-(+)-α,α-diphenyl-2-pyrrolidinemethanol, (*R*)-D2PM), (*S*)-diphenyl(pyrrolidin-2-yl)methanol ((*S*)-(−)-α,α-diphenyl-2-pyrrolidinemethanol, (*S*)-D2PM), α-(4-piperidyl)benzhydrol (PBH as internal standard), (*R*)-(−)-4-(*N*,*N*-dimethylaminosulfonyl)-7-(3-isothiocyanatopyrrolidin-1-yl)-2,1,3-benzoxadiazole ((*R*)-(−)-DBD-Py-NCS), (*R*)-(+)-4-nitro-7-(2-chloroformylpyrrolidin-1-yl) -2,1,3-benzoxadiazole ((*R*)-(+)-NBD-Pro-COCl), 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl isothiocyanate (GITC), and triethylamine were obtained from Tokyo Kasei Co. (Tokyo, Japan). Diethyl ether, dimethyl sulfoxide (DMSO), and trifluoroacetic acid (TFA) were obtained from Kanto Kagaku Co. (Tokyo, Japan). Sodium dodecyl sulfate (SDS) and hydrochloric acid (HCl) were purchased from Wako Pure Chemicals (Osaka, Japan). Acetonitrile (CH_3CN), methanol (CH_3OH), and formic acid (FA) were of LC–MS grade (Wako Pure Chemicals, Osaka, Japan). Saline was purchased from Otsuka Pharmaceutical Factory, Inc. (Naruto, Japan). All other reagents were of analytical-reagent grade and were used without further purification.

UHPLC-FL and ESI-TOF-MS conditions

A Shimadzu (Kyoto, Japan) ultra-fast liquid chromatograph system consisting of two LC-20AD pumps, a degasser (DGU-20A_3) and an auto-injector (SIL-20AC_{HT}) was used. Reversed-phase liquid chromatography was performed using TSK-gel ODS-140HTP column (2.1 mm i.d. × 100 mm, 2.3 μm, Tosoh, Tokyo). The column

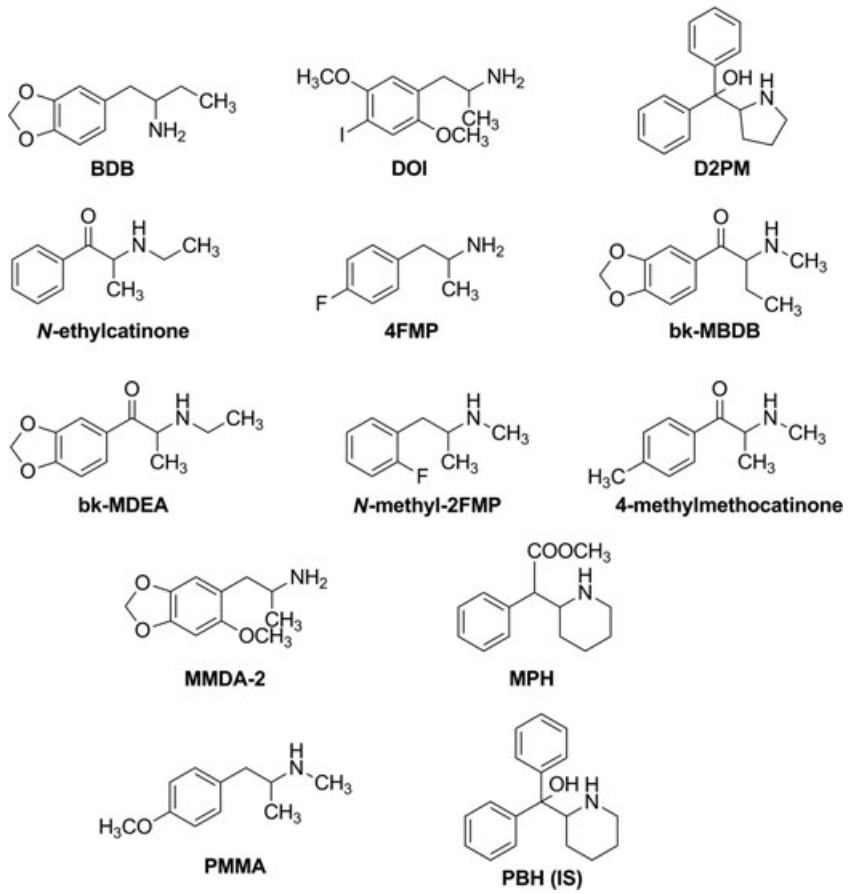

Figure 1. Chemical structures of phenethylamines used in this study.

Table 1. Separation factor (α) and resolution factor (R_s) for enantiomeric resolution of (*R*)- and (*S*)-D2PM using the chiral derivatization method[a]

Chiral derivatization reagents	α	R_s
(*R*)-(−)-DBD-Py-NCS	1.190	3.450
(*R*)-(+)-NBD-Pro-COCl	0.244	0.888
GITC	1.070	1.420

[a]Mobile phase: H_2O–CH_3OH–FA (45:55:0.1, v/v/v).

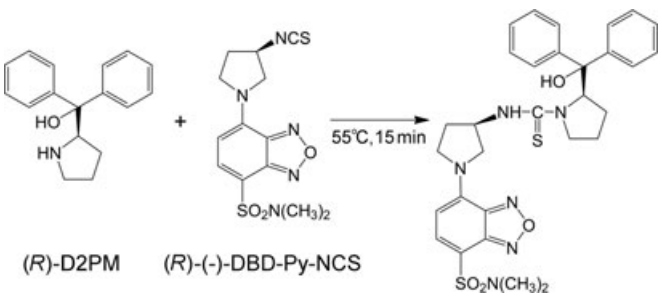

Figure 2. Derivatization reaction of (*R*)-D2PM with (*R*)-(−)-DBD-Py-NCS.

was maintained at 40°C. The eluent was monitored by an RF-10A_{XL} fluorescence detector (Shimadzu). The wavelengths of the fluorescence detector were set at 450 nm (excitation) and 560 nm (emission). The flow rate of the mobile phase was 0.45 ml/min. Isocratic separations were achieved using H_2O–CH_3OH–FA (40:60:0.1 or 45:55:0.1, *v/v/v*) as the mobile phase unless otherwise mentioned. The injection volume was fixed at 2 μl.

ESI-TOF-MS detection was performed using a Waters LCT Premier XE mass spectrometer (Waters, Milford, MA, USA). The profile data for positive ions of *m/z* 100–1000 were recorded (W-mode, mass resolution: 1.0×10^4). The capillary voltage was set at 3000 V, while the cone voltage was 10 V. Nitrogen was used as the drying gas. The desolvation gas flow rate was 650 L/h, and the cone gas flow rate was maintained at 50 L/h. The desolvation temperature was 350°C, and the source temperature was 120°C. A lock-mass of leucine enkephalin at a concentration of 2 ng/ml in H_2O–CH_3CN–FA (50:50:0.1, *v/v/v*) for the positive ion mode ($[M+H]^+ = 556.2771$) was used at a flow rate of 5 μl/min via a lock-spray ionization source. Data were collected in the centroid mode, the lock spray frequency was set at 5 s, and the lock-mass data were averaged over 10 scans for correction.

Derivatization of phenethylamines by (R)-(−)-DBD-Py-NCS

One hundred microlitres of 2 mM (*R*)-(−)-DBD-Py-NCS in CH_3CN and 5 μl of triethylamine were added to 100 μl of the sample solution containing phenethylamines (0–10 μM) in H_2O–CH_3CN (50:50, *v/v*). The mixture was heated at 55°C for 15 min.[20] The solution was cooled at 5°C, and an aliquot (2 μl) was injected into the UHPLC-FL and ESI-TOF-MS systems.

Analysis of phenethylamines from the products distributed in the Japanese market

The products distributed in the past as legal substances on the Japanese market were enantiomerically separated and analyzed for determination of phenethylamines. Product 1 (labelled as BDB, yellow powder, 1 mg) was dissolved in 1 ml of H_2O–CH_3OH (1:1, *v/v*), and product 2 (labelled as Honey Flash 2, colourless liquid) was diluted 10 times with H_2O–CH_3OH (1:1, *v/v*). The solution was sonicated for 10 min and then centrifuged at 500×g for 10 min. After centrifugation, 100 μl of the supernatant was derivatized by (*R*)-(−)-DBD-Py-NCS using the same procedure described above, and the supernatant was filtered through a Millex-LG filter (0.20 μm, 4 mm i.d.; Nihon Millipore, Tokyo, Japan). The solution was cooled at 5°C, and an aliquot (2 μl) was injected into the UHPLC–ESI-TOF-MS systems.

Experimental animals

Healthy male Dark-Agouti (DA) rats (5 weeks old) were purchased from Japan SLC, Inc. (Hamamatsu, Japan). Animal care and experiments were conducted according to the guidelines for the care and use of laboratory animals of the University of Shizuoka. The rats were housed at a constant temperature (24 ± 1°C) with an alternating 12-h light/dark cycle with free access to food and water. Racemic (*R/S* ratio =1/1), (*R*)-, and (*S*)-D2PM dissolved in saline containing 5% (*v/v*) DMSO and 0.1 M HCl were orally administered to the rats for three weeks (40 mg/kg day). The control rats were orally administered saline instead of D2PM. One week after cessation of D2PM administration, hair specimens were collected from the rats. The collected hairs were washed with 1 ml of 0.1% SDS for 1 min by vortex mixing. After three rinses with distilled water in the same manner, the hair

Table 2. Retention time (t_R), separation factor (α), and resolution factor (R_s) for enantiomeric resolution of phenethylamines using (*R*)-(−)-DBD-Py-NCS as chiral derivatization reagent

Samples	*m/z* ([M + H]$^+$)	Mobile phase[a]	t_R (min)	α	R_s
BDB	547.1797	A	4.48, 4.87	1.10	1.53
DOI	675.0920	B	17.92, 18.44	1.03	0.70
D2PM	607.2161	A	12.15, 14.52	1.19	3.45
N-Ethylcatinone	531.1848	B	13.28, 16.53	1.25	5.69
4FMP	507.1648	A	4.49, 4.79	1.07	1.24
bk-MBDB	575.1746	A	5.55, 6.83	1.16	3.84
bk-MDEA	575.1746	A	6.13, 7.58	1.25	4.43
N-Methyl-2FMP	521.1805	B	12.46, 13.15	1.06	1.40
4-Methylmethocatinone	531.1848	A	7.91, 10.02	1.28	5.28
MMDA-2	563.1746	B	6.41, 6.69	1.05	1.02
MPH	587.2110	A	8.38, 9.38	1.13	2.50
PMMA	533.2005	A	4.26, 4.52	1.07	1.13

[a]A: H_2O–CH_3OH–FA (45:55:0.1, *v/v/v*); B: H_2O–CH_3CN–FA (62:38:0.1, *v/v/v*).

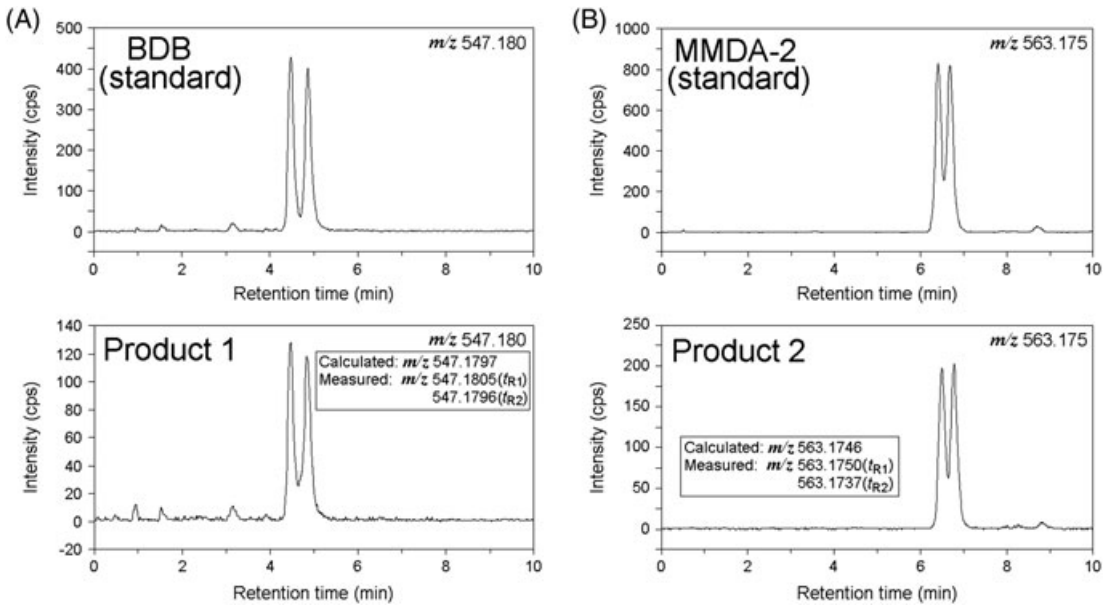

Figure 3. Typical chromatograms obtained from real products containing phenethylamines by UHPLC–ESI-TOF-MS. Analyte: (A) product 1 (yellow powder); (B) product 2 (colourless liquid). Mobile phase: (A) H_2O–CH_3OH–FA (45:55:0.1, *v/v/v*); (B) H_2O–CH_3CN–FA (62:38:0.1, *v/v/v*). Other conditions are described in Experimental section.

specimens were dried in air. The dried hairs were then cut into small pieces (approximately 2–3 mm) with scissors.

Acidic methanol extraction

A washed hair specimen (10 mg) was precisely weighed into a polypropylene tube, and 1.5 ml of CH_3OH–TFA (50:1, *v/v*) containing 5 μM of PBH as the internal standard was added for extraction. After sonication for 1 h, the solutions were allowed to stand overnight at room temperature. One millilitre of the supernatant was transferred into another tube and evaporated to dryness under a gentle stream of nitrogen at 40°C. Next, 50 μl of H_2O–CH_3CN (50:50, v/v) and 3 μl of triethylamine were serially added to the tube and reacted with 50 μl of (*R*)-(−)-DBD-Py-NCS in CH_3CN. The reaction mixture was heated at 55°C for 15 min. The supernatant was then filtered using a Millex-LG filter, and an aliquot of the filtrate (2 μl) was injected into the UHPLC-FL system.

Calibration curves and method validation by UHPLC-FL

Calibration curves were obtained by spiking a series of extraction solvents containing blank rat hair with (*R*)- and (*S*)-D2PM to give concentrations of 1.8–880 ng/mg hair. The curves were constructed by plotting the peak area ratios of (*R*)-D2PM and (*S*)-D2PM relative to the internal standard against the injected amounts. The curves were plotted for five different concentrations. Positive control hair specimens (QCL and QCH: quality control for low and high concentration, respectively) were prepared from the drug-free hair specimens according to a reported protocol with some modifications of drug concentrations,

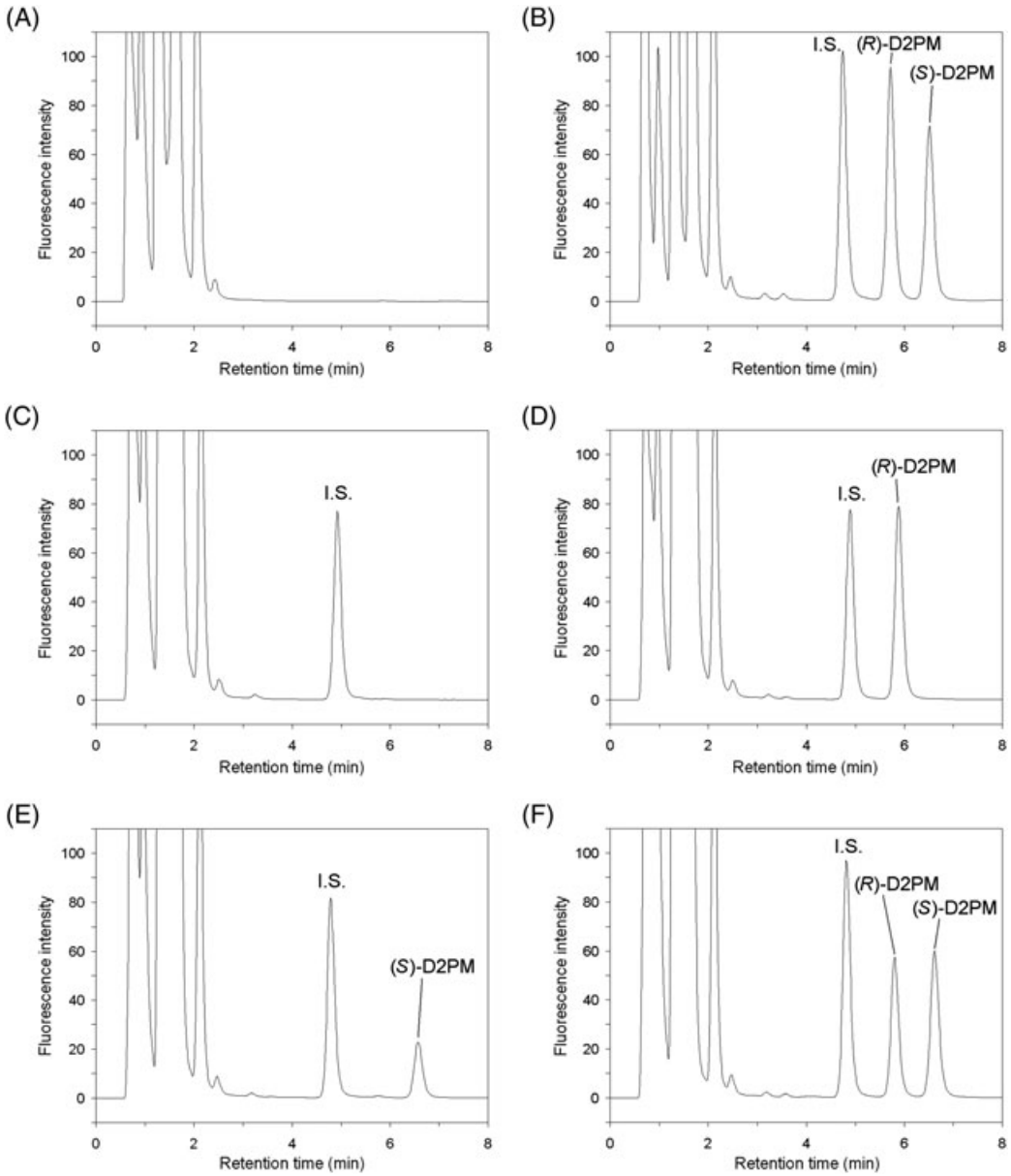

Figure 4. Typical chromatograms obtained from blank rat hair extract without addition of internal standard (A); rat hair extract spiked with 5 μmol/L (*R*)-D2PM and (*S*)-D2PM (B); rat hair extract following saline dose (C); (*R*)-D2PM (D); (*S*)-D2PM (E); and (*R,S*)-D2PM (F) by UHPLC–FL. Mobile phase: H_2O–CH_3OH–FA (40:60:0.1, v/v/v). Conditions of FL are described in Experimental section, and other conditions are the same as those in Figure 3.

Table 3. Intra- and inter-day validations of UHPLC-FL analysis of (*R*)- and (*S*)-D2PM from rat hair specimens

Samples	(*R*)-D2PM		(*S*)-D2PM	
	Amount (ng/mg hair, mean ± SD)	CV (%)	Amount (ng/mg hair, mean ± SD)	CV (%)
Intra-day assay ($n = 5$)				
QCL	051.6 ± 1.93	3.74	053.9 ± 3.52	6.53
QCH	408.1 ± 11.1	2.73	432.3 ± 28.2	6.52
Inter-day assay ($n = 5$)				
QCL	052.3 ± 3.16	6.05	052.0 ± 2.13	4.11
QCH	395.7 ± 26.3	6.64	400.9 ± 20.3	5.05

SD: standard deviation; CV: coefficient of variation.

Table 4. Amounts of (*R*)- and (*S*)-D2PM detected from rat hair by UHPLC-FL

Rat	Drug dose	Amounts of D2PM detected from rat hair (ng/mg hair, mean ± SD, $n = 5$)	
		(*R*)-D2PM	(*S*)-D2PM
1	(*R*)-D2PM	195.52 ± 9.69	Not detected
2	(*R*)-D2PM	073.89 ± 4.51	Not detected
3	(*S*)-D2PM	Not detected	133.61 ± 8.370
4	(*S*)-D2PM	Not detected	052.67 ± 2.010
5	*racemic*-D2PM	125.39 ± 7.53	187.91 ± 12.63
6	*racemic*-D2PM	052.06 ± 6.40	080.26 ± 10.98
7	–[a]	Not detected	Not detected
8	–[a]	Not detected	Not detected

[a]The rats were administered saline instead of D2PM.

soaking period, and rinse procedures.[24–26] Analyses were repeated five times a day and between days, and the precision (CVs, %) of intra- and inter-day assays was evaluated.

Results and discussion

Enantiomeric separation of phenethylamines using a chiral derivatization method

Figure 1 shows the chemical structures of phenethylamine-type abused drugs used in this study. We attempted to achieve rapid and simultaneous enantiomeric separation using a chiral fluorescent derivatization reagent, (*R*)-(−)-DBD-Py-NCS. In previous studies, (*R*)-(−)- and (*S*)-(+)-DBD-Py-NCS have proven to be very effective for total resolution of racemic mixtures of amino acids and thiol compounds by HPLC-FL.[27–29] The derivatization scheme for (*R*)-D2PM with (*R*)-(−)-DBD-Py-NCS is shown in Figure 2. Table 1 lists the separation factor (α) and resolution factor (R_s) for enantiomeric resolution of (*R*)- and (*S*)-D2PM using (*R*)-(−)-DBD-Py-NCS, (*R*)-(+)-NBD-Pro-COCl, and GITC as chiral derivatization reagents. The best resolution between the enantiomers was obtained using (*R*)-(−)-DBD-Py-NCS ($R_s = 3.45$). In addition, compared with using conventional HPLC, about 2 times faster enantioseparations of D2PM and MPH could be possible using UHPLC. Therefore, in this study, (*R*)-(−)-DBD-Py-NCS was used as the chiral derivatization reagent.

Rapid enantiomeric separation and determination of phenethylamines from the products distributed in the Japanese market by UHPLC–ESI-TOF-MS

Table 2 lists the retention time (t_R), *m/z*, α, and R_s for enantiomeric resolution of phenethylamines using (*R*)-(−)-DBD-Py-NCS as a chiral derivatization reagent. Enantiomeric separations of eight phenethylamines (BDB, D2PM, 4FMP, bk-MBDB, bk-MDEA, 4-methylmethocatinone, MPH, and PMMA) was achieved using H_2O–CH_3OH–FA (45:55:0.1, *v/v/v*) as the mobile phase. Also, DOI, *N*-ethylcatinone, *N*-methyl-FMP, and MMDA-2 were enantiomerically separated using H_2O–CH_3CN–FA (62:38:0.1, *v/v/v*) as the mobile phase.

The proposed method was applied to the determination of phenethylamine products obtained from an adult shop and via the Internet. The products were extracted with H_2O–CH_3OH, centrifuged, filtered, derivatized by (*R*)-(−)-DBD-Py-NCS, and then analyzed by UHPLC–ESI-TOF-MS. Typical chromatograms obtained from these products are shown in Figure 3. BDB and MMDA-2 were detected from products 1 and 2, respectively, and they were identified as racemic. High-resolution mass analysis by ESI-TOF-MS provided excellent accuracy in the determination of the *m/z* of the derivatives (less than 1.8 ppm). Since the products analyzed in this study contained simple racemic forms, it would be difficult to trace the manufacturing method or sources. However, in combination with impurity analysis of the products, the proposed method is expected to be applicable for such purposes.

Analysis of D2PM in rat hair by UHPLC-FL

Hair specimens are suitable for retrospective analyses when blood and urine are no longer expected to contain the illicit drugs.[30–34] Hair is typically suitable for this kind of analysis for several months up to one year after ingestion. In addition, once incorporated into hair, drugs are protected by a cuticle layer and are almost independent of daily cleaning. The developed method was also applied to the analysis of illicit drugs in hair specimens in which rats were administered oral doses (40 mg/kg day) of D2PM continuously for three weeks. The hair specimens were analyzed by FL, which has a wide dynamic rage and superior quantitative performance compared with ESI-TOF-MS.

Acidic methanol extraction was used for the analysis of D2PM-dosed rat hair specimens. CH_3OH–TFA (50:1, *v/v*) was used as an extraction solvent since D2PM was extracted efficiently, and TFA is volatile. When CH_3OH–5 M HCl (9:1, *v/v*) was used for extraction, HCl remained after removal of the solvent by nitrogen

gas. Therefore, the ratio of derivatization reaction decreased dramatically, and sufficient peak intensity of (*R*)-D2PM, (*S*)-D2PM, and PBH (internal standard) was not obtained (data not shown).

Figure 4A shows that no endogenous constituents of blank hair extracts eluted at the retention times of the peaks of (*R*)-D2PM, (*S*)-D2PM, or PBH. Adequate separation and detection were achieved within 7 min using H_2O–CH_3OH–FA (40:60: 0.1, *v/v/v*) as the mobile phase (Figures 4B–4F). Therefore, the developed method was found to be selective for (*R*)- and (*S*)-D2PM in hair specimens without interferences from normal endogenous hair constituents.

Calibration curves were obtained using blank hair spiked with (*R*)- and (*S*)-D2PM. The curves obtained by plotting the peak area ratios of (*R*)- and (*S*)-D2PM relative to the internal standard exhibited good linearity ($r^2 > 0.999$). The precisions of different concentrations (QCL and QCH) were also evaluated by intra- and inter-day assays. As shown in Table 3, the precision of the intra- and inter-day assays were 2.73–6.53% and 4.11–6.64%, respectively; thus, reasonable precisions were obtained. The detection limits of (*R*)- and (*S*)-D2PM were 0.12 ng/mg hair and 0.21 ng/mg hair, respectively (signal-to-noise ratio (S/N) = 3). Table 4 lists the amounts of (*R*)- and (*S*)-D2PM detected from rat hair. Although variability among individual rats was observed, (*R*)-form was detected from the rat dosed with (*R*)-form, (*S*)-form was detected from the rat dosed with (*S*)-form, and both (*R*)- and (*S*)-form were detected from the rat dosed with the racemic form, as expected.

Although conventional acidic methanol extraction requires long preparation times for hair specimens, by applying macropulverized extraction,[25,35] rapid enantiomeric separation and quantification of D2PM in hair was achieved. The proposed method should be useful for preventing widespread distribution of D2PM as a new illegal drug and is also expected to be appropriate for the analysis of human hair specimens from drug abusers.

Conclusion

In this study, rapid enantiomeric separation of phenethylamine-type abused drugs was accomplished using (*R*)-(−)-DBD-Py-NCS as the chiral fluorescent derivatization reagent based on UHPLC. Enantiomeric separation of 12 phenethylamines was achieved. The proposed method was successfully applied to the analysis of products containing illicit drugs distributed in the Japanese market. Among the products, BDB and MMDA-2 were detected in racemic form. The method was also applied to the analysis of rat hair specimens in which the rats were administered oral doses of D2PM. The proposed method should be useful for preventing widespread distribution of D2PM as a new illicit drug and is also expected to be applicable to the analysis of human hair specimens from drug abusers.

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