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Simultaneous determination of 11 designated hallucinogenic phenethylamines by ultra-fast liquid chromatography with fluorescence detection

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ABSTRACT

To avoid the spreading of illegal drugs, a designated drug regulation system was introduced along with revision of the Pharmaceutical Affairs Law in Japan in 2006, and 32 substances including phenethylamine-type drugs were listed in April 2007. In this study, a new simultaneous determination method, based on ultra-fast liquid chromatography coupled with fluorescence detection (UFLC–FL), was developed for the 11 designated phenethylamine drugs. The phenethylamines were labeled with 4-(*N*,*N*-dimethylaminosulfonyl)-7-fluoro-2,1,3-benzoxadiazole (DBD-F) at 60 °C for 2 h in 0.1 M borax (pH 9.3). The resulting 11 fluorophores were completely separated by reversed-phase chromatography using an ACQUITY UPLC BEH C_{18} column (2.1 mm × 100 mm 1.7 μm) and fluorometrically detected at 550 nm (excitation at 450 nm). The calibration curves obtained from the peak areas versus the injection amounts of the phenethylamines showed a good linearity. The limits of detection (signal-to-noise ratio of 3: S/N = 3) on the chromatogram were in the range from 10 fmol (PMMA) to 2.5 pmol (MMDA-2). Good accuracy (%) and precision (CV) by intra-day assay and inter-day assay were also obtained using the present procedure. The method was applied to the qualitative and quantitative analyses of phenethylamine in real products obtained from the Japanese market. As the results, BDB (0.24 mg/mg), MMDA-2 (0.98 mg/mL) and 2C-I (0.016 mg/mg) were identified from the different products (powder, liquid and mushroom like). Because the procedure is simple, selective and sensitive, the present method seems to be useful for the qualitative and quantitative analyses of the designated phenethylamines in various samples including biological specimens.

1. Introduction

Not only are many hallucinogenic phenethylamines naturally occurring, but the phenethylamines and their analogues can be easily synthesized [1–6]. 3,4-Methylenedioxyamphetamine (MDA) and 3,4-methylenedioxymethamphetamine (MDMA) are generally known as the "love drug" and "ecstasy", respectively. Although several phenethylamines are strictly controlled by the Narcotics and Psychotropic Control Law in Japan, various new psychotropic compounds possessing the phenethylamine structure have appeared on city streets, especially on the Internet. Their illegal use, especially by young persons, is one of the current serious social problems in the world. To avoid their wide spread, some of these drugs were listed as psychotropic substances controlled as "designated substances" by the Pharmaceutical Affairs Law in Japan in April 2007 [7–9]. Consequently, the qualitative and quantitative analyses are an important subject for the rapid screening of these drugs.

Many separation methods, such as HPLC-UV [10–12], GC [13] CE [14] and TLC [10] are reported for the qualitative analysis of the phenethylamine analogues. Furthermore, GC–MS [15–18] and LC–MS [19–28] are used for the preliminary study and mass screening to discriminate them from other hallucinogens. Although the individual determination of several phenethylamines have been performed [29,30], the simultaneous determination method of various phenethylamine analogues in a short run time, which is simple, sensitive, selective and quantitative, has not yet been developed. It is well known that fluorescence labeling is one of the most sensitive methods for various compounds. The phenethylamines possessing primary and secondary amino functional groups are derivatized with some fluorescence tagging reagents, e.g., 4-(*N*,*N*-dimethylaminosulfonyl)-7-fluoro-

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2,1,3-benzoxadiazole (DBD-F) and 4-(4,5-diphenyl-1*H*-imidazol-2-yl)benzoyl chloride (DIB-Cl) [31,32]. The aim of this study is to develop a rapid and simultaneous determination method for 11 hallucinogenic phenethylamines after the fluorescence labeling with DBD-F by ultra-fast liquid chromatography (UFLC). Several columns packed with small resins (1.7–2.3 μm) were tested for the rapid and high resolution of the derivatives by UFLC with fluorescence (FL) detection. Furthermore, some applications to phenethylamine products on the Japanese market are also described in this paper.

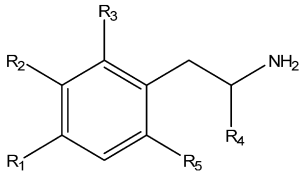
2. Experimental

2.1. Materials and reagents

Eleven designated phenethylamine hydrochlorides, i.e., 4-iodo-2,5-dimethoxyphenethylamine (2C-I), 4-chloro-2,5-dimethoxyphenethylamine (2C-C), 4-ethyl-2,5-dimethoxyphenethylamine (2C-E), 4-ethylthio-2,5-dimethoxyphenethylamine (2C-T-2), 4-isopropylthio-2,5-dimethoxyphenethylamine (2C-T-4), 2,4,6-trimethoxyamphetamine (TMA-6), 4-fluoroamphetamine (4FMP), 4-methoxymethamphetamine (PMMA), *N*-methyl-1-(3,4-methylenedioxyphenyl) butan-3-amine (HMDMA), 1-methyl-1-3,4-methylenedioxymethamphetamine (MMDA-2), and 1-(3,4-methylenedioxyphenyl)butan-2-amine (BDB) were obtained from the National Institutes of Health Sciences (NIHS, Tokyo, Japan) (Fig. 1). 4-(*N*,*N*-Dimethylaminosulfonyl)-7-fluoro-2,1,3-benzoxadiazole (DBD-F), 4-fluoro-7-nitro-2,1,3-benzoxadiazole (NBD-F), and dansylchloride (DNS-Cl) were purchased from Tokyo Kasei Co. (Tokyo, Japan). 1-Pyrenesulfonyl chloride (PSC, Molecular Probes), trifluoroacetic acid (TFA), sodium tetraborate ($Na_2B_4O_7$, Borax), methanol (MeOH), and acetonitrile (CH_3CN) were of special reagent grade (Wako Pure Chemicals, Osaka, Japan). Three products, labeled BDB, JETS, and Honey Flash 2, which were sold as illegal drugs on the Japanese market, were used for the determination of the phenethylamine(s). All other chemicals were of analytical-reagent grade and were used without further purification. De-ionized and distilled water (H_2O) was used throughout the study (Aquarius pwu-200 automatic water distillation apparatus; Advantec, Tokyo, Japan).

2.2. UFLC–FL

A Shimadzu (Kyoto, Japan) UFLC system consisting of two LC-20AD pumps, an auto-injector (SIL-20AC HT) and a degasser (DGU-20A_3) was used. The analytical columns, chosen for the optimization of the reversed-phase chromatography, were a Shim-pack XR-ODS (100 mm × 2.0 mm, i.d., 2.2 μm), a TSK-GEL ODS-140HTP (100 mm × 2.1 mm, i.d., 2.3 μm) and an ACQUITY UPLC BEH C_{18} (100 mm × 2.1 mm, i.d., 1.7 μm). The columns were maintained at 40 °C by a CTO-10A column oven (Shimadzu). The effluent was monitored by an RF-10A_{XL} fluorescence detector equipped with a



Phenethylamines

Compounds	R_1	R_2	R_3	R_4	R_5
4-Iodo-2,5-dimethoxyphenethylamine(2C-I)	I	OCH_3	H	H	OCH_3
4-Chloro-2,5-dimethoxyphenethylamine(2C-C)	Cl	OCH_3	H	H	OCH_3
4-Ethyl-2,5-dimethoxyphenethylamine(2C-E)	CH_2CH_3	OCH_3	H	H	OCH_3
4-Ethylthio-2,5-dimethoxyphenethylamine(2C-T-2)	SCH_2CH_3	OCH_3	H	H	OCH_3
4-Isopropylthio-2,5-dimethoxyphenethylamine(2C-T-4)	$SCH(CH_3)_2$	OCH_3	H	H	OCH_3
2,4,6-Trimethoxyamphetamine(TMA-6)	OCH_3	H	OCH_3	CH_3	OCH_3
4-Fluoroamphetamine(4FMP)	F	H	H	CH_3	H

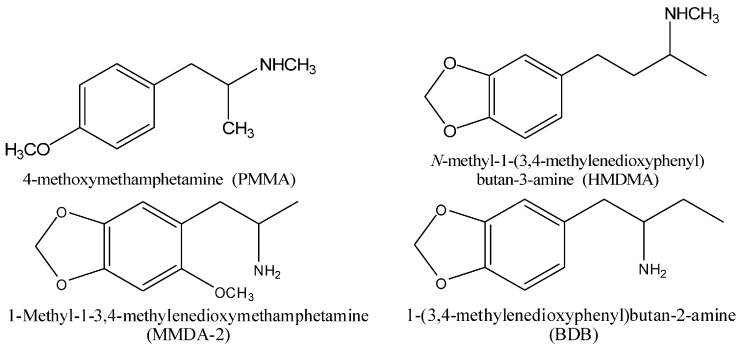


Fig. 1. Structures of tested hallucinogenic phenethylamines.

2 μL flow cell (Shimadzu). The wavelengths of the fluorescence (FL) detector were set at 450 nm (excitation) and 550 nm (emission). The flow-rate of the mobile-phase was 0.2 mL/min. The peak areas obtained from the FL detector were calculated using LC Solution chromatography software (Shimadzu).

2.3. Optimization of FL labeling of phenethylamines with DBD-F

To 100 μL of each phenethylamine in water (100 μM), 30 μL of DBD-F in CH_3CN (5 mM) and 170 μL of 100 mM Borax (pH 9.3) were added and vigorously mixed. Each solution was heated at 60 °C for 240 min. At a fixed time interval (30 min), the reaction solution was cooled on ice-water and then an aliquot (10 μL) was injected into the UFLC–FL system. The peak areas were plotted versus the sampling times. The excitation and emission spectra of each derivative were also determined by the FL detector.

2.4. Recommended procedure for the determination of phenethylamines

One hundred microliters of 2.5 mM DBD-F in CH_3CN and 170 μL of 100 mM Borax (pH 9.3) were added to 30 μL of the sample solution containing the phenethylamine(s). The mixed solution was heated at 60 °C for 2 h. The reaction solution was cooled on ice-water and then an aliquot (10 μL) was injected into the UFLC–FL system. The mobile-phases, (A) and (B), were 0.1% TFA in H_2O/MeOH (95/5) and 0.1% TFA in CH_3CN/MeOH (30/70), respectively. The derivatives were separated by an ACQUITY UPLC BEH C_{18} column (100 mm × 2.1 mm, i.d., 1.7 μm) by the isocratic elution of (A):(B) (44:56).

2.5. Validation of the method

2.5.1. Calibration curves

A series of working solutions of the phenethylamines (4.0 nM–5.0 μM) were prepared with 0.1 M borax (pH 9.3). Each solution (30 μL) at 5 different concentrations ($n = 3$) was reacted at 60 °C for 2 h with 100 μL of 2.5 mM DBD-F in CH_3CN and 170 μL of 0.1 M borax (pH 9.3). A 10-μL portion of each reaction mixture was subjected to UFLC and detected by fluorimetry, as described in Section 2.4. The peak areas of the FL derivatives were plotted versus the concentration of the phenethylamines. The injected concentration range was 0.04–50 pmol. The CV (%) was calculated for each concentration ($n = 3$).

2.5.2. Accuracy and precision by intra-day and inter-day assays

The accuracy (%) and precision (CV) by intra-day and inter-day assays were evaluated by the proposed method. They were evaluated using three different concentrations in the range of 0.1–50 nmol/mL for 11 phenethylamines. The determinations were repeated five times within a day and between days. Each 30 μL solution was reacted with DBD-F and then subjected to UPLC-FL, as described in Section 2.4. The accuracy (%) at each concentration was calculated from the calibration curves obtained from Section 2.4. The precision (CV%) for each concentration was also calculated from the SD values for 5 replicated determinations.

2.5.3. Limit of detection

The limit of detection (LOD) was defined as the calculated concentration at a signal-to-noise ratio of 3 (S/N = 3). The standard solutions of 11 phenethylamines were diluted to a series of concentrations (1.0 nM–2.5 μM). Each 30 μL solution was reacted with DBD-F and then subjected to UPLC-FL, as described in Section 2.4. The limits of detection of each phenethylamine were calculated from a comparison of the noise level and the peak height on the suitable chromatogram which had detected the target phenethylamine.

2.6. Determination of phenethylamine in real samples

The products, labeled as BDB, JETS, and Honey Flash 2, which were sold as illegal drugs on the Japanese market, were used for the determination of the phenethylamine(s). The BDB sample (1 mg) was dissolved in 1 mL of 50% methanol solution, and then centrifuged at 2000 rpm for 10 min. After the centrifugation, the separated supernatant was filtered through a 0.45 μm membrane. Thirty microliters of the filtrate was reacted with DBD-F using the recommended procedure. Similarly, 1 mg of JETS was treated as described above. Since Honey Flash 2 was a clear liq-

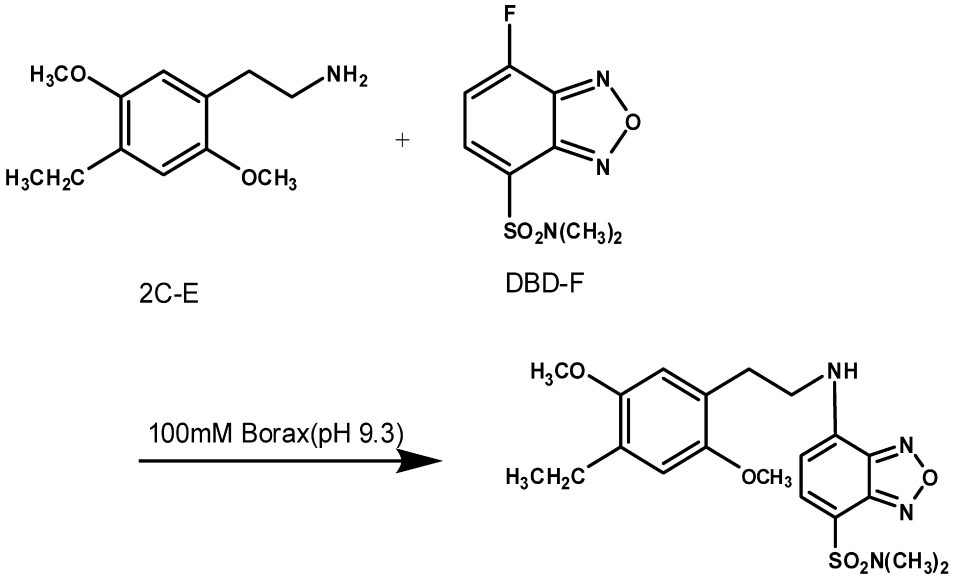


Fig. 2. Fluorescence labeling reaction of 2C-E with DBD-F.

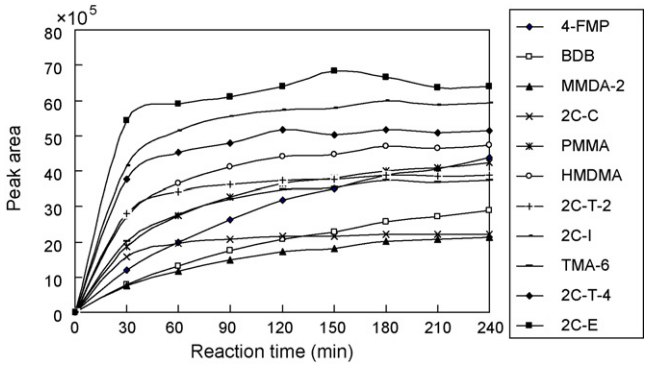


Fig. 3. Time courses of the reaction of phenethylamines with DBD-F at 60 °C.

uid sample, the solution was first diluted 10 times with water. The 30 μL aliquot was also labeled with DBD-F, separated by UFLC and detected by fluorimetry, according to Section 2.4. The phenethylamine in the sample was qualitatively and quantitatively determined from the retention time and peak area on the chromatogram.

3. Results and discussion

3.1. Optimization of fluorescence labeling reaction

Several fluorescence labeling reagents, i.e., DNS-Cl, PSC, NBD-F and DBD-F, which react with both primary and secondary amines, were first used for labeling the 11 phenethylamines. Although these reagents were essentially usable for the labeling, only DBD-F efficiently labeled all the tested phenethylamines. Therefore, DBD-F was selected for the labeling of the phenethylamines in the present study. The reaction scheme of DBD-F with 2C-E as a representative phenethylamine is shown in Fig. 2. The FL labeling effectively proceeds in basic medium due to the electrophilic substitution reaction of DBD-F for the amino group [33,34]. Higher temperatures are also important for the reaction to occur. Therefore, the reaction solution was heated at 60 °C in 0.1 M borax (pH 9.3). Fig. 3 shows the time courses of the labeling reaction of the 11 phenethylamines with DBD-F. Although the maximal peak areas were different for each phenethylamine, the reactions were almost totally completed within 2 h. Hence, the reaction condition of 60 °C for 2 h in 0.1 M borax (pH 9.3) was selected for the FL labeling of the tested phenethylamines. Table 1 shows the maximum excitation and emission wavelengths of the resulting derivatives. The excitation wavelengths in PMMA and HMDMA were slightly different from the other derivatives. However, the derivatives seem to possess the DBD moiety, judging from both the excitation and emission wavelengths. Thus, the excitation and emission wavelengths of the detector were set at 450 and 550 nm, respectively.

Table 1
Maximum excitation and emission wavelengths of DBD-phenethylamines

Phenethylamines	Ex (nm)	Em (nm)
2C-E	423	528
2C-T-2	428	543
2C-T-4	427	545
2C-C	427	551
2C-I	434	552
4-FMP	435	555
MMDA-2	430	536
BDB	436	559
TMA-6	446	552
PMMA	466	561
HMDMA	470	562

3.2. Optimization of separation and detection conditions

The simultaneous separation of the fluorescent derivatives was first tested using three different semi-micro columns which were packed with small porous resins (1.7, 2.2 and 2.3 μm). Fig. 4 shows the chromatograms of the 11 phenethylamine derivatives by UFLC separation and FL detection using an isocratic elution condition. As shown in Fig. 4A, all the derivatives were strongly retained on the 2.2 μm Shim-pack column and thus the carbon contents seem to be higher than the other two columns. Furthermore, the separation of the 2C-C and PMMA derivatives was not performed under this elution condition. On the other hand, the retention of the derivatives in the 2.3 μm TSK-Gel column was weak and the mutual separation of HMDMA and 2C-T-2 was difficult. In contrast, the 11 phenethylamine derivatives were successfully separated with a short run time (16 min) by the isocratic elution of H_2O–CH_3CN/MeOH (1/1) (40:60) containing 0.1% TFA using an ACQUITY BEH C_{18} (100 mm × 2.1 mm, i.d., 1.7 μm). Although the simultaneous separation of the phenethylamine derivatives seems to be possible by these three columns with a slight modification of the elution conditions, the BEH C_{18} column was used for further investigations. As shown in Fig. 4E, the phenethylamine derivatives were eluted very fast with H_2O–CH_3CN containing 0.1% TFA as the mobile-phase. However, the total separation was impossible without the addition of MeOH. Therefore, the existence of MeOH in the mobile-phase was important for the simultaneous separation. The slight difference in the mobile-phase increased the run time from 16 to 26 min (Fig. 4D). Although the simultaneous separation of the 11 derivatives was possible under the elution conditions shown in Fig. 4C, the conditions in Fig. 4D, i.e., H_2O/MeOH (95/5)–CH_3CN/MeOH (30/70) (44:56) containing 0.1% TFA, were adopted for the real sample analyses to minimize the chance of misreading the phenethylamines.

3.3. Method validation

Table 2 shows the results of the calibration curves and the detection limits for the 11 phenethylamines by the proposed procedure. Although the concentration ranges of the calibration curves were varied for each phenethylamine, a good linearity was obtained for all the compounds. A wide variation in the detection limits from 10 fmol to 2.5 pmol was also observed. Although the exact reason for the variance is not obvious, judging from the time courses of the labeling reaction (Fig. 2), the difference seems to be due to the fluorescent quantum yields of each derivative.

The results of the accuracy (%) and precision (CV%) for three different concentrations by the intra-day and inter-day assays are shown in Table 3. Although the CV (%) for the intra-day and inter-day were different for each phenethylamine, the values were less than 10%. Good accuracy (%) was obtained from the three different concentrations for all the phenethylamines. These results suggested that the proposed method is applicable for the qualitative and quantitative determination of any unknown phenethylamine(s) in real samples.

3.4. Application to phenethylamine products on Japanese market

The proposed method was applied to the determination of the phenethylamine products obtained from an adult shop and *via* the Internet. As show in Fig. 5, the characteristics of the products varied, i.e., pale yellow powder, colorless liquid, and dried mushroom

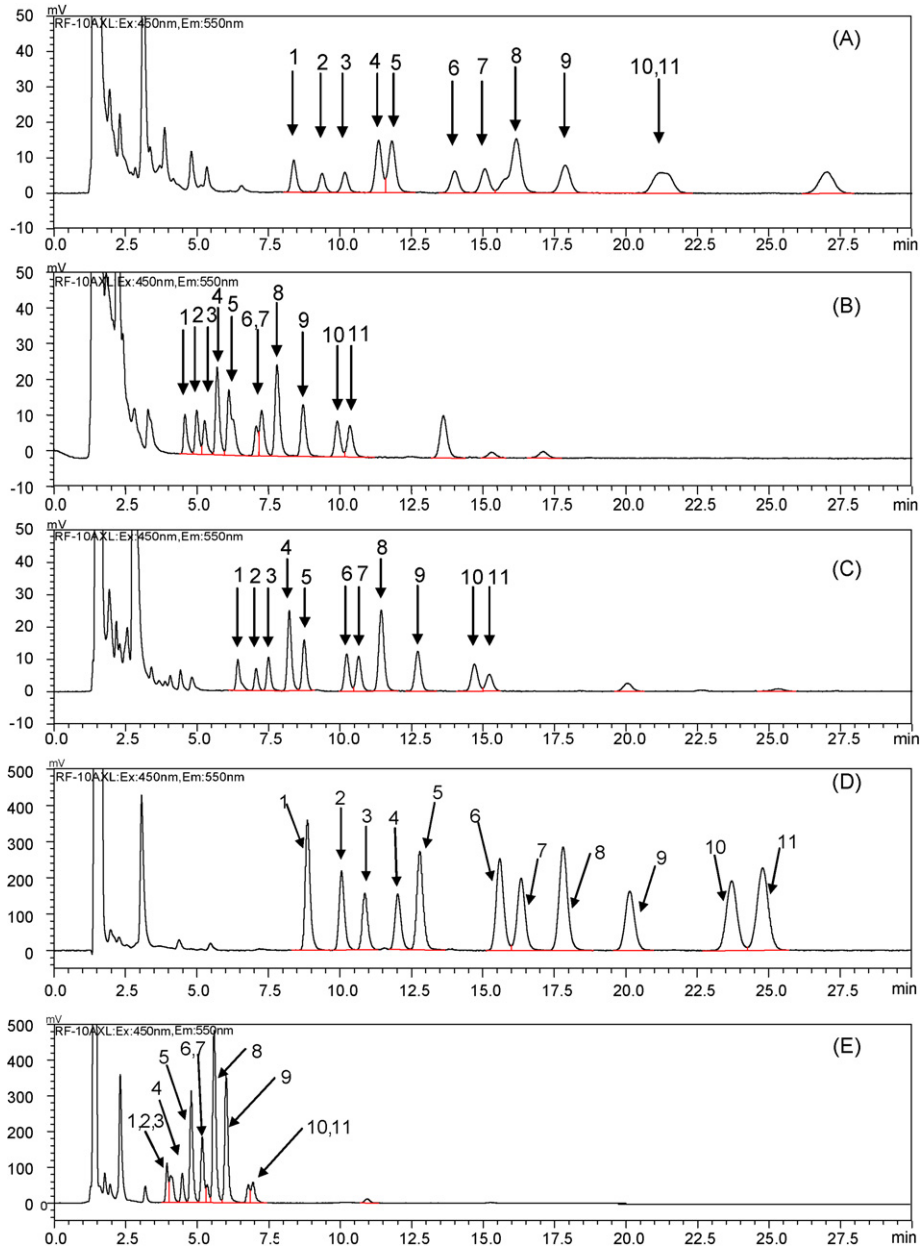


Fig. 4. Typical chromatograms of phenethylamines labeled with DBD-F by UFLC–FL. Peaks: 1, 4-FMP; 2, BDB; 3, MMDA-2; 4, 2C-C; 5, PMMA; 6, HMDMA; 7, 2C-T-2; 8, 2C-I; 9, TMA-6; 10, 2C-T-4; 11, 2C-E. Columns: A, Shim-pack XR-ODS (100 mm × 2.0 mm, i.d., 2.2 μm); B, TSK-GEL ODS-140HTP (100 mm × 2.1 mm, i.d., 2.3 μm); C, D and E, ACQUITY UPLC BEH C_{18} (100 mm × 2.1 mm, i.d., 1.7 μm). Isocratic elution: A, B, and C, 0.1% TFA in H_2O and 0.1% TFA in CH_3CN/MeOH (1/1) (40:60); D, 0.1% TFA in H_2O/CH_3OH (95/5) and 0.1% TFA in CH_3CN/MeOH (30/70) (44:56); E, 0.1% TFA in H_2O and 0.1% TFA in CH_3CN (40:60). The other UFLC–FL conditions are described in Section 2.

Table 2
Detection limits and calibration curves for DBD-phenethylamines by the proposed method

Phenethylamines	Detection limit (pmol) (S/N = 3)	Calibration range (pmol)	Linear equation	Linearity (R^2)	CV% (n = 3)
4-FMP	0.04	0.13–2.5	$y = 95005x + 3603$	0.9999	0.50–2.7
BDB	0.11	0.32–2.5	$y = 43026x - 1354$	0.9995	0.68–3.0
MMDA-2	2.5	5.0–50	$y = 2748x - 3676$	0.9997	2.2–5.3
2C-C	0.05	0.16–2.0	$y = 195780x - 11588$	0.9985	1.0–5.0
PMMA	0.01	0.04–2.0	$y = 512632x - 1798$	0.9998	1.5–4.3
HMDMA	0.03	0.08–2.0	$y = 327325x + 186$	0.9996	0.45–3.8
2C-T-2	0.83	2.5–25	$y = 12127x - 4961$	0.9986	1.4–3.3
2C-I	0.10	0.3–25	$y = 114642x - 24310$	0.9998	0.63–5.4
TMA-6	0.28	0.55–25	$y = 60369x + 811$	0.9999	0.56–4.1
2C-T-4	1.0	2.0–25	$y = 17499x + 4097$	0.9994	1.3–3.0
2C-E	1.0	2.0–25	$y = 17450x - 3909$	0.9988	1.4–7.8

Table 3
Accuracy and precision of the proposed method by intra-day and inter-day assays

Phenethylamines	Amount (nmol/mL)	Intra-day assay			Inter-day assay		
		Mean ± SD	CV% (n = 5)	Accuracy (%)	Mean ± SD	CV% (n = 5)	Accuracy (%)
4-FMP	0.5	0.51 ± 0.01	1.72	102.0	0.54 ± 0.01	1.87	108.0
	1.5	1.54 ± 0.02	1.28	102.7	1.57 ± 0.03	1.91	104.7
	2.5	2.55 ± 0.04	1.62	102.0	2.73 ± 0.07	2.66	109.2
BDB	0.5	0.51 ± 0.03	6.41	102.0	0.54 ± 0.04	7.96	108.0
	1.5	1.48 ± 0.02	1.61	98.67	1.56 ± 0.03	2.15	104.0
	2.5	2.64 ± 0.05	1.98	105.6	2.72 ± 0.08	2.96	108.8
MMDA-2	10	10.52 ± 0.28	2.67	105.2	10.79 ± 0.36	3.39	107.9
	20	19.84 ± 1.03	5.19	99.20	20.80 ± 1.12	5.42	104.0
	50	51.49 ± 1.58	3.08	102.9	53.26 ± 2.55	4.79	106.5
2C-C	0.2	0.19 ± 0.01	5.28	95.00	0.21 ± 0.02	9.67	105.0
	1.0	1.00 ± 0.05	5.00	100.0	1.01 ± 0.07	6.64	101.0
	2.0	2.03 ± 0.03	1.56	101.5	2.11 ± 0.08	3.79	105.5
PMMA	0.1	0.098 ± 0.01	8.34	98.00	0.103 ± 0.01	9.70	103.0
	1.0	1.01 ± 0.02	1.98	101.0	1.04 ± 0.03	2.86	104.0
	2.0	1.99 ± 0.01	0.56	99.50	2.14 ± 0.07	3.29	107.0
HMDMA	0.2	0.197 ± 0.01	5.08	98.50	0.21 ± 0.02	8.71	105.0
	1.0	1.04 ± 0.03	3.19	104.0	1.07 ± 0.07	6.57	107.0
	2.0	2.03 ± 0.02	1.08	101.5	2.12 ± 0.10	4.36	106.0
2C-T-2	5.0	4.81 ± 0.18	3.68	96.20	5.02 ± 0.28	5.53	100.4
	15	14.85 ± 0.42	2.85	99.00	15.40 ± 0.65	4.25	102.7
	25	26.03 ± 0.47	1.76	104.1	27.05 ± 1.30	4.81	108.2
2C-I	1.0	1.03 ± 0.06	5.83	103.0	1.07 ± 0.08	7.56	107.0
	10	9.88 ± 0.24	2.44	98.80	10.37 ± 0.43	4.16	103.7
	25	25.08 ± 0.13	0.49	100.3	26.06 ± 1.64	6.29	104.2
TMA-6	1.0	1.03 ± 0.03	2.94	103.0	1.09 ± 0.06	5.63	109.0
	10	10.19 ± 0.18	1.79	101.9	10.66 ± 0.54	5.07	106.6
	25	24.89 ± 0.16	0.64	99.56	25.83 ± 1.26	4.82	103.3
2C-T-4	5.0	4.81 ± 0.26	5.44	96.20	4.93 ± 0.37	7.51	98.60
	15	14.25 ± 0.24	1.63	95.00	14.03 ± 0.49	3.46	93.53
	25	24.79 ± 0.42	1.69	99.16	26.12 ± 1.47	5.64	104.5
2C-E	5.0	5.16 ± 0.09	1.74	103.2	5.32 ± 0.20	3.81	106.4
	15	14.91 ± 0.43	2.97	99.40	15.57 ± 1.07	6.89	103.8
	25	25.27 ± 0.31	1.21	101.1	27.21 ± 2.05	7.52	108.8

SD: standard deviation; CV: coefficient of variation.

like. The typical chromatograms obtained from these three products are shown in Fig. 6. As a result of the UFLC–FL method described herein, BDB, MMDA-2 and 2C-I were identified from the pale yellow powders (labeled BDB), colorless liquid (labeled Honey Flash 2) and the mushroom-like substances (labeled JETS), respectively. Furthermore, the concentrations of each phenethylamine were 0.24 mg/mg (BDB), 0.016 mg/mg (2C-I) and 0.98 mg/mL (MMDA-2). Since the matrix of each product is unknown, the recovery test using blank samples could not be performed. When the standard phenethylamines were spiked to the products, the corresponding phenethylamines were quantitatively recovered by the proposed method. Therefore, the present results seem to be suitable for each sample. Because the phenethylamines in real samples are easily extracted with MeOH or a H_2O–MeOH mixture and labeled with DBD-F in alkaline medium, the proposed procedure seems to be applicable to various products. Furthermore, the highly sensitive determination in biological specimens, such as blood and urine, may be possible by this method.

BDB (pale yellow powder) JETS (dried brown mushroom) Honey Flash 2 (colorless liquid)

Fig. 5. Features of tested phenethylamine products.

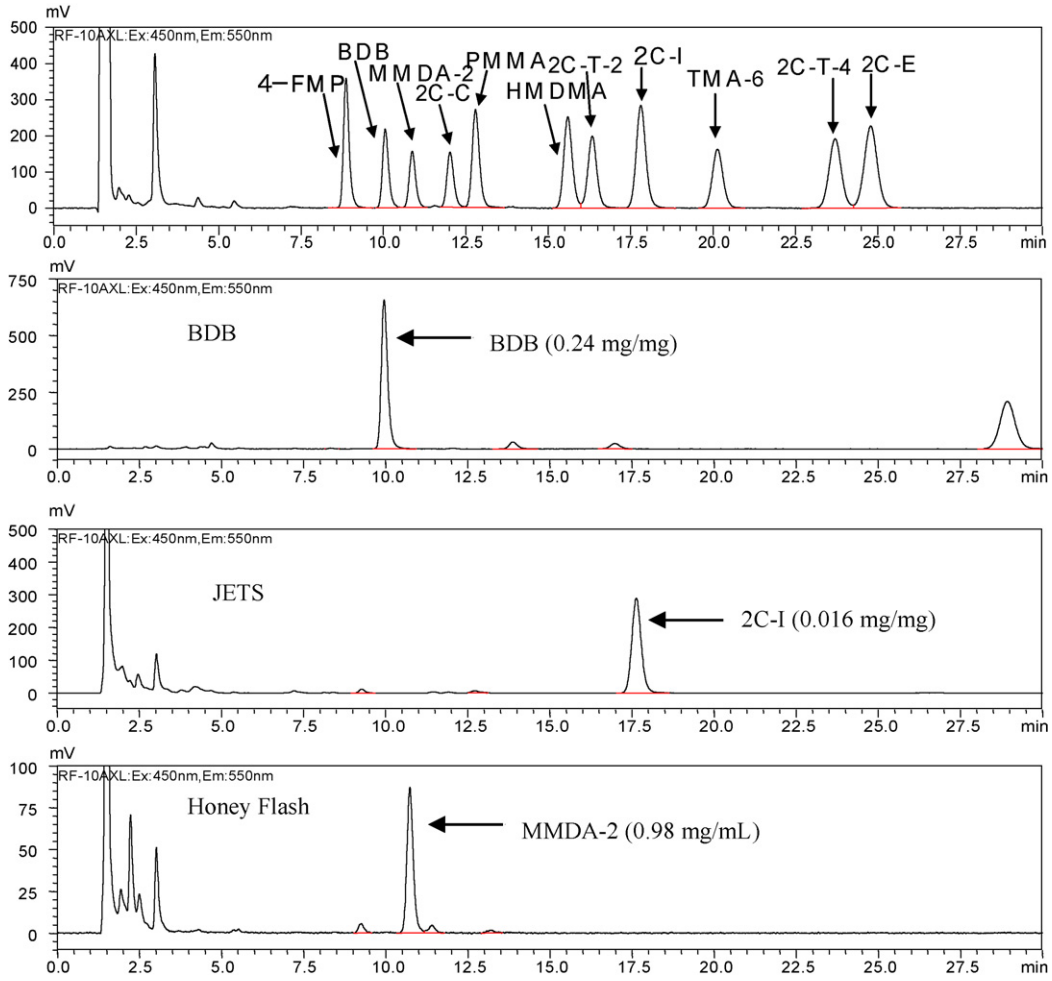


Fig. 6. Chromatograms obtained from phenethylamine products by UFLC–FL. The UFLC–FL conditions are described in Section 2.4.

4. Conclusion

In this investigation, we developed a sensitive and selective simultaneous determination method for 11 hallucinogenic phenethylamines, which are contained in 32 drugs listed as psychotropic substances controlled as "designated substances" by the Pharmaceutical Affairs Law in Japan as of April 2007. The proposed method is based upon the fluorescence labeling of primary and secondary amines in the phenethylamine structure with DBD-F. The resulting fluorophores were perfectly separated by a small resin (1.7 μm) column in a short run time and sensitively detected. The method was applied to determine the phenethylamine-containing products sold on the Japan market. Because the detection limits of the 11 phenethylamines on the chromatogram were 10 fmol–2.5 pmol, the method seems to be applicable not only for the products, but also for biological specimens, such as plasma and urine. We believe that the present method provides a useful means for the qualitative and quantitative analyses of various phenethylamines in biological specimens. Further study is currently in progress in our laboratory.

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