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TECHNICAL NOTE

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Thin Layer Chromatography/Fluorescence Detection of 3,4-Methylenedioxy-Methamphetamine and Related Compounds

ABSTRACT: A rapid and sensitive method for the detection of six methylenedioxylatedphenethylamines, 3,4-methylenedioxymethamphetamine (MDMA); 3,4-methylenedioxyamphetamine; 3,4-methylenedioxyethylamphetamine; *N*-methyl-1-(3,4-methylenedioxyphenyl)-2-butamine; *N*-methyl-1-(3,4-methylenedioxyphenyl)-3-butamine; and 3,4-methylenedioxydimethylamphetamine, by thin-layer chromatography with fluorescence detection is proposed. These compounds form fluorophores on the developing plate following spraying with a reagent consisting of sodium hypochlorite, potassium hexacyanoferrate (III), and sodium hydroxide, and heating for 3 min at 100°C. Blue fluorescent spots were observed under ultraviolet light in a wavelength range of 250–400 nm. The detection limits for MDMA and the above related compounds were 50 ng. The proposed method was effectively applied to the detection of MDMA in urine samples.

KEYWORDS: forensic science, 3,4-methylenedioxymethamphetamine (MDMA), fluorescence detection, TLC, urine

3,4-Methylenedioxymethamphetamine (MDMA) is a designer drug known as "Ecstasy" or "XTC" (Fig. 1). The casual use and smuggling of MDMA have recently increased in Japan, with significant seizures of the drug in 2001 and 2005 (1). MDMA, 3,4-methylenedioxyamphetamine (MDA), and 3,4-methylenedioxyethylamphetamine (MDEA, also called *N*-ethyl-MDA) have been listed as hallucinogens under Japanese law since 1989 and 1990, respectively, and have been strictly controlled. *N*-methyl-1-(3,4-methylenedioxyphenyl)-2-butamine (MBDB), a compound related to MDMA, has been controlled as an hallucinogen since 2006.

Several analytical methods have been reported for the detection of MDMA and related compounds, including gas chromatography with mass spectrometry (GC-MS) (2,3), liquid chromatography with fluorescence detection (LC-FD) (4–6), liquid chromatography with mass spectrometry (LC-MS) (7), thin-layer chromatography (TLC) (8,9), and immunoassay (10–12). With LC-FD methods, MDMA can be detected by its intrinsic fluorescence (λ_{ex} 285 nm, λ_{em} 320 nm) with a detection limit of 1–15 ng/mL without derivatization (4–6). Although immunoassay screening kits such as Triage® and Emit are available for MDMA, MDA, methamphetamine (MAMP), and other related phenethylamines, in practice they are not so selective and react with related compounds (10–12). Furthermore, the cutoff levels of MDMA by Triage and new Emit II are 2000 and 300 ng/mL, respectively (10,11), suggesting the poor detection sensitivities. Among the methods considered, TLC is the simplest. Although TLC is less sensitive and less quantitative either than GC-MS or LC-MS, its versatility and low running costs are advantageous for application as an initial screening test in forensic science. However, the reagents used for MDMA detection (visualization) by TLC and color test generally suffer from poor selectivity or sensitivity (e.g., Simon: no data with detection limit, acidified potassium permanganate: 0.1 μg, acidified iodoplatinate: 1 μg, Dragendorff: 5 μg, Maruqis: 1–5 μg, and Mandelin: 5–10 μg) (8,9).

Proof of MDMA use will in the future require positive detection in both urine and hair at a high analytical sensitivity (4). Our previous TLC method detected sensitively *p*-hydroxymethamphetamine (*p*OHMA, also called 4-hydroxymethamphetamine, 4HMA) as the oxidized dimer with hexacyanoferrate (III) by blue fluorescence (13). Such an approach is preferable to the reaction of hydrogen peroxide and peroxidase (14) in that the proposed reaction is faster and specific for para-substituted phenolic compounds. The reaction of MDMA with concentrated sulfuric acid and hydrogen peroxide produces 4-methoxy-3-hydroxymethamphetamine (MHMA), 4-hydroxy-3-methoxymethamphetamine (HMMA), and 3,4-dihydroxymethamphetamine (HHMA) (15). Thus, both fluorophors of HMMA and *p*OHMA can be detected sensitively on a TLC plate. Although various analytical methods (4–6) provide sensitive detection of the intrinsic fluorescence of MDMA, the emission is in the ultraviolet (UV) range (ca. 320 nm) which cannot be observed by the naked eye. TLC is useful in forensic science if the fluorescence of analytes can be detected in the visible range. In the present study, a new TLC method suitable for the fluorogenic detection of MDMA is proposed.

Materials and Methods

Materials and Reagents

3,4-MDMA hydrochloride (MDMA·HCl), 3,4-MDA hydrochloride (MDA·HCl), and 3,4-MDEA hydrochloride (MDEA·HCl) were donated by the National Research Institute of Police Science

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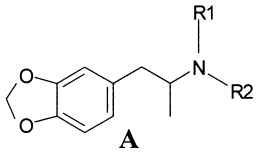

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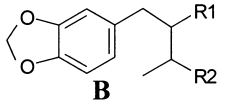

B

Compound	Structure	Abbreviation	R1	R2
3,4-Methylenedioxyamphetamine	**A**	MDA	-H	-H
3,4-Methylenedioxymethamphetamine	**A**	MDMA	$-CH_3$	-H
3,4-Methylenedioxyethylamphetamine	**A**	MDEA	$-CH_2CH_3$	-H
3,4-Methylenedioxy-*N*,*N*-dimethylamphetamine	**A**	MDDA	$-CH_3$	$-CH_3$
N-Methyl-1-(3,4-methylenedioxyphenyl)-2-butanamine	**B**	MBDB	$-NHCH_3$	-H
N-Methyl-1-(3,4-methylenedioxyphenyl)-3-butanamine	**B**	HMDMA	-H	$-NHCH_3$

FIG. 1—*Chemical structures of MDMA and related compounds.*

(Kashiwa, Japan). MBDB hydrochloride (MBDB·HCl) was purchased as 1 mg/mL in methanol solution from Wako Pure Chemical Industries (Osaka, Japan). *N*-methyl-1-(3,4-methylenedioxyphenyl)-3-butamine (HMDMA) and 3,4-methylenedioxydimethylamphetamine (MDDA, also called MDDM or MDMMA) were synthesized by the reported methods (16,17). Standard stock solutions of 10, 100, and 1000 μg/mL MDMA·HCl, MDA·HCl, MBDB·HCl, MDEA·HCl, HMDMA·HCl, and MDDA·HCl in methanol were prepared and stored in a refrigerator at −29°C.

TLC plates (silica gel 60, nonfluorescence; Lot No. 1.05721. 0009) were purchased from Merck (Darmstadt, Germany) and activated by heating in an oven at 110°C for 120 min before use.

Sodium hypochlorite solution (11.8% available chlorine), 30% hydrogen peroxide, and potassium hexacyanoferrate (III) were purchased from Wako. All other chemicals of analytical grade were from Kanto Chemical (Tokyo, Japan) or Wako. They were used as received.

Human urine samples were obtained from MDMA abusers arrested by Kanagawa Prefectural Police under "Voluntary Presentation." They were stored in a refrigerator at −29°C before use.

Analytical Instrumentation

Automated analyses were performed using a flow injection analytical (FIA) system (Shimadzu, Japan) consisting of LC pumps ($LC\text{-}10AD_{VP}$), an auto injector ($SIL\text{-}10AD_{VP}$) equipped with a 20-μL sample injection loop, a fluorescence detector (RF-10A XL) with a cell temperature controller (25°C) set at excitation 320 nm and emission 405 nm, a system controller ($SCL\text{-}10A_{VP}$), a column oven ($CTO\text{-}10AC_{VP}$), and a degasser (DGU-14A). Both system control and data analysis were carried out using LC Solutions software. The carrier solutions, the mixtures of purified water and buffers (pH 11), were supplied at a flow rate of 1.0 mL/min.

GC-MS

To compare the proposed TLC with GC-MS, the MS data of MBDB, MDEA, HMDMA, and MDDA were obtained under the following conditions: Fast atom bombardment (FAB)-MS JMS-700 (JEOL Ltd., Tokyo, Japan) was used to determine the molecular ions of the related compounds. FAB gas and the matrix were xenon and glycerol, respectively. The GC-MS system consisted of an Agilent Technologies 6890 GC system coupled with a 5973 MS system (Santa Clara, CA). A 1-μL injection was used in the split mode (50:1) with the injector held at 210°C. The analytical column was an Agilent Technologies HP-ULTRA 1 (Crosslinked Methyl Siloxane: 50 m × 0.2 mm I. D., 0.33 μm film) with a purified helium carrier gas maintained at a constant flow of 1.0 mL/min. The oven temperature was held at 190°C for 20 min. The transfer line was set at 280°C. The dried residue of several compounds was added to 100 μL of trifluoroacetic anhydride (TFA)-ethyl acetate (1:1, v/v) and heated at 55°C for 20 min. A small amount of ethyl acetate was then added to the sample, and an aliquot was then injected into the GC column.

Test Compound Treatment

In a test tube, 1 mL of 1 mM tyrosine (Tyr) or MDMA, 100 μL of 1 M sodium hydroxide, and various amounts of oxidizing reagent, 10 mM potassium hexacyanoferrate (III), 11.8% sodium hypochlorite, and 30% hydrogen peroxide were added, and the mixture was adjusted to 1.7 mL with purified water. Each test tube was heated in a boiling water bath to accomplish the oxidation reaction. After the reaction, 20 μL of the solution was injected into the FIA ($n = 3$). For the analysis of blank solutions, purified water instead of the sample solution or oxidizing reagent was used.

TLC

MDMA spotted on the TLC plate was developed with three solvent systems: DS1, isopropanol/28% ammonia solution (95:5, v/v); DS2, acetone/toluene/28% ammonia solution (20:10:1, v/v); DS3, tert-butanol/4 M ammonia solution (9:1, v/v). After development, the TLC plate was sprayed with the reagent to form the fluorophor, and then placed on a hot plate (Model: TP-35; Toyo Seisakusyo Co., Kashiwa City, Japan) at 100°C for 3 min. The sprayed reagent solution was prepared by mixing 1.0 mL of 1 M sodium hydroxide, 1.0 mL of 10 mM potassium hexacyanoferrate (III), and 500 μL–13.0 mL (increasing by 100 μL) of 1.18% sodium hypochlorite, and adjusted to a total volume of 17.0 mL with purified water. A UV lamp (PU-2; Topcon, Tokyo, Japan) was used to detect spots on the TLC plate. The fluorescent MDMA spot was observed visually under UV 250–400 nm irradiation. Retention factors (R_f) and detection limits of the analytes by the proposed method were also examined visually ($n = 5$) on the fluorescence intensity.

Urine Sample Treatment

To the urine sample (1 mL) was added 0.5 mL of 28% ammonia solution and 2 mL of diethyl ether. The mixture was mixed in a vortex mixer for a few seconds, and then centrifuged at 3000 rpm for 10 min. The same quantities of reagents were added again and the procedure repeated a total of three times. The supernatant was added to 1 μL of glacial acetic acid and evaporated to dryness. The residue was dissolved in 10 μL of methanol, and an aliquot of the solution was finally applied to a TLC plate.

Results and Discussion

Optimum Spray Reagents for the Fluorescence Reaction

FIA was used to obtain the TLC spraying reagent according to our previous report (13,14). With several oxidizing reagents examined, the fluorescence intensity of Tyr was increased by the addition of 0.59 mM hexacyanoferrate (III) as an oxidizing reagent. The maximum intensity was observed by heating at 100°C for 10 min. The fluorescence intensities obtained using sodium hypochlorite and hydrogen peroxide were approximately 10 times lower than that achieved using hexacyanoferrate (III) (Fig. 2). Although only hexacyanoferrate (III) made Tyr and *p*OHMA fluorescent (13), this reagent was ineffective for MDMA. In the meantime, the mixture of hexacyanoferrate (III) and sodium hypochlorite produced stronger fluorescence than the mixture of other oxidizing reagents (Fig. 3). Incidentally, all blank solutions examined did not show fluorescence. The mixture of 1 mL of the 1 mM MDMA solution, 100 μL of 1 M sodium hydroxide, 50 μL of 1.18% sodium hypochlorite, and 100 μL of 10 mM potassium hexacyanoferrate (III) was confirmed to give the highest fluorescence intensity. The fluorescence intensity of MDMA obtained by this reaction was six times weaker than that of *p*OHMA and 13 times weaker than that of Tyr (Fig. 4).

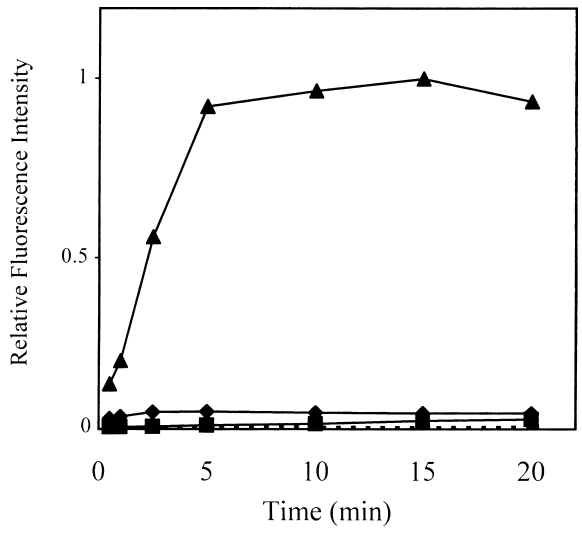

FIG. 2—*Effect of oxidizing reagent on fluorescence intensity of Tyr. Oxidizing reagent:* ▲ *0.59 mM* $K_3[Fe(CN)_6]$, ■ *0.04%* H_2O_2, ◆ *0.04% NaClO, – blank (not oxidizing reagent). The sample solution (total volume 1.70 mL) consisted of 1 mL of 1 mM Tyr, pOHMA or MDMA, 100 μL of 1 M sodium hydroxide, and various concentrations of oxidizing reagent. After oxidizing reaction, 20 μL of the solution was injected into the FIA (n = 3). To obtain the optimum conditions, the effects of the temperature, time, and concentration of oxidizing reagent were examined.*

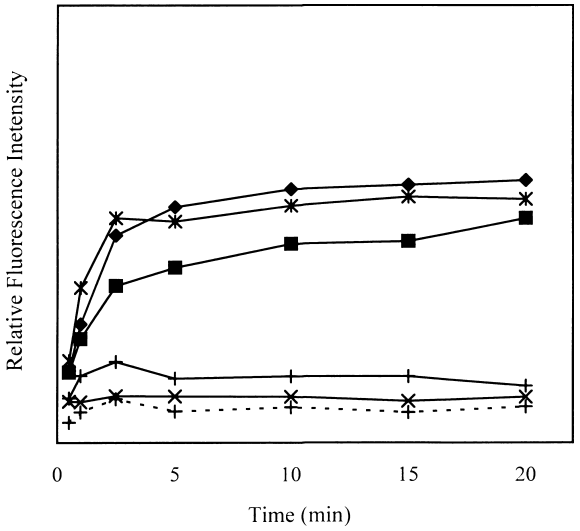

FIG. 3—*Fluorescence intensity of MDMA under various conditions. Spraying reagent solution: –+– 10 mM* $K_3[Fe(CN)_6]$, ◆ *1.18 % NaClO–10 mM* $K_3[Fe(CN)_6]$ *(1:2, v/v),* × *3%* H_2O_2*–10 mM* $K_3[Fe(CN)_6]$ *(1:5, v/v),* * *1.18 % NaClO–10 mM* $K_3[Fe(CN)_6]$ *(1:4, v/v),* ■ *1.18 % NaClO–10 mM* $K_3[Fe(CN)_6]$ *(1:1, v/v), --+-- blank (not oxidizing reagent).*

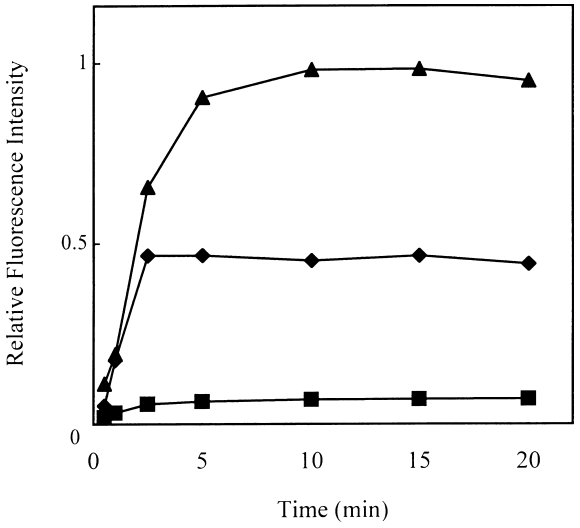

FIG. 4—*Comparison of fluorescence intensity of Tyr (▲), pOHMA (◆), and MDMA (■).*

TLC

According to the above result, the composition of the spray reagent solution was a mixture of 14.5 mL of purified water, 1.0 mL of 1 M sodium hydroxide, 1.0 mL of 10 mM potassium hexacyanoferrate (III), and 500 μL of 1.18% sodium hypochlorite. However, this spray reagent did not produce an MDMA spot on the TLC plate. It was thought that the silica gel of the TLC plate inhibited the reaction of the oxidizing reagent (18). This effect of silica gel was suppressed by the addition of more sodium hypochlorite. The intensity of MDMA fluorescence was increased depending on the amount of sodium hypochlorite added to the oxidizing reagent. The addition of the other constituents (sodium hydroxide, potassium hexacyanoferrate [III]) did not affect the fluorescence intensity of MDMA (18). The strongest fluorescence

TABLE 1—*Retention factors (R_f) and detection limits for MDMA and related compounds by the proposed method.*

Analyte	R_f value			Detection limit (μg)
	DS1	DS2	DS3	
MDMA	0.32	0.30	0.20	0.05
MDA	0.47	0.52	0.32	0.05
MBDB	0.49	0.49	0.36	0.05
MDEA	0.48	0.49	0.30	0.05
HMDMA	0.22	0.27	0.13	0.05
MDDA	0.40	0.48	0.29	0.05

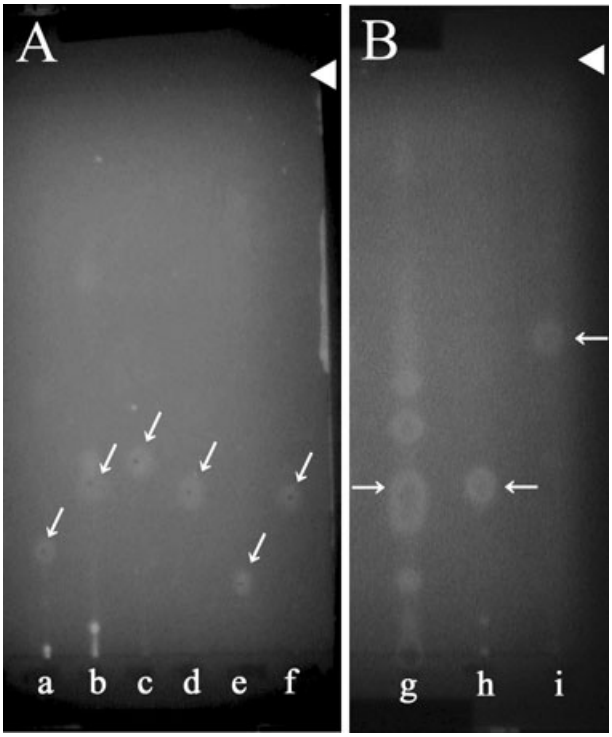

FIG. 5—*Photographs of TLC plates of (A) six standard methylendioxyphenethylamines and (B) urine sample from MDMA abuser. Developing solvent: (A) DS3; (B) DS2. Oxidizing reagent: (A and B) 10 mL of 1.18% sodium hypochlorite, 1 mL of 10 mM hexacyanoferrate (III), 1 mL of 1 M sodium hydroxide, 5 mL of purified water. Spots: (a) MDMA·HCl, (b) MDA·HCl, (c) MBDB·HCl, (d) MDEA·HCl, (e) HMDMA·HCl, (f) MDDA·HCl. One microgram of each compound was spotted on the TLC plate except for 1 μg of MDMA·HCl (h) and MDA·HCl (i). (g) Urine extract of an MDMA abuser. Arrows indicate target spots. White arrowheads indicate the fronts of the developing solvent.*

intensity on the TLC plate was obtained using the spraying solution containing 10 mL of 1.18% sodium hypochlorite. The detection limit of MDMA after TLC development was 0.05 μg. This value was the lower limit confirmed in all plates examined. It is thought that the oxidation of MDMA was more difficult than tryptamine and *p*OHMA because the concentration of sodium hypochlorite was *c*. 20 times higher than that of the test tube reaction.

The R_f values and detection limits of MDMA and related compounds by the proposed method are listed in Table 1. Six compounds could be discriminated using three developing solvents. Especially, MBDB, MDEA, HMDMA, and MDDA could be separated using DS1 and DS3, as shown in Fig. 5*A*. On the other hand, MS could not easily identify these compounds because they showed similar mass spectra (CI-free base: *m/z* 208, CI-TFA derivative: 304, EI-Free base: *m/z* 72, EI-TFA derivative: *m/z* 168), as shown in Table 2. The detection limits of MDA, MBDB, MDEA, HMDMA, and MDDA by the present TLC method were 0.05 μg. The cutoff limit of MDMA by the proposed TLC/fluorescence method is comparable to those by LC-MS (100 ng/mL) (7) and GC-MS (25–100 ng/mL) (2,3), but substantially higher than that by LC-FD (1–15 ng/mL) (4–6). Furthermore, the proposed method is capable of detecting MDMA, and is 2–100 times more sensitive than TLC using Simon, acidified potassium permanganate, acidified iodoplatinate or Doragendorff reagents (detection limits: 0.1–5 μg) (8).

After using our method, the spot of MDMA on the TLC plate could be also detected by the Simon reagent, but not by the Dragendorff or Marquis reagents. This result indicated that the acidic detection reagent might not be available after our reagent treatment. Incidentally, the detection limits of MDMA, MBDB, MDEA, and HMDMA by the TLC with the Simon reagent were 0.1 μg. A fluorescamine reagent could also be used on the TLC plate after the proposed method. MDA showed a fluorescent spot (yellow) while MDMA, MBDB, MDEA, HMDMA, and MDDA did not. As a possible season for the suppression of fluorescence, the difference in the optimum wavelengths was considered. Thus, the primary, secondary, and tertiary methylenedioxylatedphenethylamines are discriminated by spraying fluorescamine or Simon reagents after our reagent method.

MDDA is metabolized in liver and excreted mainly as MDMA and MDA (in addition to MDDA), and part of MDMA is also metabolized to HMMA and HHMA (19,20). These facts suggest that the detection of MDDA metabolites might become important in forensic science. The proposed method, which can readily distinguish MDMA and MDDA, is therefore very useful for MDDA detection.

Application to MDMA Abuser Urine Samples

The proposed method was applied to the urine samples obtained from MDMA abusers. These samples were proved to contain

TABLE 2—*MS data of MBDB, MDEA, HMDMA, and MDDA by GC-MS and FAB-MS.*

	Compounds			
	MBDB	MDEA	HMDMA	MDDA
EI (free base) (m/z)	72, 135, 77, 88, 57, 51	72, 135, 77, 51	58, 135, 77, 207, 51	72, 135, 77, 51
Retention time (EI) (minute)	8.21	7.34	8.87	7.73
EI (TFA-derivative)	168, 176, 135, 110, 77, 303	168, 162, 140, 135, 77, 303	135, 234, 303, 176, 129, 7 7	Not derived
Retention time (CI)	13.24	12.60	16.55	7.73
CI (free base)	208, 177, 206, 135,236	208, 163, 206, 236, 191	208, 236, 206, 135	208, 206, 163, 165, 236
CI (TFA-derivative)	304, 177, 332	304, 163, 332	304, 332, 135, 177	Not derived
FAB	208	208	208	208

MDMA and MDA by GC-MS. Figure 5*B* shows the TLC plate sprayed with the oxidizing reagent. The MDMA spot could be readily identified without interference by endogenous compounds. Although that of MDA could not be confirmed clearly, the proposed TLC method is useful for rapid and sensitive analysis of MDMA-abusers' urine.

Conclusion

A rapid and sensitive TLC/fluorescence method for detecting MDMA, MDA, MBDB, MDEA, HMDMA, and MDDA in human urine was demonstrated. In the proposed method, the developed silica gel plate was sprayed with a mixed oxidizing reagent of sodium hypochlorite and hexacyanoferrate (III), and heated at 100°C for 3 min. Spots of 3,4-methylenedioxyphenethylamine and its related compounds were observed as blue fluorescent spots under UV 250–400 nm irradiation.

Acknowledgments

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