HPLC with fluorescence detection of methamphetamine and amphetamine in segmentally analyzed human hair

Osama Al-Dirbashi,[a] Naotaka Kuroda,[a] Shigeko Inuduka,[b] Francesco Menichini[c] and Kenichiro Nakashima*[a]

[a] *School of Pharmaceutical Sciences, Nagasaki University, 1–14 Bunkyo-machi, Nagasaki 852-8521, Japan. E-mail: naka-ken@net.nagasaki-u.ac.jp*
[b] *Kinki Regional Narcotics Control Office, 4–1–76 Otemae, Chuo-ku, Osaka, Japan*
[c] *Department of Pharmaceutical Sciences, University of Calabria, 87036 Arcavacata di Rende, Italy*

Received 16th November 1998, Accepted 11th February 1999

A sensitive high-performance liquid chromatographic method with fluorescence detection for determining methamphetamine and its major metabolite, amphetamine, in abusers' hair segments was developed. Methamphetamine and amphetamine in hair samples collected from addicts were extracted into acidified methanol, derivatized with 4-(4,5-diphenyl-1*H*-imidazol-2-yl)benzoyl chloride, separated isocratically on an ODS column using TRIS–HCl buffer (0.1 mol dm^{-3}, pH 7.0)–methanol (30 + 70 v/v) as the mobile phase and the derivatives were detected fluorimetrically at 440 nm (λ_{ex} 330 nm). Calibration curves obtained by using control human hair spiked with standard solutions were linear ($r \geq 0.999$) up to at least 676.1 ng mg^{-1} for amphetamine and 746.1 ng mg^{-1} for methamphetamine. The detection limits at a signal-to-noise ratio of 3 were 51.4 and 74.6 pg mg^{-1} hair for amphetamine and methamphetamine, respectively. Using control hair spiked with standard solutions, the intra- and inter-day relative standard deviations ($n = 5$) were $\leq 8.6\%$ for both the target compounds. The method was successfully applied to the segmental analyses of methamphetamine abusers' hair samples.

1. Introduction

Problems related to ever-increasing substance abuse have been attracting more public attention and research efforts. Drugs of abuse including methamphetamine (MP) are known to cause serious social and law enforcement problems in addition to pathological changes in many organs and systems in the body. In Japan, MP and amphetamine (AP) are controlled by the Stimulants Drug Control Law, the former being abused by most Japanese addicts whereas the latter is detected as the main metabolite in biological samples.[1,2]

For forensic science laboratories and criminal justice agencies, hair might become the indispensable matrix for measuring exposure to xenobiotics, particularly drugs of abuse. Owing to its biological stability and physical state, hair is easy to collect and store until analysis can be carried out.[3] Moreover, the slow growth rate and the absence of drug metabolism in hair allow investigations over a lengthy period in comparison with other biosamples.[4] Hair analyses might disclose drug abuse history by providing long-term information on an individual's illegal drug consumption, in contrast to short-term information that blood or urine analysis provides. The detection of several drugs in human hair has been reported, including cannabis,[5] phencyclidine,[6] cocaine,[7–9] opiates,[8–11] tricyclic antidepressants[12] and nicotine,[10,12] in addition to MP and related compounds, referred to hereafter as methamphetamines (MPs).[1,4,8,12,13–18] Although gas chromatography–mass spectrometry (GC-MS) is the most frequently utilized method to determine drugs in hair,[3–6,8–17] other techniques such as radioimmunoassay (RIA)[7] and high-performance liquid chromatography with peroxyoxalate chemiluminescence detection (HPLC-POCL) after derivatization with a fluorescent reagent have also been reported.[1,18]

A lophine (2,4,5-triphenylimidazole) derivative, namely 4-(4,5-diphenyl-1*H*-imidazol-2-yl)benzoyl chloride (DIB-Cl), was recently synthesized in our laboratory[19] and proved to be a superior fluorescent labeling reagent for MPs owing to its reactivity with primary and secondary amines in different solvents under mild conditions, stability in acetonitrile and excellent detectability in comparison with some other commercially available fluorescent reagents.[20,21] In this paper, we report a simple and highly sensitive HPLC method with fluorescence detection for determining the concentrations of MP and AP in abusers' hair samples. Segmental analyses to reveal the drug abuse time course were performed on a 1 cm length basis.

2. Experimental

2.1. Materials and reagents

DIB-Cl was synthesized according to our reported method.[19] MP hydrochloride was obtained from Dainippon Pharmacy (Osaka, Japan). AP sulfate was synthesized in our laboratory according to known procedures.[22,23] Acetonitrile and methanol (HPLC grade) were purchased from Wako (Osaka, Japan). Analytical-reagent grade tris(hydroxymethyl)aminomethane (TRIS) was obtained from Sigma (St. Louis, MO, USA). Water was de-ionized and passed through a pure line WL21P system (Yamato Kagaku, Tokyo, Japan). All other chemicals were of analytical-reagent grade and used as received.

Stock standard solutions of MPs were prepared by dissolving suitable amounts of these compounds in water to give a final concentration of 1.0 mmol dm^{-3} per compound. These solutions were kept in the dark at 4 °C and were stable for at least 6 months. Working standard solutions were prepared by dilution in $NaHCO_3$–Na_2CO_3 buffer (10.0 mmol dm^{-3}, pH 9.0),

hereafter referred to as carbonate buffer, and in 5.0 mol dm^{-3} HCl–methanol (1 + 20 v/v) for standard sample derivatization and to spike the hair samples, respectively.

Spiked hair samples were prepared by adding aliquots of MP standard solutions to hair samples collected from healthy subjects in our laboratory who had not taken any medication over the past few months. Hair samples belonging to MP addicts were obtained from Kinki Regional Narcotics Control Office (Osaka, Japan). These samples were kept at −20 °C until needed.

TRIS–HCl buffer was prepared by adjusting the pH of TRIS aqueous solution to 7.0 with concentrated HCl and then adjusting the volume to give a final concentration of 0.1 mol dm^{-3}.

2.2. Hair sample pre-treatment and fluorescence derivatization

After marking their roots and tips, 10 strands of hair were stuck in parallel on a commercially available double-faced sticker and cut into 1 cm long segments. Each group (10 hair segments of 1 cm length each) was further cut to give 20 pieces of approximately 0.4–0.6 cm. Individually, these groups were washed by successive sonication in 10 ml of 3.5 mmol dm^{-3} aqueous sodium dodecyl sulfate (SDS) (0.1%) and 5 ml of ethanol for 1 h and 10 min, respectively. After decanting the ethanol, the hair was rinsed with 10 ml of distilled water, filtered off, thoroughly flushed with about 30 ml of distilled water and left to dry either at room temperature or in a dish dryer. Individually, the hair groups were accurately weighed, placed in clean test-tubes and portions of a mixture of 5.0 mol dm^{-3} HCl–methanol (1 + 20 v/v) were added to give a final hair concentration of 1 mg ml^{-1}. After sonication for 1 h, the hair was left to stand in the extraction solvent for 24 h at room temperature. After the hair had been filtered off, 100 μl of the filtrate were transferred into a screw-capped, brown-glass reaction vial and dried under a gentle stream of N_2 at room temperature. To the residue, 10 μl of carbonate buffer (10.0 mmol dm^{-3}, pH 9.0) and 180 μl of 0.1 mmol dm^{-3} DIB-Cl in acetonitrile were successively added, vortex mixed and kept at room temperature (*ca*. 24–27 °C). After 10 min, 10 μl of 8.0 mol dm^{-3} ammonia solution (28%) were added, vortex mixed for a few seconds and 5 μl of the resultant mixture were injected into the HPLC system. Fig. 1 shows the derivatization reaction scheme.

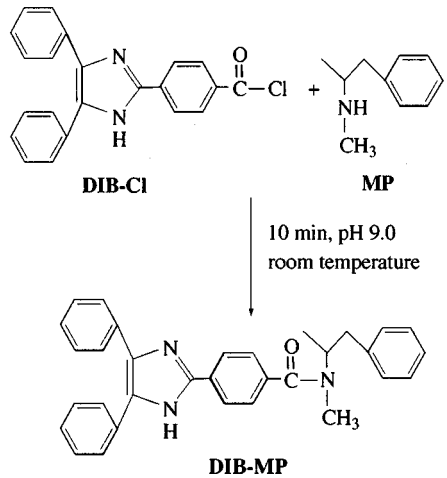

Fig. 1 Reaction scheme for labeling MP with DIB-Cl.

2.3. Efficiency of external decontamination procedure

Approximately 0.5 cm long control hair samples were soaked overnight in aqueous MP standard solutions at concentrations of 0.01, 0.5 and 10.0 μmol dm^{-3}, which are equivalent to 1.4, 67.6 and 1352.1 ng mg^{-1}, respectively, of AP and 1.5, 74.6 and 1492.1 ng mg^{-1} of hair, respectively, of MP. After filtering off and drying the hair naturally, it was extracted into 5.0 mol dm^{-3} HCl–methanol (1 + 20 v/v) with or without previous washing with SDS, ethanol and distilled water as described in Section 2.2. These samples were measured in triplicate.

2.4. Extraction efficiency

The extraction efficiency was assessed by sonicating 5 mg of abuser's hair sample D in 5 ml of 5.0 mol dm^{-3} HCl–methanol (1 + 20 v/v) for 1 h followed by incubation at room temperature for 48 h. Portions of 100 μl were taken at 0, 6, 12, 24 and 48 h after the sonication. These samples were measured in triplicate.

2.5. HPLC system and operating conditions

The HPLC system consisted of an LC10AS HPLC pump (Shimadzu, Kyoto, Japan), a Model 7125 injector with a 5 μl sample loop (Rheodyne, Cotati, CA, USA), a 250 × 4.6 mm id, 5 μm Daisopak SP-120-5-ODS analytical column (Daiso, Osaka, Japan), a CTO-6AS column oven set at 35 °C (Shimadzu), an RF-550 spectrofluorimeter (Shimadzu), set at λ_{ex} and λ_{em} of 330 and 440 nm, respectively, and a U-228-2P-500 recorder (Nippon Denshi Kagaku, Tokyo, Japan); 0.5 mm id stainless-steel tubing was used in all flow lines.

The HPLC separation was carried out isocratically by using TRIS–HCl buffer (0.1 mol dm^{-3}, pH 7.0)–methanol (3 + 7 v/v) as the mobile phase at a flow rate of 1.0 ml min^{-1}. The eluent was pre-mixed and de-gassed prior to use.

2.6. Calibration curves and reproducibility

Control human hair was treated as described in Section 2.2 except for the addition of MP standards to give final concentrations in the range 135.2 pg mg^{-1}–676.1 ng mg^{-1} for AP and 149.2 pg mg^{-1}–746.1 ng mg^{-1} of hair for MP prior to the extraction step. These samples were sonicated for 1 h, left to stand at room temperature for 24 h, then analyzed as described. Calibration curves were constructed by plotting the fluorescence intensity (FI) as peak height in arbitrary units against the concentration in ng mg^{-1}.

Intra- and inter-day reproducibilities of the proposed method were assessed by using control hair spiked with 27.0 and 29.8 ng mg^{-1} of AP and MP, respectively. These samples were analyzed as described over a period of 9 d.

3. Results and discussion

The primary purpose of this study was to establish a simple analytical method that provides information about the state of chronic stimulants abuse. For this purpose, segmental analysis of abusers' hair samples seems to be sufficiently informative if a highly selective and sensitive analytical method is employed. Several methods for the determination of MPs in hair have been reported but, to the best of our knowledge, none of them claimed a detection limit of sub-picogram level per injection. Recently, although MPs as dansyl chloride (Dns-Cl) derivatives could be determined successfully in a single human hair by HPLC with POCL detection, the detection limit was about 2 pg on-column.[1]

Using stable isotope dilution GC-MS, a detection limit of 0.5 ng mg^{-1} hair, which is equivalent to 50 pg per injection, was also reported.[15] More recently, we reported that MPs in human urine could be detected as DIB derivatives in the sub-picogram level per injection by using a simple HPLC–fluorescence system;[20,21] hence in this study we optimized a new analytical method using DIB-Cl as the fluorescent label for the determination of these drugs in abusers' hair segments to reveal the time course of substance abuse.

3.1. Pre-treatment of hair and fluorescence derivatization

It has long been known that drugs and elements found in the body are incorporated into hair *via* a mechanism or mechanisms that are not completely understood. Generally, drugs present in blood might diffuse passively into the rapidly growing hair follicles and become stably embedded in the hairshaft. In addition, the secretions of sweat, apocrine and sebaceous glands are possible vehicles for drug transfer into hair. For smokable drugs such as MPs, the analyst should consider the external exposure (*i.e.*, from air) as another potential route of contamination.[24]

Hair does not grow continuously, so it should not be treated as a uniform structure. Following an active growth and a short transitional phase, hair follicles enter a resting (*i.e.*, telogen) phase in which the hairshaft stops growing and can be removed easily. On the scalp of an adult human, approximately 15% of the hairs are in the resting phase.[25] Therefore, in our experiments we used 10 hair strands from each abuser that had the same physical properties (color, length) to avoid misleading results due to telogenic hair. After cutting into segments of 1 cm, each group of hairs was washed by sonication in 3.5 mmol dm^{-3} SDS, which has been reported to remove the external contamination on the sample,[14,15] followed by sonication in ethanol. However, when control hair samples were soaked in standard aqueous solutions of MPs overnight, significant adsorption of these compounds onto or into the hair strands took place at concentrations of ≥ 0.5 μmol dm^{-3}. As shown in Table 1, the externally adsorbed amounts of MPs could not be totally removed, which suggests that part of the adsorbed amounts might have permeated into the hairshafts rather than being adsorbed on the surface. Therefore, the external contamination should not be underestimated. However, if the metabolites together with the parent drug were simultaneously detectable in a suspect's hair, this suggests that they were incorporated through the blood or the secretions of the sweat, apocrine and sebaceous glands rather than being from an external source such as air. Sonication of hair in 5.0 mol dm^{-3} HCl–methanol (1 + 20 v/v) for 1 h followed by overnight incubation at room temperature was reported to be an efficient extraction method of MP and AP from human hair.[4,15] As shown in Fig. 2, there was a proportional relationship between the incubation time and the relative recovery; hence we employed an incubation period of 24 h.

Table 1 Effect of washing[a] on the external contamination of control hair after being soaked in MP aqueous solution overnight

Concentration of AP and MP in water/ μmol dm^{-3}	Washed before extraction/ ng mg^{-1} hair ± SD (n = 3)		Extracted without washing/ ng mg^{-1} hair ± SD (n = 3)	
	AP	MP	AP	MP
0.01	ND[b]	ND	ND	ND
0.5	0.8 ± 0.1	1.1 ± 0.3	2.1 ± 0.1	2.4 ± 0.3
10.0	12.2 ± 0.4	12.2 ± 0.5	29.5 ± 0.8	28.6 ± 0.5

[a] Washing by sonication with SDS and ethanol followed by rinsing with distilled water. For details, see Section 2.2. [b] Not detectable.

The derivatization reaction was performed according to our previously reported conditions[21] except that acetone as the reagent's solvent was changed to acetonitrile, since DIB-Cl is stable for a longer time in the latter, and the addition of 10 μl of 8.0 mol dm^{-3} ammonia solution, that reacts with the excess DIB-Cl to stop the reaction and results in faster elution of the reagent blank peaks.

The derivative's stability was monitored over 24 h when kept at room temperature in the dark. DIB-AP and -MP were sufficiently stable and no significant change in the peak heights was observed after 24 h. This suggests the possibility of the simultaneous preparation of many samples to be analyzed later or to be injected through an automated sample injector.

3.2. Chromatographic separation

Fig. 3 illustrates typical chromatograms of control human hair, control hair spiked with 27.0 and 29.8 ng mg^{-1} of AP and MP, respectively, and the first centimeter from the scalp surface of hair sample D collected from a drug abuser. As shown in Fig. 3(A), no interference due to endogenous compounds in human hair was observed and the two target compounds were well separated from the reagent blank. The retention times of DIB-AP and -MP were 35.4 and 40.5 min, respectively. The use of 5.0 mol dm^{-3} HCl–methanol (1 + 20 v/v) resulted in a more selective extraction in comparison with liquid–liquid extraction using hexane after treating the hair with 2.5 mol dm^{-3} NaOH solution. However, the peaks corresponded to compounds co-extracted with MPs and those due to the reagent blank, preventing attempts at reducing the retention time.

3.3. Calibration curves, detection limits and reproducibility

Calibration curves of AP and MP using spiked hair samples were obtained by plotting the peak height against the corresponding concentration in ng mg^{-1} hair. Linear relationships were obtained in the concentration range 135.2 pg mg^{-1}–676.1 ng mg^{-1} for AP and 149.2 pg mg^{-1} —746.1 ng mg^{-1} of hair for MP. The regression equations and correlation coefficients for the two compounds were as follows: AP, $y = 0.26x + 1.04$ (r = 1.000); and MP, $y = 0.11x + 0.54$ (r = 0.999); y is the fluorescence intensity as peak height in arbitrary units and x is the concentration in ng mg^{-1} hair. The detection limits at a signal-to-noise ratio of 3 were 51.4 (0.13) and 74.6 (0.19) pg mg^{-1} hair (pg per 5 μl injection) for AP and MP, respectively. Although it is known that spiking hair with drug substances will not result in a situation similar to those incorporated *in vivo*, unlike other biosamples such as urine,

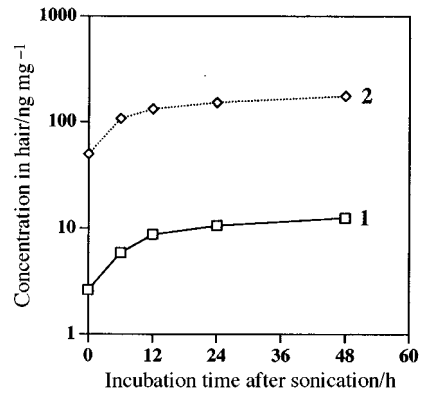

Fig. 2 Effect of incubation time of hair at room temperature after 1 h of sonication in 5.0 mol dm^{-3} HCl–methanol (1 + 20 v/v) on the efficiency of extraction of MPs from abuser hair sample D. Curves: 1, AP; and 2, MP.

adding these substances to a control hair gives information about other compounds found in normal hair that might be co-extracted and interfere with the determination of the target compound.

Intra- and inter-day variation data are summarized in Table 2. The reproducibility of the present method was investigated over 9 d using control hair samples spiked with AP and MP at concentrations of 27.0 and 29.8 ng mg^{-1}, respectively. Satisfactory intra- and inter-day reproducibility data (n = 5) with relative standard deviations (RSD) of ≤8.6% for both compounds were obtained.

In general, for the determination of MPs by GC-MS, derivatization is required to increase the volatility and it has been performed using trifluoroacetic anhydride at 55 °C for 20 min[4,15,16,26] or pentafluoropropionyl anhydride for 30 min at 60 °C[8] or 40 min at 80 °C.[17] To be sensitively detected by POCL after HPLC separation, Takayama *et al.* derivatized AP and MP with Dns-Cl at 45 °C for 1 h.[1,18] However, in our study, the derivatization of MPs with DIB-Cl for 10 min at room temperature was not only found to be simple, but also led to >10 times higher sensitivity in comparison with the HPLC-POCL method.[1] Compared with GC-MS methods,[8,15,17] the present method is still more than two orders of magnitude more sensitive in terms of the injected amount.

To carry out hair analysis for drugs of abuse, Moeller[27] recently reported that 20–50 mg of hair material are sufficient for standard procedures with electron-impact detection and single ion monitoring. An amount of hair in the range 10–50 mg was used in GC-MS methods.[8,15,17] Owing to its high sensitivity, sub-milligram amounts of hair were used in our study, which are comparable to those reported by Takayama *et al.*, who performed the analysis on a single hair.[1,18] In our procedure, however, the 1 cm-based segmental analysis of 10 hair strands from each addict is obviously more informative than a whole single hair analysis.

3.4. Segmental analysis of abusers' hair samples

Human scalp hair grows at an average rate of 1 cm per month.[8,14] Therefore, segmental analysis of hair cut into sections 1 cm long provides a monthly-based drug abuse time course as this information is stored in the keratinized hair. In this study, six hair samples from known abusers were segmentally analyzed and the concentrations of MP determined in different segments were in the range of 1.8–170.7 ng mg^{-1}. AP, the main metabolite of MP, was also detected in many of the segments and its concentrations ranged from 0.5 to 11.4 ng mg^{-1}. In addition to segment 3–4 of sample C, MP was also not detectable in segments which were more than 4 cm from the scalp surface of the 14 cm long hair sample B. This suggests that sample B corresponds to a relatively recent rather than a chronic abuse case. Interestingly, sample C contained both black and white hair in which MP was determined at the level of 5.5–10.6 ng mg^{-1} in the former whereas it was not detectable in the latter. This suggests that the presence of melanin, the principal pigment of the hair, is a determining factor for the incorporation of these compounds into hair according to their affinity to this pigment. The chemical similarity between the amino acid tyrosine, a precursor of melanin,[24,25] and MPs might significantly participate in the incorporation mechanism of these substances in pigmented hair. Similarly, Takayama *et al.* reported that MP was detected at a concentration of 10.2 ng mg^{-1} in black hair whereas it was not detectable in white hair from the same subject.[18] In a detailed study, Nakahara *et al.* also reported a high correlation coefficient (r^2 = 0.979) between the product melanin affinity × lipophilicity of 19 neutral and basic drugs, including MPs, and their incorporation rates into hair.[28] Results of the segmental analysis of six hair samples of MP abusers are summarized in Table 3. Except for sample D, which contained a maximum MP concentration of 170.7 ng mg^{-1} in segment 2–3, our results regarding the concentrations of MPs in abusers hair are, however, comparable to those reported in the literature,[1,4,12,13,18] with a maximum of 125.9 ng mg^{-1}.[12]

In conclusion, hair, as a biological sample, provides the toxicological or forensic analyst with answers corresponding to questions related to drugs of abuse that could not be clarified through conventional blood or urine analysis. Owing to its availability, ease of collection, storage, stability and stability of the drugs embedded in its keratinized strands for lengthy periods, hair is an excellent sample when chronic exposure to

Table 2 Intra- and inter-day reproducibilities of the determination of AP and MP in spiked human hair

Compound	Concentration added/ ng mg^{-1}	Intra-day (n = 5)		Inter-day (n = 5)	
		Found/ ng mg^{-1}	RSD (%)	Found/ ng mg^{-1}	RSD (%)
AP	27.0	25.8	7.7	26.9	8.0
MP	29.8	28.2	6.6	29..9	8.6

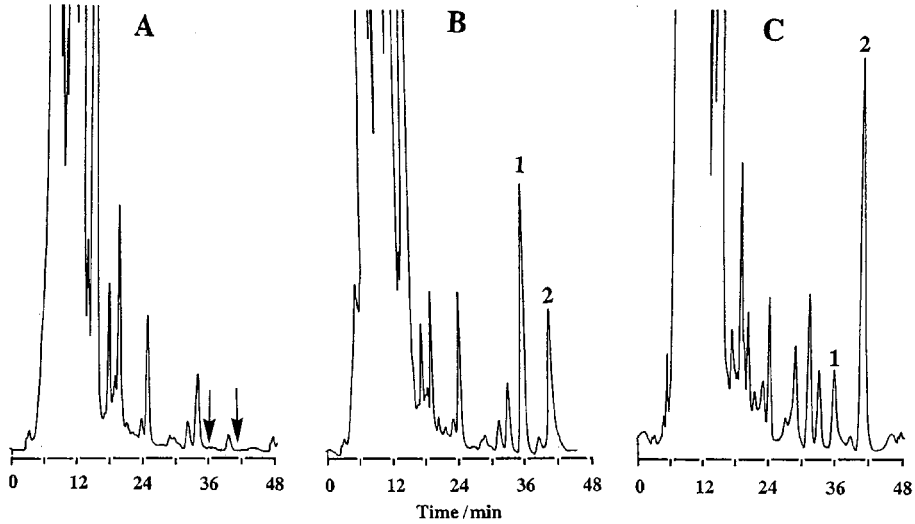

Fig. 3 Typical chromatograms with fluorescence detection corresponding to (A) control human hair, (B) control human hair spiked with 27.0 ng mg^{-1} of AP and 29.8 ng mg^{-1} of MP and (C) segment 0–1 of abuser hair sample D. Peaks: 1, DIB-AP; and 2, DIB-MP. Arrows in (A) indicate the expected retention times of DIB-AP and -MP. For other experimental conditions, see text.

Table 3 Concentration of MP and AP in abusers' hair samples determined by the present method

			Concentration[a] of MP/AP in hair segments[b]/ng mg^{-1}					
Sample	Color	Length/cm	0–1	1–2	2–3	3–4	4–5	5–14
A	Black	5	34.5/1.5	12.4/0.7	20.7/1.1	14.7/0.6	11.0/0.5	
B	Brown[c]	14	22.9/1.1	14.2/ND[e]	26.6/1.1	5.1/ND	ND/ND	ND/ND
C	Gray[d]	4	10.6/1.0	6.4/1.1	5.5/0.8	ND/ND		
D	Black	3	106.9/8.4	119.3/8.0	170.7/11.8			
E	Black	5	1.8/ND	1.8/ND	2.8/2.5	11.5/ND	3.7/ND	
F	Black	3	2.7/0.8	2.5/0.5	2.3/ND			

[a] Average of duplicate measurements. [b] Segments are numbered according to their distance from the scalp surface (cm). [c] Brown dye applied to a black hair. [d] A mixture of black and white hair. MPs were not detectable in white hair and these results correspond to the black portion. [e] Not detectable.

drugs of abuse is in question. In this paper, we have presented a new method for the determination of MP and AP in 1 cm long hair segments of abusers. Not only is the proposed method simple and highly sensitive, but also a sub-milligram amount of hair material is sufficient to carry out the analysis. The fluorescence derivatization reaction proceeds under mild conditions and the resultant derivatives are stable for at least 24 h when kept at room temperature in the dark. The present method was successfully applied to hair samples collected from MP abusers, and hence might be useful for those interested in forensic and toxicological investigations.

References

1 N. Takayama, S. Tanaka and K. Hayakawa, *Biomed. Chromatogr.*, 1997, **11**, 25.
2 T. Inoue, K. Tanaka, T. Ohmori, Y. Togawa and S. Seta, *Forensic Sci. Int.*, 1994, **69**, 97.
3 A. Pollettini, A. Groppi and M. Montagna, *Forensic Sci. Int.*, 1993, **63**, 217.
4 Y. Nakahara, K. Takahashi, Y. Takeda, K. Konuma, S. Fukui and T. Tokui, *Forensic Sci. Int.*, 1990, **46**, 243.
5 V. Cirimele, P. Kintz and P. Mangin, *Forensic Sci. Int.*, 1995, **70**, 175.
6 M. H. Slawson, D. G. Wilkins, R. L. Foltz and D. E. Rollins, *J. Anal. Toxicol.*, 1996, **20**, 350.
7 G. Koren, J. Klein, R. Forman and K. Graham, *J. Clin. Pharmacol.*, 1992, **32**, 671.
8 M. R. Moeller, P. Fey and R. Wennig, *Forensic Sci. Int.*, 1993, **63**, 185.
9 A. M. Bermejo Barrere and S. Trano Rossi, *Forensic Sci. Int.*, 1995, **70**, 203.
10 P. Kintz and P. Mangin, *Forensic Sci. Int.*, 1993, **63**, 99.
11 A. Tracqui, P. Kintz, B. Ludes, C. Jamey and P. Mangin, *J. Forensic Sci.*, 1995, **40**, 263.
12 I. Ishiyama, T. Nagai and S. Toshida, *J. Forensic Sci.*, 1983, **28**, 380.
13 O. Suzuki, H. Hattori and M. Asano, *J. Forensic Sci.*, 1984, **29**, 611.
14 Y. Nakahara, K. Takahashi and K. Konuma, *Forensic Sci. Int.*, 1993, **63**, 109.
15 Y. Nakahara, K. Takahashi, M. Shimamine and Y. Takeda, *J. Forensic Sci.*, 1991, **36**, 70.
16 S. Suzuki and T. Inoue, *J. Anal. Toxicol.*, 1989, **13**, 176.
17 M. Rothe, F. Pragst, K. Spiegel, T. Harrach, K. Fischer and J. Kunkel, *Forensic Sci. Int.*, 1997, **89**, 11.
18 N. Takayama, S. Tanaka, R. Kizu and K. Hayakawa, *Jpn. J. Toxicol. Environ. Health*, 1998, **44**, 116.
19 K. Nakashima, H. Yamasaki, N. Kuroda and S. Akiyama, *Anal. Chim. Acta*, 1995, **303**, 103.
20 O. Al-Dirbashi, J. Qvarnstrom, K. Irgum and K. Nakashima, *J. Chromatogr. B*, 1998, **712**, 105.
21 O. Al-Dirbashi, N. Kuroda, F. Menichini, S. Noda, M. Minemoto and K. Nakashima, *Analyst*, 1998, **123**, 2333.
22 N. Nagai, *Yakugaku Zashi*, 1893, **13**, 901.
23 A. Buzas and C. Dufour, *Bull. Soc. Chim. Fr.*, 1950, 139.
24 G. L. Henderson, *Forensic Sci. Int.*, 1993, **63**, 19.
25 M. R. Harkey, *Forensic Sci. Int.*, 1993, **63**, 9.
26 T. Niwaguchi, S. Suzuki and T. Inoue, *Arch. Toxicol.*, 1983, **52**, 157.
27 M. R. Moeller, *Ther. Drug Monit.*, 1996, **18**, 444.
28 Y. Nakahara, K. Takahashi and R. Kikura, *Biol. Pharm. Bull.*, 1995, **18**, 1223.

Paper 8/08912D