Available online at www.sciencedirect.com
ScienceDirect
Journal of Chromatography B, 865 (2008) 33–39
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Development of a reference material using methamphetamine abusers' hair samples for the determination of methamphetamine and amphetamine in hair

Sooyeun Lee*, Yonghoon Park, Wonkyung Yang, Eunyoung Han, Sanggil Choe, Sangwhan In, Miae Lim, Heesun Chung

National Institute of Scientific Investigation, 331-1 Sinwol-7-dong, Yangcheon-gu, Seoul 158-707, Republic of Korea

Received 26 June 2007; received in revised form 24 January 2008; accepted 25 January 2008
Available online 3 February 2008

Abstract

In the present study, we developed a reference material (RM) using authentic hair samples for the determination of methamphetamine (MA) and its main metabolite, amphetamine (AP) in human hair. MA abusers' hair samples were collected, homogenized and finally bottled. The concentration of each bottle was determined using two extraction methods, agitation with 1% HCl in methanol at 38 °C and ultrasonication with methanol/5 M HCl (20:1), followed by gas chromatography/mass spectrometry (GC–MS) after derivatization with trifluoroacetic anhydride (TFAA). Both analytical procedures were fully validated and their extraction efficiency was compared. The homogeneity of analytes was evaluated and their property values were determined with their uncertainties. The two methods were acceptable to analyze MA and AP in human hair through the validation and comparative studies using spiked and authentic hair samples as well as NIST SRM 2379 certified reference material. Satisfying homogeneity was reached for MA and AP in the prepared RM. Finally, a human hair RM containing MA and AP is prepared at the level of 7.64 ± 1.24 and 0.54 ± 0.07 ng/mg, respectively. This material can be useful in forensic laboratories for internal quality control and external quality assurance.

Keywords: Reference material; Methamphetamine; Amphetamine; Hair analysis

1. Introduction

Methamphetamine (MA) has received the most attention as a drug of abuse in Korea. It undergoes some *N*-demethylation to amphetamine (AP), its major active metabolite [1]. Thus, both MA and AP are analyzed in various specimens to prove an individual's MA abuse. Especially, hair analysis for MA is critical because it is accepted by law enforcement agencies as one of important corroborative facts for MA abuse in Korea. The hair analysis provides information not only on chronic drug use but also on drug use period according to the rate of hair growth [2], which has a key effect on legal decision. Therefore, quality assurance becomes a major issue in hair testing in the forensic field.

As part of quality control, the need for a reference material (RM) for drug analysis has rapidly increased in forensic and clinical laboratories. Several RMs have been developed in the form of lyophilized urine and powdered or segmented hair so that they can be employed to evaluate the accuracy and precision of analytical methods in the area of drugs of abuse [3–6]. In order to produce a certified reference material (CRM) assessing material homogeneity and stability as well as assigning property values based on the results of measurements are the most essential parts [7–11]. Among them, the homogeneity of the drug distribution in hair reference materials is a major concern so that hair was pulverized or cut into short segments [3,4]. On the other hand, the stability of drugs in biological fluid samples seems to be considered more significantly due to vulnerability by improper transport, handling or sample storage conditions [12]. However, the hair sample is indefinitely stable and little influenced by the

* Corresponding author at: Toxicology Unit, Division of Investigative Science, Imperial College London, Charing Cross Campus, St Dunstan's Road, London W6 8RP, United Kingdom. Tel.: +44 20 8846 7108/+82 2 2600 4937; fax: +44 20 8846 7110/+82 2 2600 4939.
E-mail address: spp26625@yahoo.co.kr (S. Lee).

1570-0232/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2008.01.039

preservation, storage and transportation condition. It is reported that the analytes spiked in hair lasted up to a year in a harsh condition [4,13]. Thus, it is assumed that stability tests in hair RMs were not considered significantly in most studies.

In 2003, the National Institute of Standards and Technology (NIST) developed two standard reference materials for drugs of abuse in human hair, SRM 2379 spiked with cocaine, benzoylecgonine, cocaethylene, phencyclidine, AP and MA as well as SRM 2380 spiked with codeine, morphine, monoacetylmorphine and tetrahydrocannabinol, which are commercially available. They were prepared by soaking drug-free human hair in DMSO solutions containing chemicals [3]. These RMs are very useful for method development and validation, quality control and proficiency tests because they have the certified values of the analytes. However, it is recommended that authentic hair should be used for quality control since spiked control samples cannot substitute for the actual hair of a drug user [14]. The spiked ones may have different characteristics, such as extraction efficiency, metabolite-to-parent drug ratios, *etc*., from authentic ones. Therefore, we planned to produce a candidate for a CRM for the determination of MA and AP in human hair using authentic hair samples. From this point of view, it was a more crucial issue to obtain the hair sample with uniformed concentration of MA and AP in a pool of MA abusers' hair samples since the amounts of MA and its metabolite, AP in the hair samples collected from actual drug users can vary drastically between individuals, between hairs of an individual or even along hair shaft, depending on drug use patterns [13].

In the present study, we developed a RM as a candidate for a CRM using MA abusers' hair samples. In order to evaluate homogeneity, the concentration of each bottle was determined using two extraction methods, one based upon agitation with 1% HCl in methanol at 38 °C and one based upon ultrasonication with methanol/5 M HCl (20:1). Finally, the property values of MA and AP in the produced RM were established with their uncertainties.

2. Experimental

2.1. Chemicals

Dichloromethane, methanol, ethyl acetate and hydrochloric acid (HCl) were analytical grade. MA, AP, MA-d_5 and AP-d_5 were obtained from Cerilliant (TX, USA) and trifluoroacetic anhydride (TFAA) was purchased from Sigma–Aldrich (MO, USA).

2.2. Preparation of a RM

Hair samples were collected from about 200 suspected drug addicts arrested by the police and submitted to the National Institute of Scientific Investigation in Korea. The specimens were first analyzed to report results to the police and then the remaining hair of each specimen was used to make a hair pool. This research was conducted according to the guideline of the National Institute of Scientific Investigation Ethics Committee. All the subjects were Korean with originally black hair and the roots of hair were removed. The specimens, where the MA concentrations ranged from 0.5 to 50 ng/mg, were mixed to create a pool. The hair was washed in a sufficient volume of dichloromethane for 2 min twice, dried at room temperature and cut into less than 2 cm. Then, it was stirred for 60 min in a large volume of distilled water to produce a homogenous mixture [13]. After drying again, the hair was segmented into about 1 mm, sieved, blended and finally bottled (103 vials, *ca.* 100 mg each). Additional 68 bottles of blank material were also prepared with drug-free hair in the same manner. The materials were kept at room temperature before analysis.

2.3. Method validation

The analytical methods were validated using spiked drug-free hair according to Eurachem Guide [15] and following parameters were evaluated: selectivity/specificity, linearity, limit of detection (LOD) and quantification (LOQ), recovery, intra- and inter-assay precision and accuracy. In order to demonstrate linearity five sets of calibrators (0.25, 0.5, 1, 2.5, 5, 7.5 and 10 ng/mg) were prepared and analyzed. For the LOD and LOQ determination, hair samples spiked with MA and AP of the concentrations below 0.25 ng/mg were evaluated and the analyte concentrations of which the signal-to-noise ratios greater than 3 and 10 were chosen for each. The recovery of MA and AP was determined by comparing the analysis of extracted and non-extracted spiked samples at low (1 ng/mg) and high (8 ng/mg) concentrations. Method precision and accuracy were examined by analyzing hair samples spiked with low (1 ng/mg), medium (4 ng/mg) and high (8 ng/mg) concentrations of MA and AP, respectively. The six aliquots of each sample were analyzed on the first day, followed by triplicates for the four consecutive days. Authentic hair from a post-mortem case and NIST SRM 2379 were used to compare two extraction methods and to verify analytical methods.

2.3.1. Agitation-based method

The agitation-based method was conducted as previously described [16]. Briefly, triplicate samples from one MA abuser's hair and NIST SRM 2379 were accurately weighed (*ca.* 10 mg) and washed twice with distilled water and methanol, respectively. After dried, they were cut into very small pieces of less than 1 mm and agitated with 3 ml of 1% HCl in methanol for 20 h at 38 °C. MA-d_5 and AP-d_5 were added as internal standards. The hair extract was evaporated to dryness at 45 °C under N_2 gas and then the residue was derivatized with 100 μl of TFAA/ethyl acetate (1:1) at 65 °C for 15 min. The excess derivatizing reagent was removed under N_2 gas at 45 °C and the residue was reconstituted in ethanol for GC–MS analysis.

2.3.2. Ultrasonication-based method

The ultrasonication-based method was carried out using a slight modification of a method described elsewhere [17]. The extraction method was performed in the same manner as shown in Section 2.3.1 except ultrasonication with 3 ml of methanol/5 M HCl (20:1) for 1 h followed by storing the solution at

room temperature overnight instead of agitation with 3 ml of 1% HCl in methanol for 20 h at 38 °C.

2.3.3. GC–MS analysis

The analysis of every hair sample was performed on an Agilent 6890/5973 GC–MS system. The GC was equipped with a 30-m-long, 0.25-mm-i.d., 0.25-μm-film-thickness HP-5MS capillary column. The inlet temperature was 260 °C and the helium flow rate was 1.0 ml/min. The oven was programmed to operate at an initial temperature of 100 °C for 1 min, to increase the temperature to 270 °C at a heating rate of 20 °C/min and to hold at 270 °C for 10 min. The MS was operated in selected ion monitoring (SIM) mode. The TFAA derivatized ions for MA, AP, MA-d_5 and AP-d_5 were as follows: MA, *m/z* 154, 118, 110, 91; AP, *m/z* 140, 118, 91; MA-d_5, 158, 122; AP-d_5, 144, 122 [16].

2.4. Homogeneity test

In order to evaluate the homogeneity among the bottles, three portions from ten bottles for each, randomly selected from a batch, were taken and the concentration of each sample was determined using the two different analytical methods as shown in Section 2.3. Statistical analysis was carried out and the uncertainties of MA and AP for the two methods were calculated using a one-way analysis of variance (ANOVA) [9,18].

2.5. Characterization

Three portions from three bottles for each, representative of the batch, were randomly chosen and analyzed using the same method as Section 2.3. The uncertainties of MA and AP for the two methods were calculated in compliance with the EURACHEM/CITAC Guide [19]. The major components contributing to their uncertainties were the amount of MA or AP in the test sample, the weight of the test sample and the method precision, which were based on the equation to calculate the mesurand from intermediate values. Statistical analysis was carried out using an ANOVA. Finally, the property values of MA and AP in the produced RM were established with their combined uncertainties [11,18].

3. Results

3.1. Method validation results

Results of the validation of the methods are summarized in Tables 1 and 2, including calibration results of five calibrations of seven standards. No interferences were detected at the retention times of the analytes and the internal standards in both methods (data not shown). Results of intra- and inter-assay precision and accuracy were satisfactory for MA and AP, less than 20% for the low-concentration samples (1 ng/mg) and less than 10% for the medium-concentration (4 ng/mg) and high-concentration ones (8 ng/mg). Recovery was over 90% and R^2 of the calibration curves was higher than 0.998 in every occasion. The LODs by the agitation-based method were 0.25 and 0.125 ng/mg for MA and

Table 1
Results of the validation of the agitation-based method for the quantification of MA and AP

Analyte (ng/mg)	Precision (CV, %)		Accuracy (bias, %)		Recovery (%, $n=5$)	Slope[a]			Intercept[a]			R^{2a}	LOD (ng/mg)	LOQ (ng/mg)
	Intra-assay ($n=6$)	Inter-assay ($n=18$)	Intra-assay ($n=6$)	Inter-assay ($n=18$)		Mean	S.D.	S.E.	Mean	S.D.	S.E.			
MA														
1	14.0	4.4	16.4	5.7	102.4									
4	8.0	2.2	9.9	2.4	–	0.020	*0.001*	*0.000*	0.072	*0.020*	*0.009*	0.998	0.25	0.25
8	3.6	2.5	4.5	2.3	97.5									
AP														
1	4.2	9.4	4.5	10.9	106.2									
4	2.2	7.4	1.3	9.6	–	0.020	*0.000*	*0.000*	0.000	*0.012*	*0.005*	0.998	0.125	0.125
8	1.6	4.9	1.2	5.6	97.2									

CV: coefficient of variation; S.D.: standard deviation; S.E.: standard error; R^2: regression coefficient; LOD: limit of detection; LOQ: limit of quantification.
[a] Calibration (0.25–10 ng/mg, $n=5$).

Table 2
Results of the validation of the ultrasonication-based method for the quantification of MA and AP

Analyte (ng/mg)	Precision (CV, %)		Accuracy (bias, %)		Recovery (%, $n=5$)	Slope[a]			Intercept[a]			R^2[a]	LOD (ng/mg)	LOQ (ng/mg)
	Intra-assay ($n=6$)	Inter-assay ($n=18$)	Intra-assay ($n=6$)	Inter-assay ($n=18$)		Mean	S.D.	S.E.	Mean	S.D.	S.E.			
MA														
1	11.0	11.0	19.0	10.0	113.3									
4	3.9	6.3	1.6	1.1	–	0.022	*0.001*	*0.000*	0.028	*0.003*	*0.001*	0.998	0.125	0.25
8	4.0	2.6	2.2	2.0	94.3									
AP														
1	1.9	6.7	15.0	11.0	113.2									
4	3.3	5.4	4.7	0.0	–	0.021	*0.001*	*0.000*	−0.009	*0.013*	*0.006*	0.999	0.125	0.25
8	3.2	4.3	4.7	0.3	95.4									

CV: coefficient of variation; S.D.: standard deviation; S.E.: standard error; R^2: regression coefficient; LOD: limit of detection; LOQ: limit of quantification.
[a] Calibration (0.25–10 ng/mg, $n=5$).

Table 3
Results of the comparison of the extraction methods using an authentic hair sample

Extraction method	Concentration (mean ± S.D., ng/mg)
Agitation ($n=3$)	
MA	7.55 ± 0.48
AP	0.59 ± 0.03
Ultrasonication ($n=3$)	
MA	8.22 ± 0.69
AP	0.57 ± 0.04

AP, respectively, and those by the ultrasonication-based method were 0.125 ng/mg for both. The LOQs by the former method were 0.25 and 0.125 ng/mg for MA and AP, respectively, and those by the latter were 0.25 ng/mg for both. The calculated LODs and LOQs were considered adequate for the purpose of the study.

To compare the extraction efficacy between agitation with 1% HCl in methanol at 38 °C and ultrasonication with methanol/5 M HCl (20:1), both MA and AP in authentic hair from a post-mortem case were determined. The average concentrations of MA and AP were 7.55 and 0.59 ng/mg by agitation and 8.22 and 0.57 ng/mg by ultrasonication for each. The average concentration of MA was slightly higher in the ultrasonication-base method and that of AP was in the agitation-based method (Table 3). However, the F-tests for comparison of variances were not significant ($p>0.05$), indicating that the two extraction procedures have no serious difference and good extraction efficacy.

Table 4 shows the certified values and their expanded uncertainties of MA and AP in NIST SRM 2379 and the calculated values and uncertainties by the two procedures. To verify the analytical methods, the following expression was used: $|\bar{x}_{\text{cert}} - \bar{x}_{\text{calc}}| \leq 2\sqrt{U_{\text{cert}}^2 + U_{\text{calc}}^2}$ where $\bar{x}_{\text{cert}}$ is the certified value, $\bar{x}_{\text{calc}}$ is the calculated value, U_{cert} is the expanded uncertainty of the certified value and U_{calc} is the expanded uncertainty of the calculated value. The average concentrations of MA and AP by the agitation-based method were 5.34 and 5.42 ng/mg and those by the ultrasonication-based method were 5.24 and 5.27 ng/mg, respectively. The results obtained using the above approaches were all acceptable.

3.2. Homogeneity

No significant differences were found for the concentration of MA and AP in homogeneity test because the calculated F-values were lower than the critical F-values (Table 5). The uncertainties of homogeneity were calculated using the following expression: $s_{\text{bb}} = \sqrt{(M_{\text{among}} - M_{\text{within}})/n}$ where s_{bb} is between-bottle homogeneity standard deviation, M is mean square (ANOVA) and n is number of observations. As a result, the uncertainties were 0.31 ng/mg (3.9%) and 0.00 ng/mg (0.72%) for MA and AP by the agitation-based method and 0.18 ng/mg (2.3%) and 0.02 ng/mg (2.8%) by the ultrasonication-based one, respectively.

Table 4
Verification of the analytical methods using a CRM (NIST SRM 2379)

	Certified value		Calculated value			
	MA[a]	AP[a]	MA[a,b]	AP[a,b]	MA[a,c]	AP[a,c]
Concentration (mean, ng/mg)	5.20	6.00	5.34	5.42	5.24	5.27
U (ng/mg)	0.27	0.32	0.38	0.34	0.59	0.34

U: expanded uncertainty.
[a] Analyte.
[b] Agitation-based method ($n = 3$).
[c] Ultrasonication-based method ($n = 3$).

Table 5
Results of homogeneity test of MA and AP (concentration (mean ± S.D., ng/mg, $n = 3$)) in the prepared RM using the agitation- and ultrasonication-based methods

	Agitation[a]		Ultrasonication[a]	
	MA[b]	AP[b]	MA[b]	AP[b]
Bottle no.				
3	7.16 ± 0.29	0.55 ± 0.01	7.47 ± 0.80	0.57 ± 0.07
15	7.06 ± 0.39	0.52 ± 0.05	8.19 ± 0.65	0.60 ± 0.08
27	7.71 ± 0.22	0.54 ± 0.06	7.48 ± 0.51	0.54 ± 0.04
31	7.92 ± 0.50	0.51 ± 0.00	7.99 ± 0.01	0.57 ± 0.02
46	7.63 ± 0.58	0.51 ± 0.04	7.52 ± 0.54	0.51 ± 0.02
52	8.09 ± 0.24	0.54 ± 0.03	7.53 ± 0.21	0.51 ± 0.03
60	8.61 ± 1.00	0.58 ± 0.04	8.02 ± 0.13	0.55 ± 0.04
76	7.59 ± 1.03	0.50 ± 0.04	7.87 ± 0.14	0.53 ± 0.03
88	7.87 ± 0.61	0.54 ± 0.03	7.21 ± 0.45	0.52 ± 0.05
95	8.09 ± 0.27	0.54 ± 0.05	7.96 ± 0.41	0.55 ± 0.03
F_{calc}	1.8	1.0	1.5	1.3
F_{crit}	2.4	2.4	2.4	2.4
u_h (ng/mg)	0.31	0.00	0.18	0.02

F_{calc}: calculated F-value; F_{crit}: critical F-value of $\alpha = 5\%$; u_h: uncertainty of homogeneity.
[a] Extraction method.
[b] Analyte.

Table 6
Results of characterization of MA and AP in the prepared RM using the agitation- and ultrasonication-based methods

	Agitation[a]		Ultrasonication[a]	
	MA[b]	AP[b]	MA[b]	AP[b]
Concentration (mean ± S.D., ng/mg, $n = 9$)	7.63 ± 0.54	0.53 ± 0.04	7.65 ± 0.57	0.54 ± 0.05
u_c	0.27	0.02	0.43	0.02

u_c: uncertainty of characterization.
[a] Extraction method.
[b] Analyte.

Table 7
Internal certification of the prepared RM

Analyte	Certified value (ng/mg)	
	Mean	U
MA	7.64	1.24
AP	0.54	0.07

U: expanded uncertainty.

3.3. Characterization and internal certification of the prepared RM

Table 6 shows the property values of MA and AP determined by the two methods and their uncertainties and Fig. 1 illustrates the SIM chromatograms from an aliquot of the prepared RM. The uncertainties were 0.27 ng/mg (3.5%) and 0.02 ng/mg (3.1%) for MA and AP by the agitation-based method and 0.43 ng/mg (5.6%) and 0.02 ng/mg (3.3%) by the ultrasonication-based method, respectively ($n = 9$ for each).

To derive the certified values of MA and AP, the arithmetic means were calculated ($n = 18$) and the expanded uncertainties were established as $U_{RM} = k\sqrt{u_{habm}^2 + u_{hubm}^2 + u_{cabm}^2 + u_{cubm}^2}$ where k is coverage factor ($k = 2$), u_{habm} is the uncertainty in the homogeneity test by the agitation-based method, u_{hubm} is the uncertainty in the homogeneity test by the ultrasonication-based method, u_{cabm} is the uncertainty in the characterization by the agitation-based

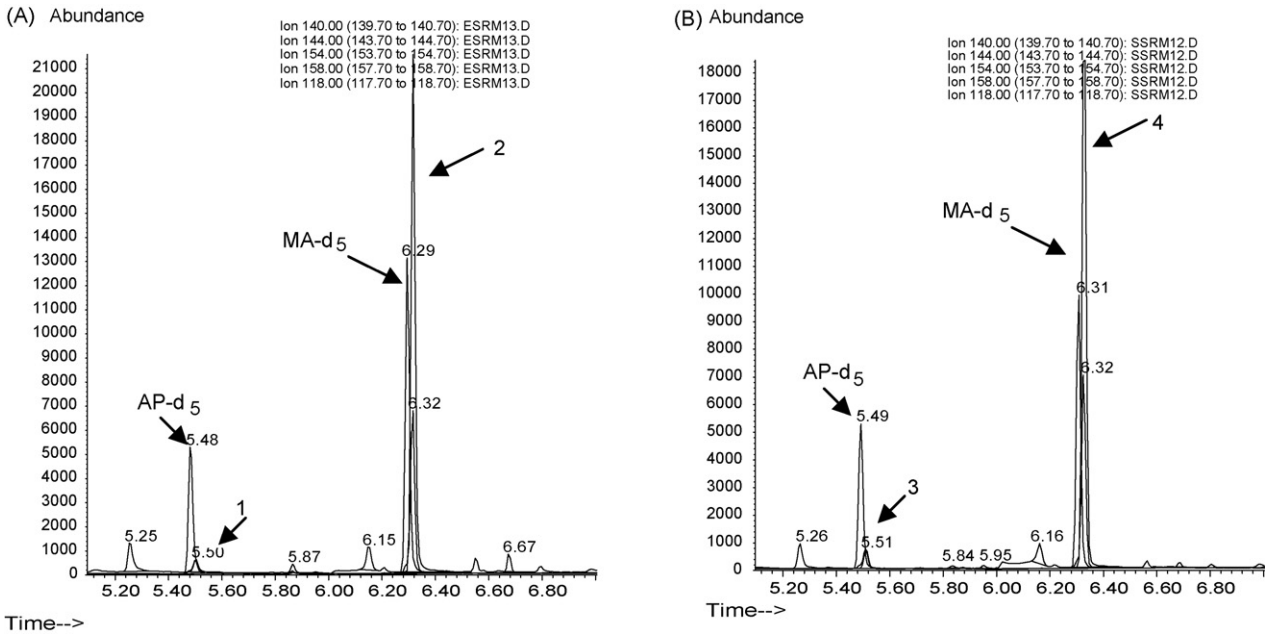

Fig. 1. SIM chromatograms of MA and AP in the prepared RM using the agitation-based (A) and ultrasonication-based (B) methods. 1, AP: 0.53 ± 0.04 ng/mg; 2, MA: 7.63 ± 0.54 ng/mg; 3, AP: 0.54 ± 0.05 ng/mg; 4, MA: 7.65 ± 0.57 ng/mg.

method and u_{cubm} is the uncertainty in the characterization by the ultrasonication-based method. The expanded uncertainties of MA and AP were 1.24 ng/mg (16%) and 0.07 ng/mg (13%), respectively. Finally, the certified values were obtained as shown in Table 7.

4. Discussion

A RM is an important tool for method validation, calibration, estimation of measurement uncertainty, training, internal quality control and external quality assurance [20]. In recent years the demand for accurate RMs has increased because the reliability of data has a great effect on administrative and legal consequences. Since 1980s, several human hair RMs have been developed and utilized to evaluate analytical methodologies but most of the RMs are for determination of heavy elements [3,21–26]. For drugs of abuse, proficiency testing programs have been accomplished since early 1990s using fortified and/or authentic hair samples that ensure homogeneity [13,27–32].

In this study, human hair RM for MA and AP was prepared using a pool of authentic hair samples with the different concentrations of MA and AP. In order to obtain uniformity, the samples were incubated in distilled water with a stirring bar for an hour [13], cut into very short pieces (about 1 mm), sieved and blended thoroughly. In both the homogeneity and characterization tests, the results from the two methods were in good agreement with no significant variance. Furthermore, the expanded uncertainties of MA and AP (16% and 13% for each) were small enough for the purpose of this study.

Hair is a solid matrix surrounded by keratinized cells. The drugs are firmly incorporated in the matrix and partly bound to intracellular components of the hair cells, such as melanin, proteins or lipids. Therefore, the extraction of drugs from hair is the most sensible step of hair testing procedures.

In practice, there are several ways of drug solubilization, such as incubation in an acidic or basic solution (e.g. NaOH), incubation in an organic solvent (generally methanol with or without hydrochloric acid) and digestion in an enzymatic solution, *etc*. After drugs are extracted by incubating the hair sample in NaOH, liquid–liquid extraction (LLE) or solid-phase extraction (SPE) is required. The NaOH incubation step destroys the protein matrix of hair completely and the additional LLE or SPE step may produce lower recovery of analytes [33]. However, our preparation method is very simple. It involves placing hair samples in the methanol solution, followed by agitation or ultrasonication, and does not need further purification. Moreover, since this method maintains the shape of hair, the effect of interferences, such as proteins, lipids, *etc*. can be minimized. Therefore, agitation or ultrasonication in the methanol solution can be considered as an effective method to extract MA and AP from hair.

The inappropriate choice of extraction or digestion method is one of the main sources to frustrate the analysis of drugs in hair. In other words, the extraction yield considerably depends on extraction manners [34]. Thus, MA and AP in hair were determined using two different extraction methods, one based upon agitation with 1% HCl in methanol at 38 °C and one based upon ultrasonication with methanol/5 M HCl (20:1) in this study. In the comparative study of the two procedures using authentic hair, the concentrations of MA and AP were not significantly different depending on the type of methods. Therefore, both procedures were included our official standard operating procedure (SOP) for analyzing MA and AP in human hair [35]. Nevertheless, the ultrasonication procedure left more dirty dry extract than the agitation one and it is also reported that methanol sonication resulted in 'dirty' chromatograms [36].

Our RM prepared by homogenizing authentic hair reflects the real condition of hair better than others by soaking drug-free hair in solution containing chemicals. Since all the specimens came from Koreans with originally black hair, the discrepancy of analytical results due to race or hair color can be eliminated for oriental people as using our RM. Moreover, although the fortified RM has a different metabolite-to-parent drug ratio from real hair, our RM has the average value of it, which can be used as one of criteria for obtaining a positive result [37]. The metabolite-to-parent drug ratio of MA was calculated from the homogeneity test: 0.07 ± 0.01 (7%) by the agitation-based method ($n = 30$); 0.07 ± 0.00 (7%) by the ultrasonication-based method ($n = 30$). These results were similar with other reports: average 9% in 2444 MA abusers and 5.9–12.8% in one post-mortem case [38,39].

Even though hair is an extremely stable matrix, further study for stability was required to set the shelf-life, which is one essential element to be included in the label of a CRM.

5. Conclusions

This research work shows that both the agitation- and ultrasonication-based method are acceptable to analyze both MA and AP in human hair through the validation and comparative studies using spiked and authentic hair samples as well as NIST SRM 2379. With these two procedures, a human hair pre-CRM containing MA and AP is prepared at the level of 7.64 ± 1.24 and 0.54 ± 0.07 ng/mg, respectively, using authentic hair specimens and satisfying homogeneity was reached for the two analytes. This material can be useful for forensic laboratories in internal quality control and external quality assurance and provided gladly to any laboratories for their internal quality control and research purposes.

References

[1] R.C. Baselt, Disposition of Toxic Drugs and Chemicals in Man, Chemical Toxicology Institute, Foster City, CA, 2000, p. 527.
[2] P. Kintz, Forensic Sci. Int. 142 (2004) 127.
[3] M.J. Welch, L. Sniegoski, S. Tai, Anal. Bioanal. Chem. 376 (2003) 1205.
[4] M.J. Welch, L.T. Sniegoski, C.C. Allgood, M. Habram, J. Anal. Toxicol. 17 (1993) 389.
[5] P. Ellerbe, T. Long, M.J. Welch, J. Anal. Toxicol. 17 (1993) 165.
[6] S.S. Tai, R.G. Christensen, K. Coakley, P. Ellerbe, T. Long, M.J. Welch, J. Anal. Toxicol. 20 (1996) 43.
[7] General requirements for the competence of reference material producers, ISO GUIDE 34, The second edition, 2000.
[8] A.M.H. van der Veen, J. Pauwels, Accred. Qual. Assur. 5 (2000) 464.
[9] A.M.H. van der Veen, T. Linsinger, J. Pauwels, Accred. Qual. Assur. 6 (2001) 26.
[10] A.M.H. van der Veen, T.P.J. Linsinger, A. Lamberty, J. Pauwels, Accred. Qual. Assur. 6 (2001) 257.
[11] A.M.H. van der Veen, T.P.J. Linsinger, H. Schimmel, A. Lamberty, J. Pauwels, Accred. Qual. Assur. 6 (2001) 290.
[12] C. Jimenez, R. De La Torre, M. Ventura, J. Segura, R. Ventura, J. Chromatogr. B 843 (2006) 84.
[13] J. Mitchell, Proceedings of Workshop on Analysis of Abused Drugs in Biological Specimen, Fooyin University, Kaohsiung, Taiwan, 2005, p. 1.
[14] Society of Hair Testing, Forensic Sci. Int. 145 (2004) 83.
[15] The fitness for purpose of analytical methods, Eurachem Guide, 1998.
[16] E. Han, W. Yang, J. Lee, Y. Park, E. Kim, M. Kim, H. Chung, Forensic Sci. Int. 147 (2005) 21.
[17] Y. Nakahara, K. Takahashi, M. Shimamine, Y. Takeda, J. Forensic Sci. 36 (1991) 70.
[18] Certification of reference materials – General and statistical principles, ISO GUIDE 35, Revision of the second edition, 2003.
[19] Quantifying uncertainty in analytical measurement, EURACHEM/CITAC Guide, Second edition, 2000.
[20] The Selection and Use of Reference Materials, European co-operation for accreditation, 2003.
[21] K. Okamoto, M. Morita, H. Quan, T. Uehiro, K. Fuwa, Clin. Chem. 31 (1985) 1592.
[22] S. Pichini, I. Altieri, M. Pellegrini, R. Pacifici, P. Zuccaro, Forensic Sci. Int. 84 (1997) 243.
[23] P.J. Moreda, R.E. Alonoso, M.P. Lopez, L.S. Muniategui, R.D. Prada, P.A. Moreda, B.P. Bermejo, Anal. Bioanal. Chem. 388 (2007) 441.
[24] T.G. Kazi, H.I. Afridi, M.K. Jamali, G.H. Kazi, M.B. Arain, N. Jalbani, R.A. Sarfraz, G.Q. Shar, J. AOAC Int. 89 (2006) 1410.
[25] S.J. Butala, L.P. Scanlan, S.N. Chaudhuri, J. Food. Prot. 69 (2006) 2720.
[26] K.W. Phinney, L.C. Sander, Anal. Bioanal. Chem. 378 (2004) 144.
[27] P. Kintz, V. Cirimele, Forensic Sci. Int. 84 (1997) 151.
[28] H. Sachs, Forensic Sci. Int. 84 (1997) 145.
[29] M. Deveaux, P. Kintz, J.P. Goulle, J. Bessard, G. Pepin, D. Gosset, Forensic Sci. Int. 107 (2000) 389.
[30] M. Montagna, A. Polettini, C. Stramesi, A. Groppi, C. Vignali, Forensic Sci. Int. 128 (2002) 79.
[31] C. Jurado, H. Sachs, Forensic Sci. Int. 133 (2003) 175.
[32] S. Pichini, M. Ventura, M. Pujadas, R. Ventura, M. Pellegrini, P. Zuccaro, R. Pacifici, R. de la Torre, Forensic Sci. Int. 145 (2004) 109.
[33] P. Kintz, in: A.C. Moffet, M.D. Osselton, B. Widdop (Eds.), Clark's Analysis of Drugs and Poisons, Pharmaceutical Press, London, UK, 2004, p. 124.
[34] F. Musshoff, B. Madea, Forensic Sci. Int. (2006).
[35] Determination of methamphetamine and amphetamine in hair, NISI SOP, KOLAS QI NM 03, Revised in April, 2007.
[36] H. Sachs, P. Kintz, J. Chromatogr. B 713 (1998) 147.
[37] R. Wennig, Forensic Sci. Int. 107 (2000) 5.
[38] E. Han, Y. Park, W. Yang, J. Lee, S. Lee, E. Kim, M. Lim, H. Chung, Forensic Sci. Int. 161 (2006) 124.
[39] T. Saito, I. Yammoto, T. Kusakabe, X.L. Huang, N. Yukawa, S. Takeichi, Forensic Sci. Int. 112 (2000) 65.