Contribution of thermal Desorption and liquid-liquid extracton for identification and profiling of impurities in methamphetamine by gas chromatography-mass spectrometry

Kenji Kuwayama[a, b,*], Hiroyuki Inoue[a], Tatsuyuki Kanamori[a], Kenji Tsujikawa[a], Hajime Miyaguchi[a], Yuko Iwata[a], Naoki Kamo[b], and Tohru Kishi[a]

[a]National Research Institute of Police Science, 6-3-1, Kashiwanoha, Kashiwa, Chiba 277-0882, Japan

[b]Laboratory of Biophysical Chemistry, Graduate School of Pharmaceutical Sciences, Hokkaido University, Sapporo 060-0812, Japan

* Corresponding author. Tel.: +81-4-7135-8001; fax: +81-4-7133-9173.

E-mail address kuwayama@nrips.go.jp (K. Kuwayama).

ABSTRACT

Impurity profiling of methamphetamine (MA) using thermal desorption (TD) and gas chromatography-mass spectrometry (GC-MS) was examined. Using TD/GC-MS, impurities were extracted and separated under various conditions. Optimal chromatograms were obtained when a 20 mg MA sample was extracted at 120˚C for 3 min using a TD instrument, followed by separation of the extracts using a nonpolar capillary column coated with (5%-phenyl)-methylpolysiloxane.

MA samples from nine different batches were analyzed under optimized conditions. Compounds related to the structure of MA, such as benzaldehyde, benzyl alcohol, amphetamine, *cis*- and *trans*-1,2-dimethyl-3-phenylaziridine, dimethylamphetamine, and *N*-acetylephedrine, were detected in the chromatograms without any laborious extraction procedure. Compounds such as ethanol, diethyl ether, and acetic acid, which are considered reagents and solvents for MA synthesis, were also detected in some of the chromatograms. The numbers and intensities of the peaks detected were different among the samples.

Impurity profiling of MA using TD was compared with that using liquid-liquid extraction (LLE). Better reproducibility of peak areas was obtained using LLE, whereas higher intensities and numbers of peaks were detected using TD. Solvents were

extracted more effectively using TD. The nine batches of MA were classified using both extraction procedures. The nine batches were divided roughly into two groups using data from LLE. Subsequently, the groups were classified in detail using data from TD.

TD can be used to provide supplemental information for LLE, and the combination of these extraction methods can be helpful for impurity profiling of MA.

KEY WORDS: thermal desorption, solid phase microextraction, methamphetamine, gas chromatography-mass spectrometry, impurity profiling

Introduction

Methamphetamine (MA) is currently the major drug of abuse in Japan [1]. The development of impurity profiling of MA is an important approach to obtaining information useful for criminal investigations, such as the relationships among seized samples, traffic routes, and sources of supply [2]. A number of methods have been reported for the impurity profiling of MA, including the use of gas chromatography (GC) with a flame ionization detector [3-9] and GC-mass spectrometry (MS) after liquid-liquid extraction (LLE) [10,11] with organic solvents under either basic or weakly acidic conditions. Methods using headspace solid phase microextraction (SPME) for the characterization of MA [12-14] and 4-methoxyamphetamine [15] have recently been reported.

Like SPME, thermal desorption (TD) is a simple, rapid, and solvent-free extraction method. It is frequently used with stir bar sorptive extraction (SBSE) for the analysis of compounds in foods, beverages, biological samples, and so on. [16-19]. Because a TD instrument enables the direct introduction of a sample without any laborious extraction procedure, it is effective for analyzing trace amounts of compounds, particularly volatiles. The analysis by direct sample introduction is applied to versatile fields [20-26].

In the present study, the impurity profiling of MA using TD/GC-MS was investigated in an attempt to develop a method that simplifies preparation and enables the detection of specific volatile compounds. In addition, LLE and TD were compared for impurity profiling of MA .

Materials and Methods

Materials

Authentic standards of *d*-MA·HCl (Philopon) and *l*-ephedrine·HCl were purchased from Dainippon Pharmaceutical Co. (Osaka, Japan). Two batches of *d*-MA·HCl were synthesized using two different methods. One batch was obtained from the direct reduction of *l*-ephedrine with hydroiodic acid and red phosphorus. The other involved the preparation from *l*-ephedrine via chloroephedrine [27]. Six batches of MA·HCl, which had been seized in Japan and which had a purity of more than 95%, were obtained from the Ministry of Health, Labor, and Welfare, Japan. *dl*-Amphetamine sulfate, *dl*-dimethylamphetamine·HCl (DMA), *N*-acetylamphetamine, *N*-acetylephedrine, *N*-formyl MA, and *cis*- and *trans*-1,2-dimethyl-3-phenylaziridine (AZ) were synthesized in our laboratory as previously reported [27-32]. All other chemicals were of analytical grade and were purchased from Wako Pure Chemical Co. (Osaka, Japan).

Glass tubes (187 mm length, 6 mm o.d. and 4 mm i.d.) for TD were purchased from Gerstel (Baltimore, MD, USA).

Sampling and extraction procedures

For TD, 20 mg of *d*-MA·HCl samples were put into a glass tube. Both ends of the tube were plugged with glass wool. The tube was set on an autosampler (TDSA, Gerstel), carried to a TD unit (TDS-2, Gerstel), and heated. The TDS-2 was programmed at 20˚C for 1 min initially, ramped at 60˚C/min to 120˚C, and held at the final temperature for 3 min. Changes in the amounts of *d*-MA·HCl (10, 20, and 50 mg) and the final temperature of the TDS-2 (50, 85, and 120˚C) were evaluated in order to optimize the detection of impurities. The extracts from the sample flowed to a cryofocussing instrument (CIS-4, Gerstel) along with nitrogen gas at 50 ml/min and were cryofocused at -150˚C. The CIS-4 was then ramped at 12˚C /sec to 260˚C and held at the final temperature for 3 min to inject the extracts to a GC-MS. The injection was performed at the solvent vent mode in order to introduce all of the extracts from a sample to the GC-MS.

For LLE, a 100 mg sample was dissolved in 1 ml of 0.1 M phosphate buffer pH 7.0 and 0.25 ml of 10% Na_2CO_3. The solution was extracted with 0.2 ml of ethyl acetate. The organic layer was diluted with ten volumes of ethyl acetate, and a 1 μl aliquot of the solution was injected into a GC-MS instrument.

After the TD conditions were optimized, a total of 27 samples, comprising 3 samples from each of the 9 batches (Philopon, 6 MA seizures and 2 synthesized

samples) were analyzed to evaluate intra- and inter-batch variations in the impurity profiles. In addition, the same 27 samples were analyzed using LLE for comparison with TD.

GC-MS analysis

The GC-MS instrument was an Agilent 6890 GC interfaced with an Agilent 5973N MSD. The columns used were an Agilent HP-5MS capillary column coated with (5%-phenyl)-methylpolysiloxane (0.25 mm i.d. × 30 m, film thickness 0.25 μm) and an Agilent DB-WAX capillary column coated with 100% polyethylene glycol (0.25 mm i.d. × 30 m, film thickness 0.25 μm). The flow rate of the carrier gas, helium, was 1.0 ml/min in constant flow mode. The oven temperature for the HP-5MS was held at 50˚C for 3 min, ramped at 15˚C/min to 150˚C and 25˚C/min to 275˚C, and then held for 2 min at the final temperature. Injection was done in the splitless mode followed by a purge after 1 min at an inlet temperature of 260˚C. The oven temperature for the DB-WAX was held at 40˚C for 1 min, ramped at 10˚C/min to 150˚C and 25˚C/min to 250˚C, and then held for 3 min at the final temperature. Injection was done in the same conditions as the HP-5MS except for an inlet temperature of 250˚C. The temperatures of the interface and MS ion source were set at 280˚C and 230˚C, respectively. The MS was

operated in the electron ionization mode with a scan range of *m/z* 33 to 400. The GC-MS, data acquisition, and data analysis were controlled using Agilent Chemstation software (G1701 DJ version). Peaks on chromatograms were identified by comparing their retention times and mass spectra with those of authentic standards.

Data processing

Some of the major peaks were selected from chromatograms and identified for the comparison of the two extraction methods. Extracted mass chromatograms with specific ions were used for integration. The compounds and the ions selected for area calculation were ethanol (*m/z* 45), benzaldehyde (*m/z* 106), *cis*-AZ (*m/z* 146), amphetamine (*m/z* 44), *trans*-AZ (*m/z* 146), and DMA (*m/z* 72). In cases where the peak was not detected, a value of 10000, which was nearly the limit of the peak area integrated automatically by the software, was assigned. The raw data were processed using Microsoft Excel and converted to their logarithms. Similarities among samples were calculated using cosine distance [14]. The equation used is shown below (Eq. 1).

$$\text{cosine distance} = \sum(X_{ik} \cdot X_{jk}) / [\sum X_{ik}^2 \cdot \sum X_{jk}^2]^{1/2} \qquad \text{(Eq. 1)}$$

X_{ik} represents the area of peak *k* in sample *i*. The classification of samples was visualized by hierarchical cluster analysis using the group average method.

Results and Discussion

Optimization of extraction conditions

Samples from the same lot were analyzed by TD/GC-MS to examine an optimal extraction temperature. Peaks of ethanol, benzaldehyde, benzyl alcohol, and *cis*-AZ, which were relatively large and characteristic peaks in the identified compounds, were selected from chromatograms for the evaluation. Table 1 shows the effects of extraction temperatures on the four peak areas. The final temperature of CIS-4 was varied from 50 to 120˚C. The extraction temperature had few effects on benzaldehyde and benzyl alcohol. The peak areas of ethanol and *cis*-AZ increased markedly as the temperature increased. Although the higher temperature might improve extraction, the temperature over the melting points of MA (*d*- or *l*-MA; 170-175˚C and *dl*-MA; 130-135˚C [33]) would make it difficult to recover the sample and prevent contamination of the instrument. Therefore, the extraction temperature of 120˚C was adopted for TD/GC-MS analysis of MA.

Samples in various amounts (10-50 mg) from the same lot were analyzed by TD/GC-MS to determine the optimal sample size. Because there was no correlation between sample size and peak area (data not shown), a large sample size was unnecessary. Considering the desirability of conserving samples and of precise sampling,

a sample size of 20 mg was used for TD/GC-MS analysis.

Polar and volatile compounds such as ethanol, diethyl ether, and acetic acid were often detected in the chromatograms obtained by TD/GC-MS. These compounds were considered specific solvents to characterize the origins of samples. Two kinds of columns (HP-5MS and DB-WAX) were compared in order to prevent solvent peaks from overlapping the CO_2 peak (Fig. 1), which is not retained on a column. Although the HP-5MS column (Fig. 1A, 1B) was inferior to the DB-WAX column (Fig. 1C, 1D) in the separation and retention of organic solvents, the numbers and intensities of the peaks using the HP-5MS column were greater than those using the DB-WAX column. Because more characteristic peaks would provide a great advantage for the classification of samples, the HP-5MS column was adopted.

MA samples from nine different origins were analyzed under optimized conditions. The peaks observed are summarized in Table 2. The numbers and intensities of the peaks detected differed from sample to sample. Compounds related to MA structure, such as benzaldehyde (peak 4), benzyl alcohol (peak 5), *cis*-AZ (peak 6), amphetamine (peak 7), *trans*-AZ (peak 9), DMA (peak 10), and *N*-acetylephedrine (peak 11), were detected in the chromatograms. Compounds such as ethanol (peak 1), diethyl ether (peak 2), and acetic acid (peak 3), which were considered reagents and solvents for MA

synthesis, were also detected in the chromatograms. Ethanol was detected from two of nine sample origins, while diethyl ether was detected from one origin. Ethanol and diethyl ether, which were rarely detected, were considered to be effective for discriminating between samples.

Comparison of extraction methods

LLE and TD were compared in terms of procedure, running cost, reproducibility, and so on. The LLE procedure involves the dissolution of the sample to alkaline buffer, the addition of extraction solvent, and the recovery of the organic phase. A series of LLE procedures is complicated. On the other hand, since the TD procedure is automated, we have only to place the sample in the tube. As for running costs, the buffer solution and extraction solvent necessary in LLE are inexpensive. The TD instrument itself is expensive, and a large amount of liquid nitrogen is consumed during analysis at all times.

Chromatographic profiles obtained using LLE were compared with those using TD (Fig. 2). In TD, the numbers and the intensities of impurity peaks detected were greater than those in LLE. TD efficiently extracted trace amounts of impurities.

The reproducibility of the peak areas on mass chromatograms obtained from LLE

and TD was evaluated using the coefficients of variation (CVs) in the peak areas of four compounds from intra- and inter-batches (Table 3). LLE showed good reproducibility where the CVs in the four peak areas from the intra-batches were less than 10%, while the CVs in intra-batches were significantly larger in TD. LLE was superior to TD in this point. Because LLE provided reproducible peak areas, it would be an appropriate extraction method for creating a database for impurity profiling of MA. Although the larger CVs in intra-batches obtained by TD imply that the peak areas are unreliable, absolute peak areas of impurities in TD are very larger than those in LLE. Therefore, TD makes sure of the identification of peaks and gives valuable information qualitatively rather than quantitatively. It sometimes enables the discrimination of samples if only to check the presences of specific compounds identified by TD. Samples used in LLE were dissolved in an extraction solvent and dispersed equally in the solvent. In contrast, samples used in TD were used as a powder or a crystal. The large difference among CVs in intra-batches between LLE and TD would be due to the state of the sample. To examine the influence of the state of the sample on the peak areas, samples were powdered in a mortar and then analyzed. This resulted in a low peak intensity, especially for volatile compounds, although the reproducibility of compounds such as *cis*-AZ and DMA was improved (data not shown). In order to make full use of the

advantage of TD, that is the detection of specific solvents to characterize the origins of samples, they were used in an intact state without grinding crystals. The CVs in inter-batches differed from compound to compounds. The greatly larger CVs in inter-batches compared to intra-batches than that in intra-batch implies that the compound can distinguish the sample from others very well. The selections of *cis*-AZ and DMA in the present study were effective for discriminating samples. The selection of appropriate compounds from each method would be important and helpful for impurity profiling.

The classification of nine batches was performed using LLE and TD (Fig. 3), and the peaks selected for the classification are shown in Table 4. At first, nine batches were classified using data from LLE, which provided the most reproducible data. The nine batches were divided roughly into two groups (batches A, F, I and batches B-E, G, H). To investigate the relationship among batches B-E, G, and H in detail, a subsequent classification was performed using data from TD. Batches C and E, in which ethanol was detected, were contained in the same group. although C and E were not very closely related in LLE. Ethanol selected for the classification reflected sufficiently the relationship between C and E. The selection of rare peaks detected by TD might be useful for the discrimination of samples. The subsequent classifications showed the

possibility of classifying groups that were difficult to discriminate using only one method.

To the best of my knowledge, this is the first report concerning the analysis of impurities in drugs of abuse by direct TD, although analyses of versatile volatiles by direct TD have been reported previously [20-26]. In previous reports [21, 23], principal component analysis (PCA) was applied to the evaluation of discrimination among groups. In PCA, major components dominate and minor ones tend to be neglected. In impurity profiling, trace amounts of impurities are also important, and their presence sometimes allows samples to be discriminated. It is necessary to investigate relationships between samples for impurity profiling. Therefore, hierarchical cluster analysis (HCA) was applied to the present study. The subsequent classifications by HCA using data from different extractions were effective for the detailed discrimination of samples.

We have reported the effectiveness of SPME for the identification of impurities in MA [14]. Both SPME and TD feature simple and rapid preparation, and both are effective for volatile compounds in GC-MS analysis. SPME was superior to TD in terms of the reproducibility of peak areas and the effective extraction of impurities without an overload of MA. However, in SPME, solvent peaks such as those of ethanol,

diethyl ether, and acetic acid were too small and broad to measure their peak areas. The intensities of impurities, especially those of volatiles, were larger in TD than in SPME. TD efficiently extracted trace amounts of impurities and enabled the identification of the peaks and measurement of the peak areas due to the increases in peak intensities. The features are the best advantage for the impurity profiling of MA using TD. TD as well as SPME can provide supplemental information for LLE, and the combination of these extraction methods will be helpful for the impurity profiling of MA.

Conclusion

TD enabled the extraction of impurities from a sample without the use of solvents or adsorbents, unlike LLE and SPME. From extraction to analysis, the procedure was automated by the TD system after the sample was placed into the tube. Moreover, because all the extracts were introduced to GC-MS, TD/GC-MS had an advantage in the extraction and analysis of trace amounts of volatile compounds. Therefore, TD/GC-MS was a simpler and more effective method than LLE for the extraction and identification of volatile compounds.

TD can provide supplemental information for LLE, and the combination of these extraction methods will be helpful for the impurity profiling of MA.

References

[1] Changes in the situation of drugs, in: National Police Agency, Government of Japan (Ed.), White Paper on Police 2003 (Excerpt), The Japan Times, Japan, pp. 60-74.

[2] B. Remberg, A.H. Stead, Drug characterization/impurity profiling, with special focus on methamphetamine: recent work of the United Nations International Drug Control Programme, Bull. Narcotics 51 (1999) 97-117.

[3] M. Perkal, Y.L. Ng, J.R. Pearson, Impurity profiling of methamphetamine in Australia and the development of a national drugs database, Forensic Sci. Int. 69 (1994) 77-87.

[4] United Nations International Drug Control Programme (Scientific Section), Scientific and Technical Notes: A Practical Guide to Methamphetamine Characterization/Impurity Profiling: Method Procedures, Mass Spectral Data of Selected Impurities, and Literature References, New York, NY, 2000.

[5] K. Tanaka, T. Ohmori, T. Inoue, Analysis of impurities in illicit methamphetamine, Forensic Sci. Int. 56 (1992) 157-165.

[6] K. Tanaka, T. Ohmori, T. Inoue, S. Seta, Impurity profiling analysis of illicit methamphetamine by capillary gas chromatography, J. Forensic Sci. 39 (1994)

500-511.

[7] T. Inoue, K. Tanaka, T. Ohmori, Y. Togawa, S. Seta, Impurity profiling analysis of methamphetamine seized in Japan, Forensic Sci. Int. 69 (1994) 97-102.

[8] H. Inoue, T. Kanamori, Y. Iwata, Y. Ohmae, K. Tsujikawa, S. Saitoh, T. Kishi, Methamphetamine impurity profiling using a 0.32 mm i.d. nonpolar capillary column, Forensic Sci. Int. 135 (2003) 42-47.

[9] F.M. Dayrit, M.C. Dumlao, Impurity profiling of methamphetamine hydrochloride drugs seized in the Philippines, Forensic Sci. Int. 144 (2004) 20-36.

[10] M. Lambrechts, T. Klemetsrud, K.E. Rasmussen, H.J. Storesund, Analysis of Leuckart-specific impurities in amphetamine and methamphetamine, J. Chromatogr. 284 (1984) 99-502.

[11] J.H. Liu, W.W. Ku, J.T. Tsay, M.P. Fitzgerald, S. Kim, Approaches to drug sample differentiation. Part Ⅲ. A comparative study of use of chiral and achiral capillary column gas chromatography/mass spectrometry for the determination of methamphetamine enantiomers and possible impurities. J. Forensic Sci. 27 (1982) 39-48.

[12] C.J. Koester, B.D. Andresen, P.M. Grant, Optimum methamphetamne profiling with sample preparation by solid-phase microextraction, J. Forensic Sci. 47 (2002)

1002-1007.

[13] D.T. Vu, SPME/GC-MS characterization of volatiles associated with methamphetamine: toward the development of a pseudomethamphetamine training material. J. Forensic Sci. 46 (2001) 1014-1024.

[14] K. Kuwayama, K. Tsujikawa, H. Miyaguchi, T. Kanamori, Y. Iwata, H. Inoue, S. Saitoh, T. Kishi, Identification of impurities and the statistical classification of methamphetamine using headspace solid phase microextraction and gas chromatography-mass spectrometry, Forensic Sci. Int. 160 (2006) 44-52.

[15] J.C. Coumbaros, K.P. Kirkbride, G. Klass, Application of solid-phase microextraction to the profiling of an illicit 4-methoxyamphetamine, J. Forensic Sci. 44 (1999) 1237-1242.

[16] L. Wennrich, P. Popp, G. Köller, J. Breuste, Determination of organochlorine pesticides and chlorobenzene in strawberries by using accelerated solvent extraction combined with sorptive enrichment and gas chromatography/mass spectrometry, J. AOAC Int. 84 (2001) 1194-1201.

[17] C. Bicchi, C. Iori, P. Rubiolo, P. Sandra, Headspace sorptive extraction (HSSE), stir bar sorptive extraction (SBSE), and solid phase microextraction (SPME) applied to the analysis of roasted Arabica coffee and coffee brew, J. Agric. Food

Chem. 50 (2002) 449-459.

[18] P. Sandra, B. Tienpont, J. Vercammen, A. Tredoux, T. Sandra, F. David, Stir bar sorptive extraction applied to the determination of dicarboximide fungicides in wine, J. Chromatogr. A, 928 (2001) 117-126.

[19] T. Benijts, J. Vercammen, R. Dams, H. P. Tuan, W. Lambert, P. Sandra, Stir bar sorptive extraction-thermal desorption-capillary gas chromatography-mass spectrometry applied to the analysis of polychlorinated biphenyls in human sperm, J. Chromatogr. B, 755 (2001) 137-142.

[20] C, Deng, J. Qian, W. Zhu, X. Yang, X. Zhang, Rapid determination of methyl salicylate, a plant-signaling compound, in tomato leaves by direct sample introduction and thermal desorption followed by GC-MS, J. Sep. Sci., 28 (2005) 1137-42.

[21] P. Zunin, R. Boggia, S. Lanteri, R. Leardi, R.D. Andreis, F. Evangelisti, Direct thermal extraction and gas chromatographic-mass spectrometric determination of volatile compounds of extra-virgin olive oils, J. Chromatogr. A, 1023 (2004) 271-6.

[22] K. Hashimoto, K.Urakami, Y. Fujiwara, S. Terada, C. Watanabe, Determination of residual solvents in pharmaceuticals by thermal desorption-GC/MS, Anal. Sci. 17

(2001) 645-8.

[23] M. Garcia, J. Sanz, Analysis of Origanum vulgare volatiles by direct thermal desorption coupled to gas chromatography-mass spectrometry, J. Chromatogr. A, 918 (2001) 189-94.

[24] E. Valero, J. Sanz, I. Martinez-Castro, Direct thermal desorption in the analysis of cheese volatiles by gas chromatography and gas chromatography-mass spectrometry: comparison with simultaneous distillation-extraction and dynamic headspace, J. Chromatogr. Sci., 39 (2001) 222-8.

[25] K. Urakami, Y. Saito, Y. Fujiwara, C. Watanabe, K. Umemoto, M. Godo, K. Hashimoto, Determination of residual solvents in bulk pharmaceuticals by thermal desorption/gas chromatography/mass spectrometry, Chem. Pharm. Bull. 48 (2000) 1894-7.

[26] S. Eri, B. K. Khoo, J. Lech, T. G. Hartman, Direct thermal desorption-gas chromatography and gas chromatography-mass spectrometry profiling of hop (Humulus lupulus L.) essential oils in support of varietal characterization, J. Agric. Food Chem., 48 (2000) 1140-9.

[27] T. Kishi, T. Inoue, S. Suzuki, T. Yasuda, T. Oikawa, T. Niwaguchi, Analysis of impurities in methamphetamine, Eisei Kagaku 29 (1983) 400-406 (in Japanese).

[28] V. Lekskulchai, K. Carter, A. Poklis, W. Soine, GC-MS analysis of methamphetamine impurities: reactivity of (+) or (-)-chloroephedrine and cis- or trans-1,2-dimethyl-3-phenylaziridine, J. Anal. Toxicol. 24 (2000) 602-605.

[29] J. Caldwell, L.G. Dring, R.T. Williams, Metabolism of (^{14}C) methamphetamine in man, the guinea pig and the rat. Biochem. J., 129 (1972) 11-22.

[30] R.P. Barron, A.V. Kruegel, J.M. Moore, T.C. Kram, Identification of impurities in illicit methamphetamine samples, J. Assoc. Off. Anal. Chem. 57 (1974) 1147-1158.

[31] C. Conn, M. Dawson, A.T. Baker, J. Keegan, B. Fryirs, Identification of N-acetylmethamphetamine in a sample of illicitly synthesized methamphetamine. J. Forensic Sci. 41 (1996) 645-647.

[32] T. Inoue, S. Suzuki, Studies on metabolism of methamphetamine analogs 6. Analysis of dimethylamphetamine and its metabolites, Rep. Natl. Res. Inst. Police Sci. 39 (1986) 228-234 (in Japanese).

[33] Standard Methods of Chemical Analysis in Poisoning-With Commentary-. Pharmaceutical Society of Japan (Ed.), Nanzandoh, Japan (1992) 446 (in Japanese).

Table 1 Effects of extraction temperatures on peak areas of four compounds

compound	peak area (*1,000)		
	50ºC	85ºC	120ºC
ethanol	17,006±14,820	22,342±1,789	309,036±159,957
benzaldehyde	10,537±10,214	6,638±5,278	7,431±2,367
benzyl alcohol	2,736±2,453	1,675±1,492	4,097±5,178
cis-AZ	76±37	331±408	4,625±6,817

The values represent the averages and the standard deviations of triplicate analyses.

Table 2 Chromatographic peaks obtained from nine batches

peak number	Retention time (min)	Number of occurrences	Major *m/z*	Tentative or identified compound
1	2.11	2	45, 46	ethanol*
2	2.20	1	59, 74	diethyl ether*
3	4.90	9	43,60	acetic acid*
	5.43	6	74,45,57	propanoic acid
4	5.92	9	106,77	benzaldehyde*
	6.45	5	60,73	butanoic acid
	6.68	3	91,126	benzylchloride*
	6.83	3	57,41	
	7.10	4	69,118	
	7.25	4	120,91,44	*N*-benzylmethanamine
	7.41	1	69,42	
5	7.42	9	108,79	benzyl alcohol*
	7.56	4	120	1,*N*-dimethylphenylmethanamine
	7.62	5	60,73	pentanoic acid
	7.79	9	120	
	7.92	1	57,98	
6	8.23	5	146,105	*cis*-1,2-dimethyl-3-phenylaziridine*
7	8.38	9	44,91	amphetamine*
	8.61	6	60,73	hexanoic acid
	8.71	5	44	
	8.73	6	120	
	8.86	2	92	1-phenyl-2-propanol
	8.95	9	58,140	benzedrex
8	9.22	9	58,91	MA*
9	9.57	8	146,105	*trans*-1,2-dimethyl-3-phenylaziridine*
	9.94	2	102,88	
	10.00	2	72,44	ethamphetamine
	10.04	1	102,87	
	10.04	1	72,87,116	
10	10.15	9	72	dimethylamphetamine*
	10.37	1	86,44,91	*N*-propylamphetamine
	10.41	4	60,73	nonanoic acid
	10.50	2	72,91,134	
	10.64	2	58	
	10.88	7	132	5-aminoindan
	10.99	4	86	
	11.11	3	120	
	11.29	4	58	
	11.33	5	150,169	
	11.39	1	138	
	11.50	9	71	
	11.56	2	60,73,129	decanoic acid
	11.73	9	71,89,56,173	
	11.79	2	58,85,148	
	12.19	1	138,181	bis-cyclohexylamine
	12.25	4	168,70,150	
	12.37	1	120	
	12.62	9	58,165,205	
	12.68	2	163,43	
	12.71	9	118,148	
	12.84	9	68,111	
	12.92	5	191,206	
	13.00	2	44,86,118	*N*-acetylamphetamine
	13.11	9	91,148	
	13.22	9	119,91,172,187	
	13.28	9	153,219	
	13.39	9	86,58,118,91	*N*-formylmethamphetamine*
	13.45	2	146,174,189	
	13.55	9	71,149	
	13.59	1	58,100	
	13.69	4	102,200,183	
	13.81	1	233,247,262	
	14.30	4	120,162	phenylpiperazine
	14.43	3	162,149	
	14.49	3	59,72,150	
11	14.56	9	58,100	*N*-acetylephedrine*
	14.61	4	132	

14.73	2	125,99
14.93	3	58,165
15.14	2	208,176,252
15.29	4	162,91,119
15.30	5	205,57
15.39	2	194,176
15.84	9	176,148,91
16.29	9	59,72
16.63	3	176,355,148
16.98	1	190,58
17.64	9	149,167,279

* indicates a compound identified by both retention time and mass spectrum compared with those of authentic standard.

Table 3 Intra- and inter-batch variations in peak areas on mass chromatograms obtained from LLE and TD

compound	CVs (%)			
	LLE		TD	
	intra	inter	intra	inter
benzaldehyde	4.7	37.9	30.0	126.1
benzyl alcohol	7.0	13.8	106.7	299.4
cis-AZ	2.5	266.8	75.7	333.1
DMA	6.5	87.1	139.8	308.3

CVs in intra-batches and inter-batches represent the averages of CVs calculated from each of nine batches and the CVs calculated from the averages of peak areas in each of nine batches, respectively.

Table 4 Compounds and *m/z* used for classification in LLE and TD

	compound	
m/z	LLE	TD
45	-	ethanol
106	benzaldehyde	benzaldehyde
146	*cis*-AZ	*cis*-AZ
44	amphetamine	amphetamine
146	*trans*-AZ	*trans*-AZ
72	DMA	DMA

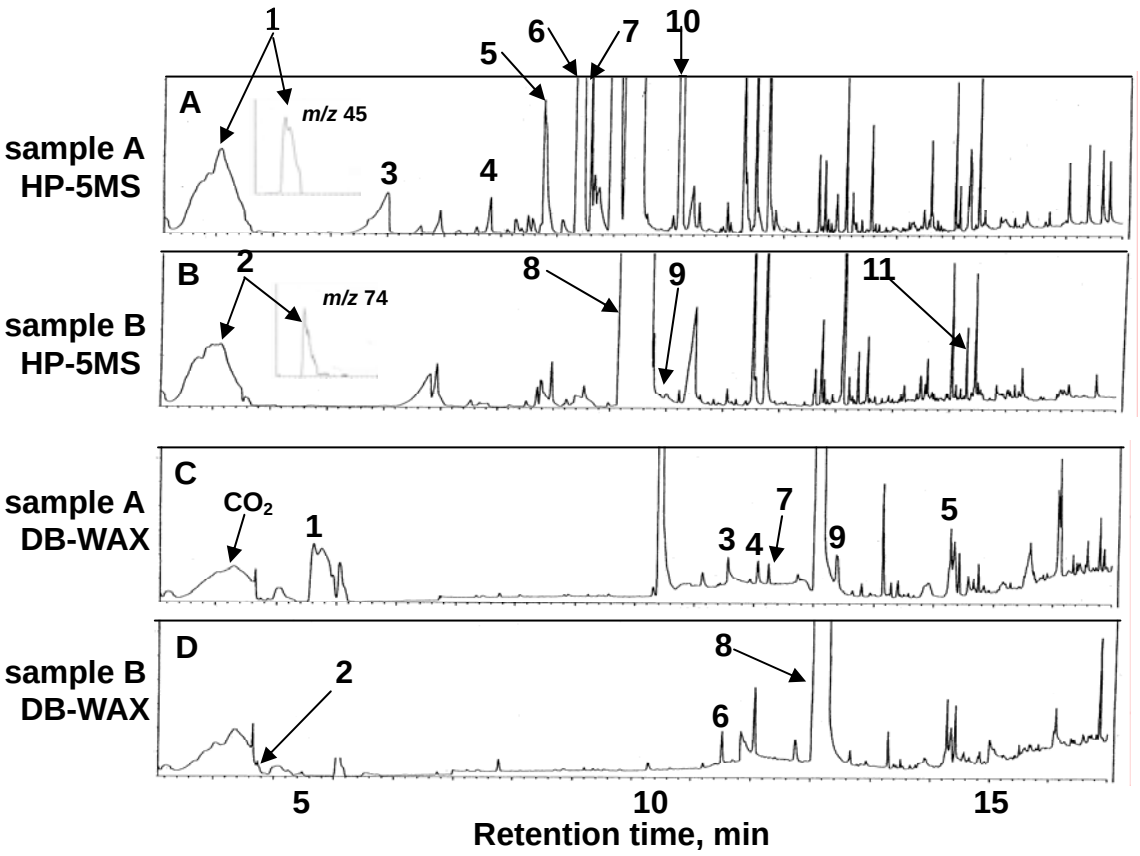

Fig. 1 Kuwayama et al

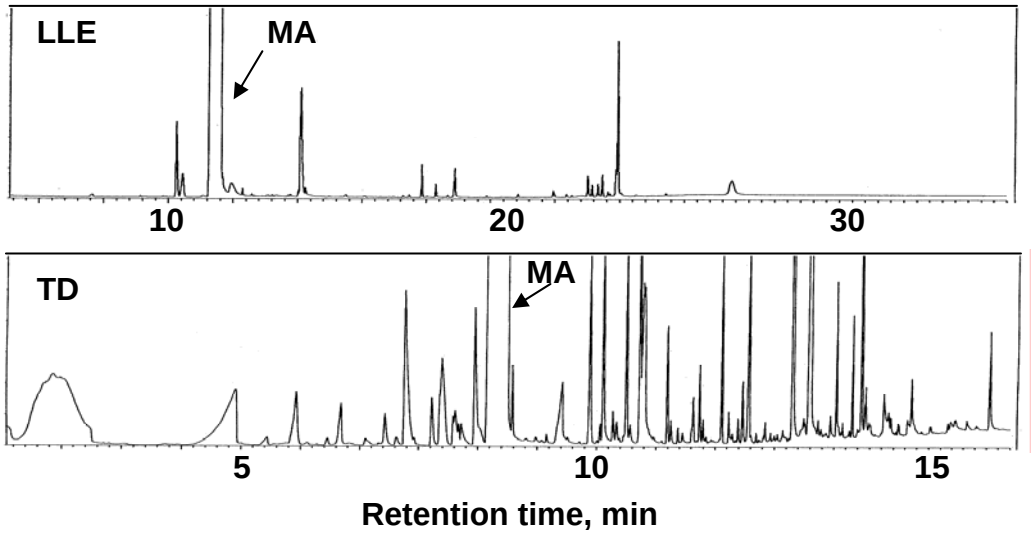

Fig. 2 Kuwayama et al

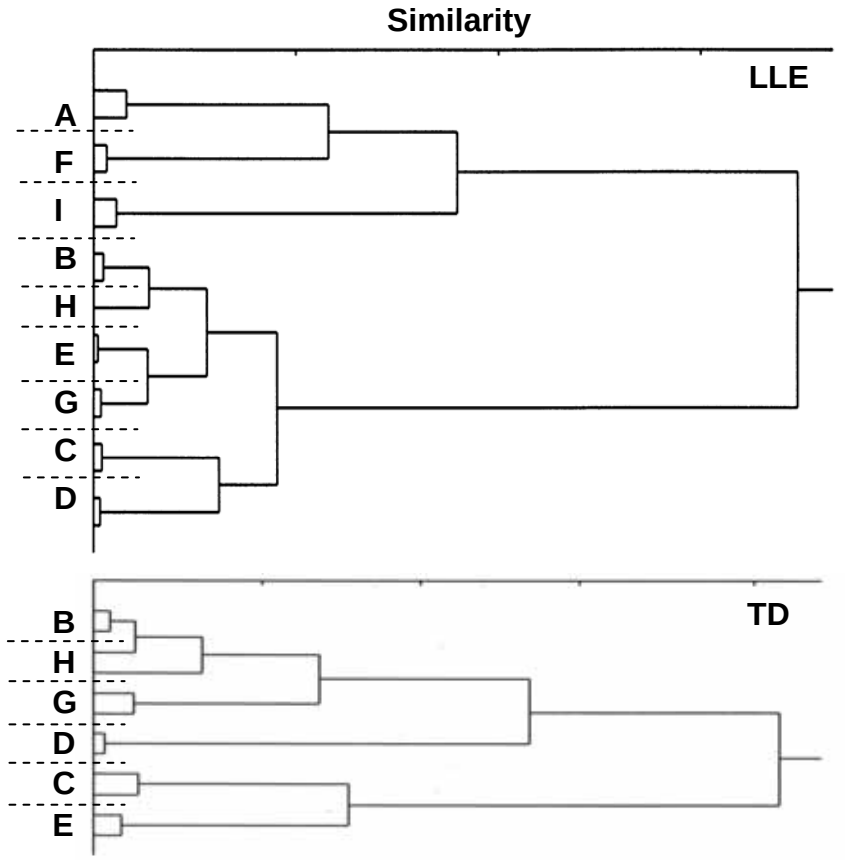

Fig. 3 Kuwayama et al

Figure legends

Fig. 1 Total ion chromatograms of MA samples from two different origins using HP-5MS and DB-WAX columns. All chromatograms are illustrated on the same scale. Extraction conditions: A 20 mg sample in a tube was heated at 120˚C for 3 min using the TD instrument and then subjected to GC-MS analysis. The compounds of peak number using HP-5MS and DB-WAX were as follows: 1, ethanol; 2, diethyl ether; 3, acetic acid; 4, benzaldehyde; 5, benzyl alcohol; 6, *cis*-AZ; 7, amphetamine; 8, MA; 9, *trans*-AZ; 10, DMA; 11, *N*-acetylephedrine.

Fig. 2 Total ion chromatograms obtained from the same sample using LLE (upper) and TD (lower). Each chromatogram is illustrated on the same scale. Extraction and analytical conditions are described in the text.

Fig. 3 Dendrogram obtained for a cluster analysis of impurity profiles from nine batches (A-I). The raw data were converted to their logarithm, and the similarity among samples was calculated using the cosine distance. At first, two samples from each of the nine batches (A-I) were classified using data from LLE. Two samples from each of six batches (B-E, G, and H) were subsequently classified using data from TD.