Hsun Lee
Chih-Sheng Jhang
Ju-Tsung Liu
Cheng-Huang Lin

Department of Chemistry,
National Taiwan Normal
University, Taipei, Taiwan

Short Communication

Rapid screening and determination of designer drugs in saliva by a nib-assisted paper spray-mass spectrometry and separation technique

A method for the rapid screening and determination of amphetamine-type designer drugs in saliva by a novel nib-assisted paper spray-mass spectrometry procedure is described. Under optimized conditions, the limit of detections for amphetamine derivatives (model samples: *o*-, *m*-, *p*-chloroamphetamine and *o*-, *m*-, *p*-fluoroamphetamine, respectively) were determined to 0.1 μg/mL by the nib-assisted paper spray-mass spectrometry method. This method is easier and has a higher sensitivity than similar methodologies, including atmospheric pressure/matrix-assisted laser desorption ionization mass spectrometry and electrospray-assisted laser desorption ionization/mass spectrometry. Data obtained using more classical separation methods, including liquid chromatography and capillary electrophoresis, are also reported.

1 Introduction

Substitutions to the amphetamine molecule give rise to a group of derivatives, and, as a result, a number of illegal, amphetamine-like drugs are produced in underground labs for sale on the street [1–3]. From the point of view of screening and the confirmation of amphetamine derivatives on the illicit market, more detailed detection and separation information would be highly desirable. Thus far, gas chromatograph-electron impact-mass spectrometer [4–7] and liquid chromatography electrospray ionization-mass spectrometry [8–10] are the most popular and powerful techniques for the analysis of illicit drugs and analogs thereof. As complementary methodologies, the use of a capillary electrophoresis-UV method [11–15], CE-laser-induced fluorescence (CE-LIF) detection [16–20], and CE-mass spectrometry (MS) [21–25] have also been reported. Although each of these above-mentioned methods has certain unique advantages and disadvantages with respect to sensitivity, precision, and simplicity of use, they can be time consuming when a separation is required. Hence, a rapid and highly accurate

screening method would be highly desirable in this area. The capabilities of ambient ionization mass spectrometry have recently been demonstrated, including atmospheric pressure-matrix assisted laser desorption ionization-mass spectrometry (AP-MALDI-MS) [26–29], electrospray-assisted laser desorption ionization-mass spectrometry (ELDI-MS) [30–34] and paper spray-mass spectrometry [35–39], respectively. These methods are useful for rapid screening, since they can be used in conjunction with a variety of samples, either extracted from blood, urine or saliva.

In this study, we selected *o*-, *m*-, *p*-chloroamphetamine and *o*-, *m*-, *p*-fluoroamphetamine as model compounds. The results obtained by the AP-MALDI-MS, ELDI-MS, and nib-assisted paper spray-mass spectrometry (NAPS-MS) methods were compared and the results are discussed. As regular analytical methods, results using the LC and CE methods were also obtained and the findings are compared.

2 Experimental

2.1 Reagents

o-, *m*-, *p*-Chloroamphetamine and *o*-, *m*-, *p*-fluoroamphetamine were generously donated by the Forensic Science Center (Military Police Command, Taiwan). The procedures for their synthesis have been described previously by Ann and Alexander Shulgin in their book entitled *TiHKAL*. Following the synthesis steps, the final products were verified by NMR, IR, and GC/MS. Chromatography paper was purchased from Advantec (Saijyo-city,

Correspondence: Professor C.-H. Lin, Department of Chemistry, National Taiwan Normal University, 88 Sec. 4, Tingchow Road, Taipei, Taiwan
E-mail: chenglin@ntnu.edu.tw
Fax: +886-2-2932-4249
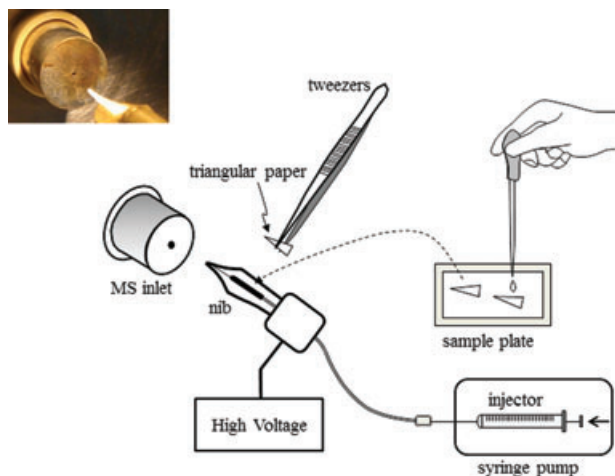
Figure 1. Schematic diagram of the nib-assisted paper spray-mass spectrometry (NAPS-MS) method used in this study.

Ehime, Japan). All the other chemicals were of analytical grade and were obtained from commercial sources.

2.2 Apparatus

A mass spectrometer (Finnigan LCQ Classic LC/MS/MS) was used in the AP-MALDI-MS, ELDI-MS, and NAPS-MS experiments, respectively. A nitrogen laser (Spectra-Physics Model: 337201–00, Ellicott city, MD, USA) was used for the former two cases and an in-house fabricated nib was specially prepared and used for the NAPS-MS (as shown in Fig. 1). A LC-Q-TOFMS system, which consisted of a Waters 1525 binary HPLC pump, a reversed-phase column (Cosmosil 5C18-MS, 5 μm, 25 cm × 4.6 mm id; Nacalai Tesque, Kyoto, Japan) and a mass spectrometer (Micromass Q-TOF) were also used in this study. As complementary methods, in-house fabricated capillary electrophoresis-UV and CE-LIF systems were also used. The CE set-ups were identical to those used in our previous studies [40–42] and are abbreviated herein.

3   Results and discussion

Figure 1 shows a schematic diagram of NAPS-MS used in this study. A piece of paper was cut into a triangular shape, 5 mm in length and 3 mm wide at the base. The sample solution was dropped on the triangular spray-paper, and then directly placed on the nib. The nib was made from brass and designed to easily connect with a capillary (id 250 μm). As a result, it was possible to continuously elute the paper with methanol at a rate of 6 μL/min. The volume of the syringe injector used was 50 μL. As shown in Fig. 2A (test sample: *p*-chloroamphetamine; concentration level: 10 μg/mL), we found that the sharpness of the portion of the tip of the triangular paper has a substantial effect on the ionization efficiency; the S/N ratios are improved dramatically when the
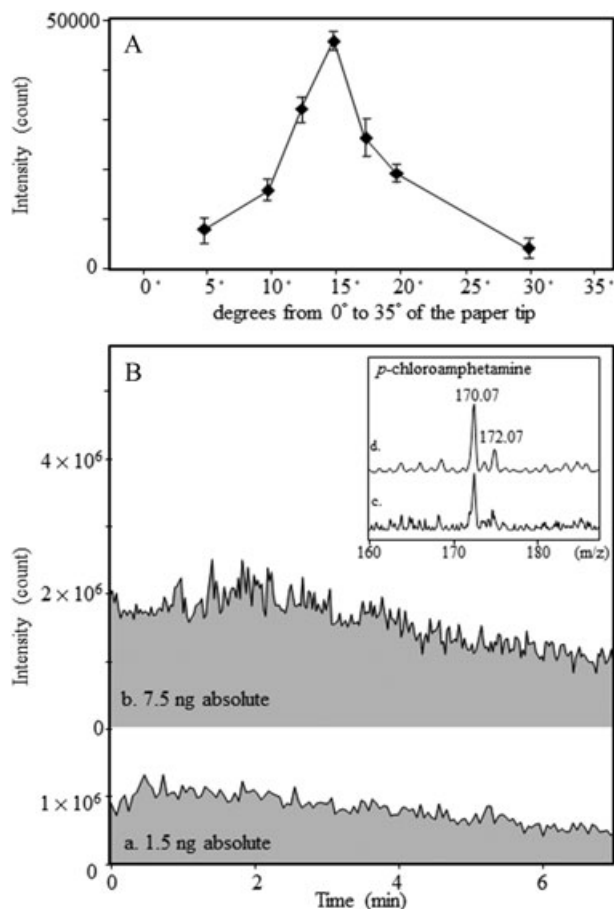
Figure 2. (A) Relationship between the sharpness of the portion of the tip of triangular paper and ionization efficiency; test sample, *p*-chloroamphetamine (10 μg/mL). (B) Relationship between the paper-spray ion intensity (total ion current; *m/z*: 169.05–170.05) and the period of ion occurring successively (time). The mass spectrum c was obtained by a single acquisition and this could be improved substantially when multiple acquisitions are used, as shown in mass spectrum d (541 acquirements).

degree is sharper than 30°. This indicates that the corona discharge also plays an important role in the process of ionization. The optimized distance from the tip of the paper to MS inlet was determined to be 6 mm, which is similar with the value reported in [36]. When a sharp (15°-tip; 5 mm in length) paper was used and a 15-μL-sample solution (concentration levels, 0.1 and 0.5 μg/mL; a and b, respectively) was dropped on it, the ion signals can be clearly observed (applied voltage, +3 kV). Figure 2B shows the relationship between the paper-spray ion intensity (total ion current; *m/z*: 169.05~170.05) and the period of ion occurring successively (time). The ion intensities were decreased very slowly. Ion intensity of ~1/5 can still be observed even 10 min later and this would be useful for rapid screening and determination of designer drugs. In this case, it can be estimated that *p*-chloroamphetamine was ionized and continuously ejected from the tip at a rate of ~1 pg/s, within the initial 5 min of the procedure. Furthermore, the mass spectrum c was obtained by a single

Table 1. Limit of detection (µg/mL; $n = 7$) values for the six amphetamines based on various screening methods

	o-F	*m*-F	*p*-F	*o*-Cl	*m*-Cl	*p*-Cl
NAPS-MS	0.11 ±0.01	0.13 ± 0.01	0.12 ± 0.01	0.10 ± 0.01	0.13 ± 0.01	0.10 ± 0.01
AP-MALDI-MS	7.00 ± 1.75	7.10 ± 2.27	7.87 ± 2.05	7.99 ± 2.32	8.10 ± 2.27	8.41 ± 1.77
ELDI-MS	3.73 ± 1.12	3.73 ± 0.93	3.10 ± 0.96	3.53 ± 0.88	4.17 ± 0.92	2.99 ± 0.87

NAPS-MS, nib-assisted paper spray mass spectrometry; AP-MALDI-MS, atmospheric pressure-matrix assisted laser desorption ionization-mass spectrometry; ELDI-MS, electrospray-assisted laser desorption ionization mass spectrometry; *o*-F, *o*-fluoroamphetamine; *m*-F, *m*-fluoroamphetamine; *p*-F, *p*-fluoroamphetamine; *o*-Cl, *o*-chloroamphetamine; *m*-Cl, *m*-chloroamphetamine; *p*-Cl, *p*-chloroamphetamine.

acquisition and this could be improved substantially when multiple acquisitions are used, as shown in mass spectrum d (541 acquirements). For *p*-chloroamphetamine, a linearity was found from 0.1∼25 µg/mL. The LODs (at S/N = 3) for the six amphetamines obtained by the NAPS-MS as well as AP-MALDI-MS, and ELDI-MS procedures are summarized in Table 1. In most conventional MALDI-MS, it is necessary to search around the sample to find what is called a "sweet spot" that is formed by the type of matrix used and the sample itself. This is time consuming and difficult to control; the LODs were determined to be 7∼8 µg/mL. In contrast to MALDI, the ELDI-MS method can be more convenient because no matrix is required. Furthermore, this method combines laser desorption and postionization by electrospray, and is suitable for the rapid analysis of solid materials under ambient conditions. However, when a pulsed nitrogen laser is used, the sample molecules are ejected from the surface, and then ionized when they encounter the electrospray-clusters. It can be imagined that the process could be intense, and, indeed, a certain amount of experimental skill is needed. The LODs obtained by the ELDI-MS method were determined to be 3∼4 µg/mL. On the other hand, in the traditional paper spray-mass spectrometry method, the sample should be preloaded onto the paper, and the wetting solution then added. However, a quantitative analysis is difficult, since the solution can evaporate during the ionization steps and, when this occurs, the electrospray process is terminated. Sometimes additional added solution is needed. In this study, a nib-assisted method was used and is described. The ionization process became more stable, which resulted in the production of a high-quality, characteristic mass spectrum. As a result, the LODs were dramatically improved to 0.1 µg/mL. In an analysis of a saliva sample, a 495-µL aliquot of a saliva sample obtained from a human volunteer was placed in a tube and then spiked with *p*-chloroamphetamine (5 µL). Although unknown matrix effects were observed, the LODs could be determined to be 0.5 µg/mL (data not shown). Hence, we conclude that NAPS-MS is, under most circumstances, the most favorable rapid "drug-screening" method for use under ambient conditions. Table 2 shows the results obtained by regular separation methods, including LC and CE, respectively. Extraction procedures were referenced and modified from [43, 44], and are abbreviated herein. In the case of LC/MS, when a gradient elution (A, 0.1% formic acid aqueous solution/pH, 2.5; B, methanol) was used, *p*-chloroamphetamine

Table 2. Comparison of limit of detection (µg/mL) values for a *p*-chloroamphetamine standard solution and saliva extracts by liquid chromatography (LC) and capillary electrophoresis (CE) methods, respectively.

Methods	*Standard*	Saliva extracts
LC-Q-TOFMS	0.5	1.0
CZE-UV	50	–
MEKC-UV	25	–
Sweeping/MEKC-UV	0.5	–
MEKC-LIF	0.05	0.25
CE-MS	0.5	0.5

LC-Q-TOFMS, liquid chromatography/electrospray ionization quadrupole time-of-flight mass spectrometry; CZE-UV, capillary zone electrophoresis-UV absorbance detection; MEKC-UV, micellar electrokinetic chromatography-UV absorbance detection; LIF, laser-induced fluorescence; CE-MS, capillary electrophoresis-mass spectrometry.

eluted at ∼3.8 min; the LODs were determined to be 0.5 and 1.0 µg/mL for the standard solution and the saliva extract, respectively. When the CZE/UV method was used, the six model samples can be completely separated within ∼20 min; buffer conditions: phosphate buffer (NaH$_2$PO$_4$, 50 mM/Na$_2$HPO$_4$, 100 mM) in acetonitrile/methanol/water (12.5:17.5:70, v/v/v) containing 7.5-mM β-CD; pH, 3.1. Using sweeping-MEKC (phosphate buffer [NaH$_2$PO$_4$, 50 mM] in acetonitrile/methanol/water [5:30:65, v/v/v] containing 75-mM SDS; pH, 2.13), or CE-LIF, dramatic increases in sensitivity are possible. Meanwhile, the LOD can be improved when the CE/LIF and CE/MS methods, respectively, are applied. Thus, we can conclude that to a LC/MS or CE/UV method, in general, it takes at least ∼3.8 and 20 min for a single measurement, respectively, whereas the use of NAPS-MS is more convenience for rapid screening.

4 Conclusions

In this study, we describe the development of a novel NAPS-MS method. It is suitable for use in the rapid screening of drugs, since it has a high degree of sensitivity, the operating procedure is simple, and the ion signal can be observed immediately and continuously. The present method can save

time because a number of illegal drugs may be screened first, without the need for any separation techniques. We believe this method has the potential for use in practical analyses and can also be regarded as a helpful tool for use in forensic and clinical analysis.

The authors have declared no conflict of interest.

5   References

[1] Rudnick, G., Wall, S. C., *J. Biol. Chem.* 1992, *31*, 6710–6718.

[2] MaronaLewicka, D., Rhee, G. S., Sprague, J. E., Nichols, D. E., *Eur. J. Pharmacol.* 1995, *287*, 105–113.

[3] Vieira, R., Mancebo, M. J., Aldegunde, M., *J. Physiol. Biochem.* 2007, *63*, 129–141.

[4] Paul, B. D., Jemionek, J., Lesser, D., Jacobs, A., Searles, D. A., *J. Anal. Toxicol.* 2004, *28*, 449–455.

[5] Rasmussen, L. B., Olsen, K. H., Johansen, S. S., *J. Chromatogr. B* 2006, *842*, 136–141.

[6] Wu, Y. H., Lin, K. L., Chen, S. C., Chang, Y. Z., *J. Chromatogr. B* 2008, *870*, 192–202.

[7] Johansen, S. S., Jornil, J., *Scand. J. Clin. Lab. Invest.* 2009, *69*, 113–120.

[8] Kronstrand, R., Nystrom, I., Strandberg, J., Druid, H. *Forensic Sci. Int.* 2004, *145*, 183–190.

[9] Concheiro, M., Simoes, S. M. D., Quintela, O., de Castro, A., Dias, M. J. R., Cruz, A., Lopez-Rivadulla, M., *Forensic Sci. Int.* 2007, *171*, 44–51.

[10] Andersson, M., Gustavsson, E., Stephanson, N., Beck, O., *J. Chromatogr. B* 2008, *861*, 22–28.

[11] Ho, T. S., Pedersen-Bjergaard, S., Rasmussen, K. E., *Analyst* 2002, *127*, 608–613.

[12] Descroix, S., Varenne, A., Geiser, L., Cherkaoui, S., Veuthey, J. L., Gareil, P., *Electrophoresis* 2003, *24*, 1577–1586.

[13] Rudaz, S., Geiser, L., Souverain, S., Prat, J., Veuthey, J. L., *Electrophoresis* 2005, *26*, 3910–3920.

[14] Iwata, Y. T., Inoue, H., Kuwayama, K., Kanamori, T., Tsujikawa, K., Miyaguchi, H., Kishi, T., *Forensic Sci. Int.* 2006, *161*, 92–96.

[15] Epple, R., Blanes, L., Beavis, A., Roux, C., Doble, P., *Electrophoresis* 2010, *31*, 2608–2613.

[16] Choi, J., Kim, C., Choi, M. J., *J. Chromatogr. B* 1998, *705*, 277–282.

[17] Choi, J., Kim, C., Choi, M. J., *Electrophoresis* 1998, *19*, 2950–2955.

[18] Kuroda, N., Nomura, R., Al-Dirbashi, O., Akiyama, S., Nakashima, K., *J. Chromatogr. A* 1998, *798*, 325–334.

[19] Ramseier, A., von Heeren, F., Thormann, W., *Electrophoresis* 1998, *19*, 2967–2975.

[20] Zhang, L., Wang, R., Yu, Y. Q., Zhang, Y. R., *J. Chromatogr. B* 2007, *857*, 130–135.

[21] Lazar, I. M., Naisbitt, G., Lee, M. L., *Analyst* 1998, *123*, 1449–1454.

[22] Ramseier, A., Siethoff, C., Caslavska, J., Thormann, W., *Electrophoresis* 2000, *21*, 380–387.

[23] Geiser, L., Cherkaoui, S., Veuthey, J. L., *J. Chromatogr. A* 2000, *895*, 111–121.

[24] Shamsi, S. A., *Electrophoresis* 2002, *23*, 4036–4051.

[25] Huck, C. W., Stecher, G., Scherz, H., Bonn, G., *Electrophoresis* 2005, *26*, 1319–1333.

[26] Pihlainen, K., Grigoras, K., Franssila, S., Ketola, R., Kotiaho, T., Kostiainen, R., *J. Mass Spectrom.* 2005, *40*, 539–545.

[27] Grasso, G., Fragai, M., Rizzarelli, E., Spoto, G., Yeo, K. J., *J. Mass Spectrom.* 2006, *41*, 1561–1569.

[28] Grasso, G., Rizzarelli, E., Spoto, G., *J. Mass Spectrom.* 2007, *42*, 1590–1598.

[29] Grasso, G., Mineo, P., Rizzarelli, E., Spoto, G., *J. Mass Spectrom.* 2009, *282*, 50–55.

[30] Peng, I. X., Shiea, J., Loo, R. R. O., Loo, J. A., *Rapid Commun. Mass Spectrom.* 2007, *21*, 2541–2546.

[31] Lin, S. Y., Huang, M. Z., Chang, H. C., Shiea, J., *Anal. Chem.* 2007, *79*, 8789–8795.

[32] Shiea, J., Yuan, C. H., Huang, M. Z., Cheng, S. C., Ma, Y. L., Tseng, W. L., Chang, H. C., Hung, W. C., *Anal. Chem.* 2008, *80*, 4845–4852.

[33] Huang, M. Z., Jhang, S. S., Cheng, C. N., Cheng, S. C., Shiea, J., *Analyst* 2010, *135*, 759–766.

[34] Peng, I. X., Loo, R. R. O., Margalith, E., Little, M. W., Loo, J. A., *Analyst* 2010, *135*, 767–772.

[35] Wang, H., Liu, J. J., Cooks, R. G., Ouyang, Z., *Angew. Chem. Int. Ed.* 2010, *49*, 877–880.

[36] Liu, J. J., Wang, H., Manicke, N. E., Lin, J. M., Cooks, R. G., Ouyang, Z., *Anal. Chem.* 2010, *82*, 2463–2471.

[37] Manicke, N. E., Yang, Q. A., Wang, H., Oradu, S., Ouyang, Z., Cooks, R. G., *Mass Spectrom.* 2011, *300*, 123–129.

[38] Wang, H., Manicke, N. E., Yang, Q. A., Zheng, L. X., Shi, R. Y., Cooks, R. G., Zheng, O. Y., *Anal. Chem.* 2011, *83*, 1197–1201.

[39] Hu, B., So, P. K., Chen, H. W., Yao, Z. P., *Anal. Chem.* 2011, *83*, 8201–8207.

[40] Tsai, C. C., Liu, J. T., Shu, Y. R., Chan, P. H., Lin, C. H., *J. Chromatogr. A* 2006, *1101*, 319–323.

[41] Yang, Y. Y., Liu, J. T., Lin, C. H., *Electrophoresis* 2009, *30*, 1084–1087.

[42] Wang, M. J., Tsai, C. H., Hsu, W. Y., Liu, J. T., Lin, C. H., *J. Sep. Sci.* 2009, *32*, 441–445.

[43] Yonamine, M., Tawil, N., Moreau, R. L. D., Silva, O. A., *J. Chromatogr. B* 2003, *789*, 73–78.

[44] Meng, P. J., Wang, Y. Y., *Forensic Sci. Int.* 2010, *197*, 80–84.