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Research Article

Rapid screening and determination of 4-chloroamphetamine in saliva by paper spray-mass spectrometry and capillary electrophoresis-mass spectrometry

A novel drug-screening system, consisting of paper spray-MS (PS-MS) and a CE-ESI-MS method was developed. This system can be easily switched either to PS-MS for rapidly screening samples or to the traditional CE-ESI-MS method for separation and to obtain detailed mass spectral information, while sharing the same mass spectrometer. In the former case, when a sharp (15°-tip) chromatography paper was used, the optimized distance from the paper tip to the mass inlet was 7.7 mm, whereas the optimized distance for the CE-ESI tip was ∼13.5 mm. Using 4-chloroamphetamine as a model compound, the LODs for PS-MS and CE-ESI-MS were determined to ∼0.1 and 0.25 ppm, respectively. Comparisons of results obtained using PS-MS and CE-ESI-MS and the experimental conditions are described.

Keywords:
4-chloro-amphetamine / CE-ESI-MS / Paper spray

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1 Introduction

Atmospheric pressure (AP)-MS is becoming more popular and several ionization techniques are now available to utilize, including AP-MALDI-MS [1–4], electrospray-assisted laser desorption ionization-MS (ELDI-MS) [5–8], and paper spray-MS (PS-MS) [9–13]. These methods can be used in conjunction with a variety of samples, either samples that are extracted from blood, urine, or salvia, and for this reason, they are useful for the rapid screening of illicit drugs. However, unknown compounds in the matrix can contaminate the sample, and a separation technique, such as LC-MS [14–16] or CE-MS [17–22], is then needed. Although all of the above methods can share one mass spectrometer, a switching system is needed, which requires that all components be optimized, a process that can be time consuming. In this study, we report on the development of a novel system that consists of PS-MS for rapid screening and a CE-ESI-MS method for acquiring detailed information on the separation and mass spectra of the analytes, respectively. System switching becomes easy and fast. 4-Chloro- and 4-fluoroamphetamines were selected as model compounds to evaluate the method. These compounds were chosen because 4-chloroamphetamine was permanently placed in Schedule III in Taiwan in 2011. It is similar to 3,4-methylenedioxymethamphetamine (MDMA) but with a substantially higher neurotoxicity [23]. Furthermore, illicit drugs are also environmental contaminants. Methods for their analysis, as well as derivatives thereof, would be highly desirable. Thus far, GC-MS [24–27] and LC-MS continue to be the officially prescribed methods, but a system that can permit both rapid screening and includes a separation technique would be highly desirable in this area. In this paper, details are provided regarding the make up and use of the novel system and results obtained by the PS-MS and CE-ESI-MS methods are compared and discussed.

2 Materials and methods

2.1 Reagents

The 4-chloro- and 4-fluoroamphetamines were generously donated by the Military Police Command, Forensic Science Center, Taiwan. The procedures for their synthesis have been described previously by Ann and Alexander Shulgin in their book entitled PiHKAL (Phenethylamines I Have Known And Loved) [28]. The chromatography paper used for PS-MS was purchased from Advantec (Japan). All the other chemicals were of analytical grade and were obtained from commercial sources.

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Abbreviations: AP, atmospheric pressure; **ELDI**, electrospray-assisted laser desorption ionization; **PS-MS**, paper spray-MS

Colour Online: See the article online to view Figs. 1 and 3 in colour.

2.2 Apparatus

A mass spectrometer (Finnigan LCQ Classic LC/MS/MS) was used in the PS-MS and CE-ESI-MS experiments, respectively. An in-house fabricated nib and CE-ESI tip were specially prepared and used for the PS-MS and CE-ESI-MS, respectively. Figure 1 shows a schematic diagram of the PS-MS and CE-ESI-MS set up used in this study. In the former case, a piece of chromatography paper was cut into a triangular shape, 5 mm in length and 1.5 mm wide at the base. A drop of sample solution was placed on the triangular-shaped spray paper, which was then placed directly on the nib. The nib was made from brass and was designed to be easily connected to a capillary (id 250 μm) for supplying additional methanol. 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. In most of the PS-MS method, triangular-shaped spray paper is simply held by a copper clip with the apex facing the inlet of the mass spectrometer. One addition of 5 μL solution to the paper allows for an analysis time of more than 30 s [9]. However, when the nib-assisted method was used, ionization process became more stable and analysis time longer than 10 min, which resulted in the production of a high-quality, characteristic mass spectrum, especially when multiple acquisitions are used. When the syringe injector is changed to a secondary capillary, a CE-ESI-MS experiment can be performed. The in-house fabricated CE-ESI-tip (as shown in the dashed-line block in Fig. 1) was also made from brass and the rate of sheath liquid (methanol) was set at a rate of 4 μL/min; the use of auxiliary gas is not necessary. The id and od of the CE capillary were 75 and 365 μm, respectively. For comparison, an in-house fabricated CZE-UV system was also used. This was identical to that used in our previous studies and is abbreviated herein [29, 30].

3 Results and discussion

3.1 PS-MS application

In our previous studies [31], we found that the sharpness of the portion of the tip of the triangular paper has a substantial effect on ionization efficiency. The S/N ratios dramatically improved when the degree is sharper than 30°. Under the same experimental conditions, a 15°-paper resulted in the maximum ion intensity. In order to investigate the optimal position for the triangular paper, a 2D moving stage (20 mm in the *y*-direction and 10 mm in the *x*-direction with a 1 mm increment for each step) was used. When a 15°-tip paper was used and a 5 μL sample solution (4-chloroamphetamine; concentration levels, 10 ppm) was dropped on it, it was possible to clearly observe the ion signals (applied voltage, +3 kV). We investigated the relationship between PS ion intensity (total ion current; m/z: 100∼300) and the period of ionization (time), and the findings show that the sample was ionized and continuously ejected from the tip when it was eluted at a rate of ∼1 pg/s, within the initial 5 min of the procedure. When the peak intensity of the protonated 4-chloroamphetamine ion (m/z 170) was recorded as a function of the position of the triangle tip, the peak intensities, from 220 different positions, were normalized and then plotted as a contour map, as shown in Fig. 2A. This shows that a stable high intensity can be obtained for PS with the paper tip is positioned in an area of about 6 × 4 mm (x by y), which is in agreement with data reported in the literature [9]. However, we also found that two specific spots, located on both sides of the mass inlet, respectively, provided the most appropriate results. By using this method, the LOD for either 4-chloro- or 4-fluoroamphetamine was determined to be 0.1 ppm, respectively. This result is much better than that obtained when the AP-MALDI-MS and ELDI-MS methods are used using the

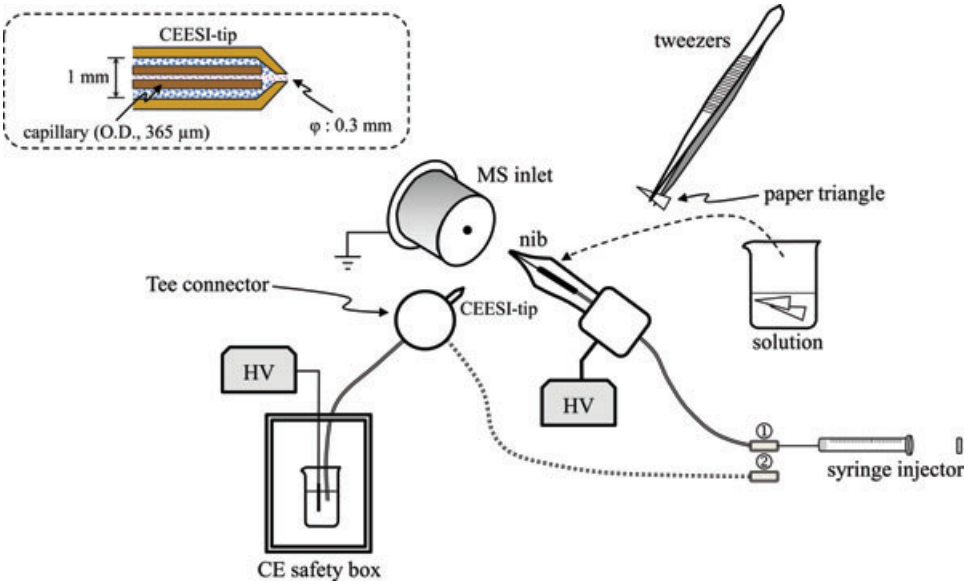

Figure 1. A schematic diagram of the system consisting of nib-assisted PS-MS and CE-ESI-MS.

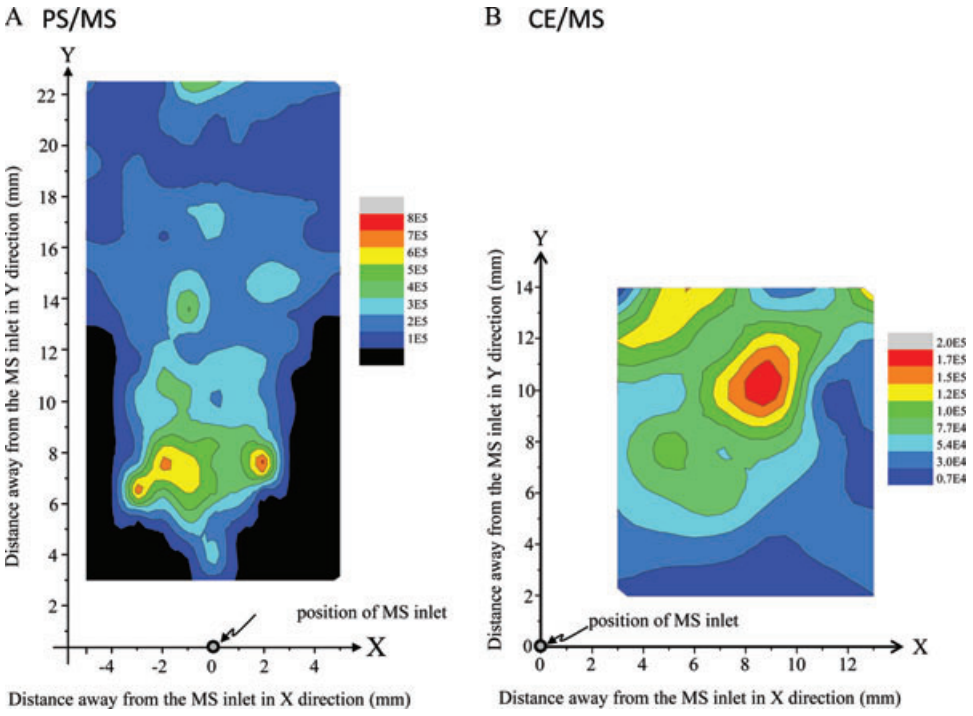

Figure 2. Characterization of the tolerance of paper spray ionization (A) and CE-ESI (B) to the position of the paper tip, using a 4-chloroamphetamine solution (10 ppm) and chromatography paper. 2D contour plot showing the relative intensity of $m/z = 170$ as a function of the paper tip location on the x–y plane.

same mass spectrometer. In using MALDI-MS, it is necessary to search around the sample to find what is referred to as a "sweet spot," which is formed by the type of matrix used and the sample itself. This is time consuming and difficult to control and the LODs using this technique were determined to be 7∼8 ppm. In contrast to MALDI, no matrix is required for the ELDI-MS method, which makes it more convenient to use. However, in this method, a pulsed nitrogen laser is used for laser desorption. The ejected sample molecules are ionized when they encounter electrospray clusters. The process can 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 ppm. On the other hand, in the traditional PS-MS method, the sample needs to be preloaded onto the paper, and the wetting solution is then added. However, carrying out a quantitative analysis is difficult, since the solution can evaporate during the ionization steps and the electrospray process is terminated when this occurs. Sometimes additional solution needs to be added. In our design, a nib-assisted method developed, in an attempt to avoid this problem. The ionization process became more stable, which resulted in the production of a high-quality, characteristic mass spectrum. Thus, we conclude that the nib-assisted PS-MS method is well suited for highly sensitive and rapid screening.

3.2 CE-ESI-MS application

In case of certain types of complex samples, a separation technique is unavoidable. Herein, the CE-ESI-MS experiment can be performed by reconnecting the syringe injector to the sec-

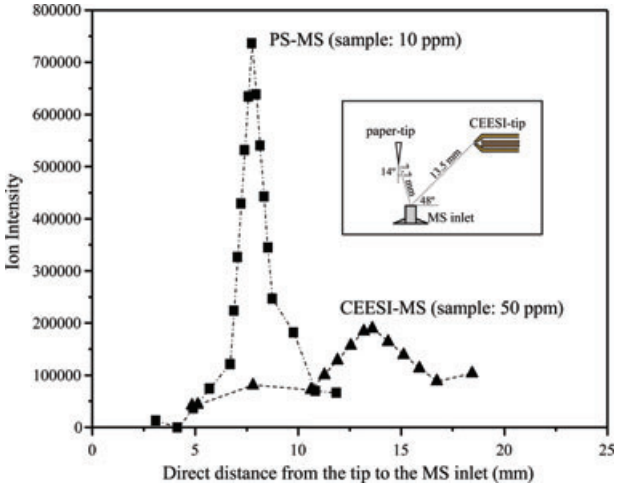

Figure 3. The relationship between the ion intensity (Y-axis) and the distance (X-axis) from the tip to the MS inlet.

ondary capillary that supplies sheath liquid to the CE-ESI tip. Although CE-MS is not a new technique, we concluded that it would be of interest to investigate the optimal position of the CE-ESI tip, to determine whether sensitivity could be further improved. A 2D moving stage (12 mm in the y-direction and 10 mm in the x-direction with a 2 mm increment for each step) was used in these tests. The test sample was 4-chloroamphetamine (concentration, 50 ppm) and CE buffer was a 10 mM ammonium acetate solution (pH 6.9); the applied voltage was +15 kV. After 60 CE runs, all of the intensities for each peak were collected and then plotted as a contour map, as shown in Fig. 2B. It is clear that a stable high

intensity can be obtained; the region that provided the most appropriate results is wider than that of PS-MS method. This is quite different from the optimized position in the case of paper spray, since the ESI process requires a longer distance for evaporation and ionization. We also found that this direct distance from the CE-ESI tip to the MS inlet is much shorter than the corresponding distances found in commercial CE-MS instruments, and as a result, the sensitivity can be dramatically improved. Figure 3 shows the relationship between the ion intensity (*Y*-axis) and the distance (*X*-axis) from the tip to the MS inlet. It is clear that, in the case of PS-MS, a specific position exists that provides maximum ion intensity. The optimized distance was 7.7 mm and the degree between the paper tip and the MS inlet was 14°. On the other hand, the region for the optimized distance (~13.5 mm) for the CE-ESI tip was wider; the angle between the CE-ESI tip and the MS inlet was ~48°. It should be noted that the concentration used for CE-ESI-MS (50 ppm) was higher than that used in PS-MS (10 ppm), but a weaker ion signal was obtained. This is because, in the case of CE-ESI-MS, a sheath liquid (6 μL/min,

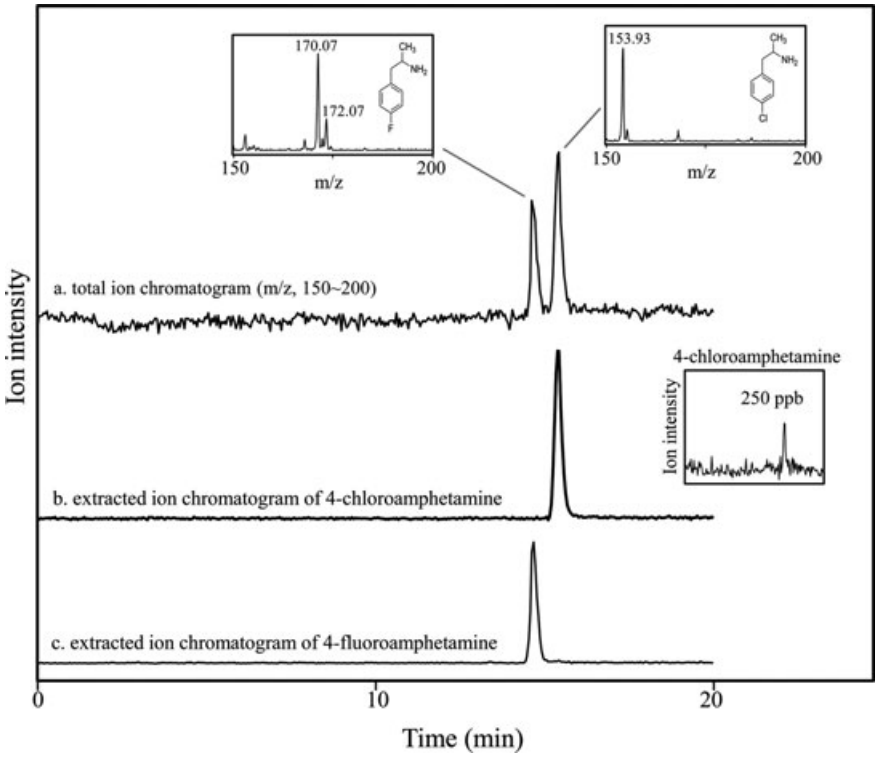

Figure 4. Typical CE electropherograms of a mixture of 4-chloro- and 4-fluoroamphetamine. Electropherogram a, total ions were collected in the range from m/z = 152 to 172; Extracted ion chromatograms b and c are assigned to 4-chloro- and 4-fluoroamphetamine, respectively.

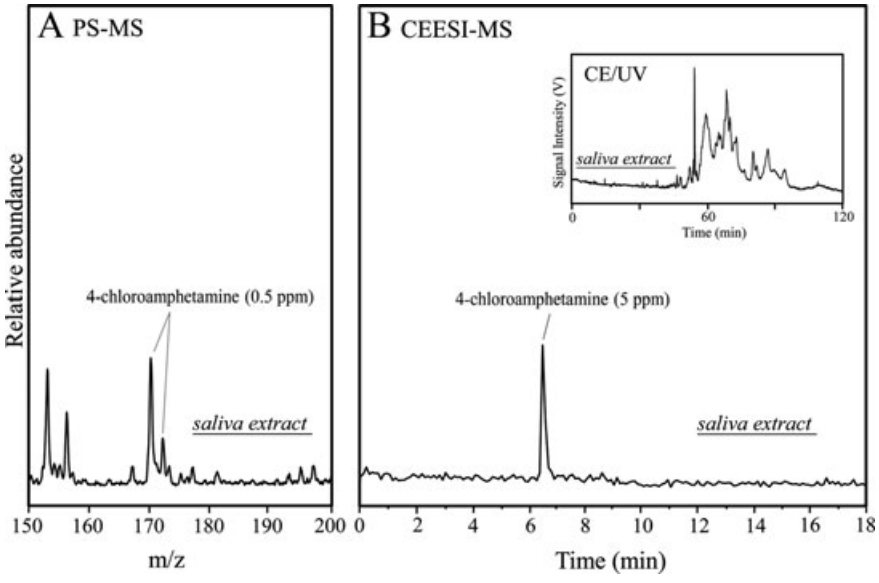

Figure 5. A, Result obtained for the analysis of a saliva extract by the PS-MS method (rapid screening); B, the result for the CE-ESI-MS method. Inset in 5B, saliva extract examined by the CZE/UV method for comparison.

methanol) was used and the distance was longer, leading to the dilution of the sample in the region of the Taylor cone. Once the optimized locations were determined, the system can be run and easily switched either to PS-MS for fast screening or to the regular CE-ESI-MS method for separation and collecting mass spectral information, while sharing the same mass spectrometer. Figure 4 shows typical CE electropherograms of a mixture of 4-chloro- and 4-fluoroamphetamine. As shown in electropherogram a, when the total ions were collected in the range from $m/z = 152$ to 172, the two analytes can be completely separated. The optimized separation buffer is simple, consisting of only 10 mM ammonium acetate in an ACN–methanol–water (12.5:17.5:70, v/v/v) solution; capillary, 75 μm id; total length, 50 cm; applied voltage, +15 kV. Methanol, at a rate of 4 μL/min, was used as the sheath liquid. The insets, in electropherogram a, show mass spectra of 4-chloro- and 4-fluoroamphetamine, respectively. At first glance, the baseline appears to be poor, but this was improved substantially when the extracted ion chromatograms were selected, as shown in electropherograms b and c, respectively. When an extracted ion chromatogram was used, even lower levels of 4-chloroamphetamine can be detected, as shown in the inset in electropherogram b (concentration level, 250 ppb). Although the LC-MS method can also be used to separate these compounds, physically installing LC-MS and PS-MS together in a small space is difficult. This is the reason for selecting the CE-MS method for this study. Finally, 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 a 4-chloroamphetamine solution (5 μL). The extraction procedures were referenced and modified from the literature [32, 33], and are abbreviated herein. As shown in Fig. 5A, although unknown matrix effects were observed, 4-chloroamphetamine still can be clearly detected, even at a level of 0.5 ppm. Hence, we conclude that the nib-assisted PS-MS method is, under most circumstances, the most favorable method for rapid "drug-screening" under ambient conditions. In the case of CE-ESI/MS (Fig. 5B), the separation buffer was consisting of only 10 mM ammonium acetate solution; capillary, 75 μm id; total length, 50 cm; applied voltage, +15 kV. 4-chloroamphetamine migrated at 6.5 min; the LOD was determined to be 1 ppm for the saliva extract. The CE electropherogram seems clear since total ions were collected only in the range from $m/z = 152$ to 172. When we examined a saliva extract using the CZE/UV method, a number of unknown peaks were found, as shown in the inset. Thus, we conclude that the CE-ESI/MS method is a very useful technique and can also be useful for simplifying spectrum. Further work still remains, such as the use of an on-line concentration technique, including a CE-stacking method, which could lead to further improvements in the LOD.

4 Conclusions

In this study, we describe the development of a novel drug-screening system that can be easily switched either to PS-MS for rapid screening or to the regular CE-ESI-MS method for achieving separation and for acquiring detailed mass spectral information, respectively, while sharing the same mass spectrometer. The distances from the paper tip and CE-ESI tip to the MS inlet were optimized and the findings reported. Saliva samples, obtained from a human volunteer, were also successfully examined by spiking the sample with 4-chloro-amphetamine (an illicit drug that has been permanently placed in Schedule III). This system constitutes a sensitive, simple, and economically complementary method for either rapid screening or the officially prescribed methods for use in forensic and clinical analysis, as well as in related work.

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The authors have declared no conflict of interest.

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