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Capillary electrophoresis–time-of-flight mass spectrometry of drugs of abuse

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The use of capillary electrophoresis (CE) for the determination of drugs of abuse was explored. A commercial CE system was interfaced with a laboratory-built time-of-flight mass spectrometer (TOFMS) which was equipped with a high-speed data acquisition system to provide accurate monitoring of efficient separations. Ionization of the CE eluent was achieved with an electrospray ionization source. Standard mixtures and seized samples were analyzed either by direct infusion of the analyte solutions or after separation by CE. Detection at the low femtomole level was obtained using CE–TOFMS.

Keywords: *Capillary electrophoresis; mass spectrometry; time-of-flight; drugs of abuse*

Capillary electrophoresis (CE), which was developed in the 1980s and rapidly expanded in the 1990s, has become a useful, complementary separation technique.[1,2] The fast development of CE is due to its unique characteristics, such as broad applicability (ionic/non-ionic, and low/high molecular mass compounds), high separation efficiency in a short analysis time, very good mass limits of detection and relatively low cost and simplicity. Rapid progress has also been made in interfacing CE with mass spectrometry (MS).[3] There are several ionization sources in use for on-line CE–MS, such as electrospray (ESI), inductively coupled plasma (ICP) and continuous flow fast atom bombardment (CF–FAB). However, it is the development of the ESI technique[4–6] which greatly facilitated CE–MS interfacing. ESI, much like CE, is applicable to both small and large molecules and ionic or ionizable compounds. CE–ICP is used in trace metal analysis, while CF–FAB is less sensitive than ESI and has less applicability to high molecular mass compounds.

A limitation of the most common mass spectrometers used as CE detectors (quadrupole, ion trap and Fourier transform ion cyclotron resonance) is the relative long time (0.1–3 s) that they need to produce a full mass spectrum, which is a direct result of the requirements of scanning or ion trapping. Time-of-flight (TOF) MS is able to produce a full mass spectrum in 50–200 µs depending on the pre-set pulsing rate. Along with speed, high ion transmission efficiency (about 10%) and high duty cycle ($>50\%$ for the off-axis configuration) result in increased sensitivity.[7,8] For fast and high-speed separations,[9–11] interfacing with MS detection will require the high speed attributes of TOFMS.

Illegal drugs normally encountered at the local or state levels are amphetamine, methamphetamine, marijuana, cocaine and heroin. Less frequent, but not uncommon, are the hallucinogens, psilocybin from mushrooms, mescaline from cactus and lysergic acid diethylamide (LSD). A wide variety of designer and commercial preparations are also encountered. Although drugs are typically found in all developed societies, their distribution and form vary from region to region. For example, in the southwest USA, methamphetamine and cocaine are the most commonly abused drugs, whereas in the eastern states, cocaine and heroin are far more common than methamphetamine. Heroin distributed in the East is usually a white powder thought to come from the Middle East, but in the western USA 'black tar heroin' from Mexico predominates.

Drugs of abuse are typically analyzed using gas chromatography (GC) or high-performance liquid chromatography (HPLC).[12–18] Certain compounds are thermally labile (*e.g.*, LSD, anabolic steroids and psilocybin), require derivatization (*e.g.*, morphine, adulterants and diluents such as sugars) or display poor chromatographic performance (*e.g.*, amphetamine and methamphetamine in their salt forms), and are not amenable to GC analysis.[19] HPLC has benefits of rugged instrumentation and methodology and good separation selectivity and it is applicable to a broad range of compounds; however, the separation times are often too long and efficiencies are much lower than in CE. Since the analytical methods used in forensic analysis must rely on widely accepted analytical techniques, and must be confirmed by at least two methods based on different physico-chemical principles with similar sensitivities,[20] CE can be very suitable for cross-validation of GC or HPLC. In the past few years, CE and especially micellar electrokinetic chromatography (MEKC) have gained interest for the analysis of illicit drugs. Numerous papers have described methodologies that exemplify increased resolving power and shorter analysis times compared with conventional methods.[19–32] Interfacing with MS offers further specificity and sensitivity. CE–MS analysis of illicit drugs has the potential to become an indispensable tool for the forensic scientist.[33]

We have recently developed an electrospray time-of-flight mass spectrometer (ESI-TOFMS) for use as a detector for fast and efficient liquid phase separations. The main features of this instrument are speed and sensitivity. Low attomole detection limits have been achieved with continuous infusion experiments, and the acquisition rate can be as high as 10 000 spectra per second.[34]

This paper describes the analysis of various mixtures of illicit drugs using CE–TOFMS. Seized drug samples were analyzed either by continuous infusion or CE–TOFMS. Using CE–TOFMS, low femtomole detection was achieved, even in the presence of non-volatile buffers.

Experimental

Reagents

Standard mixtures were prepared using HPLC grade solvents. High purity methanol and water were purchased from Mallinckrodt (Chesterfield, MO, USA). Anhydrous (99.5%) citric acid glacial acetic acid, and ammonia solution (28–30% ammonia) were obtained from EM Science (Gibbstown, NJ, USA). Tetracaine hydrochloride, procaine hydrochloride, methamphetamine hydrochloride and cocaine hydrochloride were purchased from Sigma (St. Louis, MO, USA) and amphetamine hydrochloride from Alltech (Deerfield, IL, USA). We were not able to obtain a heroin standard; however, a seized heroin sample contained enough material (about 30% m/m) to allow positive identification.

Instrumentation

Capillary electrophoresis

A Crystal CE 300 system (ATI, Madison, WI, USA) and an Applied Biosystems (Foster City, CA, USA) Model 785A UV absorption detector were used. Uncoated fused silica (50 μm id and 190 μm od) was used to perform the separations (Polymicro Technologies, Phoenix, AZ, USA). Prior to analysis, the capillaries were conditioned by rinsing for 10–15 min with sodium hydroxide solution (1 M), followed by HPLC grade water (10–15 min). Between analyses the capillaries were rinsed with running buffer for 5 min. The CE analysis of seized samples necessitated additional rinsing with sodium hydroxide solution (0.1 M) between runs in order to prevent the deterioration of the fused silica inside wall. The basic solution treatment was followed by water and buffer rinses.

Samples were prepared by dissolving the appropriate amount of compound in CH_3OH or $CH_3OH–CH_3COOH$ (100 + 0.1 v/v). Seized sample solutions (0.04 mg ml^{-1}) were filtered through Acrodisc PF 0.2 μm filters (Gelman Sciences, Ann Arbor, MI, USA).

Time-of-flight mass spectrometry

An in-house built ESI-TOFMS with orthogonal extraction was used as a detector. Using this system, the analytes from CE are brought from the liquid phase into the gas phase with the aid of an ESI source. A countercurrent heated curtain gas, fed between the sampling nozzle and a focusing lens (interface plate) placed in front of the ESI needle, is used to dry the electrosprayed droplets. Sampling of the ions from the source is accomplished using a nozzle–skimmer arrangement. The ion beam is preserved and focused by two sets of radiofrequency (rf)-only quadrupole ion optics before the pulsing region. The mass spectrometer was operated in a linear mode (non-reflecting) with detection occurring at the end of the flight tube.

Both electrospray configurations, microspray and liquid sheath, were utilized. Microspray tips were prepared by pulling 50 μm id × 190 μm od fused silica capillaries to 10–15 μm id × 40–60 μm od, which promoted the onset of electrospray at relatively low voltages (1600–1800 V). The ESI voltage was applied through a metal union placed 1–1.5 cm away from the microspray tip. For CE separations, the liquid sheath ESI source was used. The liquid sheath contained $CH_3OH–H_2O–CH_3COOH$ (80 + 20 + 0.1 v/v) supplied through a stainless steel needle (gauge 26), which had an id which closely matched the od of the fused silica capillary. Detailed descriptions of the TOFMS and the ESI source were given in previous papers.[34,35]

Continuous infusion of analytes was performed using a Harvard (South Natick, MA, USA) Model 22 syringe pump. The analyte solutions, the CE buffer and the sheath liquid were de-gassed by sonication for 5 min.

Results and discussion

Most drugs of abuse are relatively small molecules which contain basic amino functional groups that can be easily protonated in acidic aqueous solutions. If there is sufficient difference between their electrophoretic mobilities, efficient and rapid CE separations can easily be achieved. Several publications have reported the CE separation of similar drug mixtures using various buffer systems: phosphate or citrate at low pH (2.35), or borate, borate–sodium dodecyl sulfate (SDS), phosphate–borate–SDS and cyclodextrin–SDS (the last three used for MEKC) at high pH (8–9). The composition of the CE eluent can significantly alter the ionization efficiency of the ESI source. The coaxial liquid sheath flow, supplied at the CE capillary terminus, which has the combined purpose of completing the CE circuit and ensuring the ESI voltage, dilutes the CE eluent and allows it to be electrosprayed. It is especially difficult to deal with MEKC buffers in which the high concentration surfactant can result in complete analyte signal suppression. In CE, even though at low concentration, volatile buffer systems such as ammonium acetate or formate (for acidic pH conditions) are recommended; non-volatile buffer systems used at low concentrations can be electrosprayed if needed at the expense of some loss of ionization efficiency.

We performed the separation of a mixture of amphetamine (A), methamphetamine (MA), cocaine (C) and heroin (H) in 25 mM citrate buffer. The pH of the citric acid solution was adjusted to 3 with ammonia solution. The eluent flow rate was approximately 150 nl min^{-1} in the presence of a 10 mbar pressure applied to the CE inlet vial. For the 88 cm separation capillary used, this inlet pressure usually decreased the analysis time by 10–13%. The CE–UV and CE–TOF separations of this mixture are shown in Fig. 1. UV detection was applied at 210 nm, 70 cm from the inlet end. TOF detection occurred at the end of the column (90 cm from the inlet), which explains the greater migration time for the CE–TOF separation. The relative peak intensities in the two electropherograms differ for two main reasons. One is the different responses of the two detectors for the analytes. The second is that amphetamine and methamphetamine break down in the ion source as a result of the nozzle voltage setting, and the nozzle voltage had to be maintained at a lower value (lower acceleration voltage between the nozzle and skimmer), which did not produce the maximum signal intensity for all of the compounds. For later eluting components, the nozzle voltage was changed to the optimum value. These changes in nozzle voltage had to be performed with great care in order to not disturb the stability of the electrospray.

The liquid sheath was used at a flow rate of 1 μl min^{-1}. With careful adjustment of the positions of the CE capillary inside the ESI needle and the ESI needle in the ion source, a stable spray was produced even in the presence of citrate buffer. The ion source was maintained at 90 °C and the drying gas was set at a flow rate of 1200 ml min^{-1}. The CE separation efficiency was not affected by the ESI source, but was preserved at its original value of between 200 000–300 000 plates. The inconvenience of using the citrate buffer was that it produced a noisy background.

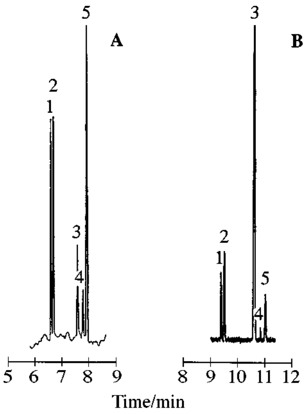

Fig. 1 CE–UV and CE-TOFMS electropherograms of drugs of abuse. Conditions: 85 cm × 50 μm id uncoated fused silica capillary, 25 mM citrate buffer (pH 3), 30 mbar × 0.2 min injection (~650–810 fmol). A, CE–UV: 27 kV, 10 mbar, 8.9 μA, 210 nm at 70 cm; B, CE-TOFMS: 30 kV, 10 mbar, 7.8 μA, 1 μl min^{-1} $CH_3OH–H_2O–CH_3COOH$ (80 + 20 + 0.1 v/v) liquid sheath; ESI (3000 V), 90 °C; MS data acquisition at 5000 Hz, 1000 spectra averaged, 5 data points s^{-1}. Peak identifications: 1, amphetamine; 2, methamphetamine; 3, cocaine; 4, impurity; 5, heroin.

A series of strong citric acid clusters (an intense ammonium citrate ion followed by its citric acid clusters) were present at *m*/*z* < 1200 (Fig. 2). A background spectrum showing the ions present during the elution of amphetamine and methamphetamine [Fig. 2(A)] was acquired at a nozzle–skimmer voltage difference of 22 V, whereas a different background spectrum [Fig. 2(B)], which was acquired at a nozzle–skimmer voltage difference of 52 V, was obtained during the elution of the later components. The flight time region in the spectrum between 16 and 24 µs became crowded with solvent–buffer cluster ions at the higher voltage; however, these ions did not overlap with the higher *m*/*z* ions. At greater liquid sheath flow rates (2–3 µl min^{-1}), the intensities of the citrate clusters decreased significantly, but the analyte signals also diminished.

Owing to the negative effects of the citrate buffer on ionization efficiency, a larger sample (650–810 fmol) was initially injected into the CE column. A similar mixture containing two additional compounds, procaine (P) and tetracaine (T), was analyzed using CE–TOFMS at two injection levels, one at 610–850 fmol [Fig. 3(A)] and the other much lower (110–135 fmol) [Fig. 3(B)]. Intense peaks were still observable. It was estimated from observing the background noise and signal intensities for the 110–135 fmol injection that detection limits of approximately 30–50 fmol in the presence of citrate buffer would be obtained (S/N = 3). All injections were performed by the hydrodynamic technique by applying the necessary pressure to the inlet vial. The TOF mass spectra for all of these analytes displayed the protonated molecular ion along with the background citrate clusters (Fig. 4).

Attomole, or more commonly, low femtomole (*i.e.*, < 10 fmol) detection limits are about the best that have been reported for CE–MS; however, most of these reported separations were performed with volatile buffer systems that permit the use of a microspray source, which leads to increased ionization efficiency. In the presence of a liquid sheath, depending on the operating conditions, the signal may decrease 10–100-fold. CE displays excellent mass detection limits; however, its poor concentration detection limits are a result of the very small injection volume required when using small CE columns (*i.e.*, < 10 nl for 50 µm id capillaries). With proper preconcentration techniques, such as membrane preconcentration, transient isotachophoresis (ITP), stacking or field amplified injection, concentration detection limits can be improved 100–1000-fold.

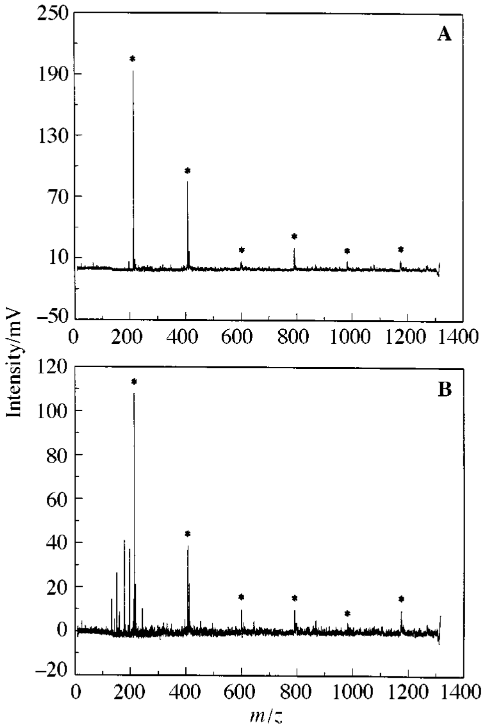

Fig. 2 TOFMS background spectra at two nozzle–skimmer voltage differences, ΔV = (A) 22 and (B) 52 V. Conditions as in Fig. 1. Asterisks indicate citrate clusters.

Selected-ion traces for CE–TOFMS analysis of drugs of abuse were obtained by integrating ion intensities within narrow *m*/*z* ranges that corresponded to the protonated molecular ions of the analytes (*i.e.*, *m*/*z* AH$^+$ 136, MAH$^+$ 150, PH$^+$ 237, TH$^+$ 265, CH$^+$ 304, HH$^+$ 370). Spectra were recorded at the rate of 5 Hz and data collection usually started approximately 30 s prior to the elution of analytes. This procedure allowed for a significant reduction in the amount of stored data and in the time required for data work-up. It is very important that data be acquired at a high enough speed to preserve the separation efficiency. A minimum of 10–15 data points should be acquired across the peak to allow precise quantification. Since each data point corresponds to a full mass spectrum, it is this instant where the ability of the mass spectrometer to produce the spectrum in a short enough time is seriously challenged.

Seized samples could easily be analyzed by continuous infusion using the microelectrospray, since the main objective in such analyses is to identify the drug present in the confiscated material. The analysis time depended only on the time needed to dissolve and filter the sample, and it was very fast, taking about 5 min. Fig. 5 shows TOF mass spectra for a standard cocaine sample and for three seized samples: cocaine, heroin and a mixture of amphetamine and methamphetamine. The spectra of the seized samples displayed mainly the protonated molecular ion of the drug. Weak signals corresponding to polymeric contaminants were also present in each of the spectra. Other components, eventually present, did not give a measurable signal in the positive ion electrospray mode. The seized sample containing a mixture of amphetamine and methamphetamine was also analyzed using CE–TOFMS (Fig. 6) under conditions identical with those used for the previously described separations. The total analysis time, including sample preparation, was 15 min.

The configuration of the commercial CE system did not permit the use of short capillaries, and a minimum length of 85 cm was necessary to achieve the interfacing through the liquid

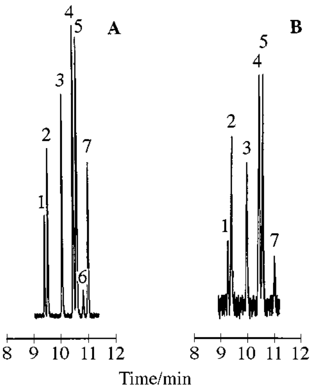

Fig. 3 CE–TOFMS electropherograms of drugs of abuse. Conditions: 85 cm × 50 µm id uncoated fused silica capillary, 25 mm citrate buffer (pH 3), 30 kV, 10 mbar, 7.8 µA; 1 µl min^{-1} CH_3OH–H_2O–CH_3COOH (80 + 20 + 0.1 v/v) liquid sheath; ESI (3000 V), 90 °C; MS data acquisition at 5000 Hz, 1000 spectra averaged, 5 data points s^{-1}. A, 30 mbar × 0.2 min injection (~650–810 fmol); B, 10 mbar × 0.1 min injection (~110–135 fmol). Peak identifications: 1, amphetamine; 2, methamphetamine; 3, procaine; 4, tetracaine; 5, cocaine; 6, impurity; 7, heroin.

sheath source. This resulted in relatively long migration times. Unfortunately, much shorter capillaries would be necessary to conduct separations in less than about 2 min.

For small molecules, ESI produces primarily the protonated molecular ion (in the positive ion mode), and the resultant ESI mass spectra are very simple and easy to interpret. The identification of the main components of an unknown sample can be achieved simply by direct infusion without prior separation of the individual components. On the other hand, for the investigation of complex mixtures, CE is an ideal technique to use prior to MS, since efficient separations can be achieved in a short time without extensive efforts at optimization.

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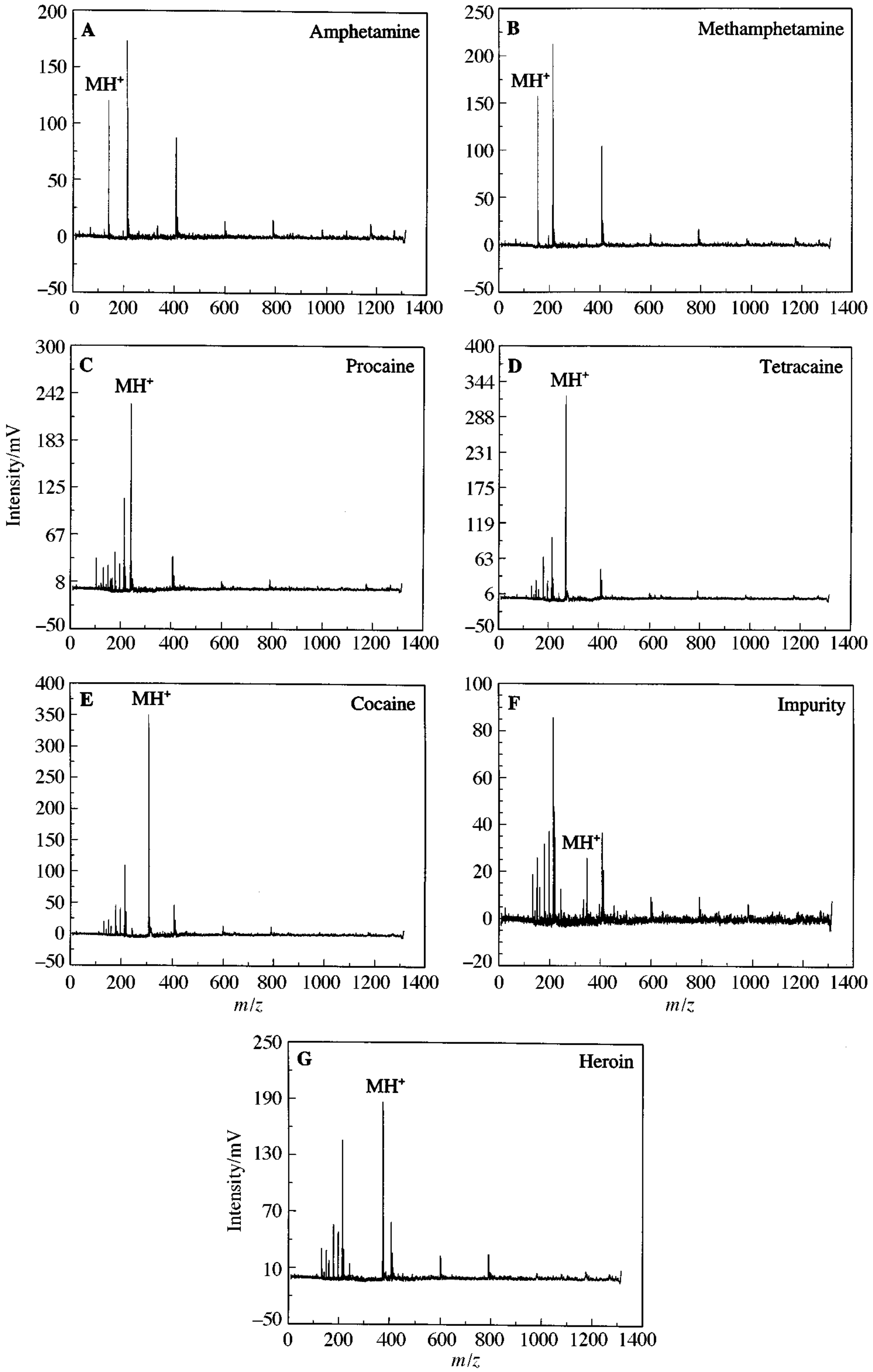

Fig. 4 TOF mass spectra of drugs of abuse (standard compounds) obtained from a CE run. Conditions as in Fig. 3. The labeled ions are the protonated molecular ions (MH$^+$) of the specific drug.

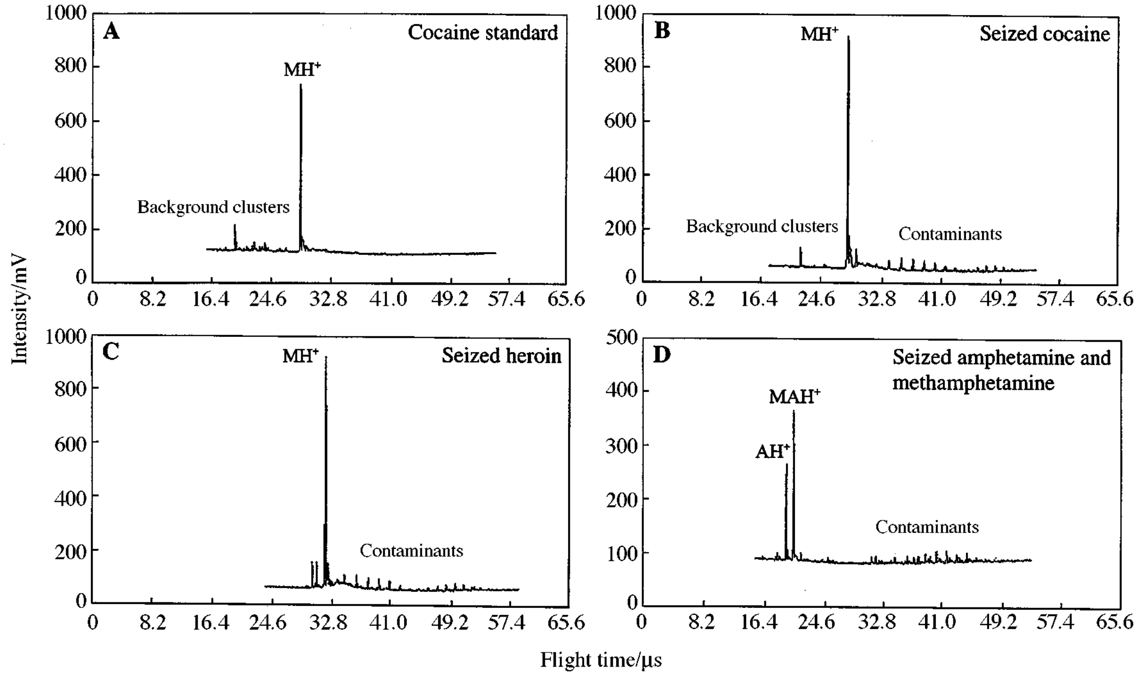

Fig. 5 TOF mass spectra of drugs of abuse (seized samples) from direct infusion. Conditions: continuous infusion (0.3 µl min^{-1}); ESI (1850 V), 80 °C; MS data acquisition at 5000 Hz, 5,000 spectra averaged, 1 spectrum s^{-1}. A, Cocaine standard [22 µM in CH$_3$OH–CH$_3$COOH (100 + 0.1 v/v)]; B, seized cocaine (0.04 mg ml^{-1} in CH$_3$OH); C, seized heroin (conditions as in B); D, seized amphetamine and methamphetamine (conditions as in B). The labeled ions are protonated molecular ions (MH$^+$). The flight time range corresponds to 0–1545 *m/z*.

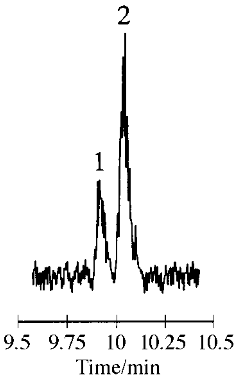

Fig. 6 CE–TOFMS electropherogram of a seized sample containing amphetamine and methamphetamine. Conditions: 85 cm × 50 µm id uncoated fused silica capillary, 25 mM citrate buffer (pH 3), 30 kV, 10 mbar, 7.8 µA, 30 mbar × 0.2 min injection (0.04 mg ml^{-1} in CH$_3$OH); 1 µl min^{-1} CH$_3$OH–H$_2$O–CH$_3$COOH (80 + 20 + 0.1 v/v) liquid sheath; ESI (3000 V), 90 °C; MS data acquisition at 5000 Hz, 1,000 spectra averaged, 5 data points s^{-1}. Peak identifications: 1, amphetamine; 2, methamphetamine.

824316-01-0) to Brigham Young University. This paper has been subjected to the EPA's peer review and has been approved as an EPA publication. Mention of trade names or commercial products does not constitute endorsement or recommendation by EPA for use.

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