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Sample-stacking techniques in non-aqueous capillary electrophoresis

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Abstract

In sample-stacking techniques, the detection limit cannot be improved by simply increasing the length of the sample solution, because the individual electrophoretic parameters must be optimized. In an attempt to increase the amount of sample injected, as well as to focus them onto a small zone, two novel methods are proposed. One of these employs an "ultra-high conductivity zone", which was inserted between the sample zone and background solution to build an unequal conductivity gradient. The other employs a "low temperature bath". A portion of the capillary (near the junction between the sample solution and the background solution) was immersed in a low temperature bath, which served as a "pseudo-high-conductivity zone" due to the fact that conductivity would increases when the temperature is decreased. As a result, a large volume of sample injection can be achieved. Using 3,4-methylenedioxymethamphetamine as a model compound, the detection limit was determined to be 1.6×10^{-6} M (S/N = 3) by means of normal non-aqueous capillary electrophoresis (NACE). This could be improved to 3.0×10^{-8} M, 4.8×10^{-9} M and 5.0×10^{-9} M, respectively, when the normal stacking, ultra-high conductivity zone NACE-stacking and the low-temperature zone NACE-stacking methods were applied.

Keywords: Non-aqueous capillary electrophoresis; Stacking; 3,4-Methylenedioxymethamphetamine

1. Introduction

Non-aqueous capillary electrophoresis (NACE) has rapidly grown in popularity and importance over the past few years [1–8]. There are several advantages of NACE including a short analysis time, high separation efficiency, better solubility and stability of some compounds in organic solvents than in water, and its ease of interfacing with mass spectrometry (NACE–MS) [9–12]. Furthermore, the detection limits can be improved due to the fact that many compounds have a higher fluorescence quantum yield in an organic solvent [13,14]. This method also offers the possibility of achieving different selectivity, compared to aqueous CE, because varying the ratio of the organic solvents (for example, the ratio of methanol: acetonitrile, etc.) alters the separation order. In addition to the above advantages, NACE, because of its possible use at lower temperatures then can be tolerated by CE and, separations can be readily performed at subzero temperatures [15–20]. However, only few reports have described the combination of NACE and on-line sample concentration techniques [21,22]. The so called "stacking", "pH-junction" and "sweeping" techniques [23–34], have rapidly grown in popularity over the past few years; these techniques have successes for many practical applications. Indeed, most of these techniques were developed to accommodate a large volume injection, since the limit of detection is proportional to the injected sample zone; a conventional CE separation provides only a low detection limit due to the fact that a short plug of sample is injected into the capillary. Unfortunately, an increase in detection limit cannot be achieved by simply increasing the injection time (in the case of electrokinetic injection) or the length of the sample plug, because the analytes must "focused" onto a small zone. For this purpose, individual electrophoretic parameters such as the injection length required for the separation, the concentration of surfactant used, buffer conductivity and even the pH value must be optimized.

In this study, we report on two novel methods, ultra-high conductivity zone NACE-stacking and a low-

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temperature-zone NACE-stacking method, compared to a normal non-aqueous capillary electrophoresis-stacking (NACE-stacking), in an attempt to increase the amount of sample injected, as well as to focus them onto a small zone. Using 3,4-methylenedioxymethamphetamine (MDMA) as a model compound, several electrophoretic parameters such as temperature, conductivity of the non-aqueous buffer, and the injection length required for the separation were optimized and these data are reported herein.

2. Experimental

2.1. Apparatus

The CE set-up was fabricated in-house and is identical to that described previously [21]. Briefly, the low temperature bath used was an insulated container, the temperature of which could be controlled via mixtures of ice/rock salt. A high-voltage power supply (Model RR30–2R, 0–30 kV, 0–2 mA, reversible, Gamma, Ormond Beach, FL, USA) was used to drive the electrophoresis and a 75 μm I.D. fused silica capillary (J&W Scientific, Folsom, CA, USA) was used for the separation. Hydrodynamic injection was achieved by raising the reservoir 30/65 cm relative to the exit reservoir (at this height, the flow rate for the sample injection was ~0.058/0.132 cm/s) to provide the injection length (depending on the specific situation). The excitation source was selected by a monochromator (ARC, Acton Research Corp., Acton, MA, USA; Model SP-150) connected to a Xe lamp (Müller Elektronik Optik, Moosinning, Germany; SVX/LAX 1450). Fluorescence data were collected at a right angle to the light source and dispersed by a second monochromator, followed by detection by means of a photomultiplier tube (ARC Model P2-R928). The analog signal was converted to a digital signal by an A/D converter (ADAM-4012 module, Advantech, Taipei, Taiwan). Electropherograms were collected with a data acquisition system connected to a personal computer. A pH meter (PHM201, Radiometer, Copenhagen) and a conductivity meter (LF320, WTW, Weilheim, Germany) were used for measurement of the pH_{app} and the conductivity of non-aqueous solutions, respectively. A gas chromatograph (Hewlett-Packard 6890 GC; Palo Alto, CA, USA) equipped with a mass spectrometer (Hewlett-Packard 5973 mass-selective detector) and an autoinjector (Model 7683) was also used for comparison. The mass conditions were identical to that described previously [35,36].

2.2. Methodology

Fig. 1 shows schematic diagrams of NACE-stacking (A), ultra-high conductivity zone NACE-stacking (UHCZ/NACE-stacking) (B), and low-temperature-zone NACE-stacking (LTZ/NACE-stacking) methods (C), respectively.

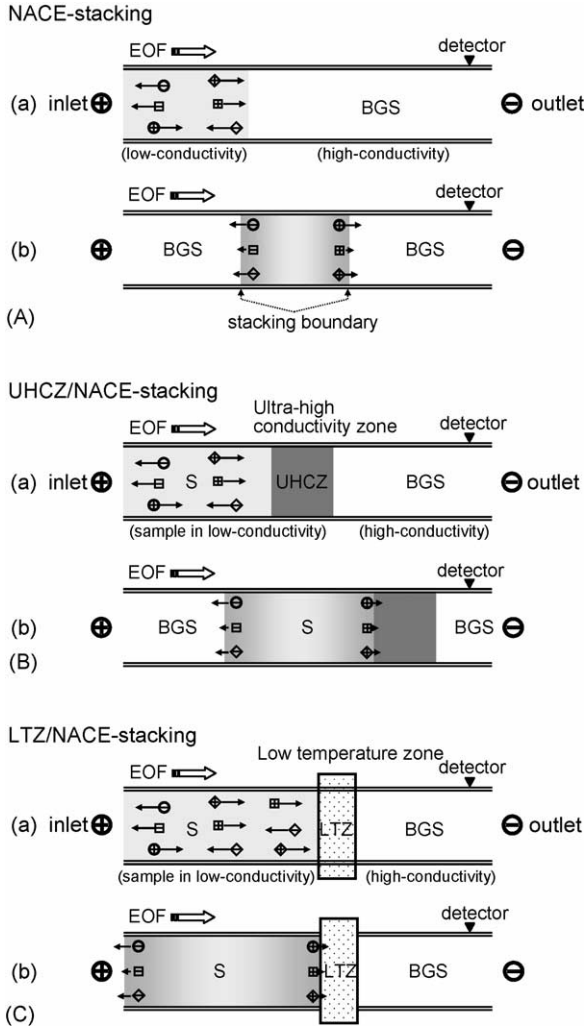

Fig. 1. Schematic diagram of NACE stacking (A), ultra-high conductivity zone NACE-stacking (UHCZ/NACE-stacking) (B) and low-temperature-zone NACE-stacking (LTZ/NACE-stacking) methods (C). BGS: Background solution; S: sample solution.

2.2.1. NACE-stacking

In NACE-stacking, the background solution (BGS) consisted of 50 mM ammonium acetate in methanol; the conductivity was 2.2 mS/cm. The sample was dissolved in a 1/100 diluted background solution resulting in a low conductivity (conductivity, 69.1 μS/cm) sample zone (S). After completion of the injection was completed, +25 kV was applied to power the CE separation. This procedure permits the MDMA cation, which is moving rapidly in the sample zone (low conductivity), to then slow down at the junction between the sample zone and the background solution (high conductivity). As a result, the sample becomes concentrated at the boundary.

2.2.2. UHCZ/NACE-stacking

In this mode, an ultra-high conductivity zone (UHCZ) was inserted between the sample zone and background solution to build a conductivity gradient [37]. Meanwhile, the sample was stacked along the capillary axis, to then reduce speed and

to permit the sample to accumulate near the junction because of the sudden increase in conductivity. This method permits a higher efficiency for sample-stacking compared to a normal NACE-stacking method.

2.2.3. LTZ/NACE-stacking

In the LTZ/NACE-stacking mode, a portion of the capillary (near the junction between the sample solution and the background solution) was immersed in the low temperature bath, which served as a "pseudo-high-conductivity zone" due to the fact that the conductivity would increases when the temperature is decreased. This is another method to build a conductivity gradient without inserting an ultra-high conductivity zone between the sample zone and background solution. As a result, when stacking was applied, the sample stacked along the capillary axis, becoming almost immobilized near the junction because of the sudden decrease in temperature. This method also permits a longer sample injection.

2.3. Reagents

3,4-Methylenedioxymethamphetamine (MDMA) was purchased from Radian International (Catalog No. M-013, 99%; 1 mg/1 mL methanol). 3,4-MDA (3,4-methylenedioxyamphetamine), DMMDA (*N*,*N*-dimethyl-3,4-methylenedioxyamphetamine) were synthesized and generously donated by the Forensic Science Center (Command of the Army Force of Military Police, Department of Defense, Taipei, Taiwan). Methanol (99.8%) was obtained from Fisher Scientific (Fair Lawn, NJ, USA). Ammonium acetate (CH_3COONH_4) was obtained from Riedel-de Haen (RdH Laborchemikalien GmbH&Co. KG, Seelze, Germany).

3. Results and discussion

3.1. NACE-stacking modes

Fig. 2 shows typical fluorescence ($\lambda_{ex}/\lambda_{em}$ = 280/320 nm) CE electropherograms of a MDMA standard when the NACE (A), NACE-stacking (B), UHCZ/NACE-stacking (C) and LTZ/NACE-stacking (D) modes were used, respectively. In the cases of NACE (A) and NACE-stacking (B) modes, the total length of the capillaries were 100 cm (94 cm to detector); the applied voltages were +25 kV and the currents were ~40 and ~2 μA, respectively. The complete, optimal experimental conditions for MDMA were achieved using a non-aqueous (methanol only) ammonium acetate (50 mM) buffer. Herein,

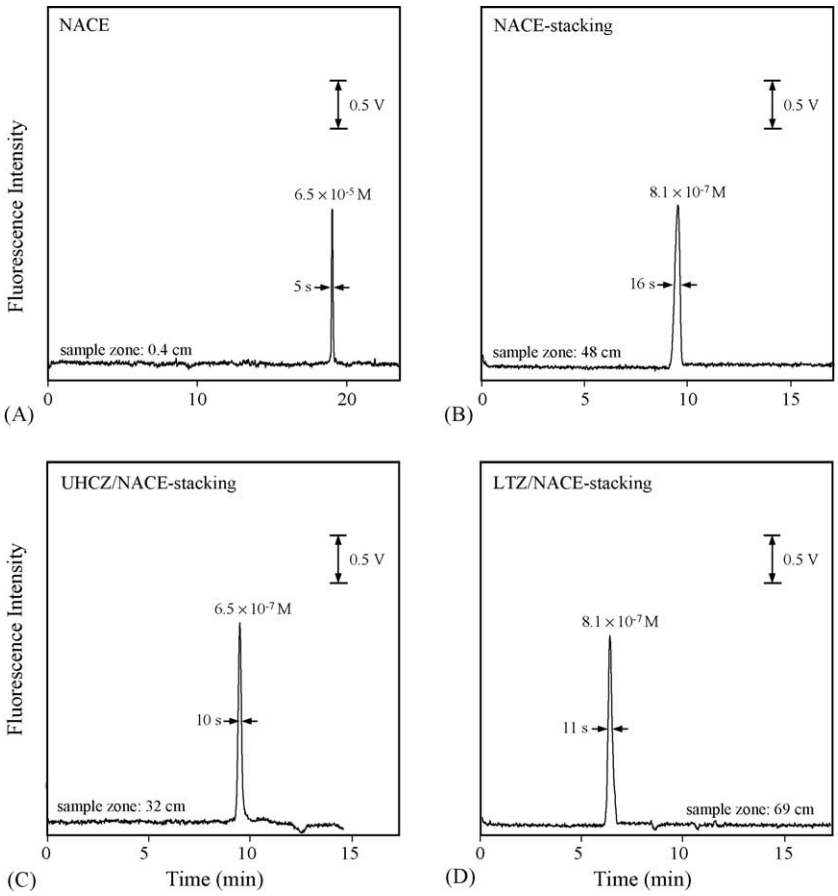

Fig. 2. Frames A–D, CE electropherograms of MDMA standard obtained by the normal NACE (A), NACE-stacking (B), UHCZ/NACE-stacking (C) and LTZ/NACE-stacking (D) modes, respectively.

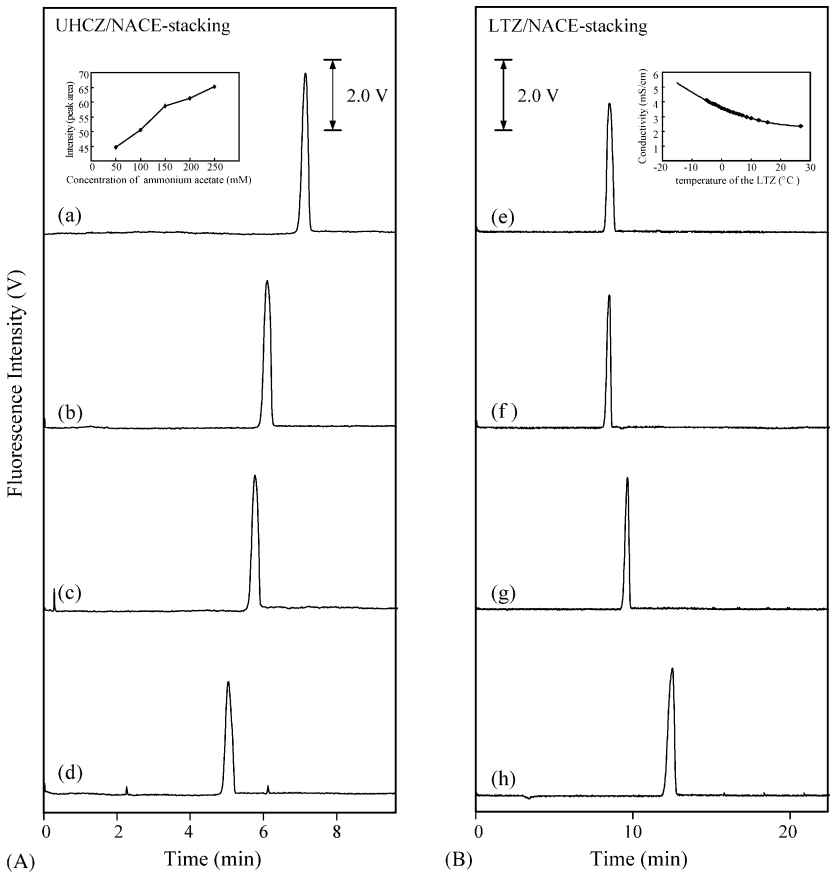

Fig. 3. (A and B) CE electropherograms of MDMA obtained by the UHCZ/NACE-stacking (C) and LTZ/NACE-stacking modes, respectively. (A) Electropherograms a–d: concentrations of ammonium acetate in the UHCZ, 250, 200, 150 and 100 mM. Inset in (A), relationship between the concentrations of UHCZ used and the corresponding intensity. (B) Electropherograms e–h: various low temperature column lengths, 2, 4, 8 and 24 cm, respectively. Inset in (B), the relationship of the LTZ temperatures and the corresponding conductivities.

in the former case (NACE mode, Fig. 2A) the test concentration and the sample injection length were 6.5×10^{-5} M and 0.4 cm, respectively. However, when the NACE-stacking (Fig. 2B) mode is used, the test concentration can be lowered to 8.1×10^{-7} M since the column injected length of the sample is longer, up to 48 cm. In comparison with the two electropherograms in frames A and B, a 120-fold sample was injected and an 80-fold improvement in detection sensitivity was obtained. It was found that when a large sample was injected, peak broadening occurred (NACE, 5 s and NACE-stacking, 16 s in peak width) and it became obvious that the sample could not be "focused" completely. This is a typical occurrence when the sample injection becomes longer.

To overcome this drawback (peak broadening when using stacking mode), two novel methods are proposed. In Fig. 2C, the electropherogram shows the result obtained using the UHCZ/NACE-stacking method. The experimental conditions were identical with those used in the NACE-stacking mode, but an ultra-high conductivity zone (ammonium acetate methanol buffer, 200 mM; conductivity, 6.7 mS/cm) was inserted between the sample zone and the background solution. The lengths of the sample zone (S), ultra-high conductivity zone (UHCZ) and the background solution (BGS) were 32, 40 and 22 cm, respectively. After completion of the injections, upon application of a high positive voltage, a proportionally greater electric field develops across the sample zone (S) causing the ions migrate more rapidly in the initial step. Once the ionic analytes reach the boundaries between the sample zone and the UHCZ, the electric field strength suddenly decreases and the migration becomes slower, causing the sample analytes to be focused near the boundaries. When the ions enter the BGS zone, the electric field strength then increases again and the migration becomes faster in the subsequent separation. Since the mobility of EOF is greater than that of the electrophoretic mobility of the charged analytes, all of the analytes will finally move toward the detection window (cations migrate faster than the anions), while the analytes are separated by the CZE mode. In this method, a larger sample injection volume can be used, compared to that for the traditional stacking method. As shown in Fig. 2C, even when the sample injection is 32 cm in length, the peak width is only 10 s. In Fig. 2D, the electropherogram shows the result obtained when the LTZ/NACE-stacking mode was used. Herein, instead of the role of UHCZ, a low temperature zone (LTZ) was used since the conductivity would be expected to increase when the temperature decreased. Fur-

thermore, the viscosity also increased. All of these result in a slower speed of migrating and aid in maintaining a sharp peak. An 8 cm in length of the capillary was immersed in a low temperature bath (−15 °C in this case). The lengths of S, LTZ and BGS were 69, 8 and 17 cm, respectively. As shown in Fig. 2D, even when the sample injection is 69 cm in length, the peak width is only 11 s. It is obvious that the sample could be injected for a longer for a period of time than for a regular stacking method and this provided a nonlinear improvement in sensitivity and the separation efficiency was also improved.

In order to investigate the appropriate concentration of UHCZ, different concentrations were selected for the following experiment; the test concentration of MDMA was 1.3×10^{-6} M. In Fig. 3A, electropherograms a–d show the results obtained from different test concentrations of UHCZ (a–d: ammonium acetate concentration, 250, 200, 150 and 100 mM; conductivity, 7.4, 6.7, 5.7, and 4.0 mS/cm). The findings show that a higher concentration provides a better detected sensitivity, as shown in the inset of Fig. 3A. In order to examine the appropriate length of the LTZ, various low temperature column lengths (2, 4, 8 and 24 cm; in Fig. 3B, electropherograms e–h) were selected for the following test. The temperature of the LTZ was −15 °C; the relationship of the LTZ temperatures and the corresponding conductivities are shown in the inset of Fig. 3B. When the temperature of LTZ was decreased to −15 °C, the calculated conductivity was ∼5.3 mS/cm. Although a LTZ plays a "pseudo-high-conductivity zone", a longer LTZ failed to improve the sensitivity further; a further colder (<−15 °C) LTZ may be necessary to create a higher conductivity zone. As a result, a length of 4–8 cm for LTZ is sufficient and this length was used in the following experiments. In order to investigate the effects of sample injection length and the corresponding signal intensity when the NACE-stacking, the UHCZ/NACE-stacking and LTZ/NACE-stacking modes were applied, respectively (Fig. 4). Under exactly the same experimental conditions, various series of column lengths were selected for comparison. Using the optimal conditions, Table 1 summarizes these results as well as the calibration curve, coefficient of correlation, detection range, limit of detection (LOD) values (at a 92.1% confidence level) for the MDMA test compound by the normal NACE, NACE-stacking, UHCZ/NACE-stacking and LTZ/NACE-stacking modes, respectively, for the above experiments.

3.2. Application

Most clandestine tablets contained multi-components, including methamphetamine, MDMA, ketamine, as well as other so-called "designer drugs". Such a complicated tablet, a mixture of multi-components, may stimulate unexpectable effects, such as hallucinogenic effects and experiences impaired perception, and, of course, such combinations are potentially dangerous. Ketamine is a non-barbiturate, rapid-acting disassociative anesthetic that is being abused by an increasing number of young people as a "club drug", and is often distributed at "raves" and parties. Although ketamine is legal, it is available by prescription for medical uses. In some case, it is marketed as Ketalar, or Ketaset, to veterinarians and medical personnel and is considered a controlled substance. However, some illicit drugs contain not only ketamine but also cocaine, methamphetamine, MDMA as well

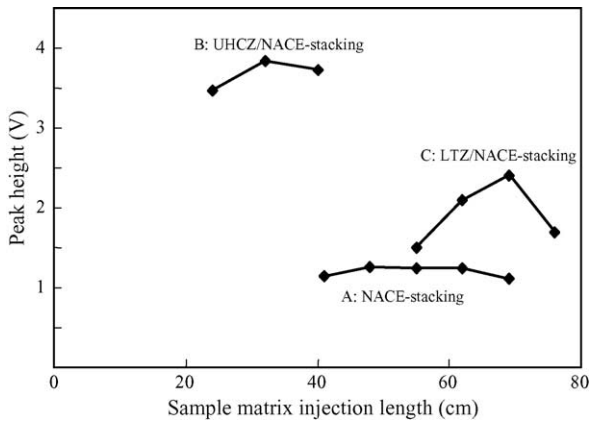

Fig. 4. The relationship between the length of sample matrix injection and the corresponding detected intensity by NACE-stacking (A), UHCZ/NACE-stacking (B) and LTZ/NACE-stacking (C) modes, respectively. 3,4-MDMA test concentration: 1.6×10^{-6} M; total length/effective length of the capillary: 100/94 cm.

Table 1
Sample injected length, calibration curve, coefficient of correlation and limit of detection (LOD) values (S/N = 3) for MDMA for NACE, NACE-stacking, UHCZ/NACE-stacking and LTZ/NACE-stacking methods

(A) NACZE	
Sample injected length	0.4 cm
Equation of the line	$y = 1.68 \times 10^5 x - 0.431$
Coefficient of correlation	$R^2 = 0.9993$
Detection range	1.3×10^{-4} to 1.3×10^{-5} M
LOD	1.6×10^{-6} M
(B) NACZE-stacking	
Sample injected length	48 cm
Equation of the line	$y = 3.55 \times 10^7 x + 0.310$
Coefficient of correlation	$R^2 = 1.0000$
Detection range	1.6×10^{-6} to 2.0×10^{-7} M
LOD	3.0×10^{-8} M
(C) UHCZ/NACE-stacking	
Sample injected length	32 cm
Equation of the line	$y = 2 \times 10^7 x + 3.491$
Coefficient of correlation	$R^2 = 0.9956$
Detection range	2.6×10^{-6} to 5.1×10^{-8} M
LOD	4.8×10^{-9} M
(D) LTZ/NACZE-stacking	
Sample injected length	69 cm
Equation of the line	$y = 3.17 \times 10^7 x - 0.128$
Coefficient of correlation	$R^2 = 0.9999$
Detection range	8.1×10^{-7} to 8.1×10^{-8} M
LOD	5.0×10^{-9} M

Light source: Xe lamp (total ∼6 W); λ_{ex} = 285 nm; λ_{em} = 320 nm. Capillary: total length/effective length = 100/94 cm.

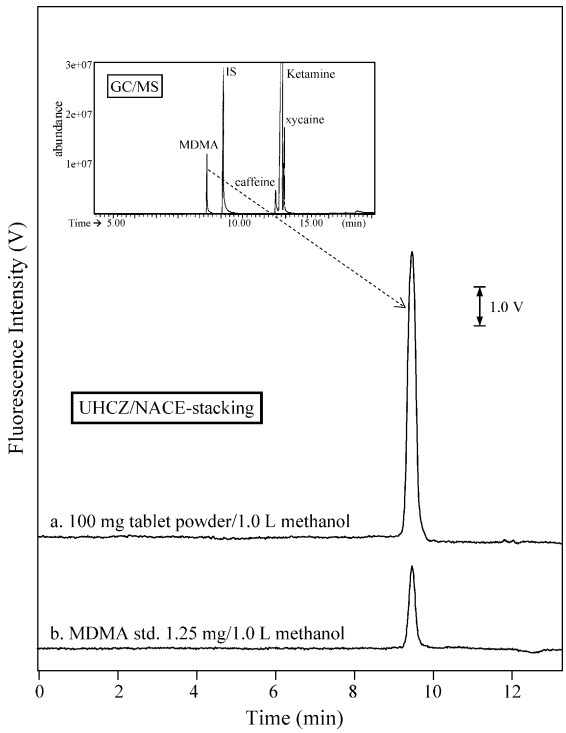

Fig. 5. Electropherograms a and b, typical CE electropherograms of the test sample (100 mg powder in 1.0 L methanol) and the MDMA standard (0.125 mg in 1.0 L methanol), respectively, by applying the UHCZ/NACE-stacking mode. The inset shows the ion chromatogram of the seized tablet, recorded using the total ion current (TIC) mode.

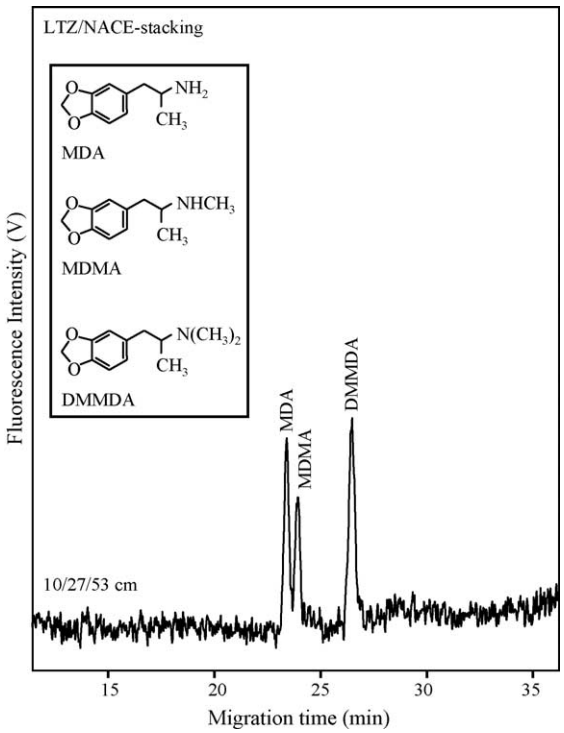

Fig. 6. Separation of a mixture of MDMA and related compounds (MDA, 3,4-methylenedioxyamphetamine; DMMDA, *N*,*N*-dimethyl-3,4-methylenedioxyamphetamine and MBDB, *N*-methyl-1-(1,3-benzodioxol-5-yl)-2-butylamine), using the LTZ/NACE-stacking mode. The test concentration of MDMA was 0.1 ppm. The sample injection length was 10 cm; the temperature of the LTZ was −4 °C.

as other of abused drugs. We selected one of the clandestine tablets, which was seized from the illicit market during 2001, for the analysis and assay of MDMA by the UHCZ/NACE-stacking mode. In Fig. 5, the electropherogram a shows a typical CE electropherogram of the test sample (100 mg powder in 1 L methanol). In order to examine this peak, we compared it to a MDMA standard (0.125 mg in 1 L methanol) under the same conditions, as shown in the electropherogram b. We assigned this peak as MDMA and by comparison with the standards the content of MDMA in the seized tablet was 0.5%. The inset shows the ion chromatogram of the seized tablet, recorded in the total ion current (TIC) mode, the peak having a migration time of 9.39 min was assigned as MDMA based on its mass spectrum (data not shown). The data also verify the actual presence of MDMA. Fig. 6 shows a typical CE electropherogram of a model mixture (MDMA, MDA and DMMDA; molecular structures are shown in the inset), when the LTZ/NACE-stacking mode was used. The CE buffer was the same as described in Fig. 2D. The test concentration of MDMA was 0.1 ppm; the sample injection length was 10 cm; the temperature of LTZ was −4 °C. The order of migration was: MDA (M_r 179.22) < MDMA (M_r 193.24) < DMMDA (M_r 207.27); basically the compounds migrated in the order of mass per charge. Thus, we conclude that the LTZ/NACE-stacking method is not only useful for a longer sample injection (normal injection, ∼1 mm; LTZ/NACE-stacking, 100 mm in this case), but would also be useful in normal CE separations. Furthermore, the location of the "low-temperature bath" can be easily moved to an ideal position to determine whether a longer capillary column is needed for on-line preconcentration strategies or for the subsequent normal CE separation. This cannot be achieved using the UHCZ/NACE-stacking method, as described above.

4. Conclusions

The NACE-stacking, UHCZ/NACE-stacking and LTZ/NACE-stacking modes, respectively, are demonstrated. The complete, optimal non-aqueous buffer used for the separation of MDMA was achieved using an ammonium acetate buffer (50 mM) in a methanol solution where the lengths of the sample zone (S), ultra-high conductivity zone (UHCZ, 200 mM ammonium acetate) and the background solution (BGS) were determined to be 32/40/22 cm. This was also used in the successful for the analysis of a seized tablet. When the UHCZ/NACE-stacking or LTZ/NACE-stacking modes were applied, a ∼300-fold improvement in detection sensitivity was obtained compared with the normal NACE method.

Acknowledgments

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