Talanta 103 (2013) 301–305

Contents lists available at SciVerse ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

On the use of capillary electrophoresis for the determination of inorganic anions and cations, and carbohydrates in residues collected after a simulated suicide bombing attack

Cédric Sarazin [a,b,c,d], Nathalie Delaunay [b,c,d,*], Christine Costanza [a], Véronique Eudes [a], Pierre Gareil [b,c,d]

[a] Laboratoire Central de la Préfecture de Police, 39 bis, rue de Dantzig, 75015 Paris, France
[b] Chimie ParisTech, Laboratory of Physicochemistry of Electrolytes, Colloids and Analytical Sciences (PECSA), 75005 Paris, France
[c] UPMC Univ Paris 06, 75005 Paris, France
[d] CNRS, UMR 7195, 75005 Paris, France

ARTICLE INFO

Article history:
Received 19 June 2012
Received in revised form
11 October 2012
Accepted 13 October 2012
Available online 23 October 2012

Keywords:
Capillary electrophoresis
Carbohydrates
Inorganic anions
Inorganic cations
Suicide bombing attack

ABSTRACT

In order to train scientist field investigators after terrorist attacks, the laboratory of the Prefecture de Police of Paris simulated a suicide bombing attack in a bus. After collection of the residues, analyses were carried out to determine the composition of the original explosive charge. This article focuses on the combined use, for the first time, of three new capillary electrophoresis methods for the determination of inorganic anions and cations, and carbohydrates in two representative extracts. Capillary electrophoresis appears as an effective tool to identify and quantify the compounds in real extracts and is fully complementary to chromatographic methods.

1. Introduction

Suicide bombing attacks in public transports (bus, train, subway, etc.) or in public areas are one of the main terrorist threats against western countries. In order to anticipate this kind of event, the Central Laboratory of the Prefecture de Police of Paris organized, in 2010, a practical exercise simulating a suicide bombing attack in a bus. This simulation, carried out in a military field, aimed at training scientist field investigators to better visualize a blast scene and to carry out first investigations and residue samplings more efficiently. The collected samples were next analyzed in laboratory, in order to determine the explosive charge composition.

For the forensic confirmation of the presence or the absence of some compounds in samples, two analytical methods, with orthogonal mechanisms, were required. Thus organic compounds were analyzed by HPLC/MS [1,2], thin layer chromatography (TLC) [3], and GC/MS [4,5]. Inorganic traces were analyzed by ion chromatography (IC) [6,7] and capillary electrophoresis (CE) [8]. New CE methods were recently developed by our group for the analyses of inorganic anions [9], cations [10], and a special formulation of both [11] in order to confirm IC results. A new CE method dedicated to carbohydrate analyses was also recently optimized [12–14]. It replaced the TLC method, and currently represents the new powerful analytical method for carbohydrate analyses in the Central Laboratory of the Prefecture de Police. Three of these new methods were involved all together for the first time for the study of residues collected after simulating a suicide bombing attack.

2. Materials and methods

2.1. Real sample preparation

In order to be as close as possible to a suicide bombing attack in a bus, a scene representing real life was created around a bus with the presence of stalls, pedestrians, and cyclists. Inside the bus, three killed butchery animals played the role of suicide bomber and passengers. The explosive charge, mainly composed of ammonium nitrate and icing sugar, was next activated. Important

Abbreviations: BGE, background electrolyte; 18-C-6, 18-crown-6-ether; DAD, diode array detector; IC, ion chromatography; CE, capillary electrophoresis; EOF, electroosmotic flow; HDMB, hexadimethrine bromide; LOD, limit of detection; PVS, polyvinylsulfonic acid sodium salt; SMIL, successive multiple ionic-polymer layers; TLC, thin layer chromatography; Tris, tris(hydroxymethyl)aminomethane

* Corresponding author at: Chimie ParisTech, PECSA, 11 rue Pierre et Marie Curie, 75231 Paris Cedex 05, France. Tel.: +33 1 55 42 63 75; fax: +33 1 44 27 67 50.
E-mail address: nathalie-delaunay@chimie-paristech.fr (N. Delaunay).

0039-9140/$ - see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.talanta.2012.10.048

damages were observed and investigations and sampling were next carried out.

The sampling method consisted of wiping water-moistened cotton swabs over residues. These cottons were beforehand purified by assisted solvent extraction using an ASE 200 instrument (Dionex, Voisins-le-Bretonneux, France), with one cycle of 5 min at 100 °C and 100 bar with water, and next with acetone. After sampling, they were next extracted in hot water in a sonication bath for 10 min. The obtained solutions were filtered through a 150 μm cellulose filter (Les Filtres Durieux, Marne-La-Vallée, France) and a 0.45 μm nylon syringe filter (Teknokroma, A.I.T. France, Houilles, France) just before analysis. In this report, the analysis of two representative extracts, collected directly in two different places inside the bus, named Extract ♯1 and Extract ♯2, are presented. Blank cotton swab extracts were regularly analyzed in lab and no analytes were detected [9,10].

2.2. Standards

Individual anionic standard solutions were prepared weekly by volumic dilution of sodium salts (Sigma-Aldrich, Saint-Quentin-Fallavier, France) in ultra-pure water delivered by a Direct-Q3 UV system (Millipore, Molsheim, France). A standard mixture of the 11 anions (chloride, nitrite, nitrate, thiosulfate, perchlorate, chlorate, thiocyanate, sulfate, carbonate, phosphate, and formate) was prepared daily (20 mg L^{-1} each in ultra-pure water).

All cationic standard samples were purchased from VWR (Fontenay-sous-Bois, France). Individual cation solutions in 3% HNO_3 were prepared weekly by volumetric dissolution in ultra-pure water. A standard mixture of the cations of interest (ammonium, potassium, monomethylammonium, calcium, sodium, magnesium, strontium, barium, and lithium) was prepared daily (15 mg L^{-1} each in ultra-pure water).

All carbohydrates used as standard samples (fructose, glucose, lactose, and sucrose) were purchased from VWR and naphthalenesulfonic acid used as internal standard was purchased from Sigma-Aldrich.

2.3. Electrolytes

All reagents used were of analytical reagent grade. The background electrolyte (BGE) for anion separations consisted of a mixture of chromium (VI) oxide (Fluka, Lyon, France), sodium chromate (Fluka), and tris(hydroxymethyl)aminomethane (Tris) (Sigma-Aldrich), and ethanol (VWR). For the cationic analysis, guanidine acetate used as chromophore was obtained from Sigma-Aldrich. Other components of the BGE were 18-crown-6-ether (18-C-6) (Sigma-Aldrich) and acetic acid (VWR). Finally, the BGE for the analysis of carbohydrates was composed of NaOH (Carlo Erba, Val-de-Reuil, France) and NaCl (Sigma-Aldrich). The reagents for electroosmotic flow (EOF) reversal and double layer coating of the capillary wall, hexadimethrine bromide (HDMB) and polyvinylsulfonic acid sodium salt (PVS), were purchased from Sigma-Aldrich.

2.4. Apparatus

CE analyses were carried out with a Beckman Coulter P/ACE MDQ system (Villepinte, France) equipped with a fixed wavelength UV detector (mercury lamp) at 254 nm for anion analyses, or with a diode array detector (DAD) set at 190 ± 4 nm (analysis wavelength) and 300 ± 40 nm (reference wavelength) for cation analyses, and 270 ± 10 nm (analysis wavelength) and 350 ± 40 nm (reference wavelength) for carbohydrate analyses. Instrument control and data acquisition were performed using the 32 Karat® software. A Dionex ICS-2000 (Voisins-Le-Bretonneux, France) ion chromatograph equipped with a suppressed conductivity detector (Dionex ASRS suppression and conductivity cell) was used for chromatographic anion analyses and a Dionex DX120 ion chromatograph equipped with a suppressed conductivity detector (Dionex CSRS-4 mm suppression and conductivity cell) was used for cation analyses. Instrument control and data acquisition were performed using the Chromeleon 6.8® software. A 25 μL loop was employed for the cation and anion injections.

2.5. Electrophoretic procedures

The three CE methods were previously developed, validated, and described (anions [9], cations [10], and carbohydrates [12–14]). Electrophoretic separations were performed using bare fused-silica capillaries purchased from Polymicro (Photonlines, Marly-Le-Roi, France). A detection window was created at 10 cm from the detection end. Before first use, capillaries were conditioned by successive percolations with 1 M NaOH for 3 min, 0.1 M NaOH for 3 min, and ultra-pure water for 3 min, each under 40 psi.

2.5.1. Anions

Electrophoretic separations were performed using 50 μm I.D. × 87 cm capillaries. After every 10 analysis, capillaries were rinsed with HDMB solution (2.5 g L^{-1} in ultra-pure water) under 40 psi for 3 min in order to keep EOF constant. Between each run, they were rinsed with the BGE, containing 25 mM CrO_3, 25 mM Na_2CrO_4, 100 mM Tris, and 6% (v/v) ethanol in ultra-pure water (aqueous pH 8.2), under 50 psi for 3 min. Injections were performed electrokinetically under −2 kV for 50 s. Separations were run at 15 °C under −30 kV.

2.5.2. Cations

Electrophoretic separations were performed using 75 μm I.D. × 80 cm capillaries. Capillaries were conditioned by successive flushes with HDMB solution (10 g L^{-1} in ultra-pure water), PVS solution (0.01% (w/w) in ultra-pure water), and finally BGE containing 15 mM guanidine acetate adjusted at pH 4.0 with acetic acid, and 3 mM 18-C-6 in ultra-pure water, each under 40 psi for 3 min (12 capillary volumes), except for PVS flushes which were under 20 psi for 5 min (10 capillary volumes) in order to have a better coating. Between each run, PVS layer was renewed, and followed by the percolation of BGE. Injections were performed hydrodynamically under 0.8 psi for 4 s (0.6% of the capillary volume). Separations were run at 20 °C under 30 kV.

2.5.3. Carbohydrates

Electrophoretic separations were performed using 50 μm I.D. × 60 cm capillaries. Capillaries were conditioned by successive flushes with HDMB solution (1 g L^{-1}) and BGE, containing 98 mM NaOH and 120 mM NaCl (pH 12.9) prepared in ultra-pure water, each under 40 psi for 3 min (12 capillary volumes). Between each run, HDMB layer was refreshed. Injections were performed hydrodynamically under 50 mbar for 5 s (0.75% of the capillary volume). Separations were run at 26.5 °C under −14 kV. BGE was changed between each run.

2.6. Chromatographic procedures

Procedures described in this part were previously optimized in the laboratory and conducted routinely (accreditation ISO/IEC 17025).

2.6.1. Anions

Chromatographic anion separations were performed at 30 °C with a Dionex AS19 column (250 × 4 mm) equipped with a

Dionex AG19 guard column (50 × 4 mm) coupled to a ASRS suppressor. Eluent gradients were generated using the Dionex ECG-KOH Elu GenII cartridge. The optimized eluent gradient was as follows: 0–2 min: 10 mM isocratic; 2–16 min: gradient from 10 to 25 mM; 16–26 min: gradient from 25 to 40 mM; 26–35 min: gradient from 40 to 45 mM; 35–40 min: 45 mM isocratic, with a flow-rate of 1 mL min^{-1}. Separations were monitored by suppressed conductivity detection at 35 °C with continuously regenerated cation trap column (Dionex CR-ATC) inserted after the separation column.

2.6.2. Cations

Chromatographic cation separations were performed at 32 °C with a Dionex CS12 column (250 × 4 mm) equipped with a Dionex CG12 guard column (50 × 4 mm) coupled to a CSRS-4 mm suppressor. Eluent gradients were generated using the Dionex ECG II-MSA cartridge, used as mobile phase. The optimized eluent gradient of the mobile phase was as follows: 0–2 min: 20 mM isocratic; 2–10 min: gradient from 20 to 35 mM; 10–25 min: 35 mM isocratic, with a flow-rate of 0.8 mL min^{-1}. Separations were monitored by suppressed conductivity detection at 35 °C with continuously regenerated anion trap column (Dionex CR-ATC) inserted after the separation column.

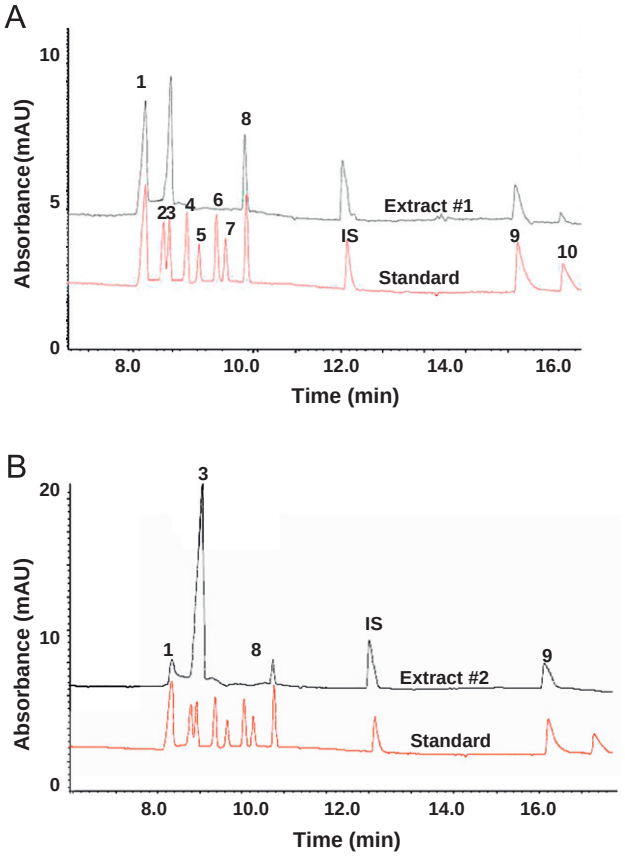

Fig. 1. CE analyses of a standard solution of 10 inorganic anions superimposed with real extract samples, named Extract ♯1 (Fig. 1A) and Extract ♯2 (Fig. 1B) collected after the simulated suicide bombing attack. Bare fused-silica capillary, 50 μm I.D. × 87 cm (detection at 77 cm) modified with HDMB at 2.5 g L^{-1} in ultra-pure water. BGE: 25 mM CrO_3, 25 mM Na_2CrO_4 and 100 mM Tris (pH 8.2), supplemented with 6% (v/v) EtOH. Temperature, 15 °C; applied voltage, −30 kV; electrokinetic injection, 50 s, −2 kV.;indirect UV detection at 254 nm. Anion concentration in standard solution, 20 mg L^{-1} each in ultra-pure water/BGE (9:1) mixture. Identification: chloride (1), nitrite (2), nitrate (3), thiosulfate (4), perchlorate (5), thiocyanate (6), chlorate (7), sulfate (8), carbonate (9), phosphate (10), and formate (IS).

3. Results and discussion

3.1. Analyses of inorganic anions

Analyses of anions were carried out from Extracts ♯1 and ♯2 with the electrophoretic method previously developed by our group [9]. This method allowed the separation of 10 inorganic anions of interest plus 7 potential interfering anions, and formate used as internal standard in 16 min and with limits of detection (LODs) around 0.5 mg L^{-1}. Fig. 1 presents the superimposition of the electropherograms obtained for a standard solution of 10 anions at 20 mg L^{-1} and for the Extracts ♯1 (Fig. 1 A) and ♯2 (Fig. 1B). Both extracts were qualitatively close: chloride, nitrate, carbonate, and sulfate anions appeared in both extracts, whereas phosphate anion only appeared in Extract ♯1. Calculated concentrations were compared with those determined by IC (Table 1). Similar results were obtained by CE and IC. CE provided the carbonate content, whereas this anion was not searched in IC. Nitrate anion appeared in both extracts.

3.2. Analyses of inorganic cations

The analysis of anions was next supplemented by the analysis of inorganic cations. Separations were carried out with the CE method previously developed by our group [10]. This method consisted of a guanidinium-based electrolyte and in order to prevent from EOF variations linked to potential effects of the real samples on capillary walls, the successive multiple ionic-polymer layers (SMIL) approach was implemented [15,16]. A HDMB-polyvinylsulfonate (HDMB-PVS) coating was optimized [11]. The separation of 8 cations of interest, 8 potential interfering cations, plus Li^+ cation as internal standard were obtained in less than 12 min. LODs comprised between 0.6 mg L^{-1} for Na^+ and 1.1 mg L^{-1} for Ba^{2+} were obtained. Fig. 2 shows the superimposition of the electropherograms obtained for a standard

Table 1
Quantitative results obtained by CE and IC for the analyses of aqueous Extract ♯1 and Extract ♯2 of cotton swabs wiped on the bus after the simulated suicide bombing attack.

Extracts	Analytes	CE		IC	
		mg L^{-1}	mM	mg L^{-1}	mM
Extract ♯1	Cl^-	24 ± 2[a]	0.70 ± 0.05[a]	28 ± 3	0.79 ± 0.08
	NO_3^-	63 ± 6[a]	1.02 ± 0.10[a]	60 ± 6	0.97 ± 0.10
	SO_4^{2-}	25 ± 2[a]	0.26 ± 0.02[a]	21 ± 4	0.22 ± 0.04
	HCO_3^-	33 ± 3[a]	0.54 ± 0.05[a]		NS
	HPO_4^{2-}	13 ± 1	0.14 ± 0.01	11 ± 2	0.11 ± 0.02
	NH_4^+	57 ± 6[a]	3.17 ± 0.30[a]	55 ± 5	3.05 ± 0.28
	K^+	12 ± 2[a]	0.31 ± 0.05[a]	11 ± 2	0.28 ± 0.05
	Ca^{2+}	39 ± 4[a]	0.98 ± 0.10[a]	36 ± 4	0.90 ± 0.10
	Na^+	27 ± 3	1.17 ± 0.13	23 ± 2	1.00 ± 0.09
	Mg^{2+}	3 ± 1	0.13 ± 0.04	3 ± 1	0.12 ± 0.04
Extract ♯2	Cl^-	8 ± 2	0.23 ± 0.06	7 ± 1	0.20 ± 0.03
	NO_3^-	183 ± 18[a]	2.95 ± 0.29[a]	176 ± 18	2.84 ± 0.29
	SO_4^{2-}	7 ± 1	0.07 ± 0.01	7 ± 1	0.07 ± 0.01
	HCO_3^-	31 ± 3[a]	0.51 ± 0.05[a]		NS
	NH_4^+	44 ± 9[a]	2.44 ± 0.49[a]	46 ± 9	2.56 ± 0.50
	K^+	3 ± 1	0.08 ± 0.02	3 ± 1	0.08 ± 0.02
	Ca^{2+}	12 ± 1	0.30 ± 0.02	11 ± 2	0.27 ± 0.05
	Na^+	7 ± 1	0.30 ± 0.04	6 ± 1	0.26 ± 0.04

NS: not searched. The confidence interval was determined at 20% for the lowest concentrations (< 15 mg L^{-1}) and 10% for the highest concentrations (> 15 mg L^{-1}).

[a] Determined after 1/10 dilution, in order to bring concentration within the linearity range.

solution of cations at 15 mg L^{-1} and for the Extracts ♯1 (Fig. 2A) and ♯2 (Fig. 2B). Both extracts exhibited the same qualitative composition in cations, except for potassium cation, which was only detected in Extract ♯1. According to Table 1, results obtained from CE and IC were not significantly different. Ammonium cation appeared as the main inorganic cation in both extracts. As this cation was not naturally present in blank samples collected before explosion, its use in the explosive charge was proved. In spite of a lack of adequacy between molar concentration of NH_4^+ and NO_3^- for Extract ♯1, results for anions and cations were well consistent with the use of NH_4NO_3 in the explosive composition.

3.3. Analyses of carbohydrates

In real cases, when an oxidizing anion such as NO_3^- is detected in large amount, analyses for the determination of reductive compounds (carbohydrates, fuel, etc) are carried out. This is why the CE method dedicated to carbohydrates was also implemented. This method consists of a direct detection of carbohydrates based on a photochemical reaction in the detection window after a separation with a BGE containing 98 mM NaOH and 120 mM NaCl [13]. Quantitative validation was already carried out including an important amount of work on matrix effects [14]. With this method, it was observed that Extract ♯1 contained only a small amount of carbohydrates with a concentration inferior to the limits of quantification (electropherogram not shown). Extract ♯2 contained a more quantitable amount of sucrose, glucose, and fructose (Fig. 3). Sucrose, the main component of icing sugar, was present at 145 mg L^{-1} (0.42 mM), whereas fructose and glucose, which probably result from the degradation of sucrose, were detected at 11 and 25 mg L^{-1} (0.06 mM and 0.14 mM), respectively. This confirmed the use of sucrose in the explosive charge.

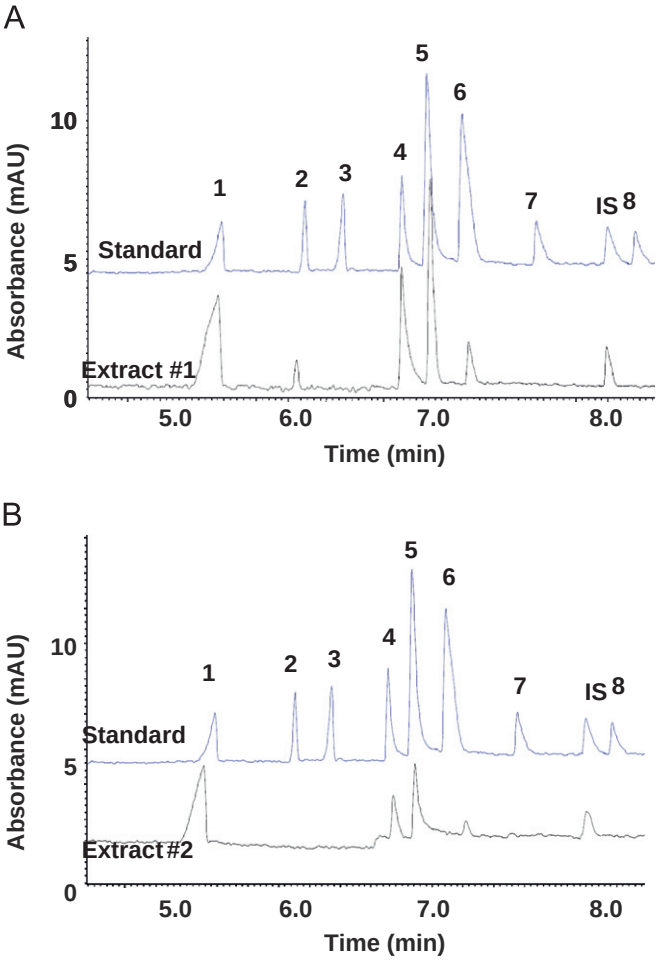

Fig. 2. CE analyses of a standard solution of 8 inorganic cations superimposed with real extract samples, named Extract ♯1 (Fig. 2A) and Extract ♯2 (Fig. 2B) collected after the simulated suicide bombing attack. Bare fused-silica capillary, 75 μm I.D. × 80 cm (detection at 70 cm) modified with (i) 10 g L^{-1} HDMB solution and (ii) 0.01% (w/w) PVS. BGE: 15 mM guanidinium acetate adjusted at pH 4.0 with acetic acid. Temperature, 20 °C; applied voltage, +30 kV; hydrodynamic injection, 4 s, 50 mbar; indirect UV detection at 190 nm; cation concentrations, 15 mg L^{-1} each, except for Li^+, 2 mg L^{-1}. Identification: NH_4^+ (1), K^+ (2), $MeNH_3^+$ (3), Ca^{2+} (4), Na^+ (5), Mg^{2+} (6), Sr^{2+} (7), Ba^{2+} (8), and Li^+ (IS).

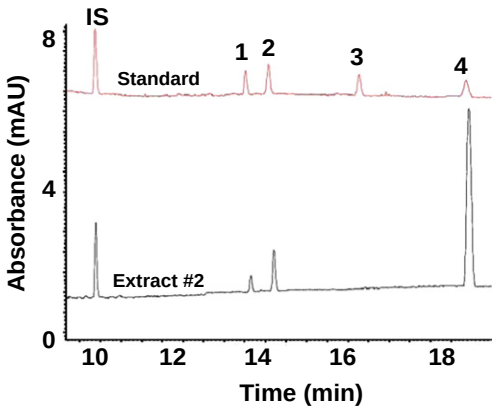

Fig. 3. CE analyses of a standard solution of carbohydrates superimposed with real Extract ♯2 collected after the suicide bombing attack. Bare fused-silica capillary, 50 μm I.D. × 60 cm (UV detection at 50 cm) modified with HDMB (1 g L^{-1} in ultra-pure water). BGE: 98 mM NaOH (pH 13.0), 120 mM NaCl; temperature, 26.5 °C. Applied voltage, −14 kV; hydrodynamic injection, 5 s, 50 mbar; direct UV detection at 270 nm; analyte concentration in standard solution, 50 mg L^{-1} each in ultra-pure water. Identification: fructose (1), glucose (2), lactose (3), sucrose (4), and naphthalenesulfonate (IS).

4. Conclusion

CE appeared as a powerful technique to detect and quantify inorganic species and carbohydrates in post-blast residue extracts. Methods previously developed by our group to analyze inorganic cations and anions, and carbohydrates allowed clear confirmation of the use of NH_4NO_3 and sucrose in the explosive charge used in this simulated suicide bombing attack. Good correlation was observed between CE and IC, and CE appeared perfectly complementary to IC. In order to acquire results more rapidly after bombing attacks , the transfer of these methods to a portable apparatus is contemplated.

References

[1] R. Tachon, V. Pichon, M. Barbe Le Borgne, J.-J. Minet, J. Chromatogr. A 1154 (2007) 174–181.
[2] R. Tachon, V. Pichon, M. Barbe Le Borgne, J.-J. Minet, J. Chromatogr. A 1185 (2008) 1–8.
[3] J. Yinon, The Analysis of Explosives, Thin-Layer Chromatography, Pergamon, Oxford, 1981, pp. 59–85.
[4] A. Beveridge, Forensic Investigation of Explosions, Recovery of Material from the Scene of an Explosion and its Subsequent Forensic Laboratory Contamination—A Team Approach, Taylor and Francis, London, 1998, pp. 100–131.
[5] J. Yinon, Modern Methods and Applications in Analysis of Explosives, Analysis of Explosive Residues, Wiley, Chichester, 1993, pp. 163–209.
[6] B.R. McCord, K.A. Hargadon, K.E. Hall, S.G. Burmeister, Anal. Chim. Acta 288 (1994) 43–56.
[7] D.T. Burns, R.J. Lewis, J. Bridges, Anal. Chim. Acta 375 (1998) 255–260.
[8] C. Sarazin, N. Delaunay, A. Varenne, C. Costanza, V. Eudes, P. Gareil, Sep. Purif. Rev. 39 (2010) 63–94.
[9] C. Sarazin, N. Delaunay, A. Varenne, J. Vial, C. Costanza, V. Eudes, J.J. Minet, P. Gareil, J. Chromatogr. A 1217 (2010) 6971–6978.

[10] C. Sarazin, N. Delaunay, C. Costanza, V. Eudes, P. Gareil, Electrophoresis 32 (2011) 1282–1291.
[11] C. Sarazin, N. Delaunay, A. Varenne, C. Costanza, V. Eudes, P. Gareil, J. Sep. Sci. 33 (2010) 3177–3183.
[12] C. Sarazin, N. Delaunay, C. Costanza, V. Eudes, J.-M. Mallet, P. Gareil, Anal. Chem. 83 (2011) 7381–7387.
[13] C. Sarazin, N. Delaunay, C. Costanza, V. Eudes, P. Gareil, J. Vial, J. Sep. Sci. 35 (2012) 1351–1358.
[14] C. Sarazin, N. Delaunay, C. Costanza, V. Eudes, P. Gareil, Talanta 99 (2012) 202–206.
[15] H. Katayama, Y. Ishihama, N. Asakawa, Anal. Chem. 70 (1998) 2254–2260.
[16] J.R. Catai, G.W. Somsen, G.J. de Jong, Electrophoresis 25 (2004) 817–824.