



Identification and determination of inorganic anions in real extracts from pre- and post-blast residues by capillary electrophoresis

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ABSTRACT

Fast, selective, and sensitive analysis of inorganic anions is compulsory for the identification of explosives in post-blast or environmental samples. For the last twenty years, capillary electrophoresis (CE) has become a valuable alternative to ion chromatography (IC) for the analysis of inorganic-based explosives because of its low running costs and its simplicity of use. This article focuses on the development and validation of a CE method for the simultaneous analysis of 10 anions (chloride, nitrite, nitrate, thiosulphate, perchlorate, chlorate, thiocyanate, carbonate, sulphate, and phosphate) which can be found in post-blast residues, plus for the first time azide anion, possibly present in the composition of detonators, and the internal standard (formate) in 20 min total runtime. Intermediate precisions were 2.11% for normalized areas and 0.72% for normalized migration times. Limits of detection close to 0.5 ppm for all anions were obtained with the use of preconcentration techniques, thanks to a fast and simple sample preparation allowing the analysis of a large variety of matrices with the developed generic CE method. The matrix effects were statistically studied for the first time in the explosive field for different matrices, containing interfering anions and cations, sometimes at high levels. In fact, no significant matrix effect occurred (tests with blank matrix extracts of soil, cloth, glass, plastic, paper, cotton, and metal). Finally, analyses of real post-blast residues and real detonator extracts were performed. The CE results were compared with those obtained with the IC method used routinely and showed excellent correlation.

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1. Introduction

Many kinds of explosives can be used to perpetrate a blast or an explosion. The main composition of these explosives consists in a mixture of organic explosives and inorganic salts such as the ammonium nitrate fuel oil (ANFO) mixture or the black powder. The detection of organic explosives can be carried out by GC coupled to a thermal energy analyzer [1], IR spectrometry [2], X-ray detection [3], and HPLC or CE coupled to UV or MS detection [4,5]. For analyzing inorganic salts, X-ray [6], IR [7], CE, or IC can be used. For many years, IC has been the most powerful technique used for these separations [8] but it has suffered from the lack of an additional technique to confirm the presence of putative ions. An approach to resolve this problem was to combine two different IC columns and detection techniques, for example on the one hand a classical ion analysis column with suppressed conductivity detection and, on the other hand, a single ion analysis column with UV detection [9].

Since 20 years, CE, with UV [10] and conductivity [11] detection, has known a real development for ion analysis and has provided a complementary technique. The complementarity of IC and CE was first studied by Hargadon and McCord for the analysis of a black powder with an indirect UV detection [12]. Afterwards, CE was considered in several papers for the analysis of different inorganic ion mixtures in various matrices [13–15]. Rapid and highly efficient separations were obtained. As most inorganic ions are UV transparent, the indirect UV detection mode was generally implemented but this approach lacks sensitivity. Different stacking methods were thus used such as field amplified sample stacking (FASS) [16] or field-enhanced sample injection (FESI) [17].

In recent years, attention was focused on developing protocols enabling fast analysis directly on the explosion site. Hutchinson et al. [18,19] described anion and cation analysis methods with portable CE and diode or contactless conductivity detection. Fast screening is also possible with the onset of microchip systems. Wang et al. [20] presented a dual opposite injection of three inorganic cations and three inorganic anions, for their simultaneous detection, in polymethylmethacrylate chip. Different papers

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reviewed the applications of CE for the analysis of explosives [21–25].

The main purpose of this work was to develop a CE method that would be complementary to the IC protocol used by French police for the analysis of a given mixture of anions in pre- and post-blast residue aqueous extracts. The chromate-based background electrolyte (BGE) proposed by Hutchinson et al. [18], which allowed the analysis of the main anions of this study, was first tested and then modified in order to separate 10 anions plus for the first time azide anion, this anion being absent in Hutchinson's mixture. Additionally, adapted, fast, and simple sample preparation procedures were studied with a view of analysing the largest variety of matrix aqueous extracts by a generic method that would simultaneously provide improved sensitivity. Finally, analyses of real pre- and post-blast residues were performed and the results were compared with those produced by IC.

2. Experimental

2.1. Standards and electrolytes

All anionic standards used were purchased from Sigma–Aldrich (L'Isle d'Abeau, France). Anionic chromophore used for the indirect UV detection was prepared from chromium (VI) oxide and sodium chromate (Fluka, Lyon, France) and the BGE was buffered with tris(hydroxymethyl)aminomethane (Tris), purchased from Sigma–Aldrich. Organic solvents, methanol and ethanol (analytical grade), were supplied by VWR (Fontenay-sous-Bois, France). Hexadimethrine bromide (HDMB) used as electroosmotic flow (EOF) reversal reagent was purchased from Sigma–Aldrich.

1000 mg L⁻¹ individual anionic standard solutions were prepared weekly by volumetric dissolution of sodium salts in ultra-pure water delivered by a Direct-Q3 UV system (Millipore, Molsheim, France). A standard mixture of the 12 anions was prepared daily (15 mg L⁻¹ each in ultra-pure water). HDMB solution was prepared by dissolving 0.25 g in 100 mL of ultra-pure water.

The final composition of the BGE was: 100 mM Tris, 25 mM CrO₃, 25 mM Na₂CrO₄ in ultra-pure water (pH 8.2), mixed weekly, and 6% ethanol was added just before the analysis.

2.2. Apparatus

The development of the CE method was carried out with an Agilent HP^{3D}CE system (Massy, France) equipped with a diode array detector (deuterium lamp) set at 254 nm, whereas the validation and the real sample analyses were achieved with a Beckman Coulter P/ACE MDQ system (Villepinte, France) equipped with a fixed wavelength UV detector (mercury lamp) at 254 nm. 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 comparison purposes. Instrument control and data acquisition were performed using Chromeleon 6.7[®] software. A 25- μ L loop was employed for the anion injection.

2.3. Electrophoretic procedures

Electrophoretic separations were performed using 50 μ m I.D. bare fused-silica capillaries purchased from Polymicro (Photon-lines, Marly-Le-Roi, France). Total capillary lengths varied from 68 to 97 cm, according to experiments, for the Agilent system and was fixed at 87 cm for the Beckman one. A detection window was created for the UV detection at 8.5 and 10 cm from the anodic end for the Agilent and Beckman systems, respectively. Before first use, capillaries were conditioned by successive percolations under 1 bar with 1 M NaOH for 20 min, 0.1 M NaOH for 20 min, ultra-pure water

for 20 min, HDMB solution for 20 min and then BGE for 10 min. Between each run, capillaries were rinsed with the BGE under 1 bar for 7 min. Then, every 10 analysis, capillaries were flushed with the HDMB solution to keep EOF constant (RSD value equal to 0.6%, but increasing up to 2.3% if the flush with the HDMB solution was done only every 15 runs). Injections were performed hydrodynamically under 50 mbar or electrokinetically at -2 kV with variable injection times. Separations were run at 15, 20 or 25 °C, according to cases, under -30 kV.

2.4. Chromatographic procedures

Chromatographic separations were performed at 30 °C with a Dionex AS19 column (250 \times 4 mm) equipped with a Dionex AG19 guard column (50 \times 4 mm) coupled to a ASRS suppressor. Eluent gradients were generated using the Dionex ECG-KOH Elu GenII cartridge. The optimized eluent gradient was: 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. All this procedure was previously optimized and conducted routinely.

2.5. Real samples preparation

Post-blast residues and blank samples were extracted in hot water placed in a sonication bath for 10 min. The obtained solutions were filtered through a 150- μ m cellulose filter (Les Filtrés Durieux, Marne-la-Vallée, France) and a 0.45- μ m nylon syringe filter (Teknokroma, A.I.T. France, Houilles, France). A small volume of high ionic strength BGE was added to the extract solutions, with a proportion of extract and BGE equal to (9/1) in order (i) to match the conductivities of the different extracts and (ii) to reach a conductivity value close to a tenth of the BGE conductivity for stacking purposes. The studied matrices were soil, plastic, paper, cloth, cotton, metal, and glass. Post-blast residues were collected after a criminal explosion either directly or via cotton swabs. The first step of this last procedure consisted in the purification of usual hydrophilic cotton swabs by assisted solvent extraction using an ASE 200 instrument (Dionex), with one cycle of 5 min at 100 °C and 100 bar with water and next with acetone. Cotton swabs moistened with water were next wiped over samples and were used for inorganic analysis, after extraction with hot water for 10 min under sonication. Blank samples were collected in or near the laboratory.

2.6. Conductivities

The conductivity values of the samples and BGEs were determined with the CE apparatus, by measuring current intensity as a function of the applied voltage. Conductivity κ was derived from Ohm's law:

$$\kappa = \frac{4LI}{\pi d_c^2 V} \quad (1)$$

where L is the capillary length, I the measured current intensity, d_c the inner diameter and V the voltage applied across the capillary. Calibration was done with solutions of 0.1 and 0.01 M KCl ($\kappa = 1.2103$ and 0.1365 S m⁻¹ at 25 °C, respectively).

2.7. Statistical approach of matrix effects

Statistical studies of matrix effects were carried out with five repeated injections for five different anion concentrations (2, 4, 5, 10, and 20 mg L⁻¹) in ultra-pure water for the standard calibration

and three repeated injections for five anion concentrations (2, 4, 6, 10, and 20 mg L⁻¹) in given blank matrix extracts. When matrices already contained some anions, additional spiking was performed. Statistical parameters of the regression lines were computed with Excel[®] software (Microsoft).

3. Results and discussion

3.1. CE method development

A method for the separation of fifteen anions (acetate, benzoate, carbonate, chlorate, chloride, chlorite, cyanate, fluoride, nitrate, nitrite, perchlorate, phosphate, sulphate, thiocyanate, and thiosulphate) potentially present in post-blast residues was developed by Hutchinson et al. [18], but azide anion, which is a primary explosive present in the composition of some detonators, was missed. Thus, the BGE developed by Hutchinson was first evaluated for the separation of an original anionic mixture differing from Hutchinson's one by the absence of acetate, benzoate, chlorite, cyanate, and fluoride anions, which are very rarely used in the explosive compositions, and conversely the presence of azide anion. The obtained electropherogram is presented in Fig. 1.

The method developed by Hutchison et al. was not adapted to the mixture of the 11 anions of interest. Indeed, comigrations were observed for azide (F), perchlorate (G), and thiocyanate (H). Some modifications of the separation conditions were thus necessary to improve the resolution and the sensitivity. The modified parameters were temperature, electric field via the applied voltage and the capillary length, BGE composition, and ionic strength.

3.1.1. Temperature

Upon decreasing temperature, the BGE viscosity increases by 2–3% per °C [26]. This increase induces a reduction of the electroosmotic and electrophoretic mobilities, and hence a slowdown of the anion migration. Separations were performed at 15, 20, and 25 °C. The decrease in temperature allowed an improvement of the resolution between thiocyanate and (perchlorate + azide) peaks ($R_s = 0.8$ at 25 °C and $R_s = 1.3$ at 15 °C), but the comigration of azide and perchlorate anions remained. Consequently, the following separations were performed at 15 °C but other parameters needed to be altered for analysis optimization.

3.1.2. Electric field

Resolution is expected to depend slightly on field strength through variation in efficiency. Electric field can be varied either by varying applied voltage at constant capillary length or by varying capillary length at constant voltage. For a capillary length fixed

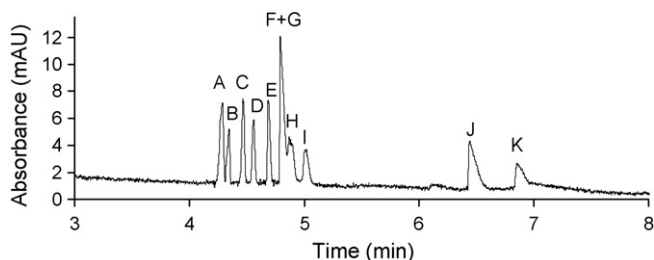


Fig. 1. CE analysis of a standard mixture of anions in ultra-pure water based on the method developed by Hutchinson et al. [16]. Bare fused-silica capillary, 50 μ m I.D. \times 68 cm (UV detection at 59.5 cm) modified with HDMB at 0.25 g in 100 mL ultra-pure water. BGE: 10 mM CrO₃, 10 mM Na₂CrO₄ and 40 mM Tris (pH 8.2). Temperature, 25 °C; applied voltage, -30 kV; hydrodynamic injection, 12 s, 50 mbar. Indirect UV detection at 254 nm. Anion concentration, 20 mg L⁻¹ each in ultra-pure water. Identification, chloride (A), thiosulphate (B), nitrite (C), nitrate (D), sulphate (E), azide (F), perchlorate (G), thiocyanate (H), chlorate (I), phosphate (J), and carbonate (K).

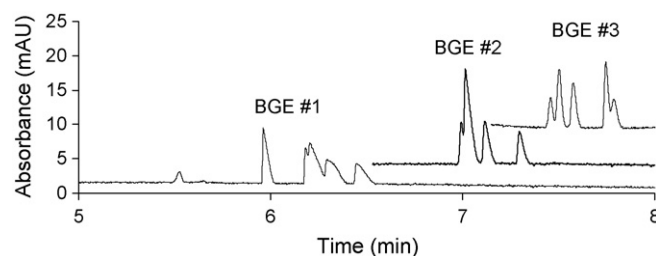


Fig. 2. Separation of sulphate, perchlorate, thiocyanate, azide, and chlorate anions with three BGEs. Bare fused-silica capillary, 50 μ m I.D. \times 96 cm (UV detection at 88.5 cm) modified with HDMB at 0.25 g in 100 mL ultra-pure water. BGE #1: 5 mM CrO₃, 5 mM Na₂CrO₄ and 20 mM Tris (pH 8.2); BGE #2: 15 mM CrO₃, 15 mM Na₂CrO₄ and 60 mM Tris (pH 8.2); BGE #3: 25 mM CrO₃, 25 mM Na₂CrO₄ and 100 mM Tris (pH 8.2). Temperature, 15 °C; applied voltage, -30 kV; hydrodynamic injection, 20 s, 50 mbar. Indirect UV detection at 254 nm. Anion concentration, 20 mg L⁻¹ each in ultra-pure water. Peaks were not identified.

at 68 cm, four applied voltages were tested (-30, -25, -20 and -15 kV) without any improvement in resolution between azide and perchlorate anions. Furthermore, peaks efficiencies were improved upon increasing voltage, with for example in the case of thio-sulphate anion plate numbers of 99,900, 105,000, 112,000, and 115,000 for $V = -15, -20, -25,$ and -30 kV, respectively. A voltage of -30 kV was thus retained as it gave the fastest separation and without observed Joule heating.

Three capillary lengths (76, 86, and 96 cm) were then tested. The comigration of azide and perchlorate anions still occurred but the resolution between the (azide + perchlorate) and the thiocyanate peaks was improved to 1.6 with the 96 cm length capillary. This value was thus retained for further optimization.

3.1.3. Ionic strength

Increasing ionic strength I is a way of decreasing the actual mobility of anions. But, according to Hutchinson et al. [18], a 1:2 ratio of Cr(VI)/Tris provided the best resolution. So, this ratio was kept constant in the different tested BGE compositions. Four BGEs were evaluated: BGE #1, 5 mM CrO₃, 5 mM Na₂CrO₄, and 20 mM Tris (pH 8.2); BGE #2, 15 mM CrO₃, 15 mM Na₂CrO₄, and 60 mM Tris (pH 8.2); BGE #3, 25 mM CrO₃, 25 mM Na₂CrO₄, and 100 M Tris (pH 8.2); BGE #4, 50 mM CrO₃, 50 mM Na₂CrO₄, and 200 M Tris (pH 8.2). Their respective ionic strengths are 30, 90, 150, and 200 mM.

Fig. 2 presents the electropherograms obtained for the five anions that were the most difficult to separate according to Fig. 1 (sulphate, perchlorate, thiocyanate, azide, and chlorate) with the BGEs #1, #2, and #3. The identification was not carried out. Increasing ionic strength resulted in increased analysis time, as expected, and improved resolution. At a 150 mM ionic strength, creating a current of 31 μ A, the overall resolution became more satisfactory. With the BGE #4 (results not shown), Joule effect appeared at -25 kV; the resulting current being equal to 79 μ A. Thus, BGE#3 was preferred.

3.1.4. Solvent composition

To complete the resolution of the anion separation, the addition of an organic solvent in the BGE was tested. According to Harakuwe and Haddad [27], Sarmini and Kenndler [28], and Descroix et al. [29], organic solvents can have different effects on the BGE. First, it may change analyte solvation, pKa values, and ion pairing phenomena, so that the electrophoretic mobilities of the anions may be modified. It may also alter the electroosmotic flow by modifying the BGE viscosity and the zeta potential.

In order to evaluate the optimal solvent composition of the BGE more easily, the relative variation of the anion effective mobilities (excepted carbonate and phosphate anions) with respect to formate

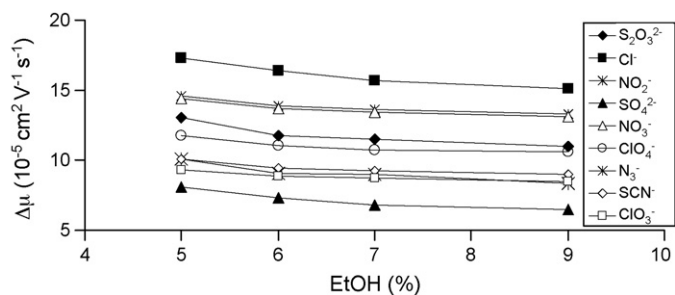


Fig. 3. Variations of the relative electrophoretic mobility ($\Delta\mu = \mu_{\text{anion}} - \mu_{\text{formate}}$) of the anions of interest as a function of the ethanol content for BGE #3.

(internal standard) mobility ($\Delta\mu = \mu_{\text{anion}} - \mu_{\text{formate}}$) was studied as a function of solvent content.

With methanol, the optimal content was 2% (v/v) but as methanol is a volatile solvent, this low content in the BGE could lead to non-robust analytical conditions. Thus ethanol was evaluated. The best resolution was obtained with 6% ethanol (Fig. 3). Indeed, with 5%, 7% or 9%, at least two anions comigrated. With 6% ethanol, the 11 anions were separated, even if azide mobility was very close to those of chlorate and thiocyanate ($R_s(\text{azide}/\text{thiocyanate}) = 1.13$; $R_s(\text{chlorate}/\text{azide}) = 1.3$). The final composition of the optimized BGE was: 25 mM CrO_3 , 25 mM Na_2CrO_4 , 100 mM Tris (pH 8.2), to which 6% (v/v) EtOH was finally added.

3.1.5. Sensitivity

In order to test the preconcentration techniques, a first injection in standard conditions was carried out with anions at 15 mg L^{-1} directly prepared in the BGE. Then, sensitivity improvement was achieved by on-line preconcentration employing either FASS or FESI. FASS consists in hydrodynamically injecting the sample in a capillary filled with a BGE of higher conductivity than that of the sample [30]. The difference between the zone conductivities creates a higher electric field in the injected sample zone, inducing a high analyte velocity in this zone. Analytes are therefore slowed down when they penetrate the BGE zone, so that they focus and preconcentrate. The optimal ratio of the BGE to sample conductivity (named γ) was shown to be around 10 [30]. Indeed, if the sample conductivity is too low, the electric field in this zone will generate Joule heating, which alters the flat velocity profile and reduces preconcentration efficiency, and may also induce bubbles and analyte degradation. Experimentally, to obtain a conductivity ratio of 10, analytes were prepared in an ultra-pure water/BGE (9:1) mixture. Such a conductivity ratio of 10 allows the injection of a larger sample volume in the capillary, so that injection time should be optimized. For 50 mbar pressure, injection times of 20, 40, 60, and 80 s were investigated. For chloride anion, from 20 to 60 s, peak height linearly varied from 1.7 to 7 mAU. For 80 s, the linearity is broken and the peak height value was 6.6 mAU. Beyond 60 s, the decrease of the peak height was related to the increase of its width due to the injection dispersion. The same phenomenon was observed for all anions. The best injection time was 60 s, which led to an injected volume representing 3% of the total capillary volume.

In FESI, analytes are introduced electrokinetically. Therefore analytes with superior apparent mobilities penetrate in larger amount in the capillary. A discrimination and preconcentration also take place during the injection. The applied voltage was fixed at -2 kV in order to limit the sample degradation. As previously, four injection times were studied (20, 40, 50, and 60 s) and chloride peak height was monitored. The peak height linearly varied with injection time from 20 to 50 s and for 60 s the linearity was broken. The optimized injection parameters were thus -2 kV for 50 s.

A constant enrichment factor of 6 was yielded for FASS whereas a factor ranging from 23 for chloride anion to 15 for phosphate

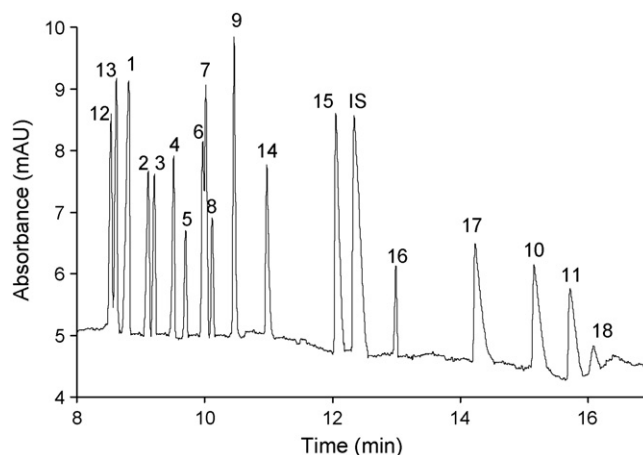


Fig. 4. Electropherogram of the 11 anions of interest + formate + 7 supplementary anions (potentially found in post-blast residues or interfering anions). Electrokinetic injection, 50 s, -2 kV . Bare fused-silica capillary, $50 \mu\text{m}$ I.D. \times 96 cm (UV detection at 88.5 cm) modified with HDMB at 0.25 g in 100 mL ultra-pure water. BGE: 25 mM CrO_3 , 25 mM Na_2CrO_4 and 100 mM Tris (pH 8.2), supplemented with 6% EtOH (v/v). Temperature, 15°C . Applied voltage, -30 kV . Indirect UV detection at 254 nm. Anion concentration, 20 mg L^{-1} each in ultra-pure water/BGE (9:1) mixture. Identification: chloride (1), nitrite (2), nitrate (3), thiosulphate (4), perchlorate (5), thiocyanate (6), azide (7), chlorate (8), sulphate (9), carbonate (10), phosphate (11), iodide (12), bromide (13), cyanate (14), fluoride (15), chlorite (16), acetate (17), oxalate (18), and formate (IS).

anion was obtained in the case of FESI. Anions with a superior apparent mobility are injected in a more important amount, their enrichment factors were also maximized. FESI led to improved detection limits, and was thus selected. Furthermore, the electrokinetic injection limited the introduction of low-mobility matrixial compounds which may reduce precision on quantitative analysis of real samples. Finally, the electropherogram obtained under optimized conditions is presented in Fig. 4.

3.2. Instrumentation and laboratory transfer

The protocol was then transferred from the university laboratory using an Agilent instrument to the police laboratory, where a Beckman instrument was available. Whereas capillary length was not limited on the Agilent apparatus, the capillary length in the Beckman instrument was limited to 87 cm due to the coolant cartridge length. Other parameters had to be also adjusted to keep the same resolution with the same electrolyte. Injection time was thus adapted to -2 kV for 40 s. The Beckman instrument led to similar resolutions for critical pairs of anions with a shorter capillary length. This could not be explained by differences in thermal regulation systems, because separations were performed with both instruments between linear range of Ohm's law. It may be linked to different geometries of optical apertures. Indeed, the resolution between nitrite and nitrate was 1.3 on Agilent HP^{3D}CCE and 1.2 on Beckman PACE. Obtained LODs on Beckman instrument (calculated at a signal to noise ratio of 3) were in a range from 0.3 to 0.7 mg L^{-1} (see Table 1). LODs were improved by a factor of 2 with the Beckman PACE instrument.

3.3. Elements of method validation

The validation of the developed method was carried out to prove the method could be applied routinely to the quantitative analysis of anions in real extracts with a quantitation limit of 2 mg L^{-1} . This validation was primarily carried out for all anions except azide anion, which has a migration time close to thiocyanate and chlorate anions. Azide anion was then validated in a mixture of all anions, except thiocyanate and chlorate anions. Precision and accuracy of

Table 1

Figures of merit for the analysis of the 11 anions of interest: intra-day ($n=3$), day-to-day ($n=3$) and intermediate precision, LODs, and detection linearity in the 2–20 ppm range.

Anions	Normalized area			Normalized t_M			LOD (mg L^{-1})	Least-squares linear regressions ($n=15$)		
	RSD (%) intra-day	RSD (%) day-to-day	RSD (%) intermediate precision	RSD (%) intra-day	RSD (%) day-to-day	RSD (%) intermediate precision		Equation	R^2	Residual standard deviation
Cl^-	0.64	0.23	0.68	0.44	0.62	0.76	0.50	$0.0585x + 0.0027$	0.995	0.027
NO_2^-	1.28	0.33	1.32	0.47	0.64	0.78	0.39	$0.0291x + 0.0011$	0.997	0.011
NO_3^-	1.94	0.65	2.07	0.97	0.51	1.09	0.61	$0.0218x - 0.0005$	0.990	0.015
$\text{S}_2\text{O}_3^{2-}$	1.75	0.85	1.94	0.61	0.55	0.81	0.29	$0.0364x - 0.0141$	0.992	0.022
ClO_4^-	2.85	2.22	3.61	0.72	0.34	0.79	0.43	$0.0203x - 0.0020$	0.993	0.011
SCN^-	1.59	0.79	1.78	0.41	0.13	0.54	0.31	$0.0358x - 0.0153$	0.998	0.014
N_3^-	0.96	0.89	1.31	0.56	0.23	0.61	0.37	$0.0418x - 0.0382$	0.992	0.022
ClO_3^-	1.36	1.41	1.96	0.57	0.25	0.63	0.33	$0.0284x + 0.0174$	0.991	0.019
SO_4^{2-}	1.21	1.34	1.81	0.54	0.22	0.59	0.64	$0.0480x - 0.0192$	0.995	0.021
HCO_3^-	2.23	1.21	2.54	0.47	0.22	0.52	0.51	$0.0345x + 0.0214$	0.987	0.017
HPO_4^{2-}	1.04	2.46	2.67	0.57	0.15	0.59	0.34	$0.0102x - 0.0140$	0.991	0.021
Pooled	1.65	1.31	2.11	0.59	0.40	0.72				

the method had to be better than 5% and 20% respectively, the usual criteria used by the French police laboratory.

3.3.1. Selectivity

The CE method was developed with a mixture containing 11 targeted anions plus an internal standard anion (formate). The study was next completed by the analysis of a more complex mixture containing 7 other anions potentially found in post-blast residues or being present in real matrices (and therefore possibly being interfering anions). Fig. 4 presents the resulting electropherogram obtained for the analysis of the 19 anions. No comigration appeared and the added anions were fully resolved, showing the good selectivity of the CE method and its versatility.

3.3.2. Precision

The precision of the method was evaluated for a mixture of about 20 mg L^{-1} concentration for each anion by making three repeated analyses ($n=3$) on three different days. Thus, standard mixtures and electrolytes were prepared daily. The responses measured on each electropherogram were the normalized area (A_i/A_{formate}) and the normalized migration time ($t_{Mi}/t_{M \text{ formate}}$) of each peak. Method repeatability was characterized by the intra-day RSD and method intermediate precision was characterized by the intermediate precision RSD. One-way analysis of variance (ANOVA) [31] was used to determine the intra-day (Eq. (2)) and the day-to-day (Eq. (3)) contributions. In the present study the day factor always appeared significant ($\alpha=0.05$). The intra-day standard deviation was denoted σ_r and the standard deviation due to the day factor was denoted σ_d . The intermediate precision RSD, corresponding to the variability of a single determination carried out on a single day (Eq. (4)) was then calculated.

$$\text{RSD}_{\text{intra-day}} (\%) = \frac{\sigma_r}{\bar{x}} \times 100 \quad (2)$$

$$\text{RSD}_{\text{day-to-day}} (\%) = \frac{\sigma_d}{\bar{x}} \times 100 \text{ with } \sigma_d^2 = \frac{q_d - q_r}{n} \quad (3)$$

$$\text{RSD}_{\text{intermediate precision}} (\%) = \frac{\sqrt{\sigma_d^2 + \sigma_r^2}}{\bar{x}} \times 100 \quad (4)$$

where \bar{x} is the mean of the response, and q_d and q_r the mean square of the day factor and the residual mean square of the ANOVA respectively.

To provide overall characterization of the method, pooled values of the RSDs for all the anions were also computed.

Results for normalized area and migration times are presented in Table 1. For migration time, overall method repeatability was 2% and overall method intermediate precision was 2.3% but when the

migration times were normalized by formate migration time, the overall method repeatability RSD went down to 0.59% and overall method intermediate precision to 0.72%. Such quite acceptable values, always inferior to 1% for normalized migrations times, should allow an easy and fast identification of peaks. If a doubt persisted on a peak identification, IC technique allowed the confirmation of its presence or absence. For normalized peak areas, overall method repeatability was 1.65% and overall method intermediate precision was 2.11%. Moreover, all the intermediate precision RSD values ranged from 0.7% to 3.6% which is always inferior to 5%. Thus, the performances of the method complied requirements.

3.3.3. Linearity

The linear range of the method response function for the anions was expected between 2 and 20 mg L^{-1} . Indeed, this is the range used with the IC certified (ISO/CEI 17025) routine method at the police laboratory because, in most cases, the targeted anions are at this concentration level in the aqueous sample extracts, except for chloride and sulphate anions which are most often more concentrated. In this last case, the sample is analyzed a second time, after dilution with water by appropriate factor. This is why eventually this linear range over 1 order of magnitude is adapted to the requested applications. As the CE method was developed here with the goal of being complementary to the IC protocol used by French police, the linearity range was kept. The linearity of the method was evaluated by plotting the normalized area for each anion against the injected concentrations. Linear regressions were performed and coefficients of determination R^2 and residual standard deviations of the regressions were calculated and are given in Table 1. It was checked that residuals of the regression were randomly distributed (for each calibration curve). This allowed to establish linearity for all the anions in the working range.

3.3.4. Limits of quantitation

For a signal to noise ratio of 10, the limits of quantitation (LOQ) were experimentally predetermined at 2 mg L^{-1} for all anions as in IC. Thus, these LOQs were checked with five solutions of all anions at 2 mg L^{-1} injected twice. At this concentration level, the maximum bias obtained for anions did not exceed 20%, the higher limit fixed by the laboratory. Precision was also estimated and values for anions ranged between 2% and 9% which fulfilled specifications. 2 mg L^{-1} level can be defined as low value of the quantitation range.

3.3.5. Sample stability

Sample stability was tested with two injections of the same sample of anions at 20 mg L^{-1} after a time space of one week. No sig-

Table 2
Matrix and aqueous extract description, evaluation of matrix effects based on statistical analysis of calibration lines and maximum bias value that could be generated on quantitation in routine analysis, expressed as a percentage.

Matrix	Composition of the matrix aqueous extract (mg L ⁻¹)		Residual Variance homogeneity	Equality of slopes	Equality of intercepts	Matrix effect	Bias (%)
	Anions	Cations					
Soil (blank 1)	NO ₃ ⁻ : 20, HCO ₃ ⁻ : 15–20, Cl ⁻ : 3, SO ₄ ²⁻ : 2	Nd ^a	Yes	No	No	Yes	15
Soil (blank 2)	NO ₃ ⁻ : 61, Cl ⁻ : 15, HCO ₃ ⁻ : 15–20	Ca ²⁺ : 28, Na ⁺ : 7	Yes	No	No	Yes	17
Paper (blank)	HCO ₃ ⁻ : 15–20, Cl ⁻ : 3, SO ₄ ²⁻ : 2	Nd	Yes	Yes	Yes	No	0
Plastic (blank)	HCO ₃ ⁻ : 15–20	Nd	Yes	Yes	Yes	No	0
Glass (blank)	HCO ₃ ⁻ : 15–20	Nd	Yes	Yes	Yes	No	0
Metal (blank)	HCO ₃ ⁻ : 15–20, Cl ⁻ : 4, SO ₄ ²⁻ : 3	Nd	Yes	No	No	Yes	18
Cloth (blank)	HCO ₃ ⁻ : 15–20, Cl ⁻ : 2, NO ₃ ⁻ : ~1, SO ₄ ²⁻ : ~1	Nd	Yes	No	No	Yes	17
Plaster (blank)	SO ₄ ²⁻ : 435	Ca ²⁺ : 760	Yes	No	No	Yes	24
Cotton (blank)	HCO ₃ ⁻ : 15–20, Cl ⁻ < LOQ	Ca ²⁺ & Na ⁺ < LOQ	Yes	Yes	Yes	No	0
Cotton wiped over metal (blank)	HCO ₃ ⁻ : 15–20, Cl ⁻ : 2, SO ₄ ²⁻ : 3	Ca ²⁺ : 36, K ⁺ : 6, Na ⁺ : 2 + interfering metal cations	Yes	No	No	Yes	15
Cotton wiped over cloth (blank)	HCO ₃ ⁻ : 15–20, Cl ⁻ : 4, NO ₃ ⁻ : 2	Na ⁺ : 6, K ⁺ : 2	Yes	No	No	Yes	9

^a Nd: not determined.

nificant modification in the normalized area was noted, except for carbonate and phosphate anions, for which the normalized areas varied from 0.83 to 1.12 and from 0.88 to 0.83, respectively. Carbonate anion can be used in some explosive mixtures, but it may be also a contaminant, appearing within time, of the water used to extract the residues. Some care must be taken to limit its natural generation, such as properly closing the sample vials and totally filling them. For the case of phosphate anion, partial precipitation is anticipated and standard solutions should be prepared every day or two days.

3.3.6. Matrix effects

To evaluate potential matrix effects, the calibration lines with standards in pure water and in a given aqueous matrix extract were compared. The comparison was carried out with a statistical approach, based on the comparison of the two regression lines obtained from least-squares linear regression [32,33]. It involves three successive steps: (i) the residual variances of the regression must be homogeneous (bilateral *F*-test), (ii) in case residual variances were homogeneous, it was then possible to compare the slopes of the regression lines (bilateral *t*-test using the pooled standard deviation of the slopes as denominator), and (iii) in case slopes were the same, it was finally possible to compare intercepts (bilateral *t*-test using the pooled standard deviation of the intercepts as

denominator). Statistically, it could be considered that two calibration lines were not significantly different only if residual variances of the regression lines, slopes, and intercepts could be considered simultaneously not significantly different. All statistical tests were carried out with a first kind risk α set at 1%. All the results and conclusions are gathered in Table 2 for the 11 tested matrices, of which the aqueous extracts varied in nature and ion contents. They were considered as blank matrices as they were not coming from post-blast places. Table 3 also presents the conductivity values of the aqueous blank matrix extracts and also of real post-blast residues, either directly collected or via a cotton swab. It appears that the conductivity of the real soil extract belongs to the range of the tested blank soil extracts. Looking at the conductivity values of the different cotton extracts, the same conclusion can be drawn. This indicates that the samples used to evaluate matrix effect are closed to real post-blast residue extracts.

In case a significant matrix effect was observed, the consequences of neglecting this matrix effect were evaluated by the maximum bias value (expressed as a percentage) that it could generate in routine analysis. This bias was calculated and the maximum acceptable value is 20%. Among the 11 tested matrices, only plaster lead to aqueous extracts showing a non-negligible matrix effect according to anion quantitation as the bias was 24%, which was eventually a value not so far from the acceptance limit, especially

Table 3
Conductivity (κ) and BGE to sample conductivity ratio (γ) values for blank matrices, crater soil extracts and several real post-blast residue extracts without and with a BGE addition to the sample. Values obtained at 25 °C ($\kappa_{\text{BGE}} = 0.709 \text{ S m}^{-1}$).

Extract	Without BGE addition		With BGE addition	
	κ (S m ⁻¹)	γ ($\kappa_{\text{BGE}}/\kappa_i$)	κ (S m ⁻¹)	γ ($\kappa_{\text{BGE}}/\kappa_i$)
Soil (blank 1)	0.097	7.3	0.159	4.4
Soil (blank 2)	0.083	8.5	0.136	5.2
Soil (real)	0.078	9.8	0.132	5.4
Paper (blank)	0.008	90.9	0.077	9.2
Plastic (blank)	0.005	157.5	0.077	9.2
Glass (blank)	0.005	157.5	0.076	9.3
Metal (blank)	0.007	109.1	0.077	9.2
Cloth (blank)	0.009	77.9	0.081	8.7
Plaster (blank)	0.202	3.5	0.341	2
Cotton (blank)	0.004	177.2	0.076	9.3
Cotton wiped over metal (blank)	0.133	5.3	0.214	3.3
Cotton wiped over cloth (blank)	0.013	54	0.089	7.9
Cotton 1 wiped over metal (real)	0.008	90.9	0.084	8.4
Cotton 2 wiped over metal (real)	0.011	67.5	0.087	8.2
Cotton 3 wiped over plastic (real)	0.018	40.1	0.091	7.8
Cotton 4 wiped over metal (real)	0.009	74.4	0.082	8.2

considering the very high concentrations of sulphate and calcium ions present in these aqueous extract.

Actually, about 75% of real residues are collected by the Police laboratory via a cotton swab, which is next extracted with water. Here, the 3 blank cotton extracts gave no or negligible matrix effect (the bias was inferior to the maximum acceptable value of 20%). So, it can be concluded that calibration lines obtained in pure water can be used for quantitative analyses of the aqueous matrix extracts by CE. For the few cases of samples having very high levels of some ions, and thus a high conductivity value inducing non-negligible bias on quantitation, only the results of IC would be considered, as the CE method is always performed in parallel with IC.

It is worthwhile to note that during the full study, more than 15 highly different matrix extracts were analyzed and no relevant migration time shift was observed. Indeed, the RSDs of normalized migration time were in the range of 0.4–0.75% for all anions, even with the extracts obtained with plaster or cotton wiped over metal (blank), which are the 2 matrices leading to high level of some ions in their aqueous extracts. The peak shape stayed also correct. The developed electrophoretic method for the identification and the quantification of anions in real post-blast residues was thus validated. Its performances were in agreement with its requirements.

3.4. Applications to real samples

3.4.1. Matrix conductivities and interest of BGE addition

The studied aqueous matrix extracts, even of similar initial matrix nature, can have very different electric conductivities, which can create stacking or destacking phenomena and so lead to non-reproducible analysis for different matrices. To insure reproducible injections, the BGE to sample conductivity ratios (noted γ) have to be close for each aqueous matrix extract. Table 3 presents the experimental conductivity (κ) and γ values of blank and post-blast residue extracts obtained at 25 °C. The κ values were very scattered but always inferior to the BGE conductivity ($\kappa_{\text{BGE}} = 0.709 \text{ S m}^{-1}$). As the sample conductivity is inferior to the BGE one, FESI can be implemented, but with variable enrichment factors, depending on matrix conductivity. The addition of 10% BGE (v/v) in the extract before its injection may lead to matching the κ values, which gives, in that case, more reproducible electrokinetic injections and pre-concentrations (see Table 3), except for matrices having initially very high conductivities, as plaster extract.

3.4.2. Real sample analysis

3.4.2.1. Detonator analysis. The analysis of detonators is only possible if the explosion fails. In order to identify the employed detonators, an anion analysis can be carried out. Fig. 5 presents the analysis of a detonator composition, after extraction in pure water, with the developed method. The presence of lead in the extract created a yellow precipitate with chromate anion in the vial after the addition of 10% (v/v) of BGE and only the supernatant was injected. The analysis of this extract showed the presence of azide anion at a

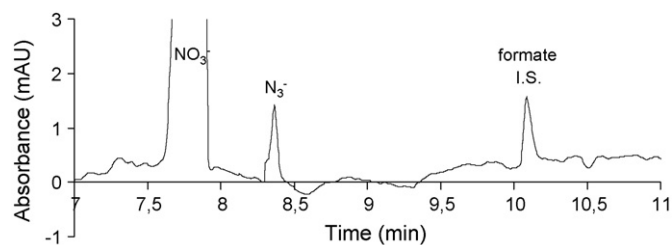


Fig. 5. Analysis of a detonator sample extract. Quantitation by this method yields 18 mg L^{-1} for azide anion and 2300 mg L^{-1} for nitrate anion. Separation conditions: see Fig. 4.

low content (18 mg L^{-1}) and a large amount of nitrate anion, possibly due to its oxidation. Azide (stemming from lead azide) is one of the main anions entering the composition of detonators.

3.4.2.2. Analysis of real pipe-bomb residue cotton swabs: comparison with IC.

After criminal explosion of pipe-bombs in public place, many residues were taken by cotton swabs which were next extracted with water. One representative extract corresponding to the swab of one pipe-bomb piece was analyzed after its dilution at 1/10 in ultra-pure water, in order to fix anion concentrations in the concentration linear range, and then after addition of 10% of BGE before the injection. The obtained electropherograms and chromatograms are presented in Fig. 6. Quantitative analysis by IC was performed with a calibration method previously developed in the police laboratory. The electropherogram from the pipe-bomb residues was characterized by the presence of chloride and chlorate anions at approximately 16 and 30 mg L^{-1} , respectively. Chlorate salts are often used in explosive compositions, their presence in a large amount clearly demonstrated its use in the explosive mixture. Other analyses performed on this sample such as cations and organic analyses determined that the explosive composition was a mixture of sodium chlorate and sugars. This sample also showed a high content of carbonate (26 mg L^{-1}). This anion, which was not searched in IC, can be used in some explosive mixtures, but it may also simply be a contaminant of the water used to extract the residues. Nevertheless, the fact that the carbonate content was superior to its natural rate suggests its use in an explosive composition. The content of sulphate and nitrate anions was so weak that it was impossible to determine if they were used in the conception of an explosive or naturally present in the matrix. Finally, quantitative results given by both techniques were close: for the chloride and chlorate peaks the obtained concentration difference between IC and CE was 5% and 13%, respectively. IC results were confirmed by CE, which also allows to reveal the potential presence of carbonate. As compared to IC, CE provides faster separation with less electrolyte consumption and under more cost-effective conditions.

3.4.2.3. Soil analysis. A soil sample originating from a crater created by explosions described previously was also directly extracted

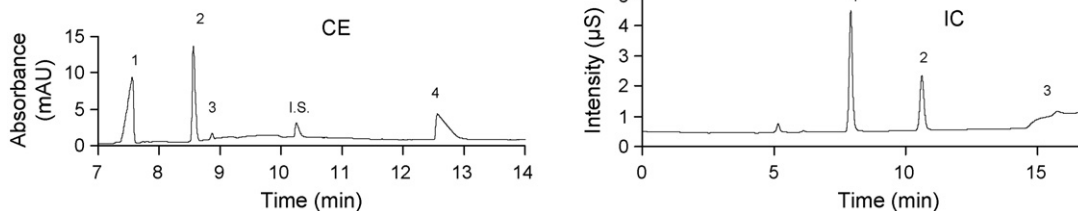


Fig. 6. CE and IC analysis of anions from an aqueous sample extract issued from post-blast residues. CE conditions, see Fig. 4. IC conditions, see Section 2.4. Identification: (1) chloride (CE, 16.5 mg L^{-1} ; IC, 15.7 mg L^{-1}), (2) chlorate (CE, 27.4 mg L^{-1} ; IC, 31.3 mg L^{-1}), (3) sulphate (CE, < LOQ; IC, masked by the baseline), (4) carbonate (CE, 26 mg L^{-1} ; IC, not searched), I.S.: formate.

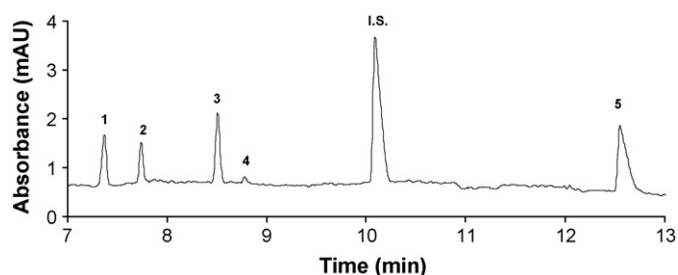


Fig. 7. CE analysis of anions in an aqueous extract of a soil originating from a pipe-bomb explosion crater. CE conditions, see Fig. 4. Identification: (1) chloride (3.8 mg L^{-1}), (2) nitrate (4 mg L^{-1}), (3) chlorate (10.4 mg L^{-1}), (4) sulphate (between LOD and LOQ), (5) carbonate (9 mg L^{-1}), I.S.: formate.

and analyzed in CE. After dilution of the aqueous extract at 1/50 in water in order to fix anion concentration in the linear range and the addition of 10% BGE, the obtained electropherogram presented in Fig. 7 showed the same qualitative anionic composition as separation presented in Fig. 6. Nevertheless, nitrate anion was detected (4 mg L^{-1}). This anion, is often naturally present in blank soil samples and thus in this analysis, nitrate anion was principally considered as an interfering anion. Chloride, chlorate, and sulphate anions were determined at about the same relative concentrations and within the same concentration range as those found in Section 3.4.2.2. Some differences can be caused by a natural presence of these anions in soil, which increased their concentration. Unfortunately, no blank soil sample was taken near the explosion to confirm the presence of these anions.

4. Conclusions

A CE method suitable for anion analysis in pre- and post-blast residues was developed. This method allowed the simultaneous separation of 19 targeted anions in less than 20 min. Enrichment factors up to 20 were obtained by FESI with the addition of 10% (v/v) of BGE in sample extracts before injection, which allowed to obtain LODs close to 0.5 mg L^{-1} . This method was validated and a substantial amount of work was dedicated to potential interfering anions and to the influence of matrix effects. Soil, metal, and cloth matrices presented significant matrix effects, but the maximum bias induced never exceeded 20%, so these effects can be tolerated, considering the method requirements. Then real pre- and post-blast residue extracts originating from detonator and

pipe-bomb explosions were analysed, showing good consistency with IC.

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