New CE methods for the determination of inorganic ions and carbohydrates in real matrices

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Analysis of post-explosion residues

- identification / quantitation of explosives used by terrorists: military, industrial, pyrotechnics or home-made formulations
  - high number of targeted inorganic and organic compounds in a large concentration range
  - great variety of matrices
Explosive formulations

**Military and industrials**
- primary: metallic salts ($\text{PbN}_3$), organics
- secondary: nitroaromatics, nitrocellulose, nitroglycerin

**Propelling powders**
nitrocellulose, nitroglycerol

**Smoke-producing, colored light-emitting, sound-emitting effects**
- anions ($\text{ClO}_3^-$, $\text{ClO}_4^-$, $\text{NO}_3^-$, $\text{SO}_4^{2-}$…)
- cations ($\text{Mg}^{2+}$, $\text{K}^+$, $\text{Ba}^{2+}$, $\text{Sr}^{2+}$, $\text{Na}^+$…)
- elements (C, S)
- carbohydrates (lactose, …)

**Home-made formulations**
- mixtures containing oxidative salts (ammonium nitrate + fuel oil, chlorate / perchlorate + carbohydrate)
- acid + aluminum foil mixtures
- peroxide-based mixtures (hydrogen peroxide + acetone / hexamethylene tetramine)

IC, GC-MS, TLC, …

❖ for forensic confirmation: complementary analytical techniques

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Aims and Scope

- CE development of
  - 2 original methods for inorganic anions and cations with LOD ~ 1 ppm, complementary to IC

<table>
<thead>
<tr>
<th>Anions</th>
<th>Cations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cl⁻, NO₂⁻, NO₃⁻, S₂O₃²⁻, ClO₃⁻, ClO₄⁻, SO₄²⁻, SCN⁻, HPO₄²⁻, HCO₃⁻, N₃⁻</td>
<td>Na⁺, K⁺, NH₄⁺, MeNH₃⁺, Sr²⁺, Ca²⁺, Ba²⁺, Mg²⁺</td>
</tr>
<tr>
<td>+ formate (internal standard)</td>
<td>+ Li⁺ (internal standard)</td>
</tr>
</tbody>
</table>

- an original method for the simultaneous analysis of anions and cations in acidic/aluminum mixtures
- an original method for carbohydrates (sucrose, lactose, glucose, fructose)
  - validation
  - application for routine analysis with real matrices

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Anion analysis

- initial conditions developed by Hutchinson et al*:
  - EOF reversed by Polybrene
  - 40 mM Tris, pH 8.2
  - 10 mM Na₂CrO₄ & 10 mM CrO₃ (anionic chromophore)

> improvement in resolution
  - ionic strength
  - solvent content
  - temperature
  - capillary length
  - voltage

Anion analysis: typical electrophoreogram

**Targeted Anions**
1. Iodide
2. Bromide
3. Chloride
4. Nitrite
5. Nitrate
6. Thiosulfate
7. Perchlorate
8. Thiocyanate
9. Azide
10. Chlorate
11. Sulfate
12. Cyanate
13. Fluoride
14. Formate (IS)
15. Chlorite
16. Acetate
17. Carbonate
18. Phosphate
19. Oxalate

**Potential Interferents**

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Bare fused-capillary 50 µm x 96 cm (detection, 87.5 cm) modified by 0.1% (w/w) polybrene. BGE: 25 mM CrO₃, 25 mM Na₂CrO₄, 100 mM Tris, pH 8.2, 6 % EtOH. Temperature, 15°C. Applied voltage, -30 kV. Electrokinetic injection, -2 kV, 50 s. Anions at 20 mg L⁻¹ injected in water/BGE (9/1, v/v). Indirect UV absorbance at 254 nm.

Anion analysis: improvement in LODs

- **IC**: LODs of ca 1 ppm for all anions (certified method ISO/CEI 17025)

<table>
<thead>
<tr>
<th>Injection</th>
<th>Anions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cl&lt;sup&gt;-&lt;/sup&gt;</td>
</tr>
<tr>
<td>Hydrodynamic, classical</td>
<td>(50 mbar, 5 s, 0.25 % V&lt;sub&gt;cap&lt;/sub&gt;)</td>
</tr>
<tr>
<td>Hydrodynamic, FASS*</td>
<td>(50 mbar, 60 s, 3 % V&lt;sub&gt;cap&lt;/sub&gt;)</td>
</tr>
<tr>
<td>Electrokinetic, FESI**</td>
<td>(- 2 kV, 50 s)</td>
</tr>
</tbody>
</table>

- **Final LODs**: < 1 ppm
- **FESI**: controlled and constant sample conductivity

*SASS: Field Amplified Sample Stacking
**FESI: Field Enhanced Sample Injection


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Anion analysis: validation (1/2)

- **LODs** (calculated for S/N = 3)
  
  from 0.3 to 0.6 mg L\(^{-1}\)

- **intermediate precision**

  (3 repeated analyses over 3 consecutive days)

  - RSD \((t_{Mi})\) 1.9 - 3.5 %
  - RSD \((t_{Mi}/t_{formate})\) 0.4 - 0.9 %
  - RSD \((A_{i})\) 2.6 - 4.8 %
  - RSD \((A_{i}/A_{formate})\) 0.6 - 2.2 %

Lab requirement < 5 %

- **linearity range**

  from 2 to 20 mg L\(^{-1}\)
matrix effects

- tested matrices: cotton, plastic, cloth, paper, soil, metal, glass
  - calibration curves for standards in water and in matrix extracts

case of NO$_3^-$

statistical comparison

- dispersions
  - Snedecor’s test
- slopes and intercepts
  - Student’s test

bias < 20% for all tested matrices ➡ negligible matrix effects

Cation analysis

- targeted cations: lack of UV absorbance
  - choice of a cationic chromophore
    - imidazole, pyridine: carcinogenic, mutagenic, reprotoxic
    - creatinine, morpholine, ephedrine: too low electrophoretic mobilities
    - guanidine
  - steepness of the UV spectrum
    ~ 190 nm

Sarazin et al., Electrophoresis, 32 (2011) 1282-1291
Cation analysis

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    - imidazole, pyridine: carcinogenic, mutagenic, reprotoxic
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    - guanidine

- steepness of the UV spectrum ~ 190 nm
- low linearity range: 0-18 mM

Guanidinium concentration fixed at 15 mM

Sarazin et al., Electrophoresis, 32 (2011) 1282-1291
Cation analysis: analysis time

- SMIL approach: Successive Multiple Ionic-polymer Layer
  - faster separation
  - more stable and pH-independent cathodic EOF

Sarazin et al., Electrophoresis, 32 (2011) 1282-1291
Targeted cations and potential interferents:

- Cations (from top to bottom): Ca\(^{2+}\), Na\(^{+}\), Mg\(^{2+}\), Fe\(^{2+}\), Co\(^{2+}\), Mn\(^{2+}\), Ni\(^{2+}\), Fe\(^{3+}\), Co\(^{3+}\), Mn\(^{3+}\), Zn\(^{2+}\), Cd\(^{2+}\), Cu\(^{2+}\), Pb\(^{2+}\), Ba\(^{2+}\), Sr\(^{2+}\), Li\(^{+}\), K\(^{+}\), MeNH\(^3\)+

Bare fused-capillary 75 µm x 80 cm (detection, 70 cm) modified (i) 1% (w/w) Polybrene and (ii) 0.01% (w/w) PVS. BGE: 15 mM guanidine acetate adjusted at pH 4.0 with acetic acid, 3 mM 18-C-6. T, 20° C. Applied voltage, + 30 kV. Indirect UV detection, 190 nm. Hydrodynamic injection (50 mbar, 4 s).

Cations at 15 mg L\(^{-1}\) (except Li\(^{+}\) (2 mg L\(^{-1}\)) and Pb\(^{2+}\) (50 mg L\(^{-1}\)) in water.

Sarazin et al., Electrophoresis, 32 (2011) 1282-1291
Cation analysis: validation

- **LODs** (calculated for $S/N = 3$)
  
  from 0.6 to 1.1 mg L$^{-1}$

- **intermediate precision**
  
  (3 repeated analyses over 3 consecutive days)
  
  - RSD ($t_{Mi}$) 1.1 - 5.9%
  - RSD ($t_{Mi}/t_{Li}^+$) 0.1 - 1.2%
  - RSD ($A_i/t_{Mi}$) 1.2 - 3.2%

- **linearity range**
  
  from 3 to 30 mg L$^{-1}$

- **matrix effects**
  
  tested: cotton, cloth, soil
  
  - bias < 20%
Analysis of acid-aluminum mixture

plastic bottle with HCl or HNO₃ acid + balls of aluminum foils

explosion = acidic gaseous release

chemical reaction

post-blast residue samples: acidic yellow liquid + metal foil pellets

❖ need for simultaneous determination of Cl⁻, NO₃⁻ and Al(III)

electrolyte containing 2,6-pyridine dicarboxylic acid (PDC)

• anionic chromophore for indirect UV detection of anions
• anionic Al(III) chelate with absorbance in UV

Bare fused silica capillary, 50 µm id x 60 cm (detection, 50 cm) coated with Polybrene (2.5 g L⁻¹ in water).
Electrolyte: 20 mM PDC adjusted at pH 4.5. Applied voltage, -30 kV. T, 20°C. UV detection, 214 nm. Hydrodynamic injection, 50 mbar, 15 s. Sample: 20 ppm Al(III), 40 ppm anions in water

simulation of the speciation diagram of aluminum with PDC

Selectivity tested with Zn(II), Cu(II), Fe(II), Ni(II), Co(II), Fe(III), Ca(II), Sr(II), Ba(II), Mg(II), S\textsubscript{2}O\textsubscript{3}\textsuperscript{2-}, SO\textsubscript{4}\textsuperscript{2-}, Cl\textsuperscript{-}, NO\textsubscript{3}\textsuperscript{-}, ClO\textsubscript{3}\textsuperscript{-}, ClO\textsubscript{4}\textsuperscript{-}, HPO\textsubscript{4}\textsubscript{2-}, SCN\textsuperscript{-}, HCO\textsubscript{3}\textsuperscript{-}

LODs (calculated for S/N = 3)
0.3 mg L\textsuperscript{-1} for Al(III) and 1.2 mg L\textsuperscript{-1} for NO\textsubscript{3}\textsuperscript{-} and Cl\textsuperscript{-}

intermediate precision (3 repeated analyses over 3 consecutive days)
- RSD (t\textsubscript{Mi}) 3.1 - 4.1 %
- RSD (t\textsubscript{Mi}/t\textsubscript{EOF}) 1.9 - 2.5 %
- RSD (A\textsubscript{i}/t\textsubscript{Mi}) 3.2 - 3.9 %

Lab requirement < 5 %

linearity range
up to 30 mg L\textsuperscript{-1}

matrix effects tested with yellow acid viscous liquid, metallic pellet extracts
- no effect

Analysis of carbohydrates

- difficult to analyze « neutral » carbohydrates in CE
  - no easily ionizable functions (pKa 12-13.5)
  - hydrophilic compounds: no interaction with micelles
  - lack of chromophore function for direct UV detection

- analytical approaches
  - derivatization
  - complexation
  - high pH and indirect UV
  - high pH and direct UV: enediolate form at 270 nm*

* Rovio et al., Electrophoresis, 28 (2007) 3129-3135
Carbohydrate analysis: method development

130 mM NaOH + 36 mM Na$_2$HPO$_4$ (Rovio) vs 130 mM NaOH

NaOH + Na$_2$HPO$_4$ vs NaOH

LOD ~ LOD
t$_{anal} > $ t$_{anal}$
N ~ N
Rs ~ Rs

NaOH

EOF inversion
- separation in 12 min
- direct UV detection
- photo-oxidation

Conditioning: Polybrene 0.1% (m/m)
Electrolyte: 130 mM NaOH

130 mM NaOH + 36 mM Na$_2$HPO$_4$ (Rovio) vs 130 mM NaOH

Capillary 50 µm x 60 cm (det. UV at 50 cm). V, +16kV. T, 20°C. Injection, 50 mbar, 4 s. Carbohydrates at 50 ppm in water.

Beckman Coulter CE-DAD system. capillary 50 µm x 60 cm (det. UV at 50 cm) modified by Polybrene 0.1% (m/m). Electrolyte: 130 mM NaOH, pH 13.1. T, 20°C. λ$_{analyse}$ = 270 nm. V, - 16 kV. Hydrodynamic injection, 50 mbar, 4 s. Carbohydrates and IS at 50 mg L$^{-1}$ in water.

Sarazin et al., Anal. Chem., 83 (2011) 7381-7387

CE Pharm 2012 – N. Delaunay et al.
Carbohydrate analysis: separation optimization

fructose, glucose, lactose, sucrose + 5 carbohydrate interferents (xylose, ribose, maltose, mannose and galactose)

Bare fused-silica capillary 50 µm id x 60 cm (det. at 50 cm) modified with Polybrene 0.1% (m/m). Electrolyte, 130 mM NaOH, pH 13.1. T, 20 °C. λdet = 270 nm. V, - 16 kV. Hydrodynamic injection, 50 mbar, 4 s (0.5 % Vcap). Carbohydrates and IS at 50 mg L⁻¹ in water.

IS = naphthalenesulfonic acid

Factors

• NaOH concentration in the electrolyte
• separation temperature
• electrolyte conductivity

Carbohydrate analysis: typical electrophoregram

Bare fused-silica capillary 50 µm id x 60 cm (det. at 50 cm) modified with Polybrene (1 g/L in water).
Electrolyte: 98 mM NaOH (pH 13.0), 120 mM NaCl. T, 26.5 °C. Voltage, -14 kV. Hydrodynamic injection, 5 s, 50 mbar. Direct UV detection at 270 nm. Concentration, 0.05 mM each in water.


CE Pharm 2012 – N. Delaunay et al.
Carbohydrate analysis: validation

- **intermediate precision** (5 repeated analyses on 3 different days)
  
  - RSD ($t_{Mi}/t_{NSA}$) 1.7-1.9 %
  - RSD (normalized corrected areas) 2.1-2.4 %

- **LOD** (S/N = 3)
  5 µM for lactose and sucrose, 7 µM for glucose, and 10 µM for fructose

- **linearity range**
  
  - 30-850 µM for glucose and fructose
  - 15-450 µM for sucrose and lactose

- **matrix effects**
  
  - tested matrices: soil, cloth, red wine, sucrose solution...
  
  - significant effects for extracts having high ionic or carbohydrate concentrations
  
  - but the resulting bias did not exceed 10 % < laboratory specifications

*Sarazin et al., Talanta, 99 (2012) 202-206*
Applications: context

- Simulation of a suicide attack in a bus

mixture of $\text{NH}_4\text{NO}_3$ & icing sugar

- residues collected on cotton swabs, extracted by water and analyzed by IC & CE
Applications: CE analysis of anions

1. Cl⁻
2. NO₃⁻
3. SO₄²⁻
4. HCO₃⁻
5. HPO₄²⁻
### Applications – anions: CE vs IC

<table>
<thead>
<tr>
<th></th>
<th>C (mg L⁻¹)</th>
<th>Cl⁻</th>
<th>NO₃⁻</th>
<th>SO₄²⁻</th>
<th>HCO₃⁻</th>
<th>HPO₄²⁻</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extract 1</td>
<td>IC</td>
<td>28 ± 2.8</td>
<td>60 ± 6.0</td>
<td>21 ± 4.2</td>
<td>NR</td>
<td>11 ± 2.2</td>
</tr>
<tr>
<td></td>
<td>CE</td>
<td>24 ± 2.4</td>
<td>63 ± 6.0</td>
<td>25 ± 2.5</td>
<td>33.4 ± 3.3</td>
<td>13.3 ± 1.3</td>
</tr>
<tr>
<td>Extract 2</td>
<td>IC</td>
<td>7.2 ± 1.4</td>
<td>176 ± 18</td>
<td>7.1 ± 1.4</td>
<td>NR</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>CE</td>
<td>8.1 ± 1.6</td>
<td>183 ± 18</td>
<td>7.4 ± 1.4</td>
<td>31 ± 3.1</td>
<td>ND</td>
</tr>
</tbody>
</table>

NR, not researched; ND, not detected

- Good correlation between CE and IC
- **NO₃⁻**: main anion present in the residue extracts
Applications – cations: CE vs IC

<table>
<thead>
<tr>
<th>C (mg L⁻¹)</th>
<th>NH₄⁺</th>
<th>K⁺</th>
<th>Ca²⁺</th>
<th>Na⁺</th>
<th>Mg²⁺</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extract 1 (dilution 1/10)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IC</td>
<td>54.5 ± 5.4</td>
<td>11.2 ± 2.2</td>
<td>36.1 ± 3.6</td>
<td>23.0 ± 2.3</td>
<td>3.4 ± 0.7</td>
</tr>
<tr>
<td>CE</td>
<td>57.3 ± 5.7</td>
<td>12.3 ± 2.4</td>
<td>39.4 ± 3.9</td>
<td>27.0 ± 2.7</td>
<td>2.7 ± 0.5</td>
</tr>
<tr>
<td>Extract 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IC</td>
<td>45.6 ± 9.1</td>
<td>3.1 ± 0.6</td>
<td>11.5 ± 2.2</td>
<td>5.6 ± 1.2</td>
<td>&lt; LOQ</td>
</tr>
<tr>
<td>CE</td>
<td>44.9 ± 8.8</td>
<td>3.5 ± 0.7</td>
<td>12.3 ± 1.2</td>
<td>7.1 ± 1.4</td>
<td>&lt; LOQ</td>
</tr>
</tbody>
</table>

NR, not researched; ND, not detected

- Good correlation between CE and IC
- NH₄⁺: main cation present in the residue extracts
Applications: CE analysis of carbohydrates

- Extract 1: no carbohydrate detected

- Quantitation:
  - Sucrose: 77 mg L\(^{-1}\)
  - Glucose: 31 mg L\(^{-1}\)
  - Fructose: 28 mg L\(^{-1}\)

- Established use of NH\(_4\)NO\(_3\) & sucrose in the explosive charge
new CE methods are available for
- inorganic anions
- inorganic cations
- neutral carbohydrates

these methods were validated for real samples and matrix effects were negligible

good correlations between IC and CE results were obtained

CE shows great interest as a method confirmation for justice inquiries
Acknowledgements

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