Organic solvent and temperature-enhanced ion chromatography-high resolution mass spectrometry for the determination of low molecular weight organic and inorganic anions

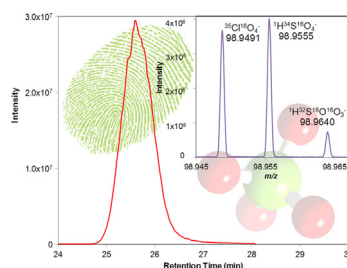Elizabeth S. Gilchrist [a], Pavel N. Nesterenko [b], Norman W. Smith [a], Leon P. Barron [a],*

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HIGHLIGHTS

- IC selectivity at high contents of organic solvent in eluent and elevated temperature is studied.
- Solvent-enhanced IC coupled to high resolution MS is beneficial for sensitive detection of ions.
- The first application of IC-HRMS to the detection of low explosives in fingermarks is shown.

GRAPHICAL ABSTRACT

ABSTRACT

There has recently been increased interest in coupling ion chromatography (IC) to high resolution mass spectrometry (HRMS) to enable highly sensitive and selective analysis. Herein, the first comprehensive study focusing on the direct coupling of suppressed IC to HRMS without the need for post-suppressor organic solvent modification is presented. Chromatographic selectivity and added HRMS sensitivity offered by organic solvent-modified IC eluents on a modern hyper-crosslinked polymeric anion-exchange resin (IonPac AS18) are shown using isocratic eluents containing 5–50 mM hydroxide with 0–80% methanol or acetonitrile for a range of low molecular weight anions (<165 Da). Comprehensive experiments on IC thermodynamics over a temperature range between 20–45 °C with the eluent containing up to 60% of acetonitrile or methanol revealed markedly different retention behaviour and selectivity for the selected analytes on the same polymer based ion-exchange resin. Optimised sensitivity with HRMS was achieved with as low as 30–40% organic eluent content. Analytical performance characteristics are presented and compared with other IC-MS based works. This study also presents the first application of IC-HRMS to forensic detection of trace low-order anionic explosive residues in latent human fingermarks.

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1. Introduction

The hyphenation of ion chromatography (IC) to mass spectrometry (MS) has been steadily gaining interest over the past two decades for a number of applications in the environmental, forensic and pharmaceutical science fields [1,2]. However even with recent developments in analytical technology, it can still represent a challenge to couple these techniques together reliably. Modern IC separations of small ions on organopolymer ion-exchange resins generally operate under aqueous conditions. For coupling to electrospray ionisation (ESI) sources, IC eluates are often modified post-suppressor with organic solvent to

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improve sensitivity [1,3,4]. Post-IC addition of ion association reagents has also been investigated to aid MS sensitivity, quantitative reliability and selectivity via the formation of ion association complexes at higher m/z, again often introduced with organic solvents [5–7]. Whilst these approaches are promising, the high mass resolution and accuracy offered by high resolution mass spectrometry (HRMS) even at the low m/z range may allow direct, sensitive and selective detection of low molecular weight anions potentially without the need for tandem MS experiments or dilution of the eluent with post-suppressor introduction of reagents/modifiers.

Early stages of IC development saw some use of organic solvents in the eluents and were mostly used to simply decrease run times, improve peak shape for hydrophobic organic anions or for column cleanup [8,9]. Conversely, their use reduced conductivity detector sensitivity (which remains the dominant approach for detection in IC) as well as having limited compatibility with some modern electrolytic devices and column packings (due to polymer particles swelling or changes in resin morphology) [10,11]. Therefore, the content of organic solvents in IC eluents was generally <30%. As a result, no significant changes were noted in the separation selectivity in these works [8]. The emergence of highly cross-linked polymer resins opened new possibilities for the optimisation of chromatographic selectivity using high organic solvent content (>50–60%) and even using entirely non-aqueous eluents [12,13]. Several papers are available from the mid-1990s on the effect of solvent on retention and selectivity in complex sample analysis [9,13,14]. However, to the best of our knowledge no recent papers comprehensively studying the chromatographic performance on modern ion-exchange resins are available. The majority of older work also used suppressed conductivity detection and, again, a reduction in eluent dielectric constant compromised sensitivity [9]. Karu et al. more recently published a series of works focusing on separations of bulky hydrophobic (logP between 2.77 and 5.77) organic anions using an anion-exchange column with eluents containing organic solvents and successfully coupled IC directly to MS [10,15–17]. In particular, this work focused on the efficiency of suppression of organic solvent-modified eluents. The organic component of these eluents was set at 40% (methanol or acetonitrile). However, these works reported a mixed-mode retention mechanism with a more pronounced contribution from hydrophobic interactions to retention, which was supported by the strong correlation between retention factors and logP values of singly charged analytes. With respect to electrostatic interactions as the dominant mode of retention, one of the only works to demonstrate the importance of eluent solvent composition on IC selectivity was published by Buchberger and Haider who showed remarkable changes in separation selectivity for inorganic anions on silica-based anion-exchangers with acetonitrile in the eluent [18]. These authors also successfully coupled IC to a single quadrupole MS using a particle beam interface.

Another condition gaining interest in IC is column temperature, and no information exists to the best of our knowledge on its effect in the presence of organic solvent-modified eluents. Unlike some other modes of liquid chromatography, the effect of temperature on retention behaviour in IC is much less predictable and several papers have discussed its associated kinetic and thermodynamic effects under aqueous conditions [19–21]. Usually, the effect of temperature, T, on chromatographic retention factor, k, can be described using the van't Hoff Eq. (1):

$$\ln k = \frac{-\Delta H}{RT} + \frac{\Delta S}{R} + \ln \varphi \tag{1}$$

where R represents the universal gas constant, ΔH and ΔS are the enthalpy and entropy change of ion-exchange respectively and ϕ is

the phase ratio. In anion exchange chromatography, ΔH depends on the type of exchangeable anion (charge, size, hydrophobicity), type of eluting ion (e.g. carbonate, hydroxide, phthalate) and its concentration [19–22]. The structure of the anion-exchanger (i.e. the type of matrix, ion-exchange capacity and structure of functional groups) also influences ΔH [20].

We recently reviewed the advancement of IC-MS technology particularly regarding the benefits of IC-HRMS for low m/z ions for low-order explosive residue detection [1]. Limited numbers of validated IC-MS methods exist for this application area [23] and none exist to the best of our knowledge using HRMS in particular. The value of HRMS in forensic analysis lies in the ability to perform non-/semi-targeted screening of samples where full scan data can be re-analysed post-acquisition [24,25]. Recent work coupling IC to HRMS was demonstrated by Burgess et al. in metabolomics using capillary-scale (0.4 mm ID) columns and suppressors resulting in fmol-pmol sensitivity for biomolecules [26,27]. A supplementary make-up flow of organic solvent was still used to aid eluent volatilisation despite the use of a heated electrospray ionisation (HESI) source [27]. Work in our laboratory has shown that capillary IC, and in contrast to other types of capillary-scale separation technologies, operates at somewhat higher flow rates ($\sim$5–10 μL min^{-1} than normal for coupling to nano-spray ionisation technologies [28]. These may also be arguably considered slightly low for ESI-MS at the micro-bore scale. Therefore, coupling micro-bore IC (2.0 mm ID) to HRMS at higher flow rates (200–500 μL min^{-1}) may still offer better performance albeit at the obvious cost of replicate analysis of samples of more limited size with capillary-scale IC.

The aim of this work was to directly couple micro-bore IC to HRMS by systematic optimisation of eluent organic solvent and hydroxide composition as well as separation temperature and assess their effects on retention, separation selectivity and efficiency, and sensitivity for small inorganic and organic anions. To the best of our knowledge, this is the first comprehensive study of the combined effects of these two variables in IC and also report the potential benefits offered when coupling to HRMS through a thorough assessment of method performance. The final objective was to apply IC-HRMS to trace forensic detection of low-order energetic materials in contaminated latent human fingermarks on glass substrates.

2. Experimental

2.1. Chemicals and reagents

All reagents were of analytical or reagent grade. Oxalate, lactate (BDH Chemicals Ltd., Poole, UK), phthalate and benzoate (Sigma–Aldrich, Gillingham, Dorset, UK) were prepared from their acids. Nitrate (BDH), perchlorate, chlorate, bromate and cyanate were prepared from their sodium salts whilst thiocyanate was prepared from its ammonium salt [29]. Bromide (Hopkin & Williams Ltd., Chadwell Heath, Essex, UK) and iodide (Alfa Aesar, Heysham, Lancashire, UK) were prepared from their potassium salts. Stock solutions were prepared at 1000 mg L^{-1}, were kept in the dark at 4 °C and were re-prepared fortnightly. Working standards were prepared daily. Eluents for anion-exchange chromatography were prepared using 50% w/v NaOH (Sigma–Aldrich). All eluents, stocks, standards and samples were prepared using ultrapure water (18.2 MΩ cm) delivered from a Millipore Synergy UV ultra-purification system (Millipore, Bedford, MA, USA), methanol or acetonitrile (Fisher Scientific, Loughborough, Leicestershire, UK) and were degassed before use. Eluents containing aqueous and organic solvents were measured in % v/v and prepared fresh every 2 days.