Method development for the redox speciation analysis of iron by ion chromatography–inductively coupled plasma mass spectrometry and carryover assessment using isotopically labeled analyte analogues

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ABSTRACT

An ion chromatography–inductively coupled plasma mass spectrometry (IC-ICP-MS) method was developed for the redox speciation analysis of iron (Fe) based on in-column complexation for Fe^{2+} and Fe^{3+} by dipicolinic acid (DPA). The effects of column type, mobile phase composition and molecular ion interference were studied in the method optimization. The carryover of the target species in the IC-ICP-MS method was uniquely and effectively evaluated using isotopically enriched analogues of the analytes ($^{54}Fe^{2+}$ and $^{57}Fe^{3+}$). Standard solutions of the enriched standards were injected into the system following analysis of a sample, and the ratios of the isotopes of iron in the enriched standards were calculated based on the chromatographic peak areas. The concentrations of the analytes carried over from the sample to the enriched standards were determined using the quantitative relationship in isotope dilution mass spectrometry (IDMS). In contrast to the routine way of evaluating carryover effect by injecting a blank solution after sample analysis, the use of isotopically enriched standards identified significant analyte carryover in the present method. Extensive experiments were carried out to systematically identify the source of the carryover and to eliminate the problem; the separation column was found to be the exclusive source. More than 95% of the analyte carryover was eliminated by reducing the length of the column. The detection limit of the IC-ICP-MS method (MDL) for the iron species was $2\,ng\,g^{-1}$. The method was used to determine Fe^{2+} and Fe^{3+} in synthetic aqueous standard solutions and a beverage sample.

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1. Introduction

The determination of iron (Fe) in terms of its redox species is important because the environmental behavior, uptake by plants, absorption by humans and other animals, transport and storage of the element depend on its oxidation states [1,2]. A variety of methods including colorimetry, spectrophotometry, chemiluminescence, potentiometry and voltammetry are known for the speciation analysis of iron [3,4]. Chromatographic methods coupled with inductively coupled plasma mass spectrometry (ICP-MS) are preferred for the determination of elemental species because of the diverse analytical advantages of the techniques which include

effective separation, wide dynamic range, superior sensitivity and selectivity, easy online coupling and fast analysis [5,6].

The redox speciation analysis of iron is extremely challenging because of the high lability, reactivity and reversibility of the species [2–4]. The challenge worsens if methods for such analysis are developed without properly addressing the key experimental parameters. Chromatographic methods for elemental speciation analyses are normally optimized by studying the effects of common parameters that include composition, pH and flow rate of the mobile phase, column type, elution mode and sample injection volume [7]. The literature shows that, in contrast to the case in bioanalytical studies such as proteomics and pharmaceutical analyses [8–10], emphasis has not been given to the effect of analyte carryover in speciation analysis. Carryover denotes detection of analyte(s) in a sample run originating from sample(s) analyzed earlier in the sequence [11,12]. It influences the accuracy and precision of a method by introducing bias in the measurement. Hence, a

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Table 1
Anion exchange columns used in the present study.

Column[a]	Length (mm)	i.d. (mm)[b]	Particle size (μm)	Capacity (μmol Cl⁻)	Working pH
Metrosep A Supp 3[c]	250	4.6	9	33	1–13
Metrosep A Supp 4[d]	250	4.0	9	71	3–12
Metrosep A Supp 5[d]	150	4.0	5	70	3–12
Metrosep A Supp 10[c]	50	4.0	4.6	43	0–14
Metrosep A Supp 10 guard[c]	5	4.0	4.6	43	0–14
Metrosep A Supp 16[c]	150	2.0	4.6		0–14
PRP ×100[c]	250	4.0	10	96	1–13

[a] All columns except PRP X100 (Hamilton) were manufactured by Metrohm.
[b] Internal diameter.
[c] Polystyrenedivinyl benzene resin with quaternary ammonium functional groups.
[d] Polyvinyl alcohol resin with quaternary ammonium functional groups.

reliable method development should properly assess the carryover of analyte(s) in the system and devise a mechanism to eliminate the effect.

Carryover in bioanalytical methods is routinely evaluated by running a blank injection following the analysis of the highest concentration calibration standard or sample [8,10]. The effect of carryover is considered to be negligible if the concentration of the analyte detected in the blank is less than 20% of the concentration in the lowest calibration standard [13]. Such evaluation, however, provides reliable information only if the blank solution effectively desorbs the residual analyte(s) from the system; erroneous conclusions may result if the blank does not elute the analyte(s). Furthermore, the carryover acceptance criterion is restricted by the dynamic range of the assay, i.e. the desorbed analyte may not be quantified accurately if its concentration is out of the calibration range.

The present study describes a unique and effective way of evaluating carryover using a solution of isotopically labeled analogue of the analyte (defined in this paper as 'enriched standard'). The enriched standard is injected following analysis of a sample, and the ratios of isotopes of the labeled element (called 'relative isotope ratios') are determined based on the peak areas of the enriched specie from its chromatogram. The concentration of the analyte carried over from the sample to the enriched standard is determined using the relative isotope ratios based on the quantitative relationship in isotope dilution mass spectrometry (IDMS) [14]. Evaluation of carryover using a solution of isotopically labeled analogue of the analyte offers several advantages. First, residuals of the analyte that may not be desorbed from the system by a blank solution can be eluted using a solution of exactly the same composition as the sample containing the analyte itself but with different isotopic composition. Furthermore, the carryover can be accurately quantified using isotopic ratios and known constants that are independent of the dynamic range of the assay.

In the present study, an ion chromatography–inductively coupled plasma mass spectrometry (IC-ICP-MS) method was developed for the determination of the redox species of iron, i.e. Fe^{2+} and Fe^{3+} based on in-column complexation of the analytes by pyridine-2,6-dicarboxylic acid, commonly known as dipicolinic acid (DPA). DPA is a known chelator for di- and trivalent metal ions including those of iron [15–17]. The two acidic protons of the ligand ($pK_a1 = 2.16$ and $pK_a2 = 4.76$) [18] enabled it to form highly stable complexes with Fe^{2+} and Fe^{3+}; $\log K_f$ 10.36 and 17.13, respectively [19]. The effects of column type, mobile phase composition and molecular ion interference were studied during the method optimization, and the carryover of the analytes was evaluated using their isotopically enriched analogues, i.e. $^{54}Fe^{2+}$ and $^{57}Fe^{3+}$. Systematic studies were undertaken to identify the source of the carryover in the system and to eliminate the effect. The IC-ICP-MS method was used to determine the two redox species of iron in synthetic aqueous standards and a beverage sample.

2. Experimental

2.1. Instrumentation and software

The ion chromatographic system was Metrohm 850 Professional IC (Metrohm). The system is metal-free and consisted of an auto-sampler (858 professional sample processor), a six-port sample injector, a pump, a column thermostat, and an eluent degasser. The columns used in this study are listed in Table 1, and the optimum conditions of the chromatographic method are described in Table 2.

An Agilent 7700 ICP-MS (Agilent Technologies) equipped with a micro-mist nebulizer, a quartz spray chamber, an octapole reaction system (ORS³), and a quadrupole mass analyzer was used. The argon and helium were of ultra high purity grade (99.999%, Airgas). The instrument was tuned on the day of every analysis using an Agilent tuning solution (1 μg L⁻¹ Li, Co, Y, Ce, and Tl in 2% HNO_3). For direct analysis by ICP-MS, samples were introduced using an auto-sampler (ASX-500 Series, Agilent Technologies),

Table 2
Optimum operating parameters of the IC and ICP-MS instruments.

IC	Metrohm 850 Professional IC
Column	Metrosep A Supp 10 guard column (anion exchange), see Table 1
Mobile phase	4.0 mM DPA and 20 mM ammonium nitrate, pH 4.3 adjusted using ammonium hydroxide
Elution mode	Isocratic
Flow rate	0.8 mL min⁻¹
Column temperature	Ambient
Injection volume	100 μL

ICP-MS	Agilent 7700
RF power	1550 W
RF matching	1.8 V
Sampling depth	8 mm
Plasma gas (Ar) flow	15 L min⁻¹
Carrier gas (Ar) flow	0.95 L min⁻¹
Makeup gas (Ar) flow	0.15 L min⁻¹
Spray chamber temperature	2 °C
ORS³ gas (He) flow	6 mL min⁻¹
Cones	Ni
Isotopes monitored	^{54}Fe, ^{56}Fe, ^{57}Fe
Data acquisition mode	Spectrum[a], time resolved analysis (TRA)[b]
Integration time per mass	0.99 s[a], 0.1 s[b]
Peak pattern	3 points per mass[a]
Replicates per analysis	4[a], 1[b]

[a] ICP-MS.
[b] IC-ICP-MS.