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Measurement of Fe(II) and Fe(III) in Groundnut by In-column and Post-column Reactions in Ion Chromatography

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The present communication deals with the development of a new specific, sensitive, rapid, and economical procedure for the determination of iron ions [Fe(III) and Fe(II)] from groundnut. Detection and measurement of both ions were performed in 25 μL of hot water leaf extract of groundnut (cv. GG 7). Both ions were detected and measured via in-column and post-column reactions with salicylic acid (SA) and 1,10-phenanthroline by ion chromatography with ultraviolet-visible detection. The Fe(III) ion was detected as the complex with salicylic acid, whereas Fe(II) ion was detected as the complex with 1,10-phenanthroline at 522 nm with different retention times. Results of the analysis were validated statistically and by recovery studies.

Keywords Fe(II), Fe(III), ion chromatography, 1,10-phenthronline, SA

Introduction

Iron (Fe) is important in the activation of several enzyme systems in plants including fumaric hydrogenase, catalase, oxidase, and cytochrome. A shortage of Fe also impairs chlorophyll production. Iron is thought to be associated with the synthesis of chloroplastic protein. Normal value of iron in groundnut is likely to be few hundred parts per million in mature leaves of normal plants (Graziano and Lamattina 2007). A deficiency of Fe first appears in the young leaves of plants, thus reducing new growth. Young leaves develop an interveinal chlorosis. In severe cases, leaves can turn completely white. In most plant species deficiencies occur when the Fe content of leaves is less than 10–80 ppm (Zhang et al. 2012).

Most of the methods are based on determination of Fe(III) or Fe(II) and/or the total Fe (Sandell 1950; Ragos, Demertzis, and Issopoulos 1998; De Costa and Araújo 2001; Paleologos et al. 2002; Ohno, Zhang, and Sakai 2003; Tesfaldet, Van Staden, and Stefan 2004; Amoli et al. 2006; De Jong et al. 2007). Saitoh and Oikawa reported determination of Fe(II) and Fe(III) by ion chromatography, where Fe(III) was reduced to Fe(II) by ascorbic acid (Kawasaki et al. 1990). Moses et al. reported determination of Fe(III) and Fe(II), but

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in determination of Fe(III) some interference occurred when Fe(II) was present (Moses et al. 1988).

For determination of heavy-metal ions, use of a complexing reagent in chromatographic separation is very popular. 8-Hydroxyquinoline (or oxine) and its derivatives have been well studied for the solvent extraction of metal ions. (Paleologos et al. 2002; Ohno, Zhang, and Sakai 2003; Starý and Smižanská 1963; Bowie et al. 1998; Takeuchi et al. 2001; Mouralian et al. 2005; Laës et al. 2005). Other examples involve bis-2-ethylhexyl succinamic acid for lanthanides (Rahni and Legube 1996), 1,5-diphenylcarbazide for Cr(VI) and/or Cr(III) after its oxidation to Cr(VI) (Weiss 1995; Šikovec et al. 1995), 1,10-phenanthroline (abbreviated as Phen in the complex) for Fe(II) and/or Fe(III) after its reduction to Fe(II) (Sandell 1950; Šikovec et al. 1995; Xu, Che, and Ma 1996), precipitation of salicylic acid (abbreviated as SA in the complex) by Fe(III) (Xu, Che, and Ma 1996), and dimethylglyoxime for Ni(II) (Sandell 1950).

In this study, Fe(III) and Fe(II) were separated and determined via in-column and post-column reaction with salicylic acid and 1,10-phenanthroline as eluent and complexing reagent. Fe(III) reacts with salicylic acid and forms a violet-colored complex that is stable for 2–3 days in the pH range of 2.5–2.7. Unlike Fe(III), 1,10-phenanthroline reacts with Fe(II) and forms an orange-colored complex in the pH range 2–9 and the material is stable for 6 months or longer. The aim of this work is to establish the most suitable conditions for routine determination of Fe(III) and Fe(II) in many plants including groundnut in a single chromatographic run.

Material and Methods

Reagents and Standards

The reagents used in this study were of analytical grade and were obtained from Sigma and Fluka. Doubly distilled water was produced in the laboratory by using a Borosil water distillation system and Milipore vacuum assembly for Mili-Q water (Millipore Corporation, Darmstadt, Germany). The standard solution of Fe(II) and Fe(III) were prepared by dissolving $FeCl_3 \cdot 6H_2O$ and $FeCl_2 \cdot 4H_2O$ in 10 mM hydrochloric acid. The eluent, 6 mM pyridine-2,6-dicarboxylic acid (PDCA) / 50 mM sodium acetate / 50 mM acetic acid, was prepared according to the standard method and the pre- and post-column reagent solutions were prepared by dissolving salicylic acid and 1,10-phenanthroline in ethanol and water. All standard solutions were stored in polyethylene containers and kept under refrigeration at 4 °C. All reagents and working standard were freshly prepared prior to use for analysis.

Ultraviolet Spectrophotometry

A U-3500 UV spectrophotometer (Hitachi, Tokyo, Japan) was used to determine maximum wavelengths for Fe(III)-SA and Fe(II)-Phen complexes. Complexes of Fe(III)-SA came from 0.26 mM Fe(III) reacted with 150 mM salicylic acid (in 40 percent ethanol) and complexes of Fe(II)-Phen came from 0.06 mM Fe(II) reacted with 20 mM 1,10-phenanthroline (in 50 percent ethanol).

Sample Preparation

Thirty-day-old groundnut (cv. GG 7) leaves were collected and kept for shed drying for 7 days. Dried leaves were ground in a mixture and a hot-water extract was made by mixing

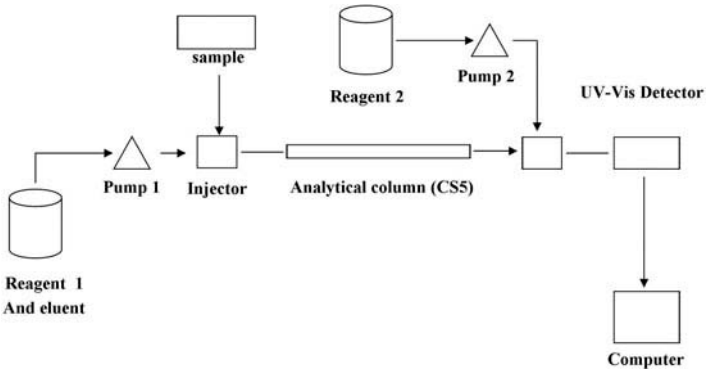

Figure 1. Flow chart of chromatographic condition for separation of Fe(III) and Fe(II) by using ion chromatography (ICS 3000).

1 g of leaf powder with hot distilled water followed by vigorously shaking. Supernatant was collected after centrifuge at 7000 rpm for 15 min. The supernatant was filtered through 0.2-μ filter paper prior to injection in the analytical column.

Chromatographic Condition

For analysis of Fe(II) and Fe(III), an ion chromatography instrument ICS 3000 series (Dionex, Sunnyvale, CA) was used. IonPac CS 5 analytical column along with guard column was used for separation of both ions. A mixture of 6 mM PDCA / 50 mM sodium acetate / 50 mM acetic acid were used as gradient eluent for the entire analysis. The flow rate for the eluent/complexing reagent 1 (150 mM salicylic acid in 40 percent ethanol) was kept at 2 mL min^{-1} and that for the complexing reagent 2 (20 mM 1,10-phenanthroline in 50 percent ethanol) was kept at 2.0 mL min^{-1}. Reagent 1 was applied along with gradient eluent whereas reagent 2 was applied after column by help of another pump. The UV-vis detector was operated at 522 nm. The chromatographic condition is shown in Figure 1.

Results and Discussion

Iron is very important micronutrient in plants for growth and development.

Optimum Wavelength for Iron Complexes

Iron complex with salicylic acid and 1,10-phenanthroline showed absorptions in the 400- to 600-nm range, whereas Fe(II)-SA and Fe(III)-Phen complexes do not have sufficient absorptions in the 400- to 600-nm range. The maximum wavelength for Fe(II)-Phen complex is 520 nm with absorbance of 0.642 and molar extinction coefficient of 1.1 ± 104 M^{-1} cm^{-1}, whereas that for Fe(III)-SA complex is 524 nm with absorbance of 0.458 and molar extinction coefficient of 1.8 ± 103 M^{-1} cm^{-1}. Figure 2 indicates that only Fe(III) forms a stable complex with salicylic acid, whereas only Fe(II) forms a stable complex with 1,10-phenanthroline ($C_{12}H_8N_2$).

Differences in the maximum wavelengths for Fe(III) and Fe(II) were very small and because of this the wavelength should be optimized. Wavelength of 522 nm was chosen for the following experiments as the mean of the two maximum wavelengths for Fe(III)-SA

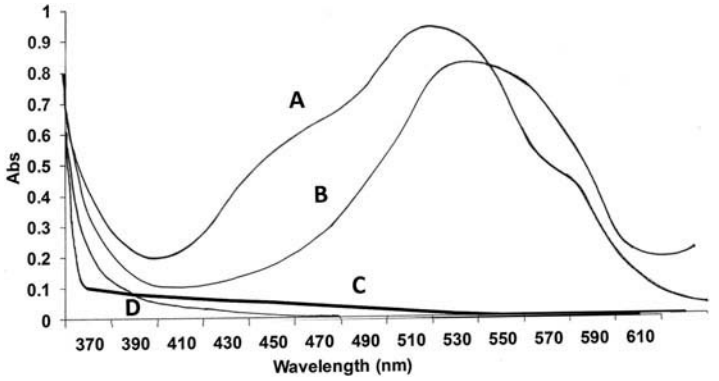

Figure 2. Absorption spectra of Fe(II)-Phen complex (A: 0.06 mM Fe(II) with 20 mM 1,10-phenanthroline in 50 percent ethanol), Fe(III)-SA (B: 0.26 mM Fe(III) with 150 mM salicylic acid in 40 percent ethanol), Fe(II)-SA (C: 0.26 mM Fe(II) with 150 mM salicylic acid in 40 percent ethanol), and Fe(III)-Phen (D: 0.06 mM Fe(III) with 20 mM 1,10-phenanthroline in 50 percent ethanol).

and for Fe(II)-Phen complexes. Similarly Oktavia, Lim, and Takeuchi (2008) found that 518 nm was the optimum wavelength for measurement of both Fe ions in their study.

In-column and Post-column Derivatization

The Fe(III)-SA complex is formed while traveling in the CS5 column (in-column derivatization), whereas Fe(II)-Phen complex is formed via post-column reaction (post-column derivatization). Silica can act as a cation exchanger by using weak acid (pH 2 to 9) as the eluent. Salicylic acid in 40 percent ethanol was supplied from micropump 1 not only as the eluent but also as the complexing reagent for Fe(III). On the other hand, 1,10-phenanthroline in 50 percent ethanol was supplied from micropump 2 for post-column reaction for Fe(II). Complexation was influenced by pH of these solutions (apparent pH of 150 mM salicylic acid in 40 percent ethanol and 1,10-phenanthroline in 50 percent ethanol employed in this research was 2.29 and 8.20, respectively) and the composition of the complexing reagents. The concentrations of salicylic acid and ethanol were important criteria and were investigated.

Figure 3 shows chromatograms for standard solutions of Fe(III) and Fe(II). The standard solution was prepared in 10 mM hydrochloric acid and the concentrations of Fe(III) and Fe(II) were 25 mg mL^{-1} each. The blank solution was 10 mM hydrochloric acid. It is observed that retention times of both complexes are different and both are completely separated within 15 min. The retention of the analytes on the column depends on their ionic charge. It is expected that Fe(III) forms a charged complex of $[Fe(C_7H_5O_3)_2]^+$ on the separation column, whereas Fe(II) travels without forming a complex in the column and forms a positive-charge complex with 1,10-phenthroline after elution from the column (post-column). Silica gel acts as a cation exchanger to separate $[Fe(C_7H_5O_3)_2]^+$ and Fe^{2+}.

Effects of Eluent Concentrations on the Retention of Complexes

It was reported in many studies that retention times of the metal complexes were strongly affected by eluent composition. To obtain the optimum eluent condition, the concentrations of SA and ethanol in the eluent were varied. Figure 4 presents the retention behavior

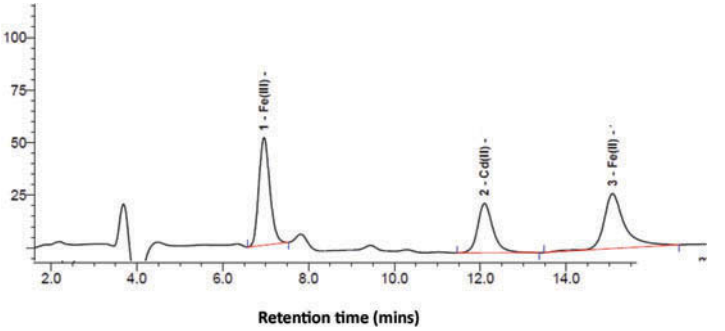

Figure 3. Chromatograms of standard (Fe(III)-SA, Fe(II)-Phen). Column, CS5; flow rate, eluent/complexing reagent 1 (150 mM salicylic acid in 40 percent ethanol), at 2 mL min^{-1} and complexing reagent 2 (20 mM 1,10-phenanthroline in 50 percent ethanol) 2.0 mL min^{-1}; injection volume, 25 μL; wavelength of detection, 522 nm. Peaks: 1, Fe(III)-SA complex; 2, other metal (cadmium); and 3, Fe(II)-1,10-Phen. Standard Fe(III) and Fe(II), 25 ppm.

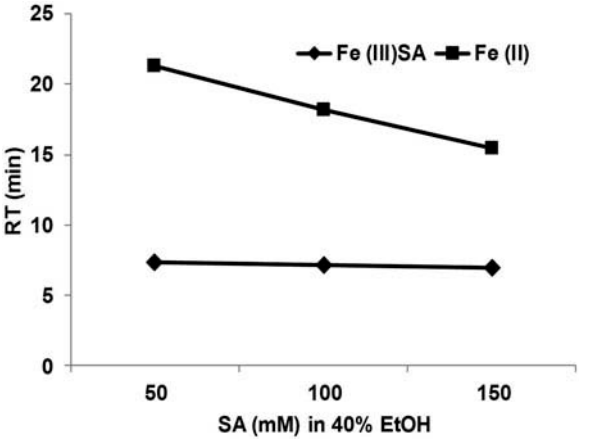

Figure 4. Effects of concentration of salicylic acid on the retention time. Operating conditions are the same as in Fig. 1 except for the eluents as indicated.

of Fe(III)-SA and Fe(II) on the CS5A column. It can be seen that when the concentration of salicylic acid increased, the retention time of the analytes decreased. This is a normal phenomenon because more H+ exists in higher concentration eluent, leading to faster elution of the analytes.

Effects of ethanol concentration in the eluent on the retention of the analytes are shown in Figure 5. When the concentrations of ethanol increased, the retention times of the analytes increased. This is because the driving force of the eluent based on cation exchange decreases with increasing ethanol concentration. Ethanol is required for dissolving high concentrations of salicylic acid. The concentration of salicylic acid around 150 mM and that of ethanol around 40 percent were chosen for the following experiment, because these conditions achieved complete separation of Fe(III)-SA and Fe(II) within a reasonable time.

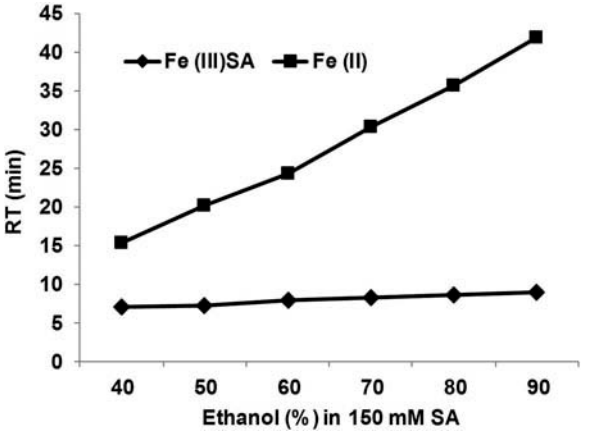

Figure 5. Effects of concentrations of ethanol in eluent on the retention time. Operating conditions are the same as in Fig. 1 except for the eluents as indicated.

Calibration Curve

The linearity of calibration for Fe(III) and Fe(II) was studied, where the concentrations of Fe(III) and Fe(II) were varied from 2.5 to 50 mg mL^{-1} under the optimum operating condition as in Figure 3. The peak areas were linear to the Fe(III) concentration up to 50 mg mL^{-1} with a correlation factor (R^2) of 0.99193 and Fe(II) concentration up to 50 mg mL^{-1} with R^2 of 0.9938.

The linearity of the standard additional method for groundnut (cv. GG 7) leaf samples has been also studied. The linear regressions for Fe(III) and for Fe(II) were $y = (9.87 \pm 103)x + 1.90 \pm 104$ with R^2 of 0.9944 and $y = (3.60 \pm 103)x + 1.86 \pm 106$ with R^2 of 0.9977, respectively.

The operating condition for the determination of Fe(III) and Fe(II) in groundnut leaf samples was validated. Table 1 shows the repeatability of the retention time and the peak signals under the conditions in Figure 3. The relative standard deviation (RSD) values for five successive measurements were less than 0.54 percent for retention time, whereas those for the peak height and peak area were less than 1.4 percent.

Application to Plant Sample

As an application to plant analysis, Fe(III) and Fe(II) contained in groundnut leaf sample collected from our experimental plots at National Research Center for Groundnut,

Table 1
Relative standard deviations (RSD) of detector signals of iron complex

		RSD, % ($n = 5$)		
Complex	Concentration of iron (mg L^{-1})	Retention time	Peak area	Peak height
Fe(III)-SA	25	0.53	1.4	1.5
Fe(II)-Phen	25	0.23	0.75	0.88

Junagadh, India, were analyzed by this method. Groundnut leaves were collected and homogenized using hot water. The extract of leaf was applied for analysis of Fe(III) and Fe(II) as these two metals are very important for activation of number of enzymes in plants. As shown in Figure 6, Fe(III) and Fe(II) in the groundnut leaf sample could be separated by using this method without any difficulties. It can be seen that there were two peaks (1 and 2) appeared from the leaf sample at the same retention time as for the standard Fe(III)-SA and Fe(II)-Phen.

The concentration of Fe(II) in the leaf sample taken in Rabi season in Table 2 was also cross-checked with a spectrometric method using 1,10-phenanthroline as the complexation reagent. The concentration of Fe(II) measured with the ion chromatography method was 254.9 ± 0.4, whereas that obtained by the spectrometric method was 254.3 ± 0.3 with student's t-test = 2.14, showing that there is no significant difference between the two data.

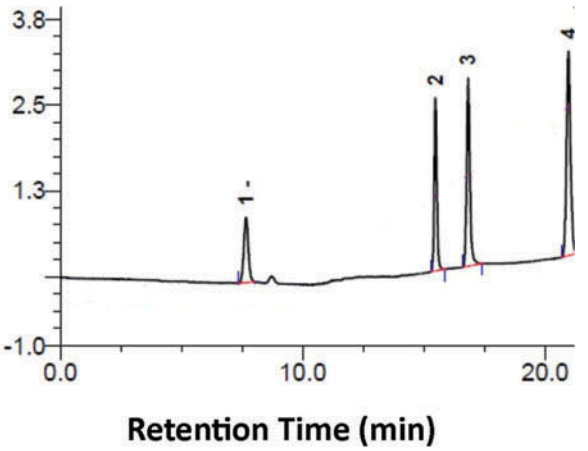

Figure 6. Chromatogram of groundnut leaf sample. Operating conditions are the same as in Fig. 3 except for the sample, groundnut leaf hot water extract 25 μL. Peak 1, Fe(III); peak 2, Fe(II); and peaks 3 and 4, other metals.

Table 2
Concentrations of Fe(III), Fe(II), and total Fe in groundnut leaf samples

		IC method[a] (mg L^{-1}) ($n = 3$)			ICP method (mg L^{-1}) ($n = 3$)	Student's
No.	Date of sample	Fe(III)	Fe(II)	Total Fe	Total Fe	t-test
1	Rabi seasons	52.47 ± 0.06	200.45 ± 0.07	254.64 ± 0.3	255.43 ± 0.5	0.87
2	(December)	56.87 ± 0.03	204.15 ± 0.01	253.47 ± 0.6	254.35 ± 1.0	1.46
3		53.9 ± 0.12	210.03 ± 0.04	256.16 ± 0.5	254.68 ± 0.4	0.74
4		54.5 ± 0.02	201.28 ± 0.02	255.36 ± 0.4	256.17 ± 0.6	0.57

Note. Student's t-test with probability 97 percent = 1.9934 (freedom = 4).
[a]IC, ion chromatography.

Conclusion

Fe(III) and Fe(II) were successfully determined via complexation with salicylic acid and 1,10-phenanthroline using in-column and post-column reactions in ion chromatography (ICS 3000) in a single chromatographic run using a CS5A analytical column. The present method provides simple routine determination of Fe(III) and Fe(II) in environmental samples including plants, soil, and water without any difficulties.

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