AgEcon SEARCH
RESEARCH IN AGRICULTURAL & APPLIED ECONOMICS

The World's Largest Open Access Agricultural & Applied Economics Digital Library

This document is discoverable and free to researchers across the globe due to the work of AgEcon Search.

Help ensure our sustainability.

Give to AgEcon Search

AgEcon Search
http://ageconsearch.umn.edu
aesearch@umn.edu

*Papers downloaded from **AgEcon Search** may be used for non-commercial purposes and personal study only. No other use, including posting to another Internet site, is permitted without permission from the copyright owner (not AgEcon Search), or as allowed under the provisions of Fair Use, U.S. Copyright Act, Title 17 U.S.C.*

Studies on the Simultaneous Determination of Cr(III) and Cr(VI) by Ion Chromatography (IC)

Guangmin LIU[1,2], Xin LIU[3], Feng GAO[3], Na SUN[1,2], Liping HU[1,2], Yaqin WANG[1,2], Hongju HE[1,2]*

1. Vegetable Research Center of Beijing Academy of Agriculture and Forestry Sciences, Beijing 100097, China; 2. Key Laboratory of Urban Agriculture (North China), Ministry of Agriculture, Beijing 100097, China; 3. Beijing Entry – Exit Inspection and Quarantine Bureau, Beijing 100026, China

Abstract The research aimed to develop and validate an ion chromatography method for the simultaneous analysis of Cr(III) and Cr(VI) from pumpkin. A new analytical method based on ion chromatography techniques was developed by the Cr(III) pre-column derivatization and Cr(VI) post-column derivatization. The ion chromatography condition was optimized and the detection sensitivity was improved. Cr(III) and Cr(VI) were determined by 365 and 530 nm, respectively. The temperature of water bath, the heating time for pre-column derivatization, and the flow rate of post-column derivative liquid were screened on the basis of single factor experiment, the effects of various factors were determined by the method of $L_{16}(4^3)$ orthogonal experiment design. Considering the results of orthogonal experiments and the variation tendency of peak area under different factors, the optimum derivatization conditions were chose as follows: the flow rate of post-column derivative liquid is 0.5 mL/min, the temperature of water bath for pre-column derivatization is 100 ℃ and the heating time is 5 min. The conditions were optimized by means of orthogonal experiments under the pH of leacheate ranged from 6.5 to 6.8 and the I^- concentration of 5 mmol/L. Under optimized derivatization conditions, the detection limits for Cr(III) and Cr(VI) were 0.17 and 0.019 mg, respectively. And the average recoveries of Cr(III) in pumpkin were in the range of 82% – 85%. Results indicated that pumpkin does not contain Cr(VI) according to this ion chromatography method of simultaneously determination for Cr(III) and Cr(VI).

Key words Pumpkin, Cr, Speciation analysis, Ion chromatography

1 Introduction

With deep studies on environmental chemistry, more and more people realize that chemical speciation of the element shows different actions on environment and organism. Behavior effect of the element does not depend on its total amount, and only specific element could act on life system and organism in a certain concentration range and existence speciation[1-2]. Biological toxicity and physiological activity of the element are closely related to its speciation, and element's speciation affects environment and human health to a different degree. Cr(III) is essential trace element in human body, which participates in glucose and fat metabolisms of human body by synergistic action with insulin or taken as an enhancer of insulin. And Cr(VI) is mobile in biological system, and induces cell lesion, which is strong carcinogen[3]. Cr belongs to the VIB family in the periodic table of elements, and inorganic chromate has three kinds of chemical forms: Cr(II), Cr(III) and Cr(VI). Among them, Cr(II) is easy to be oxidized into high-valence Cr, which is very unstable, and it is only stable under anoxic condition[4]. Hence, Cr(III) and Cr(VI) are common speciation. Cr(VI) exists in the form of H_2CrO_4 or chromate, such as $HCrO^{4-}$ or CrO_4^{2-}. Cr(III) has more complex reactions in natural environment, and it exists in the forms of $CrOH^{2+}$, $Cr(OH)_2^+$, $Cr(OH)_3$ precipitate and $Cr(OH)^{4-}$ in the solution. Additionally, there is little $Cr_2(OH)_2^{4+}$ and $Cr_3(OH)_4^{5+}$ because of very slow reaction rate at room temperature. At present, it still uses spectrophotometry to detect Cr(VI) in national standard, but there is interference when detecting the sample with deep color by spectrophotometry, and trace Cr(VI) could not be detected[5-6]. The detection method of Cr(III) is that total Cr is measured firstly, and then Cr(III) content is counted by subtraction[5]. However, these detection methods have several disadvantages: complex operation, larger error and interference. With the progress of science and technology in China, chromatography and spectroscopy techniques are also evolving, such as atomic fluorescence spectrometry[7-8], ion chromatography – UV detection[9], liquid chromatography – inductively coupled plasma mass spectrometry[10-13] and ion chromatography – inductively coupled plasma mass spectrometry[14]. The application of these new techniques greatly improve detection sensitivity of Cr(III) and Cr(VI). In this experiment, the optimal pH of extracting Cr(III) and Cr(VI), suitable heating temperature and heating time of pre-column derivatization were explored, and concentration and suitable flow rate of leacheate were optimized. The report showed that the complex generated by 2, 6-pyridinedicarboxylic acid and Cr(III) in the case of heating and boiling had UV absorption at 335 nm[15]. In the experiment, 2, 6-pyridinedicarboxylic acid chromium generated by pre-column derivatization of Cr(III) from the sample was detected at 365 nm within 0 – 4.5 min. Although it was not the maximum absorption wavelength of Cr(III), the detection at 365 nm could effectively decrease the interference of pumpkin matrix.

Received: February 13, 2017 Accepted: April 7, 2017

Supported by Science and Technology Innovation Ability Construction Special Item of Beijing Academy of Agriculture and Forestry Sciences (KJCX20140302, KJCX20140111).

* Corresponding author. E-mail: hehongju@nercv.org

Via post-column derivatization, Cr(VI) of the sample became 1, 5-diphenylcarbazide chromium, and the detection of 530 nm within 4.5 – 8.0 min was conducted. The method has completed determination of Cr(III) and Cr(VI) from multiple pumpkin varieties, with good sensitivity. At present, there is not report on ion chromatography applying in speciation analysis of Cr element from pumpkin and even vegetable, and the research could provide detection method and reference basis for vegetable nutrition and safety quality.

2 Materials and methods

2.1 Materials and reagents Materials: test materials were from Vegetable Research Center Base of Beijing Academy of Agriculture and Forestry Sciences. Pumpkin variety with higher total Cr content was screened by atomic absorption spectrophotometry.

Reagents: 2, 6-pyridinedicarboxylic acid (PDCA, purity 99%); 1, 5-diphenylcarbazide (DPC, AR); disodium hydrogen phosphate dodecahydrate ($NaHPO_4 \cdot 12H_2O$, AR); potassium iodide (KI, GR); ammonium acetate ($CH_3CO_2NH_4$, AR); lithium hydroxide (LiOH, 56%); methanol (GR); sodium carbonate anhydrous (Na_2CO_3, AR); sodium bicarbonate anhydrous ($NaHCO_3$, AR); sulfuric acid (GR); hydrochloric acid (BV-III Grade); Cr(III) standard reagent (1000 mg/L), Cr(VI) standard reagent (100 mg/L); pH reference powder; ultra pure water (18.2 MΩ · cm).

2.2 Instruments and devices DIONEX ICS-5000 DC-5 type of ion chromatograph; ICS-5000 EG-5 type of leacheate automatic generator; DIONEX AS-AP automatic sampler; DIONEX ICS-SERIES VWD UV detector; ICS-3000 DP-1 type of pump; MILLI-Q pure water instrument; pH meter (PB-10, Sartorius Scientific Instrument Beijing Co. Ltd.); BECKMAN high-speed centrifuge; Sartorius TE612-L electronic balance; OnGuard IIRP pretreatment column (1.0cc); 0.45 μm of nylon filter membrane; IonPac CS5A analytical column (4 mm × 250 mm); IonPac CG5A protective column (4 mm × 50mm)

2.3 Reagent preparation Mixed standard solution: Cr(III) and Cr(VI) reserve solutions (mass concentrations were 1.0 and 0.1 mg/L respectively) were diluted into five groups of mixed standard solutions with different mass concentrations: 0.4 and 0.04 mg/L, 0.2 and 0.02 mg/L, 0.1 and 0.01 mg/L, 0.05 and 0.005 mg/L, 0 and 0 mg/L.

Leacheate reserve solution: 1.67 g of PDCA, 3.58 g of Na_2HPO_4, 4.65 g of $NaI \cdot 2H_2O$, 19.27 g of $CH_3CO_2NH_4$ and 0.59 g of LiOH were taken in 500 mL of ultra pure water, and pH of PDCA reserve solution was 6.5. PDCA reserve solution was diluted for 10 times, and then ion chromatography leacheate was obtained.

Post-column derivatization solution: 0.24 g of DPC was taken in 50 mL of methanol, and 12 mL of concentrated sulfuric acid was added in 100 mL of ultra pure water. After sulfuric acid solution cooled, the two kinds of solutions were mixed, and ultra pure water was used to fix the volume to 500 mL.

2.4 Sample pretreatment Three same-variety pumpkins were taken randomly. After freezing and drying, they were grinded into powder, and then the three treated samples were mixed, thereby obtaining detection sample of a pumpkin variety. 2.5 g of sample was taken in 50 mL of centrifuge tube, and the prepared PDCA leacheate was used to fix the volume to 25 mL. It was centrifuged for 20 min at the velocity of 8000 r/min. After total supernatant was filtered by filter paper, organic matter was removed by RP pre-treatment column, and then 0.45 μm of nylon filter membrane was used to remove impurity. 5 mL of treated sample solution was taken in 10 mL of colorimetric tube with the plug, and PDCA leacheate was used to fix the volume to the graduate. It was heated for 5 min in 100 ℃ of water bath. After cooling, 2 mL of solution was filtered by 0.45 μm of nylon filter membrane, which was collected in sample bottle for determination. Meanwhile, blank control was prepared.

2.5 Ion chromatography conditions Leacheate: the mixed solution of 2 mmol/L PDCA, 2 mmol/L Na_2HPO_4, 5 mmol/L NaI $\cdot 2H_2O$, 50 mmol/L $CH_3CO_2NH_4$ and 2.8 mmol/L LiOH. Post-column derivatization reagent: the mixed solution of 2 mmol/L DPC, 10% (*V/V*) methanol and 1.8 mol/L sulphuric acid. Flow rate of leacheate: 1.0 mL/min. Flow rate of post-column derivatization solution: 0.5 mL/min. UV detection wavelength: 0 – 4.5 min, wavelength is 365 nm; 4.5 – 8.0 min, wavelength is 530 nm. Retention time was used for qualitation, and peak area was used to quantify.

3 Results and analyses

3.1 Optimization of ion chromatography conditions

3.1.1 Selection of separation manner. Cr(III) reacted with PDCA into $Cr(PDCA)^{2-}$ under pH≥6.5, while Cr(VI) mainly existed in the form of CrO_4^{2-} but did not react with PDCA, and they were separated by anion exchange column. In this experiment, CS5 separation column with good chemical stability and high column capacity was used. Cr(III) needed pre-column derivatization because of slow derivatization reaction velocity. Before injecting sample, sample solution was mixed with adequate amount of PDCA, and then it was heated for 5 min in boiling bath.

3.1.2 Effect of leacheate pH. pH could affect the existing forms of Cr(III) and Cr(VI) and the stability of $Cr(PDCA)^{2-}$, thereby affecting peak occurrence time, peak shape, dead space peak occurrence and peak area of ion chromatogram, which is key of Cr speciation analysis. The existing form of Cr(VI) depends on solution's pH and its own concentration. When pH is less than 1, Cr(VI) exists in the form of H_2CrO_4; when pH is more than 6, Cr(VI) exists in the form of CrO_4^{2-}; when pH is between 1 and 6, Cr(VI) exists in the form of $HCrO_4^-$. When Cr(VI) concentration exceeds 1 g/L, $Cr_2O_7^{2-}$ could be formed. For Cr(III), it mainly exists in the forms of $CrOH^{2+}$ and $Cr(OH)_2^+$ under acidic condition, generates $Cr(OH)_3$ precipitate under alkaline condition, and

forms $Cr(OH)_4^-$ under strong alkaline condition. It is clear that pH difference induces that the existing valences of Cr(III) and Cr(VI) in the solution change. When there are large amounts of antioxidants in the solution, pH decline is easy to cause that Cr(VI) transforms into Cr(III). To guarantee the reliability of analytic result, speciation stability of Cr(III) and Cr(VI) should be ensured firstly[16].

When determining Cr(III) and Cr(VI) standard solutions with the same concentration, pH could affect peak occurrence time (retention time), peak shape, dead space peak occurrence and peak area of ion chromatogram. The effect of pH on Cr(III) and Cr(VI) retention time was shown as Fig. 1. Seen from Fig. 1, pH had little impact on Cr(VI) retention time, while Cr(III) retention time showed the tendency of rising firstly and then declining with pH increased. The effect of pH on Cr(III) and Cr(VI) peak areas was shown as Fig. 2. Seen from Fig. 2, pH had great impact on peak area of Cr(III). Besides, pH also affected dead space peak occurrence. Both Cr(III) and Cr(VI) started to have dead space peak occurrence under pH = 7, and the peak was high, which was not conducive to quantitation. For Cr(VI), peak shape changed when pH was 9 and 10, and it started to show asymmetrical shape. When pH was less than 6, chromate could transform into dichromate, and dichromate could harm column. In a word, considering system's stability, separation and detection of Cr(III) and Cr(VI), leacheate pH was controlled between 6.5 and 6.8.

3.1.3 Leacheate selection. By reviewing lots of literatures, the mixed solution of 2 mmol/L PDCA, 2 mmol/L Na_2HPO_4, 10 mmol/L NaI · $2H_2O$, 50 mmol/L $CH_3CO_2NH_4$ and 2.8 mmol/L LiOH was taken as leacheate for determining Cr(III) and Cr(VI) contents. The experiment result of pumpkin sample was shown as Fig. 3. It was clear that chromatographic peak could not separate from system peak, and peak shape was not very symmetric, thereby affecting detection result of Cr(III). In the leacheate, NaI mainly played the role of leaching. In the experiment, by appropriately declining NaI concentration, Cr(III) and Cr(VI) retention time was prolonged, thereby separating chromatographic peak of Cr(III) from system peak well, and guaranteeing stable pressure and datum line when detecting Cr(III) and Cr(VI). Via adjustment, the mixed solution of 2 mmol/L PDCA, 2 mmol/L Na_2HPO_4, 5 mmol/L NaI · $2H_2O$, 50 mmol/L $CH_3CO_2NH_4$ and 2.8 mmol/L LiOH was taken as leacheate, and pH was adjusted as 6.5 – 6.8 by $CH_3CO_2NH_4$ and LiOH.

3.1.4 Orthogonal experiment of ion chromatography conditions. Pre-column derivatization of Cr(III) has a heating process, and heating temperature and time directly affect sensitivity of Cr(III) analysis. Additionally, flow rates of leacheate and post-column derivatization solution also affect the experiment result. Flow rate of post-column derivatization reagent is closely related to post-column derivatization efficiency of Cr(VI). In this experiment, factor levels of water bath heating temperature, heating time of pre-column derivatization and flow rate of post-column derivatization solution were made according to prior work (Table 1).

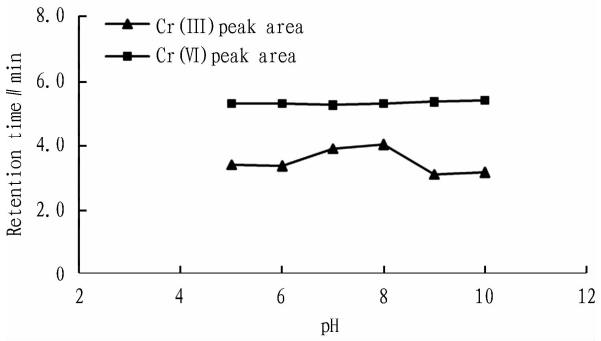

Fig. 1 Effect of pH on Cr(III) and Cr(VI) retention time

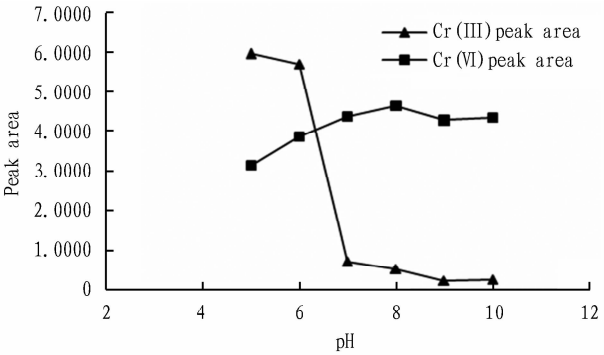

Fig. 2 Effect of pH on Cr(III) and Cr(VI) peak area

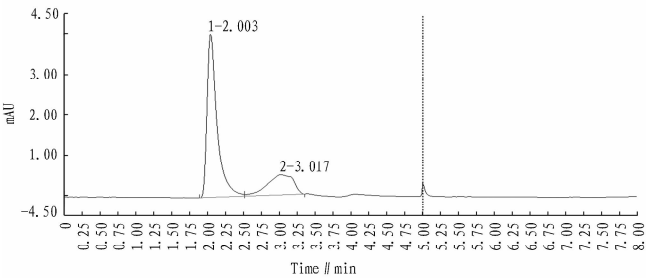

Fig. 3 Chromatogram of pumpkin sample

Table 1 Orthogonal design factors and levels of optimizing ion chromatography conditions

Level	Factor		
	Water bath heating temperature A//℃	Water bath heating time B//min	Flow rate of post-column derivatization solution C//mL/min
1	40	1	0.5
2	60	3	0.8
3	80	5	1.0
4	100	7	1.2

Table 2 The results of the orthogonal experiments[$L_{16}(4^3)$] about optimizing IC conditions

No.	A	B	C	Cr(III) peak area	Cr(VI) peak area
1	1	1	1	0.0426	3.6998
2	1	2	2	0.0534	3.8361
3	1	3	3	0.0728	3.7659
4	1	4	4	0.0633	3.8997
5	2	1	3	0.1358	3.7286
6	2	2	4	0.4762	3.7455
7	2	3	1	0.6330	3.8034
8	2	4	2	0.6189	3.7960
9	3	1	4	0.2934	3.6975
10	3	2	3	3.7937	3.7217
11	3	3	2	5.7280	3.7764
12	3	4	1	5.6986	3.6887
13	4	1	2	0.3705	3.7996
14	4	2	1	4.8011	3.7148
15	4	3	4	5.1655	3.8130
16	4	4	3	5.7521	3.8079
Cr(III) K_1	0.2321	0.8423	11.1753		
K_2	1.8639	9.1244	6.7708		
K_3	15.5137	11.5993	9.7544		
K_4	16.9892	12.1323	5.9984		
Cr(VI) K_1	15.2015	14.9255	14.9067		
K_2	15.0375	15.0181	15.2081		
K_3	14.8843	15.1587	15.0241		
K_4	15.1353	15.1923	15.1557		
Cr(III) k_1	0.0580	0.2106	2.7938		
k_2	0.4660	2.2811	1.6927		
k_3	3.8784	2.8998	2.4386		
k_4	4.2473	2.8670	1.4996		
Cr(VI) k_1	3.8003	3.7314	3.7267		
k_2	3.7594	3.7545	3.8020		
k_3	3.7211	3.7897	3.7560		
k_4	3.7838	3.7980	3.7889		
Cr(III) R	4.1893	2.8224	1.2942		
Cr(VI) R	0.0729	0.0666	0.0753		

The experiment was conducted by randomly arranging level of each factor and using $L_{16}(4^3)$ orthogonal experiment table, thereby optimizing water bath heating time, heating temperature and flow rate of post-column derivatization reagent (Table 2). Seen from Table 2, range sequence of Cr(III) was $R_A > R_B > R_C$, that is to say, water bath heating temperature before derivatization was the most main influence factor of Cr(III) analysis sensitivity, followed by water bath heating time, and flow rate of post-column derivatization solution had the minimum influence. Range sequence of Cr(VI) was $R_C > R_A > R_B$, that is to say, flow rate of post-column derivatization solution was the most main influence factor of Cr(VI) analysis sensitivity. Because that Cr(VI) was post-column derivatization, conditions A and B had little influence on Cr(VI) analysis and detection. Fig. 4 – Fig. 6 respectively showed change trend of peak area under each factor.

Seen from Fig. 4 – Fig. 6, Cr(III) peak area increased with water bath heating temperature rose. Cr(III) peak area was the minimum at 80 ℃; when temperature reached 80 ℃, peak area

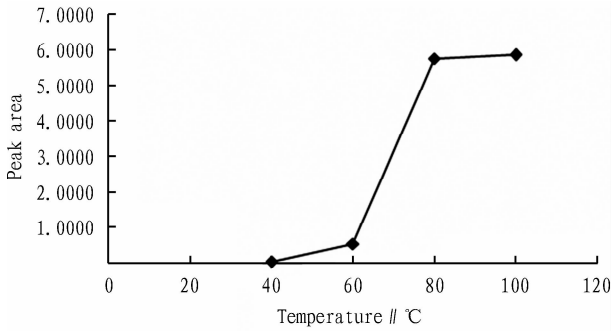

Fig. 4 The curve of Cr(III) peak area versus water bath temperature

increased slowly. Cr(III) peak area also increased with water bath heating time prolonged. When heating time reached 5 min, although heating time continued to increase, peak area changed slowly. Cr(VI) peak area changed slowly with flow rate of post-column derivatization solution increased. When flow rate of post-

column derivatization solution increased to 1.2 mL/min, Cr(III) peak area became smaller. When flow rate of post-column derivatization solution was 0.5 mL/min, it had higher detection SNR. By comprehensive considering, flow rate of post-column derivatization solution selected 0.5 mL/min, while pre-column derivatization used 100 ℃ as the optimal water bath temperature, and 5 min was the best heating time.

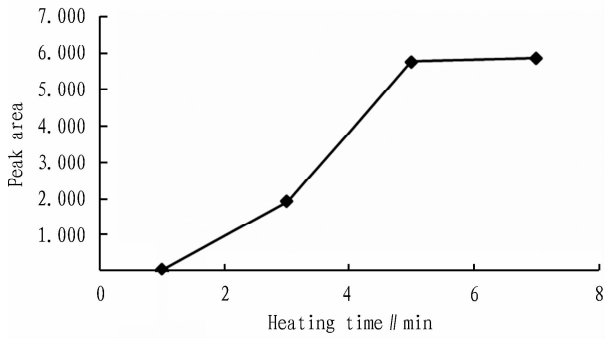

Fig. 5 Effect of water bath time on Cr(III) analysis

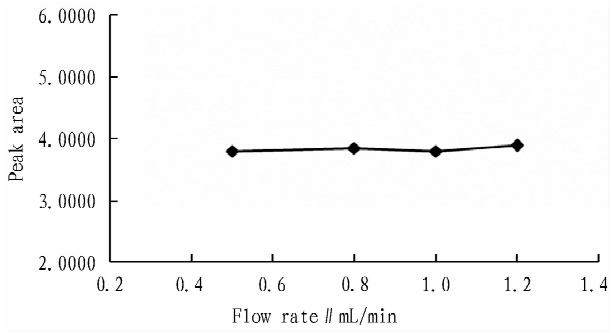

Fig. 6 Effect of flow rate of post-column derivation liquid on Cr(VI) analysis

3.2 Reliability of the method

3.2.1 Determination of ion chromatographic peak. 100 μg/mL of Cr(III) standard solution, 1 μg/mL of Cr(VI) standard solution and the mixed standard solution of Cr(III) and Cr(VI) were prepared for the determination of ion chromatographic peak. Peak occurrence time of Cr(III) standard solution was 3.3 s, while peak occurrence time of Cr(VI) standard solution was 5.4 s, and peak occurrence time of the mixed solution of Cr(III) and Cr(VI) was consistent with that of single standard solution (Fig. 7 – 9).

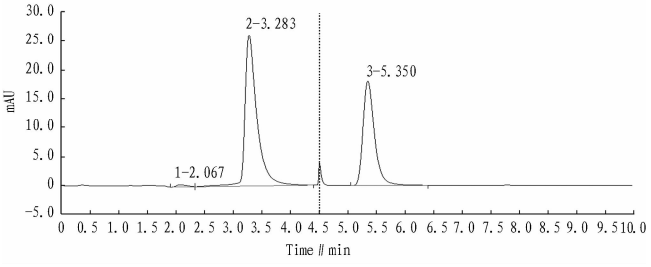

Fig. 7 Chromatogram of Cr(III) and Cr(VI) mixed standard solution

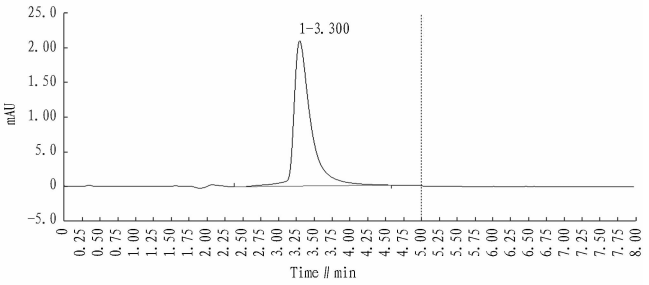

Fig. 8 Chromatogram of Cr(III) standard solution

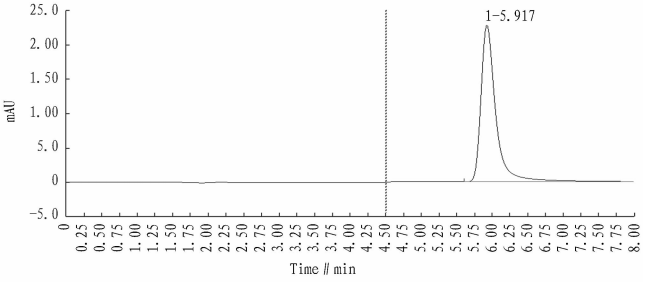

Fig. 9 Chromatogram of Cr(VI) standard solution

3.2.2 Linear relationship and method detection limit. Instrument parameters were set according to above mentioned conditions. After the instrument was preheated and became stable, ion chromatography detection of the mixed standard solution with different concentrations was conducted. Taking Cr(III) or Cr(VI) standard solution concentration as transverse coordinate and the corresponding peak area of Cr(III) or Cr(VI) as vertical coordinate, standard curve was drawn. Result showed that Cr(III) linear regression equation was $y = 0.56 \times 10^{-4}x - 0.31 \times 10^{-5}$, and correlation coefficient $R^2 = 0.9994$; Cr(VI) linear regression equation was $y = 0.73 \times 10^{-2}x - 0.11 \times 10^{-3}$, and correlation coefficient $R^2 = 0.9996$. According to three times of SNR, the lowest detection limit was counted. Detection limit of Cr(III) was 0.17 mg/kg, while detection limit of Cr(VI) was 0.019 mg/kg.

3.2.3 Sample determination result and recovery rate. Due to not detecting Cr(VI) in the sample, only recovery rate of Cr(III) was counted. 2.5 g of sample was weighed accurately, and 0.15, 0.30 and 0.45 μg/mL of Cr(III) standard solution was added respectively, and average recovery rate was counted (Table 3).

Table 3 Recovery rates and the relative standard deviations of Cr(III) at different levels ($n = 3$)

Adding concentration // μg/mL	Recovery rate // %	RSD // %
0.15	82.3	2.5
0.30	85.1	3.2
0.45	81.9	4.0

By determining total Cr content in 200 copies of different pumpkin varieties, pumpkin variety with high total Cr content was screened out for ion chromatography detection. The determination results were shown as Table 4.

Result showed that Cr(VI) was not detected in pumpkin sample, which was consistent with Zayed's conclusion that only Cr

(III) was detected in seven kinds of vegetables by XAS[17], but was different from Cheng Yongan's conclusion that Cr(III) existed in more than 95% of pumpkin[18], and the difference may be induced by different determination methods or materials[19]. Cr(VI) was

Table 4 The determination results of real samples Cr(III) and Cr(VI)

Sample name	Determination component	Actual determination value//μg/mL	Cr(III) content in pumpkin//mg/kg
36	Cr(III)	0.1232	0.2835
	Cr(VI)	ND	
41	Cr(III)	0.0835	0.2336
	Cr(VI)	ND	
47	Cr(III)	0.1057	0.2685
	Cr(VI)	ND	
48	Cr(III)	0.1241	0.3464
	Cr(VI)	ND	
50	Cr(III)	0.0930	0.1901
	Cr(VI)	ND	
51	Cr(III)	0.0893	0.2445
	Cr(VI)	ND	
52	Cr(III)	0.1009	0.1941
	Cr(VI)	ND	
58	Cr(III)	0.0968	0.2723
	Cr(VI)	ND	
66	Cr(III)	0.0614	0.1772
	Cr(VI)	ND	
82	Cr(III)	0.0916	0.3472
	Cr(VI)	ND	
103	Cr(III)	0.1222	0.3543
	Cr(VI)	ND	
122	Cr(III)	0.1000	0.2268
	Cr(VI)	ND	
176	Cr(III)	0.1424	0.2715
	Cr(VI)	ND	
233	Cr(III)	0.1591	0.4316
	Cr(VI)	ND	

Note: ND showed not detection.

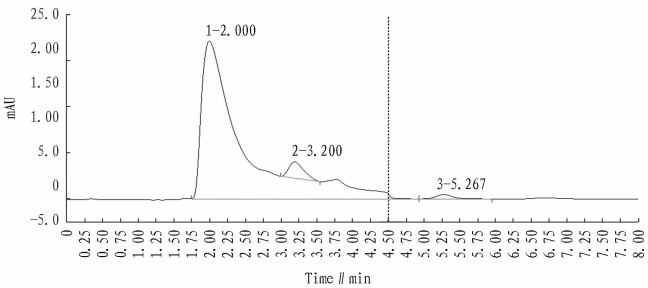

Fig. 10 Chromatogram of pumpkin extract containing 1 μg/mL of Cr(VI) standard solution

not detected in the sample, which was because that pumpkin contained a large number of reducing components. Experiment showed that the treated pumpkin aqueous solution reacted with nearly saturated potassium permanganate, with the volume ratio was 1: 4.5. It was speculated that Cr(VI) in pumpkin could be reduced into Cr(III). When adding high-concentration Cr(VI) standard solution in pumpkin extraction solution, Cr(VI) peak area on ion chromatogram was still very small and even there was no peak. Fig. 10 was ion chromatogram of the treated pumpkin aqueous solution adding 1 μg/mL of Cr(VI) standard solution. Compared with Fig. 9 (chromatogram of 1 μg/mL Cr(VI) standard solution), it was further proved that Cr(VI) could not exist in pumpkin.

The determination of Cr(III) and Cr(VI) in pumpkin by ion chromatography had following advantages: convenience, good reproducibility, quick peak occurrence and good selectivity, which could simultaneously determine Cr(III) and Cr(VI), and complete sample element speciation analysis within 8 min. Due to complex pumpkin sample composition and more interference, the selected separation column should have good stability and high capacity, and could effectively isolate $Cr(PDCA)^{2-}$ and CrO_4^{2-}, exclude the interference of other substances and improve detection sensitivity. Concentration range of ion chromatography analysis was between ppb and ppm. Via one-time sample analysis, it not only played the advantage of ion chromatography in separating anion and cation but also combined the advantages of ion chromatography and UV visible detector in detecting chromogenic complexation reaction[20]. Except higher RP column fee, ion chromatography had relatively lower operation fee.

4 Conclusions

(i) Ion chromatography could simultaneously determine Cr (III) and Cr(VI) in pumpkin. Cr(III) reacted with PDCA into $Cr(PDCA)^{2-}$ under suitable pH, while Cr(VI) mainly existed in the form of CrO_4^{2-} and did not react with PDCA. They were isolated by anion exchange column. In this experiment, CS5 separation column with good chemical stability and high column capacity was used.

(ii) By considering existence forms of Cr(III) and Cr(VI) under different pH, the effects of pH on peak occurrence time (retention time), peak shape, dead space peak occurrence, peak area of ion chromatogram and system stability, leacheate pH was controlled between 6.5 and 6.8 in the experiment.

(iii) When detecting pumpkin sample, it was found that chromatographic peak could not be separated from system peak well, and peak shape was not very symmetric, which had greater impact on Cr(III) detection result. In this experiment, by adjusting I^- concentration as 5 mmol/L and prolonging Cr(III) and Cr (VI) retention time, chromatographic peak of Cr(III) was separated from system peak well, thereby guaranteeing stable pressure and datum line when detecting Cr(III) and Cr(VI).

(iv) Based on prior work, $L_{16}(4^3)$ orthogonal design test on water bath heating temperature, heating time of pre-column derivatization and flow rate of post-column derivatization solution was made. By combining change trend of peak area under each factor, flow rate of post-column derivatization solution selected 0.5 mL/min, while pre-column derivatization used 100 ℃ of optimal water bath temperature, and the optimal heating time was 5 min.

(v) In this experiment, Cr(III) pre-column derivatization-ion chromatography method and Cr(VI) post-column derivatization-ion chromatography method in pumpkin were established. Via pre-column derivatization, Cr(III) in pumpkin became 2, 6-pyridinedicarboxylic acid chromium; via post-column derivatization, Cr(VI) in pumpkin became 1, 5-diphenylcarbazide chromium, and they were detected at 365 and 530 nm respectively. Ion chromatography conditions were optimized, and detection sensitivity was improved, and method detection limits of Cr(III) and Cr(VI) were 0.17 and 0.019 mg/kg respectively. And the average recoveries of Cr(III) in pumpkins were in the range of 82% – 85%.

References

[1] CARUSO JA, MONTES-BAYON M. Elemental speciation studies new directions for trace metal analysis[J]. Ecotoxicology and Environmental Safety, 2003(56): 148 – 163.

[2] ANDREWES P, DEMARINI DM, FUNASAKA K, *et al.* Do arsenosugars pose a risk to human health? The comparative toxicities of a trivalent and pentavalent arsenosugar[J]. Environmental Science and Technology, 2004, 38(15): 4140 – 4148.

[3] SCHMEISSER E, GOESSLER W, KIENZL N, *et al.* Volatile analytes formed from arsenosugars: Determination by HPLC – HG – ICPMS and implications for arsenic speciation analyses[J]. Analytical Chemistry, 2004, 76(2): 418 – 423.

[4] KOTAS J, STASICKA Z. Chromium occurrence in the environment and methods of its speciation[J]. Environmental Pollution, 2000, 107(3): 263 – 283.

[5] HUA L, AN H, WU FS, *et al.* Determination and factor analysis of trace hexavalent chromium in plastics for RoHS directive[J]. Acta Chimica Sinica, 2008, 66(6): 662 – 668. (in Chinese).

[6] MA LN, LI ZH, ZHANG S, *et al.* Experimental study on extraction methods of chromium(VI) in soil[J]. Hydrology, 2010, 30(2): 11. (in Chinese).

[7] CHENG T, XING WB, LIU WX, *et al.* Determination of chromium(VI) in electrical and electronic equipment by atomic fluorescence spectrometric method[J]. Information Technology & Standardization, 2011, 1(2): 55. (in Chinese).

[8] CAI SX, YAN MJ, HUANG DF, *et al.* Analysis of content of As, Hg, Pb, Cd and Cr absorbed in water hyacinth[J]. Fujian Agricultural Science and Technology, 2005(3): 49 – 50. (in Chinese).

[9] DAO P, ZHANG LL, LI LH, *et al.* Determination of hexavalent chromium in atmospheric particles(PM2.5, PM10) by ion chromatography with post-column derivatization and UV/visible spectrophotometer detection [J]. Environmental Chemistry, 2014, 33(7): 1194 – 1200. (in Chinese).

[10] XU M, WANG B, LIU YQ, *et al.* Determination of extractable chromium(III) and chromium(VI) in textiles with HPLC – ICP – MS method [J]. Dyeing and Finishing, 2014(9): 44. (in Chinese).

[11] LI DK, FAN GL, YAO HM, *et al.* Analysis of chromium speciation in tobacco by HPLC – ICP – MS[J]. Acta Tabacaria Sinica, 2016, 22 (2): 1 – 7. (in Chinese).

[12] GUO SF, WANG P, WANG F, *et al.* Determination of chromium(VI) in silty cosmetics using high performance liquid chromatography – inductively coupled plasma mass spectrometry[J]. Inspection and Quarantine Science, 2012, 22(4): 8 – 13. (in Chinese).

[13] HIGHT SC, CHENG J. Determination of methyl mercury and estimation of total mercury in seafood using high performance liquid chromatography (HPLC) and inductively coupled plasma – mass spectrometry (ICP – MS): Method development and validation[J]. Analytica Chimica Acta, 2006, 567(2): 160 – 172.

[14] CHEN G, LIN L, QIAN C, *et al.* Determination on the trivalent chromium and hexavalent chromium in the drinking water using ion chromatography coupled with inductively coupled plasma mass spectrometry[J]. Farm Machinery, 2012(4): 127 – 130. (in Chinese).

[15] LI J, WANG Y, CHEN HB, *et al.* Simultaneous extraction and determination of chromium (III) and chromium (VI) in milk[J]. Food Science, 2010, 31(10): 250 – 253. (in Chinese).

[16] HUA HJ, LI YH, FENG WY, *et al.* Simultaneous determination of trace Cr(III) and Cr(IV) in water using ion-pairing reversed phase chromatography – inductively coupled plasma mass spectrometry[J]. Chinese Journal of Analytical Chemistry, 2009, 37(3): 433 – 436. (in Chinese).

[17] ZAYED A, TERRY N. Chromium accumulation, translocation and chemical speciation in vegetable crops[J]. Planta, 1998, 206(2): 293 – 299.

[18] CHENG YA, GAO SB, WANG F, *et al.* Study on the contents of Chromium (Cr) and its chemical configuration in pumpkin[J]. Journal of Northwest A&F University(Natural Science Edition), 2004, 32(z1): 91 – 93. (in Chinese).

[19] SHEWRY PR, PETERSON PJ. The uptake and transport of chromium by barley seedlings (*Hrodeum vulgare* L.)[J]. Journal of Experimental Botany, 1974, 25(4): 785 – 797.

[20] YANG LL, XU AH, XU ML, *et al.* On the determination of hexavalent chromium in the milk using ion chromatography with UV detector detection [J]. China Metrology, 2012(10): 83 – 84, 100. (in Chinese).