Simultaneous Determination of Chloride, Bromide and Iodide in Foodstuffs by Low Pressure Ion-Exchange Chromatography with Visible Light Detection

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Abstract

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An ion-exchange chromatography method with visible light detection was developed for the simultaneous determination of chloride, bromide, and iodide in foodstuffs. They were separated by means of low pressure ion-exchange chromatography using 4.0mM sodium carbonate solution as the eluent and a low pressure ion-exchange chromatography column as the separation column. The detection limits of chloride, bromide and iodide were 0.011 mg/l, 0.002 mg/l, and 0.023 mg/l, respectively. The relative standard deviations (RSDs) of the peak area were smaller than 2.9%. The recoveries were between 98.61% and 105.65%. Unlike the traditional methods, this validated method is inexpensive and stable.

Keywords: LPIEC; spectrophotometry; halide

Most analytical approaches reported for chloride, bromide, and iodide determination have shortcomings to some extent. The traditional methods, such as titration and colorimetric measurement, with a relatively low sensitivity and using lots of toxic reagents (e.g., silver nitrate, mercuric thiocyanate), can determine one substance only.

Ion-exchange chromatography (IEC) was first introduced about 33 years ago and has become a well-established technique. The simultaneous analysis of common anions such as chloride (Cl^-), bromide (Br^-), iodide (I^-), nitrate, phosphate, or sulfate is the most important IEC routine application. These solutes are usually determined by ion-exchange separation with suppressed conductivity detection (Tucker & Flack 1998; Kapinus *et al.* 2004; Zhe *et al.* 2006). IEC with suppressed conductivity detection permits the use of higher capacity stationary phases and higher ionic strength eluents than those which use conductivity without chemical suppression detection methods, and provides a greater variation in ion-exchange selectivity. The other common means of detection are conductivity without chemical suppression (Zhou & Guo 2000) and direct or indirect UV detection (Quattrocchi *et al.* 2001; Hu *et al.* 2002). However, the visible light detection has not been used to detect Cl^-, Br^- and I^- simultaneously.

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Commonly, the analyte ions monitored by optical (visible light) detector are visible light-absorbing ions such as Cu^{2+} or Fe^{3+}. For non-visible light-absorbing ions, the indirect detection and/or post-column derivatisation with subsequent visible light measurement has been considered to be a useful detection method.

This paper describes a low pressure ion-exchange chromatography (LPIEC) with an optical detector to detect simultaneously Cl^-, Br^-, and I^- by indirect detection. LPIEC is a type of ion chromatography without the suppressor column and working at a low pressure of 1.96×10^5 Pa to 2.94×10^5 Pa (Jiang *et al.* 1999; Zhou *et al.* 2007). Cl^-, Br^-, and I^- are separated by LPIEC column and then each of them catalyses the oxidative discoloration of indigo carmine by potassium bromate ($KBrO_3$) in acidic media and heating condition. The spectrophotometric measurement can be applied satisfactorily to the detection of Cl^-, Br^-, and I^-.

Table 1. Experimental conditions for Cl^-, Br^-, and I^- determination in LPIEC

Analytical separation	
LPIEC column	Anion-exchange resin column (0.6 cm × 13 cm, I.D. 30–35 μm), exchange capacity 0.001 meq/g
Eluent	4.0mM Na_2CO_3
Flow rate	0.70 ml/min
Injection volume	300 μl
Chemical reaction variables	
R1	0.15mM Indigo Carmine and 1.1M H_2SO_4
R1 Flow rate	0.25 ml/min
R2	32.0mM $KBrO_3$
R2 Flow rate	0.25 ml/min
Reaction coil	1.4 m
Optical detector	
Wavelength	610 nm

MATERIAL AND METHODS

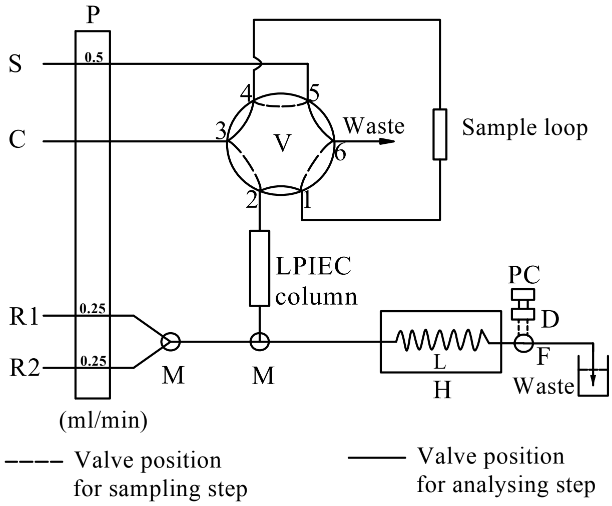
All the analyses were conducted on Low Pressure Ion Chromatographic Instrument (Jiang *et al.* 1999; Zhou *et al.* 2007) consisting of a low pressure four-way peristaltic pump (Shanghai Huxi Analytical Instrument Plant, Shanghai, P.R. China), LPIEC column, six-way automatic injection valve, optical flow cell, optical detector, and homothermic heater (installing a reaction coil). A HW-2000 Chromatography workstation (Qianpu software Co., Ltd., Zhehang, P.R. China) was used for the system control and data collection. The experimental conditions used for the determination of Cl^-, Br^-, and I^- are given in Table 1.

Reagents. All the chemicals used were of analytical grade quality (Chengdu Kelong Chemical Reagent Factory, Sichuan, P.R. China). The standards were prepared daily by diluting 1000 mg/l ICP standard solutions. The eluent (4.0mM Na_2CO_3) was prepared from 1M sodium carbonate aqueous solution. The mixture of Indigo Carmine and sulfuric acid solution was marked as colour solution R1 and was prepared from 1mM Indigo Carmine aqueous solution and 6M sulfuric acid (H_2SO_4). Concentration $KBrO_3$ solution was marked as colour solution R2 and was prepared from 0.1M $KBrO_3$ aqueous solution. The above solutions were prepared using deionised water with a resistance of 18.2 MΩ (Molecular, Shanghai, P.R. China).

Sample preparation. All samples including fortified cookies (No. 1), kelps (No. 2), and radishes (No. 3) were bought in the morning market of Chengdu city. The fortified cookies were pulverised and sieved (80 mesh); fresh kelps and radishes were carefully rinsed with deionised water, and then the deionised water on the surface of the kelps and radishes was removed by a centrifuge, at last the kelps and radishes were separately and completely ground by a mill. In the sample preparation, all the deionised water was freed from dissolved oxygen by boiling and was adjusted to pH 10, to prevent the oxidation of Br^- and I^-.

The processed samples weighing 25.00 g each were added to 150 ml flasks with approximately 80 ml water, which were subsequently shaken for 30 min in supersonic wave, thereafter, the mixtures were diluted to a final volume of 100 ml with deionised water. All samples were filtered before use.

Procedure. The schematic representation of the LPIEC setup is shown in Figure 1. The sample was loaded via the four-way peristaltic pump and six-way automatic injection valve on a 300 μl sample loop. After injection, the separation of the analyte ions took place in the ion-exchange column by passing the Na_2CO_3 eluent (4.0mM Na_2CO_3). The separated ions with the eluents *R*1 and *R*2 mixed by mixing coil then came into the reaction coil, in which the separated ions accelerated the reaction of *R*1 and *R*2 at 85 ± 0.5°C. The reaction solu-



S – sample; C – Na_2CO_3 eluent; R1 – Indigo Carmine and H_2SO_4 solution; R2 – $KBrO_3$ solution; P – four-way peristaltic pump; V – six-way automatic injection valve; L – reaction coil; M-Mixing coil; H – homothermic heater; F – optical flow cell; D – optical detector

Figure 1. Schematic diagram of the LPIEC system

tion passed through the optical detector, where the peaks of Cl^-, Br^-, and I^- were recognised at a wavelength of 610 mm.

RESULTS AND DISCUSSION

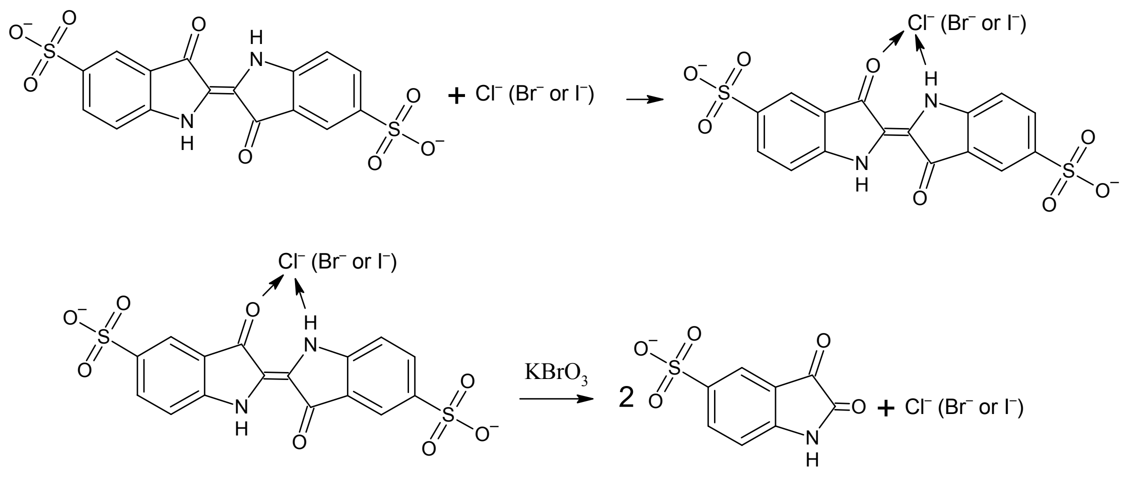
Detection principle

The catalytic reaction was monitored spectrophotometrically by measuring the change in absorbance of the reaction mixture. Only the changes in the ions (Cl^-, Br^-, and I^-) concentrations influenced the rate of the oxidative, discoloration of indigo carmine.

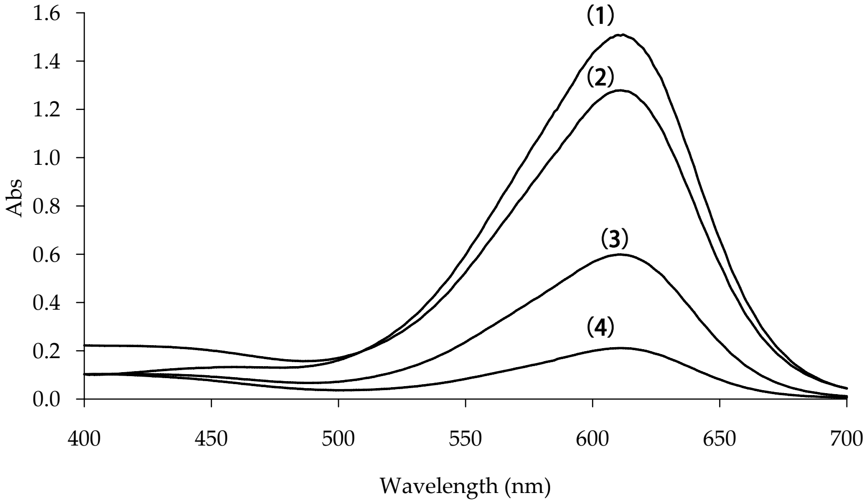
The method is based on the observation of discoloration. Without catalysts, the rate of oxidative discoloration of indigo carmine by $KBrO_3$ is very slow in acidic media. The rate of discolouring oxidation of indigo carmine by $KBrO_3$ becoms faster when Cl^-, Br^-, or I^- is added into the acidic reaction solution. The reason is that all of Cl^-, Br^-, and I^- coordinate with Indigo Carmine and form a six-member ring intermediate, which can weaken the double bond force of the two 3-sulfo indoles full-ketone. Therefore, the indigo carmine easily oxidises by $KBrO_3$ when Cl^-, Br^-or I^- is present in the acidic reaction solution (Schema 1).

In order to find the most appropriate wavelength for the analysis on the LPIEC, the effect of wavelength on the peak height was studied in the range from 400 nm to 700 nm using the Spectrum UV2800 (You Nike Ltd. Co., Shanghai, P.R. China). Figure 2 shows the absorbance spectra of three different solutions. The standard solutions of Cl^-, Br^-, and I^- respectively, accelerated the fading through oxidation reaction of *R*1 and *R*2 in the heating condition. The greatest absorbance intensity of the solutions was at 610 nm, which was selected for further work.

Effect of the length of LPIEC column. The relatively short LPIEC column (invented by Professor Xinshen Zhang) was selected in the study for the following two advantages: the reduced system pressure, and improved sensitivity and precision. The column lengths of 11–15 cm were tested, the results are shown in Figure 3. When the length of the column was shorter than 13 cm, the separation of Cl^- and Br^- was poor. However, if the length of the column exceeded 13 cm, the retention time



Schema 1



(1) Mixture solution of 0.15 mM Indigo Carmine solution, 1.1M H_2SO_4 and 32.0mM $KBrO_3$

(2) Mixture solution of 0.15mM Indigo Carmine solution, 1.1M H_2SO_4, 32.0mM $KBrO_3$ and 10.00 mg/l I^-

(3) Mixture solution of 0.15mM Indigo Carmine solution, 1.1M H_2SO_4, 32.0m M $KBrO_3$ and 5.00 mg/l Cl^-

(4) Mixture solution of 0.15mM Indigo Carmine solution, 1.1M H_2SO_4, 32.0mM $KBrO_3$ and 2.00 mg/l Br^-

Putting the above mixture solution into a temperature of 85 ± 0.5°C constant temperature water bath, it was heated for 2 min, and then quickly taken out and put in the cold water for 3 min to end the reaction. At last, the absorbance spectra were obtained by the Spectrum UV2800

Figure 2. Absorbance spectra

increased. Thus, the length of the column of 13 cm was chosen for further work.

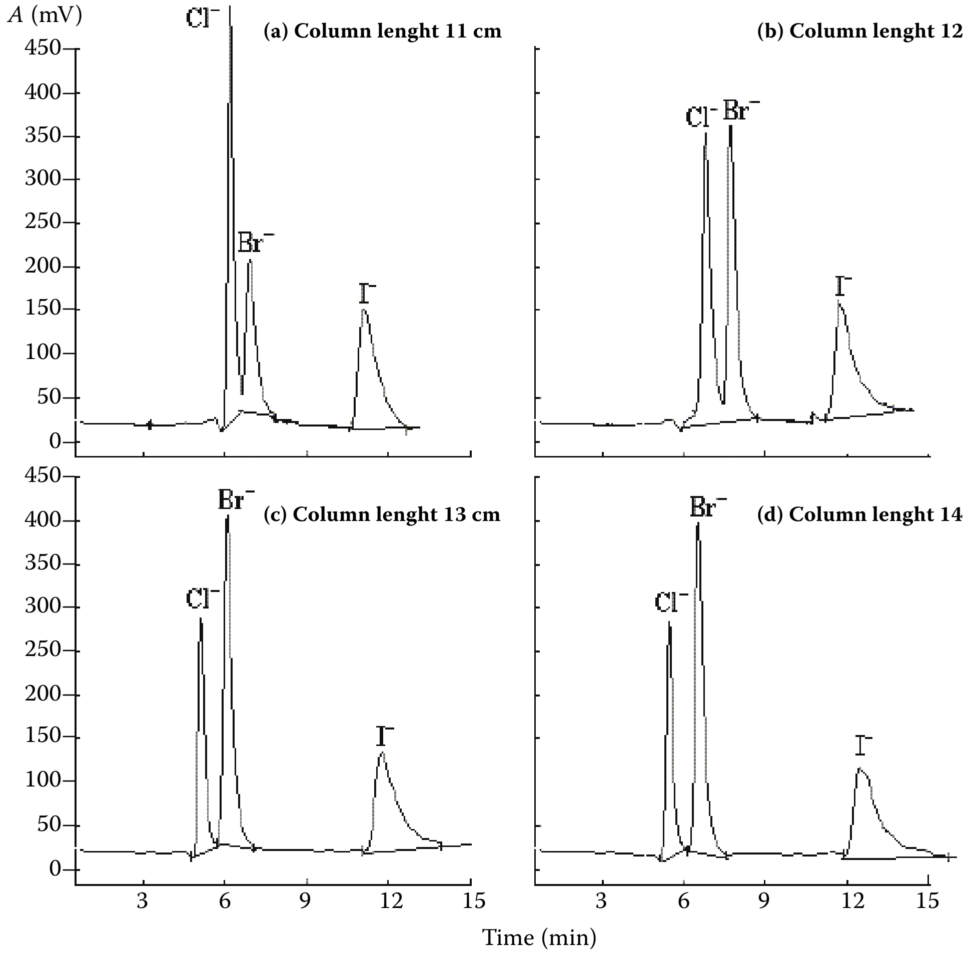
Optimisation of separation conditions. The eluent is critical for the analysis by ion-exchange chromatography. Sodium carbonate (Na_2CO_3) was selected as the eluting solution in this study. Different concentrations of Na_2CO_3 (2.0, 3.0, 4.0, 5.0, and 6.0mM) were studied to determine the optimum concentration, with an injection volume of 300 µl and using the visible light detection at 610 nm. The results showed that the separation of Cl^-, Br^-, and I^- was worse at higher concentrations while the low concentrations resulted in a broad peak shape. The separation of these ions with 4.0mM Na_2CO_3 appeared to be optimum. Under the optimum concentration, different flow rates (0.5, 0.6, 0.7, 0.8, and 0.9 ml/min) were set to investigate the impact of the flow rate on the separation. A faster rate resulted in peaks, overlapping while the lower was the rate, the longer was the overall time, as well as the worse was the peak shape. Therefore, it was suggested to use a flow rate of 0.7 ml/min.

Effect of reaction temperature. To obtain the best overall reaction, the effect of the reaction temperature was also investigated in the range of 65–87°C (Figure 4). The peaks became higher with the reaction temperature increased from 65–87°C. When the temperature was higher than 85°C, the reproducibility of the system peak height became poor, and the flow path was prone to bubbles formation, which impacted on the stability of the system determination. To maintain the system stability, the temperature of 85 ± 0.5°C was chosen for further study.

As a consequence, we chose the length 13 cm for the LPIEC column, 4.0mM Na_2CO_3 as the eluent with a flow rate of 0.7 ml/min, and 85 ± 0.5°C as the reaction temperature. Under the optimised experimental conditions, the analytes in the solution were well separated within 14 min (Figure 3c).

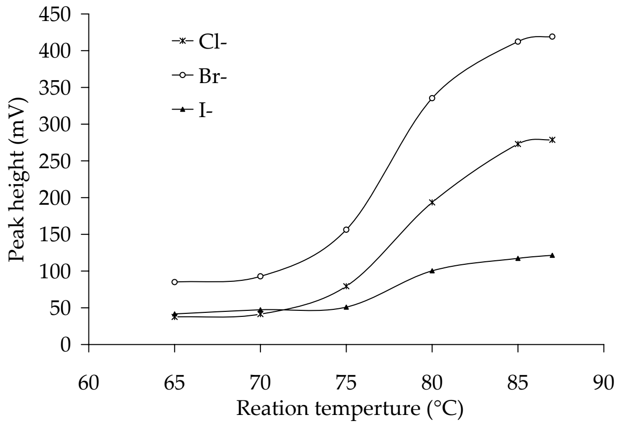
Method validation

Linearity was investigated using the stock solution containing the analytes, which was diluted serially. Eight concentrations of the three analytes solution were injected in triplicate and the calibration curves were constructed by plotting the peak height (A, mV) versus the concentration (C, m/l) of each analyte. The detection limits were estimated as the mean of the blank sample plus three



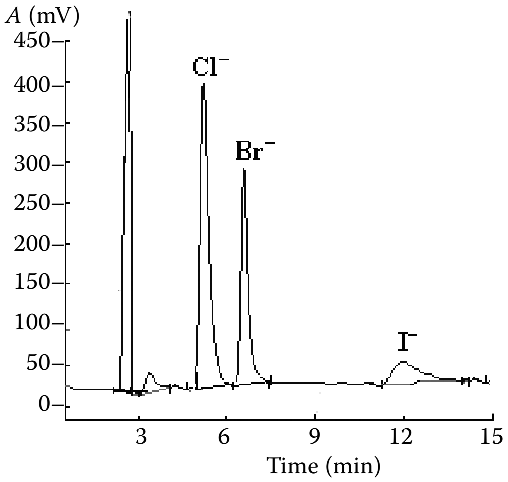
Conditions: Cl^- 10.0 mg/l, Br^- 2.0 mg/l, and I^- 10.0 mg/l; eluent 4.0mM Na_2CO_3 with a flow rate of 0.7 ml/min; temperature 85 ± 0.5°C

Figure 3. Effect of LPIEC column length



Conditions: Cl^- 10.0 mg/l, Br^- 2.0 mg/l, and I^- 10.0 mg/l; eluent 4.0mM Na_2CO_3 with a flow rate of 0.7 ml/min

Figure 4. Effect of reaction temperature



Conditions: Cl^- 10.0 mg/l, Br^- 2.0 mg/l, and I^- 10.0 mg/l; eluent 4.0mM Na_2CO_3 with a flow rate of 0.7 ml/min; temperature 85 ± 0.5°C

Figure 5. Chromatogram of mineral water

Table 2. Analytical characteristic of Cl^-, Br^-, and I^-

Analyte	Linear range (mg/l)	Calibration equation	Correlation coefficient	Detection limit (mg/l) (S/N = 3)	*RSD* (%) (*n* = 10)	Retention time (min)
Cl^-	0.15–35.0	$A = 45.51C - 1.45$	0.9992	0.011	2.96	6
Br^-	0.03–5.0	$A = 486.24C - 74.33$	0.9991	0.002	2.12	7.5
I^-	0.10–25.0	$A = 49.71C - 59.64$	0.9990	0.023	2.45	14

RSD = relative standard deviation

Table 3. The concentrations and recovery data of Cl^-, Br^-, and I^- in the samples

Analyte	Concentrations (mg/l)			Recovery (%)		
	No. 1	No. 2	No. 3	No. 1 added (4.0 mg/l)	No. 2 added (2.0 mg/l)	No. 3 added (1.0 mg/l)
Cl^-	23.48	20.04	15.93	105.65	103.37	105.21
Br^-	0.91	1.69	1.06	101.97	99.47	100.47
I^-	4.62	2.42	0.71	98.61	100.12	101.75

times the standard deviation obtained on the blank sample. The precision was evaluated by performing ten replicate analyses of the standard solution at the concentrations of 10.0 mg/l for Cl^-, 2.0 mg/l for B^-, and 10.0 mg/l for I^-. The results are illustrated in Table 2. Under the optimised experimental conditions, all three analytes showed perfect linearity.

Sample determination

To illustrate the application of the method developed, three aquatic samples (No. 1 Fortified Cookies, No. 2 kelps, and No. 3 radishes) from Chengdu, P.R. China were collected for the determination. Figure 5 shows the chromatogram of the No. 3 radishes. The contents of the analytes in real samples were determined using the external calibration method. Suitable amounts of Cl^-, Br^-, and I^- standards were added to the real samples of known contents and the mixtures were analysed using the proposed procedure. Recovery was expressed for each component as the mean percentage ratio between the measured and the added amounts. The results are shown in Table 3.

CONCLUSIONS

This method proves that with the use of visible light detection, ion-exchange chromatography can detect Cl^-, Br^-, and I^- at the same time; the method has shown good correlation between and high accuracy of the results, and it is also easily operated in comparison to other methods; it provides a simple and efficient procedure for analysing the above ions in foodstuffs. The Low Pressure Ion Chromatographic Instrument employed in this study not only has a low price ($4000 per instrument), but is also portable occupying a small volume (dimension of 25 cm × 20 cm × 20 cm).

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