

## Ion chromatographic determination of chlorite and chlorate in chlorinated food using a hydroxide eluent

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**Abstract:** This study was conducted to develop an analytical technique for determination of chlorite and chlorate concentrations in fresh-cut food and dried fish products by an ion chromatography/conductivity detection method using a hydroxide mobile phase. Deionized water was added to homogenized samples, which were then extracted by ultrasound extraction and centrifuged at high speed (8,500 rpm). Subsequently, a Sep-Pak tC18 cartridge was used to purify the supernatant. Chlorite and chlorate ions were separated using 20 mM KOH solution as the mobile phase and Dionex IonPac AS27 column as the stationary phase. Ethylenediamine was used as sample preservative and dibromoacetate was added to adjust for the disparity in extraction efficiencies between the food samples. The method detection limit) for chlorite and chlorate were estimated to be 0.2 mg/kg and 0.1 mg/kg, respectively, and the coefficient of determination ( $r^2$ ) that denotes the linearity of their calibration curves were correspondingly measured to be 0.9973 and 0.9987. The recovery rate for each ion was 92.1 % and 96.3 %, with relative standard deviations of 7.47 % and 6.18 %, respectively. Although neither chlorite nor chlorate was detected in the food samples, the analytical technique developed in this study may potentially be used in the analysis of disinfected food products.

**Key words:** chlorinated food, chlorite, chlorate, hydroxide eluent, ion chromatography

### 1. Introduction

Chlorine-based food disinfectants include chlorine dioxide ( $\text{ClO}_2$ ), sodium hypochlorite ( $\text{NaClO}$ ), and hypochlorous acid ( $\text{HClO}$ ). When used with water, these disinfectants undergo redox reactions to form chlorite ( $\text{ClO}_2^-$ ), chloride ( $\text{Cl}^-$ ), and chlorate ( $\text{ClO}_3^-$ ).<sup>2-4</sup>

Thus, such anionic products may remain in food products unless sufficiently rinsed with water.

Both chlorite and chlorate exhibit toxic properties and may cause weight loss in the adrenal gland, spleen, liver, and kidneys.<sup>5</sup> Negative neurological or anemic effects were also observed in infants who consumed water with a contaminant level greater

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than the maximum contaminant level (MCL) of 1.0 mg/L.<sup>5</sup> Despite this, countries that permit the use of chlorine-based food disinfectants tend to designate only the application standards for such disinfectants but not standards with respect to the residual quantity of disinfectants or its decomposition products. Hence, potential health effects may arise with the increasing consumption of disinfected food products.

While analytical techniques to determine chlorite and chlorate in disinfected water are well established,<sup>6-8</sup> the same cannot be said in case of food products. Aside from being rich in organic substances, the matrix of food products contains various types of anions. To date, the ion chromatography-conductivity detection (IC-CD) method was widely used in the determination of chlorite and chlorate in potable water<sup>6-11</sup> and food products,<sup>12-13</sup> using AS9-HC or AS9-SC columns for analysis.

Anions present in food products were extracted by cutting the food sample into smaller pieces, after which the cut samples were either shaken in water<sup>13</sup> or immersed in deionized water before the water was separated.<sup>14-15</sup> However, while it was possible to extract chlorite and chlorate on the surface of the samples, ions embedded in the interior of the food samples could not be extracted using this method. This necessitated the use of a blender or a homogenizer to homogenize the sample.<sup>12</sup> In addition, a process to eliminate numerous types of organic products present in the extract is required.

The method detection limit (MDL) for the determination of chlorite and chlorate in food samples was found to be approximately 1 mg/kg in vegetables, eggs, and seafood products,<sup>12,14</sup> with > 90 % recovery rate.<sup>12</sup> However, these studies did not provide any relevant data on the linearity of calibration curves or the accuracy and precision of the experimental results related to quality assurance and quality control (QA/QC). Hence, the reliability of the results obtained could not be verified. Further, ethylenediamine (EDA) must be used as a preservative for chlorite as chlorite oxidizes to chlorate over time.<sup>6-8</sup> However, none of the previous studies pertaining to food product analysis used EDA.

In this study, an experimental technique that allows a reliable quantitative analysis of chlorite and chlorate ions in dried meat products and fresh-cut food—which have seen drastic increase in consumption over the years—was established. In addition, the health impacts of chlorine-based disinfectants were evaluated by analyzing commercially available food samples using the technique established.

## 2. Materials and Methodology

### 2.1. Materials, samples, and standard solution

Fresh-cut food and dried meat products were selected as food samples to be used for the experiment. A total of 120 types of fresh-cut food (sprouts and salads) and dried meat products (dried fish, seasoned dried fish, and other dried meat) that are typically distributed in large departmental stores were purchased and kept at -24 °C until the commencement of the experiment.

Methanol and dibromoacetic acid (DBA)—used as the surrogate standard (SS)—were purchased from TEDIA (Fairfield, OH, USA) and Fluka (St. Louis, USA), respectively. One M KOH solution was bought from Fischer Chemical (Waltham, USA), while sodium chlorite (NaClO<sub>2</sub>), sodium chlorate (NaClO<sub>3</sub>), sodium carbonate (NaCO<sub>3</sub>), and EDA were purchased from Sigma-Aldrich (St. Louis, USA). Sep-Pak tC18 cartridges (6 mL, 1 g) were acquired from Waters (Milford, USA). Deionized water (> 18 MΩ) was prepared using the reverse osmotic system from Shinhan ScienTech (Daejeon, Korea), 0.2 μm PVDF filters (Whatman, Little Chalfont, UK) were used for filtration.<sup>6</sup>

Stock solutions (100 mL) of chlorite and chlorate with concentration 1000 mg/L were prepared by dissolving 0.1676 g of NaClO<sub>2</sub> and 0.1275 g of NaClO<sub>3</sub> in deionized water. Standard solutions required to plot the calibration curves were prepared using these stock solutions and used within 2 days of preparation. The standard solutions were wrapped in aluminum foil to prevent exposure to sunlight and kept refrigerated until use. The EDA preservative solution was prepared by dissolving 2.8 mL of EDA reagent in deionized

water to form a 25 mL solution with concentration 100 mg/mL,<sup>6</sup> while the concentration of DBA solution prepared was 755 mg/L.

### 2.2. Sample extraction and purification

Frozen food samples (10 g) were cut into smaller pieces using scissors; the fresh-cut food were homogenized using a homogenizer (SciLabTis SHG-15A, SciLab Korea, Seoul, Korea), while the dried meat products were blended using a blender (Hanil HMF-2100S, Hanil, Seoul, Korea). To verify the separation of chlorite and chlorate from the samples, combined sprout vegetable samples were used. The blended sample (1 g) was transferred to a glass container and was mixed with 8  $\mu$ L of EDA and 15 mL of deionized water, after which 0.30 mL or 0.12 mL of the 50 mg/L standard solution was added, so that the concentration of chlorite (or chlorate) in the sample was set to be either 15 mg/kg or 6 mg/kg. Ionic components were extracted using an ultrasound extractor (Powersonic 510, Hwashin Tech, Daegu, Korea) for 30 min at 30 °C; the extract was centrifuged at 8,500 rpm for 30 min (LaboGene 1580, Gyrogen, Incheon, Korea). The analytical sample was prepared by passing the supernatant obtained through tC18 cartridge (6 mL, 1 g) and filtering through a 0.2  $\mu$ m syringe filter.

### 2.3. Instrumental analysis

The IC used for this analysis consisted of a 717 plus Autosampler, 512 HPLC pump, and a conductivity detector from Waters (*Table 1*). IonPac<sup>TM</sup> AG9-HC (50 mm  $\times$  4 mm) and IonPac<sup>TM</sup> AS9-HC (250 mm  $\times$

4 mm), which are typically used for anion analysis, were selected as the guard and analytical columns (option A). To improve the resolution between chlorite and chlorate, the optimum resolution condition was sought by varying the fluid velocity of the 9 mM Na<sub>2</sub>CO<sub>3</sub> mobile phase through 1, 0.9, 0.7, 0.6, and 0.5 mL/min. The resolution increased with decreasing fluid velocity. Hence, the optimum mobile phase concentration was determined by varying the concentration of Na<sub>2</sub>CO<sub>3</sub> from 9 mM to 8, 6, 4, and 2 mM at the fluid velocity of 0.5 mL/min.

Improvement of the resolution was also attempted using IonPac<sup>TM</sup> AG27 and AS27 columns, which have the same equipment standards as the columns in option A (option B, *Table 1*). An AS27 column—which is used with a hydroxide-based mobile phase—typically uses two pumps, but the conventional IC method uses a single pump. To find the optimum concentration level that gives maximum resolution at a single fluid velocity, the concentration of the KOH solution was varied from 10 to 30 mM at 1 mL/min fluid velocity.

### 2.4. Significance of the analytical technique

For quality assurance and quality control, combined sprout vegetable and dried squid were selected as representative samples for fresh-cut food and dried meat products, respectively. Qualitative analysis was conducted to verify their selectivity. The MDL and the limit of quantitation (LOQ) were estimated while the linearity, accuracy, and precision of the calibration curves were examined.<sup>16</sup>

*Table 1.* Analytical conditions of ion chromatographic system

|                    | Option A   | Option B   |
|--------------------|--|--|
| Auto sampler       | 717 plus Autosampler, Waters                             | 717 plus Autosampler, Waters                     |
| Pump               | 525 HPLC Pump, Waters                                    | 525 HPLC Pump, Waters                            |
| Guard column       | IonPac <sup>TM</sup> AG9-HC (50 mm $\times$ 4 mm)        | IonPac <sup>TM</sup> AG27 (50 mm $\times$ 4 mm)  |
| Analytical column  | IonPac <sup>TM</sup> AS9-HC (250 mm $\times$ 4 mm)       | IonPac <sup>TM</sup> AS27 (250 mm $\times$ 4 mm) |
| Injection volume   | 200 $\mu$ L  | 50 $\mu$ L                                       |
| Eluent (flow rate) | 9 mM Na <sub>2</sub> CO <sub>3</sub> solution (1 mL/min) | 20 mM KOH solution (1 mL/min)                    |
| Suppressor         | AERS <sup>TM</sup> 500 (4 mm), Waters                    | AERS <sup>TM</sup> 500 (4 mm), Waters            |
| Detector           | Conductivity cell, Waters                                | Conductivity cell, Waters                        |

Table 2. Analytical conditions of LC-MS/MS

|                  |   |
|------------------|---|
| System           | Agilent technologies 1260 Infinity Binary LC system and AB SCIEX API 3200™ system mass spectrometer |
| Column           | Dionex™ IonPac™ AS27 (250 mm × 4 mm)  |
| Eluent           | 20 mM KOH aqueous solution  |
| Flow rate        | 0.5 mL/min  |
| Column temp.     | 30 °C   |
| Injection vol.   | 50 µL   |
| Ion source       | Electrospray ionization (ESI, turbo spray), 200 °C  |
| Cone voltage     | 40 V  |
| Cone Gas         | 50 L/h  |
| Collision energy | 30 eV   |

#### 2.4.1. Qualitative analysis

Due to the complexity of the matrix of food samples, chlorite and chlorate peaks in the ion chromatogram may not be easily distinguishable from the peaks of other ions. Hence, a standard solution was added to the samples to verify if the chlorite and chlorate peaks increased.

Chlorite and chlorate peaks were identified using liquid chromatography-tandem mass spectrometry (LC-MS/MS); the conditions for this instrumental analysis are specified in Table 2. Standard solutions (200 µg/L) of chlorite and chlorate were added to the fully processed food samples, and its analysis result was compared to the result obtained using only the standard solutions. Analysis of the two anions was conducted in multiple reaction monitoring (MRM) mode.

#### 2.4.2. Accuracy and precision

Certified reference materials (CRMs) for chlorite and chlorate could not be purchased. Hence, the accuracy of the results was examined using the recovery rate as an indicator while the precision of the experiment was evaluated from the reproducibility of the results. For each of the combined sprout vegetable and dried squid samples, 7 samples were prepared and tested at each of three concentration levels: low concentration (0.6 and 1 mg/kg), moderate concentration (3 mg/kg), and high concentration (6 mg/kg).

#### 2.4.3. Method detection limit and limit of quantitation

Each MDL was estimated using the following

method.<sup>16</sup> The estimated detection limit corresponding to  $S/N = 3$  in the ion chromatogram was determined and 7 samples with concentrations double the value of the detection limit found were prepared and analyzed. The standard deviation of the concentrations calculated from the analysis was found, and the MDL value was obtained by multiplying with the Student's  $t$  value (3.143) for 6 degrees of freedom at the 99% level of confidence. The LOQ was obtained by multiplying the standard deviation value by 10.

#### 2.4.4. Linearity

Calibration curves were plotted using five different data points, the lowest point being the LOQ. Samples to be used for the plotting of the calibration curves were mixed with constant volumes of standard solutions before extraction and purification. After analysis, the calibration curves were plotted. For combined sprout vegetables, the data points selected were 0.3, 3.0, 4.5, 6.0, and 7.0 mg/kg, while the lowest concentration selected for dried squid was 1 mg/kg. Additionally, 100 µL of DBA solution was added to each of the samples prior to extraction. Each ratio obtained by dividing the area of the target ions by that of the DBA was assigned as the value for the vertical axis. The regression equation for a straight line was found with respect to the concentration within the sample (horizontal axis) to plot the calibration curve.

#### 2.4.5. Cross-validation

Cross-validation of results was carried out in two

laboratories using the same analytical method established in this study to obtain results for the aforementioned categories. The significance of the analytical technique was also verified.

### 2.5. Sample analysis

One hundred and twenty samples of commercially available fresh-cut food and dried meat products were purchased from October 2015 to December 2015, and kept frozen (at  $-24\text{ }^{\circ}\text{C}$ ) until the start of analysis. Frozen samples (10 g) were cut using scissors and homogenized using either a homogenizer (fresh-cut food) or a blender (dried seafood products); homogenized samples (1 g) were transferred into 40 mL glass containers. EDA (8  $\mu\text{L}$ , 100  $\mu\text{L}$ , and 15 mL), DBA, and deionized water were added to each sample and the samples were sonicated for 30 min at  $30\text{ }^{\circ}\text{C}$ . The extract was centrifuged for 30 min at 8,500 rpm and 3 mL of the supernatant was purified using the tC18 cartridge. The purified sample was filtered through a  $0.2\text{ }\mu\text{m}$  syringe filter and analyzed via IC-CD (option B, Table 2).

## 3. Results and Discussion

### 3.1. Separation of peaks

Upon analyzing the standard solutions (50 mg/L) according to the conditions specified in Table 1 option A, the peak retention times of chlorite and chlorate were measured to be 5.6 min and 12.4 min, respectively (Fig. 1). When the combined sprout vegetable samples extracted and purified as per the method described in section 2.2 were analyzed, chlorite and chlorate peaks were not detected. When standard solutions of the target ions were added to the food sample to determine if the chlorite and chlorate peaks in the food sample overlapped with those of other anions present in the food samples, significant overlaps between chlorite and formate (peak retention time measured at 5.6 min), and between chlorate and nitrate (peak retention time measured at 13.4 min) were found (Fig. 1). In contrast, a large chloride peak found at 7.5 min did not affect the resolution of the peaks of the target ions, due to the large difference in retention times

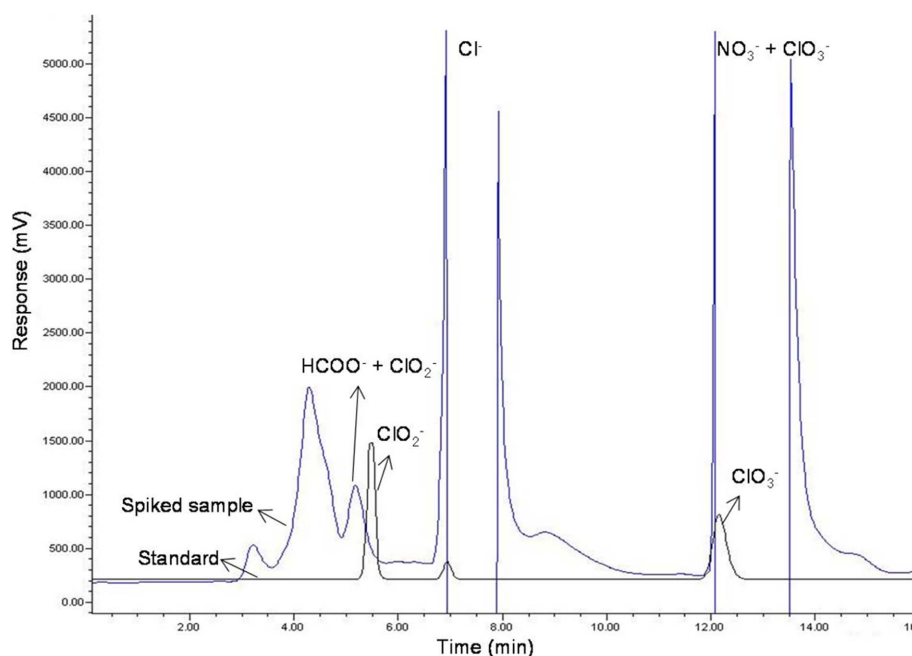


Fig. 1. Chromatograms of a standard solution (50 mg/L) and a spiked mixed sprouts sample (15 mg/kg). The column used was Dionex IonPac AS9-HC and the mobile system was 9 mM sodium carbonate (1 mL/min). The chlorite and chlorate peaks were overlapped with formate and nitrate peaks, respectively.

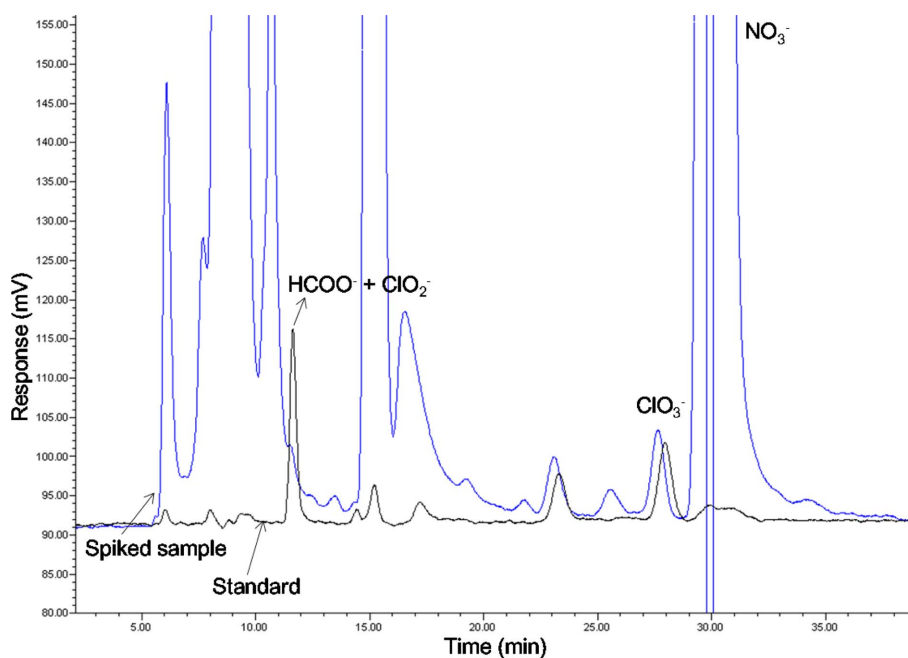


Fig. 2. Chromatograms of a standard solution (0.4 mg/L) and a spiked mixed sprouts sample (6 mg/kg). The chlorite peak was overlapped with the formate peak, whereas the chlorate peak was separated from the nitrate peak with a retention time of 27.9 min. The column used was Dionex IonPac AS9-HC and the mobile system was 6 mM sodium carbonate (0.5 mL/min).

(Fig. 1).

To find the optimum resolution condition, the concentration and the fluid velocity of the sodium carbonate mobile phase were varied, as suggested in section 2.3. Chlorate and nitrate peaks could be separated at concentrations and fluid velocity of 6 mM and 0.5 mL/min, respectively. However, at this condition, the retention time for chlorate was delayed considerably to 27.9 min, and was concluded to be impractical as the total time taken for complete analysis using this condition was 125 min.

Thus, the experiment was carried out using a hydroxide-based mobile phase and the AS27 column from Dionex.<sup>17</sup> By changing the concentration of KOH from 10 to 30 mM while keeping the fluid velocity parameter constant at 1 mL/min, the resolution between the two pairs of interfering peaks (chlorite and formate; chlorate and nitrate) using 20 mM KOH were measured to be 2.33 and 5.96, which are greater than 1 and so were uninterrupted by nearby peaks (Fig. 3(b)). In contrast, the separation of peaks

could not be achieved using 10 mM KOH mobile phase (Fig. 3(a)). It was able to separate the chlorite and formate peaks using 30 mM KOH but not the chlorate and nitrate peaks (Fig. 3(c)). Hence, the concentration and fluid velocity parameters were fixed at 20 mM KOH and 1 mL/min, respectively; the retention times of chlorite and chlorate using these values were 6.2 min and 11.8 min, respectively. In addition, the retention time of DBA used as the SS was 10.6 min. Extraction and purification conditions were determined in accordance with the conditions of the stationary and mobile phases established here.

### 3.2. Extraction and purification

Since using deionized water on cut samples can only extract ions present on the surface of the samples, extraction was conducted on food samples homogenized when frozen. Comparing the peak areas of the ion chromatograms of cut samples and homogenized samples, the latter was shown to have an area at least 1.2 times greater than the former;

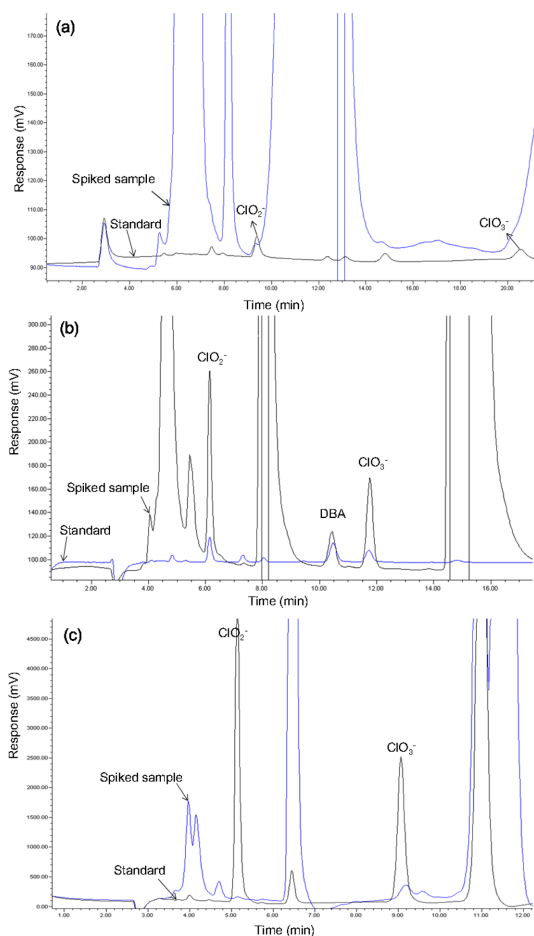


Fig. 3. Chromatograms of a standard solution (0.4 mg/L) and a spiked mixed sprouts sample (30 mg/kg). The column was Dionex IonPac AS27 and the mobile system was KOH (1 mL/min) with different concentrations: (a) 10 mM, (b) 20 mM, and (c) 30 mM. Chlorite and chlorate peaks were separated from formate and nitrate peaks in the 20 mM KOH with retention times of 6.2 and 11.8 min for chlorite and chlorate, respectively. DBA was used as a surrogate standard.

hence, it was chosen as the extraction method. In fact, homogenization was commonly used in previous studies.<sup>12,18-21</sup>

However, the presence of residual organic macromolecules in the columns increased the pressure within the system when the extract was centrifuged at 3,500 rpm (Centrifuge HA-12, Hanil Science Industry, Incheon, Korea) and the resultant supernatant was filtered through a 0.2  $\mu\text{m}$  syringe filter before being

injected to the IC. Large organic molecules contained in the samples include proteins, fats, and carbohydrates were retained in the column, causing pressure elevation in the system. To remove the organic substances present in the samples, an additional purification procedure using an SPE cartridge was introduced.<sup>18-19,22</sup> Cartridges used for this process include Sep-pak tC18 (hereafter referred to as Waters), C18, C18 plus, coconut charcoal (hereafter referred to as Sigma-Aldrich), Supelclean ENVI-Carb SPE tube, Carboxen 569, Carboxen 572, SCX, Strata NH2, Strata SI-1 (hereafter referred to as Phenomenex, Torrance, USA). The extract and ion chromatogram obtained were shown to be the clearest when a tC18 cartridge (6 mL, 1 g) was used, hence it was selected as the cartridge to process the food samples.

Despite such efforts, the problem of increasing pressure within the system persisted. To resolve the issue, ethanol or acetone was added to the extract to precipitate large biomolecules such as proteins or carbohydrates, and liquid-liquid extraction using hexane was carried out to remove lipids. The improvement achieved using this method was marginal. The problem was solved by increasing the rotational velocity of the centrifuge to 8,500 rpm.

### 3.3. Verification of the significance of the analytical technique

#### 3.3.1. Qualitative analysis

Qualitative analysis carried out in this study can be broadly categorized into two methods. Standard solutions of chlorite and chlorate ions were analyzed via IC-CD to identify the retention time of each ion. When the standard solutions were added to the fully processed combined vegetable samples via extraction and purification, the peak retention times of the two ions increased.

The retention times of chlorite and chlorate ions in standard solutions and fragment ions were identified using LC-MS/MS. The combined sprout vegetable samples mixed with the standard solutions were analyzed (Fig. 4). The retention times of chlorite and chlorate in the standard solutions were measured to be 12.4 and 24.5 min, respectively, and the mass-to-

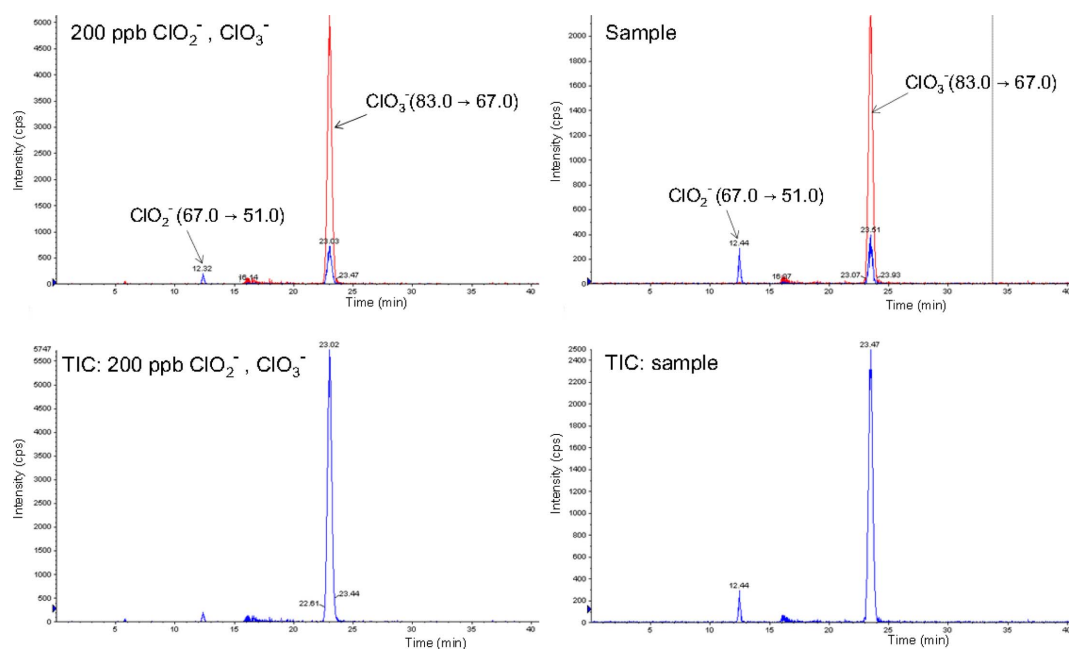


Fig. 4. Chromatograms of a standard solution (200 µg/L, left) and a spiked mixed sprouts sample (right) using LC-MS/MS.

charge ( $m/z$ ) ratio of each of their product ions/daughter ions was 67.0/51.0 and 83.0/67.0. Samples mixed with standard solutions exhibited the same retention times and  $m/z$  values as the standard solutions.

### 3.3.2. Accuracy and precision

The accuracy of the analysis was indicated using the recovery rate, and was measured at three concentration levels: low concentration (0.6 mg/kg for combined sprout vegetables, 1 mg/kg for dried fish products),

moderate concentration (3 mg/kg), and high concentration (6 mg/kg). While the recovery rate for dried fish products was relatively higher than in vegetable samples, all samples in general showed high recovery rates ranging between 91.5 and 99.0% (Table 3). Reproducibility of the results, which is an indicator of the precision of the analysis, was carried out by repetitively testing 7 samples at each of the concentration levels; the relative standard deviation (RSD) obtained ranged from 1.57% to 10.7% (Table 3).

Table 3. Results of recovery and repeatability for the established method

|                                    | Concentration (mg/kg) | Chlorite      |            | Chlorate      |            |
|------------------------------------|-----------------------|---------------|------------|---------------|------------|
|                                    |                       | Mixed sprouts | Dried fish | Mixed sprouts | Dried fish |
| Recovery (%) <sup>b</sup>          | 0.6 (1) <sup>a</sup>  | 94.1          | 91.5       | 97.7          | 96.6       |
|                                    | 3                     | 93.3          | 93.0       | 98.3          | 94.1       |
|                                    | 6                     | 98.5          | 91.6       | 99.0          | 98.1       |
|                                    | Average               | 95.3          | 92.1       | 98.3          | 96.3       |
| Repeatability (RSD %) <sup>b</sup> | 0.6 (1) <sup>a</sup>  | 10.7          | 9.95       | 3.21          | 6.10       |
|                                    | 3                     | 3.09          | 7.69       | 1.57          | 9.79       |
|                                    | 6                     | 4.14          | 4.77       | 1.73          | 2.66       |
|                                    | Average               | 5.99          | 7.47       | 2.17          | 6.18       |

<sup>a</sup>0.6 mg/kg for mixed sprouts and 1 mg/kg for dried fish.

<sup>b</sup>Seven replicate samples for each concentration level were analyzed.



### 3.3.3. MDL and LOQ

The MDLs of chlorite and chlorate for combined sprout vegetable samples were 0.2 and 0.1 mg/kg, respectively, while the corresponding LOQs were calculated to be 0.7 and 0.3 mg/kg. This was in agreement with the LOQs of chlorite and chlorate in potato peels, found to be approximately 0.1 mg/kg in previous studies.<sup>23</sup>

In contrast, the MDLs of both chlorite and chlorate for dried fish samples were 0.2 mg/kg, and the LOQs were estimated to be 0.7 mg/kg. The MDL obtained in this research was one-fifth the MDL obtained using seafood products in previous studies, which were recorded at 1 mg/kg.<sup>12</sup>

### 3.3.4. Linearity

The linearity of fresh-cut food, analyzed using combined sprout vegetable samples, was expressed using the coefficients of determination ( $r^2$ ) (Fig. 5), which were calculated to be 0.9973 and 0.9987 for chlorite and chlorate, respectively. Both the  $r^2$  values of chlorite and chlorate for dried squid samples were 0.9999. Hence, it was concluded that both types of food samples exhibit high levels of linearity with respect to chlorite and chlorate.

### 3.3.5. Cross-validation

The results of quality assurance and quality control

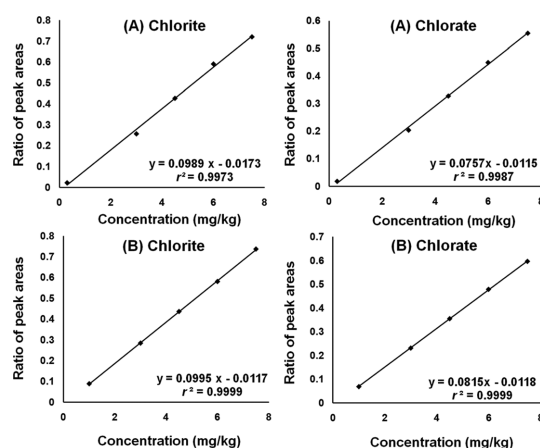


Fig. 5. Calibration curves of chlorite and chlorate in (A) ready-to-eat vegetables and (B) dried fish.

Table 4. Comparison of recovery, repeatability, and linearity for chlorite and chlorate in mixed sprouts samples between this study (A) and two other laboratories (B and C) using the established method

| Analyte <sup>a</sup> | Institution | Recovery (%) <sup>b</sup> | Repeatability (RSD%) <sup>b</sup> | $r^2$  |
|----------------------|-------------|---------------------------|-----------------------------------|--------|
| Chlorite             | A           | 92.1                      | 7.47                              | 0.9973 |
|                      | B           | 97.3                      | 8.55                              | 0.9979 |
|                      | C           | 95.5                      | 8.47                              | 0.9975 |
| Chlorate             | A           | 96.3                      | 6.18                              | 0.9987 |
|                      | B           | 103                       | 6.33                              | 0.9982 |
|                      | C           | 109                       | 6.84                              | 0.9975 |

<sup>a</sup>The tested concentration level was 4.5 mg/kg.

<sup>b</sup>Seven replicate samples for each concentration level were analyzed.

acquired in this laboratory (A) were cross-validated in two other laboratories (B and C). The results obtained in all three labs were compared (Table 4). At the 4.5 mg/kg concentration level, the recovery rates of chlorite and chlorate for combined sprout vegetables were determined to be over 95.5 % in both of labs B and C, while the RSD was found to lie between 6.33-8.55 %, which is similar to the level found in lab A. The  $r^2$  values of the calibration curves calculated in all three laboratories were over 0.997. Hence, the statistical significance of the analytical technique established in this study could be verified in other laboratories.

### 3.4. Food sample analysis

Of the 60 samples of fresh-cut food and 60 samples of dried meat products acquired and analyzed, chlorite and chlorate ions were not detected in any sample. Ion chromatograms of three representative samples are illustrated in Fig. 5. The main reason why the target substances were not detected in the food samples may be that food disinfectants are rarely used domestically. This deviates from the predictions that the use of food disinfectants would have increased following the research carried out in 2009, in which general bacteria, staphylococcus, and bacillus bacteria<sup>24</sup> (including aerotropic bacteria<sup>25</sup>) were detected in 101 commercially distributed dried meat samples and seasoned dried meat products

available in local markets and traditional markets. Another possibility was that the food products were rinsed sufficiently with water after disinfection, and this was confirmed by enquiring with some of the food manufacturers. Hence, the negative health impacts of residual chlorite and chlorate that may exist in fresh-cut food and dried meat samples due to the usage of chlorine-based disinfectants was concluded to be negligible in this study.

#### 4. Conclusions

An analytical technique using a hydroxide-based mobile phase and AS27 to determine the quantity of chlorite and chlorate—formed as byproducts of the decomposition of chlorine-based food disinfectants—via IC-CD was developed. The stability of chlorite ions was ensured using ethylenediamine. Target ions present in the interior of the food samples could also be examined by homogenizing the food samples prior to analysis. The difference in the extraction efficiencies due to differences between the matrices of food samples could be corrected for using dibromoacetate as the surrogate standard. Using an ultracentrifuge (8,500 rpm) and a tC18 cartridge purification procedure, analysis of the target ions could be carried out without eliminating the chloride and nitrate ions present in high concentrations within the food samples. The analytical technique developed was found to have a high degree of accuracy, precision, and linearity. The significance of the technique was verified by conducting cross-validation studies in other laboratories. While the target anions were not detected in food samples being distributed commercially, it is expected that the analytical technique devised in this study will be widely used in evaluating the health hazards of using chlorine-based food disinfectants.

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