

## Determination of Chromium(III) Picolinate Using High Performance Liquid Chromatography-Ultraviolet Spectrophotometry

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Chromium(III) is very well known as an essential mineral.<sup>1,2</sup> It is suggested as a cofactor in the maintenance of both normal lipid and carbohydrate metabolism by assisting the action of insulin on a cell membrane.<sup>3,4</sup> According to the National Research Council, the daily recommended intake of chromium(III) is 50-200  $\mu\text{g}$ .<sup>5</sup> Several organic chromium(III) complexes have been reported to have significantly higher absorption and tissue incorporation activity than inorganic salts such as chromium(III) chloride.<sup>6,7</sup>

Chromium(III) picolinate (Scheme 1) is lipophilic, which facilitates its entry into and through the plasma membrane of cells.<sup>8,9</sup> In the skeletal muscle cells cultured in media containing chromium(III) picolinate, the rate of insulin internalization was increased and the uptake of both glucose and leucine was improved.<sup>10</sup> Chromium(III) picolinate as a nutritional supplement and weight-loss agent has been commercially successful and known to have beneficial effects on carbohydrate and lipid metabolism alleviating symptoms associated with adult-onset diabetes.<sup>11,12</sup>

The identification of chromium(III) picolinate has been carried out using FT-IR,<sup>13</sup> NMR spectroscopy,<sup>13-15</sup> and X-ray diffraction spectrometry.<sup>16</sup> FT-IR usually detects the functional groups of the investigated material and is generally

compared with the standard material. Analyses of chromium(III) picolinate have demonstrated the use of the mass spectrometer with fast atom bombardment ionization and electron ionization without HPLC. The identification of chromium(III) picolinate using HPLC-MS has been reported.<sup>17</sup>

Until now, the determination method of chromium(III) picolinate has not been reported.

In the present research, we studied the determination method of chromium(III) picolinate using ESI-MS on-lined with HPLC and determined the chromium(III) picolinate in feed products.

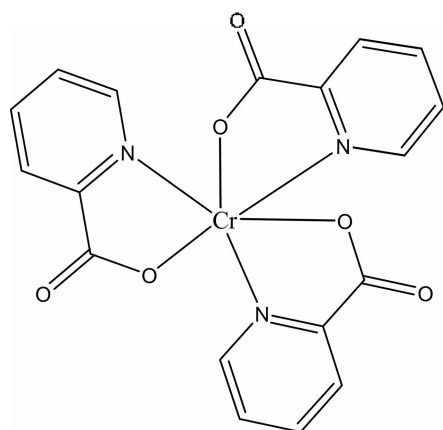
### Experimental Section

**Reagents & apparatus.** Chromium(III) nitrate nonhydrate (purity: 99.99%), picolinic acid (purity: 99%), chromium(III) picolinate and sodium hydroxide were purchased from Aldrich. Acetonitrile used for HPLC analysis was of chromatographic grade and purchased from Merck. Water was purified by a Milli-Q system from Millipore (Milford, MA, USA). The octadecyl bonded column (total coverage B191A, octadecyl silica, 5  $\mu\text{m}$  particle size, 4.6  $\times$  250 mm I.D.) was purchased from Separation Methods Technologies.

HPLC was performed using Jasco High Performance Liquid Chromatography with a UV-1575 detector, DG-1580-54 degaser, PU-1580 pump. Optimal conditions of high performance liquid chromatograph were found to be: flow rate = 1.0 mL/min, wavelength = 264 nm, column oven temperature = 50  $^{\circ}\text{C}$ , and mobile phase composition = acetonitrile : H<sub>2</sub>O = 6 : 4.

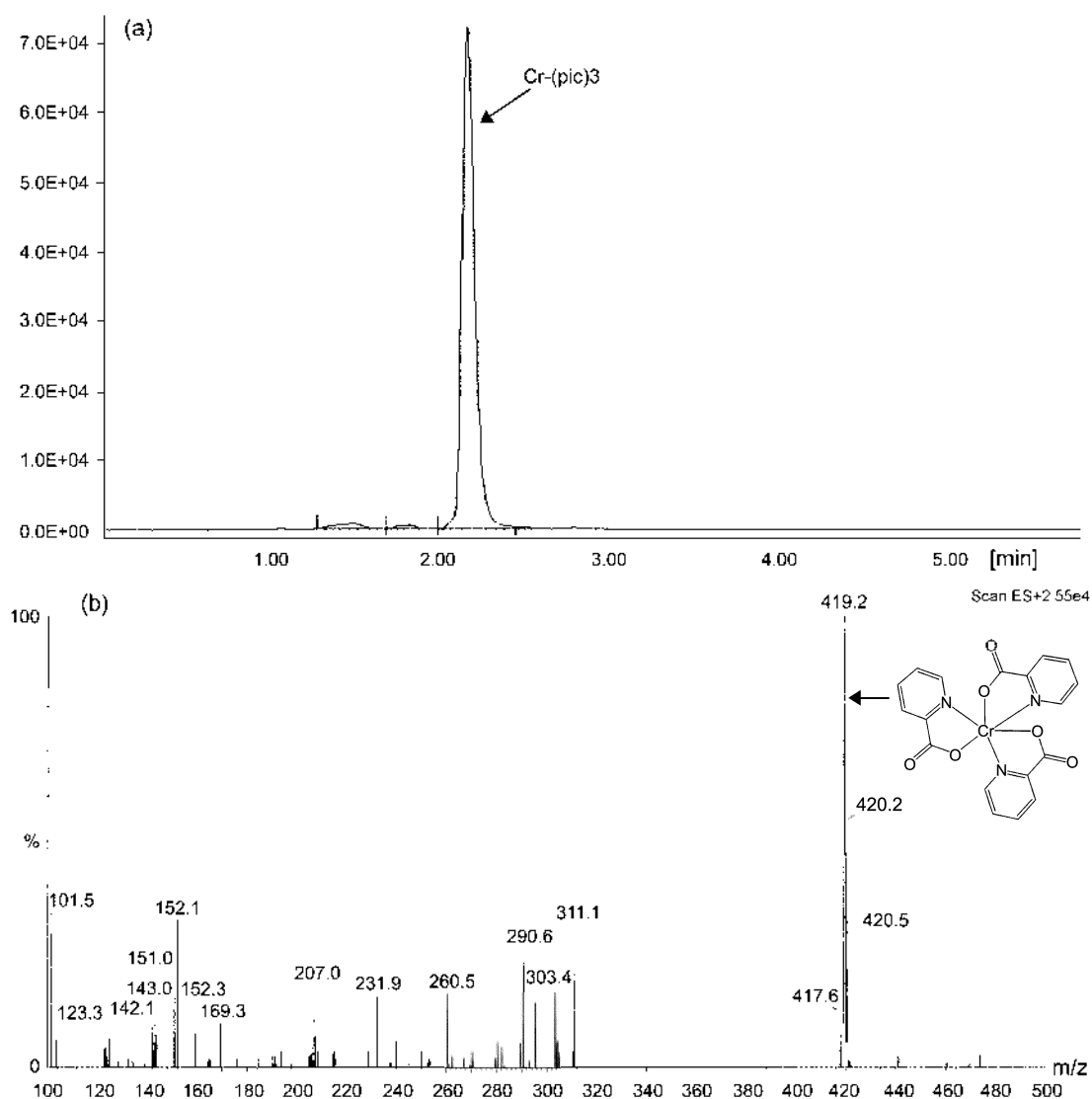
Mass spectra were made with A Quattro LC Triple Quadrupole Tandem Spectrometer equipped with an electrospray ionization source. The operative parameters were set as follows; tuning parameters; ES-, capillary voltage: 3.92 volt, conc voltage: 30 volt, extractor voltage: 4 volt, Rf lens voltage: 0.28 volt, source block temperature: 600  $^{\circ}\text{C}$ , desolvation temperature: 180  $^{\circ}\text{C}$ . The column was connected to a Quattro LC Triple Quadrupole Tandem Mass Spectrometer.

**Sample solution.** Each feed product containing chromium(III) picolinate was collected from three companies. 200 mg of each sample was weighed accurately and then dissolved in the mobile phase solution and diluted to mark of a 100 mL

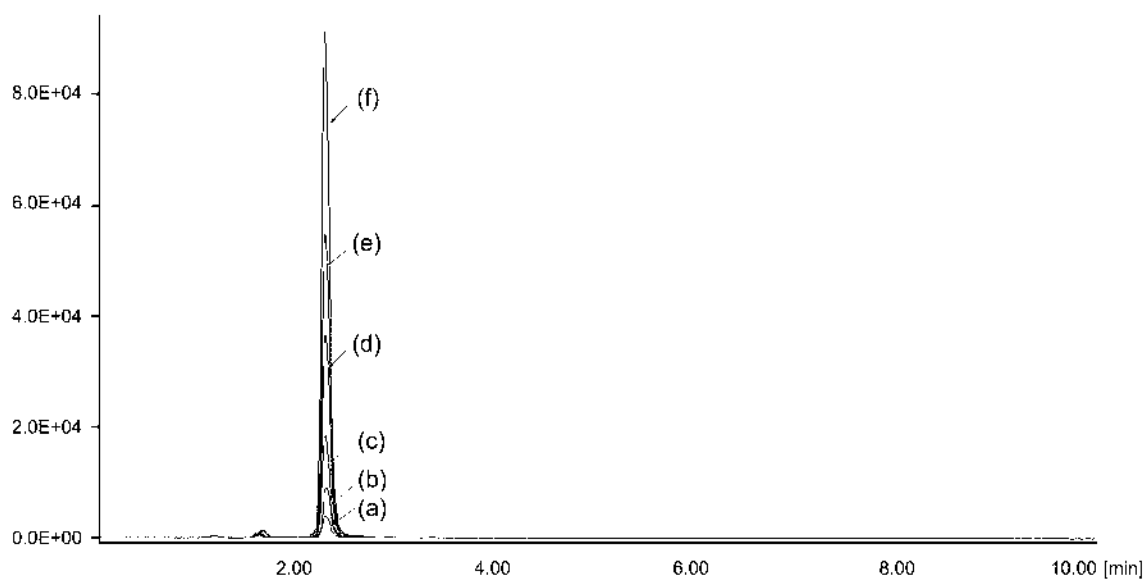


**Scheme 1.** Structure of chromium(III) picolinate (Cr-(pic)<sub>3</sub>).

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**Figure 1.** (a) Chromatogram of chromium(III) picolinate, concentration:  $10 \mu\text{g mL}^{-1}$  in mobile phase (AcCN : H<sub>2</sub>O = 6 : 4); flow rate:  $1 \text{ mL min}^{-1}$  (b) ESI mass spectrum of the above pure compound directly after LC separation, peak:  $419 \text{ m/z [M+H]}$ .



**Figure 2.** Chromatograms of chromium(III) picolinate in various concentrations; a:  $0.55 \mu\text{g mL}^{-1}$ ; b:  $1.37 \mu\text{g mL}^{-1}$ ; c:  $2.74 \mu\text{g mL}^{-1}$ ; d:  $5.48 \mu\text{g mL}^{-1}$ ; e:  $8.22 \mu\text{g mL}^{-1}$ ; f:  $13.7 \mu\text{g mL}^{-1}$  chromium(III) picolinate.

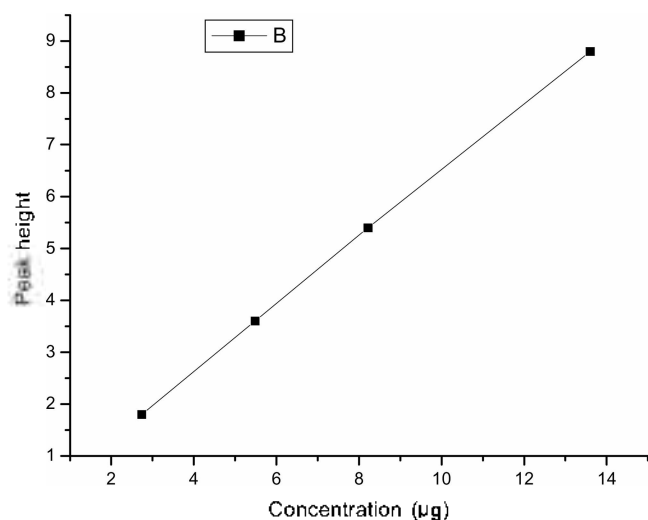


Figure 3. Calibration curve of chromium(III) picolinate.

Table 1. Recovery of chromium(III) picolinate in various matrices

Matrix	Added (µg)	Found (µg), n = 5	Recovery (%)
Yeast powder	50	54.3, 53.2, 55.6, 52.7, 51.9	107.1 ± 2.9
CaCO <sub>3</sub>	50	50.4, 50.8, 50.1, 49.8, 50.3	100.6 ± 0.7
Whey mill	50	55.3, 54.7, 56.8, 52.4, 56.7	110.4 ± 3.6

Table 2. The assay of chromium(III) picolinate in feed products manufactured by several companies

Company	Guaranteed (%)	Found (%), n = 5
A	0.33	0.34 ± 0.007
B	0.33	0.37 ± 0.013
C	0.34	0.36 ± 0.011

volumetric flask with mobile phase. After sonication for 30 min, the solution was vortexed for 30 min and then filtered with 0.45 µm nylon for HPLC analysis.

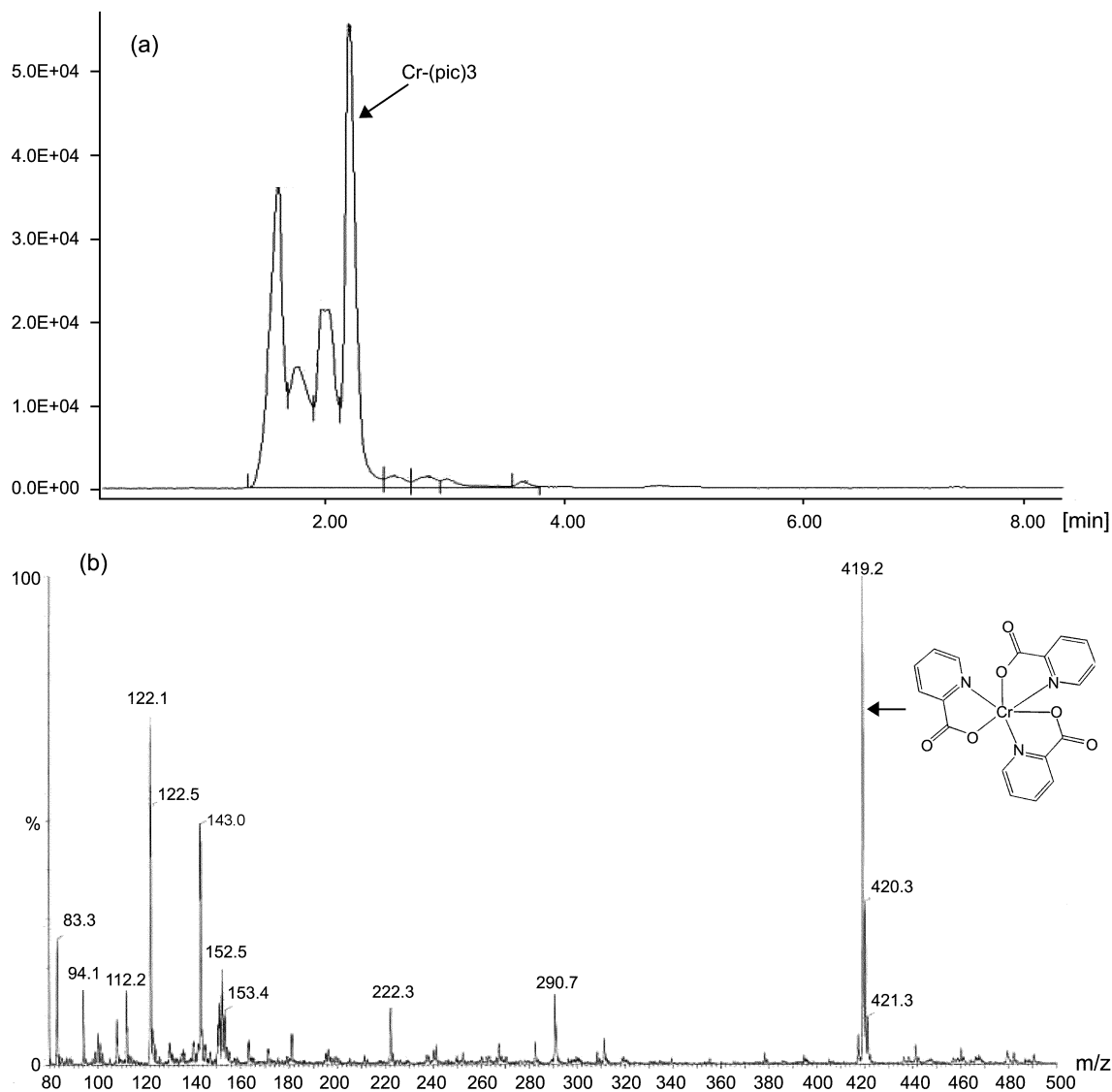


Figure 4. (a) Chromatogram obtained from feed product (company C); same conditions as described in Figure 1(b) the ESI mass spectrum detected directly after I.C separation (peak time = 2.15 min), peak; 419 m/z [M·H].

## Results and Discussion

**Chromatogram & mass spectrum of chromium(III) picolinate.** 20  $\mu\text{L}$  of the chromium(III) picolinate solution (10  $\mu\text{g}/\text{mL}$ ) were injected into the HPLC column and eluted with the mixture solution of various ratios of acetonitrile and water as an eluent. In the eluent of 60% acetonitrile and 40% water, a single dominant peak was observed at 2.15 min on the chromatogram as shown in Figure 1(a). This eluent indicates the optimal chromatographic result according to the signal to background and symmetry. Figure 1(b) shows the ESI positive ion mass spectrum of the peak. The intact molecular cation,  $[\text{Cr}(\text{pic})_3 + \text{H}]^+$ , for the compound ( $m/z$  419) was observed as the base peak. The characteristic isotopic pattern of Cr for the molecular cation as well as the nominal molecular mass (418Da) shows that the peak is due to  $\text{Cr}(\text{pic})_3$ .

**Calibration curve.** The solubility of chromium(III) picolinate in water is known to be 0.6 mM.<sup>13</sup> The stock solution of chromium(III) picolinate (125  $\mu\text{g mL}^{-1}$  mobile phase) was prepared, and then diluted to each concentration before injection. The calibration curve (Figure 3) was drawn out in the concentration range of 0.1-13.7  $\mu\text{g mL}^{-1}$  using data of HPLC chromatogram (Figure 2). The calibration curve shows excellent linearity with  $R^2 = 0.9999$ . The detection limit ( $S/N = 3$ ) was 20  $\text{ng mL}^{-1}$ , and the relative standard deviation (R.S.D.,  $n = 7$ ) was 2.5%.

**Effects of co-existing substance.** To study the effects of the foreign substance, 0.1 g of each sample of different matrix spiked with 50  $\mu\text{g}$  chromium(III) picolinate was determined (Table 1). The sample solution was made according to the preparation process. Those matrices are the drug carrier. The recovery ranged from 100.6% to 110.4% and the average recovery was 106.0%. Among the matrices used in this experiment, a 10% error was characteristic of the whey mill matrix, and the calcium carbonate matrix gave excellent recovery (100.6%).

**Application.** The present method was applied to the determination of chromium(III) picolinate in feed products. Each feed product for animal diets was determined as shown in (Table 2). In this table, the data obtained by the present method is in a good agreement with the guaranteed values. This method can be applied to other products, such as foods, medicine, human serum, and various matrices containing chromium(III) picolinate.

## Conclusions

$\text{Cr}(\text{pic})_3$  has been widely used as food additives, drugs, and feed additives. Accordingly, its determination method should be established. In the present paper, we have studied the determination method of chromium(III) picolinate accurately using ESI-MS on-lined with HPLC. Chromium(III) picolinate in feed products was determined successfully.

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