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# Speciation of chromium in edible animal oils after microwave extraction and liquid chromatography inductively coupled plasma mass spectrometry

Ya-An Lin [a], Shiuh-Jen Jiang [a,b,*], A.C. Sahayam [c], Yeou-Lih Huang [b]

[a] Department of Chemistry, National Sun Yat-sen University, Kaohsiung 80424, Taiwan
[b] Department of Medical Laboratory Science and Biotechnology, Kaohsiung Medical University, Kaohsiung 80708, Taiwan
[c] National Centre for Compositional Characterisation of Materials (CCCM), Hyderabad, India

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ABSTRACT

Speciation of chromium has been carried out using reversed phase HPLC and ICP-MS in tandem. Isocratic elution using 0.5 mmol $L^{-1}$ tetrabutyl ammonium phosphate (TBAP) and 0.3 mmol $L^{-1}$ EDTA in 1% methanol (pH 6.9) as mobile phase separated Cr(III) and Cr(VI) in less than 110 s. Spectral interferences in ICP-MS at m/z 52 and 53 were reduced using $NH_3$ in the DRC. Detection limits were 0.045 and 0.052 ng Cr $mL^{-1}$ for Cr(III) and Cr(VI), respectively. This method has been applied to determine Cr compounds present in selected edible animal oils and NRCC SLRS-3 Riverine Water reference material. The animal oils selected are used for different applications including cooking. Chromium species were extracted from oils using microwave heating with 0.4% v/v HF and 2% Triton X-100 in mobile phase. HPLC-ICP-MS results showed good agreement with total chromium concentrations in extracts obtained by ICP-MS analysis. An unknown chromium compound was found in all oil samples with the absence of Cr(VI).



## 1. Introduction

The environmental and toxicological effects of an element often depend on the form or species of the element present in the original sample. The determination of the total element content by most spectroscopic detectors does not provide this crucial information. However, interfacing chromatography with element-specific detectors can provide speciation analysis involving identification and determination of individual physical–chemical forms of an element present in the sample of interest. Elemental speciation has become a part of the environmental monitoring where the level of toxicity of an element differs from one form to the other. Chromium is one such element which needs no introduction as it is well known that its species, Cr(III) and Cr(VI), have opposing toxicities [1]. Hence, speciation analysis of chromium in foods is vital [2] to evaluate their suitability for human consumption. The raw material for the manufacture of edible animal oils is collected from leather industries also. Animal oils might be contaminated with Cr(VI) due to the extensive use of Cr in leather industries. Hence, it is important to determine the concentration of Cr and its species in edible animal oils.

Inductively coupled plasma mass spectrometer (ICP-MS) is a trace-element detection tool with superior analytical capabilities. Several procedures based on liquid chromatography (LC) [3–9] and capillary electrophoresis (CE) [10,11] coupled to ICP-MS have been reported for chromium speciation analysis. Coupling of LC to ICP-MS gained much attention due to the easiness of sample preparation, simplicity of the interface, availability of isotope ratio information and also specificity of the signal intensity of the determined element [12]. The determination of chromium by quadrupole ICP-MS is typically compromised by isobaric interferences from $^{40}Ar^{12}C^+$ and $^{35}Cl^{16}OH^+$ on $^{52}Cr^+$ and $^{40}Ar^{12}CH^+$ and $^{37}Cl^{16}O^+$ on $^{53}Cr^+$. These interferences are reported to be alleviated by the use of dynamic reaction cell (DRC) technique with $O_2$ [2] and/or $NH_3$ [13–15] as the reaction gas.

The aim of the present work is to develop a rapid and accurate method for the speciation analysis of Cr(III) and Cr(VI) in edible animal oils using liquid chromatography DRC-ICP-MS. The optimization of the LC-DRC-ICP-MS operating conditions, analytical figures of merit of the developed procedure as well as its application to chromium speciation analysis in selected oil samples are described.

* Corresponding author at: Department of Chemistry, National Sun Yat-sen University, Kaohsiung 80424, Taiwan.
*E-mail address:* sjjiang@faculty.nsysu.edu.tw (S.-J. Jiang).

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## 2. Experimental

### 2.1. Apparatus and conditions

An ELAN 6100 DRC II ICP-MS (Perkin-Elmer SCIEX, Concord, ON, Canada) was used for the experiments. Samples were introduced by a cross-flow pneumatic nebulizer with a Scott-type spray chamber. The operating conditions of ICP-MS were optimized by continuous introduction of a solution containing 5 ng $mL^{-1}$ Cr in mobile phase. Operating conditions of ICP-MS used in this work are summarized in Table 1.

A HPLC pump (Hitachi, Model CM-5110), an injector (Rheodyne 7725i) and reversed phase HPLC column (Perkin-Elmer C8 silica based, 3 μm diam. particles, 3.0 mm i.d. × 33 mm length) comprised the LC system. Samples were loaded with a syringe onto a 100 μL sample loop. All separations were performed at room temperature under isocratic conditions. The conditions listed in Table 1 were those that yielded the best chromatogram, with respect to best resolution and lower retention times, of various sets tested. The column outlet was connected to the pneumatic nebulizer of the ICP-MS device through polytetrafluoroethylene (PTFE) tubing of 0.18 mm internal diameter and 47 cm length.

### 2.2. Reagents

Analytical-reagent grade chemicals were used without further purification. Purified water (18.2 MΩ-cm), from a Milli-Q water purification system (Millipore, Bedford, MA, USA), was used to prepare all solutions. Methanol, acetic acid, $NH_4OH$ and Suprapur nitric acid (65%) were purchased from Merck (Darmstadt, Germany). Tetra-n-butylammonium phosphate (TBAP) was from TCI (Tokyo, Japan). The disodium salt of ethylenediamine tetraacetic acid (EDTA) was obtained from Fisher (Fair Lawn, NJ, USA). Chromium(III) chloride was procured from Alfa Chemicals (Danvers, MA, USA). Potassium dichromate(VI) was obtained from Merck (Darmstadt, Germany). Cr(III) and Cr(VI) stock standard solutions were prepared using $CrCl_3 \cdot 6H_2O$ and $K_2Cr_2O_7$ in 0.2 mmol $L^{-1}$ EDTA and deionized water, respectively. The Ar gas used was supplied by Hsin E Li Gases Co., Ltd. (Kaohsiung, Taiwan). $O_2$ and $NH_3$ gas used were of 99.999% purity (Air Liquide, Taiwan). To prepare mobile phase solutions, suitable amounts of EDTA and TBAP were dissolved in HPLC-grade methanol and pure water (Milli-Q, 18.2 MΩ-cm resistivity) to the desired concentration. The pH of mobile phase was adjusted to 6.9 with acetic acid and $NH_4OH$.

**Table 1**
Equipment and operating conditions.

| ICP-MS instrument | Perkin-Elmer SCIEX ELAN 6100 DRCII |
|---|---|
| *ICP parameter* | |
| RF power | 1300 W |
| Plasma gas flow rate | 15 L $min^{-1}$ |
| Auxiliary gas flow rate | 1.325 L $min^{-1}$ |
| Nebulizer gas flow rate | 0.97 L $min^{-1}$ |
| Mass spectrometer settings | |
| Dwell time | 100 ms |
| Sweeps/Reading | 5 |
| Reading/Replicate | 300 |
| Replicates | 1 |
| Isotope monitored | $^{50}Cr$, $^{52}Cr$, $^{53}Cr$ |
| DRC parameter | |
| Reaction gas | $NH_3$ |
| Reaction gas flow rate | 0.6 mL $min^{-1}$ |
| Rpq | 0.65 |
| Rpa | 0 |
| Axial field voltage | 175 V |
| *HPLC system* | |
| Pump | Hitachi Model CM-5110 LC pump |
| Injector | Rheodyne model 7725i |
| Stationary phase | Perkin Elmer C8, 3.0 mm i.d. × 33 mm length 3 μm diameter particles |
| Mobile phase | 0.5 mmol $L^{-1}$ TBAP and 0.3 mmol $L^{-1}$ EDTA in 1% methanol (pH 6.9) |
| Flow rate | 1.0 mL $min^{-1}$ |
| Sample loop | 100 μL |

### 2.3. Sample preparation

The applicability of the developed procedure to the real samples has been carried out on three chromium contaminated oil samples [Oil 1 (buffalo/cow/cattle), Oil 2 (mixed animal oil) and Oil 3 (fish oil)] obtained locally. The accuracy of the method has been validated by comparing the sum of the concentrations of individual species obtained by the present procedure, with total concentration of chromium certified in NRCC SLRS-3 Riverine Water reference material (National Research Council of Canada, Ottawa, Canada).

A microwave-assisted extraction procedure was used for the extraction of various chromium compounds from oil samples. A MARS 5 open microwave digester (CEM, Matthews, NC, USA) was used as the extracting device. 0.1 g each of oil samples was weighed accurately into 15 mL polyethylene centrifuge tubes and a 5 mL solution of 0.4% v/v HF and 2% Triton X-100 in mobile phase was added. The tubes were inserted into a 250 mL beaker (Pyrex glass) having 75 mL water. The microwave system was programmed to maintain the water temperature at 90 °C for 45 min with a ramp time of 10 min. The extraction solution and temperature have been selected based on our earlier experience [7] and time of exposure is selected to obtain maximum recovery of species. After microwave heating, the samples were allowed to cool and directly centrifuged for 10 min at 3743 g. The supernatants were filtered through a PVDF filter (Pall Corporation, Ann Arbor, MI, USA) of 0.2 μm porosity before HPLC separation. The concentrations of Cr species were determined by external calibration method based on peak area. The recoveries of individual species were determined by spiking oil samples with suitable amounts of chromium standards [50 ng each of Cr(III) and Cr(VI) in 0.1 g of Oil 1 and Oil 2 samples; 25 ng each of Cr(III) and Cr(VI) in 0.2 g of Oil 3 sample] and then extracted by the extraction solution.

Since there is no reference value for the real-world samples, the extraction efficiency of chromium has been verified by comparing the total chromium concentrations in the extracts using the present procedure with those obtained from the complete dissolution method. For complete dissolution, samples were digested in a closed microwave oven (MARS 5, CEM) using 5 mL $HNO_3$ for 0.1 g of samples. The microwave dissolution was carried out at 170 °C for 10 min with a ramp time of 20 min and then the solution was heated again at 200 °C for 15 min with a ramp time of 15 min. The analysis was carried out for total Cr concentration by DRC ICP-MS using solution nebulization after suitable dilution. The chromium concentration in the sample was quantified by means of external calibration with 1 ng $mL^{-1}$ of rhodium as the internal standard.

## 3. Results and discussion

### 3.1. Selection of chromatography operating conditions

As reported in our previous paper [15], the species of Cr(VI) is anionic ($HCrO_4^-$ or $CrO_4^{2-}$) at pH 6–7 and Cr(III) is converted to anionic species by complexing with EDTA. The anionic species are separated using C8 reversed phase column with TBAP as ion pairing reagent. Preliminary studies were carried out using conditions similar to the previous work. Several parameters including the concentrations of EDTA and TBAP in mobile phase affected the separation of Cr(III)–EDTA and Cr(VI).

The effect of the concentration of EDTA in mobile phase on the chromatogram was studied in the range 0.1–0.3 mmol $L^{-1}$ at pH 6.9. The mobile phase flow rate was set at 1.2 mL $min^{-1}$. As shown in Fig. S1 (Supplementary data), the retention times of Cr(VI) and Cr(III)–EDTA were decreased with EDTA concentration, which could