# Analysis of Bromine Pesticide Residue in Australian Grain Export Cargoes Using ICP-MS

## Utilizing an existing Agilent 8900 ICP-QQQ instrument for quantitative analysis of Br at lower levels than traditional techniques

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## Introduction

Pesticide residues in food products are subject to Maximum Residue Levels (MRLs) established by the Codex Alimentarius Commission (CAC). Worldwide agencies such as the United Nations Food and Agriculture Organization (UN FAO) and the World Health Organization (WHO) adopt these limits to ensure protection of consumers and to encourage free trade. Bromide ion (Br) is currently referenced in Codex Alimentarius as a generic limited pesticide in 29 categories of food, with target MRLs between 20 mg/kg for fruits and 500 mg/kg for garden peas (*1*). The variation in MRLs is partly due to differences in natural levels in different crop plants. For example, green leafy vegetables such as spinach and lettuce can take up large amounts of bromide ion.

At their 2021 meeting, the Codex Committee on Pesticide Residues (CCPR) recommended that the MRLs for Br ion in cereals, fruit, vegetables, and herbs and spices should be revoked. However, as of September 2022, CAC has not approved the recommendation.

The natural bromide level in freshwater is typically about 0.05 mg/L (50 ppb), but seawater contains Br at around 65 mg/L. As a result, bromide can be found at elevated levels in marine biota, so marine origin foods may provide significant natural dietary intake. Bromide can also enter foodstuffs via Br-containing pesticides, including the active substance methyl bromide ($CH_3Br$, also known as bromomethane). Methyl bromide is used as a fumigant to control pests and diseases in cereal grains, for soil fumigation during the commercial production of strawberry runners, and as part of the rice packaging process. Methyl bromide is a known ozone depleting substance and so is banned for general use, with fumigation only permitted under specific exception categories. Methyl bromide is no longer authorized for use as a pesticide in the European Union, but other countries can still apply the compound to foods in quarantine or pre-shipment. Pre-shipment application occurs 21 days before export.

The MRL for methyl bromide in foodstuffs has been established as 0.01 mg/kg (*2*). The separate – much higher – limits that currently apply to bromide ion include Br from all sources excluding covalently bound bromine. The safe limit for dietary intake of bromide ion has been evaluated by the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) and has been tentatively set as 1 mg/kg body weight per day (*3*).

Metals analysis is a standard requirement for food safety monitoring, with the required analytes typically including the toxic elements As, Cd, Hg, and Pb. ICP-MS is commonly used for the determination of these trace metals, often together with micronutrient elements and even major elements. However, bromine and the other halogens are not easy to measure by ICP-MS, due to their high first ionization potential (1st IP), which translates into low ICP-MS sensitivity. Also, Br and I are chemically incompatible with the nitric ($HNO_3$) and hydrochloric (HCl) acids used to prepare samples for multi-element analysis by ICP-MS, and Cl cannot be determined when HCl is used.

As a result, bromide in foods is traditionally determined by ion selective electrode (*4*) or by colorimetric techniques. However, for food laboratories that use an ICP-MS for elemental (heavy metal) testing, the ability to also measure bromine by ICP-MS would offer additional flexibility in workflows. Having ICP-MS available as a backup technique for Br analysis would also provide cover in the case that the standard Br technique was not available, for example due to servicing or maintenance. In the Australian Superintendence Company (ASC) food laboratory, when the standard Br technique was unavailable for use, the question was asked whether the ICP-MS could be used to determine Br. This note details the evaluation that we performed to answer that question.

Maximum permitted total Br ion residue levels are currently set at an MRL of 50 mg/kg for cereals. The industry therefore requires a simple, routine procedure for the analysis of total bromine residues on cereals. When methyl bromide gas is used as an insecticidal fumigant on cereals, the concentration and exposure period may need to be carefully controlled to avoid exceeding the allowable level of bromide residues. Almost all methyl bromide will be converted to inorganic bromide within a few days of fumigation. Figure 1 illustrates this conversion as decay curves for organic and inorganic bromide content following fumigation of wheat flour. The plots show how the proportion of inorganic and organic Br alters over the period of a week, but the total bromine content is stable after the first 24 hours or so (*5*).

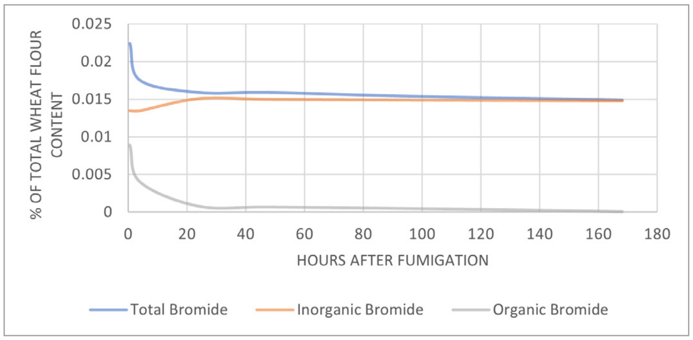

**Figure 1.** Conversion and loss of bromine species after wheat flour fumigation. Modified from Reference 5.

A procedure for the extraction and quantitative determination of Br in cereal grains has been developed by ASC as an in-house method. The method determines the total bromine—sum of both methyl bromide and inorganic bromide—in an aqueous extraction of a finely ground grain sample. The powdered sample is extracted with water and an aliquot of the extraction solution undergoes protein coagulation in a water bath. Total bromine is detected and quantified using a triple quadrupole Inductively Coupled Plasma Mass Spectrometer (ICP-QQQ).

The new method extracts and quantifies the total bromine concentration in the sample. If sampling occurs immediately after fumigation, there is a relatively high level of organic bromine present, as shown in Figure 1. As a result, for about a week after fumigation, the reported results are high—giving an overestimation of the total bromide content—until the methyl bromide is lost by volatilization. During the same period, a portion of the methyl bromide is hydrolyzed to methyl alcohol and hydrobromic acid, which is absorbed onto the grain, giving a slight increase in the level of inorganic Br. This absorbed bromide is readily soluble in water and so is extracted along with the residual methyl bromide. While the methyl bromide is very soluble in water (16.1 g/L at 25 °C), its high vapor pressure (1893 kPa) causes it to rapidly evaporate from the water extract.

## Experimental

### Calibration standards

Bromine standards (AccuStandard, New Haven, CT, USA) were prepared in 0.01 M high purity ascorbic acid ($C_6H_8O_6$, Merck, Germany). The acids that are typically used for stabilization of samples for multi-element ICP analysis, such as $HNO_3$ and HCl, often contain high levels of Br. This contamination precludes the use of these reagents for analysis of Br at the low ppm levels required for this method. The use of ascorbic acid to stabilize the solutions ensured the bromine was retained in solution without the risk of elevated backgrounds due to Br contamination. Br calibration standards were prepared to cover the concentration range from 0.01 to 10.0 mg/L.

An internal standard (ISTD) solution was chosen to ensure compatibility with existing trace metal analysis methods, to facilitate routine switching between trace metals analysis and bromine analysis. Iridium (Ir) was selected as the ISTD element, as it has a higher 1$^{st}$ IP than most other mid-mass ISTD elements that are commonly used in ICP-MS. Ir therefore gives better correction for ionization suppression of Br, which is also poorly ionized. The ISTD was prepared at a concentration of 50 µg/L Ir in 20% acetic acid, 6% $HNO_3$, and 1% HCl. The ISTD solution was added to the sample flow automatically using the standard online mixing tee. The ISTD flow rate was approximately 16 times lower than the sample flow rate, giving an Ir concentration as measured of approximately 3 µg/L.

### Samples and sample preparation

Representative samples of export wheat were collected from cargoes passing through the Port of Brisbane and Port of Esperance, Australia. The samples were ground to 0.75 µm, then sub-samples of approximately 10 g were weighed to the nearest 0.01 g and diluted 5-fold with de-ionized water. The samples were shaken for a couple of minutes to ensure they were fully homogenized. An aliquot of 5 mL was taken and diluted to volume in 0.01 M ascorbic acid, then thermally treated to coagulate the co-extracted proteins before centrifugation and analysis. The concentration of total Br in the extraction solution was determined by ICP-QQQ and corrected for the dilution factor to provide the concentration in the original grain sample for final reporting.

### Fortified analytical portions

For each sample, three fortified analytical portions (FAPs) were prepared to test the robustness and recovery of the sample preparation method. Before extraction, the FAP sub-samples were spiked (fortified) with Br at levels of 1, 5, and 20 mg/kg, with all spike levels being below the MRL of 50 mg/kg.

### Instrumentation

An Agilent 8900 Standard configuration ICP-QQQ was used. The instrument was fitted with a PEEK Mira Mist parallel path nebulizer, to ensure tolerance to any suspended particulates remaining in the sample extracts. The remainder of the sample introduction system was standard, comprising the Peltier cooled, Scott type, double-pass, quartz spray chamber, Ultra-High Matrix Introduction (UHMI) gas connector, and quartz torch with 2.5 mm id injector. Pt-tipped interface cones were used in place of the standard Ni cones. The 8900 ICP-QQQ was operated in single quad (SQ) mode and with the collision/reaction cell (CRC) pressurized with He cell gas to attenuate the transmission of polyatomic ions. In SQ mode, the Q1 mass filter—before the CRC—is operated as an ion guide, not as a true mass filter as in MS/MS mode. SQ mode emulates the performance of a single quadrupole ICP-MS system, suggesting that the method described could be applied to a single quadrupole instrument. The main 8900 ICP-QQQ operating conditions are shown in Table 1.

**Table 1.** Agilent 8900 ICP-QQQ operating conditions.

| Parameter | Value |
|---|---|
| ICP-QQQ Mode | Single Quad |
| Plasma RF Power (W) | 1550 |
| Sampling Depth (mm) | 9 |
| Carrier Gas Flow Rate (L/min) | 1.00 |
| Dilution Gas Flow Rate (L/min) | 0.15 |
| Spray Chamber Temp. (°C) | 2 |
| He Cell Gas Flow Rate (mL/min) | 5.0 |
| Kinetic Energy Discrimination (V) | 5.0 |

## Results and discussion

Br has two isotopes, $^{79}$Br and $^{81}$Br, with almost equal abundance of 50.69 and 49.31%, respectively. The $^{79}$Br isotope is preferred for quantitative determinations, as it is less affected by contributions from polyatomic ions such as $Ar_2H^+$, $ArCaH^+$, and $S_2OH^+$, as well as having slightly higher abundance. The Br calibration was prepared in 0.01 M ascorbic acid to ensure that the Br remained stable in solution. The calibration plot for $^{79}$Br (using $^{193}$Ir as the ISTD) is shown in Figure 2. The figures of merit—linearity, detection limit (DL), and background equivalent concentration (BEC)—taken from the $^{79}$Br calibration are presented in Table 2.

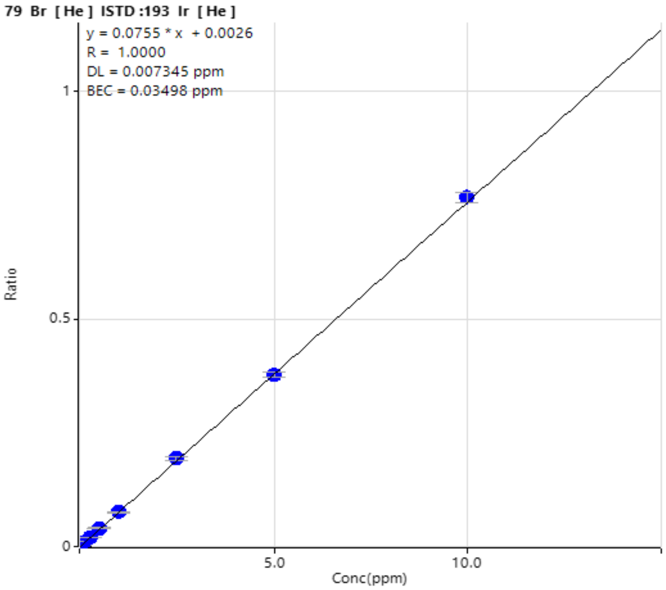

**Figure 2.** Calibration plot for $^{79}$Br showing low BEC and DL and excellent linearity (r = 1.000) demonstrating effective stabilization of Br with 0.01 M ascorbic acid.

**Table 2.** Instrument detection limit (IDL), background equivalent concentration (BEC), and R value of the calibration curve of $^{79}$Br. The low BEC (<50 ppb) confirms the low level of contamination from 0.01 M ascorbic acid.

|  | 8900 Calibration Performance Figures of Merit for Br |
|---|---|
| R | 1.0000 |
| DL (mg/L) | 0.0073 |
| BEC (mg/L) | 0.0350 |

### Instrument robustness and stability: ISTD recovery (%)

The sample extracts were run twice over a 4-hour period. The Ir ISTD recovery plot showed that the Ir signals were easily within ±20% of the signal in the reference sample (calibration blank). There were no internal standard failures throughout the run, as shown in Figure 3. The results demonstrate the robustness of the plasma and the good matrix tolerance of the 8900 ICP-QQQ for routine analysis of large batches of sample extracts that contain co-extracted starch and sugars.

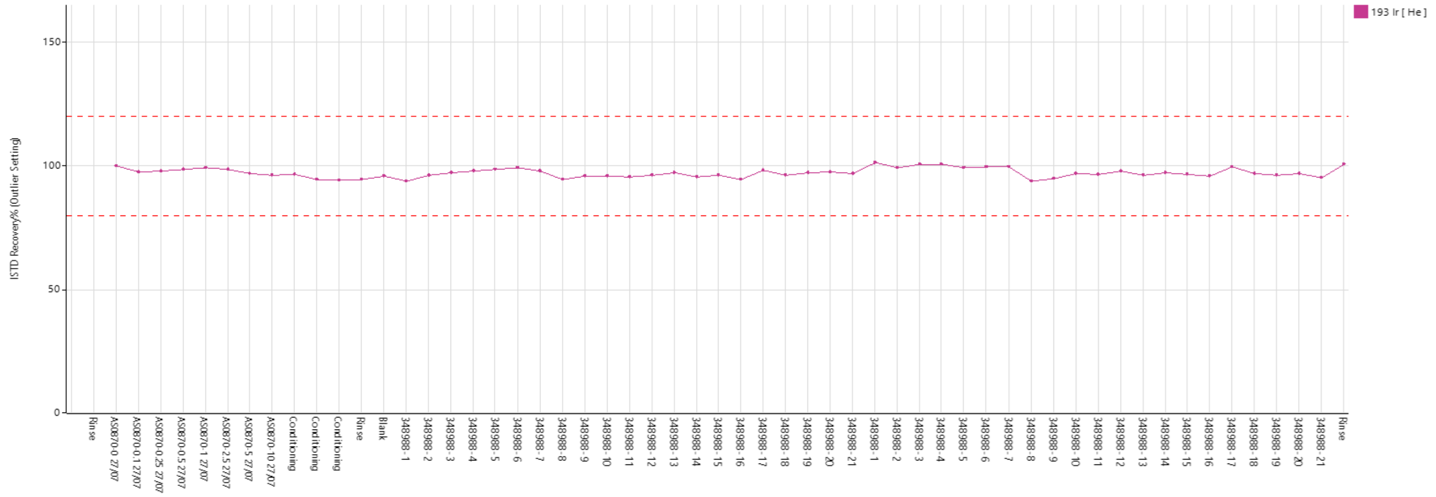

**Figure 3.** ISTD $^{193}$Ir signal in all samples, normalized to the calibration blank, showing stability of the ISTD signal over four hours. All ISTD recoveries were within the method requirements of 60–120%. The limit lines (red dotted lines) indicate ±20%.

## Spike recovery test

A spike recovery (fortified analytical portion – FAP) test was carried out to further evaluate the Br extraction capability and matrix tolerance of the method at levels below the MRL of 50 mg/kg for wheat samples. A wheat sample with a measured Br content of 2.1 mg/kg was selected as the target for the spike recovery test. Spikes at 1, 5, and 20 mg/kg of Br were added to multiple replicates of the ground grain subsample. The spikes were recovered with accuracies between 107–119% and standard deviations between 0.14–0.96 mg/kg for the three fortification levels, as shown in Table 3. The good accuracy and precision confirm the quantitative extraction performance and the accurate quantification capability of the ICP-MS method.

**Table 3.** Replicates of a wheat sample run unspiked and fortified with Br at concentration levels of 1, 5, and 20 mg/kg, showing accurate recovery results and excellent reproducibility.

| Sub-Sample Replicate | Fortification Level (mg/kg) | Concentration for Replicate (mg/kg) | Average (mg/kg) | Recovery of Fortification (%) | Standard Deviation (mg/kg) |
|---|---|---|---|---|---|
| 1 | Not spiked | 1.997 | 2.10 | NA | NA |
| 2 | Not spiked | 2.030 | | | |
| 3 | Not spiked | 2.128 | | | |
| 4 | Not spiked | 2.071 | | | |
| 5 | Not spiked | 1.987 | | | |
| 6 | Not spiked | 2.033 | | | |
| 7 | Not spiked | 2.201 | | | |
| 8 | Not spiked | 2.317 | | | |
| 9 | 1.0 | 3.248 | 3.19 | 107 | 0.14 |
| 10 | 1.0 | 3.324 | | | |
| 11 | 1.0 | 3.187 | | | |
| 12 | 1.0 | 3.155 | | | |
| 13 | 1.0 | 2.877 | | | |
| 14 | 1.0 | 3.321 | | | |
| 15 | 1.0 | 3.075 | | | |
| 16 | 1.0 | 3.320 | | | |
| 17 | 5.0 | 9.113 | 7.64 | 111 | 0.60 |
| 18 | 5.0 | 7.795 | | | |
| 19 | 5.0 | 7.609 | | | |
| 20 | 5.0 | 7.537 | | | |
| 21 | 5.0 | 7.133 | | | |
| 22 | 5.0 | 7.241 | | | |
| 23 | 5.0 | 7.197 | | | |
| 24 | 5.0 | 7.459 | | | |
| 25 | 20.0 | 23.970 | 25.80 | 119 | 0.96 |
| 26 | 20.0 | 24.834 | | | |
| 27 | 20.0 | 25.551 | | | |
| 28 | 20.0 | 26.421 | | | |
| 29 | 20.0 | 26.158 | | | |
| 30 | 20.0 | 27.047 | | | |
| 31 | 20.0 | 26.789 | | | |
| 32 | 20.0 | 25.658 | | | |

*NA = not applicable.*

### External laboratory tested samples

In addition to development of this in-house procedure for the analysis of bromine residues, eight samples were sent out to an external laboratory for confirmatory analysis. A comparison of the results for these eight samples is shown in Table 4.

**Table 4.** Comparative data showing good agreement between the Br content in wheat samples measured using the newly developed, in-house ICP-MS method, and the results obtained using a traditional technique at an independent external laboratory.

| Sample ID | In-House Laboratory Result (mg/kg) | External Laboratory Result (mg/kg) |
|---|---|---|
| A | 1.7 | <5 |
| B | 1.7 | <5 |
| C | 6.7 | 7 |
| D | 1.2 | <5 |
| E | 2.0 | <5 |
| F | 3.1 | <5 |
| G | 2.7 | <5 |
| H | 3.4 | <5 |

The high sensitivity of the ICP-MS method provides accurate quantitative analysis at concentrations below the levels required for compliance with current export testing for Br. In cereal grains, the new ICP-MS method provides much lower detection limits than traditional techniques, future-proofing food laboratories' analytical capability should regulated levels be reduced in the future.

## Conclusion

The Agilent 8900 ICP-QQQ operating in He KED mode is a highly effective tool for the routine determination of toxic heavy metals in foods. This study showed that the same ICP-MS configuration can also be easily applied to low-level quantitative analysis of Br in cereal grains. This capability provides food testing laboratories with a high throughput, high-performance alternative to traditional techniques used for Br testing in grain cargoes.

- Br was measured accurately and consistently at levels below the current 50 mg/kg MRL, following a simple aqueous extraction approach.
- Compared to conventional ion selective electrode and colorimetric techniques, the ICP-MS method provided much lower detection limits and greatly improved the reproducibility of the analysis. The method used the 8900 ICP-QQQ in single quad mode, so a similar method could also be implemented on an Agilent single quadrupole ICP-MS, such as the 7850 or 7900.
- The ICP-MS method easily met the requirements for Br determination in pre- and post-fumigation grain samples, providing an effective method for assessment of pesticide residues in export grain cargoes.

## References

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DE48188566

Printed in the USA, October 3, 2022
5994-5349EN