Application Note
Food

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High Sensitivity Determination of Lead in Soy sauce

Measured using the Agilent 280Z graphite furnace atomic absorption spectrometer and Omega platform tube

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Introduction

Soy sauce is a liquid condiment and according to the fermentation process can be divided into light (thin), dark, and thick soy sauce. Soy sauce is a traditional Chinese condiment, with a unique flavor and delicious taste. The China National Health Commission and the State Administration of Market Regulation have prepared The National Standard for Food Safety for the Maximum Levels of Contaminants in Foods GB 2762-2017 (*1*). This standard specifically states the limits for heavy metals in food for lead, cadmium, mercury and arsenic, tin, nickel and chromium. The analysis of food samples can prove challenging due to the complex matricies that are present, often being high in salts and sugars.

In this study, Pb was analyzed in soy sauce by the Agilent 280Z AA Graphite Furnace Atomic Absorption Spectrometer (GFAAS).

Experimental

Instrumentation

The Agilent 280Z AA features patented Transverse Zeeman Background Correction technology to ensure the highest sensitivity for Pb in soy sauce. The Agilent PSD 120 autosampler automatically prepares the calibration curve, adds modifier and accurately and reliably delivers samples to the furnace. The Hot Injection capability in the software was used to control the conditions of injection for effective delivery of the sample droplets to the Omega platform tube. The Omega platform tube ensures improved performance and the highest sensitivity for Pb. The Tube-CAM furnace viewing camera, standard on the 280Z AA, provides real time viewing inside the graphite tube. This is used to confirm the deposit position of the sample. It is also used to control the drying program, which is essential when analyzing samples like soy sauce that have a complex matrix. To assist in the development of a robust furnace program, the Surface Response Methodology (SRM) Wizard in the Spectra 5.5 software was used to find the optimum ash and atomization temperature (2). The wizard guides the operator to find the maximum absorbance in just a few experiments, significantly simplifying method development and saving time.

An important consideration when handling samples containing potentially toxic heavy metals is to limit the contact with the operator or laboratory environment. The optional local fume extraction accessory ensures that any harmful vapors are removed at the source ensuring they don't escape into the lab. It also includes a convenient LED lighted mirror to provide a clear view of the graphite tube injection hole assisting in the easy insertion and removal of graphite tubes (Figure 1).

Figure 1. Agilent's optional extraction/LED accessory for the Agilent GTA 120 graphite tube atomizer.

Reagents

The following reagents were used for the preparation of calibration standards solution and samples:

- Magnesium nitrate salt (AR grade)
- 1% Palladium nitrate solution (Agilent, p/n 5193-8336)
- 1000 mg/L Lead (Pb) stock standard (Agilent, p/n 5190-8475)
- High purity Nitric Acid, (EMSURE, Merck)
- Ultrapure 18.2 Ωcm^{-1} Milli-Q water (Millipore)
- Dilution solution: 1% Nitric acid

Standard and modifier preparation

A 50 µg/L bulk standard of Pb was prepared from the 1000 mg/L Pb stock standard and diluted with 1% nitric acid (HNO_3). The working calibration standards were prepared with the PSD 120 Autosampler with the concentration 0, 10, 20, 30 and 40 µg/L.

The prepared chemical modifier consisted of 1% magnesium nitrate + 0.2% palladium nitrate in 1% HNO_3.

Sample preparation

An accurately weighed soy sauce samples of 0.10 g and was diluted to 5.0 g using 1% HNO_3 and thoroughly mixed with a vortex mixer for one minute, to have a final dilution factor of 50.

Instrument conditions

Table 1 lists the instrument operating conditions. Table 2 lists the furnace settings used for the analysis.

Table 1. Agilent 280Z AA instrument operating conditions.

Element	Pb
Lamp current (mA)	10
Wavelength (nm)	283.3
Slit Width (nm)	0.5
Type of tube	Omega platform tube
Background correction	Zeeman
Modifier	5 µl 1% Magnesium nitrate + 2% Palladium nitrate (Co-inject)
Sample Volume (µL)	10
Lamp type	Hollow Cathode Lamp
Hot injection Temp (°C)	60
Injection Speed	8

Table 2. Furnace program for the analysis of Pb in soy sauce.

Step	Temp (°C)	Time (s)	Argon Flow (L/min)	Reading
1	95	50.0	0.3	-
2	120	20.0	0.3	-
3	400	30.0	0.3	-
4	500	20.0	0.3	-
5	805	15.0	0.3	-
6	805	10.0	0.3	-
7	805	1.0	0.0	-
8	2132	0.6	0.0	Yes
9	2132	2.0	0.0	Yes
10	2500	2.0	0.3	-

Results and discussion

Optimization

To ensure accuracy and precision of analysis in GFAAS, control over the furnace temperature program is critical. The Tube-CAM on the 280Z AA provides real time viewing inside the Omega platform tube to correctly optimize drying step, this is valuable for the high salt matrix of the soy sauce samples. The Tube-CAM showed an additional heating step, was required to sufficiently remove salts and sugars common with food type samples, to 400 °C, prior to ashing and to complete the matrix removal.

The ash and atomize temperatures in GFAAS are not independent variables. The SRM Wizard is unique in its ability to simultaneously find the relationship between ashing and atomization. This ensures the maximum absorbance, highest sensitivity and excellent %RSD of replicates. Once the initial Ash and Atomize temperatures and steps were defined (Table 3), the wizard automatically derived the experiments needed to find the optimum ash and atomization temperature with the schematic showing the stability of the vapor phase (Figure 2).

Table 3. SRM, experimental design factors.

Step	Temperature (°C)	Step (°C)
Ash	1000	300
Atomization	2000	300

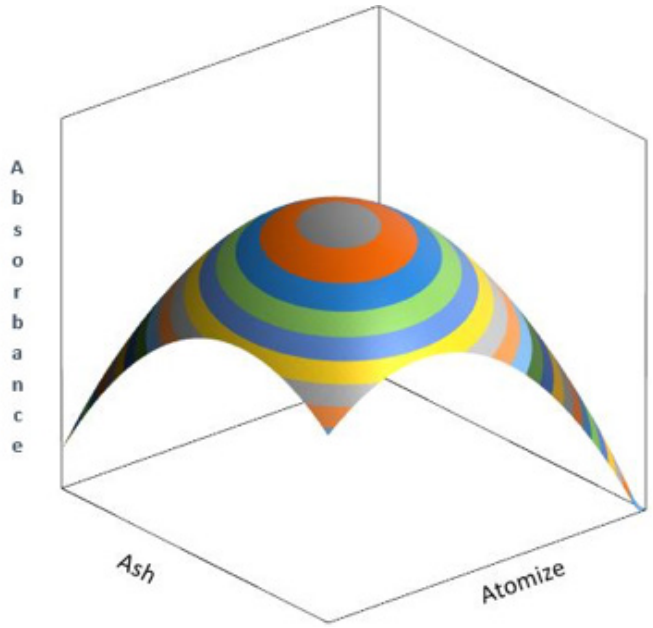

Figure 2. The SRM tool in the SpectrAA software uses a mathematical model to automatically optimize ash and atomize temperatures. Shown here the schematic for a spiked soy sauce sample.

The optimum temperatures found by the SRM Wizard were:

- Ash: 805 °C
- Atomization: 2132 °C

Calibration

The Agilent 280Z AAS comprises patented longitudinal graphite tube heating and transverse Zeeman background correction to delivers outstanding sensitivity and maximum performance for all types of complex sample matrices. The calibration curve for Pb shows excellent linearity with correlation coefficient >0.9996. Sensitivty can be measured by the characteristic mass. The characteristic mass in peak area is defined as the mass that gives 1% absorbance *(3)*. The characteristic mass, in peak area, for Pb is 22.3 pg for an injection of 10 µL, this demonstrates the excellent sensitivity of the method.

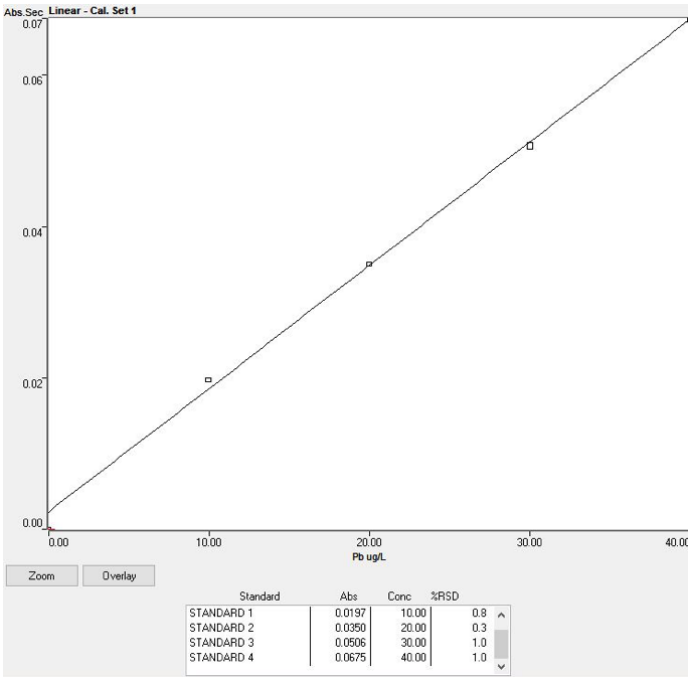

Figure 3. Absorbance peak for a soy sauce sample spiked with 10 µg/kg Pb.

Method detection limit

The Method detection limit (MDL) was determined from the 3 sigma measurement of 10 matrix blank solutions and multiplying by the dilution factor. The food safety standard GB 2762-2017 specifies the maximum allowable content of Pb in condiments to be ≤1.0 mg/kg. The limits show that the 280Z AA are well below the requirements of the standard (Table 4).

Table 4. TBC.

	MDL in solution (µg/kg)	MDL in sample (µg/kg)	LOQ in sample (µg/kg)	Maximum allowable Limit GB 2762 2017 (µg/kg)
Limit	0.35	18	59	1000

Sample analysis and method robustness

The stability of the 280Z AA was investigated by analyzing a soy sauce sample spiked with 10 µg/kg of Pb, each sample was measured in triplicate. Figure 3 shows the Pb analyte absorbance peak in the spiked soy sauce sample, recovered accuracy and precisely (1.0 %RSD) even with in the complex background (light grey line).

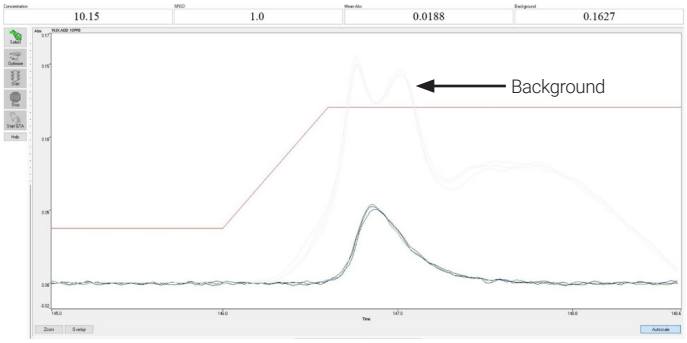

Figure 4. Absorbance peak for a soy sauce sample spiked with 10 µg/kg Pb (dark lines) in a complex background (light grey line).

The method robustness was examined by repeating it in four Centers of Excellence (COEs) in China with different operators/instruments. The results across the four sites were almost identical (Table 5.) with excellent precision of <2.0 %RSD and recovery of the spike 98 ±4% demonstrating the robustness of the method and reliability of the Agilent 280Z AA.

Table 5. Repeatability Pb analysis in soy sauce across the four COEs.

COE	# of Samples Measured	Unspiked Soy Sauce (µg/kg)	Spiked Sauce (µg/kg)	RSDs	Recovery
Shanghai HS	n=10	<MDL	9.66	1.7%	97%
Shanghai LYX	n=10	<MDL	10.4	1.2%	102%
Chubang	n=7	<MDL	9.62	0.7%	98%
LiJinji	n=7	<MDL	9.35	1.2%	94%

Conclusion

The Agilent 280Z AA using the Omega platform tube provided high sensitivity, accuracy and precision quantify Pb in soy sauce according to GB 2762-2017. The SRM Wizard ensured the optimum ash and atomization temperatures, producing a robust method for the maximum absorbance. The method robustness was validated across four different laboratories where it was shown that the method exceeds the GB 2762:2017 requirements for Pb in soy sauce.

With its excellent precision and accuracy for such a difficult matrix, the Agilent 280Z is ideally suited for laboratories that require sensitive and cost-effective instrumentation.

References

1. GB 2762:2017 National Standard for Food Safety – Limits of contaminants in foods,
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4. GB 5009.12-2017 National Food Safety Standard – Determination of lead in foods

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