Application Note: 43060

The Analysis of Trace Elements in Honey by Flame and Graphite Furnace Atomic Absorption Spectrometry

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Key Words

- Atomic Absorption
- Flame
- Deuterium
- Graphite Furnace
- Honey
- Zeeman

Key Benefits

- The robust flame sample introduction system allows dissolved honey samples to be run without blockage or contamination.
- The advanced furnace autosampler speeds up analysis by automatically preparing the working standards from a single master standard.
- The permanently aligned true dual atomizer enables rapid switching between flame and furnace methods.
- Deuterium and Zeeman background correction offer a flexible solution for the analysis of challenging matrices such as honey.

Summary

The Thermo Scientific iCE 3500 Atomic Absorption Spectrometer is the ideal solution for the analysis of major, minor and toxic elements in honey. The permanently aligned true dual atomizer allows robust and reliable analysis of major elements by flame, followed by accurate and precise determination of minor and toxic elements by graphite furnace.

Introduction

Honey is a sweet and viscous substance produced from the nectar and secretions of plants and flowers. The nectar is transported to a beehive by honey bees, where worker bees then add enzymes to create honey. Most honey is created from a variety of plants and flowers, though in some areas, where a particular plant or flower is in abundance, monofloral honey can be produced, and this is particularly valuable. Honey is typically advertised to the consumer by floral source or geographical location, however many honey products are blended from a variety of sources. This has resulted in a global market with hundreds of types of honey, each with unique taste, color and crystallization properties. In the EU, honey must adhere to strict composition criteria, including sugar, moisture and hydroxymethylfurfural (HMF) content.[1]

Sugar is often substituted with honey in the making of cake products. Not only is honey sweeter than sugar, and therefore used as a sugar alternative, it is also hygroscopic. This causes it to attract and hold water, resulting in deliciously moist baking products. Honey is predominantly fructose and glucose, combined with a mixture of other natural ingredients such as organic acids and enzymes. It also contains a small percentage of metals, including potassium, sodium, magnesium and calcium. The metal composition is geographically significant, as the majority of metals in honey are transferred from the soil to the plant or flower from which the nectar is collected. Metals can also be transferred from other sources such as water aerosol spray and atmospheric pollution. The metal profile of honey is therefore significantly important on three levels – for evidence of provenance, nutritional benefit and toxicological implications.

The viscous and sugary nature of honey makes it a difficult substance for quantitative trace elemental analysis. Honey can be dissolved in water; however this can result in contamination of sample introduction systems, such as graphite furnace cuvettes. In addition, standards may require matrix matching to take into account the change in viscosity and the increased organic content. As a simpler alternative, acid digestion can be used to remove the organic material from the sample prior to dilution with water.

This application note presents two methods for the analysis of trace elements in honey. A simple dissolution technique is used for the analysis of two typical major elements by flame atomic absorption spectrometry, while a microwave-assisted digestion protocol is implemented for the analysis of two toxic contaminants by graphite furnace atomic absorption spectrometry.

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Sample Preparation

Reagents

- Nitric acid, 69 %, trace metal grade
- Hydrogen peroxide, > 30 % w/v, trace metal grade
- 1000 ppm cadmium, lead, magnesium and sodium master standards
- Magnesium nitrate

All standards and reagents purchased from Fisher Scientific.

Preparation by dissolution for flame analysis

Three honey samples (Spanish Orange Blossom, Australian Eucalyptus and Brazilian Pure Set Honey) were purchased from a local supplier. The honey samples were warmed with rotation in a water bath (in their original containers) at approximately 60 °C in order to homogenize each sample. Aliquots of approximately 10 g of honey were transferred to clean glass beakers and weighed. Approximately 1 g of honey was transferred from the beaker to a volumetric flask and the mass determined by difference. The sample was diluted to approximately 100 g with 1 % nitric acid and the % m/m concentration and dilution factor determined. It was necessary to sonicate the honey/water sample to ensure complete dissolution.

Preparation by microwave-assisted digestion for furnace analysis

Honey samples were warmed with rotation in a water bath (in their original containers) at approximately 60 °C in order to homogenize each sample. Aliquots of approximately 10 g of honey were transferred to clean glass beakers. Approximately 0.25 g portions of honey were weighed into clean, dry Teflon microwave digestion vessels. (NB: the hygroscopic nature of honey and the insulating properties of Teflon make it a difficult substance to weigh, as the honey is attracted to the Teflon surface. A pipette was used to deposit honey directly onto the base of the digestion vessel. It was also necessary to use an anti-static gun to discharge the digestion vessels prior to weighing). 4 ml nitric acid and 2 ml hydrogen peroxide were added to the honey samples which were left uncovered for 15 mins. The samples were sealed and digested via temperature ramping (ramped to 120 °C for 10 minutes, held for 5 minutes, then ramped to 200 °C over 10 minutes, then held for 15 minutes). A sample blank containing only nitric acid and hydrogen peroxide was prepared in the same way. The digested samples were quantitatively transferred to 100 ml volumetric flasks.

Standard and Reagent Preparation

Flame calibration standards were prepared on a v/v basis in 1 % nitric acid. 1000 ppm standard stock solutions of magnesium and sodium were used to prepare 1.0, 2.0 and 10 ppm multi-element standards by pipetting 0.1, 0.2 and 1.0 ml of stock solutions into 100 ml volumetric flasks and making to the mark with 1 % nitric acid. The 10 ppm multi-element standard was used to prepare 0.3 and 0.5 ppm multi-element standards by pipetting 3.0 and 5.0 ml of the 10 ppm sub-standard into 100 ml volumetric flasks and making to the mark with deionsed water. A blank of 1 % nitric acid was also prepared. Standards of 0, 0.3, 0.5, 1.0 and 2.0 ppm were used to generate calibration curves for magnesium and sodium. (NB: corresponding calculated dilution factors were used to quantify the prepared samples using the v/v flame calibration standards).

Furnace calibration standards were prepared on a v/v basis in 4 % nitric acid, to ensure acid matrix matching to the samples. 1000 ppm standard stock solutions of cadmium and lead were used to prepare a 1 ppm multi-element sub-standard by pipetting 0.1 ml aliquots into a 100 ml volumetric flask and diluting to the mark with 4% nitric acid. The sub-standard was subsequently used to prepare a method master standard of 20 ppb by pipetting 2 ml into a 100 ml volumetric flask and making to the mark with 4 % nitric acid. The furnace autosampler was used to automatically prepare calibration standards at 2, 5, 10, 15 and 20 ppb. A calibration blank of 4 % nitric acid was also prepared.

Magnesium nitrate was prepared as a matrix modifier for use with lead analysis by dissolving 1 g in 100 ml deionised water, such that a 5 µl aliquot added 50 µg magnesium nitrate to the sample.

Method Development and Analysis

Flame Method

Magnesium and sodium were analyzed by flame atomic absorption spectrometry. The lateral and rotational burner positions and the impact bead were optimised manually using the 1 ppm multi-element standard. Burner height and gas fuel flow were optimized individually and automatically for each element as part of the analytical method. Spectrometer parameters are shown in Table 1.

Spectrometer Parameter	Magnesium	Sodium
Flame type	Air/Acetylene	
Fuel flow, l/min	1.0	
Burner height / mm	8.6	6.2
Wavelength / nm	285.2	589.0
Bandpass / nm	0.2	
Background correction	Deuterium	None
Measurement time / s	4	
Number of resamples	3	
Calibration type	Segmented curve fit	

Table 1: Spectrometer parameters for the analysis of magnesium and sodium in honey by flame atomic absorption spectrometry.

Furnace Method

Cadmium and lead were analyzed by graphite furnace atomic absorption spectrometry. Default spectrometer parameters were found to be suitable for each element and lead required the addition of 50 µg magnesium nitrate matrix modifier for optimum peak shape and recovery as recommended in the Solaar software cookbook. Spectrometer parameters are shown in Table 2.

Spectrometer Parameter	Cadmium	Lead
Wavelength / nm	228.8	217.0
Cuvette	Electrographite	ELC (Extended Life Cuvette)
Dry temperature / °C	100	
Ash temperature / °C	800	
Atomize temperature / °C	1000	1200
Bandpass / nm	0.5	
Background correction	Zeeman	
Signal measurement	Transient Height	
Number of resamples	3	
Calibration type	Quadratic least squares fit	

Table 2: Spectrometer parameters for the analysis of cadmium and lead in honey by graphite furnace atomic absorption spectrometry.

Results and Discussion

Analysis by Flame

Honey samples were diluted in 1 % nitric acid as described above and analyzed by flame atomic absorption spectrometry following automated optimization of the burner height and gas fuel flow. Analysis was found to be straightforward. Calibration curves for magnesium and sodium are shown in Figure 1.

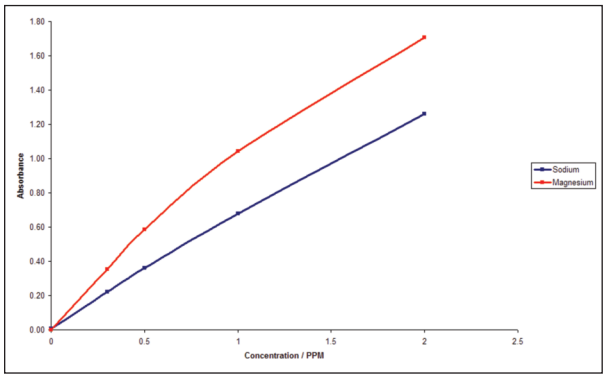

Figure 1: Calibration curves for the analysis of magnesium and sodium in honey samples by flame atomic absorption spectrometry.

To verify the method, an additional honey sample was prepared with a spike equal to 0.2 ppm in the diluted sample and the percentage recovery calculated. Results are shown in Table 3.

Sample	Concentration in original honey sample / ppm	
	magnesium	sodium
Spanish Orange Blossom	9.26	16.9
Australian Eucalyptus	25.33	94.08
Brazilian Pure Set	25.14	42.83
	Spiked Honey	
Measured concentration in solution / ppm	0.196	0.187
% Recovery	96	92

Table 3: Results following the analysis of honey samples by flame atomic absorption spectrometry for magnesium and sodium.

This testing revealed the presence of magnesium and sodium in each of the analyzed honey samples. According to the literature, these elements are expected in honey, however the levels vary greatly, depending upon the country of origin, local environment and flower type.[2] Excellent recoveries on the spiked samples were obtained, demonstrating the suitability of this method for the analysis of majors in honey by flame atomic absorption spectrometry. Analysis took only 12 seconds for a triplicate reading on a single sample.

Analysis by Furnace

Honey samples were digested in a high pressure microwave digestion system and analyzed by graphite furnace atomic absorption spectrometry. The furnace autosampler was used to automatically prepare standards from a single master standard and the matrix modifier was automatically injected into the cuvette for the analysis of lead. Cadmium and lead were not detected in analyzed honey samples. To verify the method, an additional honey sample was prepared with a spike equal to 5 ppb in the diluted sample. The spike was added prior to microwave digestion and the spiked sample was subsequently digested in the same way as described above. The percentage recovery was calculated and results are shown in Table 4.

	Cadmium	Lead
Measured Concentration in solution / ppb	4.66	5.49
% Recovery	93	110

Table 4: Spiked recoveries following the analysis of honey samples by graphite furnace atomic absorption spectrometry for cadmium and lead.

Conclusion

This application note has demonstrated how trace elements in honey can be quantitatively determined using atomic absorption spectrometry. A simple dissolution method was used to prepare samples for the analysis of magnesium and sodium by flame, while a microwave-assisted digestion procedure was used for the accurate and precise analysis of cadmium and lead by furnace. Spiked samples were used to verify each method and recoveries were found to be very good. The iCE 3500 Atomic Absorption Spectrometer proved to be a robust and reliable solution for the analysis of trace elements in honey.

References

1. Official Journal of the European Communities, Council Directive 2001/110/EC of December 2001 relating to honey.
2. P. Pohl, Trends in Analytical Chemistry, Vol. 28, No. 1, 2009

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