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Article

# Fast and Interference Free Determination of Calcium and Magnesium in Honeys by Solid Phase Extraction Followed by Flame Atomic Absorption Spectrometry

***Pawel Pohl,* Helena Stecka and Piotr Jamroz***

*Department of Analytical Chemistry, Faculty of Chemistry, Wroclaw University of Technology, Wybrzeze Stanislawa Wyspianskiego 27, 50-372 Wroclaw, Poland*

Um procedimento para preparo de amostras, baseado na extração em fase sólida e eluição em duas etapas foi desenvolvido para a determinação da concentração total de Ca e Mg em méis de abelha. As amostras de mel foram tratadas como segue: soluções de amostra (50 mL) a 5,0% (m/v) foram passados a uma vazão de 20 mL $min^{-1}$, através de resina Dowex 50W×8-400, para reter íons Ca(II) e Mg(II) e separá-los da matriz contendo glicose e frutose, e dos minerais aniônicos. Depois, 20 mL de uma solução de $HNO_3$ 0,5 mol $L^{-1}$ foram usados para remover os íons K e Na. Finalmente, Ca e Mg foram eluídos com 5 mL de solução de HCl 2,0 mol $L^{-1}$ antes da medida de suas concentrações por espectrometria de absorção atômica em chama (FAAS). O procedimento proposto para o preparo da amostra foi rápido (30 min) e permitiu medir as concentrações de Ca e Mg em mel, com precisão de 1-5% e exatidão de 1-3%. Os limites de detecção de 24 ng $g^{-1}$ de Ca e 4,4 ng $g^{-1}$ de Mg foram obtidos para FAAS com este procedimento.

A sample preparation procedure based on the solid phase extraction and the two-step elution was developed for the determination of the total concentrations of Ca and Mg in bee honeys. The honey samples were treated as follows: the 5.0% (m/v) sample solutions (50 mL) were passed at the flow rate of 20 mL $min^{-1}$ through the resin beds of Dowex 50W×8-400 to retain the Ca(II) and Mg(II) ions and separate them from the matrix of glucose and fructose in addition to the anionic minerals. Afterwards, 20 mL of a 0.5 mol $L^{-1}$ $HNO_3$ solution was used to remove the K and Na ions. Finally, Ca and Mg were eluted prior to the measurements of their concentrations by flame atomic absorption spectrometry (FAAS) using 5 mL of a 2.0 mol $L^{-1}$ HCl solution. The proposed sample preparation procedure was fast (30 min) and enabled to measure the concentrations of Ca and Mg in honey with the precision of 1-5% and the accuracy of 1-3%. The limits of detection of 24 ng $g^{-1}$ of Ca and 4.4 ng $g^{-1}$ of Mg were obtained for FAAS with this procedure.

**Keywords**: Ca, Mg, honey, FAAS, solid phase extraction, ion exchange, sequential elution

## Introduction

Calcium and magnesium, within the typical concentration range of 1-200 μg $g^{-1}$, are nutritionally important minor elements of honey.[1-5] Both elements are quite often determined in this matrix using air-acetylene flame atomic absorption spectrometry (FAAS), however, due to the complexity of the samples, *i.e.*, the very high content of the monosaccharides, the easily ionized elements like K and Na, phosphates and the other anions, their measurements with the aid of FAAS may encounter some problems related to the matrix effects.[6] In reference to the possibility of the routine analyses and the quality and food safety control measurements of honey, the selection of a suitable procedure for the sample treatment is of a special importance in this case because it decides about the time of the analysis and the dependability of the results.

The possible chemical and physical interferences linked to the presence of the aforementioned main organic and inorganic components of honey may be eliminated when the analyzed samples are decomposed using the different mineralization procedures, namely the dry ashing and the wet digestion with the oxidative reagents, while the concentrations of Ca and Mg are measured later on by FAAS in the resulting solutions of digests.[1,2,4,5,7-13] Although both pointed sample preparation strategies, *i.e.*, the calcination in the air and the wet decomposition, enable to extract the determined elements from the matrix of honey into the solutions and prevent the physical interferences related to the changes in the nebulization rate of the sample

*e-mail: pawel.pohl@pwr.wroc.pl

solutions, they are simply time-consuming and laborious. The subsequent stages of these procedures can additionally be responsible for losses (by the sample overheating and sputtering) or gains (by the contamination from the reagents and the vessels) of the elements.[6]

The direct analysis of honey on the content of Ca and Mg, predated by the dilution of the respective samples in water or diluted solutions of the oxidizing reagents ($HNO_3$ mostly, $H_2O_2$) and the aspiration of the resulting solutions into FAAS, is less frequent due to profound matrix effects originating from the presence of fructose and glucose in addition to the mineral elements like P or S. Although this method seems to be straightforward, a series of dilutions has to be done for each honey in order to assure that the selected content of honey in the analyzed solutions and the constituents that could potentially interfere with the Ca and Mg measurements would not affect the analytical response for these elements in FAAS.[3,4,14-16] What is more, although the 2-5% (m/v) honey solutions can directly be measured by FAAS, the load of the organic matter and the main mineral constituents in the solutions of such untreated samples may still be high. So that, the flow injection manifolds have to be used to decrease it[3,15] or the analyzed sample solutions are further diluted.[4]

Irrespective of that if the solutions of the digested or the undigested samples of honey are analyzed, serious non-spectral interferences in the flame can be observed due to the enhanced ionization of Ca and Mg in the nitrous oxide-acetylene flames or the occlusion of these elements into the refractory or the less volatile molecules produced in the reactions with the phosphates in the air-acetylene flames. As a result, the releasing buffers like NaCl or KCl[1,5] and/or La containing compounds like $La_2O_3$, $La(NO_3)_3$ or $LaCl_3$[1-5] are commonly added to the analyzed sample and standard solutions. This preparation step certainly increases the time of the analysis and the included labor investment. With respect to the described problems of the prolonged analysis of honey when the samples are digested by the dry or wet ashing procedures and subsequently diluted or mixed with the solutions of the aiding substances, the development of the alternative approaches that enable the fast and the interference free analysis of honey by FAAS would seem to be valuable in terms of the simplification of the whole analytical procedure.

The objective of this work was to develop a solid phase extraction (SPE) procedure for the preparation of honey prior to the determinations of Ca and Mg using the common air-acetylene FAAS. Initially, the sorption behavior of the commercially available strongly acidic cation exchangers, including Amberlite IRP-69, Dowex 50W×8-200, Dowex 50W×8-400 and Dowex HCR-W2, toward the Ca(II), K, Mg(II) and Na ions as well as fructose and glucose was studied in the conditions of the different solution pH and the flow rates across the SPE columns. For the selected Dowex 50W×8-400 resin, the conditions of the two-step elution aimed at the removal of the K and Na ions in the first step and the recovery of the Ca(II) and Mg(II) ions in the second step before the determination of their concentrations by FAAS were established. The reliability of a devised SPE sample preparation procedure was verified by the comparison of its results with those obtained with the other sample preparation procedures. The spiking experiments were conducted as well and the respective recoveries were assessed. The procedure was applied for the determination of the concentrations of Ca and Mg in six raw mono- and multi-flower honeys.

## Experimental

### Reagents and materials

Ultra-pure water from a WIGO (Wroclaw, Poland) PRO-11G reverse osmosis water purification system was used throughout. The ACS grade concentrated reagents, *i.e.*, 30% (m/m) $H_2O_2$, 37% (m/m) HCl, 65% (m/m) $HNO_3$ and 96% (m/m) $H_2SO_4$ solutions, were purchased from J. T. Baker (Deventer, Netherlands). D-fructose (purity > 98%) and D-glucose (purity > 98%) were supplied by POCH (Gliwice, Poland). The other solid reagents were also taken from POCH and were used for the preparation of alkaline 6.0 g $L^{-1}$ $CuSO_4$, 50 g $L^{-1}$ $(NH_4)_6Mo_7O_{24}$, 6.0 g $L^{-1}$ $Na_2HAsO_4$, 0.5 (m/v) $LaCl_3$ and 0.5% (m/v) CsCl solutions. A solution of the arseno-molybdate reagent was prepared by reaction of the $(NH_4)_6Mo_7O_{24}$ and $Na_2HAsO_4$ solutions in the medium of a 0.8 mol $L^{-1}$ $H_2SO_4$ solution. The Titrisol single-element AAS standards from Merck KGaA (Darmstadt, Germany) were applied to prepare 1000 mg $mL^{-1}$ single-element stock standard solutions of Ca, K, Mg and Na. These solutions were used to prepare the standard solutions of Ca, K, Mg and Na for the calibration as well as the 50 mL working standard solutions containing 2.0, 50, 0.5 and 2.0 mg $mL^{-1}$ of Ca, K, Mg and Na, respectively, in addition to 20 mg $mL^{-1}$ of fructose and glucose. This composition represented an average content of the studied constituents in the 5.0% (m/v) water solutions of Polish honeys.[17,18] The latter solutions were acidified with a 0.01 mol $L^{-1}$ HCl solution to reach the pH of 3.5, 4.0 and 4.5 and then used in the study of the cation exchange behavior of Ca, K, Mg, Na, fructose and glucose toward the resins applied.

The strong cation exchange styrene-divinylbenzene resins with the sulphonic acid functional groups, *i.e.*,

Amberlite IRP-69 (100-500 mesh size, $Na^+$ form), Dowex 50W×8-200 (100-200 mesh size, $H^+$ form), Dowex 50W×8-400 (200-400 mesh size, $H^+$ form) and Dowex HCR-W2 (16-40 mesh size, $H^+$ form), were supplied by Sigma-Aldrich (Saint Louis, MO, USA). The SPE columns (1.0 cm of the inner diameter) from Sigma-Aldrich were equipped with the glass coarse frits and the polytetrafluoroethylene (PTFE) stopcocks. They were filled with the water slurries of the cation exchange resins (the 1.0 g portions) and then, the resin beds formed were washed with 10 mL of a 2.0 mol $L^{-1}$ HCl solution at the flow rate of 5.0 mL $min^{-1}$. Afterward, the resin beds were rinsed with 50 mL of water to remove the excess of HCl.

The Cole-Parmer (Vernon Hill, IL, USA) 4-channel MasterFlex L/S peristaltic pumps were used to control and measure the flow rates of the solutions that were passed through the columns.

### Instrumentation

A Perkin Elmer single-beam air-acetylene flame atomic absorption spectrometer model 1100B with standard accessories, *i.e.*, a wettable plastic coated burner mixing chamber, a drain interlock assembly, a 10 cm long Ti single slot burner head and a stainless steel nebulizer, was used in the measurements. The determinations of the Ca and Mg concentrations were carried out in an absorption mode (FAAS) while the concentrations of K and Na were determined using the same instrument but operated in an emission mode (flame optical emission spectrometry, FOES). The absorbance and the emission intensities were recorded using a time-average integration mode. In each read cycle, 3 readings integrated at 0.1 s intervals over a 1 s integration time were taken. The spectrometer was operated under the standard working parameters given in Table 1.

A Thermo Scientific (Bremen, Germany) single-beam visible spectrophotometer model Spectronic 20D$^+$ was used to measure the sum of fructose and glucose with the aid of the Somogyi-Nelson (arseno-molibdate) method.[19] In brief, 500 µL of an alkaline $CuSO_4$ solution were added to the 10 µL sample portions. The resulting mixtures were incubated in a water bath at the temperature of 90 °C for 10 min to reduce the ions of Cu(II) to Cu(I). After cooling, a solution of the arseno-molybdate reagent was added to react with the Cu(I) ions and produce a polymolybdate complex (molybdenum blue) of the intensive blue color. The absorbance readings of the final solutions containing the molybdenum blue were taken at 520 nm. The sums of the concentrations of fructose and glucose were determined against the standard solutions of glucose being within the concentration range of 1.0-50 mg $mL^{-1}$.

### Preparation of honey and analysis by FAAS

The samples of freshly ripened honeys, including acacia, goldenrod, heather, lime, multi-floral, lime and rape, were from an apiary placed in the suburbs of Wroclaw (Lower Silesian Province, Poland). Honeys were kept in the original glass jars used for their shipping.

Four different means of the sample treatment were used to prepare the sample solutions before the measurements of the concentrations of Ca and Mg in the analyzed honeys by FAAS. In case of the wet oxidative digestion in the mixture of the concentrated $HNO_3$ and 30% (m/m) $H_2O_2$ solutions (the procedure A), the 2.5 g samples of the analyzed honeys were placed in the 250 mL beakers, dissolved in 10 mL of water, poured with 10 mL of $HNO_3$ and heated under the cover at the temperature of 85-90 °C for about 2.5 h. Afterward, 10 mL of $H_2O_2$ were added to the solutions and the heating was extended for next 1 h to decompose the oxidant and reduce the volume of the sample solutions to about 1-2 mL. The resulting aliquots were re-constituted with water to 50 mL. In a similar way, the samples of the analyzed honeys were digested using two 10-mL portions of a 30% (m/v) $H_2O_2$ solution only (the procedure B). In case of the water dissolution (the procedure C), the 2.5 g samples of the analyzed honeys were placed in the 250 mL beakers, dissolved in about 10 mL of water and finally diluted with it

**Table 1.** The operating conditions for the measurements of Ca and Mg (by FAAS) and K and Na (by FOES)

| | Ca | K | Mg | Na |
|---|---|---|---|---|
| Air flow rate / (L $min^{-1}$) | 8.0 | 8.0 | 8.0 | 8.0 |
| $C_2H_2$ flow rate / (L $min^{-1}$) | 1.2 | 1.2 | 1.2 | 1.2 |
| Lamp current / mA | 10 | 7 | 6 | 10 |
| Wavelength / nm | 422.7 | 766.5 | 285.2 | 589.0 |
| Slit / nm | 0.7 | 0.4 | 0.7 | 0.2 |
| Upper calibration range / (mg $mL^{-1}$) | 10.0 | 1.0 | 2.0 | 1.0 |
| Instrumental limit of detection[a] / (ng $mL^{-1}$) | 18 | 0.4 | 2.5 | 1.1 |

[a]3×SD of the mean signals for a water blank (n = 5).

to 50 mL. The sample solutions achieved after the execution of the procedures A, B and C were diluted 2 times with 0.5% (m/v) $LaCl_3$ and 0.5% (m/v) CsCl solutions and measured by FAAS against the standard solutions containing the same amounts of both chloride salts.

In case of the SPE procedure (the procedure D), the 2.5 g samples of the analyzed honeys were placed in the 250 mL beakers, dissolved in 10 mL of water and diluted with it to 50 mL. The resulting sample solutions were passed through the SPE columns of Dowex 50W×8-400 at the flow rate of 20 mL $min^{-1}$ to retain Ca and Mg and separate these elements from the main monosaccharides and the anionic minerals (mostly phosphates). Next, 20 mL of a 0.5 mol $L^{-1}$ $HNO_3$ solution was passed through them at the flow rate of 2.0 mL $min^{-1}$ to completely remove K and Na. Finally, 5 mL of a 2.0 mol $L^{-1}$ HCl solution at the flow rate of 2.0 mL $min^{-1}$ was used to recover Ca and Mg from the SPE columns. The respective eluates were diluted with water to 25 mL with water and subjected to the measurements by FAAS *versus* the simple standard solutions.

## Results and Discussion

All the retention, the separation and the elution efficiencies were mean values for six independent replicates. The respective column blanks were run at each step and considered in the final results. Three independent analyses of honey samples were made using different sample preparation procedures. The respective procedural blanks were prepared, analyzed and considered in the final results of the analysis.

### Cation exchange behavior of element ions and monosaccharides

The cation exchange behavior of the studied resins toward the Ca(II), K, Mg(II) and Na ions and fructose and glucose was investigated by passing the 50 mL working standard solutions (pH 3.5, 4.0 and 4.5) through the SPE columns at the flow rate of 2.0 mL $min^{-1}$ and analyzing the respective column effluents (each time, the 10 mL portions were collected by the end of the solution loading). The concentrations of the elements in these effluents were determined by FAAS (Ca, Mg) and FOES (K, Na) *versus* the calibration with the matrix matching standard solutions (with fructose and glucose at the concentration of 20 mg $mL^{-1}$ of each). The concentrations of the sum of fructose and glucose were measured using the Smogoyi-Nelson method.[19] The retention efficiencies of Ca, K, Mg and Na (in %) and the separation efficiencies of fructose and glucose (in %) were calculated considering the concentrations of these constituents found in the column effluents and their initial concentrations in the working standard solutions.

In this experiment it was found that all the cation exchange resins, *i.e.*, Amberlite IRP-69, Dowex 50W×8-200, Dowex 50W×8-400 and Dowex 50W HCR-W2, completely retain the Ca(II) and Mg(II) ions and separate them from fructose and glucose irrespectively of the studied solution pH. The latter monosaccharides passed in these conditions through the resin beds unretained. Accordingly, the retention efficiencies established for both elements were within 99.2-100.0% (Amberlite IRP-69), 99.6-99.9% (Dowex 50W×8-200), 99.8-100.0% (Dowex 50W×8-400) and 99.0-99.8% (Dowex 50W HCR-W2) with the coefficients of variance (CV) for 6 parallel experiments varied from 0.1 to 0.7%. The separation efficiencies for fructose and glucose in these conditions were within 96.3-101.9% (Amberlite IRP-69), 97.8-101.3% (Dowex 50W×8-200), 96.0-101.5% (Dowex 50W×8-400) and 98.8-101.6% (Dowex 50W HCR-W2) with the CV values for all the results within the range of 0.3-4.9%. Unfortunately, it was also established that the K and Na ions are exhaustively retained by all the resins at the studied pH range (3.5-4.5). The retention efficiencies of K and Na in these conditions were within 96.9-100.0% (Amberlite IRP-69), 99.8-100.0% (Dowex 50W×8-200), 99.9-100.0% (Dowex 50W×8-200) and 99.5-99.7% (Dowex 50W HCR-W2). The reproducibility of these results were in the range of 0.1-0.7% (as CV, n = 6).

In a similar way, the effect of the flow rate (in the range from 2.0 to 20 mL $min^{-1}$) with which the working standard solutions were driven through the SPE columns was assessed on the retention efficiencies of Ca, K, Mg and Na and the separation efficiencies of fructose and glucose. Here, the working standard solutions (pH 4.0) were passed through the SPE columns at the different flow rates and the respective column effluents were collected and analyzed on the content of the elements (Ca, K, Mg, Na) and the monosaccharides (fructose and glucose).

Apparently, it was determined that all the Dowex resins exhaustively retain the Ca(II) and Mg(II) ions at the flow rates much higher than 2.0 mL $min^{-1}$, *i.e.*, 4.0, 6.0, 8.0, 10, 14 and even 18 and 20 mL $min^{-1}$. The average retention efficiencies of Ca and Mg calculated for the individual retention efficiencies obtained at the different flow rates corresponded to 99.6 ± 0.2 and 100.0 ± 0.1% (Dowex 50W×8-200), 99.6 ± 0.2 and 99.9 ± 0.1% (Dowex 50W×8-400) and 99.5 ± 0.3 and 99.6 ± 0.5% (Dowex 50W HCR-W2). Unfortunately, it was not possible to use the flow rates higher than 14 mL $min^{-1}$ for the resin

Amberlite IRP-69. A strong resistance in the flow of the solution was observed in this case, likely due to the small particle size of this resin, *i.e.*, 25-180 mm. Nevertheless, the average retention efficiencies of Ca and Mg evaluated for this resin on the basis of the individual retention efficiencies achieved at the flow rate within the range of 2.0-14 mL $min^{-1}$ were 99.7 ± 0.2 and 100.0 ± 0.1%, respectively. In case of all the resins, fructose and glucose were separated from the Ca(II) and Mg(II) ions irrespectively of the flow rate used. Accordingly, the sums of the concentrations of both monosaccharides determined in the column effluents were practically the same as in the working standard solutions loaded onto the columns. Unfortunately, in these conditions, the K and Na ions were quantitatively retained by the resins as well. The retention efficiencies established for these elements at the different flow rates were within 99.3-100.0% (Amberlite IRP-69), 99.8-100.0% (Dowex 50W×8-200), 99.7-100.0% (Dowex 50W×8-400) and 99.6-100.0% (Dowex 50W HCR-W2) with the reproducibility of these results within 0.1-0.6% (as CV, n = 6).

Considering the cation exchange behavior of the studied resins, Dowex 50W×8-400 with the particle size of 38-74 mm was selected for further experiments.

### Removal of K with Na and elution of Ca and Mg

Although the analytical response of slightly ionized Mg in the air-acetylene flame is recognized not to be changed much with the varying content of K and Na, the response of Ca is recognized to significantly increase in the presence of the alkali metals, especially when they are at the moderately high levels in the solutions (up to 250 mg $mL^{-1}$).[20] Therefore, to eliminate the possible non-spectral interferences in the air-acetylene flame used here and originating from the presence of K and Na in the analyzed sample solutions, it was decided to find out the conditions of the removal of both alkali metals before the elution of the Ca(II) and Mg(II) ions and the measurements of the concentrations of these elements by FAAS. Considering the cation exchange behavior of the monovalent metal ions on the strong cation exchange resins, it was presumed that the diluted solutions of the common mineral acids could elute the K and Na ions not removing such divalent metal ions as Ca(II) and Mg(II), which are usually retained on this type of the resins much stronger.[21]

To evaluate the suitability of the 0.2 and 0.5 mol $L^{-1}$ HCl and $HNO_3$ solutions for the removal of the K and Na ions from the selected cation exchange resin Dowex 50W×8-400, the working standard solutions (pH 4.0) were driven at first through the SPE columns at the flow rate of 20 mL $min^{-1}$. Then, the tested solutions in three portions of 10 mL each were passed at the flow rate of 2.0 mL $min^{-1}$ through the SPE columns to strip off the K and Na ions. The respective 10 mL portions of the column eluates were collected and the concentrations of Ca, K, Mg and Na were determined against the simple standard solutions of these elements.

It was found that the quantitative recovery of the K and Na ions from the resin beds of Dowex 50W×8-400 was obtained using a 0.5 mol $L^{-1}$ $HNO_3$ solution. With 20 mL of this solution, already 99.7 ± 3.7% of the total K and 97.3 ± 3.4% of the total Na retained by the resin were recovered while Ca and Mg remained in these conditions practically unchanged on the SPE columns (the recoveries of Ca and Mg were 1.7 ± 0.2% and 0.4 ± 0.4%, respectively). A less concentrated solution of $HNO_3$ (0.2 mol $L^{-1}$) was established to be useless since its 20 mL resulted in the recovery of 0.4 ± 0.1% of the total K only and 75.7 ± 3.4% of the total Na. The use of a 0.5 mol $L^{-1}$ HCl solution was also discarded. Although 20 mL of this solution led to the quantitative recovery of the total Na retained (102.5 ± 4.4%), K was stripped with the efficiency lower than 90%.

In next turn, the effectiveness of 1.0, 2.0, 3.0 and 4.0 mol $L^{-1}$ HCl solutions was studied in respect to the elution of the Ca(II) and Mg(II) ions retained on the resin. For that purpose, the working standard solutions (pH 4.0) were passed through the SPE columns of Dowex 50W×8-400 at the flow rate of 20 mL $min^{-1}$ to separate fructose and glucose and subsequently, the retained K and Na ions were removed by passing 20 mL of a 0.5 mol $L^{-1}$ $HNO_3$ solution. At the end, 5-mL portions of given HCl solutions were passed through the SPE columns to recover Ca and Mg; the respective eluates were collected, diluted to 25 mL with water and subjected to the analysis on the content of Ca and Mg by FAAS *versus* the simple standard solutions. The recovery efficiencies (in %) of Ca, K, Mg and Na were calculated relating the concentrations of the elements determined in the respective eluates to their original concentrations in the working standard solutions.

Apparently, it was found that except for a 1.0 mol $L^{-1}$ HCl solution, the others lead to the quantitative release of Ca and Mg. The respective recovery efficiencies were 99.8 ± 2.7% (Ca) and 99.6 ± 1.9% (Mg) in case of a 2.0 mol $L^{-1}$ HCl solution, 101.3 ± 2.4% (Ca) and 98.2 ± 3.5% (Mg) for a 3.0 mol $L^{-1}$ HCl solution and 99.2 ± 1.8% (Ca) and 101.2 ± 3.1% (Mg) in case of a 4.0 mol $L^{-1}$ HCl solution.

Considering all these results, it was decided that 20 mL of a 0.5 mol $L^{-1}$ $HNO_3$ solution and 5 mL of a 2.0 mol $L^{-1}$ HCl solution would be applied for the two-step elution in the proposed sample preparation SPE procedure.

### Analytical characteristics and application

Considering the ion exchange capacity of the Dowex 50W´8-400 resin toward the Ca(II) and Mg(II) ions, which was determined to be 66 and 72 mg of Ca and Mg, respectively, *per* g of the resin as received, and the highest possible concentrations of K and Na in honey samples, reaching even 6.5 and 1.2 mg $g^{-1}$, respectively, it was decided that 5.0% (m/v) sample solutions of the analyzed honeys would be prepared and treated with the SPE procedure proposed.

The accuracy of this procedure was evaluated by the recovery of the known amounts of the Ca(II) and Mg(II) ions (0.5, 1.5 and 3.5 mg $mL^{-1}$ in case of Ca and 0.3, 0.6 and 1.2 mg $L^{-1}$ in case of Mg) added to the 5.0% (m/v) solutions of goldenrod, heather and rape honeys to double the original concentrations of these elements. The recoveries achieved were in the range of 97.1-102.2% for Ca and 98.6-101.7% for Mg. The precision (as CV) obtained was within 2.0-5.1% for Ca and 0.7-3.2% for Mg. The intermediate precision within three consecutive days was better than 6.8 and 5.3%, respectively in case of Ca and Mg. The limits of detection (LODs) of Ca and Mg achieved for FAAS after the execution of this procedure were expressed as the concentrations of these elements that correspond to the absorbance signals of 3 standard deviations of the means for the repeated (n = 5) measurements of the respective procedural blanks. These LODs were found to be 1.2 and 0.2 ng $mL^{-1}$, respectively for Ca and Mg. In the relation to the solid matrix it was 24 and 4.4 ng $g^{-1}$, respectively. The limits of quantification (LOQs) for Ca and Mg (10σ criterion) were 4.1 and 0.8 ng $mL^{-1}$, correspondingly. The linearity ranges were 200 μg $g^{-1}$ (Ca) and 40 μg $g^{-1}$ (Mg).

Next, the proposed sample preparation procedure based on the SPE separation and the two-step elution (procedure D) was used to determine the concentrations of Ca and Mg in six mono- and multi-flower honeys. Three other sample preparation procedures, namely the decomposition in

**Table 2.** The concentrations of Ca and Mg determined by FAAS in the samples of bee honeys prepared using the different sample treatment

| Honeys | Concentration / (μg $g^{-1}$) | | $t_{calculated}$ [a] | |
|---|---|---|---|---|
| | Ca | Mg | Ca | Mg |
| Procedure A (the open vessel digestion in mixture of $HNO_3$ and $H_2O_2$) | | | | |
| Acacia | 12.96 ± 0.55 (4.2) | 6.49 ± 0.17 (2.6) | +0.121 | +3.687 |
| Goldenrod | 65.24 ± 4.06 (6.2) | 18.68 ± 0.24 (1.3) | –0.130 | +1.586 |
| Heather | 74.20 ± 3.41 (4.6) | 19.05 ± 0.20 (1.0) | +0.560 | +2.044 |
| Lime | 62.39 ± 2.10 (3.4) | 17.89 ± 0.18 (1.0) | +0.592 | –3.795 |
| Multi-flower | 31.19 ± 1.08 (3.5) | 10.37 ± 0.29 (2.8) | +1.443 | +3.223 |
| Rape | 38.58 ± 2.85 (7.4) | 14.29 ± 0.25 (1.7) | –0.152 | –0.479 |
| Procedure B (the open vessel digestion in $H_2O_2$) | | | | |
| Acacia | 12.29 ± 0.25 (2.0) | 6.33 ± 0.54 (8.5) | +1.791 | +1.777 |
| Goldenrod | 65.95 ± 1.07 (1.6) | 18.11 ± 1.19 (6.6) | –1.069 | +1.186 |
| Heather | 76.60 ± 3.45 (4.5) | 20.94 ± 1.59 (7.6) | –0.370 | –1.124 |
| Lime | 63.02 ± 2.95 (4.7) | 17.12 ± 1.09 (6.4) | +0.118 | +0.202 |
| Multi-flower | 32.27 ± 2.07 (6.4) | 10.75 ± 1.32 (12.3) | +0.208 | +0.524 |
| Rape | 39.02 ± 1.12 (2.9) | 13.47 ± 1.30 (9.7) | –0.866 | +0.939 |
| Procedure C (the dissolution in water) | | | | |
| Acacia | 12.53 ± 0.63 (5.0) | 6.97 ± 0.07 (1.0) | +0.930 | –1.303 |
| Goldenrod | 64.54 ± 1.47 (2.3) | 18.82 ± 0.12 (0.6) | +0.329 | +1.077 |
| Heather | 74.89 ± 2.51 (3.4) | 20.30 ± 0.27 (1.3) | +0.340 | –1.188 |
| Lime | 62.57 ± 1.77 (2.8) | 17.17 ± 0.16 (0.9) | +0.524 | +0.495 |
| Multi-flower | 32.74 ± 0.26 (0.8) | 10.88 ± 0.06 (0.6) | –0.246 | +1.536 |
| Rape | 39.21 ± 0.67 (1.7) | 14.49 ± 0.18 (1.2) | –1.435 | –1.601 |
| Procedure D (the dissolution in water followed by the SPE and the two-step elution) | | | | |
| Acacia | 13.02 ± 0.66 (5.1) | 6.89 ± 0.08 (1.2) | - | - |
| Goldenrod | 64.92 ± 1.28 (2.0) | 18.93 ± 0.13 (0.7) | - | - |
| Heather | 75.64 ± 2.88 (3.8) | 19.83 ± 0.63 (3.2) | - | - |
| Lime | 63.24 ± 1.33 (2.1) | 17.25 ± 0.23 (1.3) | - | - |
| Multi-flower | 32.56 ± 1.24 (3.8) | 11.16 ± 0.31 (2.8) | - | - |
| Rape | 38.32 ± 0.84 (2.2) | 14.19 ± 0.27 (1.9) | - | - |

Average values (n = 3) with standard deviations behind (±SDs) and coefficients of variance (CVs) in brackets. [a]Values of the *t*-test calculated for the comparison of the average results obtained for the procedures A, B and C with those obtained for the procedure D ($t_{critical}$ = 4.303, p = 0.05).

$HNO_3$ and $H_2O_2$ (procedure A), the decomposition in $H_2O_2$ (procedure B) and the dissolution in water (procedure C), were used and the resulting 5.0% (m/v) honey solutions were analyzed as well. The results obtained with the aid of these procedures (A, B, C) were compared to those obtained with the proposed procedure D using the *t*-test at the 95% level of the significance.[22]

As can be seen from Table 2, the proposed sample preparation procedure combining the SPE separation of the monosaccharides and the anionic minerals (especially the phosphates), followed by the removal of K with Na and the elution of Ca and Mg, offers the precision (as CV) within 2.0-5.1% in case of Ca and 0.7-3.2% in case of Mg. The re-usability of the resin was high since the reproducibility of the results obtained using one SPE column for 25 analyses was 6.5% (as CV). The time investment required for this procedure was 30 min. The analyzed solutions resulted from the dilution of the eluents did not require to be admixed with the solutions of the aiding substances.

The differences between the results achieved with the proposed SPE sample preparation procedure (procedure D) and the other sample preparation procedures are statistically insignificant. The values of the calculated parameter *t* are lower than the critical value of 4.303. Nevertheless, as compared to the other procedures, it seems that the precision (as CV) achieved for the repeated analysis of the samples (n = 3) for the wet oxidative digestion procedures is lower (see the results in Table 2 for Ca in the procedure A and Mg in the procedure B).

Besides, the time investment required to complete the decomposition of the samples and the dilution of their digests with the releasing buffers was high, *i.e.*, 240 and 180 min, respectively for the procedure A and B. The precision (as CV) assessed for the direct analysis of the solutions resulted from the dissolution of the honey samples is comparable to this achieved with the SPE procedure in case of Ca (0.8-5.0%) and a slightly better for Mg (0.6-1.3%). The time investment required for the preparation of the sample solutions and their dilution with the solutions of the releasing buffers before the measurements of Ca and Mg by FAAS was also comparable to this assessed for the SPE procedure, *i.e.*, about 30 min. However, it should be noted that since the overall content of the organic substances, especially fructose and glucose, in the analyzed samples is not known, as a part of the quality assurance and control, the individual samples of honey have to be differently diluted, *e.g.*, 100, 50 and 20 times, what corresponds to the preparation of their 1.0, 2.0 and 5.0% (m/v) sample solutions, and analyzed. This is aimed at finding out such a suitable content of honey that dissolved in water would not result in the physical interferences in the transport and the aspiration rate of the prepared solutions. In consequence, more samples have to be analyzed and the analysis time is much longer than stated 30 min.

## Conclusions

This paper reports on the development of the sample preparation procedure that employs the solid phase extraction and the two-step extraction for the determination of the concentrations of Ca and Mg in honey by FAAS. The latter elements are separated from fructose, glucose and the anionic minerals in addition to K and Na what makes that the prolonged decomposition of the samples or the usage of the additional aiding substances can be freely omitted before the measurements. The procedure was demonstrated to be fast, straightforward and interference free. In this way, it could be a useful alternative to the time-consuming and laborious wet digestion procedures.

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## References

1. Baroni, M. V.; Arrua, C.; Nores, M. L.; Faye, P.; Diaz, M. P.; Chiabrando, G. A.; Wunderlin, D. A.; *Food Chem.* **2009**, *114*, 727.
2. Lachman, J.; Kolihova, D.; Miholova, D.; Kosata, J.; Titera, D.; Kult, K.; *Food Chem.* **2007**, *101*, 973.
3. Hernandez, O. M.; Fraga, J. M. G.; Jimenez, A. I.; Jimenez, F.; Arias, J. J.; *Food Chem.* **2005**, *93*, 449.
4. Lopez Garcia, I.; Vinas, P.; Blanco, C.; Hernandez Cordoba, M.; *Talanta* **1999**, *49*, 597.
5. Uren, A.; Serifoglu, A.; Sarikahya, Y.; *Food Chem.* **1998**, *61*, 185.
6. Pohl, P.; Sergiel, I.; Stecka, H.; *Crit. Rev. Anal. Chem.* **2009**, *39*, 276.
7. Juszczak, L.; Socha, R.; Roznowski, J.; Fortuna, T.; Nalepka, K.; *Food Chem.* **2009**, *113*, 538.
8. Silva, L. R.; Videira, R.; Monteiro, A. P.; Valentao, P.; Andrade, P. B.; *Talanta* **2009**, *93*, 73.
9. dos Santos, J. S.; dos Santos, N. S.; dos Santos, M. L. P, dos Santos, S. N.; Lacerda, J. J. J.; *J. Braz. Chem. Soc.* **2008**, *19*, 502.
10. Silici, S.; Uluozlu, O. D.; Tuzen, M.; Soylak, M.; *J. Hazard. Mater.* **2008**, *156*, 612.

11. Stankovska, E.; Stafilov, T.; Sajn, R.; *Environ. Monit. Assess.* **2008**, *142*, 117.
12. Tuzen, M.; *Fresenius Environ. Bull.* **2002**, *11*, 366.
13. Tuzen, M.; Duran, M.; *Adv. Food. Sci.* **2002**, *24*, 125.
14. Sergiel, I.; Pohl, P.; *J. Agric. Food Chem.* **2010**, *58*, 7497.
15. Vit, P.; Rodriguez-Malaver, A.; Rondon, C.; Gonzalez, I.; di Bernardo, M. L.; Garcia, M. Y.; *Arch. Latinoam. Nutr.* **2010**, *60*, 405.
16. Pohl, P.; Prusisz, B.; *Talanta* **2006**, *69*, 1227.
17. Madejczyk, M.; Baralkiewicz, D.; *Anal. Chim. Acta* **2008**, *617*, 11.
18. Chudzinska, M.; Baralkiewicz, D.; *Food Chem. Toxicol.* **2010**, *48*, 284.
19. Fournier, E. In *Handbook of Food Analytical Chemistry*; Wrolstad, R. E.; Acree, T. E.; Decker, E. A.; Penner, M. H.; Reid, D. S.; Schwartz, S. J.; Shoemaker, C. F.; Smith, D.; Sporns P., eds.; John Wiley & Sons: New York, 2005, pp. 651-660.
20. Pinta, M.; *Atomic Absorption Spectrometry*, Halstead Press: New York, 1975.
21. Zagorodni, A. A.; *Ion Exchange. Materials, Properties and Applications*, Elsevier: Amsterdam, 2007.
22. Miller, J. N.; Miller, J. C.; *Statistics and Chemometrics for Analytical Chemistry*, Pearson Education: Harlow, UK, 2005.

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