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A validated flame AAS method for determining magnesium in a multivitamin pharmaceutical preparation

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Abstract

A rapid method for determining magnesium in a multivitamin pharmaceutical preparation has been validated. This element is analysed by flame atomic absorption spectrometry after dissolution of the sample in acid medium. Linearity, precision, accuracy and robustness of the method have been determined, and detection and quantification limits have been calculated. Linearity of response was verified for concentrations ranging from 0.05 to 0.40 mg l^{-1} of magnesium. Correlation coefficient of the calibration straight lines was always ≥ 0.9999. Repeatability of the method gave relative standard deviation (R.S.D.) of 0.6%. Reproducibility of the method calculated after analysis of samples by the same analyst in different days (day-to-day fluctuation) and by two different analysts in different days (analyst-to-analyst fluctuation) gave relative standard deviation of 1.1 and 1.6%, respectively. Mean recoveries of magnesium obtained after spiking sample placebos with increasing amounts of magnesium chloride ranged from 98.9 to 100.8%. Robustness of the method evaluated by changing different experimental conditions under which analyses were performed, gave relative standard deviation from 0.2 to 0.5%. Limits of detection and quantification were 3.8 and 7.0 μg of Mg per gram of sample, respectively. Results show the suitability of the method for direct measurement of magnesium in a water-soluble multivitamin pharmaceutical preparation.

Keywords: Flame atomic absorption spectrometry; Magnesium; Multivitamin preparation; Method validation

1. Introduction

Validation of an analytical method is a necessary step in controlling the quality of quantitative analysis. Validation can be defined as the process by which it is established, by laboratory studies, that the analytical parameters of the method meet the requirements for the intended analytical applications. Thus, with the background knowledge of linearity, detection and quantification limits, precision, accuracy, specificity and robustness of the analytical method, it is relatively easy to derive

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the confidence and reliability of the analytical data obtained with it.

Magnesium is an essential nutrient mainly found in foods like cereals, nuts, cacao, meat, milk, and vegetable [1]. It is involved in bone and tissue metabolism and its deficiency occurs, in general, as complication of other diseases like alcoholism, diabetes, kidney failure and in some post-operative periods. Magnesium deficiency can be treated by oral or parental administration of some magnesium salts [2,3].

Multivitamin pharmaceutical preparations may contain magnesium in their composition, so analytical laboratories need validated methods of analysis for determining magnesium in these preparations. A great variety of methods can be used for the magnesium analysis in different matrix samples, with the choice often depending on the precision and sensitivity required. Thus, a validated titrimetric method for the determination of magnesium in drugs (including multivitamin with minerals preparations) can be found in the Official Methods of Analysis of AOAC International [4]. More modern instrumental methods used for the magnesium analysis are flame atomic absorption spectrometry (FAAS) and inductively coupled plasma emission spectrometry. Flame atomic absorption spectrometry methods generally, are applicable at moderate concentrations in clean and complex matrix samples, so this analytical technique is the most frequently used for determining this element in different samples [5].

Preliminary treatment of the sample is often required to present the metal to the analytical methodology in an appropriate form. Dry ashing and wet digestion with mineral acid are the most commonly used treatments. However, these digestion methods are very laborious, and could be avoided when the sample is soluble in acid medium and no interferences due to the organic matter are found. The purpose of the present work was to validate a rapid and easy analytical method for the determination of magnesium in a water-soluble multivitamin granulated preparation using FAAS.

2. Experimental

2.1. Instrumentation

The determination of Mg was carried out by FAAS using a Perkin Elmer Model 1100 B atomic absorption spectrophotometer with a Mg hollow cathode lamp (Perkin Elmer, Norwalk, CT). An air (compressed air)/acetylene (N 26 quality, Air Liquide, Spain) flame was employed. The instrumental settings of the spectrophotometer for the Mg determination are summarised in Table 1.

2.2. Reagents

All reagents were of analytical grade. Water (resistance 18 MΩ cm^{-1}) used in the preparation of standard solutions and samples was obtained from a Millipore Milli-Q-System (Waters, Millipore, Medford, MA). Concentrated HCl (37% w/w) was obtained from Panreac (Barcelona, Spain).

A commercially prepared Mg standard solution (1 mg ml^{-1}) was obtained from Merck (Darmstadt, Germany). A Mg standard stock solution (20 mg l^{-1}) was prepared by diluting 10 ml of the commercial Mg standard solution to 500 ml in a volumetric flask with Milli-Q water. l0 ml of the Mg stock solution were diluted to 100 ml in a volumetric flask with Milli-Q water to obtain a 2 mg l^{-1} Mg standard solution.

In order to obtain Mg standard working solutions (0.05–1.50 mg l^{-1}), appropriate amounts of the 2 mg l^{-1} Mg standard solution were

Table 1
Instrumental conditions for the measurement of magnesium by FAAS

Parameter	
Wavelength (nm)	285.2
Slit width (nm)	0.7
Light source	Magnesium holow lamp
Power supply (mA)	4
Flame, flow setting (1 min^{-1})	Air (8), Acetylene (2.5)
Integration time (s)	3

transferred into 100 ml volumetric flasks, 40 μl of HCl were added and volume was completed with Milli-Q water. Blank solutions were also prepared by adding 40 μl of HCl into a 100 ml volumetric flask and completing volume with Milli-Q water.

2.3. Samples

Abelló Farmacia S.L. (Spain) furnished a multivitamin granulated formulation. The preparation contained two B-group vitamins (vitamin B_1 and B_6), vitamin C, magnesium that was present in the formulation as $MgCl_2 \cdot 6H_2O$, and other components such as aminoacids and excipients. The pharmaceutical company also supplied a placebo of the multivitamin granulated formulation (all the components except the analyte to be determined) and $MgCl_2 \cdot 6H_2O$.

2.4. Procedures

Approximately 1.5 g of product were weighted in an analytical balance, dissolved with Milli-Q water and put into a 250 ml volumetric flask. 1 ml of HCl was added and volume was completed to 250 ml with Milli-Q water. The solution was sonicated for 5 min until dissolution. Then 10 ml of this solution were transferred into 100 ml volumetric flask and completed with Milli-Q water. This solution was ready to be read in the spectrophotometer.

Blanks, standard working solutions and samples were measured in the spectrophotometer, and magnesium was determined under the instrumental conditions described in Table 1.

3. Results and discussion

To assess the validity of the proposed method, analytical performance characteristics for determination of magnesium in the multivitamin pharmaceutical preparation were estimated.

Linearity of response was studied by using magnesium standard solutions containing 0.05, 0.10, 0.20, 0.40, 0.80, and 1.50 mg l^{-1}. By plotting absorbance for each solution versus its magnesium concentration, a linear relationship was obtained until 0.40 mg l^{-1}. Thus, in order to obtain the calibration straight line, standard solutions containing 0.05, 0.10, 0.20 and 0.40 mg l^{-1} of Mg were employed. Equation of the calibration straight line and correlation coefficient (r) after least squares analysis were $Y = 0.9365\ X + 0.0003$, $r = 1.000$.

Precision of the instrument was checked in order to show if the instrument response for a Mg standard solution was always the same. This parameter considers only the error attributable to the operating system and not the error attributable to sample handling and preparation. The instrumental precision was calculated from ten consecutive measurements of a 0.20 mg l^{-1} magnesium standard solution. A good precision, expressed as R.S.D., %, was obtained since relative standard deviation was equal to 0.3%.

To evaluate the precision of the method, measurements were performed under conditions of repeatability and reproducibility. Repeatability of the method was estimated from the analysis of ten sample solutions prepared individually by the same operator with the same equipment within 1 day (as indicated in the Section 2). Relative standard deviation (R.S.D. = 0.6%) indicated a good repeatability of the procedure. Reproducibility of the method (internal reproducibility) was studied by carrying out analysis on two different lots of the multivitamin preparation (lots 1 and 2) under various conditions. As it can be seen in Table 2, when four samples from lot 1 were analysed (by duplicate) by the same analyst on 4 different days over a period of a month (day-to-day fluctuation), relative standard deviation obtained was equal to 1.1%. A slightly higher R.S.D. = 1.6% was obtained when five samples from lot 2 were analysed by duplicate in different days over a period of 45 days by two different analysts (analyst-to-analyst fluctuation).

The reproducibility of calibration straight lines obtained on 7 different days (over a period of a month) by using different Mg standard solutions was also investigated. After linear regression analysis, correlation coefficient (r) for the straight lines was always ≥ 0.9999 and R.S.D. for slopes was equal to 1.1%.

Table 2
Reproducibility in the determination of magnesium in two different lots of the multivitamin preparation

Lot 1			Lot 2		
Analyst	Days	Mg (mg g^{-1})[a]	Analyst	Days	Mg (mg g^{-1})[a]
A	1	0.324	A	5	0.292
A	2	0.317	A	6	0.295
A	3	0.320	A	7	0.304
A	4	0.325	B	8	0.294
			B	9	0.295
Mean		0.322	Mean		0.296
R.S.D., %		1.1	R.S.D., %		1.6

[a] Results are the mean of two determinations.

A method is accurate if it gives results that are close to the true value. The accuracy of the method should be estimated by measuring a reference standard containing a well-known amount of the analyte in a similar matrix to the real sample. However, when a reference standard with these characteristics does not exist, the accuracy of the method can be expressed as the percent of analyte recovered from a spiked sample placebo (% R). Accuracy of the method was calculated by preparing samples containing the same quantity of placebo (all the components except the analyte to be determined) as the real sample, and increasing amounts of $MgCl_2 \cdot 6H_2O$ (50, 75, 100, 125 and 150% of the theoretical Mg content in the multivitamin preparation). Mean recoveries (% R) of magnesium for two prepared samples with identical spike ranged from 98.9 to 100.8%.

Robustness was evaluated in order to know how sensitive is the method to small changes introduced in the procedure. Changes introduced in the method were: (a) changes in the volume of HCl added during sample dissolution, (b) changes in the slit width used for magnesium measurement by FAAS, and (c) changes in the sample matrix. To demonstrate the robustness of the method to changes in the volume of HCl added during sample dissolution, analyses of the multivitamin preparation were carried out by using 1 ml of HCl (as indicated in the Section 2) and 2 ml of HCl. As it can be seen in Table 3, relative standard deviation of the obtained results was equal to 0.4%. To assess the influence of the slit width in the magnesium measurement by FAAS, measurements were carried out by using three different slit width: 0.7 nm (as indicated in the Section 2), 0.2 and 2.0 nm. Relative standard deviation of the obtained results was equal to 0.5%. Robustness of the method was also demonstrated by analysing a sample with a different matrix. This sample had a similar composition as the multivitamin preparation, with the exception that its vitamin C and arginine aspartate content was twice. As it can be seen in Table 3, relative standard deviation obtained in this case was equal to 0.2%.

The limit of detection of an analyte in a sample, may be described as the concentration that gives an instrument signal significantly different from the blank or background signal. The limit of quantification is the lowest concentration of analyte that can be determined with acceptable precision and accuracy under the stated experimental conditions. In order to obtain the detection and quantification limits, the blank signal was measured twenty times. The detection limit was calculated as $[(Y_b + 3 \text{ S.D.}) - b]a^{-1}$ and the quantification limit as $[(Y_b + 10 \text{ S.D.}) - b]a^{-1}$, where Y_b is the mean of the blank signal, S.D. is the standard deviation of the blank signal, b is the intercept of the calibration straight line, and a is the slope of the calibration straight line [6]. Results obtained were 3.8 μg of Mg per gram of sample for the detection limit and 7.0 μg of Mg per gram of sample for the quantification limit.

Table 3
Robustness in the determination of magnesium in the multivitamin preparation

HCl volume added		Slit width		Matrix sample	
ml	Mg (mg g^{-1})[a]	nm	Mg (mg g^{-1})[a]	Component added	Mg (mg g^{-1})[a]
1	0.320	0.7	0.320	None	0.316
2	0.318	0.2	0.323	Vitamin C and arginine aspartate	0.317
		2.0	0.322		
Mean	0.319	Mean	0.322	Mean	0.316
R.S.D., %	0.4	R.S.D., %	0.5	R.S.D., %	0.2

[a] Results are the mean of two determinations.

4. Conclusions

A simple method for the determination of magnesium in a multivitamin preparation by FAAS has been developed and validated. Linear range of calibration, precision, accuracy, robustness and detection and quantification limits obtained for the proposed method show its suitability for determining magnesium in a multivitamin granulated pharmaceutical preparation containing water-soluble vitamins. The procedure is very practical and useful for routine laboratory analyses of a large number of samples.

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