DEVELOPMENT AND VALIDATION OF AN ASSAY METHOD FOR MAGNESIUM IN SOFT CAPSULES BY AAS

Dr. Le Thi Huong Hoa[1], MA. Tran Duc Lai[2]
[1]Hoa Binh University
[2]International Institute of Drug Quality Control
Corresponding Author: lthhoa@daihochoabinh.edu.vn

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Abstract

A procedure for quantitative analysis of trace element magnesium (Mg) in soft capsules (mainly containing vitamins, trace elements and herbal extracts) using atomic absorption spectroscopy (AAS) has been established.

The sample was treated to convert the Mg compound to Mg+2 ion form in solution by heating the sample on a boiling water bath with 10% hydrochloric acid solution, with the addition of Polysorbate 80. The sample was diluted with 1% hydrochloric acid solution, with the addition of LaCl3 in the final solution for AAS measurement. The standard solutions were prepared by diluting the 1000 µg/ml magnesium stock standard solution with 1% hydrochloric acid solution. Using an atomic absorption spectrometer equipped with a Magnesium hollow cathode lamp and an acetylene-air flame, measure the atomic absorption of the standard and test solutions at the maximum spectral line of Magnesium at 285.2 nm. Calculate the results using the standard curve method.

The method validation results showed that the method has high specificity, linear range from 0.1 µg/ml to 0.5 µg/ml with correlation coefficient R2 = 0.9972, high accuracy (from 99.19 -101.39%), good repeatability with small CV% values (0.44 to 0.91%), the method's determination range is from 0.18 to 0.32 µg/ml.

Keywords: *Soft capsules, Magnesium quantification in soft capsules, Mg quantification, AAS-Mg.*

Xây dựng phương pháp định lượng Magnesi trong viên nang mềm bằng quang phổ hấp thụ nguyên tử (AAS)

TS. Lê Thị Hường Hoa[1], ThS. Trần Đức Lai[2]
[1]Trường Đại học Hòa Bình
[2]Viện Kiểm nghiệm thuốc Trung ương
Tác giả liên hệ: lthhoa@daihochoabinh.edu.vn

Tóm tắt

Một quy trình phân tích định lượng nguyên tố vi lượng Mg trong viên nang mềm bằng phương pháp quang phổ hấp thụ nguyên tử AAS đã được thiết lập.

Xử lý mẫu để chuyển hợp chất của Mg về dạng ion Mg+2 trong dung dịch bằng cách đun trên cách thủy sôi mẫu thử đã được thêm dung dịch acid hydrochloric 10% và chất Polysorbat 80. Pha loãng mẫu bằng dung dịch acid hydrochloric 1%, có thêm chất LaCl3 ở dung dịch cuối để đo AAS. Các dung dịch chuẩn được chuẩn bị bằng cách pha loãng dung dịch chuẩn gốc magnesi 1000 µg/ml với dung dịch acid hydrochloric 1%. Sử dụng máy quang phổ hấp thụ nguyên tử có trang bị đèn cathod rỗng Magnesi, đầu đốt sử dụng ngọn lửa acetylene - không khí nén. Tiến hành đo độ hấp thụ nguyên tử của các dung dịch chuẩn và dung dịch thử tại vạch phổ cực đại của Magnesi 285,2 nm.

Tính kết quả theo phương pháp đường chuẩn.

Kết quả thẩm định phương pháp cho thấy, phương pháp có độ đặc hiệu cao, khoảng tuyến tính từ 0.1 µg/ml đến 0.5 µg/ml với hệ số tương quan R2 = 0,9972, có độ đúng cao (từ 99,19 -101,39%), độ lặp lại tốt với các giá trị CV% nhỏ (0,44 to 0,91%), khoảng xác định của phương pháp là từ 0,18 đến 0,32 µg/ml.

Từ khóa: *Nang mềm, định lượng Magnesi trong viên nang mềm, định lượng Mg, quang phổ hấp thụ nguyên tử - Mg.*

1. Problem Statement

In recent years, pharmaceutical manufacturers have tended to produce products containing many active ingredients to enhance the effectiveness of the preparation and to be convenient and suitable for the needs of users. Tablets containing many ingredients, especially tablets containing vitamins and trace elements, extracts from medicinal herbs have been researched and produced quite abundantly, soft capsules are also one of these dosage forms. In order to standardize and control the quality of the above dosage forms, analytical methods need to be studied and applied to check the presence and content of active ingredients in the mixed tablets. Magnesium in the form of glucolate or chloride salt is one of the minerals commonly found in tablets containing vitamin and trace element mixtures. Magnesium is an essential mineral, playing an important role in the human body, is a coordinating factor in about 250-300 enzyme systems that regulate the body's biochemical processes. Magnesium is necessary for the metabolism of calcium, phosphorus, sodium, potassium, vitamins B, C... regulating the absorption, use and excretion of these substances. Helps bones and teeth stay healthy, prevents calcium deposits that cause kidney stones, reduces indigestion and constipation. Magnesium regulates the nervous system and motor function of the muscular system... [1]. In this article, we would like to introduce the results of a study on the method of quantifying magnesium (Mg) in the form of magnesium glucolate in soft capsules using atomic absorption spectroscopy (AAS).

2. Materials and Research Methods

Chemicals and Standards: Pure type used for AAS measurement (Ion-exchange water with resistivity ≥18 MΩ.cm. Merck's polysorbate 80, polysorbate solution: Dissolve 100 ml of polysorbate 80 in 1000 ml of absolute ethanol. Hydrochloric acid: Merck's ultra-pure type, hydrochloric acid solution in ion-exchange water with concentrations: 1% and 10% (v/v); Lanthan chloride (Merck), Lanthan chloride 2.7% solution: Dissolve 2.7 g of Lanthan chloride in 1% hydrochloric acid solution to 100 ml

Magnesium stock standard solution with concentration of 1000 mg/L, SKS: HC 258003 (Merck)

Research Subjects

Samples: *Blank sample:* Pipet 10 ml of 2.7% Lanthan chloride solution into a 100 ml volumetric flask, add 1% hydrochloric acid solution to the mark, shake well. Standard sample: From the original Magnesium standard solution with a concentration of 1000 µg/ml, dilute with 1% hydrochloric acid solution to obtain solutions with appropriate concentrations. Test sample: Amorvita ginseng soft capsule enteric solution. Placebo sample: Amorvita ginseng soft capsule enteric solution but without magnesium compounds. Self-made sample: Add Mg standard solution to Placebo sample (used in the method's accuracy verification).

Equipment and Instruments: Calibrated according to ISO/IEC 17025

Thermo scientific iCE 3500 atomic absorption spectrometer; SARTORIUS ED224S analytical balance, accuracy 0.1 mg; Argon gas cylinder; Water bath, Volumetric flask, beaker made of type A glass, Micropipette 10-100 µl; 100-1000 µl …

Research Method

Analysis Process Building

Sample treatment: Add a certain amount of 10% hydrochloric acid solution to the sample, add Polysorbate 80 surfactant, stir well to increase the acid's ability to contact with the sample. Heat the mixture in a water bath, the sample digestion time is faster. Then, dilute the sample with 1% hydrochloric acid solution to a suitable concentration, add 2.7% LaCl3 solution to the final solution to measure AAS.

Procedure: Using an atomic absorption spectrometer equipped with a Magnesium hollow cathode lamp, the burner uses an acetylene-compressed air flame. Measure the atomic absorption of standard solutions and test solutions at the maximum spectral line of Magnesium 285.2 nm [2], [5], [6]. The appropriate measurement parameters and methods will be investigated and selected.

Evaluation of Analytical Procedures: Based on reference to documents of ICH [7], USP [8], AOAC [4], Circular No. 32/2018/TT-BYT dated November 12, 2018 of the Ministry of Health regulating the registration of drugs and pharmaceutical ingredients [3], The evaluated indicators: Specificity; Linearity; Accuracy; Precision; Method determination range.

Data processing method: By statistical method.

3. Results and Discussion

Development of Analysis Procedure

AAS measurement parameters: Through field survey, combining default parameters of the device, machine parameters and optimal measurement temperature program are presented in Table 1.

Fig7. Effect of excipients to create NR3 on the viability and death of HL-6

Condition	Parameters
Type of lamp	Mg hollow cathode
Lamp intensity (mA)	5
Wavelength (nm)	285.2
Slit width (nm)	0.5
Graphite cuvette	Tube A
Sample volume (µl)	10

Sample preparation: To select the appropriate hydrochloric acid concentration, we mixed HCl solutions with different concentrations (5%; 10% and 20%) and kept the other components the same, then surveyed the recovery ability of Mg in the sample matrix. The results showed that: At 5% acid concentration, the amount of Mg recovered was not complete, while at 10% and 20% concentrations, the amount of Mg recovered was from 99.0 to 101.0%, we chose 10% hydrochloric acid solution. With a similar survey, we found that using 0.5 ml of Polysorbate solution for a 0.25 g sample was appropriate.

Prepare Standard Solution: Dilute the 1000 µg/ml Magnesium stock standard solution with 1% hydrochloric acid solution to obtain a standard solution with a Magnesium concentration of 10 µg/ml. In 4 100 ml volumetric flasks, add 1 ml, 2 ml, 3 ml, 4 ml of 10 µg/ml Magnesium solution to each flask, add 10 ml of 2.7% Lanthan chloride solution, then add 1% hydrochloric acid solution to the mark, shake well. The obtained standard solutions have Magnesium concentrations of 0.1 µg/ml; 0.2 µg/ml; 0.3 µg/ml and 0.4 µg/ml, respectively.

Test Sample Preparation: Take 20 soft capsules, determine the average mass of the drug in the capsule, mix well. Weigh accurately about 0.25 g of the drug into a 100 ml volumetric flask. Add 10 ml of 10% hydrochloric acid solution, 0.5 ml of Polysorbate solution to the flask, heat on a boiling water bath, shake occasionally until completely dissolved, heat for another 15 minutes, let cool, add ion exchange water to the mark, shake well to get solution A. Pipette exactly 10.0 ml of solution A into a 100 ml volumetric flask, add 1%

hydrochloric acid solution to the mark, shake well to get solution B. Pipette exactly 1.0 ml of solution B into a 50 ml volumetric flask, add 5 ml of 2.7% LaCl3 solution, add 1% hydrochloric acid solution to the mark, shake well.

Blank sample: Pipet 10 ml of 2.7% Lanthan chloride solution into a 100 ml volumetric flask, add 1% hydrochloric acid solution to the mark, shake well.

Procedure: Measure the atomic absorption of the blank sample solution, standard solution and test solution at the maximum spectral line of Magnesium 285.2 nm. From the absorbance of the standard solutions, establish an experimental standard curve showing the dependence of absorbance on Magnesium concentration and calculate the Magnesium concentration in the test solution based on the standard curve.

Magnesium content (%) compared to label is calculated according to the formula:

$$X(\%) = \frac{C}{1000} * \frac{D * \overline{M}_{tb}}{m_t * 9,8} * 100$$

In which:

C (µg/ml): Magnesium concentration of the test solution measured on the machine.

mt (g): mass of the drug to be quantified.

Mtb (g): Average mass of the drug in the capsule.

D: Dilution factor of the test solution.

39.8: Theoretical Mg content in 1 capsule.

Evaluation of the Magnesium Analysis Process in the Sample

Method Specificity: Evaluate the influence of the sample matrix on the measurement results.

- *Blank sample*: Prepare as stated in the section on developing the analytical procedure.
- *Test sample:* Weigh accurately about 0.25 g of the preparation into a 100 ml volumetric flask, process the sample as stated above. The test solution has a Magnesium concentration of about 0.2 µg/ml.
- *Placebo sample:* Weigh a 0.25 g placebo sample without Magnesium into a 100 ml volumetric flask, add the reagents, and process as the test sample stated above.

The actual sample weight is shown in Table 2.

Table 2. Actual Sample Weight

Numerical order	Placebo sample (g)	Test sample (g)
1	0,2534	0,2575
2	0,2567	0,2567
3	0,2519	0,2625

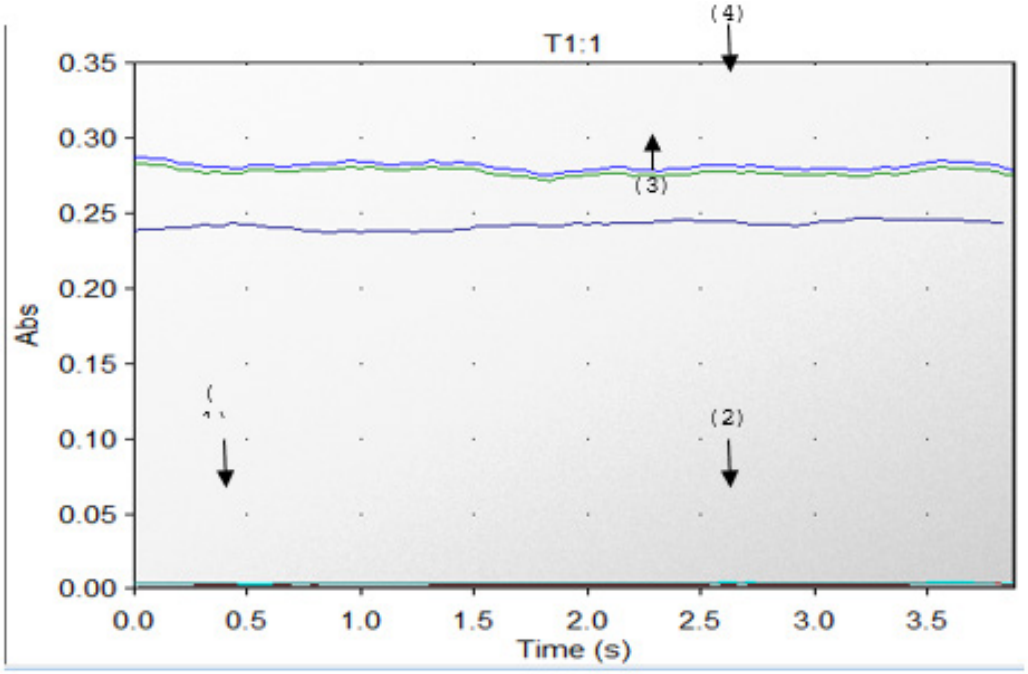
Magnesium standard solution 0.2 µg/ml: Is a Magnesium standard solution with a concentration of 0.2 µg/ml in the linear range survey.

Measure the absorbance of blank, placebo, test samples and standard solutions under the stated conditions. The results showed that blank, Placebo gave negligible absorbance (< 2% of the absorbance of Magnesium at the measured concentration), standard and test gave absorbance greater than 0.2 at the characteristic wavelength of Magnesium 285.2 nm, as shown in Table 3, Figure 1.

Table 3. Results of the Study on the Influence of Sample Matrix Solution

Absorbance / Number	Blank	Placebo	Mg Standard solution 0.2 mg/ml	Test	% affect
1	0,0032	0,0036	0,2413	0,2787	1,49 %
2	0,0030	0,0036	0,2424	0,2766	1,49 %
3	0,0030	0,0036	0,2402	0,2745	1,50 %
Mean	0,0031	0,0036	0,2413	0,2756	1,49 %

Figure 1. Absorbance of Magnesium at 285.2 nm: (1): blank solution; (2): Placebo solution; (3): 0.2µg/ml Magnesium standard solution; (4): sample solution.



Linear Range

Dilute the 1000 µg/ml Magnesium standard stock solution with 1% hydrochloric acid solution to obtain a 10 µg/ml Magnesium standard solution. From this standard solution, continue diluting as shown in Table 4 to obtain the Magnesium standard series solutions.

Measure AAS of standard series solutions under the stated conditions, the results are shown in Table 5 and Figure 2.

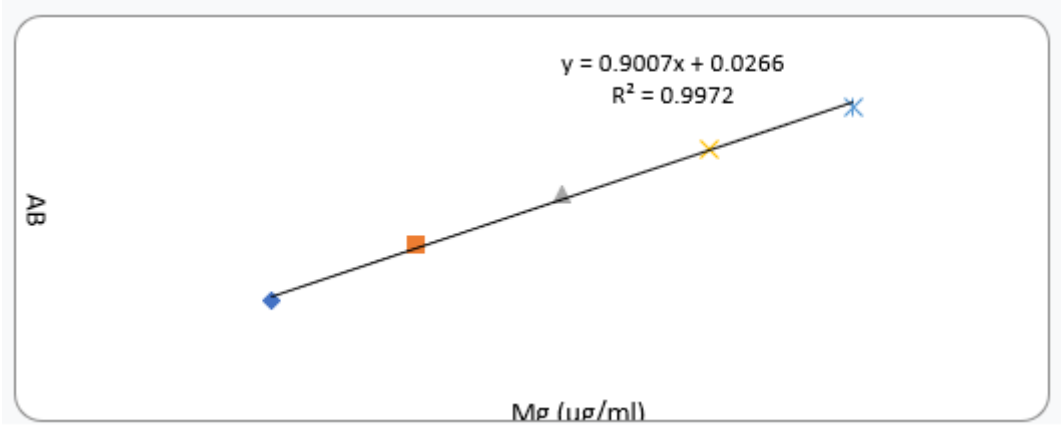
Table 4. Preparation of Standard Series Solutions

Number	Mg concentration (µg/ml)	Volume (ml) of Mg solution 10 µg/ml	Volume (ml) of $LaCl_3$ solution 0,27%	Add enough volume with 1% hydrochloric acid solution
1	0,1	1	10	100 ml
2	0,2	2	10	100 ml
3	0,3	3	10	100 ml
4	0,4	4	10	100 ml
5	0,5	5	10	100 ml

Table 5. Results of Linear Range Survey of the Method

Standard Solution	1	2	3	4	5
Concentration of Mg (µg/ml)	0,1	0,2	0,3	0,4	0,5
Absorbance (Abs)	0,1091	0,2098	0,3049	0,3917	0,4685
Regression equation: $y = 0,9007 x + 0,0266$					
Linear correlation coefficient: $R^2 = 0,9972$					

Figure 2. Graph Illustrating the Linear Correlation between Absorbance and Mg Concentration



The results from Table 5 and Figure 2 show that, in the investigated concentration range, there is a close linear correlation between the Mg concentration in the standard solution and the absorbance, with the correlation coefficient R2 = 0.9972.

Precision of the Method

Two analysts on two different days, each performed 6 independent analyses on the same soft capsule sample with an average drug mass in the capsule of mtb = 0.8532g, the results are shown in Table 6. The results show that the method has high repeatability and intermediate precision with Mg content reaching from 97.99% to 100.87% compared to the content on the label, RSD value from 0.41% to 0.91%. Thus, the developed method has appropriate precision for quantifying Magnesium in the preparation.

Table 6. Survey of the Precision of the Method

First inspector					Second inspector			
Number	Sample weight (g)	Dilution factor	Mg Concentration of test solution (µg/ml)	Mg content in the preparation (%)	Sample weight (g)	Dilution factor	Mg Concentration of test solution (µg/ml)	Mg content in the preparation (%)
1	0,2575	50000	0,2354	97,99	0,2555	50000	0,2354	98,75
2	0,2567	50000	0,2362	98,63	0,2593	50000	0,2381	98,42
3	0,2625	50000	0,2426	99,06	0,2645	50000	0,2441	98,92
4	0,2532	50000	0,2338	98,97	0,2547	50000	0,2397	100,87
5	0,2586	50000	0,2390	99,06	0,2508	50000	0,2336	99,84
6	0,2528	50000	0,2337	99,09	0,2561	50000	0,2366	99,02
$\bar{X}$ = 98,80 % RSD = 0,44 %					$\bar{X}$ = 99,30 % RSD = 0,91 %			
Results of two analyses (n=12) **$\bar{X}$ = 99,05 % ; RSD= 0,73 %**								

Accuracy of the Method

Weigh accurately about 0.25 g of placebo sample into a 100 ml volumetric flask, repeat the procedure 9 times in 9 separate flasks. Add accurately the Magnesium stock standard solution with a concentration of 1000 µg/ml into the test flasks as follows: 9.0 ml for flasks 1 to 3; 12.0 ml for flasks 4 to 6; 16.0 ml for flasks 7 to 9. Process the sample as described above. Determine the Magnesium concentration in the test solutions under the AAS measurement conditions stated. The results are presented in Table 7.

The results showed that the recovery rate was from 99.19 to 101.39% (within the range of 97.0% - 103.0 being the limit given by the research group when referring to the document on the evaluation of analytical methods of the Association of Analytical Chemists AOAC

[4], because the theoretical Mg content in the tablet is 4.6%; The requirement for the content of elements in the tablet is wide (90.0% - 125.0% according to USP; The quantitative concentration of small Mg elements (≈ 0.23 µg/ml)) and the largest RSD is 0.4% (< 2%). Therefore, the quantitative method meets the accuracy requirements.

Table 7. Results of Method Accuracy Survey

Sample	*Placebo sample weight (g)*	*Amount of Mg added (µg)*	**Mg concentration in measuring solution (µg/ml))**	*Amount of Mg recovered (µg)*	*% Recall*	*Statistics*
80%	0,2479	9000,00	0,1825	9125,00	101,39	TB: 101,11 % RSD: 0,24%
80%	0,2546	9000,00	0,1817	9085,00	100,94	
80%	0,2581	9000,00	0,1818	9090,00	101,00	
100%	0,2516	12000,00	0,2400	12000,00	100,00	TB: 99,99% RSD: 0,19%
100%	0,2573	12000,00	0,2395	11975,00	99,79	
100%	0,2591	12000,00	0,2404	12020,00	100,17	
140%	0,2576	16000,00	0,3197	15985,00	99,91	TB: 99,47% RSD: 0,39%
140%	0,2495	16000,00	0,3174	15870,00	99,19	
140%	0,2488	16000,00	0,3178	15890,00	99,31	
Mean (%)					**99,77**	
RSD (%)					**0,77**	

Method Range

From the research data on linearity, precision and accuracy stated above, the method range is: From 0.18 µg/ml to 0.32 µg/ml.

4. Conclusion

A method for quantifying trace element magnesium (Mg) in soft capsules (mainly containing vitamins, trace elements and herbal extracts) using atomic absorption spectroscopy (AAS) has been established. The sample was treated by boiling in a water bath with 10% hydrochloric acid solution, with the addition of surfactant Polysorbate 80. Then the sample was diluted with 1% hydrochloric acid solution, with the addition of LaCl3 to the final solution for AAS measurement. The standard solutions were prepared by diluting the 1000 µg/ml magnesium stock standard solution with 1% hydrochloric acid solution.

Using a Thermo atomic absorption spectrometer equipped with a Magnesium hollow cathode lamp, the burner uses an acetylene-compressed air flame. Measure the atomic absorption of the standard solutions and the test solutions at the maximum spectral line of Magnesium 285.2 nm. Calculate the results according to the standard curve method. The method validation results show that the method has high specificity, linear range from 0.1 µg/ml to 0.5 µg/ml with a correlation coefficient R2 = 0.9972, high accuracy (from 99.19 -101.39%), good precision with a small CV% value (0.44 to 0.91%), the method's determination range is from 0.18 µg/ml to 0.32 µg/ml. With the results of this study, we hope to be able to apply the quantification of Mg in preparations with similar composition.

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