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Development of a procedure for the determination of magnesium and calcium in pharmaceutical preparations by atomic absorption spectrometry (AAS)

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Abstract

Objective: To establish and validate an F-AAS procedure for the simultaneous quantification of Mg and Ca in oral solutions.

Subjects and Methods: An oral solution containing calcium glycerophosphate and magnesium gluconate was used. Sample treatment (mineralization) was optimized using Response Surface Methodology (RSM) with a Box-Behnken Design (BBD), surveying HNO_3 concentration (1–3 M), digestion time (10–30 min), and temperature (50–70°C) for maximum recovery Method validation was performed in accordance with the ICH Q2 (R1) guidelines and Ministry of Health standards.

Results: Optimal sample treatment conditions were 1.7 M HNO_3, 11 minutes, and 54°C. F-AAS showed high specificity and was unaffected by excipients. The linearity range had $r \geq 0.998$. Accuracy (recovery) was 99.64%–103.76% for Mg and 97.04%–106.22% for Ca. Precision (RSD) was ≤ 2%. F-AAS demonstrated higher precision and fewer errors than complexometric titration.

Conclusion: This study successfully established a high-precision F-AAS protocol for the simultaneous quantification of Ca and Mg in complex oral solutions. The primary novelty lies in the application of a Box-Behnken Design (BBD) to rigorously optimize the wet digestion process, ensuring total analyte release from organic matrices with minimal acid consumption and time. By addressing the specific challenges of complex organic matrices and simultaneous determination, this method provides a superior and more efficient alternative to traditional pharmaceutical analysis, significantly enhancing quality control standards for such liquid formulations.

Keywords: Calcium, Magnesium, Atomic absorption spectrometry, Complexometric titration, Design-Expert, Box-Behnken design.

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1. INTRODUCTION

Magnesium (Mg) and calcium (Ca) are essential cations in human physiology. Calcium is paramount for bone mineralization, blood coagulation, and signal transduction [1-3], while magnesium is a vital cofactor for over 300 enzymes driving energy and biosynthetic processes, crucially regulating neuromuscular function and cardiovascular stability [1, 4, 5]. Deficiencies in these two macrominerals can lead to detrimental health effects. Consequently, calcium and magnesium supplements are now widely consumed. However, the therapeutic window for these minerals requires careful monitoring. Hypercalcemia can lead to renal calculi, calcification of soft tissues, and cardiac arrhythmias [6], while hypermagnesemia can induce hypotension and bradycardia, and coma in severe cases [4]. Accurate quantification of Ca and Mg in

pharmaceutical supplements is therefore imperative to ensure labeled compliance and consumer safety.

The F-AAS method has been studied for the analysis and quantification of various preparations containing Ca or Mg. Abarca et al. established a robust F-AAS framework for magnesium analysis in multivitamin supplements, highlighting the reliability of acid dissolution for simple pharmaceutical matrices [7]. Yebra further advanced the field by integrating flow injection analysis with ultrasound-assisted preparation, demonstrating the feasibility of simultaneous Ca and Mg estimation in effervescent tablets with significantly enhanced throughput [8]. Sbahi et al. expanded the application of F-AAS to evaluate magnesium not only as an active ingredient but also as a functional excipient within solid-state formulations, ensuring precise quantification despite the presence of complex tablet lubricants [9]. Patel et al. standardized a closed-vessel mineralization technique using an HNO_3/H_2O_2 mixture, emphasizing the necessity of thorough sample digestion for accurate calcium determination in herbomineral product [10]. Research on the simultaneous quantification of Ca and Mg using F-AAS remains limited, particularly regarding the optimization of wet digestion parameters.

This study aims to develop and validate an optimized F-AAS method for the simultaneous quantification of magnesium and calcium in pharmaceutical oral solutions. Wet acid digestion parameters (HNO_3 concentration, time, and temperature) were optimized using a BBD in Design-Expert software. The method was validated in accordance with the Ministry of Health and ICH Q2(R1) guidelines. The F-AAS method was benchmarked against the pharmacopoeial complexometric titration procedure. The applicability of the F-AAS method was confirmed through the analysis of commercial pharmaceutical products available on the Vietnamese market.

2. MATERIALS AND METHODS

2.1. Research materials

A commercial calcium-magnesium oral solution (100 mL, containing calcium glycerophosphate and magnesium gluconate) and its corresponding placebo were provided by MEBIPHAR (Vietnam). The sample matrix consists of a complex mixture of pharmaceutical excipients, including sorbitol, sucrose, sucralose, sodium methylparaben, and sodium benzoate. All experiments were conducted at Pham Ngoc Thach University of Medicine.

2.2. Chemicals and reagents

High-purity calcium and magnesium stock standards, HNO_3 and EDTA (all AAS grade) were purchased from Merck (Germany). Calcium glycerophosphate and magnesium gluconate, were obtained from Givaudan-Lavirotte (France). The metal indicator Calcon was supplied by Shanghai Zhanyun (China). Eriochrome Black T, NaOH, NH_4Cl, NH_3, NaCl, and Na_2SO_4, were sourced from Xilong (China).

2.3. Instrumentation

Multiwave PRO sample digestion machine (Anton Paar, Austria), PinAAcle 900T atomic absorption spectrometer (PerkinElmer, USA), Stuart UC152 heated magnetic stirrer (Stuart, UK), WaterPro PS ultra-pure water filtration (Labconco, USA).

The AAS system was equipped with single-element hollow cathode lamps (HCL). The instrument was operated in flame mode with an air-acetylene mixture, maintaining a slit width of 0.7 nm for both analytes. Specific operating wavelengths were set at 422.67 nm for calcium and 285.21 nm for magnesium. A deuterium (D2) lamp was employed for background correction to eliminate potential interferences

from the complex pharmaceutical matrix.

2.4. Sample preparation for the F-AAS method

Calibration standards were prepared from 1000 mg/L Mg and Ca stock solutions by dilution with HNO_3 to appropriate concentrations.

The concentration of HNO_3 was diluted based on the test concentration (1 - 3M).

Test samples (0.5 mL) were digested with approximately 30 mL of HNO_3 under controlled temperature and time conditions. After digestion, the solution was quantitatively transferred to a 50 mL volumetric flask and diluted to volume with dilute HNO_3 to obtain Solution A. An aliquot of Solution A (1.0 mL) was further diluted to 50 mL with HNO_3 before analysis.

F-AAS was used to quantify the concentrations of magnesium and calcium in the test samples. The metal content was determined by comparing the absorbance of the sample to be analyzed with standard solutions of known concentrations, with each solution being measured at least twice. Metal concentrations were calculated using the equation:

$$X_{(mg/mL)} = \frac{C}{1000} \times D$$

Where:

C: concentration of the metal in the test sample derived from the calibration (µg/mL)

D: dilution factor of the test sample

2.5. Optimization of the sample preparation procedure

Wet sample mineralization was optimized using the BBD model in Design-Expert software, a RSM technique. BBD reduces experiments compared to CCD while accurately evaluating factor interactions. Based on references and liquid matrix properties, three independent variables (X) and their ranges were selected: HNO_3 concentration (X_1: 1–3 M), digestion time (X_2: 10–30 min), and digestion temperature (X_3: 50–70°C). The dependent variables (Y) were the recovery percentages of Mg (Y_1) and Ca (Y_2).

2.6. Validation of the F-AAS Method

The optimized F-AAS procedure was validated in accordance with the ICH Q2(R1) [11] and the Ministry of Health Handbook for Drug Registration [12]. The validation protocol encompassed the evaluation of specificity, linearity, precision, and accuracy.

2.7. Determination of magnesium and calcium by complexometric titration

To benchmark the F-AAS method, Ca and Mg were quantified using complexometric titration as described in the United States Pharmacopeia (USP 46), the British Pharmacopoeia (BP 2022), and the Vietnamese Pharmacopoeia V [13-15].

Calcium determination: complexometric titration with 0.1 M disodium edetate at high pH (> 12) using Calcon indicator. At this pH, Mg precipitates as $Mg(OH)_2$ and theoretically does not interfere. The endpoint is a color change from wine-red to blue.

Magnesium determination: complexometric titration with 0.1 M disodium edetate at pH 10 (ammonia buffer) using Eriochrome Black T indicator. This complexometric titration determines the total concentration of divalent cations (Ca + Mg). The magnesium content is derived by subtracting the calcium content (determined from the first complexometric titration) from the total content.

Following the Ministry of Health Drug Registration Guidelines, the method was evaluated for specificity, repeatability, and accuracy.

To compare the results with the F-AAS method, the same pharmaceutical samples provided by MEBIPHAR were quantified using the validated complexometric titration procedure. The resulting data were then cross-referenced with the AAS measurements

for comparison.

2.8. Application to Market Samples

The validated F-AAS procedure was applied to analyze two commercial oral solution batches (labeled A and B) available in the Vietnamese market.

3. RESULTS

3.1. Optimization of the sample preparation procedure

The BBD generated a dataset relating the digestion variables (X_1, X_2, X_3) to the recovery of magnesium (Y_1) and calcium (Y_2). The Design-Expert software fitted the experimental data to quadratic polynomial models. The analysis of variance (ANOVA) indicated that the models for both Mg and Ca were statistically significant ($p < 0.05$).

3D response surfaces **(Figure 1 and Figure 2)** showed a strong interaction between temperature and acid concentration. Recovery increased with temperature, plateauing at the average level, which is typical for liquid organic-containing samples (gluconate/glycerophosphate) easier to decompose than solid ores. Acid concentration and time had lesser positive effects.

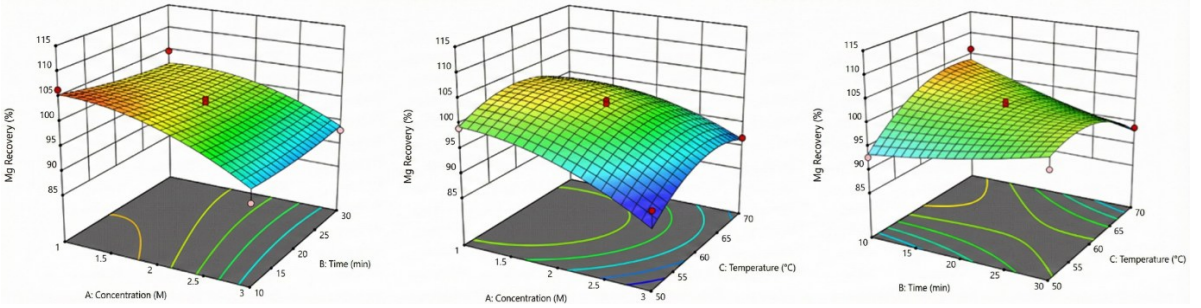

Figure 1. 3D graph illustrating the influence of independent variables on the magnesium recovery

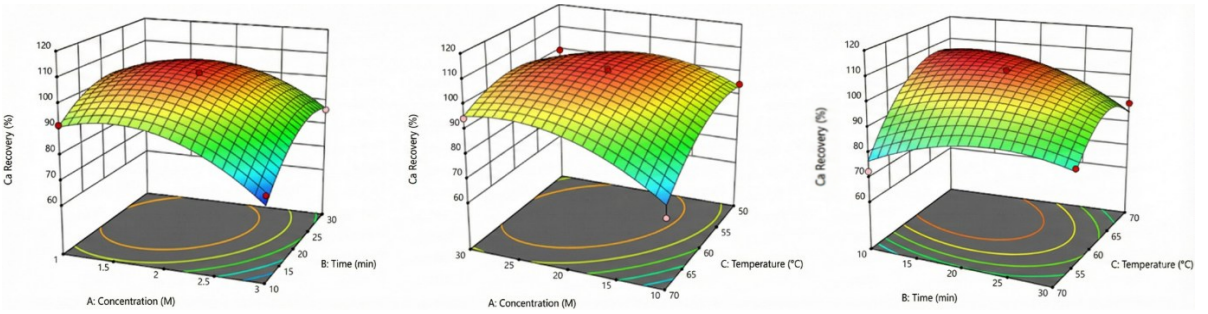

Figure 2. 3D plot showing the effect of independent variables on calcium recovery

Numerical optimization using the desirability function in Design-Expert predicted the global optimum for the simultaneous recovery of both metals at an HNO_3 concentration of 1.7 M, a digestion time of 11 minutes, and a digestion temperature of 54°C.

To validate the predicted optimum, six independent replicate digestions were performed under the conditions of 1.7 M HNO_3, 11 mins, and 54°C. The results are summarized in **Table 1**.

Table 1. Verification results of optimized conditions (n=6)

Analyte	Mean recovery (%)	RSD (%)	95% Prediction interval	Conclusion
Mg	98.84	0.63	95.07–104.93	Valid
Ca	100.61	1.13	92.42–107.58	Valid

The experimental mean recoveries (98.84% for Mg and 100.61% for Ca) fell well within the 95% prediction intervals generated by the model. The low RSD values (< 1.2%) indicate that the optimized sample preparation method is highly reproducible and robust.

3.2. Method validation results (F-AAS)

The analytical procedure was validated for specificity, linearity, precision, and accuracy in accordance with ICH Q2(R1) Guidelines [TLTK] and Vietnamese Ministry of Health Regulatory Standards [TLTK].

3.2.1. Specificity

The specificity analysis showed that the blank solution (dilute HNO_3) and the placebo matrix (excipients) produced negligible absorbance values (< 0.002 Abs) at the specific resonance lines of Mg (285.21 nm) and Ca (422.67 nm). The test samples and standard solutions yielded distinct, stable absorbance peaks. This confirms that the excipients in the oral solution do not spectrally interfere with the analysis after digestion.

3.2.2. Linearity

Calibration curves were constructed using five concentration levels for each element. The regression analysis demonstrated excellent linearity.

Table 2. Linearity parameters

Analyte	Linear range (µg/mL)	Regression equation	Correlation coefficient *(r)*	*p*-value (slope)
Mg	0.05–0.9	$\hat{y} = 0.7259x$	0.9985	< 0.05
Ca	0.5–4	$\hat{y} = 0.0296x + 0.0066$	0.9990	< 0.05

The correlation coefficients (r > 0.998) comply of the ICH guidelines and the Vietnamese Pharmacopoeia V.

Table 3. Linearity Results for Magnesium and Calcium

Metal	Concentration (µg/ml)	Mean Absorbance
Mg	0.05	0.0480
	0.1	0.0915
	0.3	0.2568
	0.6	0.4739
	0.9	0.6630
Regression equation: $\hat{y} = 0.7259x$ Correlation coefficient: r = 0.9985		

Metal	Concentration (µg/ml)	Mean Absorbance
Ca	0.5	0.0199
	1	0.0374
	2	0.0652
	3	0.0980
	4	0.1230
Regression equation: $\hat{y} = 0.0296x + 0.0066$ Correlation coefficient: r = 0.9985		

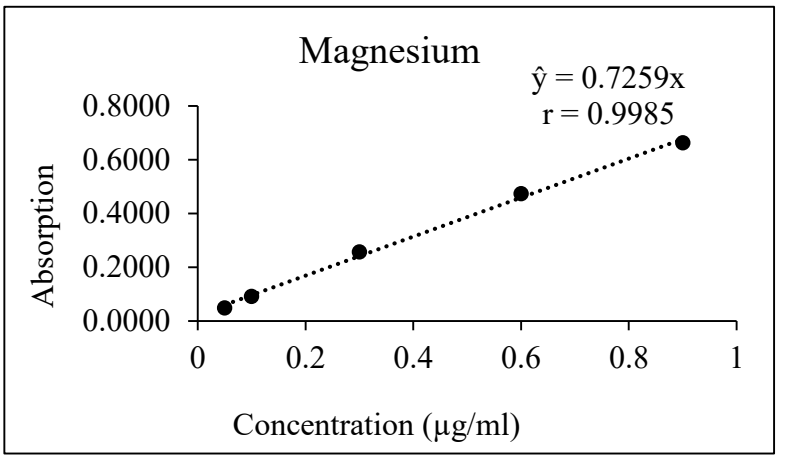

Figure 3. Calibration curve for Magnesium

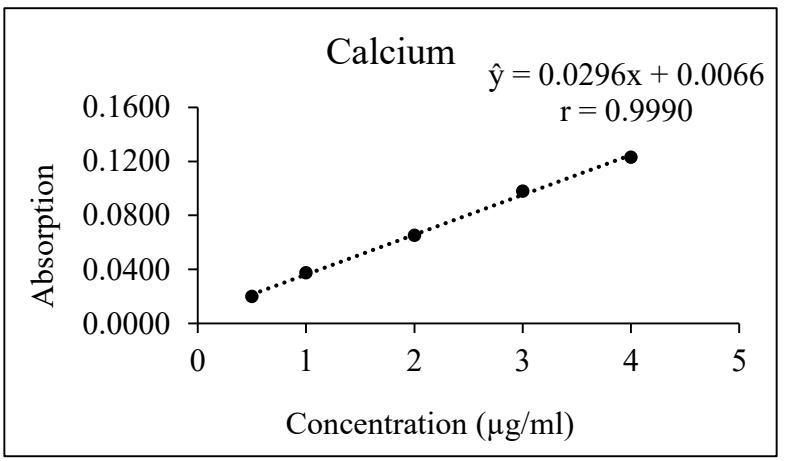

Figure 4. Calibration curve for Calcium

3.2.3. Precision

Precision was evaluated at two levels: repeatability (same day, same analyst) and intermediate precision (different days, different analysts).

Table 4. Precision data

Analyte	Parameter	Mean recovery (%)	RSD (%)	Acceptance criteria
Mg	Repeatability	98.73	0.38	≤ 2.0%
	Intermediate Precision	98.94	0.48	≤ 2.0%
Ca	Repeatability	100.35	1.71	≤ 2.0%
	Intermediate Precision	100.67	1.44	≤ 2.0%

All RSD values were significantly below the 2% threshold commonly applied to drug assay methods. The intermediate precision results demonstrate the method's reliability over time and across different operating conditions.

3.2.4. Accuracy

Accuracy was determined by the standard addition method at three levels (80, 100, and 120%).

Table 5. Accuracy (recovery studies)

Analyte	Spike level	Mean recovery (%)	RSD (%)	AOAC limit
Mg	80%	103.76	2.25	90–110%
	100%	100.38	1.70	
	120%	99.64	1.13	
Ca	80%	106.22	1.51	90–110%
	100%	101.46	2.22	
	120%	97.04	0.97	

The recovery rates for both metals spanned from approximately 97% to 106%, falling within the rigorous 90–110% range stipulated by AOAC for pharmaceutical preparations. This confirms the method is free from significant systematic errors or bias.

3.3. Comparison with complexometric titration

The proposed F-AAS method was compared with the pharmacopoeial complexometric titration method using the same batch of samples. The comparative results between the F-AAS and complexometric titration methods are presented in **Table 5.**

Table 6. Method comparison (F-AAS and complexometric titration)

Analyte	Method	Mean content (%)	Precision (%RSD)	Comparison
Mg	F-AAS	98.84	0.63	F-AAS is more precise
	Complexon	97.24	1.00	
Ca	F-AAS	100.61	1.13	F-AAS is more precise
	Complexon	104.12	1.17	

The F-AAS method demonstrated superior precision for both analytes, with RSD values (0.63% for Mg and 1.13% for Ca) being lower or comparable to those of complexometric titration (1.00% and 1.17%, respectively). Statistical analysis (F-test and t-test) confirmed that the F-AAS method provides more reliable and consistent results by eliminating the inherent visual errors and endpoint subjectivity associated with traditional titration. This is particularly significant for magnesium, where complexometric determination is often affected by the propagation of errors from indirect calculation.

3.4. Application to market samples

Two different commercial batches of calcium-magnesium oral solution (A and B) were analyzed using the validated F-AAS method.

Table 7. Quantification of commercial samples

Analyte	Sample Code	Label claim (%)	RSD (%)
Ca	A	97.48	0.95
	B	100.73	0.92
Mg	A	103.94	0.44
	B	105.24	0.28

Two different commercial batches of calcium-magnesium oral solution (A and B) were analyzed using the validated F-AAS method. All results complied with the standard pharmacopoeial limit of 90–110% of the labeled amount. The magnesium content was found to be 103.94% in Sample A and 105.24% in Sample B. To verify the significance of this difference, an independent t-test was performed, yielding a p-value < 0.05. This statistical evidence confirms that the magnesium content in Sample B is significantly higher than in Sample A.

This discrepancy likely stems from variations in the manufacturing process or differences in the excipient matrix, such as sweeteners or thickening agents, which affect the viscosity and surface tension of the formulation. Such physical properties can influence the aspiration rate and nebulization efficiency within the F-AAS system, highlighting the necessity of considering matrix effects when analyzing complex liquid dosage forms.

4. DISCUSSION

The research findings effectively demonstrate the superior performance of the F-AAS method compared to traditional complexometric titration for the simultaneous quantification of Magnesium and Calcium, particularly in addressing the limitations of selectivity within complex multi-component pharmaceutical matrices. Although titration remains a common technique due to its instrumental simplicity and cost-effectiveness, it faces significant scientific hurdles because magnesium determination often relies on indirect calculation—subtracting the calcium content from the total divalent cation volume. This process inherently leads to the propagation of analytical errors, compromising the overall reliability of the results. In contrast, F-AAS allows for the direct and independent quantification of each element at specific resonance lines (285.21 nm for Mg and 422.67 nm for Ca), thereby eliminating mutual interference and subjective visual errors associated with endpoint detection, which are frequently obscured in colored or highly viscous formulations. The transition to F-AAS aligns with "Green Chemistry" principles by eliminating the need for toxic masking agents such as cyanide or triethanolamine. The enhanced precision, evidenced by lower RSD values and the stability of an objective instrumental measurement system, confirms that F-AAS is the optimal alternative for meeting modern, stringent pharmaceutical quality control standards.

5. CONCLUSION

The research successfully developed and validated a robust F-AAS procedure for the simultaneous quantification of Magnesium and Calcium in pharmaceutical oral solutions. By optimizing the wet acid digestion protocol through Design of Experiments (DoE), the method ensures maximum recovery while significantly minimizing resource consumption and analysis time. The validated procedure fulfilled all regulatory requirements of the ICH Q2(R1) guidelines and the Vietnamese Ministry of Health, demonstrating excellent specificity, linearity, precision, and accuracy.

Furthermore, the F-AAS method exhibited superior analytical reliability and operational safety compared to traditional complexometric titration, effectively eliminating subjective visual errors and the need for toxic reagents. The successful application of this method to commercial samples confirms its readiness for deployment in pharmaceutical quality control laboratories as a precise and efficient alternative for routine analysis.

REFERENCES

1. Lê Thành Phước. Hóa đại cương - vô cơ tập 1. Nhà xuất bản Y học; 2015.
2. Vannucci L, Fossi C, Quattrini S, Guasti L, Pampaloni B, Gronchi G, et al. Calcium intake in bone health: a focus on calcium-rich mineral waters. Nutrients. 2018;10(12):1930.
3. Cormick G, Belizán J.M. Calcium intake and health. Nutrients. 2019; 11(7):1606.
4. Razzaque M.S. Magnesium: are we consuming enough? Nutrients. 2018; 10(12):1863.
5. Schwalfenberg G.K, Genuis S.J. The importance of magnesium in clinical healthcare. Scientifica. 2017.
6. Walker M.D, Shane E. Hypercalcemia: a review. JAMA. 2022;328(16):1624-1636.
7. Abarca A, Canfranc E, Sierra I, Marina M. A validated flame AAS method for determining magnesium in a multivitamin pharmaceutical preparation. Journal of pharmaceutical and biomedical analysis. 2001; 25(5-6): 941-945.
8. Yebra M.C. Flow injection determination of calcium and magnesium in soluble pharmaceutical tablets by flame atomic absorption spectrometry using ultrasound for sample preparation. Atomic Spectroscopy. 2011;32(2):80-84.
9. Sbahi A, Abdelwahed W, Sakur A.A. A New Flame AAS Application for Magnesium Determination in Solid Pharmaceutical Preparations as an Active Ingredient and an Excipient. International Research Journal of Pure and Applied Chemistry. 2020;21(23): 89-95.
10. Patel P.H, Shah J.R, Soni H, Patel V, Patel J. Development and validation of analytical method for estimation of calcium in herbomineral formulation by atomic absorption spectrophotometry. Journal of Natural Remedies. 2022; 22(4):597-606.
11. ICH Harmonised Tripartite Guideline. Validation of analytical procedures: text and methodology. Q2 (R1). 2005;1(20):05.
12. Bộ Y tế. Sổ tay hướng dẫn đăng ký thuốc. 2013.
13. United States Pharmacopeial Convention. United States Pharmacopeia-National Formulary (USP 46-NF 41).United States Pharmacopeial Convention; 2023.
14. British Pharmacopoeia Commission. British Pharmacopoeia 2022. TSO;2022.
15. Bộ Y tế. Dược điển Việt Nam V. Nhà xuất bản Y học; 2017.