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ORIGINAL ARTICLE

The application of atomic absorption spectrometry for the determination of residual active pharmaceutical ingredients in cleaning validation samples

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Abstract

The objective of this work was the development and validation of atomic absorption spectrometric (AAS) methods for the determination of residual active pharmaceutical ingredients (API) in rinse samples for cleaning validation. AAS as an indirect method for the determination of API in rinse samples can be applied when it is in the form of salt with metal ions or when the metal ion is a part of the API's structure. The electrothermal AAS methods (aqueous and ethanol medium) for the determination of magnesium in esomeprazole magnesium and the flame AAS method for the determination of lithium in lithium carbonate in rinse samples were developed. Various combinations of solvents were tested and a combination of 1% aqueous or ethanol solution of nitric acid for esomeprazole magnesium and 0.1% aqueous solution of nitric acid for lithium carbonate were found to be the most suitable. The atomization conditions in the graphite furnace and in the flame were carefully studied to avoid losses of analyte and to achieve suitable sensitivity. The cleaning verification methods were validated with respect to accuracy, precision, linearity, limit of detection, and quantification. In all the cases, the limits of detection were at the microgram level. The methods were successfully applied for the determination of esomeprazole magnesium and lithium carbonate in rinse samples from cleaning procedures.

Key words: Electrothermal atomic absorption spectrometry, esomeprazole magnesium, flame atomic absorption spectrometry, lithium carbonate, recovery, validation

Introduction

The production of active pharmaceutical ingredients (API) requires well-defined and validated cleaning procedures to ensure that the contamination from one product to another is reduced to safe and acceptable levels. The current available methods for setting cleaning validation acceptance limits are based on different criteria[1,2]. The calculation can be done according to toxicological or therapeutic data if available. The fraction of API allowed to carry over is referred to as the maximum allowable carryover and is based on the acceptable daily intake of the API being cleaned (toxicological criterion) or on the principle that an API should not be present in a subsequently produced product at levels higher than 1/1000 (oral preparations) of the minimum daily dose of the API in a maximum daily dose of the subsequent product (pharmacological criterion). Both calculations take into consideration the batch size of the subsequent product and the safety factor that depends on the formulation type. For oral preparations, safety factors from 100 to 1000 are recommended, for parenteral preparations they range from 1000 to 10,000. If data are not available for either of these calculations, the more stringent 10 ppm criterion should mostly be used (not more than 10 ppm of any API should appear in any other product). The Food and Drug Administration does not set acceptance specification or methods for their determination. The US Food and Drug Administration's guidance for determining

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residue limits states that residue limits should be logical, practical, achievable, and verifiable[3].

The analytical methods applied in cleaning validation studies should be based on previously established limits. There are many analytical techniques available that can be used in cleaning validation. These choices include specific and nonspecific methods. Currently, high-performance liquid chromatography (HPLC)[4,5] and ultraviolet–visible spectrophotometric procedures[6] are two of the most common methods used in cleaning validation. The HPLC methods are time-consuming but more specific and sensitive. The ultraviolet-visible spectrophotometric methods are less specific but faster. However, both methods may have difficulty reaching a limit of quantification (LOQ) of 1–10 ppm[7]. Other specific but less used methods are capillary electrophoresis[8,9], gas chromatography[10,11], and ion chromatography[12]. Some examples of nonspecific methods are total organic carbon (TOC)[13,14], pH, titration, and conductivity[15]. Atomic absorption spectrometry (AAS)[16] and inductively coupled plasma atomic emission spectrometry are used mainly for inorganic contaminants down to extremely low levels.

In the United States (USP) and European (EP) Pharmacopoeia there is a HPLC method for the assay determination of esomeprazole magnesium and a titrimetric method for the assay determination of lithium carbonate presented. The main disadvantage of both methods when used for cleaning validation samples is the relatively high limit of detection (LOD) and quantification regarding AAS. When HPLC or titrimetric methods for the determination of API in rinse samples are applied, it must be established that the active substance has not changed its chemical nature during the cleaning process and is still detectable and quantifiable using analytical methods. All mentioned requirements are irrelevant when the AAS is applied instead.

The most commonly used atomic spectrometric technique for Mg[17] and Li[18] determination is flame atomic absorption spectrometry (FAAS). For lower concentrations, electrothermal atomic absorption spectrometry (ETAAS) is suggested[19,20]. In USP and EP for Mg determination in esomeprazole, magnesium FAAS method is recommended.

The goal of this work was to demonstrate that AAS is an appropriate indirect method for the determination of API in samples (rinse water or organic solvents) for cleaning validation. The method can be applied if the API is in the form of salt with metal ions (such as Ca, Mg, K, and Na) or when the metal ion is a part of its structure (lithium carbonate, silver sulfadiazine, cisplatin, auranofin). In the first case, the concentrations of metal ions are much lower and range mostly between 1% and 10%. As the concentration of Mg in esomeprazole magnesium is about 5× lower than the concentration of Li in lithium carbonate, the ETAAS methods (for aqueous and ethanol medium) for Mg and the FAAS method for lithium determination were developed. It was shown that ETAAS can be applied for either a water medium or organic solvents (ethanol medium), which can be an advantage when a low solubility of API in an aqueous medium is expected.

Experimental

Reagents and samples

The Mg and Li working standard solutions were prepared from a standard stock solution (1000 mg/L Merck, Darmstadt, Germany). For Mg determination, the standards were prepared over a range of 5–40 μg/L in a 1% aqueous and ethanol solution of nitric acid. For Li determination, the standards were prepared over a range of 60–1000 μg/L in a 0.1% aqueous solution of nitric acid.

For the preparation of standards and samples, suprapur nitric acid of 65% (Merck), suprapur hydrochloric acid of 30% (Merck), and ethanol absolute (Merck) were used.

The potassium and cesium modifier solutions [$c(K^+)$ = 5.0 g/L and $c(Cs^+)$ = 5.0 g/L] were prepared by dissolving 9.5 g of potassium and 6.3 g of cesium chloride (Merck)—suprapur grade in 1000 mL of Milli-Q water.

High-purity deionized water from a Milli-Q system (Millipore, Bedford, MA, USA) was used throughout.

For method validation, the samples of esomeprazole magnesium and lithium carbonate obtained from LEK pharmaceuticals were applied. The amount of Mg in the esomeprazole magnesium was determined by the reference FAAS method described in USP and was 3.11%. The amount of Li in the lithium carbonate was calculated on the basis of the theoretical content of Li in the lithium carbonate and was 18.79%. For dissolving samples of esomeprazole magnesium, a 1% aqueous and ethanol solution of nitric acid and for lithium carbonate a 0.1% aqueous solution of nitric acid were used. Different amounts of samples of esomeprazole magnesium (30, 60, 100, 140, and 190 mg) and lithium carbonate (20, 65, 110, 155, and 200 mg) were dissolved in 100 mL of the media as described above. The samples were further diluted so that the dissolution factor for the esomeprazole magnesium samples was 1667 and for lithium carbonate 400. To the final diluted lithium carbonate samples, 200 mg/L of K^+ was added.

Twenty milliliter of the rinse water or ethanol sample was transferred to a 25-mL flask; 65% nitric acid was added and diluted to volume so that the final concentration of nitric acid was 1% for Mg and 0.1% for Li determination. To the lithium carbonate samples, 200 mg/L of K^+ was added. The blank was prepared in the same medium as the sample.

Apparatus

Atomic absorption measurements were carried out using a Varian SpectrAA 280 Z atomic absorption spectrometer equipped with a GTA graphite furnace, PSD 120 autosampler, Zeeman background correction, and an Mg

Table 1. Graphite furnace temperature program for the determination of Mg in aqueous and ethanol medium.

	Aqueous medium			Ethanol medium			
Step	Temperature (°C)	Ramp (s)	Hold (s)	Temperature (°C)	Ramp (s)	Hold (s)	Air flow Rate (L/min)
1	40–85	5	—	75	—	10	0.3
2	85–95	40	—	75–80	40	—	0.3
3	95–120	30	—	80–100	20	—	0.3
4	120–900	15	30	100–1000	20	30	0.3
5	900	—	2	1000	—	2	0
6	900–2100	0.6	2	1000–2100	0.5	2	0
7	2100–2700	3	—	2100–2700	3	—	0.3

hollow cathode lamp as a radiation source with a current of 4.0 mA. The main analytical line at 202.6 nm was used for all determinations with a spectral bandwidth of 1.0 nm. Argon was used as the inert transport gas for all analyses. The peak height absorbance was used exclusively for signal evaluation and qualification. Measurements were performed by using pyrolytical-coated graphite tubes and the sample volume was 10 µL. The optimized graphite furnace temperature program is given in Table 1. Measurements with flame atomization were carried out using a Varian SpectrAA 280 FS equipped with lithium and magnesium hollow cathode lamps with a current of 4.0 mA for Mg and 5.0 mA for Li determination. The main analytical line at 285.2 nm with a spectral bandwidth of 0.5 was used for Mg determination and the line at 670.8 with a spectral bandwidth of 1.0 nm for Li determination. For Mg determination, a deuterium background corrector was applied. For all measurements an air-acetylene flame was used. The calibration graph method was used for all determinations.

Establishing cleaning limits

We used an approach where the 10 ppm criterion is compared with a criterion based on the pharmacological activity of the substances. The lower of the two values was established as the residue acceptance limit.

The calculation formula based on the pharmacological criterion is

$$MACO = \frac{TD \cdot BS}{LDD \cdot SF},$$

MACO is the maximum allowable carryover in milligrams, *TD* the minimum daily dose of previous product, *SF* the safety factor (1000 for oral preparation), *BS* the smallest batch size of the subsequent product, and *LDD* the maximum daily dose of the following product. The calculated *MACO* for esomeprazole magnesium was 4000 mg (the following product was amlodipine besylate; *TD* was 20 mg, *LDD* 10 mg, and *BS* 2 kg) and for lithium carbonate 75,000 mg (the following product was lovastatin; *TD* was 150 mg, *LDD* 80 mg, and *BS* 40 kg). Both values are greater than the values obtained from the 10 ppm criterion (for esomeprazole magnesium it was 20 mg and for lithium carbonate 400 mg); therefore, the more stringent 10 ppm criterion was used in further studies. The acceptance limit for the residue in the rinse medium (LA) was calculated according to the following equation:

$$LA = \frac{MACO}{V_r},$$

where V_r is the volume of rinse. The *LA* value for both substances was 1333 µg/L.

The corresponding acceptance limits for residues expressed in micrograms per square centimeter of equipment were 0.400 for esomeprazole magnesium and 1.333 for lithium carbonate.

The calculated acceptance limits for both APIs in the rinse samples were far below the quantification limits of the pharmacopoeial HPLC method for esomeprazole magnesium determination (the LOQ for the HPLC method was 2.3 mg/L) and the titrimetric method for lithium carbonate determination (the LOQ was 200 mg/L). Therefore, the pharmacopoeial methods in our case cannot be applied. We expected that the limits of quantification for the new AAS methods would be lower than 50% of the acceptance concentration which was 667 µg/L for both substances.

Method validation

The methods were validated according to the International Conference on Harmonization guidelines for the validation of analytical procedures[21].

Limit of detection and quantification

The LOD and LOQ were estimated by analyzing 10 replicates of the calibration blanks as the samples. The LOD was calculated on the basis of 3× the standard deviation (SD) of the blank divided by the slope of the calibration curve and the LOQ as 10× the SD of the blank[22].

Linearity

The linearity of the method was evaluated by analyzing a series of Li and Mg standards over the selected range. The linear relationship between the signal and the concentration was confirmed by determining the concentration of analyte in different amounts of samples at five

concentration levels. Measurements at all concentration levels were carried out in triplicate.

The linearity was evaluated using linear regression analysis, which was calculated by the least square regression method.

Accuracy

The accuracy of the methods was evaluated by measuring the absorbance of Mg and Li in samples of esomeprazole magnesium and lithium carbonate with known amounts of analyte. The measurements were performed in triplicate at five concentration levels over the entire concentration range. The accuracy of the methods was expressed in a percentage form as the recovery between the determined value of analyte and the true value.

Additionally, the recovery studies of rinse samples were performed. The stainless steel plates (5×5 cm^2) were spiked with known quantities of samples of esomeprazole magnesium and lithium carbonate in the range of 50%, 100%, and 150% of the MACO concentration (the MACO concentration was 0.400 μg/cm^2 for the esomeprazole magnesium and 1.333 μg/cm^2 for the lithium carbonate). After drying and rinsing the plates with water (water is used as a last rinse medium for equipment cleaning), the accuracy of the procedure was determined by comparing the analyte amount in the rinse samples versus the known amount of the spiked analyte on stainless steel plates at three concentration levels with five replicates for each concentration level investigated. Parallel blank samples were prepared. The rinse volume was calculated on the basis of the ratio between the surface area and the volume that was used in the actual equipment. Some authors[23] recommended a minimum of 50% recovery. According to our internal prescriptions, recoveries over 70% are acceptable. When the average recovery is less than 70%, this usually means that the cleaning procedure should be improved. The correction factor based on the lowest recovery value (worst case) was applied for the calculations made in the sample analysis.

Precision

The precision of the methods was assessed by the repeated determination of Mg and Li in samples of esomeprazole magnesium and lithium carbonate. Precision experiments were carried out at five different concentration levels, in three replicates covering the entire specific range of the procedure. The level of precision was expressed in terms of the SD and the relative SD (RSD).

The precision of the rinsing procedure was examined by the RSD of the recovery data at three concentration points with five replicates for each point. A precision of less than 12% was acceptable[23].

Results and discussion

Esomeprazole magnesium is the S isomer of omeprazole, which is a mixture of S and R isomers. It is in the form of magnesium salt, with an Mg concentration of between 3% and 4%. The content of lithium in lithium carbonate amounts to 18.79%. As both substances are soluble in water or in diluted mineral acids and esomeprazole magnesium also in ethanol, for the cleaning procedure, an aqueous or ethanol medium can be applied. According to the solubility of esomeprazole magnesium in ethanol and water, two ETAAS methods for Mg were developed: one in an aqueous and another in an ethanol medium. Hence, the basic investigations were concerned with the choice of the best dissolution medium for Mg and Li determination in the rinse samples, the choice of the best modifier for the FAAS determination of Li, and the optimization of atomization conditions in the graphite furnace and in the flame.

For AAS determination of metals, a nitric acid (ETAAS) or hydrochloric acid (FAAS) water medium is recommended. The effect of the concentration of nitric acid on the Mg signal and hydrochloric and nitric acid on the Li signal was investigated. The measurements were performed for different concentrations of nitric acid and hydrochloric acid in the water or ethanol solutions of the samples and standards (the concentration of acids ranged between 0% and 2%). Compared to pure aqueous or ethanol solutions the signal increased considerably when a 1% water or ethanol solution of nitric acid was used for Mg ETAAS determination. Increasing the nitric acid concentration over 1%, the signal was decreased (Figures 1 and 2). The reason for better sensitivity when nitric acid ethanol or water medium is used is the better oxidation of the organic matrix in the graphite tube or the better solubility of samples when nitric acid is added to the water or ethanol solution. For Li FAAS determination, the signal was measured in different concentrations of nitric and hydrochloric acid aqueous solutions of the samples and

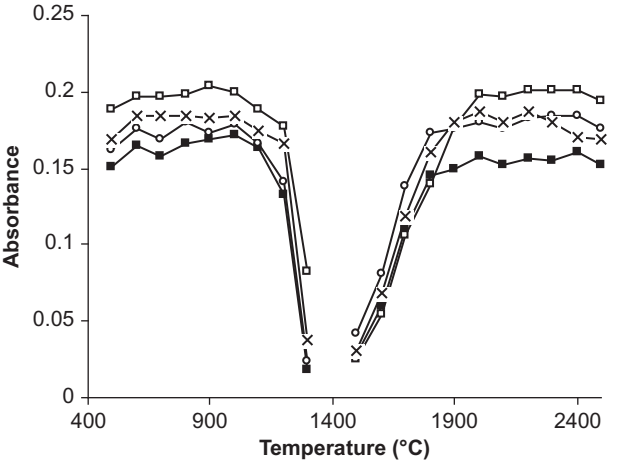

Figure 1. Pyrolisis and atomization curves for samples of esomeprazole magnesium diluted in an aqueous medium (–•–sample diluted in water, –○–sample in 0.1% HNO_3, –▫–sample in 1% HNO_3, –×–sample in 2% HNO_3). The temperature of the pyrolisis was 900°C and atomization 2100°C.

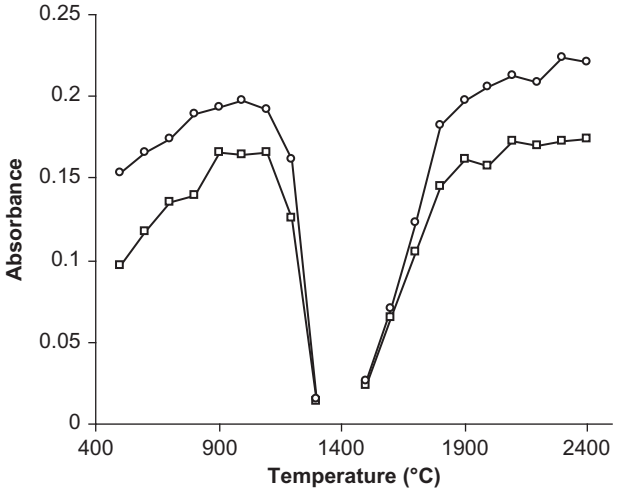

Figure 2. Pyrolisis and atomization curves for samples of esomeprazole magnesium diluted in an ethanol medium (—∘—sample diluted in ethanol, —∘—sample diluted in 1% HNO_3). The temperature of the pyrolisis was 1000°C and atomization 2100°C.

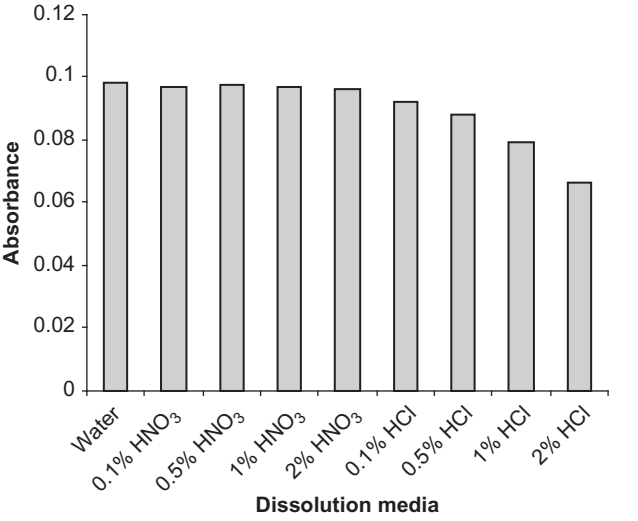

Figure 4. Effect of different concentrations of HCl and HNO_3 on absorbance of Li in lithium carbonate samples (50 mg of sample/100 mL, dilution factor 400).

standards. From Figures 3 and 4 it is evident that compared with hydrochloric acid solutions, the signal in nitric acid increased significantly. Increasing the nitric acid concentration over 0.1%, the signal did not change considerably. Based on experimental data for Mg ETAAS determination, a 1% water or ethanol solution of nitric acid and for Li FAAS determination a 0.1% water solution of nitric acid were found to be the most suitable solvents.

As the temperature of the acetylene–air flame caused appreciable ionization of Li, it has been suggested[24] that the addition of an excess of ionization suppressant should be used. Those most commonly used are cations having a lower ionization potential than that of the analyte. Two different suppressants in different concentrations were tested: potassium and cesium chloride. In spite of the recommendations in the literature (the addition of 2000 mg/L of K^+ is suggested)[25], the addition of 200 mg/L was found to be the most suitable. It is suspected that the decrease in sensitivity as the K^+ concentration increased was because of changes in transport efficacy, namely the viscosity and the surface tension increased with higher salt concentrations that resulted in a worse aerosol formation in the nebulizer.

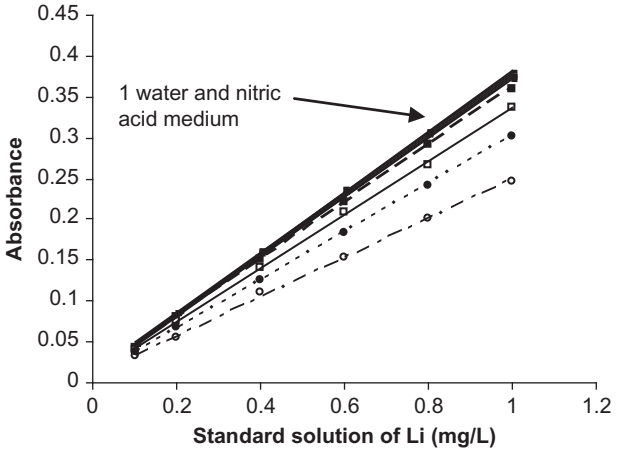

Figure 3. Effect of different concentrations of HCl and HNO_3 on the absorbance of Li standard solutions; 1 standard curve in aqueous and HNO_3 medium (0.1%, 0.5%, 1%, and 2% HNO_3; —•—0.1% HCl; —∘—0.5% HCl; —•—1% HCl; —∘—2% HCl).

For the resulting matrix solutions, pyrolysis and atomization temperatures were optimized using a standard solution of magnesium (10 μg/L) prepared in the same medium as the sample and the samples (50 mg of esomeprazole magnesium diluted in 100 mL of a 1% aqueous or ethanol solution of nitric acid; the dilution factor was 1000). Temperatures over the range of 500–1300°C were tested at a constant atomization temperature of 2100°C. As shown in Figures 5 and 6, no significant change in magnesium absorption was observed within the temperature range from 500°C to 1000°C for the standard solution of magnesium as well as for samples of either in ethanol or in aqueous solutions of nitric acid. In aqueous solutions of nitric acid, pyrolysis temperatures above 1000°C resulted in losses of Mg. In ethanol solutions, the losses of Mg were detected at temperatures above 1100°C. Based on these considerations, the pyrolysis temperature was fixed at 900°C for aqueous solutions and at 1000°C for ethanol solutions of 1% nitric acid to ensure maximum matrix removal without signal loss.

The atomization temperature of magnesium was studied over the range of 1500–2500°C at a constant pyrolysis temperature of 900°C and 1000°C for aqueous and for ethanol solutions of 1% nitric acid, respectively. Within this range, the atomic absorption signal increased with the increasing atomization temperature

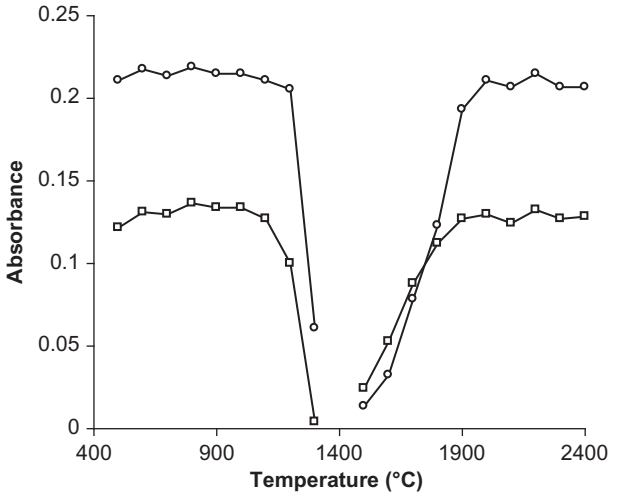

Figure 5. Pyrolisis and atomization curves obtained with a standard solution of Mg (10 µg/L) in a 1% aqueous solution of HNO_3 (—○—) and sample in a 1% aqueous solution of HNO_3 (—□—).

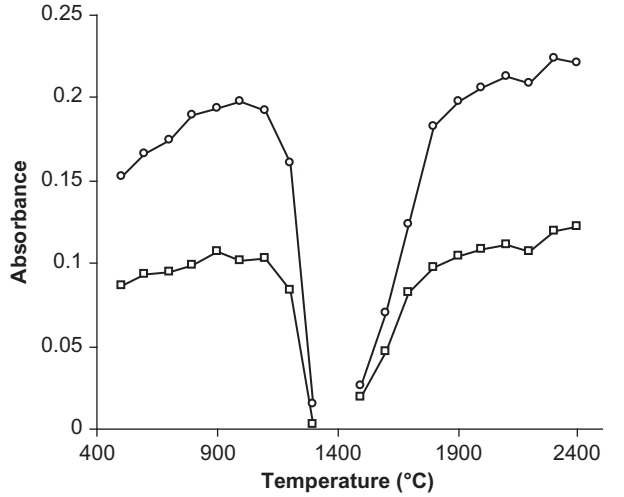

Figure 6. Pyrolisis and atomization curves obtained with a standard solution of Mg (10 µg/L) in a 1% ethanol solution of HNO_3 (—○—) and sample in a 1% ethanol solution of HNO_3 (—□—).

and reached a plateau at 2000°C (Figures 5 and 6). Although the sensitivity could be slightly improved with a higher atomization temperature, it was set at 2100°C to prolong the life span of the graphite furnace tubes. The graphite tube was cleaned at 2700°C with the argon protection gas turned on after the atomization step. The lower surface tension of ethanol tends to cause the injection solution to spread over a much larger surface area of the tube, which results in a poorer precision relative to the aqueous solution of nitric acid. To improve the precision, a 'hot injection' was used. We preheated the tube to a temperature just below the boiling point of the solvent (75°C) and then we injected the solution at a slower rate. In this way, the sample dried as it touched the warm surface of the tube and the creepage of the sample was prevented.

By using peak height instead of peak area measurements, the sensitivity improved considerably.

Methods validation

Limit of detection and quantification

The LOD for Mg ETAAS determination was 1.2 µg/L for the aqueous and 1.3 µg/L for the ethanol 1% nitric acid solution. The LOD for Li FAAS determination was 17.8 µg/L. LOQs of 4.1 and 4.4 µg/L were obtained for Mg in the aqueous and the ethanol solution, respectively. For Li, the LOQ was 59.3 µg/L. Taking into account the assay of Mg in esomeprazole magnesium, which is 3.20% on average, the corresponding LOQ for esomeprazole magnesium amounted to 128.1 and 137.5 µg/L for the aqueous and ethanol solution, respectively. The corresponding LOQ for lithium carbonate (the assay of Li in lithium carbonate is 18.79%) was 315.6 µg/L. The resulting limits of quantification are low enough to enable the calculation of residuals in the rinse samples in the cases when the batch size of the subsequent product is low (production for research and development purposes) or the high-dosing products are following (in both cases the low values of MACO are calculated).

Linearity of the methods

The linearity of the methods was studied by measuring a series of Mg and Li standards prepared at concentrations of 5, 10, 20, 30, and 40 µg/L for Mg and 60, 200, 400, 600, 800, and 1000 µg/L for Li and ensuring that the correlation coefficient was better than 0.99. The linearity of the range was confirmed by analyzing the samples of esomeprazole magnesium and lithium carbonate at five concentrations levels prepared according to the instructions in the 'Experimental' section. The linear relationship between the absorbance and the concentration of standard solutions of Mg was observed in the range from 5 to 40 µg/L with correlation coefficients 0.9962 and 0.9981 for the aqueous and ethanol medium, respectively. For the standard solution of Li, the linear relationship between the absorbance and the concentrations ranged from 60 to 1000 µg/L with a correlation coefficient of 0.9998. In the same ranges as for the standard solutions, the linear relationship between the absorbance and the analyte concentration for esomeprazole magnesium and lithium carbonate samples was confirmed. The correlation coefficient for the samples of esomeprazole magnesium was 0.9992 and 0.9971 for the aqueous and ethanol medium, respectively. For samples of lithium carbonate the correlation coefficient was 0.9999.

Accuracy

Suitable amounts of esomeprazole magnesium and lithium carbonate with a known content of analyte were weighed and prepared as described in the 'Experimental' section. The absorbance of Mg and Li was measured at five concentration levels. Table 2 summarizes the recovery results for various samples. The recoveries based on the

Table 2. Accuracy of the methods.

	Mg determination (aqueous medium)		Mg determination (ethanol medium)		Li determination (aqueous medium)		
Sample weigh (mg)	Mg found ± SD (μg/g)	Recovery (%)	Mg found ± SD (μg/g)	Recovery (%)	Sample weigh (mg)	Li found ± SD (μg/g)	Recovery (%)
30	3.06 ± 0.08	98.4	3.06 ± 0.01	98.4	20	18.99 ± 0.06	101.1
60	3.07 ± 0.16	98.7	3.12 ± 0.05	100.3	65	19.12 ± 0.10	101.8
100	3.33 ± 0.13	107.1	3.07 ± 0.06	98.7	110	18.86 ± 0.09	100.4
140	3.16 ± 0.19	101.6	2.89 ± 0.12	92.9	155	18.86 ± 0.12	100.4
190	3.18 ± 0.04	102.3	3.16 ± 0.06	101.6	200	18.59 ± 0.07	98.9

The certified value for Mg in esomeprazole magnesium was 3.11% and for Li in lithium carbonate was 18.79%. The results are the mean of three determinations.

average of triplicate measurements ranged from 92.9% to 107.1% for Mg and from 98.9% to 101.8% for Li determination.

The recovery studies of the rinsing procedure were performed by spiking 0.2 mL of samples of esomeprasole magnesium and lithium carbonate on stainless steel plates at three concentration levels (0.200, 0.400, and 0.600 μg/cm^2 for esomeprazole magnesium and 0.667, 1.333, and 2.000 μg/cm^2 for lithium carbonate). The plates were allowed to dry (~4 hours) at room temperature and then rinsed with 7.5 mL (esomeprazole magnesium determination) and 25 mL (lithium carbonate determination) of water. An adequate volume of the sample was transferred to a volumetric flask and a 65% nitric acid solution was added so that the final acid concentration for the esomeprazole magnesium samples was 1% and for the lithium carbonate samples 0.1%. For Li determination, 200 mg/L K^+ was added. The concentration of Mg was determined by ETAAS and Li by FAAS according to the procedures described in the 'Experimental' section. The obtained results (Table 3) revealed a good recovery of the rinsing procedure with recovery factors of over 80% for esomeprazole magnesium and 90% for lithium carbonate. Higher recoveries of lithium carbonate can be explained with the better solubility of lithium carbonate in water and a larger volume of water used for rinsing the equipment.

Precision

Suitable amounts of esomeprazole magnesium and lithium carbonate were weighed and prepared as described in the 'Experimental' section. The precision experiments were carried out at five different concentrations levels in three replicates each.

The results summarized in Table 4 present a good level of precision with the RSD below 5% for ETAAS determination of Mg and below 1% for FAAS determination of Li.

The results for the precision study (Table 3) provided a good indication regarding the reproducibility of the rinsing procedure with the RSD values below 8%.

The new ETAAS methods for Mg determination in the rinse samples (aqueous and ethanol medium) were compared with the compendial FAAS method for Mg determination in esomeprazole magnesium. With the *F*-test (at a confidence level of 95%) it was established that there

Table 3. Recovery and precision data for rinse samples.

Esomeprazole magnesium ETAAS				Lithium carbonate FAAS			
Added (μg/cm^2)	Found ± SD (μg/cm^2)	Recovery (%)	RSD (%)	Added (μg/cm^2)	Found ± SD (μg/cm^2)	Recovery (%)	RSD (%)
0.200	0.203 ± 0.005	101.5	2.5	0.664	0.633 ± 0.010	95.3	1.6
0.400	0.357 ± 0.028	89.3	7.8	1.334	1.308 ± 0.016	98.1	1.2
0.597	0.575 ± 0.043	96.3	7.5	2.020	2.035 ± 0.021	100.7	1.0

The results are the mean of five determinations.

Table 4. Precision of the methods and statistical evaluation of new ETAAS methods for Mg determination compared with the compendial FAAS method.

Method	Mean (%)	SD (%)	RSD (%)	Welch' *t*-test	*F*-test
Reference FAAS method for Mg determination	3.11	0.043	1.4	—	—
ETAAS Mg determination (aqueous medium)	3.16	0.148	4.7	1.15 (2.12)*	11.91 (2.98)*
ETAAS Mg determination (ethanol medium)	3.06	0.110	3.6	1.73 (2.10)*	6.54 (2.98)*
FAAS Li determination	18.88	0.196	1.0	—	—

The results are the mean of 15 determinations.
*The figures in parenthesis are the theoretical *t* and *F* values ($P = 0.05$).

is significant difference in the precision between both new methods and the compendial method. A higher RSD for the ETAAS methods was expected, which could be attributed to the better sensitivity of the methods and to an error resulting from the dissolution factor that was 1667 for ETAAS and 167 for the FAAS determination of Mg. The values of the means of both ETAAS methods do not differ significantly (Welch' *t*-test at a confidence level of 95%) from the mean result obtained by the compendial FAAS method (Table 4). It can be concluded that the compendial FAAS method could also be used for Mg determination in rinse samples of esomeprazole magnesium. The disadvantage of the method is the higher LOQ (665.6 μg/L of esomeprazole magnesium) in comparison with the ETAAS methods (128.1 and 137.5 μg/L for the aqueous and ethanol medium, respectively) and the fact that for safety reasons the organic solvents cannot be used when FAAS methods are applied.

The pharmacopoeial HPLC method for esomeprazole magnesium determination and the titrimetric method for lithium carbonate determination can also be used for cleaning validation purposes. They are appropriate for higher concentration ranges, for example, when the batch size of the subsequent product is higher or for the equipment used in in-process stages. Because of the low batch size of the subsequent product and the consequently low MACO values this is not possible in our case. The alternative method for esomeprazole magnesium determination could be TOC and for lithium carbonate inductively coupled plasma atomic emission spectrometry. The first is distinguished by the low LOQ (40 μg/L), but is nonspecific and can be used only when water as a rinse medium is applied. The second is much more expensive and not so widely applicable in the pharmaceutical industry. The reported LOD for lithium is 3 μg/L[26].

It can be concluded that the main problem when developing the methods for the determination of residuals in rinse samples is the low LOQ, especially when the batch size of the following batch is low (low BS) and the product that is cleaned is highly active (low TD). In some cases when the solubility of the API in water is worse, the final rinse mediums could be organic solvents, which additionally make the development of analytical methods more difficult.

In comparison to the HPLC and titrimetric methods, the AAS is distinguished for its low LOQ. Its advantage over TOC is the possibility of using organic solvents when the electrothermal technique is applied.

The new methods are simple and quick procedures for the determination of residual esomeprazole magnesium in water or ethanol medium and for lithium carbonate determination in a water medium. The average time for esomeprazole magnesium determination is about 1.5 hours and for lithium carbonate less than 1 hour, which make them applicable for everyday work in analytical laboratories.

Application of the methods on production samples

The described methods were used for the determination of esomeprazole magnesium and lithium carbonate in rinse waters from different manufacturing equipment (milling, blending) in the last step of the manufacturing process. In all the cases, demineralized water was used for cleaning. The last rinse water was sampled and analyzed according to procedure described in the 'Experimental' section. The water used for cleaning was analyzed as a reference sample for Mg and Li content at the same time. The assay of analyte in all samples was below the LOQ. The obtained results gave the confidence that the cleaning procedures provided sufficient removal of the residues from the equipment.

Conclusion

The developed ETAAS methods for the indirect esomeprazole magnesium determination and the FAAS method for lithium carbonate determination in rinse samples are accurate, precise, and simple procedures, appropriate for control laboratories in the pharmaceutical industry. In comparison to TOC, which is widely used for rinse samples, they are distinguished for selectivity and the possibility of using of organic solvents when ETAAS is applied. The advantage of AAS over HPLC or spectrophotometric techniques is the lower LOD. Therefore, the developed methods can be recommended in particular when the batch size of the subsequent product is low (trial batch) or when the manufacturing of a low-dose product is followed by a high-dose product (in both cases low values of MACO are expected). The methods were successfully applied for the determination of esomeprazole magnesium and lithium carbonate in rinse samples.

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Declaration of interest

The authors report no conflict of interest. The authors alone are responsible for the content and writing of this paper.

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