Development and Validation of Simple Titrimetric Method for the Determination of Magnesium Content in Esomeprazole Magnesium

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Haddadin and Issa: Titrimetric Method for Magnesium Content in Esomeprazole Magnesium

A simple and inexpensive titrimetric method for the determination of magnesium ion in esomeprazole magnesium raw material was developed and validated according to International Conference on Harmonization guidelines and the United States Pharmacopoeia. The method depends on complex formation between EDTA and magnesium ion. The method was proven to be valid, equivalent and useful as an alternative method to the current pharmacopeial methods that are based on atomic absorption spectrometry.

Key words: Atomic absorption, disodium edetate, esomeprazole magnesium, International Conference on Harmonization, titrimetric method, United States Pharmacopoeia, validation

Esomeprazole (fig. 1) is the S-isomer of the proton pump inhibitor omeprazole. It is used in the treatment of peptic ulcers and NSAID-associated ulceration, in gastro-oesophageal reflux disease, and the Zollinger-Ellison syndrome[1]. It is given orally as the magnesium salt or intravenously as the sodium salt but doses are calculated in terms of esomeprazole[1]. Alkaline salts of proton pump inhibitors including magnesium salt of esomeprazole are responsible for the high stability of the drug in alkaline conditions

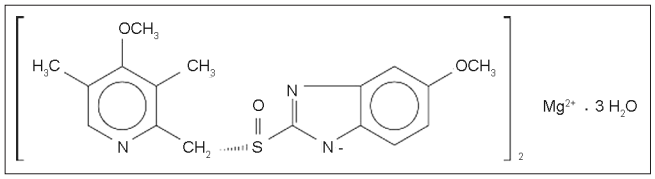

Fig. 1: Chemical structure of esomeprazole magnesium trihydrate

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and make it exceptionally suited for further processing in oral dosage forms[2,3].

Esomeprazole magnesium is a white to slightly coloured powder and it is slightly hygroscopic. It is soluble in methanol, slightly soluble in water and practically insoluble in heptane[4,5]. In February 2001, US FDA has approved AstraZeneca's product Nexium® (esomeprazole magnesium) for the treatment of duodenal ulcers, erosive esophagitis, and gastroesophageal reflux disease[6]. Currently, esomeprazole magnesium trihydrate has an official monograph for the active substance in the United States Pharmacopoeia (USP) and European Pharmacopoeia (EP)[4,5]. An important quality parameter presented in USP and EP for esomeprazole magnesium active substance is the quantitative determination of the magnesium ion. In the official monographs (USP 32 and EP 6.7), the content of magnesium in esomeprazole magnesium has an acceptance criterion that is between 3.30% and 3.55% calculated on an anhydrous basis. The official pharmacopeial method for assaying the magnesium in esomeprazole magnesium is by atomic absorption spectrometry using a magnesium lamp with an emission line at 285.2 nm. The pharmacopeial methods demand the availability of an atomic absorption spectrometer and are, subsequently, a relatively expensive methods as they require costly solutions for the preparation of calibration curve.

Several methods have been reported for the determination of esomeprazole magnesium as such[4,5,7] or in pharmaceutical dosage forms[8-10] using spectrophotometric or HPLC methods. These methods detect the esomeprazole molecule itself rather than the corresponding magnesium ion. To our knowledge, no volumetric method has been reported so far for the determination of magnesium ion content in the esomeprazole magnesium active substance or raw material. This paper reports a quantitative procedure for the determination of magnesium content in esomeprazole magnesium using a simple and inexpensive complexation titrimetric method. The proposed titrimetric method is based on complex formation between EDTA (added in excess) and the magnesium ions in solution buffered at pH 9. EDTA forms chelates (complexes) with nearly all metal ions, with a 1:1 metal-legand ratio. The complex formation equilibrium is affected by the pH of the medium[11]. Hence, the pH for the formation of EDTA-magnesium complex must be controlled at a pH of 9. The extra amounts of EDTA are then back titrated by a volumetric solution of zinc sulphate.

This method is validated here to prove the accuracy, precision, ruggedness and specificity of this proposed technique in order that it may be used as an alternative to the pharmacopeial method.

Esomeprazole magnesium from three sources was used in this study (Hetero Drugs Ltd, Hyderabad, India; Glenmark Pharmaceuticals, Mumbai, India; Precise Chemipharma Ltd, Mumbai, India). Esomeprazole sodium from Glenmark Pharmaceuticals, Mumbai, India. Magnesium standard solution traceable to SRM from NIST was purchased from Merck, Darmstadt, Germany. All chemicals used were American Chemical Society grade or analytical grade.

In order to determine the content of magnesium in esomeprazole magnesium, about 500 mg of esomeprazole magnesium accurately weighed were transferred to 250 ml Erlenmeyer flask and dissolved in 50 ml ethanol. Ammonium hydroxide (5 ml) was added, mixed and followed by ammonium chloride pH 10 buffer (3 ml; 5.4 g of ammonium chloride, 20 ml of ammonium hydroxide, water up to 100 ml) and 30.0 ml of 0.1M EDTA. One to two drops of eriochrome black solution (200 mg of eriochrome black and 2 g of hydroxylamine hydrochloride in methanol up to 50 ml) was added and mixed. The excess EDTA was titrated with 0.1M zinc sulphate solution until the solution colour changes from blue to violet. Each one millilitre of 0.1M zinc sulphate is equivalent to 2.431 mg magnesium. Blank determination was performed.

The content % of magnesium=(Vb-Vs)×2.431×100/Wt…(1), where, Vb is the volume (ml) of zinc sulphate consumed by blank determination; Vs is the volume (ml) of zinc sulphate consumed by the sample and Wt is the weight of the sample (mg) on anhydrous basis.

The methods for determining magnesium content in esomeprazole magnesium described in the EP monograph under the test *Magnesium* or in the USP monograph under the test *Content of magnesium* are very similar with only minor differences. However, the USP method is more detailed in the monograph and was applied in this study. In brief, accurately weighed quantity of esomeprazole magnesium (250 mg) was dissolved in 1M HCl (20 ml), water

was added up to a final volume of 100 ml. Serial dilutions were made in water to obtain a concentration of 12.5 µg/ml of esomeprazole magnesium. Appropriate dilutions of magnesium standard solution (1000 µg/ml) were made to obtain standard solutions with 0.1, 0.2, 0.3, 0.4 and 0.5 µg/ml magnesium. At the final dilutions of each of the samples and standard solutions, a suitable volume of lanthanum solution (58.7 g lanthanum oxide, 250 ml hydrochloric acid, water up to 1000 ml) was added to have 4 ml per 100 ml final solution. Suitable blank solution was prepared. The absorbencies of the standard solutions of magnesium were determined concomitantly with the sample solutions and blank solution at the magnesium emission line 285.2 nm using flame atomic absorption spectrometry (Varian, SpectrAA-250 Plus; Varian, Mulgrave, Victoria, Australia). The linear equation generated from the calibration curve was Y=0.838X+0.031 with a correlation coefficient r=0.999. From this equation the content of magnesium in the samples was calculated and results reported on the anhydrous basis. The developed method was validated according to USP and International Conference on Harmonization (ICH) Q2R1[12,13].

The accuracy for a drug substance can be determined by comparing the results obtained by a novel procedure with those obtained by a well characterized procedure such as a pharmacopeial procedure[12,13]. Three different raw materials each obtained from different supplier were assayed using titration method and the official USP 32 method described above respectively. For each testing method, five samples from each supplier were assayed and results reported. In addition, the accuracy of our procedure was evaluated across the testing range covering 250 to 750 mg, by assaying three replicates at each of 250, 500 and 750 mg according to ICH guideline recommendation.

The precision of an analytical procedure expresses the closeness of agreement between a series of measurements obtained when the procedure is applied repeatedly to multiple sampling of the same homogenous sample[12,13]. Usually it is expressed as the standard deviation (SD) or relative standard deviation (RSD %). To assess precision, three replicates at each of 250, 500 and 750 mg were analyzed and relative standard deviation for each level was calculated.

Ruggedness or also known as intermediate precision, expresses the variation within a laboratory, such as techniques performed on different days, or with different equipment or different analysts within the same laboratory[12,13]. Three replicates of three amounts at three levels, 250, 500 and 750 mg were performed on two different days by two different analysts. Relative standard deviation was calculated for each level at each day and compared with that of the second day.

Specificity is the ability of the method to assess unequivocally the analyte in the presence of components that are expected to be present such as degradation products or structurally related compounds[12,13]. In order to evaluate specificity, samples of esomeprazole magnesium were exposed to forced degradation by acid and base. Three samples containing accurately weighed amounts of approximately 500 mg esomeprazole magnesium were degraded by adding 0.1M HCl Similarly three samples containing accurately weighed amounts of approximately 500 mg esomeprazole magnesium were degraded by adding 0.1M NaOH. The content of magnesium was then determined by titration. The results obtained were compared with the nominal content of magnesium. Furthermore, to prove that positive responses are not obtained from materials that are structurally similar to the drug, samples of esomeprazole sodium were assayed for magnesium content to prove specificity of the titration method to magnesium ion. Esomeprazole sodium (500 mg) was weighed and analysed by applying the titration method.

The range of an analytical procedure is the interval between the upper and lower amounts of analyte in the sample where a suitable level of accuracy, precision and linearity have been demonstrated[12,13]. In this work, since the analytical procedure relies on stoichiometric reaction, linearity was considered not significant as long as the other validation parameters can be demonstrated acceptable. The validity of a range covering 50 to 150% of the test amount was studied, where triplicate preparations of each of 250, 500 and 750 mg representing 50, 100 and 150% levels, respectively were analysed and precision and accuracy were evaluated.

One way analysis of variance (ANOVA analysis) with equal variance at 99% confidence level was used to evaluate the equivalence of different sets of data. Minitab 14 was used to perform the statistical analysis. Each analysis was performed at least in triplicate, standard deviation and relative standard deviation were calculated and reported.

TABLE 1: MAGNESIUM CONTENT % DETERMINED BY THE TITRIMETRIC METHOD AND THE USP METHOD

Sample/method	Supplier 1		Supplier 2		Supplier 3	
	USP method Mg %	Titration method Mg %	USP method Mg %	Titration method Mg %	USP method Mg %	Titration method Mg %
1	3.39	3.43	3.39	3.37	3.49	3.41
2	3.38	3.47	3.38	3.31	3.40	3.47
3	3.41	3.42	3.35	3.41	3.45	3.39
4	3.36	3.48	3.36	3.35	3.50	3.48
5	3.41	3.32	3.36	3.40	3.41	3.30
Mean	3.39	3.42	3.37	3.37	3.45	3.41
SD	0.02	0.06	0.02	0.04	0.05	0.07
RSD%	0.63	1.85	0.49	1.20	1.31	2.12
Accuracy % (titrimetric/USP method)	-	100.88	-	100.00	-	98.84

Determination is performed for three batches of esomeprazole magnesium from different suppliers. SD - standard deviation; RSD% - relative standard deviation

Table 1, shows the accuracy of the titrimetric method by comparing the magnesium content of three batches from three different suppliers with those obtained from USP method. The accuracy was in the range of 98.8 to 100.9% with %RSD of up to 2.1%. ANOVA analyses indicate no significant difference between the titrimetric method and the USP method at 0.01 significance level where *P* was 0.29, 1.00 and 0.33 for suppliers 1, 2 and 3 respectively.

Accuracy was also evaluated at three different levels (250, 500 and 750 mg representing 50%, 100% and 150% levels respectively) as per the ICH guideline and USP recommendation, and %RSD indicating precision of the method at each level was calculated (Table 2). The results demonstrated good intra- and inter-day accuracy and precision. ANOVA analysis of the titration results indicates no significant difference among the results of the three levels for each day ($P>0.01$), where $P=0.074$ and $P=0.630$ for day one and day two respectively. In other words, the results presented for the two days by two analysts indicate that the method has a valid range from 250 to 750 mg, where the method is rugged within this range. Esomeprazole magnesium samples which were exposed to forced degradation by acid or base were assayed by titration method and the results were compared to the nominal content of magnesium in these samples (Table 3). The presence of esomeprazole magnesium degradation products in the solution did not result in significant interferences on the analysis of magnesium as shown in Table 3, where the error did not exceed 1.5%, indicating good specificity of the method. On the other hand, and as would be expected, the titration of esomeprazole sodium produced zero content of magnesium (Table 3) indicating that structurally related compounds do not interfere with the titration method.

TABLE 2: ACCURACY AND PRECISION OF THE TITRIMETRIC METHOD

Sample/level	Magnesium content %					
	Day 1/analyst 1			Day 2/analyst 2		
	50%	100%	150%	50%	100%	150%
1	3.39	3.39	3.39	3.41	3.36	3.34
2	3.47	3.34	3.35	3.33	3.43	3.41
3	3.44	3.38	3.38	3.37	3.39	3.34
Mean	3.43	3.37	3.37	3.37	3.39	3.36
SD	0.04	0.03	0.02	0.04	0.04	0.04
RSD%	1.18	0.79	0.62	1.19	1.04	1.21
Accuracy (%) (actual/nominal)	101.18	99.41	99.41	99.41	100.00	99.11

Accuracy and precision determined at three levels on two days by two analysts. Samples from supplier one were used (nominal magnesium content = 3.39% as determined by USP method). SD - standard deviation; RSD% - relative standard deviation

TABLE 3: SPECIFICITY OF THE TITRIMETRIC METHOD

Sample type	Nominal Mg content %	Actual Mg content % (SD)	Error %
HCl degradation	3.39	3.35 (0.02)	−1.18
NaOH degradation	3.39	3.41 (0.02)	0.59
Esomeprazole sodium	0.00	0.00	0.00

Samples of esomeprazole magnesium (from supplier one) exposed to acid (0.1 M HCl) or base degradation (0.1 M NaOH), as described in the text, and samples of esomeprazole sodium were titrated and actual magnesium content was calculated. Error %: percentage variability between the actual content and the nominal content divided by the nominal content. SD - standard deviation. Each result represents the average $n=3$

In conclusion, we were able to develop a simple titration method for the determination of magnesium content in esomeprazole magnesium active substance and the method was found accurate, precise, rugged and specific and can be used as alternative to the current pharmacopeial methods with confidence.

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