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Residual thiosulfate determination in omeprazole by liquid chromatography

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Abstract: An HPLC-UV method for the determination of thiosulfate in aqueous media was modified and validated in this study to quantify the amount of residual thiosulfate in omeprazole samples. Absorption of thiosulfate ion was monitored at 215 nm in alcoholic tetrabutylammonium hydroxide solution as a mobile phase, in which the pH was adjusted to 6.20 by using dilute hydrochloric acid. A method detection limit of 0.08 mg/L was achieved for thiosulfate ion in 1000 mg/L omeprazole solution. The precision, accuracy, and the expanded uncertainty of the method were 2.7 %, 92.4-102.6 %, and 2.2 % (at 95 % confidence level (k = 2) assuming 1.0 mg/L thiosulfate in the sample solution), respectively.

Keywords: HPLC-UV; omeprazole; residual thiosulfate; method validation.

1. Introduction

Omeprazole is one of the important proton-pump inhibitor to regulate acid production in the stomach. It is also used to treat infection caused by *Helicobacter pylori* [1-7].
Many chemicals are involved in the synthesis of drugs. The possible impurities for Omeprazole originate from the route of synthesis or from degradation, especially from heat, moisture or light; these should be below the limits in the monograph of the Pharmacopoeia. In all finished products, the specification for a drug substance should include a list of impurities [8-15]. If the substance for which a certificate is requested is not on the market already (new process, new source) in medicinal products licensed in Europe, the impurity profile in the representative batches needs to be more thoroughly discussed in order to determine if the impurities can be considered as qualified [9-12]. Sodium thiosulfate is one of the chemicals that is used in the synthesis of omeprazole. Thus, the authority of the European Agency for the Evaluation of Medicinal Products [9] and European Directorate for the quality of medicines [10] requested the levels of thiosulfate in the omeprazole even though it is not included in the monographs. Many methods have been described for the determination of thiosulfate and other anions using liquid chromatography [16-20]. However, there is not any method in the literature for the determination of thiosulfate in the medicinal products. Thiosulfate anion is UV-absorbing. In this study, a method developed by Connolly and Paull [16] for the determination of UV-

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absorbing anions in aqueous media was modified and validated for the analysis of omeprazole for residual thiosulfate. This rapid and easy method can be applied to other medicinal drugs to determine thiosulfate anion for the routine analyses in pharmacy.

2. Experimental

2.1. Chemicals

Omeprazole (5-methoxy-2-{[(4-methoxy-3,5-di-methyl-2-pyridinyl)methyl]sulphinyl}-1H-benzimidazole) was supplied from the manufacturer company, Milen İlaç Hammaddeleri Sanayi ve Ticaret A.Ş. Tuzla, Istanbul, Turkey. Tetrabutylammonium hydroxide (TBAH) solution (40% in water) was purchased from Fluka and sodium thiosulfate-pentahydrate (Suprapur® 99.999%) was purchased from Merck. Purex analytical grade Methanol was purchased from Lab-Kim.

2.2. Apparatus

Analyses were carried out on an Agilent 1100 Series HPLC equipped with Variable Wavelength UV Detector, Quaternary Pump, thermostatic ALS auto sampler and ChemStation software (Rev. A.10.01 (1635). The HPLC column was Perkin Elmer-BROWNLEE PECOSFERE, C-18, 33x4.6 mm (3 μm).

2.3. Chromatographic conditions

The wavelength and oven temperature was 215 nm and 45 ^{o}C, respectively. Mobile phase flow rate was kept at 2.0 mL/min and 10 μL of samples were injected. Mobile phase was 50 mM TBAH (80%) and methanol (20%). To prepare 50 mM TBAH solution, 33 mL of 40% aqueous TBAH solution was first diluted to 500 mL with HPLC grade water. A 100 mL of methanol was added and final solution was diluted to 1000 mL with water. The pH of the resultant solution was adjusted to 6.20 with dilute (6.0 M) hydrochloric acid. The final mobile phase was 80% 50 mM TBAH and 20% methanol during the analyses. The sample cooler temperature and the column temperature were kept at 15 ^{o}C and 45 ^{o}C, respectively.

2.4. Preparation of standards and calibrations

A 1000 mg/L of omeprazole was prepared by dissolving 100 mg of omeprazole in 100 mL of mobile phase. For the thiosulfate ion, 1000 mg/L stock standard solution was prepared in water and for the calibration curve two types of calibrations were done. For the first calibration, working standards were diluted with the mobile phase and average peak areas were used to plot the calibration curve for the calibration levels of 0.5, 1.0, 5.0, and 10 mg/L thiosulfate ion and this calibration was named as *direct calibration*. For the second type of calibration, addition calibration was used. As a matrix, freshly prepared 1000 mg/L omeprazole solution in mobile phase was used. The calibration standards were 0.0 (matrix itself), 1.0, 3.0, 5.0 and 10.0 mg/L. Results obtained from these two calibrations are discussed in the section of results and discussion.

3. Results and discussion

Omeprazole is not stable when it is dissolved in mobile phase or methanol. The stability of omeprazole was studied very extensively in literature and recently by Riedel and Leopold [21] and it was reported that omeprazole is very stable in alkaline media. Due to acidic media (pH = 6.20), in this study, for each of the replicate analysis a new omeprazole solution was prepared and used as soon as possible (less than 15 minutes including sample preparation and analysis). Omeprazole solutions older than 20 minutes change the results of the analysis significantly. The short lifetime of the omeprazole solution was the only disadvantage of the validated method.

The method detection limit (MDL) was found as 0.08 mg/L for thiosulfate ion in omeprazole determined by considering a value, which was 3 times the standard deviation of concentrations obtained from the ten replicates of the zero solution (1000 mg/L omeprazole in mobile phase). The

limit of quantification (LOQ) was calculated as 0.15 mg/L and determined as 6 times the same standard deviation used to calculate the method detection limit.

Both addition calibration and direct calibration curves were linear from 0.5 to 50.0 mg/L, however, for this study the highest working standard was kept at 10.0 mg/L due to very low levels of residual thiosulfate in the omeprazole. Correlation coefficients (r) were 0.999 for both of the calibrations and the linear regression equations were y = 14.62x + 1.85 for the addition calibration and y = 10.37x – 1.42 for the direct calibration, where y is the peak area and is the concentration of the thiosulfate ions in mg/L. The difference between the slopes of two calibration types was large enough to propose addition calibration as the suitable plot for this method. Addition calibration is more sensitive than the direct calibration as shown in the regression equations. Besides, this slope change encountered significant recovery problems in direct calibration due to the present matrix interferences.

Precision of the method was determined as the percent relative deviation (RSD%) of the calibration standards. The calculated RSD%s from the six replicates of the addition calibration standards and the direct calibration standards were found as 2.7 % and 2.5 %, respectively.

To determine the accuracy of the method, known amounts of thiosulfate standards were added to the 1000 mg/L of freshly prepared omeprazole solutions and addition calibration was used to analyze the spiked samples. Percent recoveries of 1.0 mg/L of thiosulfate ion were found in the range of 92.4-102.6 %. When omeprazole is dissolved in methanol instead of mobile phase, then the percent recoveries for the replicates of 1.0 mg/L of thiosulfate were found in the range of 72.5-88.3 %. Percent recoveries from the direct calibration were not included due to the matrix interferences from both of the omeprazole solutions prepared in mobile phase and methanol.

For the determination of uncertainty budget EURACHEM/CITAC Guide 2012 [22], was used and the percent relative uncertainty of thiosulfate in omeprazole solution was 2.2 % at 95 % confidence level (k = 2) assuming 1.0 mg/L of thiosulfate in sample solution. It was observed that the maximum contributions to the total uncertainty budget of the validated method was originating from repeatability and the calibration curve. Sample weighing and purity of the standard (thiosulfate) did not cause significant uncertainties.

Sample chromatograms of an unspiked omeprazole (A) and a spiked omeprazole sample containing 1.0 mg/L thiosulfate ion (B) were presented in Fig. 1. As can be seen from the figure, the amount of residual thiosulfate ion in the omeprazole sample is below the method detection limit, that is, lower than 0.08 mg/L. As a result, the validated method in this study is suitable for the reliable detection of residual thiosulfate in the omeprazole samples.

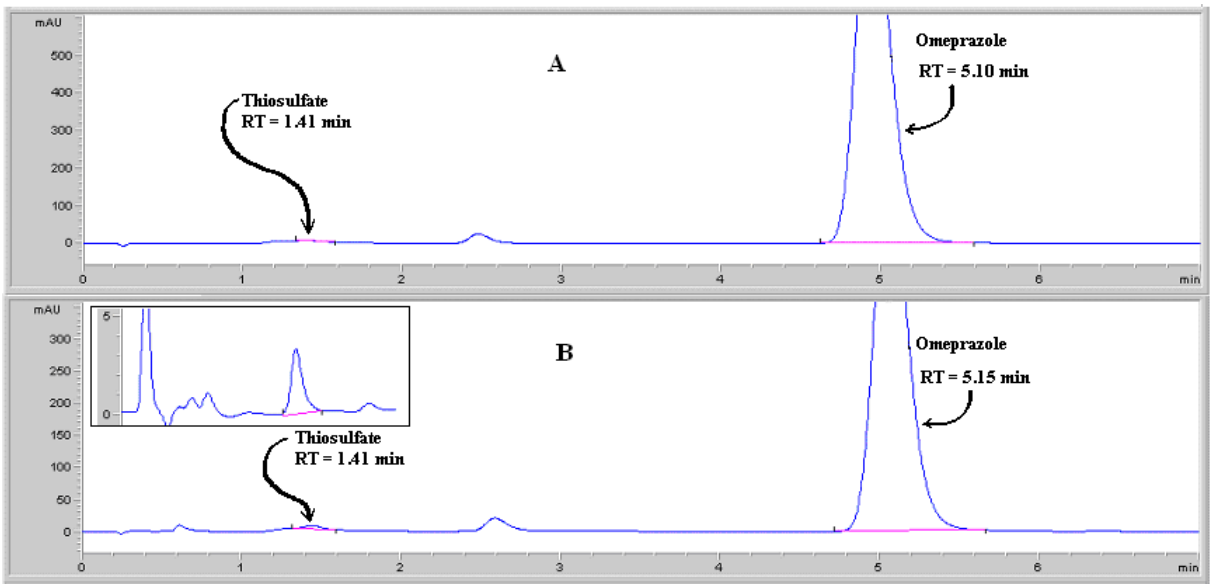

Figure 1. Sample chromatograms of an unspiked omeprazole (A) and a spiked omeprazole sample containing 1.0 mg/L thiosulfate ion (B).

4. Conclusion

The method developed by Connolly and Paull [16] was modified and validated for the residual thiosulfate determination in omeprazole samples. Method detection limit was calculated using addition calibration and found as 0.08 mg/L of thiosulfate in 1000 mg/L of omeprazole solution. Recoveries greater than 92 % were observed with the method and no residual thiosulfate was observed in the omeprazole samples supplied by the customer. The method validated in this study can be applied for the routine determination of residual thiosulfate in omeprazole samples efficiently due to its simplicity and low method detection limit.

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