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A New Ultra Performance Liquid Chromatographic Method for Determination of Omeprazole in Capsule Dosage Form

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Omeprazole is a widely used proton pump inhibitor prescribed for the treatment of dyspepsia, peptic ulcer disease, gastro esophageal reflux disease, laryngo pharyngeal reflux and Zollinger-Ellison syndrome. A new ultra performance liquid chromatographic (UPLC) method was developed and validated for the quantitative analysis of omeprazole in a capsule dosage form. The separation and analysis of the related drug in the presence of ondansetron as internal standard (IS) were performed on Waters UPLC BEH C18 column (50 mm × 2.1 mm i.d., 1.7 µm) using a mobile phase consisting of acetonitrile and 0.05 M H_3PO_4 (28:72 v/v). Flow rate of the used mobile phase was 0.28 mL/min. The retention time for omeprazole and internal standard was found to be 0.787 and 1.060 min, respectively. A calibration graph for omeprazole in the concentration range of 4-46 µg/mL was obtained by using peak area ratio of omeprazole and internal standard in their chromatogram obtained by the detection at 302 nm. In the method validation process, percent mean recovery and relative standard deviation was found as 101.6 % and 1.20 %, respectively. It was observed that the application of the newly developed UPLC method gave us successful results for the quantitative estimation of omeprazole in capsules.

Keywords: Ultra performance liquid chromatography, Determination, Omeprazole, Capsule.

INTRODUCTION

Omeprazole, 5-methoxy-2-[[(4-methoxy-3,5-dimethyl-2-pyridinyl)methyl]sulphinyl]-1*H*-benzimidazole, is a substituted benzimidazole compound which structural formula is given in Fig. 1.

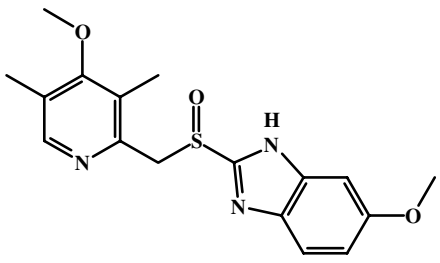

Fig. 1. Chemical structure of omeprazole

Omeprazole was the first drug released on the market within the group of proton pump inhibitors. Due to its highly targeted mechanism of action, this group of drugs is the most potent suppressors of gastric acid secretion. Likewise the rest of the proton pump inhibitors, omeprazole is a prodrug that activates in the gastric parietal cells and binds covalently to the proton pump (H^+/K^+-ATPase) which secretes gastric acid. By inhibiting the final step of gastric acid formation, omeprazole causes elevation of gastric pH and a relief of symptoms in gastric acid related disorders. It is widely used for the treatment of gastro-oesophageal reflux disease, reflux oesophagitis, Zollinger-Ellison syndrome and also for the treatment and prevention of gastric and duodenal ulcers[1-3].

In previous studies, determination of omeprazole was performed by spectrophotometry[4-6], HPLC[6-9], UPLC[10], LC-MS[11], LC-MS/MS[12,13] and voltammetry[14,15] in pharmaceutical and biological samples.

Recent applications of liquid chromatography indicate that ultra performance liquid chromatography, has many advantages over traditional high performance liquid chromatography such as short analysis time, high separation efficiency, high sensitivity, less solvent consumption. The fact that UPLC analysis requires short analysis time and economically use of solvents and chemicals, makes it a very feasible application especially for drug industry.

The development of fast, accurate and precise methods for quantification of omeprazole in its pharmaceutical formulations is important due to its wide usage and need for quality control and routine analysis procedures.

The aim of this study is to develop a new, rapid, sensitive, accurate and precise UPLC method to quantify omeprazole in commercial capsule dosage form. The validation of the proposed UPLC method was performed using independent sample containing omeprazole, analyzing inter-day and intra-day samples and standard addition technique. The developed UPLC method was successfully applied to the quantification of omeprazole in capsules.

EXPERIMENTAL

Methanol and acetonitrile was of HPLC grade (Sigma Aldrich, USA). Phosphoric acid (Sigma Aldrich, USA) was of analytical grade. Ultrapure water obtained from Milli-Q water purification system (Millipore, Bedford, MA, USA)was used during chromatographic analysis. Standard materials of omeprazole (99.8 % purity) was kindly gifted from Sandoz Pharmaceuticals (Istanbul, Turkey) and ondansetron (99.7 % purity) was kindly provided from Adeka Inc. (Istanbul, Turkey). A commercial capsule dosage form (Omeprol Micropellet Capsule) containing 20 mg omeprazole per capsule was purchased from local pharmacy and investigated in this study.

The chromatographic analysis was carried out on a Waters Corp. (Milford, MA) ACQUITY UPLC™ H-Class system, with a quaternary solvent manager, photodiode array (PDA) detector, an oven enabling control of the temperature of the analytical column and cooling auto-sampler. Chromatographic separation was achieved on a Waters UPLC BEH C18 column (50 mm × 2.1 mm i.d., 1.7 µm). Chromatographic data were collected and processed by Waters®Empower2 software. Calibrations and related calculations were performed using MATLAB 7.12 (Math Works Inc.) and Microsoft Excel Software.

Chromatographic conditions: In the presented study, the chromatographic separation was accomplished on a Waters UPLC BEH C18 (50 mm × 2.1 mm i.d., 1.7 µm) column system. Mobile phase composition was a mixture of 28 % acetonitrile and 72 % 0.05 M H_3PO_4. Flow rate of the mobile phase was 0.28 mL/min. Column temperature was kept at 40 °C. Injection volume was 1 µL for all samples.Quantification was carried out by measuring the peak areas at 302 nm (Fig. 2A).

Preparation of standard, test and sample solutions: Stock solutions of omeprazole and ondansetron as an internal standard were separately prepared by dissolving 10 mg of each compound in 100 mL calibrated flask in methanol. Calibration set of omeprazole was obtained by diluting the stock solutions with methanol to get solutions in the concentration range of 4-46 µg/mL. A constant amount of internal standard stock solution was added to all sample solutions prepared in this study to obtain a concentration of 20 µg/mL internal standard. A validation set of 5 mixture solutions in the linear concentration range of omeprazole was prepared from the stock solutions and used to predict accuracy and precision of the method. For inter-day and intra-day studies, the test samples containing omeprazole at three different concentration levels were freshly prepared within working concentration range. In order to check the specificity of the method, standard addition samples with three different concentration levels were prepared within the concentration range.

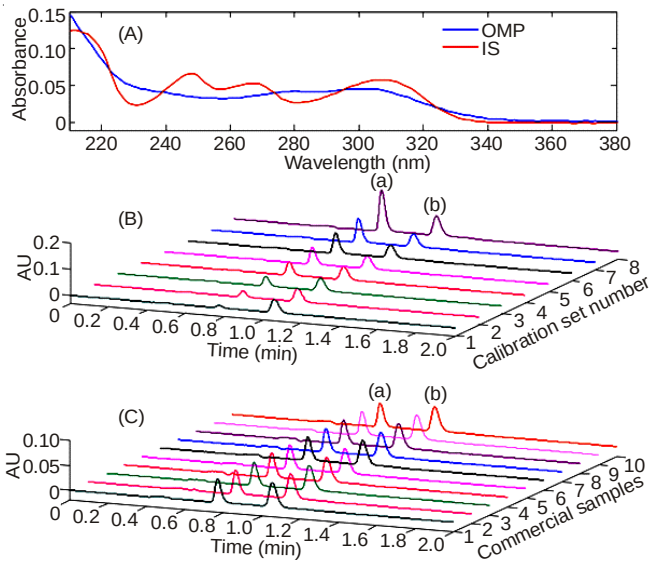

Fig. 2. (A) Absorbance spectra of omeprazole and internal standard obtained by PDA detector B) UPLC chromatograms of the calibration set C) UPLC chromatograms of the commercial samples. (a) and (b) corresponds to omeprazole and internal standard in the chromatograms, respectively

In the analysis procedure of capsules, content of 20 capsules was accurately weighed and finely powdered in mortar. A homogenous powder sample containing omeprazole equivalent to one capsule content was dissolved in methanol in flasks and sonicated for 0.5 h. The content of the flasks made up to 100 mL in calibrated flasks and filtered through a 0.20 µm syringe filter. This procedure was repeated 10 times. Afterwards, the method was applied to the final commercial sample solutions after dilution to the working concentration range.

All calibration and sample solutions were prepared in methanol and were filtered through a 0.20 µm syringe filter before injecting to chromatographic system.

RESULTS AND DISCUSSION

In this study, we developed a new UPLC method to quantify omeprazol in its capsule dosage form. Our goal was to establish an accurate, precise, sensitive, selective and fast method which provides satisfactory system suitability results. In order to improve the quality of the collected data, ondansetron was used as an internal standard. All calculations were made according to ratio of the peak area of omeprazole to the peak area of internal standard.

Calibration set of omeprazole was obtained by diluting the stock solutions with methanol to get solutions in the concentration range of 4-46 µg/mL. A constant amount of internal standard stock solution was added to all sample solutions prepared in this study to obtain a concentration of 20 µg/mL internal standard. UV absorbance spectra of omeprazole and internal standard obtained from PDA detector was illustrated in Fig. 2A. UPLC chromatograms of the calibration set was recorded by PDA detection at 302.0 nm using a mixture of acetonitrile and 0.05 M H_3PO_4 (28:72, v/v) as a mobile phase and illustrated in Fig. 2B. Calibration curves were constructed by plotting the peak area ratios *versus* the concentration. Each

response used in the calibration curve was obtained from the average of three repetitions. As it can be seen in Fig. 2B and 2C, the retention times of omeprazole and internal standard were 0.787 and 1.060 min, respectively.

Validation of the method: Linear regression analysis and its statistical results including slope, intercept and correlation coefficient of calibration curve, LOD and LOQ calculated from the data obtained from calibration set were indicated in Table-1. The quantity of omeprazole in capsules was determined by using the computed calibration curve. In the working concentration range of omeprazole, good linearity with high correlation coefficient was observed as shown in Table-1. The limit of detection (LOD) and the limit of quantitation (LOQ) were calculated using the standard deviation of the regression intercept and slope of regression equation and were shown in Table-1.

TABLE-1
LINEAR REGRESSION ANALYSIS AND STATISTICAL RESULTS OF OMEPRAZOLE

Parameter	
Slope of the linear regression equation (m)	0.0340
Intercept of the linear regression equation (n)	0.0451
Correlation coefficient of the linear regression equation (r)	0.9994
Standard error of slope (m)	0.0005
Standard error of intercept (n)	0.0132
Standard error of correlation (r)	0.0056
Limit of detection (µg/mL)	1.16
Limit of quantitation (µg/mL)	3.88

Accuracy and precision tests were performed by the analysis of test samples consisting of omeprazole at five different concentration levels and inter-day intra-day samples. Recovery results for the test set were stated in Table-2. The recovery results for inter-day and intra-day samples with their standard deviation and standard error values were summarized in Table-3. These results show the precision and accuracy of the developed method.

TABLE-2
RECOVERY RESULTS OF THE TEST SAMPLE

Exp. No.	Added (µg/µL)	Found (µg/µL)	Recovery (%)
1	4	4.10	102.4
2	16	16.08	100.5
3	28	28.66	102.4
4	40	40.04	100.1
5	20	20.55	102.7
	Mean		101.60
	Standard deviation (%)		1.22
	Relative standard deviation (%)		1.20

Standard addition technique was used to observe the presence or the absence of the interference of excipient on the analysis of capsules. As seen in Table-4, no matrix interference on the analysis results was reported.

TABLE-4
RECOVERY RESULTS FOR STANDARD ADDITION STUDY

Added (µg/mL)	Found (µg/mL)	Recovery (%)	Standard deviation (%)	Relative standard deviation (%)
8	8.19	102.4	1.59	1.55
16	16.67	104.2	0.70	0.67
24	24.75	103.1	0.62	0.60
Mean	16.50	103.23	0.97	0.94

According to these validation results, developed UPLC method was found to be suitable to quantify omeprazole in commercial capsule samples.

Application of the method on commercial samples: After method validation procedures, the method was applied for the quantitative estimation of omeprazole in capsule dosage forms. The solutions of the commercial samples were prepared as described earlier. The chromatograms of the commercial sample solutions were illustrated in Fig. 2C. The ratios of the peak areas of omeprazole to internal standard were placed into the regression equation to calculate the amount of omeprazole. The analysis results of the commercial capsule samples were calculated from the average of ten replicate experiments. The determination results were summarized in Table-5.

TABLE-5
ANALYSIS RESULTS OF COMMERCIAL CAPSULE SAMPLES

Replicate	mg/capsule	Replicate	mg/capsule
1	20.35	6	20.26
2	20.52	7	20.21
3	20.25	8	20.43
4	20.10	9	19.33
5	20.03	10	19.76
	Mean		19.84
	Standard deviation (%)		0.35
	Relative standard deviation (%)		1.77

Conclusion

A new UPLC method for the quantitative determination of omeprazole was developed and validated. The method involved the use of ondansetron as an internal standard. This simple, rapid method was found to be specific, precise and accurate for the analysis of the related drug in capsules. The method was successfully applied to the quantification of omeprazole in commercial capsule dosage forms without interfering excipients of capsule. It is concluded that the developed method provides economic and reliable analysis of the related drug.

TABLE-3
RECOVERY RESULTS OF THE INTER-DAY AND INTRA-DAY SAMPLES

Added (µg/mL)	Found (µg/mL)		Recovery (%)		Relative standard deviation (%)		Standard error (%)	
	Inter-day	Intra-day	Inter-day	Intra-day	Inter-day	Intra-day	Inter-day	Intra-day
4	4.01	3.97	100.2	99.4	1.46	1.27	1.46	1.27
22	22.68	22.73	103.1	103.3	0.71	0.76	0.71	0.76
34	34.37	34.92	101.1	102.7	1.44	0.43	1.44	0.43
			Mean	101.8	1.20	0.82	1.20	0.82

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