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A New Fast Reversed-Phase Ultra-Performance Liquid Chromatographic Approach for Quantitative Analysis of Pantoprazole in Tablets

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A new fast reversed-phase ultra-performance liquid chromatographic (RP-UPLC) approach was developed for the determination of pantoprazole in tablets. Chromatographic separation and quantitation was achieved isocratically on a Waters UPLC BEH C_{18} column (50 mm × 2.1 mm i.d., 1.7 µm) using a mobile phase containing the mixture of methanol and 0.05 M H_3PO_4 (40:60, v/v) at a flow rate of 0.25 mL/min with a PDA detection at 288 nm. Ondansetron was used as an internal standard. The retention time of pantoprazole and ondansetron was reported as 1.69 and 1.15 min, respectively. Calibration curve for the analyzed drug was obtained by using the linear concentration range of 2.0-40.0 µg/mL with eight different concentration levels. The developed RP-UPLC method was validated using recovery studies, inter-day, intra-day experiments and standard addition assays. It was concluded that the proposed and validated RP-UPLC approach can be used for the quality control and routine analysis of pantoprazole in commercial tablets.

Keywords: Reversed phase ultra-performance liquid chromatography, Pantoprazole.

INTRODUCTION

Pantoprazole is an acid inhibitory agent belonging to the group of proton pump inhibitors. In pharmaceutical formulation, it is widely used for the treatment of acid-related gastrointestinal disorders such as reflux esophagitis, duodenal and gastric ulcers. Pantoprazole suppress gastric acid secretion by specific inhibition of the H^+/ K^+-ATPase (the proton pump) in the gastric parietal cells. This results in inhibition of the acid secretion and elevation of the intragastric pH[1-4].

Pantoprazole is a substituted benzimidazolesulphoxide and is chemically known as 5-(difluoromethoxy)-2-[[(3,4-dimethoxypyridin-2-yl)methyl]sulfinyl]-1*H*-benzimidazole. Its chemical structure formula is given in Fig. 1. In literature, various analytical methods including spectrophotometry[5,6], HPLC[7-9], voltammetry[10,11], potentiometry[12] and LC-MS/MS[13,14] have been reported for the quantification of pantoprazole in pharmaceutical and biological samples.

In recent years, the use of the ultra-performance liquid chromatography (UPLC) method instead of the conventional HPLC method has been preferred for the chemical and pharmaceutical analysis. This UPLC technique provides relatively new approaches in liquid chromatography, particularly short analysis time with higher chromatographic peak resolution. In this study, the quantity of pantoprazole in tablets was determined by newly developed fast RP-UPLC method. The validation of

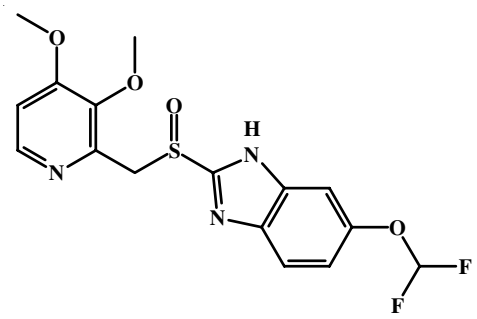

Fig. 1. Chemical structure of pantoprazole

the developed RP-UPLC approach was carried out by analyzing the test samples containing pantoprazole, inter-day and intra-day experiments and using standard addition technique. The analysis of the related drug with the total runtime of 3 min was performed by newly developed and validated fast RP-UPLC method. It was concluded that the developed RP-UPLC method is most suitable for the quantitative estimation and routine analysis of pantoprazole in commercial tablets.

EXPERIMENTAL

Chromatographic analysis and quantitation were performed by Acquity UPLC system equipped with a Waters UPLC BEH C_{18} column (50 mm × 2.1 mm i.d., 1.7 µm), a quaternary solvent

delivery pump, an auto sampler and PDA detector. The chromatographic data were acquired and processed using Empower2 software (Waters Corporation, Milford, USA). Calibration and quantitation of pantoprazole were computed by using the Matlab and Microsoft Excel software.

Methanol (Merck) and acetonitrile (Merck) were of HPLC grade. Active compounds (pantoprazole and ondansetron) were obtained a gift from the National Pharm Ind. Tablets analyzed in this study were purchased from a local drug store in Ankara.

Ultra-performance liquid chromatographic condition: An UPLC™ BEH C18 column (2.1 mm × 5 mm, 1.7 µm particle size) and flow rate of 0.28 mL/min, an injection volume of 1.0 µL and acetonitrile and 0.05 M H_3PO_4 mobile phases were used. Mobile phase was filtered through 0.20 µm nylon membranes (Sartorius Minisart).The separation was performed using an isocratic elution with mobile phase. Mobile phase was acetonitrile/0.05 M H_3PO_4 (28:72, v/v) delivered at a flow rate of 0.25 mL/min and column temperature of 40 ºC. The chromatograms were monitored at the wavelength 288.0 nm, which corresponds to the λ_{max} of the related drug.

Standard solution: Stock solutions of 10 mg/100 mL of pantoprazole and 10 mg/100 mL of ondansetron were individually prepared in methanol. A standard series of pantoprazole in the concentration range 2-40 µg/mL in the presence of a constant amount (20 µg/mL) of ondansetron was prepared from the above stock solutions. An independent test set of the samples containing 4-40 µg/mL at five different concentration levels was prepared by using the stock solutions of pantoprazole with ondansetron for the validation of the developed RP-UPLC method. From the stock solutions of the analyzed drug and ondansetron, it was prepared the validation samples for inter-day and intra-day analyses and standard addition experiments to check the applicability of the proposed UPLC method.

Analysis procedure of tablet: Twenty tablets were weighted and powdered. An amount equivalent to one tablet was transferred into a 100 mL volumetric flask. The flask content was dissolved in 50 mL of methanol by shaking for 15 min and completed to volume with methanol. The tablet solution was filtered through 0.20 µm membrane filter. The resulting solution was diluted with methanol into the working concentration range and then it was injected to UPLC system. Chromatograms of samples were recorded. The analysis procedure was repeated ten times for each tablet.

RESULTS AND DISCUSSION

The main aim of this study is to develop and validate a new, fast, precise and accurate RP-UPLC method for the quantitative estimation of the quantity of pantoprazole in a commercial solid dosage form. To perform the aim of this study, it was tested several mobile phase systems in different compositions of organic and inorganic phases on Waters UPLC BEH C_{18} column (50 mm × 2.1mm i.d., 1.7 µm) in order to get desirable chromatographic conditions of pantoprazole with appropriate internal standard (ondansetron in our case). From the mentioned experiments, it was found the optimal chromatographic conditions as a mobile phase consisting of methanol and 0.05 M H_3PO_4 (40:60, v/v) at a flow rate of 0.25 mL/min with a PDA detection at 288 nm to reach the best analysis results of the related drug with short runtime, adequate precise and accurate.

Application of RP-UPLC method: As explained above the calibration solutions of pantoprazole in the presence of 20 µg/mL ondansetron as an internal standard were prepared in the concentration range of 2-40 µg/mL, starting from the stock solutions of pantoprazole and ondansetron. The UPLC chromatograms of pantoprazole's calibration solutions were recorded by using the PDA detection at 288 nm as depicted in Fig. 2B. In the analysis of the related drug, the detection wavelength was chosen as 288 nm, which corresponds to maximum point in the spectral range of 210-380 nm (Fig. 2A). As it can be seen from Fig. 2B and 2C, the retention time of pantoprazole and ondansetron was observed as 1.71 and 1.15 min, respectively. Calibration curve was obtained by the regression of concentration on the peak area-ratio of the analyte to ondansetron at 288 nm. The statistical results for calibration curve of pantoprazole were summarized in Table-1. Pantoprazole in tablets was analyzed using the mentioned computed calibration curve.

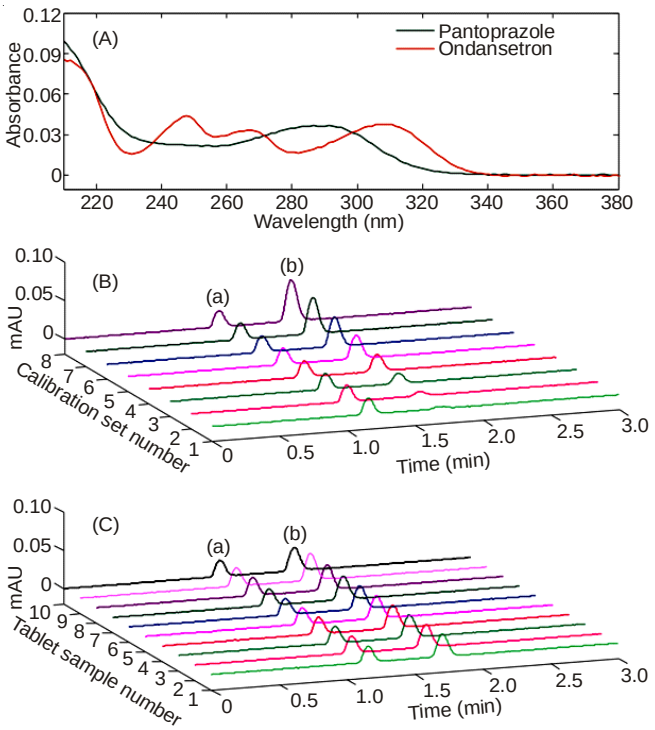

Fig. 2. (A) UV absorption spectra of pantoprazole (—) and ondansetron (—) (B) chromatograms of calibration set containing pantoprazole (b) in the concentration range of 2-40 µg/mL with ondansetron (a) and (C) chromatograms of tablet samples containing pantoprazole (b) and ondansetron (a)

Validation of RP-UPLC method: The validity of proposed RP-UPLC method was verified by using the validation parameters consisting of the linearity, precision, accuracy and specificity, limit of detection and limit of quantitation. In the application of the RP-UPLC method, a good linearity was observed for the calibration curve in the concentration range of 2-40 µg/mL with higher correlation coefficient 0.9998 as indicated in Table-1. The precision and accuracy of the appli-

TABLE-1
LINEAR REGRESSION ANALYSIS AND STATISTICAL RESULTS OF PANTOPRAZOLE

Parameter	Numerical values
Wavelength (nm)	288.0
Concentration range (µg/mL)	2-40
Slope of linear regression equation (m)	0.0701
Intercept of linear regression equation (n)	0.0381
Correlation coefficient (r)	0.9998
Standard deviation of slope (m)	5.35×10^{-4}
Standard deviation of incept (n)	1.25×10^{-2}
Standard deviation of correlation coefficient (r)	3.11×10^{-3}
Limit of detection (µg/mL)	0.54
Limit of quantitation (µg/mL)	1.79

cation of the proposed RP-UPLC method to the independent test solutions explained in the section of standard solution were confirmed by recovery assays with relative standard deviation. The results were illustrated in Table-2. Precision and accuracy of the RP-UPLC method was evaluated by the analysis of the intra-day samples containing three different levels (4, 22 and 34 µg/mL) with intervals of 1 h within the same day. The same procedure was adopted for inter-day experiments to evaluate the inter-day precision of the UPLC method. From the inter-day precision and accuracy for the consecutive three days we computed the percentage recovery, relative standard deviation (RSD %) and relative standard error (SE %). The numerical values for the recoveries, RSD % and SE % for intra-day and inter-day assays of pantoprazole were summarized in Table-3. Specificity of the proposed RP-UPLC was determined using standard addition technique. In the application of the standard addition technique, samples were obtained by adding the pure stock solution of pantoprazole (at different concentration levels 8, 16 and 24 µg/mL) to the solution of commercial tablets as shown in Table-4. After that, it was calculated the recoveries with standard deviation and relative standard deviation for pantoprazole and then results were given in Table-4. From the numerical results listed in Table-4, it was not observed any

TABLE-2
RECOVERY RESULTS OBTAINED FROM THE TEST SAMPLES BY THE DEVELOPED RP-UPLC METHOD

Exp. No.	Added (µg/µL)	Found (µg/µL)	Recovery (%)
1	4	3.93	98.3
2	16	15.66	97.9
3	28	28.19	100.7
4	40	40.23	100.6
5	20	20.19	101.0
		Mean	99.7
		SD	1.47
		RSD	1.47

TABLE4
ANALYSIS RESULTS OF PANTOPRAZOLE IN THE STANDARD ADDITION SAMPLES BY THE DEVELOPED RP-UPLC METHOD

Added (µg)	Found (µg/mL)	Recovery (%)	SD (%)	RSD (%)
8	8.15	101.9	2.47	2.43
16	16.71	104.4	1.80	1.73
24	24.34	101.4	1.70	1.68
	Mean	102.57	1.99	1.94

interference of excipients on the analysis of pantoprazole in tablets by the developed and validated RP-UPLC method.

Analysis of commercial tablet: The samples of commercial tablets containing pantoprazole in the presence of ondansetron were prepared and injected into UPLC system. As can be seen in Fig. 2C, the chromatograms of commercial tables were recorded between 0.0-3.0 min. The peak area of pantoprazole and ondansetron were estimated using Waters Empower2 software and then the peak-area ratio were computed. Replacing the calculated peak-area ratio into the calibration equation given in Table-1, the quantity of pantoprazole in commercial tablets was determined. Analysis results (mg/tablet) with relative standard deviation and relative standard deviation were illustrated in Table-5. In the short runtime of the RP-UPLC analysis, a good agreement was observed for the assay results of pantoprazole.

TABLE-5
DETERMINATION SAMPLES OF PANTOPRAZOLE IN COMMERCIAL TABLETS BY DEVELOPED RP-UPLC METHOD

Replicate	mg/tablet	Replicate	mg/tablet
1	42.1	6	42.2
2	41.4	7	40.8
3	41.2	8	41.2
4	40.7	9	40.1
5	42.1	10	40.2
Mean			41.21
Standard deviation			0.38
Relative standard deviation			0.93

Conclusion

The analysis of pantoprazole in commercial tablets was accomplished by using a new fast RP-UPLC method. In application of the validated RP-UPLC method, the determination was performed within the total runtime of 3 min with adequate precision and accuracy. It was concluded that the optimized and validated RP-UPLC method can be used for the quantitative estimation and routine analysis of pantoprazole in tablets.

TABLE-3
ASSAY RESULTS OF PANTOPRAZOLE IN THE INTER-DAY AND INTRA-DAY SAMPLES BY THE DEVELOPED RP-UPLC METHOD

Added (µg)	Found (µg/mL)		RSD (%)		SE (%)		Recovery (%)	
	Inter-day	Intra-day	Inter-day	Intra-day	Inter-day	Intra-day	Inter-day	Intra-day
4	3.93	3.90	1.15	1.95	-1.84	-2.57	98.2	97.4
22	22.44	22.27	0.45	0.25	2.00	1.23	102.0	101.2
34	33.89	34.19	1.97	0.83	-0.34	0.56	99.7	100.6
				Mean	-0.06	-0.26	99.9	99.7

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