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Research Article

A Validated HPLC-DAD Method for Simultaneous Determination of Etodolac and Pantoprazole in Rat Plasma

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A simple, sensitive, and accurate HPLC-DAD method has been developed and validated for the simultaneous determination of pantoprazole and etodolac in rat plasma as a tool for therapeutic drug monitoring. Optimal chromatographic separation of the analytes was achieved on a Waters Symmetry C18 column using a mobile phase that consisted of phosphate buffer pH~4.0 as eluent A and acetonitrile as eluent B in a ratio of A : B, 55 : 45 v/v for 6 min, pumped isocratically at a flow rate of 0.8 mL min^{-1}. The eluted analytes were monitored using photodiode array detector set to quantify samples at 254 nm. The method was linear with $r^2 = 0.9999$ for PTZ and $r^2 = 0.9995$ for ETD at a concentration range of 0.1–15 and 5–50 μgmL^{-1} for PTZ and ETD, respectively. The limits of detection were found to be 0.033 and 0.918 μgmL^{-1} for PTZ and ETD, respectively. The method was statistically validated for linearity, accuracy, precision, and selectivity following the International Conference for Harmonization (ICH) guidelines. The reproducibility of the method was reliable with the intra- and interday precision (% RSD) <7.76% for PTZ and <7.58 % for ETD.

1. Introduction

Pantoprazole (5-(difluoromethoxy)-2-[(3,4-dimethoxy-2-pyridyl)methylsulfinyl]1*H*-benzimidazole, PTZ, Figure 1(a)) is a selective long-acting proton pump inhibitor [1]. It is used for peptic ulcers, gastroesophageal reflux disease (GERD), Barrett's esophagus, and Zollinger-Ellison syndrome, as well as the eradication of *Helicobacter pylori* as part of combination regimens [2]. Etodolac (1,8-diethyl-1,3,4,9-tetrahydropyrano[3,4-*b*]-indole-1-acetic acid, ETD, Figure 1(b)) is a potent and well-tolerated nonsteroidal anti-inflammatory drug (NSAID), and is indicated for the treatment of acute pain and for the signs and symptoms of rheumatoid arthritis and osteoarthritis [3]. NSAIDs have been reported to cause gastrointestinal (GI) lesions and result in dyspeptic symptoms and ulcerations and lead to increased risk of serious GI complications [4, 5]. Approximately 20 million patients in the US consume NSAIDs on a regular basis; the risk for hospitalization for serious GI adverse effects is 1-2%, resulting in approximately 200,000 to 400,000 hospitalizations per year [6]. Hence, many patients are likely to receive both NSAIDs (either nonselective [7, 8] or selective COX-2 inhibitors [9]) and proton pump inhibitors.

Monitoring the combinations of NSAIDs and proton pump inhibitors in plasma helps to improve the effectiveness of therapy by minimizing drug toxicity and ensuring an appropriate dosage regimen. Therefore, detailed specific, reproducible, and accurate method for the quantitation of PTZ and ETD as candidates representing those classes of medications is of valuable importance.

A thorough review of the literature has revealed that several methods have been reported for the determination of pantoprazole alone or in combination in dosage forms and/or plasma. These methods included UV-spectrophotometry [10–13], HPLC-UV [14–18], LC-MS [19–21], capillary electrophoresis [22], thermogravimetric analysis [23], voltammetry [24, 25], densitometry [26], and biamperometric analysis [27]. Additionally, various methods have also been developed for the determination of etodolac solely or in combinations in pharmaceutical dosage forms and/or biological fluids.

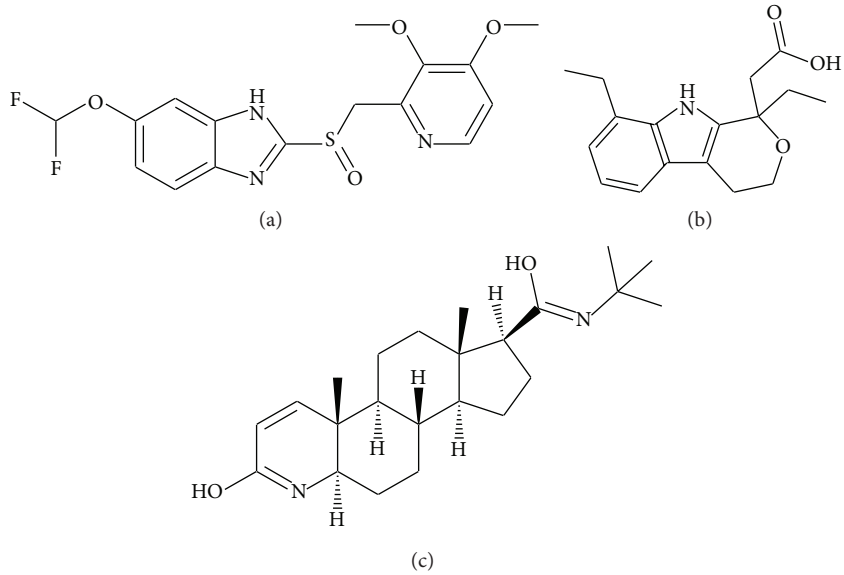

Figure 1: Chemical structures of (a) pantoprazole, (b) etodolac, and (c) finasteride (IS).

Such methods include spectrophotometry [28–30], spectrofluorimetry [31], HPLC-UV [32–34], LC-MS [35], GC-MS [36], voltammetry [37], and capillary electrophoresis [38]. However, simultaneous analysis of NSAIDs and proton pump inhibitors (PPIs) has only been reported once for the determination of ketoprofen, pantoprazole, and valsartan in human plasma using HPLC-UV [15]. Hence, this paper describes for the first time the development and validation of a sensitive, specific, and accurate HPLC-DAD method for the simultaneous determination of pantoprazole and etodolac in plasma.

2. Experimental

2.1. Chemicals and Reagents. Pantoprazole sodium sesquihydrate and etodolac both are European Pharmacopoeia (EP) Reference Standards (ETD CRS batch number 1 and PTZ; CRS batch number 1), were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA), and used as they are. HPLC-grade solvents and reagents were purchased from Merck (Darmstadt, Germany). Phosphate buffer solution (pH 4) was prepared by dissolving disodium hydrogen orthophosphate and potassium dihydrogen orthophosphate in distilled water, adjusting the pH to 4.0 ± 0.1 with glacial acetic acid and completing the volume. Deionized water was purified using cartridge system (Millipore, Bedford, MA, USA). Ultrapure water of 18 $\mu\Omega$ was obtained from Milli-Q plus purification system (Millipore, Bedford, MA, USA). Rat plasma was obtained from the animal house facility at College of Pharmacy, King Saud University (Riyadh, KSA) and was kept frozen until use after gentle thawing.

2.2. Apparatus. The LC system consisted of a Waters Breeze system (Waters Corporation, Milford, MA, USA) equipped with 1525 binary pump with on-line degasser, 717+ autosampler, 5CH thermostatted column compartment, and 2996 photodiode array (DAD) detector. Binary chromatography was carried out on a Symmetry C18 column (3.5 μm, 75 mm × 4.6 mm i.d) manufactured by Waters Corporation, Milford, MA, USA. The column temperature was kept constant at 25 ± 2°C. The system control and on-line data acquisition were performed using Waters Breeze software (Waters Corporation, Milford, MA, USA).

2.3. Chromatographic Conditions. The most suitable chromatographic conditions were achieved at a flow rate of 0.8 mL min^{-1} with a mobile phase that consisted of phosphate buffer (pH~4) : acetonitrile (45 : 55, v/v). The mobile phase was filtered through a Millipore vacuum filtration system equipped with a 0.45 μm pore size filter and degassed by ultrasonication. The samples (30 μL each) were injected by the aid of the autosampler. Quantification of PTZ and ETD was achieved with the DAD detector set at 254 nm. Prior to each run, the HPLC-DAD system was allowed to warm up for nearly 30 min and the baseline was monitored until it becomes stable before the samples were injected. For optimization of quantification wavelength, the photodiode array detector was used in scan mode with a scan range of 210–400 nm. Peaks identities were confirmed by retention time comparison and comparison of the spectra obtained from the DAD detector. The relation between the peak areas of PTZ and ETD and their concentrations was used as the basis for the quantification.

2.4. Standard Solutions. Stock solutions of PTZ and ETD (1 mgmL^{-1}) were prepared in methanol. Stock solutions were stored at −20°C. The working standard solutions were prepared by diluting aliquots (1 mL) of stock solutions into 10 mL volumetric flasks with methanol to give concentrations of 100 μgmL^{-1} for both PTZ and ETD. The internal standard (IS) finasteride stock solution was prepared in methanol to produce a concentration of 1.0 mgmL^{-1}. One mL of stock

solution (IS) was prepared into 10 mL measuring flask in methanol to produce a working solution of a 100 μgmL^{-1} concentration. All working solutions were stored at 4°C until required for analysis. The solutions were stable for at least two months when stored in refrigerator, and no evidence of degradation of the analytes was observed on the chromatograms during this period.

2.5. Sample Processing. Samples were prepared with volumes of 500 μL of rat plasma spiked with 50 μL of finasteride (IS, 100 μgmL^{-1}) and appropriate volumes of PTZ and ETD (according to different concentrations) in a 1.5 mL microcentrifuge tube (Eppendorf) thoroughly vortex-mixed for 1 min and then mixed with 200 μL of acetonitrile for deproteinization. Analytes were extracted with 5 mL ethyl acetate and centrifuged at 5,000 rpm for 10 min. The supernatant layer was separated and evaporated to dryness under a gentle stream of nitrogen using the 27-port Reacti-Vap evaporator (Thermo Fisher Scientific Inc., MA, USA). The residues were reconstituted in 1 mL mobile phase and filtered through a 0.45 μm Millex filter (Millipore, Bedford, MA, USA), and 30 μL of the filtrate was injected onto the analytical column.

2.6. Bioanalytical Method Validation. The described method was validated in terms of linearity, limit of detection (LOD), limit of quantification (LOQ), recovery, specificity, stability, precision, and accuracy according to international guidelines regarding bioanalytical method validation [39–41]. LOD and LOQ were calculated from the residual standard deviation of the regression line (σ) of the calibration curve and its slope (S) in accordance to the following equations:

$$\text{LOD} = 3.3\left(\frac{\sigma}{S}\right), \qquad \text{LOQ} = 10\left(\frac{\sigma}{S}\right). \tag{1}$$

2.6.1. Calibration and Control Samples. Appropriate volumes of PTZ and ETD working standard solutions (100 μgmL^{-1}) were added to drug-free rat plasma (20 mL) to prepare eight nonzero standard drug concentration of PTZ of 0.1, 0.5, 1, 3, 5, 7, 10, and 15 μgmL^{-1} and 5, 10, 15, 25, 30, 35, 45, and 50 μgmL^{-1} of ETD. Additionally, three quality control (QC) samples were prepared at concentrations of 0.3, 6, and 12 μgmL^{-1} of PTZ and 8, 20, and 40 μgmL^{-1} of ETD. Standard drug concentrations used for the preparation of the calibration curves were different from those employed in the quality control studies. A calibration curve was constructed from blank plasma sample, a zero sample (a plasma spiked with IS), and eight nonzero samples covering the total ranges (0.1–15 μgmL^{-1} for PTZ and 5–50 μgmL^{-1} for ETD). Each validation run consisted of system suitability sample, blank sample, a zero sample (a plasma processed with IS) calibration curves consisting of eight nonzero samples, and QC samples (n = 5, at each concentration). Such validation samples were generated on six consecutive days. Calibration samples were analyzed from low to high concentrations at the beginning of each validation run and the other samples were distributed randomly throughout the run. The peak area ratios of PTZ, ETD, and IS and their concentrations were used as the basis for the quantification. The calibration curves had correlation coefficients (r^2) = 0.9999 for PTZ and (r^2) = 0.9995 and for ETD.

2.6.2. Specificity. To evaluate the specificity of the method, drug-free plasma samples were examined throughout the assay procedure to ascertain the absence of any endogenous interference at the retention times of PTZ, ETD, and IS. Specificity of the method was assessed to test the matrix influence between different plasma samples.

2.6.3. Recovery. The absolute recoveries of PTZ and ETD were evaluated by comparing drug peak area ratios to IS of the spiked analytes samples to the unextracted analytes of stock solution that has been injected directly into the HPLC system. The assay absolute recovery for each drug, at five replicates of each concentration, was computed using the following equation:

$$\begin{aligned}&\text{absolute recovery}\\ &= \left(\frac{\text{peak area ratio (to IS) of extract}}{\text{mean peak area ratio (to IS) of direct injection}}\right)\\ &\quad \times 100,\\ &\text{relative recovery} = \left(\frac{\text{conc. of extract}}{\text{theoretical conc.}}\right) \times 100.\end{aligned} \tag{2}$$

2.6.4. Accuracy and Precision. Intraday accuracy and precision evaluations were performed by repeated analysis of PTZ and ETD in rat plasma. The run consisted of a calibration curve along with five replicates of each low, medium, and high QC samples. Interday accuracy and precision were also assessed by the analysis of samples consisting of calibration curves and five replicates of low, medium, and high QC samples for PTZ and ETD on three consecutive days. The overall precision of the method was expressed as relative standard deviation and accuracy of the method was expressed in terms of bias (percentage deviation from true value). Relative error (accuracy) was estimated as percent error [Mean determined value − theoretical (added amount)]/theoretical × 100.

2.6.5. Stability. The stability of QC sample solutions of PTZ and ETD was evaluated under several conditions. The solutions were stored in tightly capped volumetric flasks, on a laboratory bench at room temperature (6 h) and autosampler (24 h). Recoveries of samples solutions stored in the refrigerator for 7 days and one month in −80°C were checked against freshly prepared solutions. Freeze-thaw stability of the samples was determined over three freeze-thaw cycles, by thawing at room temperature for 6 h and refreezing for 12–24 h. For each QC sample, six replicates were analyzed in one analytical batch. The concentration of ETD and PTZ after each freeze-thaw cycle was related to the initial concentration as determined for the samples.

2.6.6. Robustness and Ruggedness. In order to measure the extent of method robustness, the most critical parameters were interchanged within the range of 1–10% of the optimum

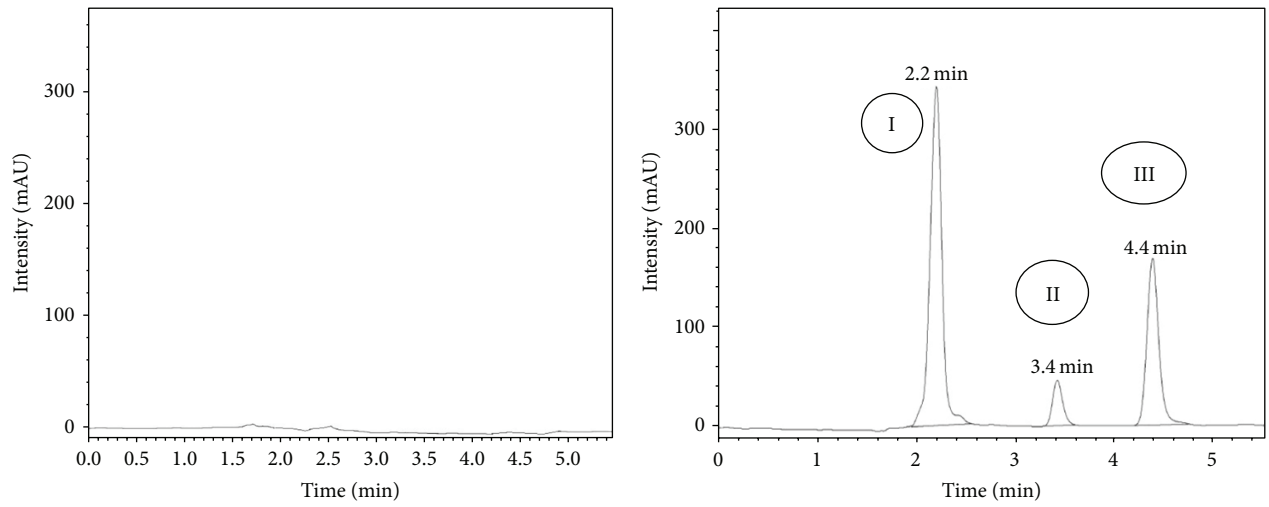

Figure 2: Representative HPLC chromatogram for the analysis of PTZ and ETD in rat plasma: (a) blank rat plasma, (b) rat plasma spiked with ETD (RT = 2.2 min) (I), IS (RT = 3.4 min) (II), and PTZ (RT = 4.4 min) (III), respectively.

recommended conditions while keeping the other parameters unchanged and in parallel the peak areas and retention times of PTZ and ETD were observed and recorded. The studied parameters were the composition of the mobile phase, pH, flow rate, and column temperature. Ruggedness of the method was determined using the mobile phase components from two different manufacturers and two different analysts.

3. Results and Discussion

3.1. Chromatographic Separation. Typical chromatograms of blank and plasma samples spiked with internal standard, PTZ and ETD are shown in Figure 2. Under the aforementioned described chromatographic conditions, IS PTZ and ETD were well resolved in plasma after efficient extraction procedure. The peaks were of good shape and completely resolved and eluted at a retention time of 3.4, 4.4, and 2.2 min, for PTZ, ETD, and IS, respectively. Optimization was achieved by monitoring various reversed-phase columns, mobile phase systems, and flow rates.

3.2. Method Validation. The proposed method was fully validated in terms of sensitivity, linearity, selectivity, accuracy, intra- and interday precision, and system suitability. Method validation was conducted according to recommendations of the International Conference on Harmonisation (ICH) [39] and the guidelines of the Food and Drug Administration (FDA) for validation of analytical procedures and methods [40].

3.2.1. Linearity and Sensitivity. Using the previously mentioned optimum chromatographic conditions, three independent calibration curves were constructed, correlating the calculated peak area ratios of PTZ and ETD to IS versus their corresponding concentrations. Calibration plots for PTZ and ETD were prepared daily at eight nonzero concentrations,

Table 1: Analytical parameters for determination of PTZ and ETD via the proposed method.

Parameter	PTZ	ETD
Concentration range (μgmL^{-1})	0.1–15	5–50
Intercept ± SD	0.0369 ± 0.0045	0.07229 ± 0.01911
Slope ± SD	0.45537 ± 0.00242	0.06867 ± 0.000619
Correlation coefficient (r^2)	0.9999	0.9995
LOD (μgmL^{-1})	0.033	0.918
LOQ (μgmL^{-1})	0.0988	2.783
Linearity range	0.1–15	5–50
Retention time (min)	4.4	2.2

and each concentration was injected in five replicates. The peak area ratios of PTZ and ETD to IS in rat plasma were linear with respect to the analytes concentrations over the concentration ranges of 0.1–15 μgmL^{-1} and 5–50 μgmL^{-1} for PTZ and ETD, respectively. The mean linear regression equation of the peak ratio (y) versus drug concentration (μgmL^{-1}) in rat plasma samples (x) showed correlation coefficient r^2 = 0.9999 and r^2 = 0.9995 for ETD over the concentration ranges used (Table 1). The high r^2 value was indicative for the good linearity, and the low values of standard deviations of the intercept and the slope were indicative for the significant validity of the calibration points used for constructing the calibration curve. The method is selective as no interference was observed in drug-free plasma at the retention times of PTZ and ETD.

3.2.2. Limits of Detection and Quantification. The limit of quantitation (LOQ) is the lowest concentration that can be measured with acceptable accuracy and precision for

Table 2: Data of back-calculated PTZ and ETD concentrations of the calibration standards in rat plasma.

Drug	Nominal concentration (μgmL^{-1})	Mean[a] ± SD (μgmL^{-1})	Precision (% RSD)	Accuracy (% error)
PTZ	0.1	0.11 ± 0.01	9.09	10
	0.5	0.50 ± 0.01	2.78	0.67
	1.0	1.09 ± 0.07	6.88	8.68
	3.0	3.14 ± 0.22	7.01	4.67
	5.0	4.93 ± 0.39	7.91	−1.43
	7.0	7.44 ± 0.38	5.11	6.31
	10.0	9.77 ± 0.79	8.08	−2.23
	15.0	14.39 ± 0.68	4.72	−4.07
ETD	5.0	4.97 ± 0.24	4.83	−0.57
	10.0	10.64 ± 0.80	7.52	6.40
	15.0	14.90 ± 0.52	3.49	−0.67
	25.0	24.13 ± 1.21	5.01	−3.48
	30.0	32.51 ± 1.99	6.12	8.37
	35.0	36.93 ± 1.39	3.76	5.51
	45.0	42.25 ± 2.88	6.82	−6.11
	50.0	52.03 ± 1.91	3.67	4.06

[a]Average of six replicates.

Table 3: Intraday and interday accuracy and precision of quality control samples of PTZ and ETD obtained by HPLC-DAD.

Day of analysis	PTZ			ETD		
	Low QC (0.3 μgmL^{-1})	Medium QC (6 μgmL^{-1})	High QC (12 μgmL^{-1})	Low QC (8 μgmL^{-1})	Medium QC (20 μgmL^{-1})	High QC (40 μgmL^{-1})
Day 1	0.296	5.94	13.27	8.36	21.24	42.63
	0.286	5.67	12.47	8.15	20.37	41.65
	0.312	6.23	12.84	8.91	20.56	42.19
	0.326	6.84	12.08	7.62	19.62	39.41
Day 2	0.249	6.45	11.99	7.98	19.10	43.54
	0.281	5.86	12.61	9.11	20.28	39.16
	0.274	5.34	11.83	7.64	21.71	39.08
	0.296	6.94	11.26	8.17	19.37	40.42
	0.315	6.41	12.47	8.78	19.88	40.37
Day 3	0.292	6.36	13.16	8.94	19.74	41.28
	0.295	6.14	12.61	9.64	21.13	38.68
	0.308	5.72	11.45	9.82	20.09	39.46
	0.291	6.15	11.27	8.42	20.54	39.44
	0.318	5.73	11.43	8.68	21.81	42.18
	0.315	6.89	12.51	8.28	19.37	41.72
Mean ± SD (μgmL^{-1})	0.29 ± 0.02	6.18 ± 0.48	12.22 ± 0.66	8.56 ± 0.65	20.32 ± 0.85	40.75 ± 1.52
Precision (% RSD)	6.89	7.76	5.40	7.59	4.18	3.73
Accuracy (% error)	−3.33	2.97	1.83	7.08	1.60	1.87

the analytes. LOQ values were calculated to be 0.0988 and 2.783 μgmL^{-1} for PTZ and ETD, respectively. The limit of detection (LOD) values were 0.033 and 0.918 μgmL^{-1} for PTZ and ETD, respectively. The limit of detection and LOQ were determined at 3 and 10 times the baseline noise, respectively, following the United States of pharmacopoeia procedure [42]. Table 2 summarizes the back-calculation of PTZ ad ETD concentrations of the calibration standards in rat plasma. The accuracy (% error) for the analytes covering the concentration ranges varied from −4.07 to 10% for PTZ and −6.11–8.37% for ETD, while precision ranged from 2.78–9.09% for PTZ and from 3.49–7.52% for ETD.

3.2.3. Accuracy and Precision-Quality Control (QC) Samples. The precision and accuracy at low, medium, and high QC samples of PTZ and ETD in rat plasma were within the acceptable limits (Table 3). Intra- and interdays relative standard deviations (precision, % RSD) ranged <7.76% for PTZ and <7.58% for ETD. Accuracy was estimated as percent error (relative error) [(measured concentration − spiked

Table 4: Data of freeze-thaw stability of ETD and PTZ plasma samples for QC samples.

Sample number	PTZ						ETD					
	Low QC (0.3 μgmL^{-1})		Medium QC (6 μgmL^{-1})		High QC (12 μgmL^{-1})		Low QC (8 μgmL^{-1})		Medium QC (20 μgmL^{-1})		High QC (40 μgmL^{-1})	
	Initial conc.	3rd cycle	Initial conc.	3rd cycle	Initial conc.	3rd cycle	Initial conc.	3rd cycle	Initial conc.	3rd cycle	Initial conc.	3rd cycle
Mean[a]	0.32	0.31	6.14	5.96	11.95	11.68	8.31	8.22	21.23	20.12	40.62	40.08
SD	0.02	0.02	0.42	0.48	0.68	0.49	0.57	0.41	0.76	0.95	2.31	1.54
Precision (% RSD)	6.25	6.45	6.84	8.05	5.69	4.19	6.86	4.99	3.58	4.72	5.69	3.84
Recovery (%)	106.67	103.33	102.33	99.33	99.58	97.33	103.88	102.75	106.15	100.60	101.55	100.20
Stability (%)		96.87		97.07		97.74		98.91		94.77		98.67

[a]Average of six replicates.

Table 5: Recovery of QC samples for determining the concentration of PTZ and ETD in plasma.

Sample number	PTZ			ETD		
	Low QC (0.3 μgmL^{-1})	Medium QC (6 μgmL^{-1})	High QC (12 μgmL^{-1})	Low QC (8 μgmL^{-1})	Medium QC (20 μgmL^{-1})	High QC (40 μgmL^{-1})
Mean[a]	0.31	5.98	12.73	8.24	20.44	40.79
Recovery (%)	103.33	99.67	106.08	102.96	102.20	101.98
Standard deviation (SD)	0.025	0.38	0.61	0.51	0.88	1.92
Precision (% RSD)	8.06	6.35	4.79	6.19	4.31	4.71

[a]Average of six replicates.

concentration)/spiked concentration] × 100, while precision was reported as % relative standard deviation (%RSD) = (S.D./mean) × 100 (Table 3).

3.2.4. Specificity. There were no interfering peaks present in six different randomly selected samples of drug-free rat plasma used for the analysis at the retention times of either analytes or internal standard (Figure 2).

3.2.5. Stability. The stability of QC sample solutions of PTZ and ETD evaluated under several conditions revealed that all samples were found to be stable with no evidence of samples degradation under the studied conditions. Three freeze-thaw cycle (Table 4) for QC samples indicated that ETD and PTZ were stable in rat plasma under the experimental condition.

3.2.6. Recovery. The recoveries of PTZ and ETD from rat plasma reported in Table 5, were determined at concentrations of QC samples by comparing each peak area ratio (to IS) of extracted samples with mean peak area ratio (to IS) of unextracted standard solutions containing the corresponding concentrations in the mobile phase that represented 100% recovery.

3.2.7. Robustness and Ruggedness. The studied parameters were the composition of the mobile phase, pH, and flow rate at sample concentrations of 6 and 20 μgmL^{-1} for PTZ and ETD, respectively. Results revealed relative standard deviation (RSD) values of less than 8% for PTZ and 6% for ETD for peak areas ratio to the internal standard. Moreover, RSD was 0.42% for PTZ and 0.52% for ETD for retention times for consecutive measurements and different analysts. Additionally, ruggedness was determined by using mobile phase components from two different manufactures and two different analysts. There was no significant change observed in the retention times of PTZ; RSD values ranged from 0.32 to 0.49% for PTZ and from 0.42 to 0.57 for ETD. The results indicated that small change in the chromatographic conditions did not have significant effect on the determination of PTZ and ETD. This proves that the method is highly rugged and capable of producing results with high precision.

4. Conclusions

Concurrent administration of proton pump inhibitors and nonsteroidal anti-inflammatory drugs (NSAIDs) has been previously reported due to the gastrointestinal problems arising from the chronic administration of NSAIDs alone. The aim of the current study was to develop a reliable, sensitive, and reproducible HPLC-DAD method for simultaneous determination of pantoprazole (PTZ, proton pump inhibitor) and etodolac (ETD, NSAID) in rat plasma. The method was extensively validated for the assay of PTZ and ETD in rat plasma which allows the quantification of PTZ and ETD in biological plasma samples for the purpose of bioequivalence study in the range of 0.1–15 μgmL^{-1} and 5–50 μgmL^{-1} for PTZ and ETD, respectively. Monitoring of the concentration of PTZ and ETD in biological fluids such as plasma is important to enable pharmacokinetic studies of both drugs to be undertaken in a hospital. Moreover, the method described herein can be readily used in any

clinical laboratory for routine application because of the simple sample preparation procedure and high specificity. The proposed method showed that acceptable accuracy, precision, sensitivity, and good linear concentration ranges cover the reported plasma concentration levels of both drugs. The method has demonstrated that it can be easily and reliably used in pharmacokinetic studies and deems to be suitable for use in all laboratories.

Conflict of Interests

The authors of this paper report no conflict of interests and have no financial and personal relationships with other people or organizations that could influence their work.

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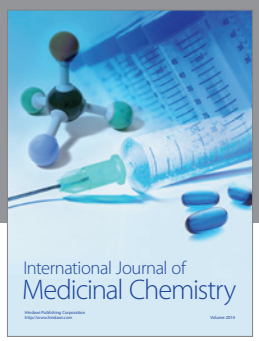

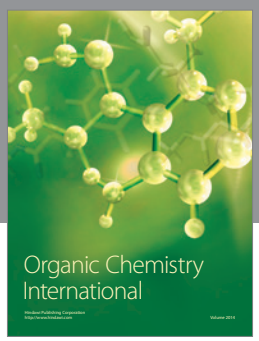

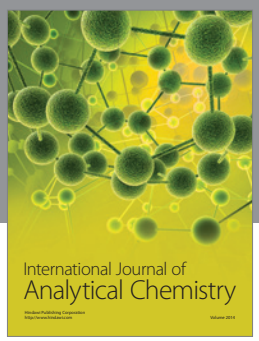

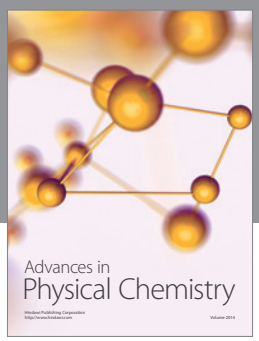

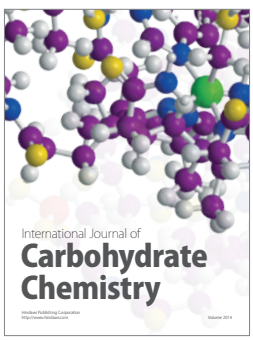

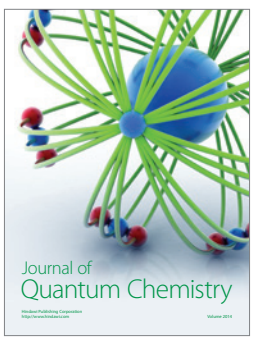

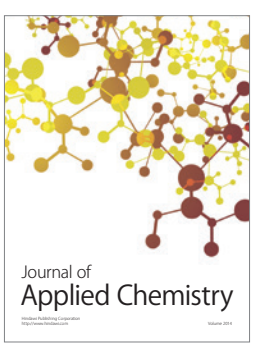

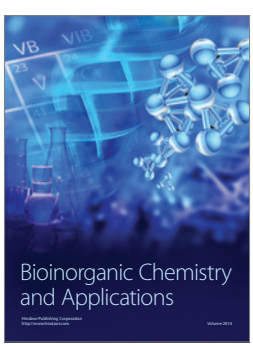

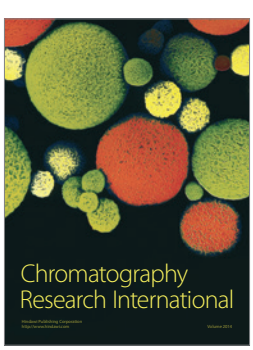

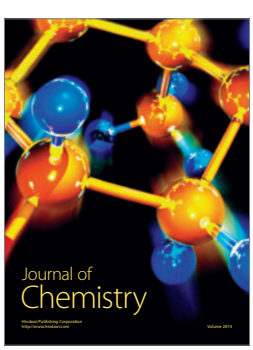

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