Original Article

Thai Journal of Pharmaceutical Sciences

Development and validation of RP-HPLC method for simultaneous determination of ondansetron hydrochloride and granisetron hydrochloride in their admixtures with pantoprazole sodium

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Received: Feb 14, 2019
Accepted: Jan 13, 2020
Published: Apr 19, 2020

ABSTRACT

Objective: Development of rapid and sensitive high-performance liquid chromatography method for the quantification of ondansetron hydrochloride and granisetron hydrochloride in their admixtures with pantoprazole sodium in its dosage form using ketorolac tromethamine as internal standard. **Materials and Methods:** Effective chromatographic separation of ondansetron hydrochloride or granisetron hydrochloride with pantoprazole sodium was achieved using Hypersil BDS-C18 column with isocratic elution of the mobile phase composed of acetonitrile:10 mM acetate buffer:trimethylamine (20:80:0.5, v/v/v), pH 3.5. Detection was adjusted at dual wavelengths 290 and 305 nm for pantoprazole sodium and ondansetron hydrochloride or pantoprazole sodium and granisetron hydrochloride, respectively. **Results:** The correlation coefficient for pantoprazole sodium, ondansetron hydrochloride, and granisetron hydrochloride was 0.9973, 0.9996, and 0.9988, respectively, limits of detection were 1.94, 1.21, and 0.67 µg/mL and limits of quantitation were 2.03, 3.66, and 5.90 µg/mL, respectively, which indicated high sensitivity of the proposed method. **Conclusion:** The developed method can be used for quanti-cation of ondansetron hydrochloride and granisetron hydrochloride in their admixtures with pantoprazole sodium in its dosage form.

Keywords: High-performance liquid chromatography, Ondansetron hydrochloride, Granisetron hydrochloride, Pantoprazole sodium

INTRODUCTION

The serotonin (5-HT_3) antagonists, ondansetron hydrochloride (OND) and granisetron hydrochloride (GRN) [Figure 1], have become first-line therapy for the treatment of postoperative nausea and emesis as well as emetogenic side effects of cancer chemotherapy.[1-4] The biotransformation of these drugs is mediated by multiple cytochrome P-450 enzymes, among them the polymorphic CYP2D6 and CYP1A2 resulting in a high interindividual variability in plasma concentrations and effectiveness.[5,6] Control of emetogenic side effects is of major importance for the success of cancer chemotherapy.

British Pharmacopoeia reports non-aqueous titrimetric and HPLC methods[7] for the studied drugs. Methods based on several techniques such as spectrofluorimetry,[8,9] UV spectrophotometry,[10-14] potentiometry,[15] voltammetry,[16-18] radioimmunoassay,[19] and flow injection[20,21] have been developed for assay of the studied (5-HT_3) antagonists. Separational methods based on thin-layer chromatography[22-24] and high-performance liquid chromatography[25-29] have also been employed extensively for the estimation of these drugs.

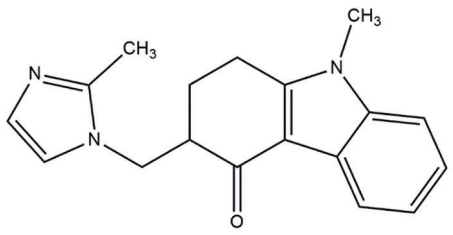
Pantoprazole sodium (PAN) is chemically known as 5-(Difluoromethoxy)-2-{[3, 4-dimethoxy-2-pyridinyl Methyl}

sulfinyl]-1H-Benzimidazole [Figure 1]. It suppresses gastric acid secretion by H^+/K^+-ATPase enzyme system at the secretary surface of the gastric parietal cell. This drug is used for the treatment of duodenal, gastric, and esophageal ulceration. PAN is official drug in the United States Pharmacopoeia and British Pharmacopoeia. A combination regimen of OND or GRN plus PAN has been suggested in patients with peptic ulcer and gastroesophageal reflux disease (GERD) to prevent nausea. Literature reveals that various spectrophotometric,[30,31] colorimetric,[32] thin-layer chromatographic,[33] spectroflouorimetric,[34] and HPLC[35,36] have been reported for the determination of PAN in pharmaceutical preparations. In the present work, we hope to develop a simple accurate and validated RP-HPLC method for simultaneous determination of PAN and OND or GRN in bulk and their admixtures.

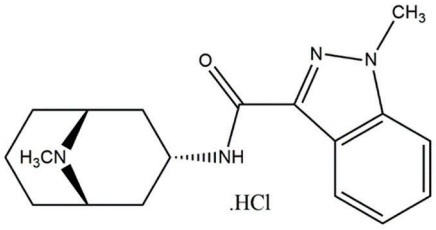
MATERIALS AND METHODS

Reagents and Chemicals

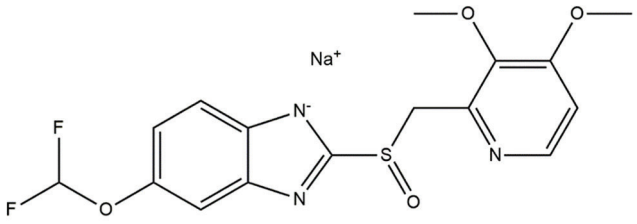
All solvents and reagents used were of analytical reagent grade. Ondansetron HCl (OND) (98.0%) was kindly supplied by Global Napi Pharmaceuticals Co. (October 6, Egypt). GRN (98.5%) was obtained from European Egyptian Pharmaceuticals. PAN (99.96%) was obtained from EI-Nasr Pharmaceutical. Ketorolac tromethamine (KET) (99.0%) was obtained from European Egyptian Pharmaceuticals. Sodium acetate trihydrate was obtained from EI-Nasr Pharmaceutical Chemical Co. (Abu-Zaabal, Egypt). Acetic acid obtained from EI-Nasr Pharmaceutical Chemical Co. (Abu-Zaabal, Egypt). Methanol and acetonitrile HPLC grade were obtained from Sigma-Aldrich (U.S.A). Pharmaceutical formulations, Danset® ampoules labeled to contain 4 mg OND per ampoule, Granitryl® ampoules labeled to contain 1 mg GRN/ampoule, and Pantoloc® tablet labeled to contain 20 mg PAN per tablet.



Ondansetron hydrochloride dihyrate



Granisetron hydrochloride



Pantoprazole sodium

Figure 1: Chemical structures of the investigated drugs

Instrumentation

A Younglin Autochro-3000 HPLC system (Younglin, Korea) with UV detector, a Rheodyne injection valve with a 20 µL loop was used. Hypersil BDS-C18 (150 mm × 4.6 mm, 5 µm i.d.) (Shandon Scientific, Cheshire, UK) was used.

Chromatographic Conditions

Compounds were separated isocratically on Hypersil BDS-C18 column (150 mm × 4.6 mm, 5 µm i.d.) (Shandon Scientific, Cheshire, UK) that was maintained at ambient temperature (25 ± 2° C). Column washing was done using acetonitrile:water in ratio (50:50, v/v). The mobile phase was acetonitrile:10 mM acetate buffer:trimethylamine (20:80:0.5, v/v/v), pH 3.5. The mobile phase was filtered and degassed by sonication before use. The flow rate was 1 mL min^{-1}. Detection was adjusted at dual wavelengths 290 and 305 nm for PAN and OND or GRN, respectively.

PREPARATION OF SOLUTIONS

Preparation of Buffer

A 10 mM acetate buffer pH 3.5 was prepared by dissolving 0.34 g of sodium acetate trihydrate in 250 mL of distilled water; then, 1.5 mL triethylamine was added and adjusted to pH 3.5 with acetic acid

Preparation of Standard Solutions

An accurately weighed amount (25 mg) of OND, GRN, and PAN was transferred quantitatively to 100 mL standard flask. About 60 mL of methanol was added to the contents of the flask and shacked well for about 10 min. The solution was then completed to the mark with methanol to give stock standard solution of OND or GRN and PAN containing 250 µg/mL. The working standard solutions were prepared by further dilution of the stock solutions with the same solvent to obtain concentrations ranging from 5 to 25 µg/mL. The stock and working standard solutions were kept in refrigerator at about 4–8°C in light protected flasks.

PROCEDURES

Analysis of PAN Tablets (Pantoloc Tablets)

Ten tablets were weighed, finely powdered, and mixed thoroughly. An accurately weighed amount equivalent to the average weight of one tablet was transferred into a 100 mL volumetric flask. The contents of the flask were mixed with

about 80 mL of methanol and sonicated for about 15 min and then the volume was made to the mark with the same solvent and filtered. The first portion of the filtrate was rejected. Further dilution of the stock solution to prepare solutions containing the desired concentrations of PAN was added.

Analysis of OND and GRN Ampoules

The sample solutions were prepared by mixing the contents of 5 ampoules and diluting quantitatively to 25 mL with methanol in a measuring flask. The working sample solutions were prepared by further dilution of stock sample solutions with methanol immediately before use to prepare solutions containing the desired concentrations of OND or GRN.

Analytical Method Validation

The procedure was fully validated in accordance with ICH guidelines (ICH, 2005),[37] for linearity, range, sensitivity, accuracy, precision, and robustness, and according to the US Pharmacopeia guidelines[38] as well as ICH guidelines for limits of detection and quantification (LOD and LOQ) (*United States Pharmacopeia*, 2016), for the statistical analysis, Excel 2016 (Microsoft Office) was used. About 5% significance level was selected.

Linearity and range

Calibration curves were constructed by plotting peak area ratio (y) of OND, GRN, and PAN to the internal standard versus their concentrations (x). The concentrations range from 5 to 25 μg/mL. Linear regression equations were obtained, the required parameters were calculated using Microsoft Excel 2016 and the range for linearity was computed for both drugs.

Sensitivity parameters

The sensitivity of the method was estimated by examining the limit of detection (LOD; the lowest concentration of an analyte in a sample that can be detected but not necessarily quantified) and the limit of quantification (LOQ; the lowest concentration of analyte in a sample that can be determined with reasonable precision and accuracy under the stated operational conditions of the method).

The signal-to-noise ratio (S/N) was calculated according to the guidelines of the *United States Pharmacopeia* (2016) using the following expression:

$$5/N = 2H/h_n$$

Where, H is the height of the peak, related to the average base signal (baseline) calculated from the distance between the maximum of the peak and the extrapolated baseline of the signal observed over a distance equal to 20 times the width at half-height ($w_{0.5}$); h_n is the maximum spread of the baseline signal observed over a distance equal to 20 times the width at half-height of the peak. Limits of detection and quantification are then defined as the concentration offering an S/N of 3 or 10, respectively.

Accuracy

Accuracy was calculated using standard addition method, by adding a known amount of each drug standard to a certain concentration of dosage form. Three different concentration levels were measured covering low, medium, and high levels of calibration curves for each drug.

Precision

Intraday precision was determined by three replicate analysis (n = 3) of standard solutions of the admixture at three concentration levels (5, 15, and 25 μg/mL) covering the low, medium, and higher ranges of the calibration curve. The interday precision was conducted by repeating the analysis of standard solutions of the admixture at three concentration levels (5, 15, and 25 μg/mL) covering the low, medium, and higher ranges of the calibration curve over a period of 3 consecutive working days. The overall precision of the method was expressed as percentage relative standard deviations (%RSD).

Robustness

The robustness of the proposed methods was examined by studying the effects of minor changes in the optimized experimental parameters such as mobile phase composition ratio, wavelength, flow rate, pH, and concentration of buffer on the peak area ratio, retention time, and the method performance.

System suitability parameters

The system suitability parameters were calculated for a representative chromatogram, including retention factor, separation factor, resolution, number of theoretical plates, height equivalent to theoretical plates, and tailing factor.

RESULTS AND DISCUSSION

Method Validation

The developed method was validated for the following parameters:

Linearity

The linearity of the method was checked by analyzing six solutions, each solution was prepared in triplicate in the range of 5–25 μg/mL for all drugs [Figures 2 and 3, Table 1]. Calibration curves were constructed by plotting peak area ratio (y) of OND, GRN, and PAN to the internal standard versus OND, GRN, and PAN concentrations (x) and fitted to the equation $y = a + bx$ [Figure 4].

Limits of detection and quantification

Limits of detection were 0.5, 1.0, and 1.5 μg/mL and limits of quantitation were 1.5, 3.0, and 5.0 μg/mL for OND, GRN, and PAN, respectively, which indicated high sensitivity of the proposed method [Figure 5].

Accuracy

Method accuracy was determined by addition of known amounts of standard OND, GRN, and PAN to a sample solution of known concentration and calculating the recovery percentages. The results in Table 2 revealed a good accuracy and recovery percentages ranged from 98.17 to 100.50%, 97.87 to 100.84%, and 98.27 to 99.68% for OND, GRN, and PAN, respectively.

Precision

The results in Table 3 show good precision and repeatability of the proposed method where the % RSD ≤ 3.13, 3.05, and 2.46

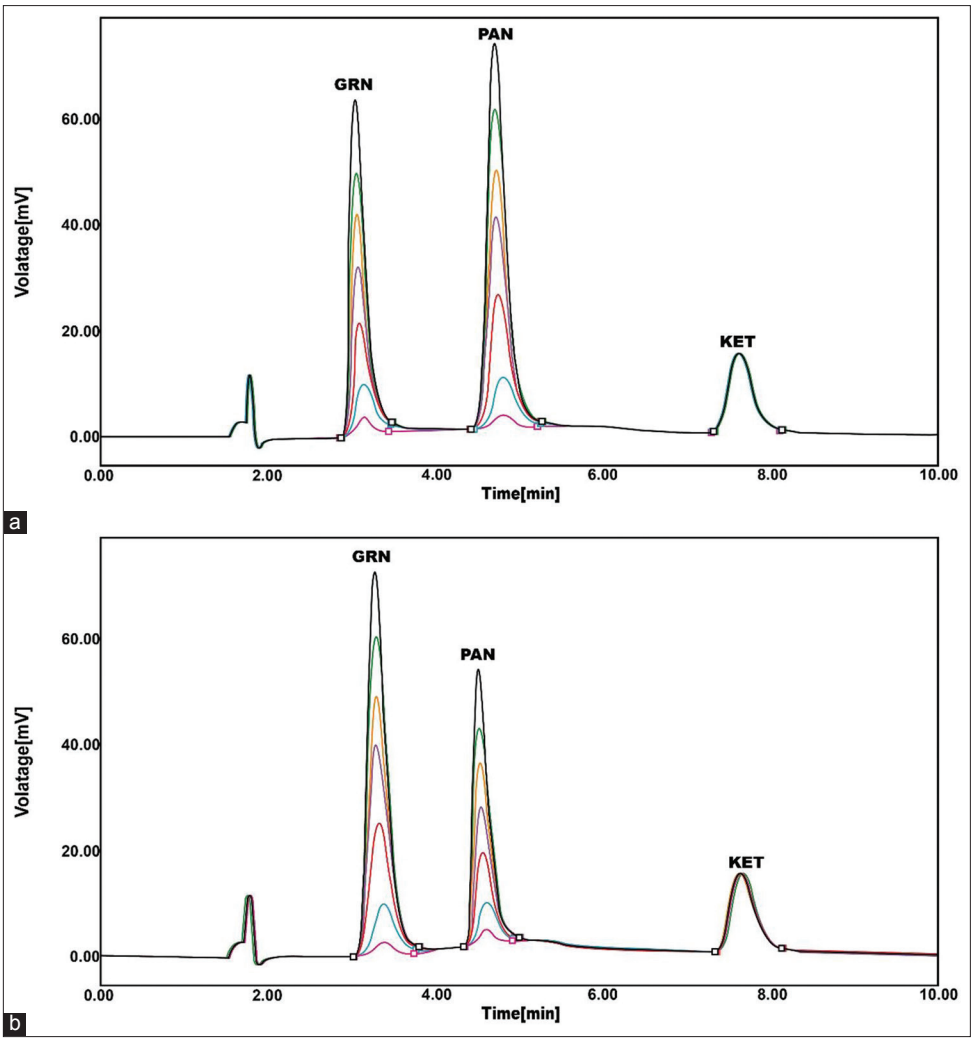


Figure 2: Overlaid chromatograms illustrating the calibration curve of GRN and PAN admixture in concentrations range of 5–25 µg/mL of each drug in the admixture using the proposed HPLC method (a) at 290 nm and (b) at 305 nm

Table 1: Quantitative parameters and statistical data of the proposed HPLC method for the determination of OND/GRN and PAN admixture

Drug	Linearity range (µg/mL)	Intercept±SD	Slope±SD	r
OND	5–25	0.01±0.015	0.07±0.001	0.9996
GRN	5–25	0.01±0.02	0.05±0.001	0.9988
PAN	5–25	−0.07±0.08	0.13±0.004	0.9973

Table 2: Standard addition method for the assay of OND, GRN, and PAN admixture using the proposed HPLC method

Dosage form (content)	Drug amount taken (µg)	Pure drug added (µg)	Pure drug found (µg)	%Recovery±ecover[a]
Danset® ampoules (OND)	5.00	00.00	4.91	98.17±1.29
(4 mg/amp)		5.00	5.05	100.50±2.21
		10.00	9.87	99.17±0.74
Pantoloc® tablet (PAN)		00.00	4.91	98.27±1.60
(20 mg/tablet)		5.00	4.97	99.68±1.37
		10.00	9.87	99.14±1.78
Granitryl® ampoules (GRN)		00.00	5.04	100.84±2.51
(1 mg/amp)		5.00	4.79	97.87±0.82
		10.00	9.89	99.27±1.55

[a]Average of three determinations

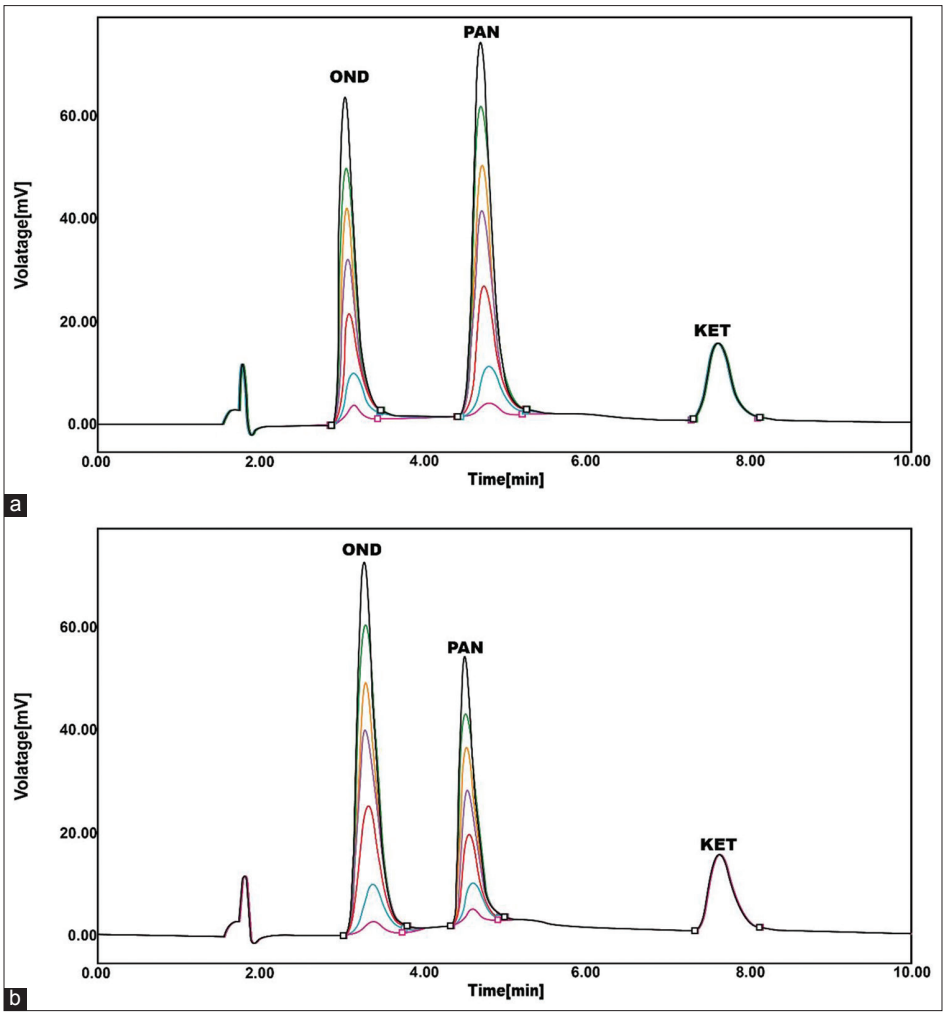


Figure 3: Overlaid chromatograms illustrating the calibration curve of OND and PAN admixture in concentrations range of 5–25 μg/mL of each drug in the admixture using the proposed HPLC method (a) at 290 nm and (b) at 305 nm

Table 3: Intraday and interday precision of the proposed HPLC method for simultaneous determination of OND, GRN, and PAN admixtures

Drug	Conc. (μg/mL)	Intraday precision (n=3)		Interday precision (n=9)	
		%Found	%RSD	%Found	%RSD
OND	5	96.55	3.13	97.43	3.09
	15	97.63	1.38	97.48	1.10
	25	99.32	1.75	99.72	1.10
PAN	5	99.05	2.21	97.81	1.82
	15	99.75	2.46	99.99	2.45
	25	98.77	1.80	98.86	1.59
GRN	5	100.93	3.05	101.73	2.94
	15	99.97	1.82	100.01	1.49
	25	102.09	2.27	101.78	1.94

for OND, GRN, and PAN, respectively, which makes it adequate for application in the quality control laboratories.

Selectivity and specificity

Peak purity and identity can be evaluated by comparison of the retention time values of the pure substances and drugs presented in tablet and ampoule. Since retention time value is characteristic for any given compound provided that the same stationary and mobile phases are used, it can provide corroborative evidence to the identity of a compound. In the determination of the mixture of OND and PAN, retention times were; in pure 3.30 ± 0.02 and 5.05 ± 0.04 for OND and PAN, respectively, while in

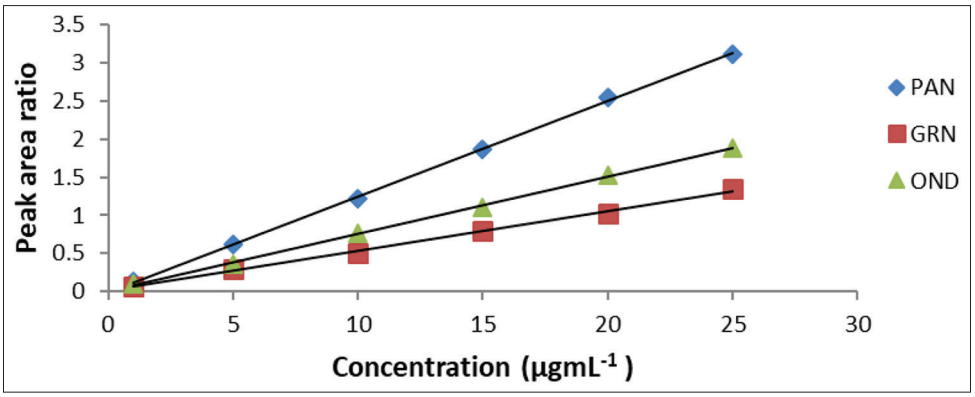


Figure 4: The calibration curves for the HPLC analysis of the studied admixtures

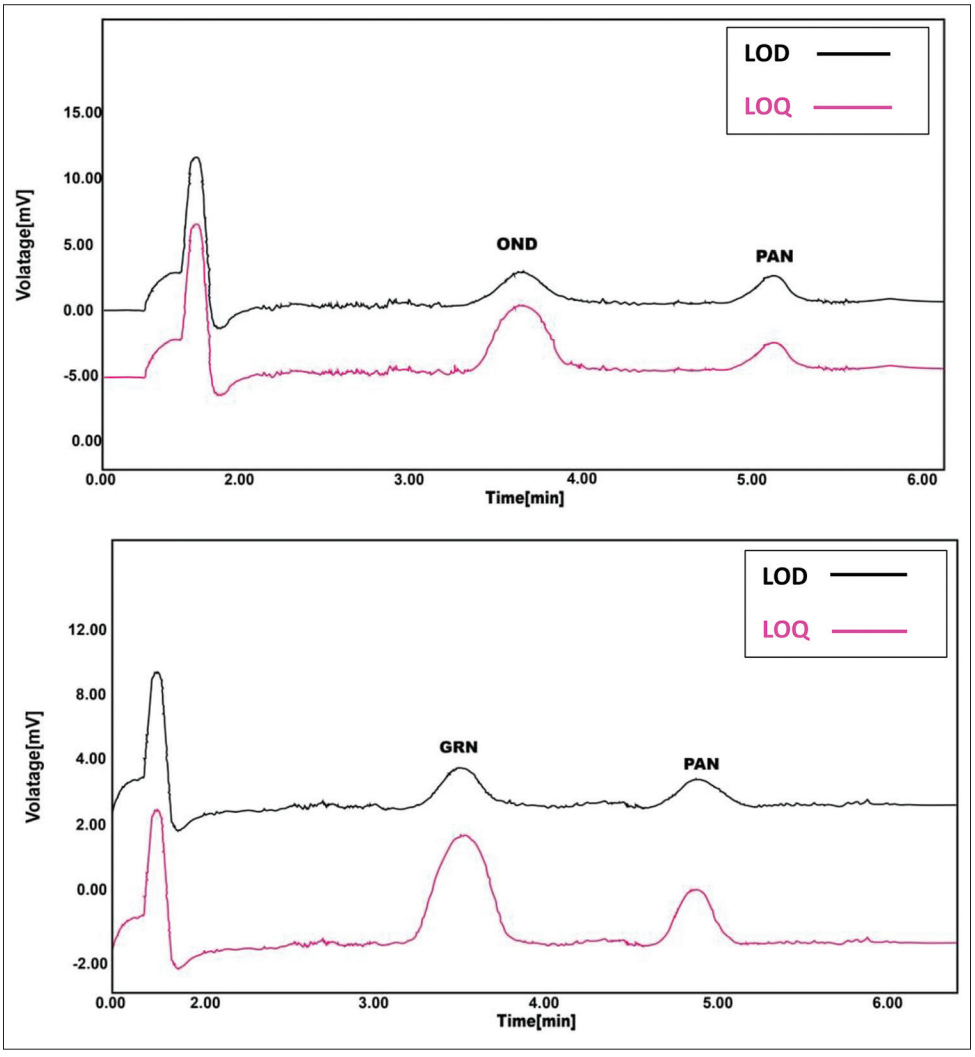


Figure 5: HPLC chromatogram representing LOD and LOQ for OND, GRN, and PAN calculated based on S/N approach (0.5, 1.0, and 1.5 µg mL^{-1} and 1.5, 3.0, and 5.0 µg mL^{-1}, respectively).

dosage forms were 3.35 ± 0.01 and 5.07 ± 0 for OND and PAN, respectively [Figure 6]. In the determination of the mixture of GRN and PAN, retention times were; in pure 2.91 ± 0.02 and 4.56 ± 0.04 for GRN and PAN, respectively, while in dosage forms were 2.94 ± 0.01 and 4.58 ± 0 for GRN and PAN, respectively [Figure 6]. There is no significant difference in the retention time values at different positions between the compared peaks.

Robustness

The studied parameters were pH (±0.1) and mobile phase composition; acetonitrile (±1%), acetate buffer conc. (±2.5 mM), determination wavelength (±2 nm), and flow rate (±0.1). It was found that slight variation of these variables did not significantly affect the performance of the

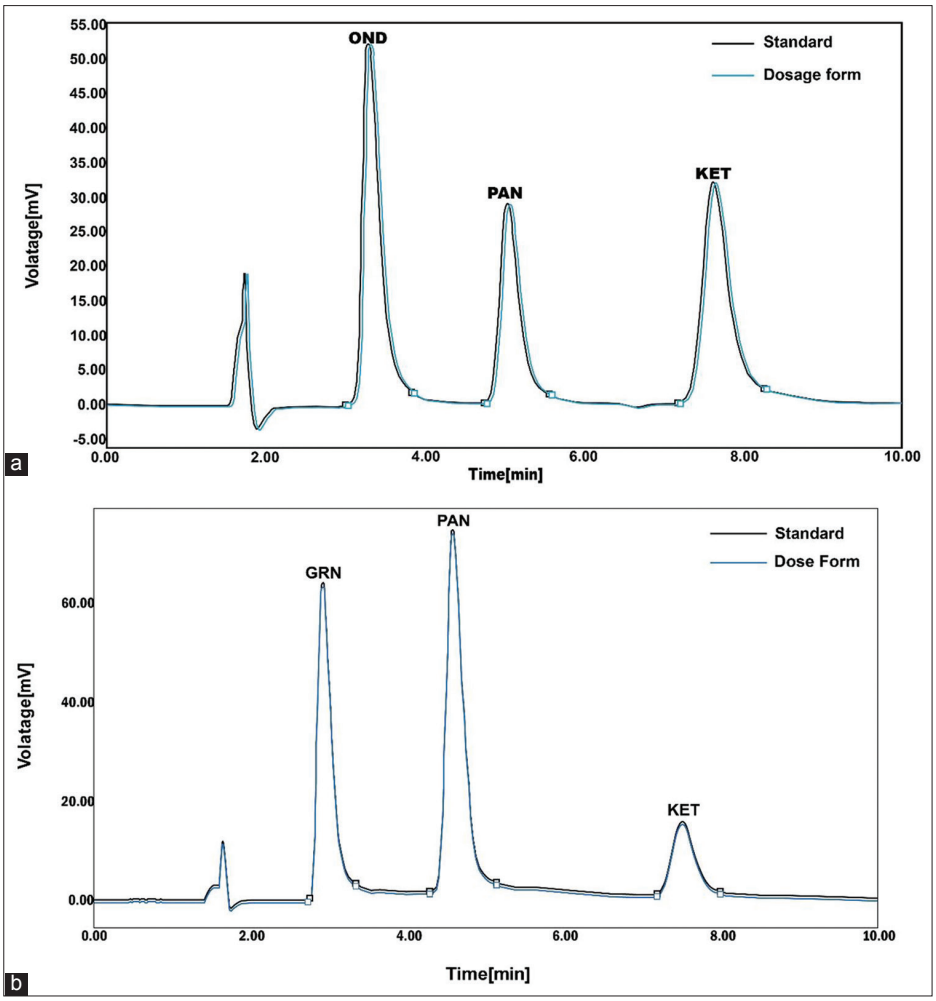


Figure 6: Overlaid chromatograms for HPLC separation of (a) standard and dosage form of OND and PAN admixture (20 µg/mL of OND and 20 µg/mL of PAN) and (b) the standard and dosage form of GRN and PAN admixture (20 µg/mL of GRN and 20 µg/mL of PAN)

Table 4: Robustness of the proposed HPLC method for the determination of OND/GRN and PAN admixture

Experimental parameters	%Found±%RSD[a]		
	PAN	OND	GRN
Results under optimized conditions	98.45±2.36	99.43±0.95	99.82±2.10
1 – pH (3.5)			
3.4	97.66±0.69	97.75±0.46	100.25±0.46
3.6	101.15±1.34	100.80±0.75	102.90±1.03
2 – Acetonitrile (20%)			
19	96.34±0.87	99.12±1.56	99.93±0.73
21	99.08±1.36	102.89±3.20	99.61±0.79
3 – Buffer conc. (10.0 mM)			
7.5	99.09±1.11	100.64±0.62	101.68±0.97
12.5	97.09±2.13	100.38±0.19	99.84±0.81
4 – Flow rate (1.0 mL/min)			
0.9	96.66±1.28	99.62±1.02	101.00±0.78
1.1	96.84±1.78	98.55±0.19	101.41±0.95
5 – Detection wavelength (290/305 nm)			
(288/303)	97.45±2.24	99.42±0.64	101.60±1.28
(292/307)	96.57±1.92	97.47±1.60	100.30±2.83

[a]Average of three determinations

Table 5: System suitability parameters for the investigated drugs by the developed isocratic HPLC method

System suitability test parameters[a]	GRN	OND	PAN	Reference value
Retention time (min)	2.91	3.30	4.56/5.05	
Tailing factor (asymmetric factor)[b]	1.0	1.0	1.0	1.0
Retention factor (k')[c]	0.85	0.85	1.78/1.68	1-10
Number of theoretical plates (N)[f]	1075.84	1223	1980.25	-
Height equivalent to a theoretical plate (H.E.T.P.)[g]	139.42	122.65	75.75	-
	OND-PAN	GRN-PAN		-
Resolution (R_s)[d]	3.71	4.21		1.5
Selectivity factor (α)[e]	2.09	1.98		1.25

[a]Average of three determinations, [B]calculated at 10.00% peak height, [c]$k' = (t_r - t_0)/t_0$, where t_r is the retention of analytes and t_0 is the column dead time, [d]$R_s = 2(t_2 - t_1) / (w_2 + w_1)$. Where, t_2 and t_1 are the retention of the second and first peaks, w_2 and w_1 are the peaks widths of the second and first peaks. [e]Selectivity factor, calculated as k_2/k_1., [f]$N = 16 (t_R/w)^2$, [g]H.E.T.P. = L/N where, L is the length of the column.

Table 6: Application of the proposed HPLC method for simultaneous determination of OND/GRN and PAN in dosage forms

Dosage forms (content)	% Recovery±Recov[a]		*t*-value[b]	*F*-value[b]
	Proposed Method	Reported Method		
Danset® ampoules (OND) (4 mg/amp)	100.84±0.86	101.18±0.74	0.73	1.34
Pantoloc® tablet (PAN) (20 mg/tablet)	101.29±0.88	101.08±0.50	0.49	3.16
Granitryl® ampoules (GRN) (1 mg /amp)	100.79±0.39	101.01±0.21	1.21	3.37

[a]Average of six determinations, [B]theoretical value for *t* and *F* at 95% confidence limit, *t*=2.30, *F*=5.05(39)

proposed method, as shown in Table 4. Hence, the proposed method could be considered robust and reliable during the normal usage.

System suitability test

The test was performed by applying the standard or sample mixtures 3 times and the parameters were calculated as reported by the USP. The obtained parameters under the optimum chromatographic conditions are reported in Table 5.

APPLICATION OF THE PROPOSED HPLC METHOD FOR ANALYSIS OF THE STUDIED ADMIXTURE IN DOSAGE FORMS

The proposed method was applied successfully for the determination of the studied drugs in the pharmaceutical dosage forms. Three replicate measurements were made; the results obtained for tablet and ampoule analysis were validated by comparison with a previously reported method. No significant difference was found by applying *t* and *F* tests at 95% confidence level indicating good accuracy, precision, and suitability of the proposed method for the determination of the investigated drugs in pharmaceutical dosage forms, as shown in Table 6.[39]

CONCLUSION

The present work aims to develop an efficient, fast HPLC method for simultaneous determination of granisetron in its admixtures with pantoprazole. The developed method is simple, selective, and sensitive. The method is reliable for the accurate determination of studied drugs in bulk, in commercial tablets and could be recommended for the routine use in quality control laboratories. Enhancement of sensitivity, precision, and accuracy of methods used for analysis of the previously studied admixtures (ondansetron and pantoprazole) using HPLC method.

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