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Development and Validation of analytical method for the determination of Rabeprazole and Ondansetron in pharmaceutical dosage form by Reversed-phase HPLC

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Abstract: The present work describes a validated reverse phase high performance liquid chromatographic method for simultaneous estimation of Rabeprazole and Ondansetron in pharmaceutical dosage form. Chromatography was performed on a ODS Hypersil C18 (250 mm x 4.6 mm i.d., 5 µm particle size) column with mobile phase containing Buffer (Ammonium Acetate, pH-5.5): Water: Methanol (25: 15: 60 v/v/v). The flow rate was 1.0 ml/min and the eluent was monitored at 275 nm. The selected chromatographic conditions were found to effectively separate Rabeprazole (RT- 5.941 min) and Ondansetron (RT- 3.541 min). Linearity for Rabeprazole and Ondansetron were found in the range of 1-20 µg/ml. The values obtained of LODs were 0.093 and 0.16 µg/ml, LOQs were 0.28 and 0.49 µg/ml for Rabeprazole and Ondansetron, respectively. The proposed method was found to be fast, accurate, precise, reproducible and rugged and can be used for simultaneous analysis of Rabeprazole and Ondansetron in combined pharmaceutical formulations.
Keywords: Rabeprazole, Ondansetron, Reversed-phase HPLC.

Introduction

Ondansetron (ONDA) hydrochloride is chemically 1, 2, 3, 9-tetrahydro-9-methyl-3-[(2-methyl-1H-imidazol-1-yl) methyl]-4H-carbazol-4-one, monohydrochloride, dihydrate. It represents the class of selective $5HT_3$ antagonists which is commonly employed as anti-emetic in combination with anti-ulcer and anti-cancer agents[1-5]. Literature survey revealed that very few analytical methods have been reported for the estimation of ONDA which includes HPLC [6-11], HPTLC [12]. Second drug, Rabeprazole (RABE) a sodium salt of 2-[[[4-(3-methoxypropoxy)-3-methyl-2-pyridinyl]-methyl] sulfinyl]-1H–benzimidazole, represents the class of orally active H^+ - K^+ ATPase Inhibitors (Proton Pump Inhibitor) employed in the management of gastric ulcer[13-15]. The individual determination of Rabeprazole has been carried out in formulations by HPLC[16-19], HPTLC[20], Capillary Electrophoresis[21], LC-MS/MS[22] and Derivative Spectroscopy [23]. Literature review did not reveal any method for simultaneous determination of RABE and ONDA in combined pharmaceutical dosage form. So,

we decided to work towards development and validation of simple, sensitive, accurate, precise, rugged and economic method for simultaneous determination of these drugs in combined dosage forms. The present work describes a validated reverse phase HPLC method for simultaneous determination of these drugs in combined dosage form.

Figure 1. Structure of Ondansetron

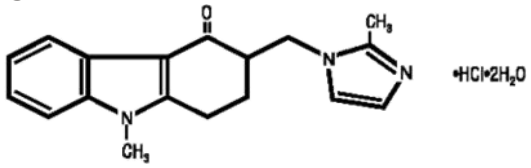
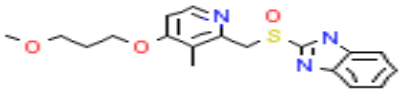


Figure 2. Structure of Rabeprazole



Experimental

Apparatus

A Shimadzu RP-HPLC instrument (LC-10AT vp) equipped with an photodiode array detector, manual injector with 20 µl loop, and Phenomenex (Torrance, CA) C18 column (250 mm × 4.6 mm id, 5 µm particle size) and Class-VP software were used. Sartorius CP224S analytical balance (Gottingen, Germany), and ultra sonic cleaner (Frontline FS 4, Mumbai, India) were used during the study.

Reagents and materials

Rabeprazole and Ondansetron were received as gift samples from Torrent Research Centre, Ahmedabed. Methanol used in mobile phase was of HPLC grade, Ammonium Acetate buffer was of AR grade and water for RP-HPLC was prepared by triple glass distillation and filtered through a nylon 0.45 µm membrane filter (Gelman Laboratory, Mumbai, India). Pharmaceutical formulation of RABE and ONDA were purchased from local pharmacy.

Chromatographic conditions

Phenomenex C18 column (250 mm x 4.6 mm i.d., 5 µm particle size) was used at ambient temperature. The mobile phase consisted of Buffer (Ammonium Acetate, pH-5.5): Water: Methanol (25: 15: 60 v/v/v) at a flow rate of 1.0 ml/min. The mobile phase was filtered through a nylon 0.45 µm membrane filter and degassed before use. The elution was monitored at 275 nm, and the injection volume was 20 µl.

Preparation of solutions

Ammonium Acetate Buffer

Accurately weighed Ammonium Acetate (2.5 gm) was transferred to a beaker (500 ml) and dissolved in tripled distilled water (500 ml).

Mobile phase

The mobile phase was Buffer (Ammonium Acetate, pH-5.5)-Water-Methanol (25-15-60).

RABE and ONDA standard stock solutions

Accurately weighed RABE (50 mg) and ONDA (50 mg) were transferred to two separate 100 ml volumetric flask. 50 ml methanol was added to the flask. The drug was dissolved with sonication and the final volume was adjusted with methanol up to the mark to prepare a 500 □g/ml stock solution of both drugs.

RABE and ONDA working standard solution (100 µg/ml)

From the above stock solutions (500 □g/ml) of both drugs, an accurately measured 20 ml volume of the stock solutions were transferred into separate 100 ml volumetric flasks and the final volume were adjusted with methanol to prepare 100 □g/ml working solutions.

Sample solutions

Powder was collected from 20 capsules and accurately weighed quantity of the powder equivalent to about 10 mg of RABE and 2 mg of ONDA was transferred in 100 ml measuring flask. Powder was dissolved in methanol and sonicate for 20 minutes. The solution was filtered through Whatman filter paper No. 41 and the residues were washed thoroughly with methanol. The filtrate and washings were combined in a 100 ml volumetric flask and diluted to the mark with methanol to get final concentrations of 100 □g/ml of RABE and 20 □g/ml of ONDA.

Determination of wavelength of maximum absorbance

The standard solutions of RABE and ONDA were scanned in the range of 200 -400 nm against mobile phase as a blank. RABE and ONDA showed maximum absorbance at 275 nm. So the wavelength selected for the determination of RABE and ONDA was 275 nm.

Method Validation

Calibration curve (Linearity)

Calibration curves were plotted over a concentration range of 1-20 µg /ml for RABE and ONDA. Accurately measured standard stock solutions of RABE and ONDA (0.1, 0.5, 1, 1.5, 2, 2.5 and 3 ml) were transferred to a series of 10 ml volumetric flasks and the volume in each flask was adjusted to 10 ml with mobile phase. The resulting solutions were injected into the column and the peak area obtained at flow rate of 1.0 ml/min for RABE and ONDA

respectively. Calibration curves were constructed for RABE and ONDA by plotting peak area versus concentration at 275 nm. Each reading was average of three determinations.

Accuracy (% Recovery)

Accuracy of the method was assured by use of the standard addition technique, involving analysis of formulation samples to which certain amounts of authentic drugs were added. The resulting mixtures were assayed, and the results obtained for both drugs were compared to those expected. The recovery experiments were carried out in triplicate by spiking previously analyzed samples of the tablets (RABE 4 µg/ml and ONDA 4 µg/ml) with three different concentrations of standards (RABE 1,2,4, µg/ml and ONDA 1,2,4, µg/ml). The good recoveries with the standard addition method (Table 4) prove the good accuracy of the proposed method.

Method precision

For evaluation of precision, repeatability of the results for a concentration of 4 µg/ml was evaluated by six replicate determinations. For evaluation of intermediate precision, the results over the concentration range 1 - 20 µg/ml were evaluated by three replicate determinations to estimate intraday variation and another replicate determination on three different days to estimate interday variation. The coefficients of variation (CV) values at these concentration levels were calculated. Relative standard deviation of all the parameters was less than 2%, which indicates that the proposed method is repeatable.

Limit of detection and limit of quantification

The limit of detection (LOD) and the limit of quantification (LOQ) for both drugs were derived by calculating the signal-to-noise ratio (S/N, i.e., 3.3 for LOD and 10 for LOQ) and using following equations as per International Conference on Harmonization (ICH) guidelines.

$LOD = 3.3 \times \sigma/S$

$LOQ = 10 \times \sigma/S$

Where σ = the standard deviation of the responses and S = Slope of calibration curve.

Specificity

The ICH document define specificity as the ability to assess unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products, and matrix components. In the case of assay, demonstration of specificity requires that it can be shown that the procedure is unaffected by the presence of impurities or excipients.

Robustness

The robustness of an analytical method is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

System suitability

System suitability parameter is established to ensure that the validity of the analytical method is maintained whenever used. Typical variations are the stability of analytical solution, different equipment, and different analyzer. In case of liquid chromatography typical variations are the pH of the mobile phase, the mobile phase composition, different lots or supplier of columns, the temperature and flow rate.

Analysis RABE and ONDA in combined dosage forms

Pharmaceutical formulation of RABE and ONDA was purchased from local pharmacy. The responses of formulations were measured at 275 nm for quantification of RABE and ONDA by using RP-HPLC. The amounts of RABE and ONDA present in sample solution were determined by fitting the responses into the regression equation for RABE and ONDA. Results are given in Table 5.

Table 1. Common chromatographic parameters

Parameters	RABE	ONDA
Retention Time	5.941	3.541
Tailing factor	1.01	0.96
Asymmetry factor	0.87	1.02
Theoretical plate	2258.94	2070.63

Table 2. Statistical analysis of parameters required for system suitability testing of the HPLC method

Parameters	*Mean*		S.D.		% CV	
	RABE	ONDA	RABE	ONDA	RABE	ONDA
Retention Time	5.941	3.541	0	0	0	0
Area	40972.67	34438.67	211.62	195.97	0.5165	0.569
Asymmetric Factor	0.8733	1.02	0.0052	0	0.595	0
Tailing Factor	1.003	0.958	0.0051	0.0040	0.051	0.417

Table 3. Optical and Regression characteristics and validation parameters of HPLC method for analysis of RABE and ONDA

Parameters	RABE	ONDA
Calibration range	1-20µg/ml	1-20µg/ml
Detection limit	0.093 µg/ml	0.16 µg/ml
Quantitation limit	0.28 µg/ml	0.49 µg/ml
Slope	25989	20261
Intercept	14185	24739
Mean	100.105	100.534
Standard deviation	1.6448	1.5117
Coefficient of variance	1.643	1.5037
Correlation coefficient	0.9976	0.9902
Intraday RSD, %	0.109-1.28	0.083-1.29
Interday RSD, %	0.061-0.622	0.129-1.03

[a]Intraday and interday relative standard deviation(RSD) values of the whole concentration range

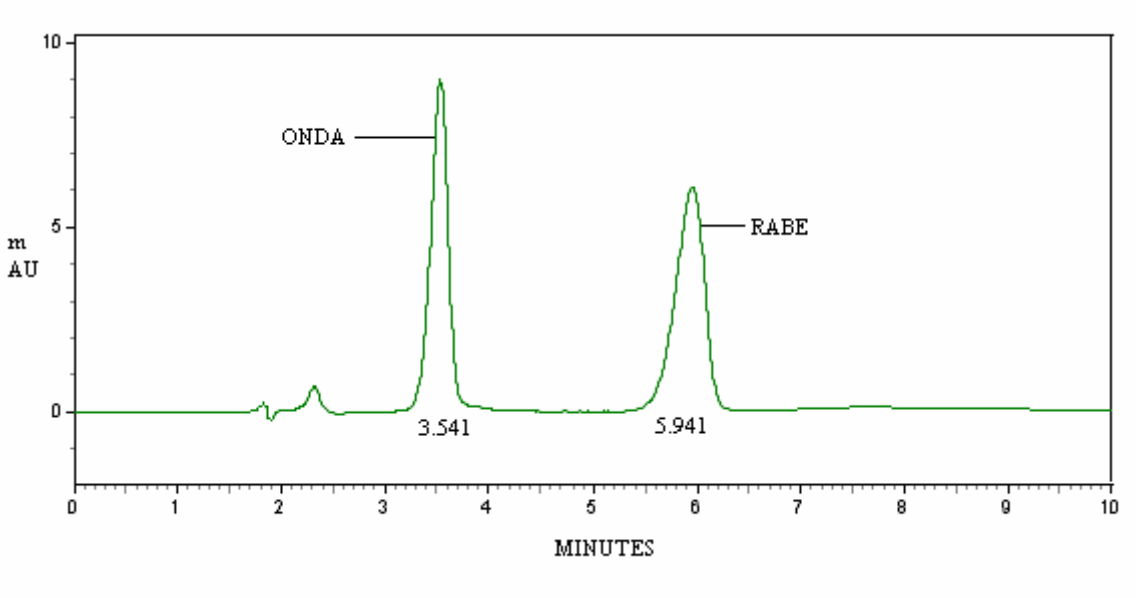
Table 4. Data of recovery study for RABE and ONDA by HPLC method

Drug	Amount taken (µg/ml)	Amount added (µg/ml)	Amount found (µg/ml)	% Recovery ± S.D (n=3)
RABE	4	1	5.056	101.515 ± 0.582
	4	2	6.038	100.552 ± 1.063
	4	4	7.859	98.23 ± 0.830
ONDA	4	1	5.155	103.107 ± 0.727
	4	2	6.228	103.83 ± 0.907
	4	4	8.273	103.412 ± 1.260

Table 5. Application of the proposed method to the pharmaceutical dosage forms

RABE			ONDA		
Amount taken (µg /ml)	Amount found (µg /ml)	% Amount found S.D. (n=3)	Amount taken (µg /ml)	Amount found (µg /ml)	% Amount found S.D. (n=3)
10	9.85	98.52 ± 1.59	2	1.96	98.208 ± 0.895
15	15.28	101.86 ± 0.586	3	2.93	97.624 ± 0.338
20	20.24	101.20 ± 0.298	4	3.94	98.624 ± 1.46

Figure 3. A typical RP-HPLC chromatogram RABE (10 µg/ml) and ONDA (10µg/ml) with corresponding retention time



Result and Discussion

To optimize the RP-HPLC parameters, several mobile phase compositions were tried. A satisfactory separation and good peak symmetry was found in a mixture of Ammonium Acetate, methanol and water (25:60:15) at 1.0 ml/min flow rate. The optimum wavelength for detection was set at 275 nm at which much better detector responses for both drugs were obtained. As it was shown in Fig. 3 the retention times were 5.9412 min for RABE and 3.541 min for ONDA. Common Chrimatographic parameters are outlined in table 1.The calibration graphs for RABE and ONDA were constructed by plotting the peak area versus their corresponding concentrations, good linearity for both was found over the range 1-20 µg/ml. Results obtained by applying the RP-HPLC method showed that the concentrations of RABE and ONDA can be simultaneously determined in prepared mixtures. The proposed method has been applied to the assay of RABE and ONDA in pharmaceutical dosage form. The validity of the method was further assessed by applying the standard addition technique. The results obtained indicate the additives present do not interfere with analysis of the studied mixtures. System suitability test parameters for RABE and ONDA for the RP-HPLC method are reported in Table 2. The optical and regression characteristics and validation parameters are reported in Table 3.

Conclusion

All these factors lead to the conclusion that the proposed method is accurate, precise, simple, sensitive, rugged and rapid and can be applied successfully for the estimation of RABE and ONDA in pharmaceutical formulations without interference and with good sensitivity.

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