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Development and validation of Q-absorbance ratio spectrophotometric method for simultaneous estimation of ondansetron HCl and esomeprazole magnesium in bulk and formulation

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ABSTRACT: The current study describes a Q- Absorption Ratio spectrophotometric method development and validation for the simultaneous determination of esomeprazole magnesium and ondansetron hydrochloride. The concentration of the drugs was determined by using the ratio of absorbance at the iso-absorptive point (λ_1 = 275 nm) and the λ_{max} of esomeprazole magnesium (λ_2 = 302 nm). This method is linear for both drugs in the range of 5–25 µg/mL at λ_1 (R^2 = 0.999) and at λ_2 (R^2 = 0.9998) for ondansetron hydrochloride, and esomeprazole magnesium found at λ_1 (R^2 = 0.9995) and λ_2 (R^2 = 0.9997). According to ICH recommendations Q2(R1), statistical analysis and recovery studies have been used to validate the analysis findings. The % Recovery was 97.78% – 100.81 % of ondansetron and 100.33% – 102.03 % of esomeprazole by the standard addition method. The method was found to be precise as the % relative standard deviation was less than 2.00 in interday and intraday precision for ondansetron and esomeprazole. The results of the current study indicated that the developed method is simple, linear, precise, accurate, and sensitive, hence may be used for quality control purposes.

KEYWORDS: Ondansetron hydrochloride; esomeprazole; UV-spectrophotometer; validation; accuracy; precision.

1. INTRODUCTION

Ondansetron (OND) is a 9-methyl-3-[(2-methyl-1H-imidazol-1-yl)-methyl]-1,2,3,9-tetrahydro-4H-carbazol (Fig. 1) [1], is a selective 5-HT_3 receptor antagonist. It works centrally in the region postrema's chemoreceptor trigger zone as well as peripherally on vagal nerve terminals. Common uses of ondansetron include the prevention of chemotherapy-induced and radiation-induced nausea and vomiting, the prevention of postoperative nausea and vomiting, and off-label use for the prevention of nausea and vomiting associated with pregnancy. It belongs to the class of antiemetics and has the chemical formula $C_{18}H_{19}N_3O$ and a molecular weight of 293.4 g/mol, respectively [2,3]. Esomeprazole (ESO) is [5-methoxy-2-[(S)-[(4-methoxy-3,5-dimethyl-2-pyridinyl) methyl]sulfinyl] in its chemical makeup magnesium trihydrate (1H-benzimidazole-1-yl) (Fig. 2). It has a molecular weight of 767.2 g/mol and the chemical formula $(C_{17}H_{18}N_3O_3S)_2$ $Mg.3H_2O$ [4]. The gastric parietal H+/K ATPase, which is involved in the generation of hydrochloric acid in the stomach, is irreversibly inhibited by esomeprazole, the S-isomer of omeprazole. It functions as a proton pump inhibitor and is used to treat gastric ulcers, erosive esophagitis, and gastroesophageal reflux disease (GERD) [5].

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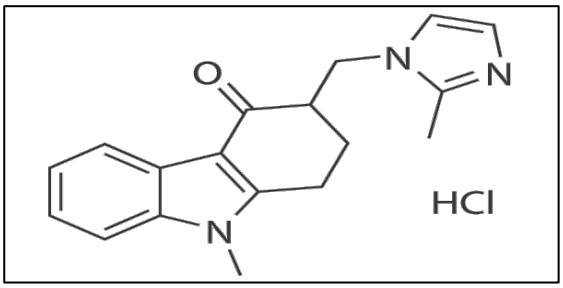

Figure 1. Structure of ondansetron hydrochloride

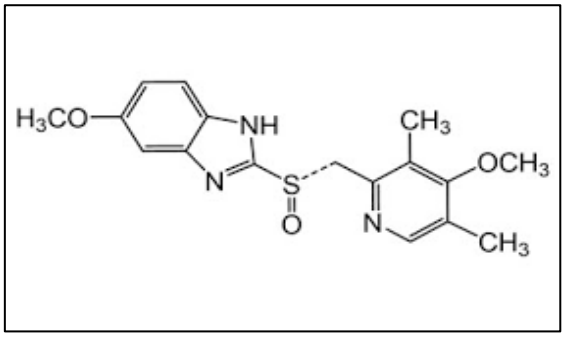

Figure 2. Structure of esomeprazole magnesium

Ultraviolet and visible spectrophotometry is one of the most frequently employed analytical tools in the pharmaceutical industry. The absorbance ratio approach relies on the property that the ratio of absorbances at any two wavelengths is a constant number irrespective of concentration or path length to simultaneously estimate two components [5].

A literature review revealed several methods, including UV [1], HPLC [6, 7], HPTLC [8], LC-ESI-MS/MS [9], and spectro-fluorimetry [10] for the analysis of individual drugs and combinations of drugs, but no method for the simultaneous determination by absorptive ratio method for ondansetron hydrochloride and esomeprazole magnesium was published. Therefore, the goal of the current work was to design and validate a spectrophotometric approach called the Q-absorbance ratio for simultaneously estimating ondansetron hydrochloride and esomeprazole magnesium.

2. RESULTS AND DISCUSSION

2.1. Wavelength identification

Distilled water was used to create standard solutions of OND and ESO that were scanned between 200 and 400 nm at a concentration of around 20 µg/mL. Following analysis, the λ_{max} was discovered to be 248 nm and 302 nm, respectively, for the solution of 20 µg/mL of both OND and ESO (Fig. 3 and 4).

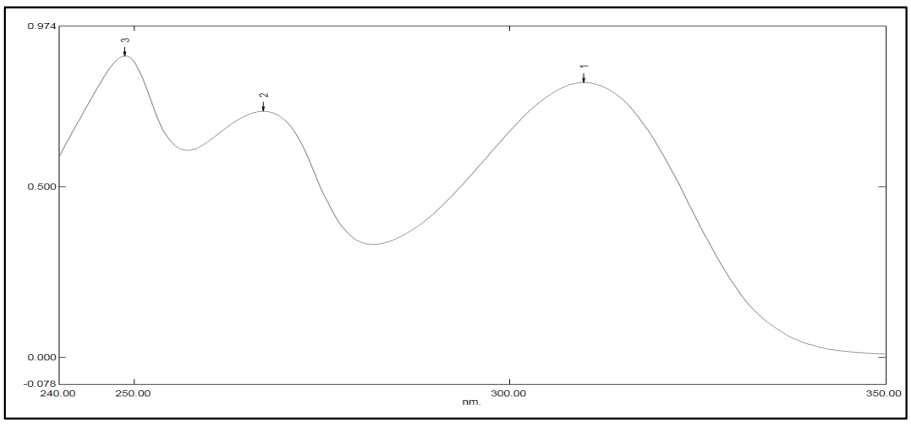

Figure 3. UV spectrum of pure ondansetron

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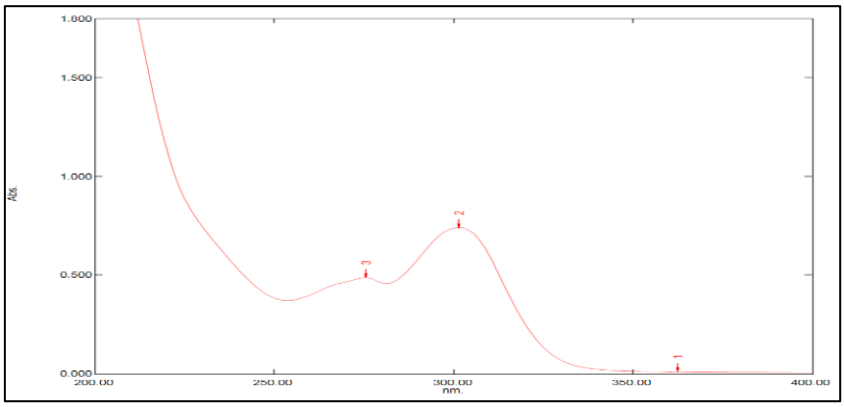

Figure 4. UV spectrum of pure esomeprazole

2.2. Isoabsorptive point determination

Both medications were produced as 20 µg/mL solutions from standard stock solutions, and they were scanned in the 200–400 nm range with distilled water used as a blank. Additionally, the overlying spectrum was acquired to establish the iso absorptive point. Three iso absorptive points: 305 nm, 275 nm, and 239 nm were found in overlaying spectra (Fig. 5) and the iso absorptive point 275 nm was selected for further analysis.

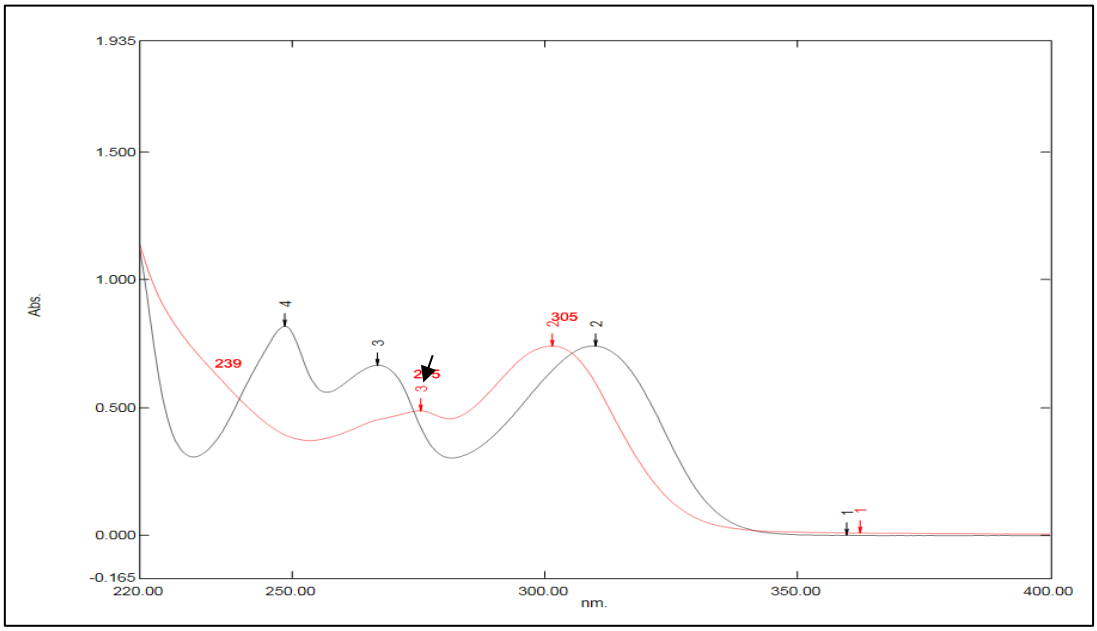

Figure 5. Overlay spectrum of ondansetron and esomeprazole

2.3. Method validation

ICH validation criteria were used to validate the analytical method.

2.3.1. Linearity

According to ICH, linearity is the capacity of an analytical technique in order to get test outcomes that are proportionate to the amount of analyte in the sample. The spread between the analyte's higher and lower concentrations in the sample is the range of an analytical procedure, which shows that the method was followed with an appropriate level of precision, accuracy and linearity [11]. Five independent levels of the calibration curve, ranging from 5 to 25 µg/mL, were examined to determine the linearity (Table 1). Absorbance of each solution was recorded at 275 and 302 nm. The calibration curve was obtained, and the regression line equations and correlation coefficient for OND and ESO was calculated (Fig. 6 and 7).

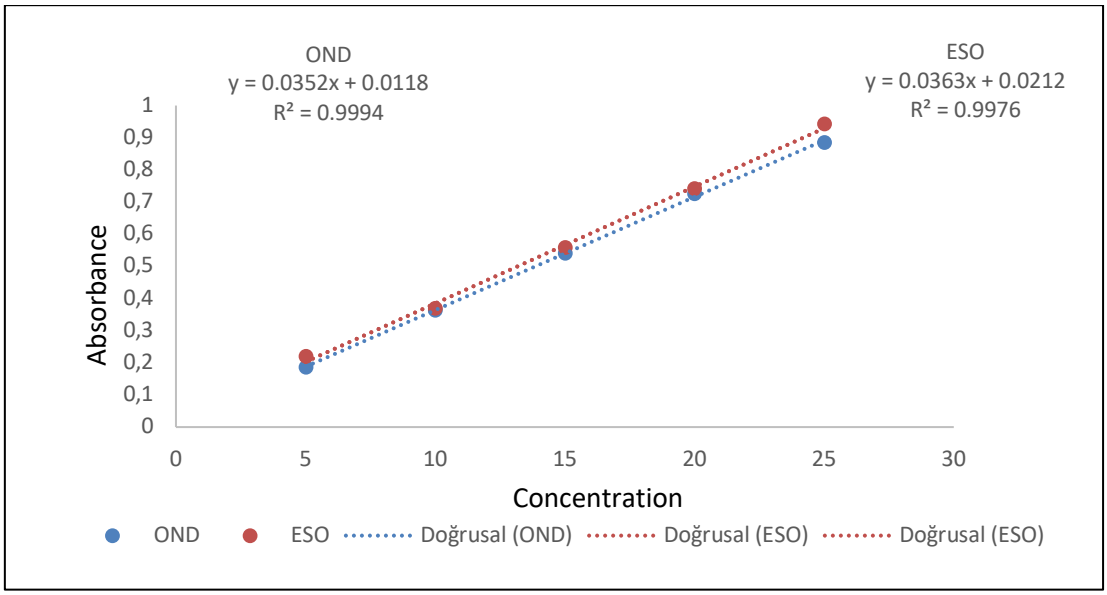

Figure 6. Standard plot of ondansetron and esomeprazole at 275 nm

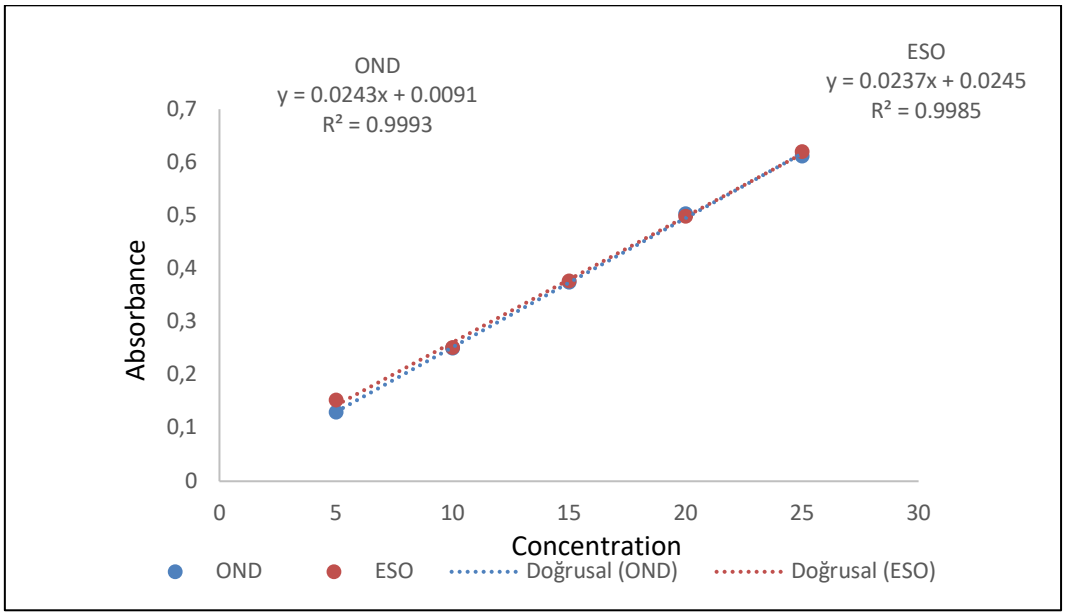

Figure 7. Standard plot of ondansetron and esomeprazole at 302 nm

The calibration curve of OND and ESO at 275 nm (λ_1) and 302 nm (λ_2) were plotted (Fig. 6 & 7). At both wavelengths of 275 nm and 302 nm, it was discovered that the relationship between the absorbance and the concentration of OND and ESO was linear in the range of 5 to 25 µg/ml. The least squares method was used to generate the sample linear equations, and the correlation coefficients showed very strong linearity (Table 1).

Table 1. Calibration points of standard curve with standard deviation (sd) and % relative standard deviation(rsd)

Concentration (µg/ml)	Abs at 275nm				Abs at 302nm			
	OND		ESO		OND		ESO	
	Mean Abs ± SD (n=3)	%RSD	Mean Abs ± SD (n=3)	%RSD	Mean Abs ± SD (n=3)	%RSD	Mean Abs ± SD (n=3)	%RSD
5	0.13 ± 0.001	1.17	0.152 ±0.003	2.00	0.186 ±0.003	1.67	0.22 ±0.004	1.80

10	0.25 ±0.003	1.50	0.251 ±0.003	1.19	0.362 ±0.003	0.83	0.369 ±0.002	0.73
15	0.374 ±0.003	0.82	0.376 ±0.003	0.80	0.541 ±0.003	0.65	0.558 ±0.004	0.73
20	0.503 ±0.002	0.50	0.498 ±0.002	0.50	0.726 ±0.004	0.57	0.741 ±0.003	0.40
25	0.611 ±0.003	0.57	0.62 ±0.003	0.49	0.884 ±0.004	0.45	0.942 ±0.001	0.16

2.3.2. Accuracy

Experiments measuring percent recovery at three distinct levels (e.g., 80, 100, and 120% accuracy) were used to assess the method's efficacy. To the pre-analyzed sample solutions, known quantities of standard OND and ESO solutions were added. Utilizing determined drug amounts in the following formula, absorbances were measured and % recovery was estimated [12].

% Recovery = A–B/C×100

where, A indicates the expected total amount of medication,

B represents the amount of substance discovered via pre-analysis,

C resembles amount of pure medication added.

Good percent recovery in the conventional addition approach served as evidence of the proposed method's high accuracy. For OND, it ranged from 98.14% to 100.80% and 97.78% to 100.81% at 275 nm and 302 nm respectively (Table 2(a)). For ESO, it was between 100.33% to 101.20% and 101.16% to 102.03% at 275 nm and 302 nm respectively (Table 2(b)).

Table 2(a). Results of accuracy studies of OND.

Amount of sample (µg/ml)	Amount of drug added (µg/ml)	Percent of spiked	Amount recovered* (µg/ml)		Percent recovery	
			275 nm	302 nm	275 nm	302 nm
15	12	80%	26.50	26.40	98.14%	97.78%
15	15	100%	29.79	29.58	99.30%	98.60%
15	18	120%	33.28	33.26	100.80%	100.81%

*Mean of three replicates n=3.

Table 2(b). Results of accuracy studies of ESO

Amount of sample (µg/ml)	Amount of drug added (µg/ml)	Percent of spiked	Amount recovered* (µg/ml)		Percent recovery	
			275 nm	302 nm	275 nm	302 nm
15	12	80%	27.32	27.55	101.18%	102.03%
15	15	100%	30.10	30.35	100.33%	101.16%
15	18	120%	33.39	33.43	101.20%	101.32%

*Mean of three replicates n=3

2.3.3. Precision

In the intraday study, three separate calculations of the drugs concentration per replication were made on the same day. In an inter-day study, the drug concentration was calculated over three days that followed one another, expressing the laboratory variation over the course of the day. In the approaches intraday and interday precision studies, the % relavitve standard deviation (RSD) was calculated[12,13].

Evaluation of repeatability, intraday and interday precision, and percent relative standard deviation (% RSD) were calculated. Indicating good precision, these values were found to be less than two % RSD (Table 3(a&b) and 4(a&b)).

Table 3(a). Results of OND intraday precision

Conc (µg/ml)	Absorbance of OND						%RSD					
	Morning*		Afternoon*		Evening*		Morning		Afternoon		Evening	
	275 nm	302 nm	275 nm	302 nm	275 nm	302 nm	275 nm	302 nm	275 nm	302 nm	275 nm	302 nm
5	0.113	0.172	0.132	0.178	0.107	0.177	1.85%	0.34%	1.58%	1.42%	1.94%	1.13%
15	0.374	0.532	0.373	0.531	0.370	0.527	0.15%	0.19%	0.46%	0.38%	0.41%	0.40%
25	0.608	0.870	0.607	0.867	0.601	0.850	0.09%	0%	0.58%	0.18%	0.35%	0.18%

*Mean of three replicates n=3

Table 3(b). Results of ESO intraday precision

Conc (µg/ml)	Absorbance of ESO						% RSD					
	Morning*		Afternoon*		Evening*		Morning		Afternoon		Evening	
	275 nm	302 nm	275 nm	302 nm	275 nm	302 nm	275 nm	302 nm	275 nm	302 nm	275 nm	302 nm
5	0.135	0.191	0.133	0.184	0.129	0.179	1.19%	0.85%	0.87%	0.54%	0.43%	0.52%
15	0.360	0.528	0.362	0.524	0.360	0.522	0.28%	0.29%	0.32%	0.11%	0.30%	0.22%
25	0.604	0.901	0.608	0.888	0.605	0.872	0.83%	0.34%	0.34%	0.28%	0.10%	0.17%

*Mean of three replicates n=3

Table 4(a). Results of inter-day precision of OND

Conc (µg/ml)	Absorbance of OND						% RSD					
	DAY I*		DAY II*		DAY III*		Morning		Afternoon		Evening	
	275 nm	302 nm	275 nm	302 nm	275 nm	302 nm	275 nm	302 nm	275 nm	302 nm	275 nm	302 nm
5	0.113	0.172	0.133	0.161	0.085	0.161	1.85%	0.34%	1.90%	1.29%	1.80%	2.00%
15	0.374	0.532	0.378	0.507	0.348	0.449	0.15%	0.19%	1.05%	0.46%	0.17%	0.27%
25	0.608	0.870	0.660	0.831	0.582	0.775	0.09%	0%	0.11%	0.05%	0.17%	0.15%

*Mean of three replicates n=3

Table 4(b). Results of inter-day precision of ESO

Conc (µg/ml)	Absorbance of ESO						% RSD					
	DAY I*		DAY II*		DAY III*		Morning		Afternoon		Evening	
	275 nm	302 nm	275 nm	302 nm	275 nm	302 nm	275 nm	302 nm	275 nm	302 nm	275 nm	302 nm
5	0.135	0.191	0.128	0.171	0.127	0.142	1.19%	0.85%	0.45%	0.34%	0.79%	0.70%
15	0.360	0.528	0.354	0.504	0.272	0.543	0.28%	0.29%	0.99%	0.70%	0%	0.10%
25	0.604	0.901	0.592	0.851	0.449	0.909	0.83%	0.34%	0.10%	0.07%	0.34%	0.06%

*Mean of three replicates n=3

2.3.4. Limits of quantification (LOQ) and detection (LOD)

The ICH defines the limit of quantitation of an analytical procedure as the lowest amount of analyte in a sample that can be determined quantitatively with adequate precision and accuracy, as opposed to the limit of detection of an analytical method, which is the lowest amount of analyte in a sample that can be detected but not necessarily quantitated as an exact value [14,15,16].

LOD and LOQ were determined using the formula below:

LOD = 3.3×σ/S and LOQ = 10×σ/S

where, is the regression line's y-intercept standard deviation,

S represents the calibration curve's slope.

At the iso-absorptive point (275 nm), it was discovered that OND and ESO had detection limits of 0.054 µg/mL and 0.052 µg/mL, respectively. For OND and ESO, the LOD at 302 nm was determined to be 0.136 µg/mL and 0.139 µg/mL, respectively. At the iso-absorptive point (275 nm), it was discovered that OND and ESO had limits of quantification of 0.164 µg/mL and 0.159 µg/mL, respectively. For OND and ESO, the LOQ at 302 nm was determined to be 0.412 µg/mL and 0.422 µg/mL, respectively.

3. CONCLUSION

The simultaneous study of OND and ESO was carried out using the UV spectrophotometric Q-absorption ratio method, which was developed and validated. The combined results demonstrated that the method is simple, precise, repeatable, fast, and sensitive.. At both wavelengths of 275 nm and 302 nm, it was discovered that the relationship between the absorbance and the concentration of OND and ESO was linear in the range of 5 to 25 µg/ml. The least squares method was used to generate the sample linear equations, and the correlation coefficients showed very strong linearity. The method could be applied successfully and economically for the simultaneous estimation of OND and ESO in laboratory samples for efficient data generation and for combination formulations of these two drugs in the future.

4. MATERIALS AND METHODS

4.1. Instrument

UV experimentation were performed using double beam UV/vis spectrophotometer, Shimadzu model UV- 1800 having a spectral bandwidth of 1 nm and a thickness of 1 cm quartz cells was used and the calibrated analytical balance (Mettler Toledo) were used.

4.2. Materials

The bulk drug Ondansetron hydrochloride was obtained as a gift sample from M/s ZIM Laboratories, B-21/22, MIDC Area, Kalmeshwar, Nagpur, Maharashtra 441501 and esomeprazole magnesium was purchased from Yarrow Chem Products (Dhamtec Pharma and Consultants, Navi Mumbai). Methanol of the analytical grade was purchased from Finar Ltd., Ahmedabad.

4.3. Methods (Preparation of stock solutions)

A precisely weighed amount of 100 mg each of OND and ESO was put into a volumetric flask of 100 ml, dissolved in 10 ml of methanol, and then diluted with methanol until the desired strength was reached to produce a stock solution with a 1000 µg/mL concentration.

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