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Research Article

# SIMULTANEOUS DETERMINATION OF ONDANSETRON HYDROCHLORIDE AND PANTOPRAZOLE SODIUM IN THEIR BULK DOSAGE FORM BY RATIO DERIVATIVE SPECTROSCOPY

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### ABSTRACT

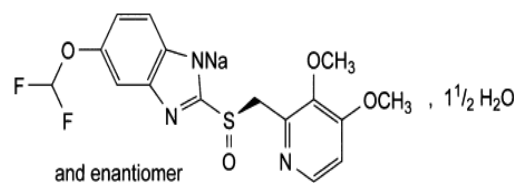
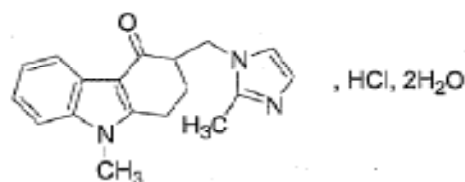
Simple, economical, precise and accurate ratio derivative spectrophotometric method for simultaneous determination of Ondansetron Hydrochloride (OND) and Pantoprazole Sodium (PAN) in bulk form have been developed and validated. The method utilize methanol as a solvent. The amplitudes in the first derivative of the corresponding ratio spectra at 271.80 nm and 300.20 nm were selected to determine Ondansetron Hydrochloride and Pantoprazole Sodium respectively. Beer's law is obeyed in the concentration range of 1-6 µg/ml ( $r^2=0.9991$ ) for OND and 10-60 µg/ml ( $r^2=0.9994$ ) for PAN. The results of analysis have been validated statistically. The results were found to be within acceptance criteria according to ICH Q2 R1 guideline. Thus, the proposed method can be applied successfully in routine analysis for the estimation of PAN and OND in bulk dosage form.

**Keywords:** Ondansetron Hydrochloride, Pantoprazole Sodium, Ratio Derivative Spectroscopy, Methanol.

### INTRODUCTION

Ondansetron (OND) is a 5-HT<sub>3</sub>-receptor antagonist used as an antiemetic. Chemically it is 9-methyl-3-[(2-methyl-1H-imidazol-1-yl)-methyl]-1, 2, 3, 9-tetrahydro-4H-carbazol-4-one hydrochloride dihydrate<sup>1</sup>. It acts both, peripherally on vagal nerve terminals and centrally in the chemoreceptor trigger zone of the area postrema. It is indicated for the prevention of nausea and vomiting associated with cancer chemotherapy, radiotherapy or anesthesia and surgery<sup>2</sup>. Literature survey revealed that few spectrophotometric methods have been reported for the estimation of OND with other drugs<sup>3-6</sup>. Second drug, Pantoprazole (PAN) is an anti-ulcerative drug and used in the treatment of peptic ulcer and gastro-esophageal reflux disease (GERD)<sup>7</sup>. Chemically it is 5-(difluoromethoxy)-2-[[[(3,4-dimethoxy-2-pyridinyl)methyl]sulfinyl]-1H-benzimidazole<sup>8</sup>. Literature describes RP-HPLC<sup>9-10</sup> for determination of PAN in plasma and pharmaceutical preparations and spectrophotometric methods<sup>11-13</sup> for estimation of PAN with other drugs. Literature review reveals few methods for simultaneous determination of PAN and OND 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 three validated spectroscopic methods for simultaneous determination of these drugs in bulk dosage form.



Ondansetron Hydrochloride

## MATERIALS AND METHODS

### Instrumentation:

- A double-beam UV-Visible spectrophotometer, model Shimadzu UV-2450 having two matched cells with 1-cm light path
- Semi micro analytical balance (Sartorius CD2250, Germany)
- Sonicator (Trans-O-sonic)
- Volumetric flasks – 10ml, 50ml, 100ml
- Pipettes – 1ml, 5ml, 10ml, beakers, measuring cylinders.

### Chemicals and Reagents:

- Authentic samples of Pantoprazole (PAN) and Ondansetron (OND).
- Methanol

### Experimental condition:

According to the solubility characteristics, the common solvent for the two drugs was found to be methanol.

### Preparation of standard stock solution:

Standard stock solution containing 100 µg/ml of OND and 1000 µg/ml of PAN were prepared by dissolving 10 mg of pure OND and 100 mg of pure PAN in 50 ml of methanol in a 100 ml volumetric flask and sonicated for 15 mins. The drugs were dissolved and the final volume was adjusted with methanol up to the mark. The working standard solutions of these drugs were obtained by dilution of the respective stock solution with methanol.

### Theory of Ratio Derivative Spectrophotometry:

The method involves dividing the spectrum of mixture by the standardized spectra of each of the analyte and deriving the ratio to obtain spectrum that is independent of concentration of analyte used as a divisor. The concentration of active compounds are then determined from calibration graph obtained by measuring amplitude at points corresponding to minima or maxima. The calibration curves were checked for linearity and linear behavior was observed for each of the drugs.

### Ratio Derivative Method:

The working standard solutions of these drugs were scanned in the wavelength range 200-400 nm. For the determination of OND, the spectra of OND at increasing concentrations in methanol were divided by previously stored absorption spectrum standard solution of PAN (40 µg/ml) to obtain the corresponding ratio spectra. Then the first derivative of the obtained ratio spectra were traced with interval of  $\Delta\lambda = 4$  nm. In the binary mixtures, content of OND was determined by measuring the first derivative amplitude at 271.80 nm, where there is no contribution or interference from PAN. On the other hand, for the determination of PAN, an analogous procedure was followed. The spectra of PAN at increasing concentrations were divided by previously stored spectrum of 4 µg/ml solution of OND and the first derivative of the developed ratio spectra were traced with  $\Delta\lambda = 4$  nm. In the binary mixtures, content of PAN was determined by measuring the first derivative amplitude at 300.20 nm, where there is no contribution or interference from OND. First-

derivative technique (D1) traced with  $\Delta\lambda = 4$  nm was used to resolve the spectral overlapping.

### Method Validation:

The method was validated with respects to linearity, LOD (Limit of detection), LOQ (Limit of quantitation), precision and accuracy and ruggedness<sup>14-15</sup>.

### Linearity:

Linearity was checked from the ratio derivative spectra of six different concentrations of both drugs. OND was linear with the concentration range of 1-6 µg/ml and PAN showed linearity in the range of 10-60 µg/ml and calibration curves [ $n=6$ ] were plotted between concentration and absorbance of drugs.

### Sensitivity:

The limit of detection (LOD) and limit of quantification (LOQ) parameters were calculated, in accordance with ICH guidelines,  $LOD = 3.3\sigma/S$  and  $LOQ = 10\sigma/S$  respectively, where  $\sigma$  is the standard deviation of the response (y-intercept) and  $S$  is the slope of the calibration plot.

### Accuracy:

To check the accuracy of the developed method, analytical recovery experiments were carried out by using standard addition method in three different concentrations. From the total amount of drug found, the percentage recovery was calculated. This procedure was repeated for three times for each concentration. The % RSD was calculated.

### Precision:

The precision of the method was confirmed by repeatability and intermediate precision. The repeatability was performed by the analysis of bulk mixture and it was repeated for three times with the same concentration. The amount of each drug present in the bulk mixture was calculated. The % RSD was calculated. The intermediate precision of the method was confirmed by intraday and inter day analysis i.e. the analysis of bulk mixture was repeated three times in the same day and on three successive days. The amount of drugs was determined and % RSD also calculated.

### Ruggedness:

The ruggedness test of analytical assay method is defined as the degree of reproducibility of test results obtained by the analysis of the same samples under a variety of normal test conditions such as different labs, different analysts, different lots of reagents etc. Ruggedness is a measure of reproducibility of test results under normal expected operational conditions from laboratory to laboratory and from analyst to analyst. In present study, determination of OND and PAN were carried out by using different analysts.

## RESULTS AND DISCUSSION

### Linearity:

The response for Ondansetron was linear in the concentration range of 1-6 µg/ml with coefficient of correlation  $r^2 = 0.9991$  and the response for Pantoprazole was linear in the concentration range of 10-60 µg/ml with coefficient of correlation  $r^2 = 0.9994$  for their bulk mixture. The results are shown in Table 1 and Figures 1 to 4.

**Table 1: Linearity Study Data of Ondansetron And Pantoprazole for Ratio Derivative Spectroscopy**

Conc. (µg/ml)	Ondansetron 271.80 nm (Δλ=4, SF#1)	Conc. (µg/ml)	Pantoprazole 300.20 nm (Δλ=4, SF#1)
	$\partial A/\partial \lambda^* \pm \%CV$		$\partial A/\partial \lambda^* \pm \%CV$
1	0.0022 ± 0.1076	10	0.0986 ± 0.0261
2	0.0062 ± 0.0489	20	0.2045 ± 0.0221
3	0.0113 ± 0.0231	30	0.3257 ± 0.0191
4	0.0164 ± 0.0337	40	0.4372 ± 0.0207
5	0.0211 ± 0.0199	50	0.5421 ± 0.0128
6	0.0258 ± 0.0237	60	0.6458 ± 0.0097

\* Mean of six determinations

# scaling factor

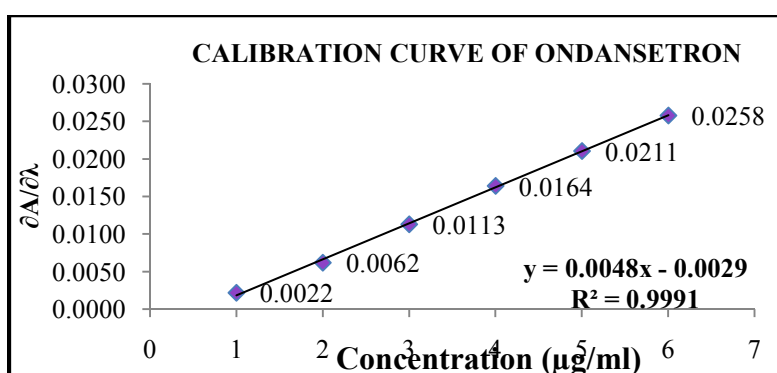


Figure 1: Calibration Curve of Ondansetron by Ratio Derivative Spectroscopy

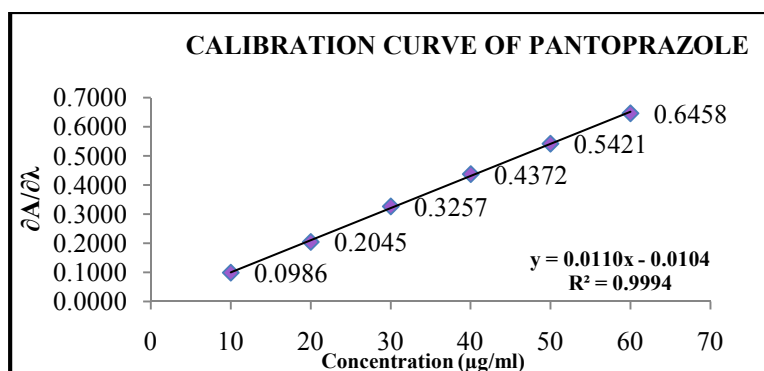


Figure 2: Calibration Curve of Pantoprazole by Ratio Derivative Spectroscopy

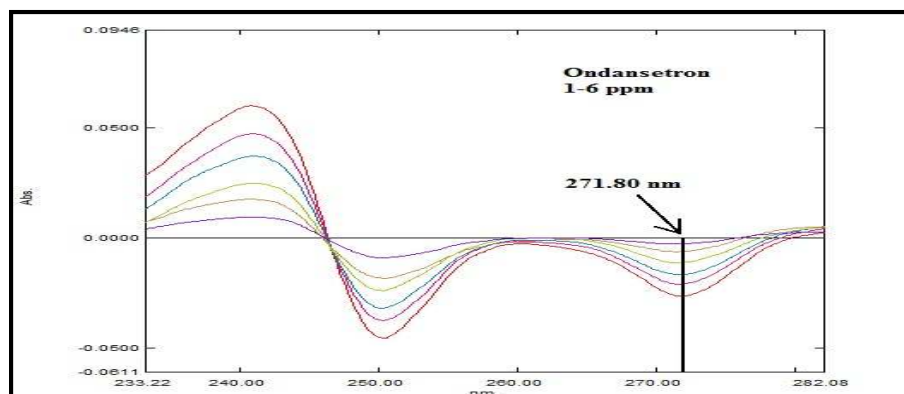


Figure 3: Ratio Derivative Spectra of Ondansetron

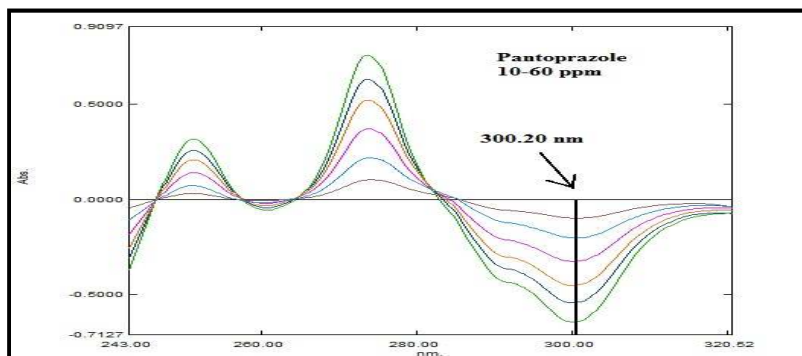


Figure 4: Ratio Derivative Spectra of Pantoprazole

**Precision:**

Precision evaluated through intraday and interday of the bulk mixture are presented in Table 2. Intermediate precision using second analyst is presented in Table 3.

**Accuracy (Recovery):**

Accuracy evaluated through spiking of pure drugs to the bulk mixture is presented in Table 4.

**Limit of detection (LOD) and Limit of quantification (LOQ):**

The LOD was determined as the lowest detectable amount of drug and LOQ was determined as the lowest concentration for drug shown in Table 5.

**Table 2: Intraday & Interday Precision Data of Developed Spectroscopic Method**

Mixture Conc. (µg/ml)	Ondansetron	Pantoprazole
	$\partial A/\partial \lambda^* \pm \%RSD$	$\partial A/\partial \lambda^* \pm \%RSD$
<b>Intraday Precision</b>		
2:20	0.0061 ± 0.8229	0.2011 ± 0.6470
4:40	0.0157 ± 0.9709	0.4289 ± 0.2682
6:60	0.0249 ± 0.6126	0.6365 ± 0.9074
<b>Interday Precision</b>		
2:20	0.0059 ± 0.6341	0.2097 ± 0.4697
4:40	0.0162 ± 0.9410	0.4351 ± 0.9762
6:60	0.0268 ± 0.2152	0.6442 ± 0.1966

\*Mean value of three determinations

**Table 3: Intermediate Precision Data of Developed Spectroscopic Method**

Mixture Conc. (µg/ml)	Ondansetron	Pantoprazole
	$\partial A/\partial \lambda^* \pm \%RSD$	$\partial A/\partial \lambda^* \pm \%RSD$
<b>Intermediate Precision</b>		
2:20	0.0060 ± 0.2188	0.2058 ± 0.6945
4:40	0.0168 ± 0.3443	0.4308 ± 0.5859
6:60	0.0255 ± 0.5982	0.6427 ± 0.3391

\*Mean value of three determinations

**Table 4: Accuracy Study Data for Ratio Derivative Spectroscopic Method**

Initial mixture conc. (µg/ml)	Quantity spiked (µg/ml)		Total Amount (µg/ml)		Ondansetron	Pantoprazole
	OND	PAN	OND	PAN	%Recovery ± S.D	%Recovery ± S.D
2:20	1.6	16	3.6	36	99.7299 ± 0.8840	98.7710 ± 0.4534
2:20	2	20	4	40	100.3472 ± 0.7956	99.9015 ± 0.5587
2:20	2.4	24	4.4	44	98.8005 ± 0.7233	99.7452 ± 0.4960

**Table 5: Limit of Detection (Lod) and Limit of Quantification (Loq) for Developed Method**

Parameters	Ondansetron	Pantoprazole
LOD (µg/ml)	0.2520	0.8529
LOQ (µg/ml)	0.7636	2.5845

**Table 6: Summary of Validation Parameters of Ondansetron and Pantoprazole for Ratio Derivative Spectroscopy**

Sr. No.	Parameters	Ondansetron Hydrochloride	Pantoprazole Sodium
1.	$\lambda$ max (nm)	271.80	300.20
2.	Linearity range ( $\mu\text{g/ml}$ )	1 – 6	10 – 60
3.	Regression equation	$y = 0.0048x - 0.0029$	$y = 0.0110x - 0.0104$
4.	Correlation coefficient ( $r^2$ )	0.9991	0.9994
5.	Precision		
	Intraday % RSD (n = 9)	0.8021	0.6075
	Inter day % RSD (n = 9)	0.5967	0.5475
6.	Reproducibility % RSD (n=9)		
	Analyst 2	0.3871	0.5398
7.	% Recovery	99.6259	99.4726
8.	Limit of detection ( $\mu\text{g/ml}$ )	0.2520	0.8529
9.	Limit of quantification ( $\mu\text{g/ml}$ )	0.7636	2.5845

### CONCLUSION

From the results obtained by applying the suggested procedure, it is obvious that the proposed method is accurate, precise, simple, sensitive, selective and rapid and can be applied successfully in routine analysis for the estimation of PAN and OND in bulk dosage form.

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