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STABILITY INDICATIµG ASSAY METHOD DEVELOPMENT AND VALIDATION FOR ONDANSETRON HYDROCHLORIDE AND PANTOPRAZOLE SODIUM IN BULK AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

RP-HPLC method showed adequate linearity from 100-600 µg/mL for PAN (Pantoprazole sodium) and 10- 60 µg/ml for OND (Ondansetron). The mean recoveries for all methods were found in between 98 %-102 % for both the drugs. The RP-HPLC method successfully separated Pantoprazole and Ondansetron from degradation products formed under stress conditions like acidic, alkali, oxidative, photolytic and thermal. PAN degraded significantly under acidic, neutral, oxidative, photolytic and thermal conditions and gave 1 degradation products respectively, whereas OND degraded significantly under oxidative conditions and gave 1 degradation product each condition. Both the drugs were found to be stable under alkali conditions. HPTLC method showed adequate linearity from 200-1200 µg/band for OND and 2000-12000 µg/band for PAN.

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INTRODUCTION

The RP-HPLC method developed for quantitative and qualitative analysis of pantoprazole and Ondansetron tablets was rapid, simple, accurate, precise and specific. Here conclude that this method is one of best method available in market because it is Economically proved. Recovery study on tablet formulation gave accurate prediction of good relation between label claim and practical value. Method is specific due to it shows no interference in analyte peak of any unknown or known compounds. Method was validated as per USP acceptance criteria and ICH guidelines. Hence present validated method can be confidently utilize as an alternative method to reporting of result in routine practice or release of product report in quality control compliance (1).

Ondansetron hydrochloride is the prototype of new class of antiemetic drugs used for the prevention of nausea and vomiting associated with highly emetogenic cancer chemotherapy, radiotherapy or anesthesia and surgery.

Ondansetron is serotonin 5-HT3 receptor antagonist used in mainly as an antiemetic. Its effects are through to be on both peripheral and central nerves. It reduce the activity of the vagus nerve which activates the vomiting center in medulla oblongata, and also block serotonin receptor in chemotherapy trigger zone.

Pantoprazole sodium is proton pump inhibitor. It is used in treatment of gastro esophageal reflux disease, gastric ulcer and duodenal ulcer. It accumulates in the acidic compartment of parietal cells and is converted to the active form, which binds to hydrogen-potassium- ATPase at the secretory surface of gastric parietal cells. Inhibition of hydrogen- potassium- ATPase blocks the final step of gastric acid production, leading to inhibition of both basal and stimulated acid secretion.

MATERIAL AND METHOD

In RP-HPLC method, chromatographic separation was achieved on Phenomenex, C_8 (250× 4.6) mm 5µm column using Methanol: ACN: Water (20:30:50%) as the mobile phase with detection at 216 nm. Both the drugs were subjected to acid, alkali, oxidative, thermal and photolytic stress conditions individually and in combination whereas tablet formulation was subjected to thermal and photolytic stress conditions. Both the methods were validated as per ICH guidelines. In HPTLC method, optimized on TLC plate pre-coated with silica gel 60F 254 using Dichloromethane: Methanol (9.0:0.7) as

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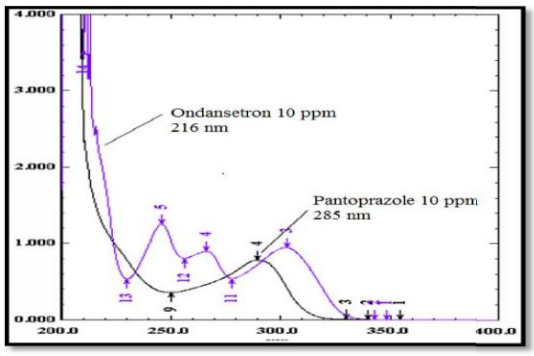
mobile phase and scanning the plate at 290 nm.

DETERMINATION OF λmax



Mode: Spectrum Scan, Speed: Medium

Wavelength range: 400-200 nm

Absorbance scale: 0.00A - 2.00A

Initial base line correction: Methanol

Concentration: 10 ppm of OND & 10ppm PAN

CHROMATOGRAPHIC CONDITIONS

Column- Phenomenex Luna C_8 (250 mm × 4.6 mm, 5 µm

HPLC system: LC 2010CHT, Shimadzu UV detector

Mobile phase: Methanol : Acetonitrile: Water (20:30:50 % v/v/v)

Flow rate: 1.0 mL/min

Detection wavelength: 216 nm

Column Temperature: 40°C

Total run time: 20 min

Injection Volume: 20 µL

Diluent: All the final dilution of sample and standard done with Mobile Phase

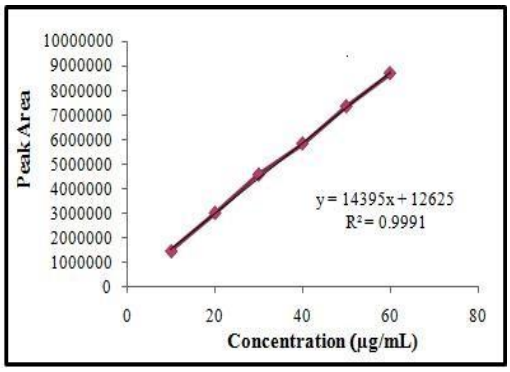
VALIDATION OF DEVELOPED HPLC METHOD AND DISCUSSION

LINEARITY AND RANGE

The linearity of PAN and OND were found between 100-600 µg/mL and 10-60 µg/mL respectively. The calibration data is presented in Table and correlation coefficient and regression line equation analysis presented in Figure

Concentration (µg/mL)		**Mean Peak Area* ± S.D**		**% RSD**	
PAN	OND	PAN	OND	PAN	OND
100	10	1639082 5 ± 91490	147182 5±526 4	0.56	0.17
200	20	3248762 1 ± 100265	302548 2±589 7	0.31	0.20
.300	30	5215489 6 ± 122569	458795 4±998 5	0.24	0.22
400	40	7012458 9 ± 201548	584875 0±197 45	0.29	0.34
500	50	8241258 6 ± 358745	735483 2±201 59	0.43	0.27
600	60	1025482 1 ± 458562	869874 5±298 54	0.45	0.34

Calibration Curve of PAN



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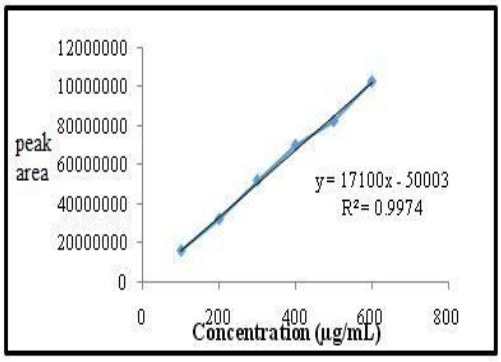
Calibration Curve of OND



LOD AND LOQ

PAN		OND	
LOD µg/mL	LOQ µg/mL	LOD µg/mL	LOQ µg/mL
17.65	53.50	1.21	3.67

The Limit of detection (LOD) was found to be 17.65 µg/mL and 0.03 µg/mL; while the Limit of quantification (LOQ) was found to be 5.48 µg/mL and 0.09 µg/mL for PAN and OND, respectively.

FORCED DEGRADATION STUDY

% Degradation of PAN and OND in various stress condition are shown in table. From degradation study it was found that PAN was marginally degraded in acidic, oxidative, photolytic and thermal condition and stable in alkali conditions whereas OND was significantly degraded in oxidative conditions and marginally stable in acidic, alkali, photolytic and thermal and conditions.

Sr	Stress Type	Stress Conditions
1	Acid hydrolysis	0.1 N HCl at RT for 5 hrs
2	Alkali hydrolysis	0.1 N NaOH at RT for 5 hrs
3	Neutral hydrolysis	Water at RT for 24 hrs
4	Oxidative Degradation	3 % H O at RT for 5 hrs
4	Photolytic Degradation	UV 254 nm for 8 hrs
5	Thermal Degradation	At 60 for 48 hrs

Acid hydrolysis
Degrade about 19.2 %.
Alkali hydrolysis
No significant degradation observed.
Neutral hydrolysis
Degrade about 6.01 %.
Oxidative Degradation
Degrade about 22 %.
Photolytic Degradation
Degrade about 91.3 %.
Thermal Degradation
Degrade about 19.96.

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PRECISION

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Repeatability (Intra-day Precision):

Reproducibility was determined by analyzing PAN and OND standard solution in the range of 200, 400, and 600 µg/mL of PAN and 20, 40 and 60 µg/mL of OND in different laboratories. Calculate % RSD for PAN and OND.

Conclusion

The % RSD for Intra-day precision was found to be 0.18-0.63 % for PAN and 0.12- 0.16 % for OND.

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Inter-day Precision

Inter-day precision was determined by analyzing of PAN and OND standard solutions in the range 200, 400, and 600 µg/mL of PAN and 20, 40 and 60 µg/mL of OND in triplicate in different days. Calculate % RSD for PAN and OND.

Conclusion

The % RSD for Inter-day precision was found to be 0.11-0.80 % for PAN and 0.17-0.43% for OND.

SPECIFICITY

Specificity is the term extent that analyte may be exist without interference from other related compound in a mixture. Specificity able to differentiate all possible impurities by applying forced stress testing. When in any chromatogram analyte peak not affected from other known or unknown impurity it indicates that chromatographic parameters good.

a) There should not be any interference from blank, excipient and reagent peaks with main peak.
b) The peak purity index for the main peaks and degradation product peaks in standard preparation and sample preparation should be equa to or more than 0.990.

ACCURACY AS RECOVERY

Accuracy of the method was confirmed by recovery study from marketed formulation at three level of standard addition. Percentage recovery was found to be in range, for PAN 99-101.1 % and for OND 99-101.41 %.

Level	Amount of Test Solution (µg/mL)	Amount of Std added (µg/mL)	Mean Peak area ± SD	Amount found (µg/mL)	% Recovery ± RSD
% Recovery data of PAN					
80%	100	80	3045854 5±12356	178	99.11±0.40
100%	100	100	3356897 4±19874 5	196.6	99±0.59
120%	100	120	3801456 1±38014 56	222	101.1±0.68
% Recovery data of OND					
80 %	10	8	26403 85±54 66	18.2	101.41± 0.20
100 %	10	10	28554 22±80 24	19.7	99±0.28
120 %	10	12	32120 64±10 125	22.2	101.0±0.31

ANALYSIS OF MARKETED FORMULATION

Applicability of proposed method was tested by analysing the commercially available Vomizen P tablet. The results are shown in table

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Analysis of market formulation

VomizenP	Amount of drug (mg/tablet)	%Assay (n=6)	% RSD
PAN	40	101.01	0.13
OND	4	101.3	0.17

Preparation of standard stock solution of PAN

Accurately weighed quantity of 10 mg of PAN was transferred into 10 mL volumetric flask, dissolved and diluted up to mark with methanol. This was a stock solution having strength of 1000 µg/mL of PAN. From this solution, 5 mL of solution was pipetted out and diluted up to 10 mL to get 500 µg/mL of PAN

Preparation of standard stock solution of OND

Accurately weighed quantity of 1mg of OND was transferred into 10 mL volumetric flask, dissolved and diluted up to mark with methanol.

This was a stock solution having strength of 100 µg/mL of OND. From this solution, 5 mL of solution was pipetted out and diluted up to 10 mL to get 50 µg/mL of OND.

Preparation of stock solution of synthetic mixture of PAN and OND

Accurately weighed quantity of 10 mg of PAN and 1 mg of OND was transferred into 10 mL volumetric flask, dissolved and diluted up to mark with methanol. This was a stock solution having strength of 1000 µg/mL of PAN and 100 µg/mL of OND. From this solution, 5mL of solution was pipetted out and diluted up to 10 mL to get 500 µg/mL of PAN and 50 µg/mL of OND.

Test solution of Pantoprazole and Ondansetron

10 tablets were weighed and powdered; a quantity of tablet powder equivalent of 40 mg PAN and 4 mg of OND was weighed accurately and transferred to a 100 mL volumetric flask. The tablet powder was dissolved in methanol with aid of ultra- sonication, diluted up to mark with same and filtered through a whatman filter paper

CONCLUSION

The suitable chromatographic methods (RP-HPLC, HPTLC) were developed and validated for estimating OND and PAN in tablet dosage form. HPLC method was stability indicating as it achieved separation of both drugs from potential degraded products. More degradation has been

observed for either of drugs in combination than degradation of such single drug

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