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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF PARACETAMOL AND ONDANSETRON COMBINED DOSAGE FORM BY RP-HPLC

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ABSTRACT

Here we are performing an Analytical Method Development and Validation of Paracetamol and Ondansetron Combined Dosage Form by RP-HPLC method. A combination of Paracetamol and Ondansetron is clinically used in the treatment of analgesic effect. The present work deals with the stability indicating RP-HPLC methods for simultaneous spectroscopic determination of Paracetamol and Ondansetron in pharmaceutical formation in the presence of their degradation products. Method validation is the process used to confirm that the analytical procedure employed for a specific test is suitable for its intended use. Results from method validation can be used to judge the quality, reliability and consistency of analytical results; it is an integral part of any good analytical practice.

KEYWORDS: Paracetamol, Ondansetron, RP-HPLC Validation.

INTRODUCTION

Analytical chemistry deals with quantitative analysis of composition of substances and complex materials in various matrices by measuring a physical or chemical property of a distinctive constituent of the components of interest. Analytical methods are classified according to the property of the analyte measured.[1] the pharmaceutical analysis is one of the most important fields in analytical chemistry. Modern analytical chemistry is dominated by instrumental analysis. There are so many different types of instruments used today that, it seems like a confusing array of acronyms rather than a unified field of study.[2] The analytical methods should be accurate as required and not as accurate as possible.[3]

Method development and optimization in liquid chromatography is still an attractive field of research for theoreticians. Complex mixtures or samples required systematic method development involving accurate modeling of the retention behavior of the analyte. Among all, the liquid chromatographic methods, the reversed phase systems based on modified silica offers the highest probability of successful results. However, a large number of (system) variables (parameters) affect the selectivity and the resolution.

Method validation is the process used to confirm that the analytical procedure employed for a specific test is suitable for its intended use. Results from method validation can be used to judge the quality, reliability and consistency of analytical results; it is an integral part of any good analytical practice. It is the process of defining an analytical requirement, and confirms that the method under consideration has performance capabilities consistent with what the application requires. Use of equipment that is within specification, working correctly and adequately calibrated is fundamental to the method validation process. Likewise the operator carrying out the studies must be competent in the analysis under study and have sufficient knowledge of the method/analysis to draw conclusions from the observations as the validation work proceeds. Quite often method validation evolves from method development and so the two activities are often closely tied, with the validation study employing the techniques and steps in the analysis as defined by the method development.

OBJECTIVE

- To develop method for Analgesic effects (combination) in bulk and solid dosage form.
- To validate accuracy, precision , linearity, robustness as per ICH guidelines.
- The method provides selective quantification of Paracetamol and Ondensartan. This developed RP-HPLC method for estimation of Paracetamol and Ondensartan is accurate, precise, robust and specific.
- The method has been found to be better than previously reported method, because of its less retention time Gradient mode and use of an

economical and readily available mobile phase, readily available column, UV detection and better resolution of peaks.

- The Analgesic effects of Paracetamol and Ondensartan results from a decrease in systemic vascular resistance and the parent compound Paracetamol And Ondensartan is primary responsible for the Analgesic effects activity.

RESULT AND DISCUSSION

UV Spectroscopy

UV absorption of 10 µg/mL solution of Paracetamol and Ondansetron in methanol was generated and absorbance was taken in the range of 200-400 nm. Λ max of Paracetamol and Ondansetron in Methanol was found to be 286nm respectively.

Table 1: Chromatographic behavior of Paracetamol and Ondansetron mobile phase of various compositions.

Sr. No.	Mobile Phase	Retention Time (min)		Remark
		PARARI	ONDA	
1.	Methanol + water (80:20% v/v) 1ml	3.7833	4.1667	No sharp peak
2	Acetonitrile + Water) (70 : 30% v/v) 1.1ml	4.5833	-	No sharp peak
3	Acetonitrile +Water (75:25%v/v)	4.0167	4.9500	No sharp peak
4	Acetonitrile +Water (60:40%v/v)	1.9667	-	No peak
5	Acetonitrile +Water (60:40%v/v)	1.5000	-	No peak
6	Acetonitrile + Water (40:60%v/v)-WL-248 samp.in CAN	2.4333	4.0667	No sharp peak
7	Acetonitrile +Water (40:60%v/v) 248nm,samp in mob phase	2.4333	4.1667	No sharp peak
8	Water +Acetonitrile (70:30%v/v)-248nm.	2.2333	3.2000	No sharp peak
9	Water + Acetonitrile (90:10%v/v)-248	7.8833	-	No peak
10	Methanol+water (70:20% v/v) 1ml-286nm	3.5333	8.7500	sharp peak

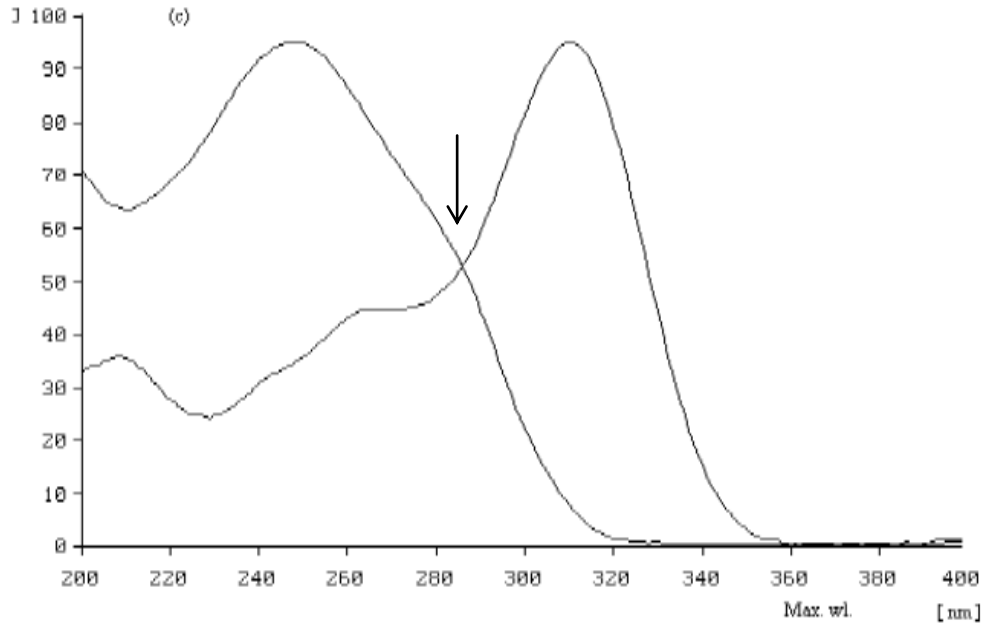

Fig. 1: Iso-absorptive point of Paracetamol and Ondansetron.

Studies on the chromatographic behavior of Paracetamol and Ondansetron TABLE NO-1: Chromatographic behavior of Paracetamol and Ondansetron mobile phase of various compositions.

Thus, from the above, it has been observed that, using mobile phase of Methanol + 0.05% OPA PH 2.5(70:30%)v/v) 225nm,1.0ml, pH 2.5 gave adequate retention time at 3.5333min and 8.7500min. with good peak shape (Theoretical plates of 4085.3of Paracetamol & 11229.0 of Ondansetron.

Chromatogram of Trial 1

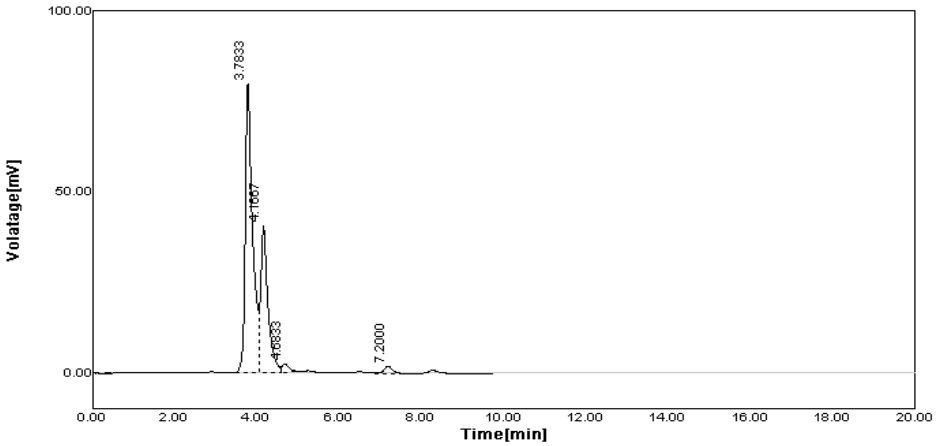

Fig. 2: Representative Chromatogram of Paracetamol and Ondansetron using Methanol +water (80:20% v/v) 1ml as mobile phase, showing the no retention time 3.7833min and 4.1667min.

Chromatogram of Trial 2

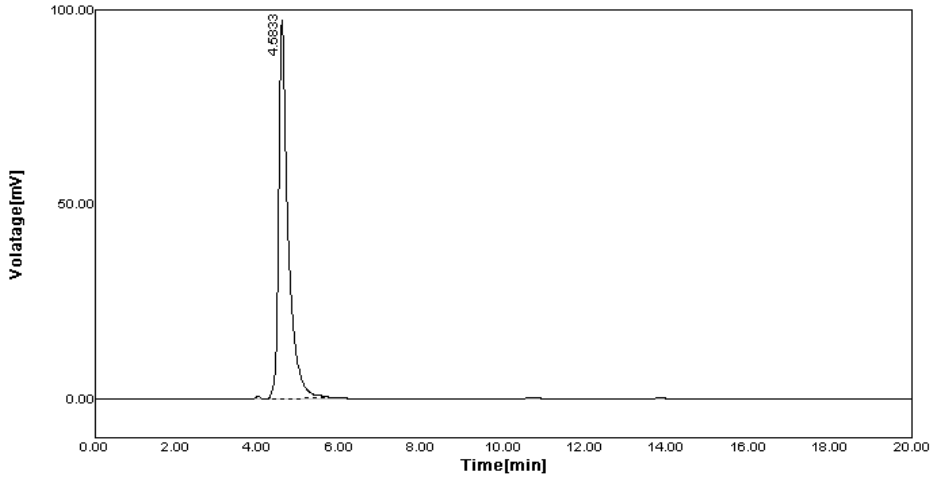

Fig. 3: Representative Chromatogram of Paracetamol and Ondansetron using Acetonitrile + Water) (70: 30 % v/v) 0.7ml) as mobile phase, showing retention time 4.5833 min.

Chromatogram of Trial 3

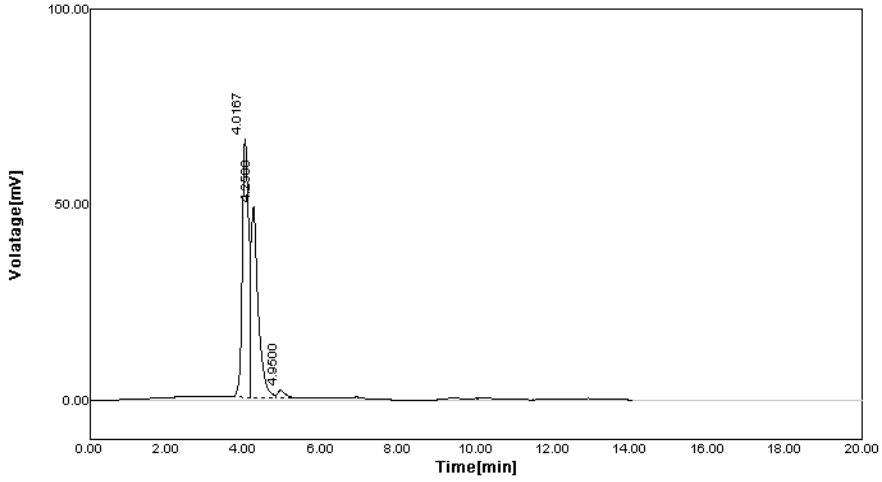

Fig. 4: Representative Chromatogram of Paracetamol and Ondansetron using Acetonitrile +Water (75:25%v/v) as mobile phase, showing retention time 4.0167min and 0 min.

Chromatogram of Trial 4

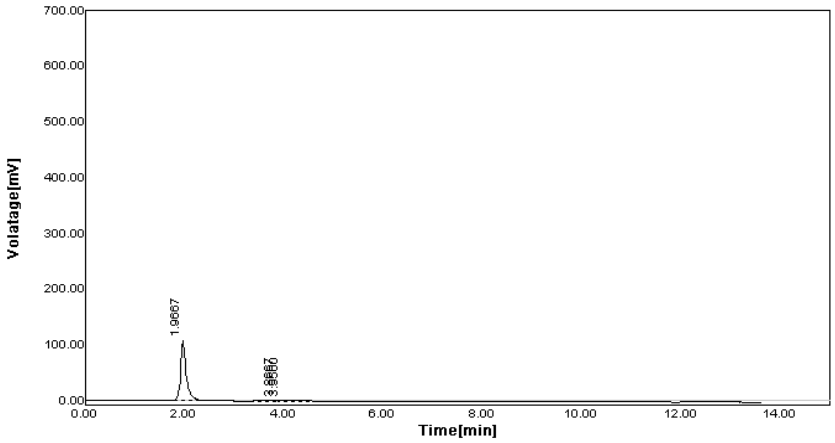

Fig. 5: Representative Chromatogram of Paracetamol and Ondansetron using ACN + 0.05% OPA PH 2.5 (60:40v/v) as mobile phase, showing retention time 1.9667 min and 0min.

Chromatogram of Trial 5

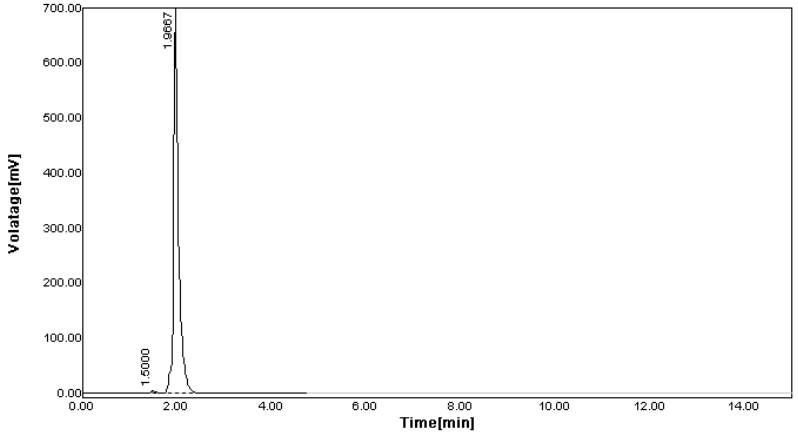

Fig. 6: Representative Chromatogram of Paracetamol and Ondansetron using ACN + 0.05% OPA PH 2.5 (60:40v/v) WL 248nm as mobile phase, showing retention time 1.9667 min and 0min.

Chromatogram of Trial 6

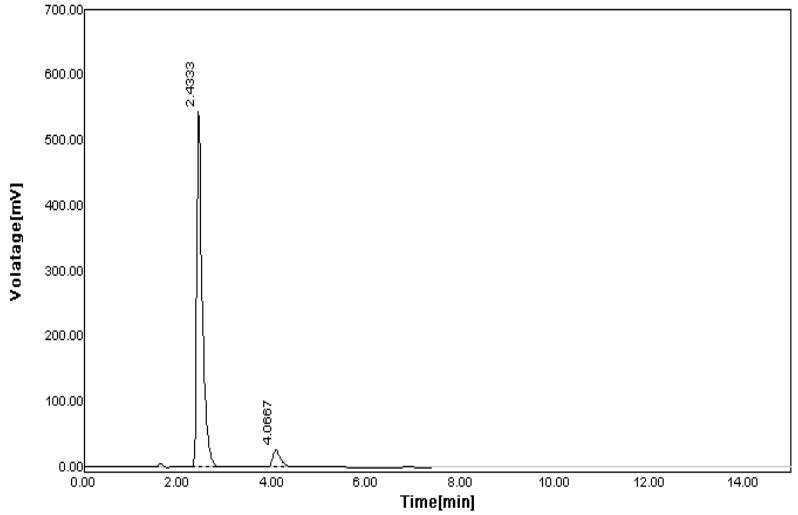

Fig. 7: Representative Chromatogram of Paracetamol and Ondansetron using ACN + 0.05% OPA PH 2.5 (40:60v/v) WL 248nm as mobile phase, showing retention time 2.433min and 4.0667min.

Chromatogram of Trial 7

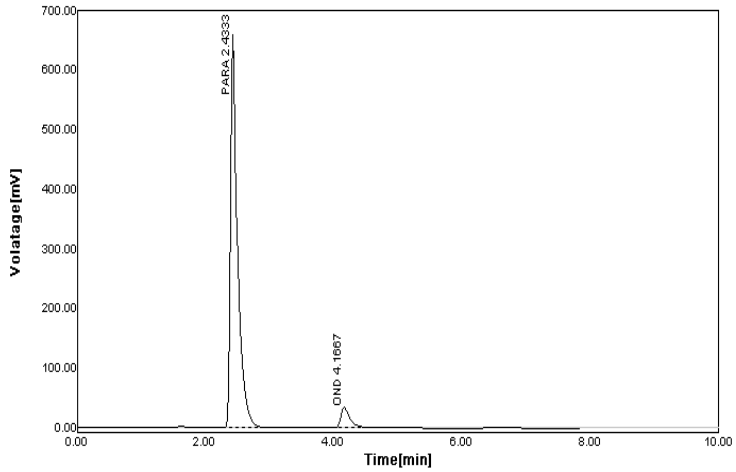

Fig. 8: Representative Chromatogram of Paracetamol and Ondansetron using ACN + 0.05% OPA PH 2.5 (40:60v/v) WL 248nm as mobile phase, showing retention time 2.433min and 4.1667min.

Chromatogram of Trial 8

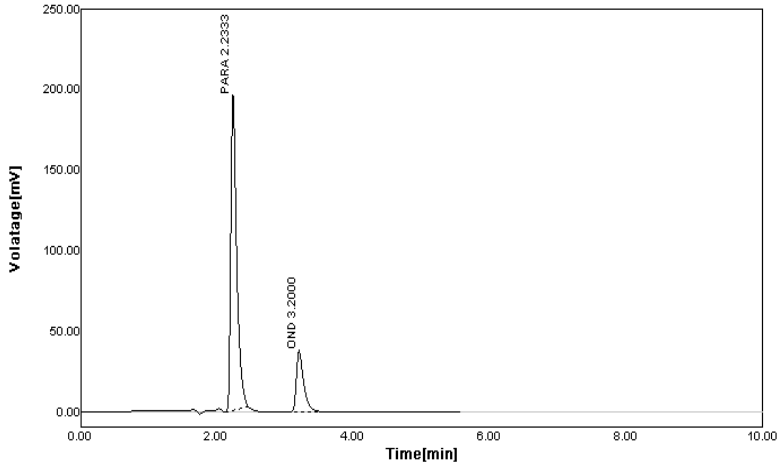

Fig. 9: Representative Chromatogram of Paracetamol and Ondansetron using ACN + 0.05% OPA PH 2.5 (30:70v/v) WL 248nm as mobile phase, showing retention time 2.233min and 3.2000min.

Chromatogram of Trial 9

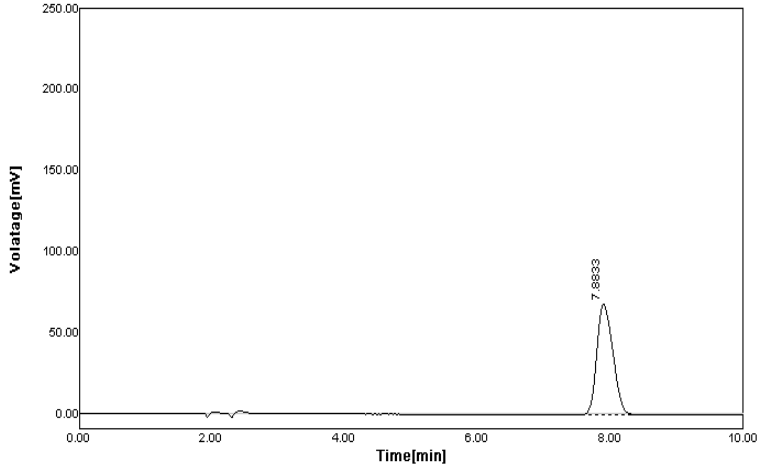

Fig. 10: Representative Chromatogram of Paracetamol and Ondansetron using ACN + 0.05% OPA PH 2.5 (10:90v/v) WL 248nm as mobile phase, showing retention time 7.8833min and 0min. *Chromatogram of Trial 10:*

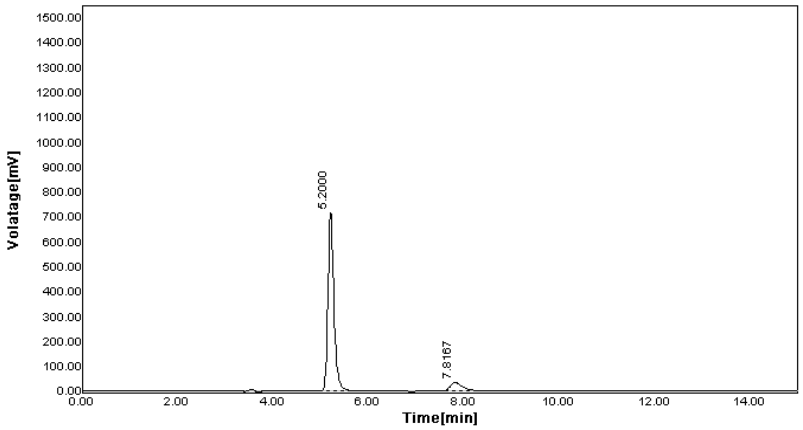

Fig. 11: Representative Chromatogram of Paracetamol and Ondansetron using Methanol + 0.05% OPA PH 2.5 (70:30v/v) WL 286nm as mobile phase, showing retention time 5.2000min and 7.8167min.

- **The final chromatographic conditions selected were as follow**

Mobile phase: Methanol +0.05% OPA PH 2.5 (70+30% v/v)

- Analytical column: Agilent C18 Column (150mm x 4.6mm, 5µm partical size).
- Injection volume: 20µl
- Flow rate: 1.0ml/min
- Detection: 286 nm
- Run Time: 15 min

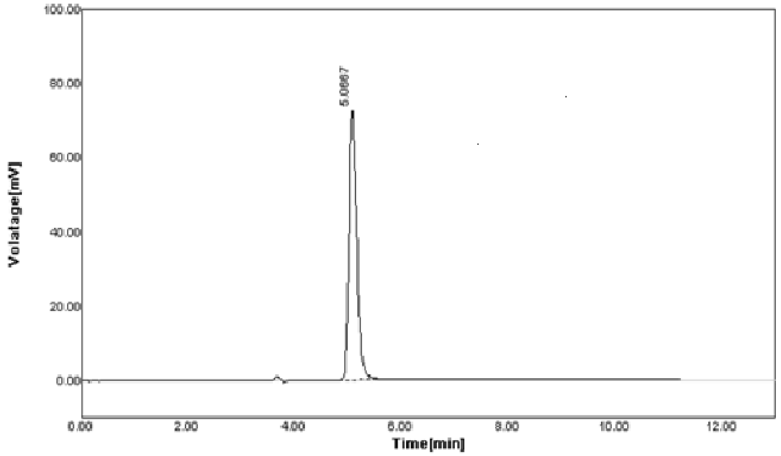

Fig. 12: Chromatogram of standard Paracetamol.

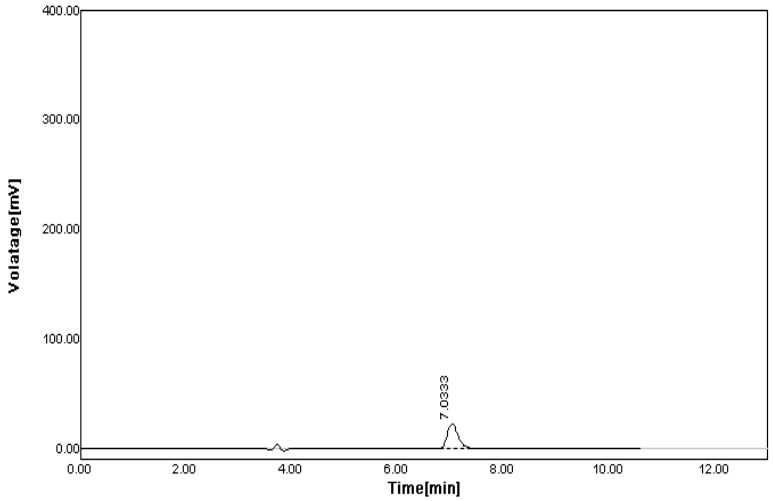

Fig. 13: Chromatogram of standard Ondansetron.

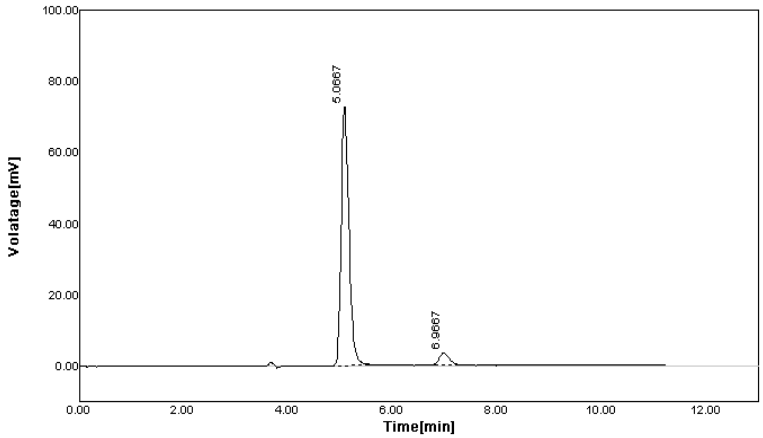

Fig. 14: Chromatogram of standard Combination of Paracetamol and Ondansetron.

Table 2: Details of chromatogram of standard Combination containing Paracetamol and Ondansetron.

No.	RT[min]	Area[mV*s]	Area%	TP	TF	Resolution
1	5.0667	749.0005	94.00	5124.7	1.3750	0.0000
2	6.9667	47.7730	6.00	4943.3	1.2917	4.7500
Sum		796.7736				

In the standard mixture of Paracetamol and Ondansetron theoretical plates were found above 2000 i.e. for Paracetamol 5124.7 and Ondansetron 4943.3 at minimum RT 5.0667and 6.9667respectively**.**

Calibration experiment

RP-HPLC Method

The data obtained in the calibration experiments when subjected to linear regression analysis showed a linear relationship between peak areas and concentrations in the range62.5-312.5 µg/mL for Paracetamol and 1-5µg/mL for Ondansetron (**Table No: 3 and Table No: 4)** depict the calibration data of Paracetamol and Ondansetron The respective linear equation for Paracetamol was y = 12.13x-14.99 and Ondansetron equation y = 69.34x-25.44 where x is the concentration and y is area of peak. The correlation coefficient was 0.999. The calibration curve of Paracetamol and Ondansetron is depicted in **(FigNo.14 and Fig No.15)**

Table 3: Linearity data for Paracetamol.

Method	Conc. µg/ml	Peak area(µV. sec)		Average peak area (µV. sec)	S.D. of Peak Area	% RSD of Peak Area
		1	2			
RP-HPLC Method	62.5	749	738.86	743.93	7.17	0.96
	125	1531.76	1539.77	1535.77	5.66	0.37
	187.5	2226.18	2219.92	2223.05	4.43	0.20
	250	2998.05	2990.73	2994.39	5.18	0.17
	312.5	3780.11	3835.79	3807.95	39.37	1.03
	Equation		Y=12.13x-14.99			
	R^2		0.999			

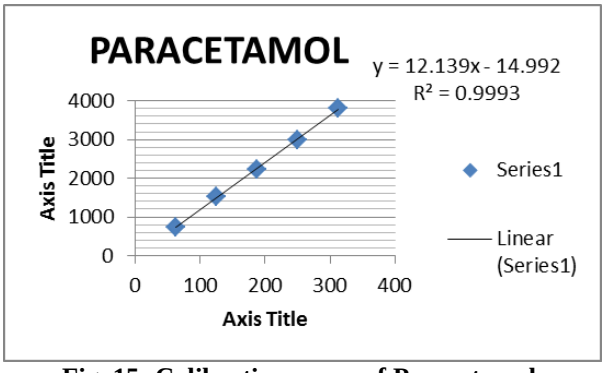

Fig. 15: Calibration curve of Paracetamol.

The RP-HPLC Method for respective linear equation for Paracetamol was Y=12.13X+14.99 where x is the concentration and y is area of peak. The correlation coefficient was 0.999. The calibration curve of Paracetamol is depicted in **Fig. 15.**

Table 4: Linearity data for Ondansetron.

Method	Conc. µg/ml	Peak area(µV. sec)		Average peak area (µV. sec)	S.D. of Peak Area	% RSD of Peak Area
		1	2			
RP-HPLC Method	1	46.77	46.63	46.70	0.10	0.21
	2	111.22	110.52	110.87	0.49	0.45
	3	182.75	179.54	181.15	2.27	1.25
	4	249.52	251.83	250.68	1.63	0.65
	5	326.43	320.57	323.50	4.14	1.28
	Equation		Y=69.34X-25.44			
	R^2		0.999			

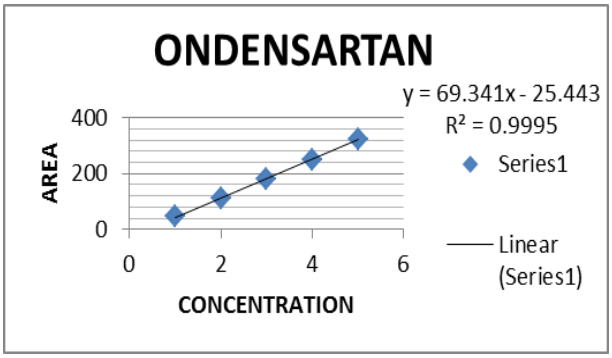

Fig. 16: Calibration curve of Ondansetron.

The RP-HPLC method for respective linear equation for Ondansetron was Y=69.34X-25.44where x is the concentration and y is area of peak. The correlation coefficient was 0.999. The calibration curve of Ondansetron is depicted in **Fig. 16.**

7.2. Analytical of Method Validation

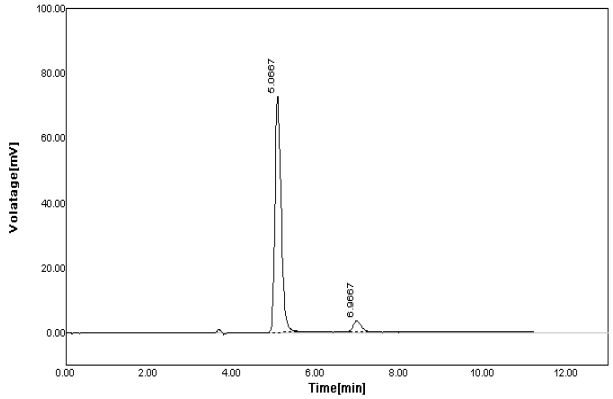

Fig. 17: Chromatogram of linearity.

Table 5: Linearity of Paracetamol.

Concentration µg/ml	Area Paracetamol
62.5	743.93
125	1535.77
187.5	2223.05
250	2994.39
312.5	3807.95

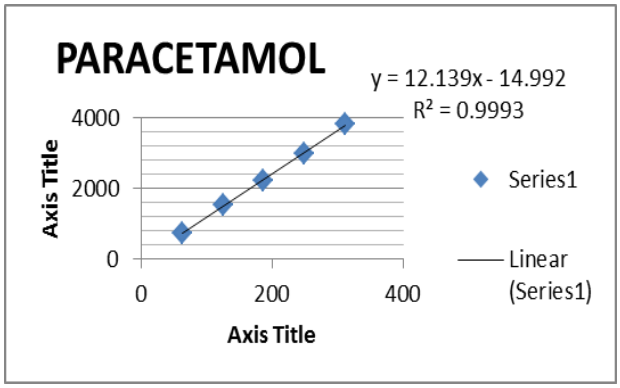

Fig. 18: Calibration curve of Paracetamol for HPLC method.

Table 6: Regression equation data for Paracetamol.

Regression Equation Data Y=mx+c	
Slope(m)	12.13
Intercept(c)	14.99
Correlation Coefficient	0.999

Table 7: Linearity of Ondansetron.

Concentration µg/ml	Area Ondansetron
1	46.70
2	110.87
3	181.15
4	250.68
5	323.50

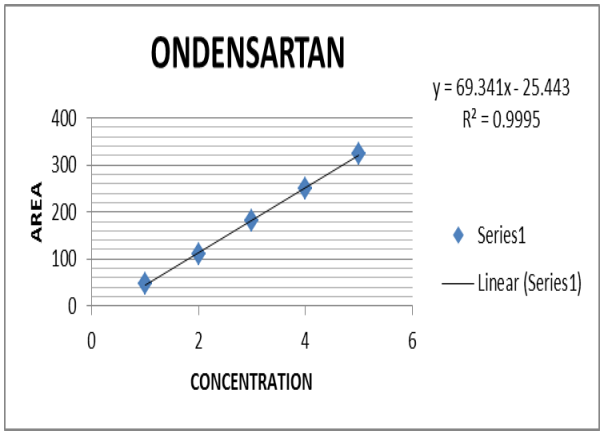

Fig. 19: Calibration graph of Ondansetron for HPLC method.

Table 8: Regression equation data for Ondansetron.

Regression Equation Data Y=mx+c	
Slope(m)	69.34
Intercept(c)	25.44
Correlation Coefficient	0.999

Linearity of of Paracetamol and Ondansetron was observed in the range of 10-50µg/ml and 1-5µg/ml. Detection wavelength used was 286nm. **(Table No. 6 & 8).**

The plot should be linear passing through the origin; Correlation Coefficient should not be less than 0.999.that concluded. **(Table No. 7& 8).**

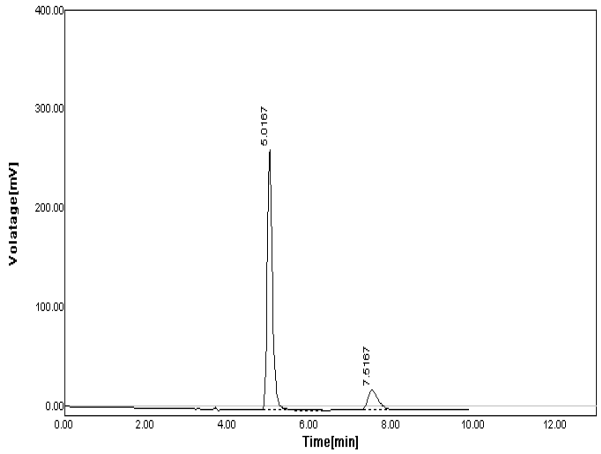

Fig. 20: Chromatogram of Accuracy 80%.

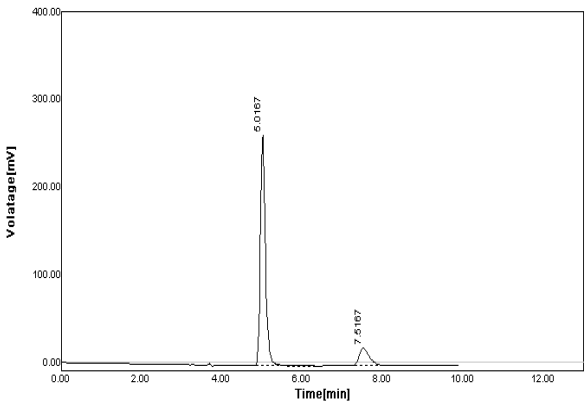

Fig. 21: Chromatogram of Accuracy 100%.

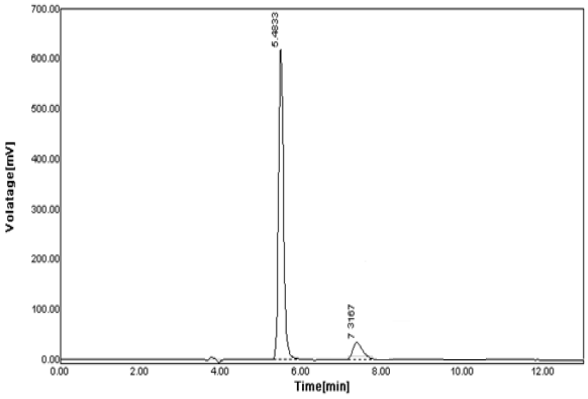

Fig. 22: Chromatogram of Accuracy 120%.

Table 9: Result of Recovery data for Paracetamol and Ondansetron.

Method	Drug	Level (%)	Amt. taken (µg/ml	Amt. Added (µg/ml	Area Mean* ± S.D.	Amt. recovered Mean *±S.D.	%Recovery Mean *± S.D.
RP-HPLC Method	**Para**	80%	125	100	224.30± 0.19	99.30± 0.19	99.30 ±0.19
		100%	125	125	252.83± 0.89	127.8 ±0.89	102.2 ±0.71
		120%	125	150	275.1±0.88	150.1 ±0.88	100.07±0.5
	Onda	80%	2	1.6	3.58 ±0.01	1.58±0.01	98.8± 0.73
		100%	2	2	3.99 ±0.03	1.99 ±0.03	99.50 ±1.41
		120%	2	2.4	4.37± 0.03	2.37±**0.03**	98.75± 1.18

***mean of each 3 reading for RP-HPLC method.**

Table 10: Statistical Validation of Recovery Studies Paracetamol and Ondansetron.

Method	Level of Recovery (%)	Drug	Mean % Recovery	Standard Deviation*	% RSD
Rp-HPLC Method	80%	PARA	99.30	0.19	0.19
		ONDA	98.86	0.73	0.74
	100%	PARA	102.26	0.71	0.70
		ONDA	99.50	1.41	1.42
	120%	PARA	100.07	0.58	0.58
		ONDA	98.75	1.18	1.19

***Denotes average of three determinations for RP-HPLC and UV method**

Accuracy of RP-HPLC method is ascertained by recovery studies performed at different levels of concentrations (80%, 100% and 120%). The % recovery was found to be within 99-101% **(Table No. 9&10).**

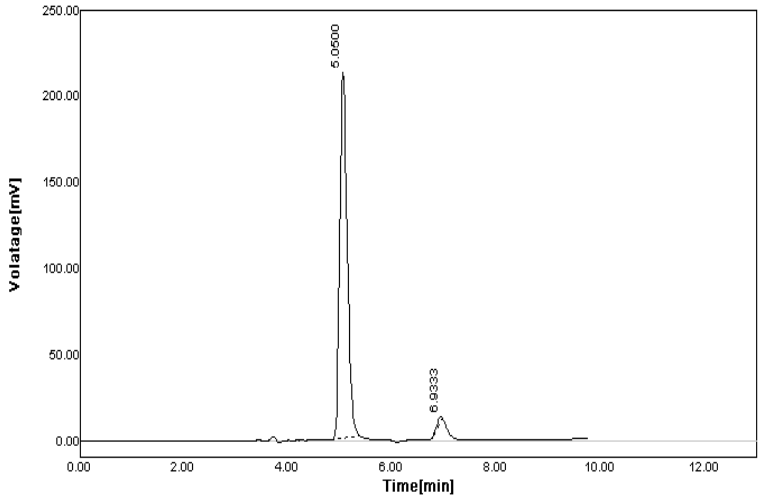

Fig. 23: Chromatogram of System suitability-1.

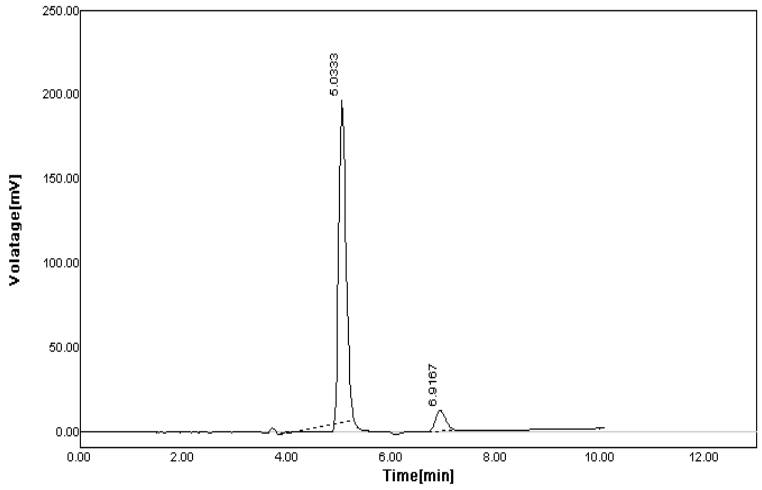

Fig. 24: Chromatogram of System suitability No- 2.

Table 11: Repeatability studies on RP-HPLC for Paracetamol and Ondansetron.

Method	Concentration of Paracetamol and Ondansetron (mg/ml)	Peak area	Amount found (mg)	% Amount found
RP-HPLC Method for PARA	187.5	2226.18	184.76	98.54
	187.5	2219.92	310.02	98.26
	187.5	2261.72	39.35	100.10
	187.5	2297.65	190.65	101.68
		Mean	**181.20**	
		SD	**1.58**	
		%RSD	**1.59**	
RP-HPLC Method for ONDA	3	182.75	3.00	100.00
	3	179.54	2.96	98.54
	3	183.07	3.00	100.00
	3	185.37	3.04	101.48
		Mean	**3.00**	
		SD	**0.03**	
		%RSD	**1.14**	

Chromatogram of Precision

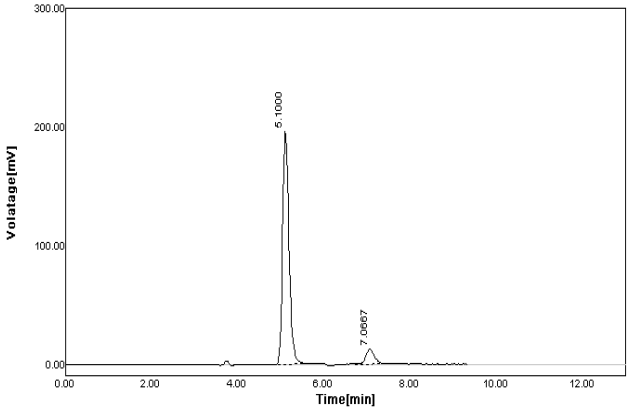

Fig. 25: Chromatogram of Precision.

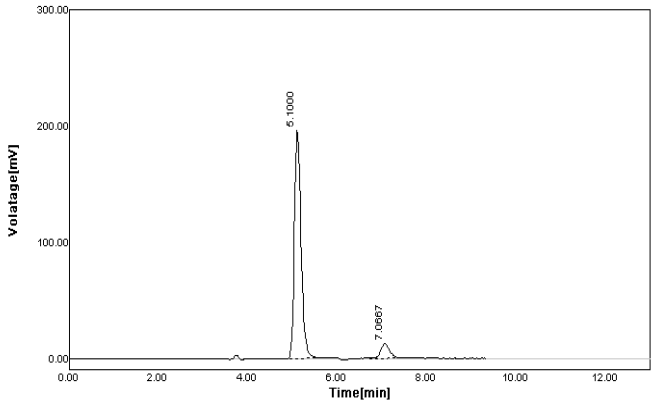

Fig. 26: Chromatogram Intra-day precision.

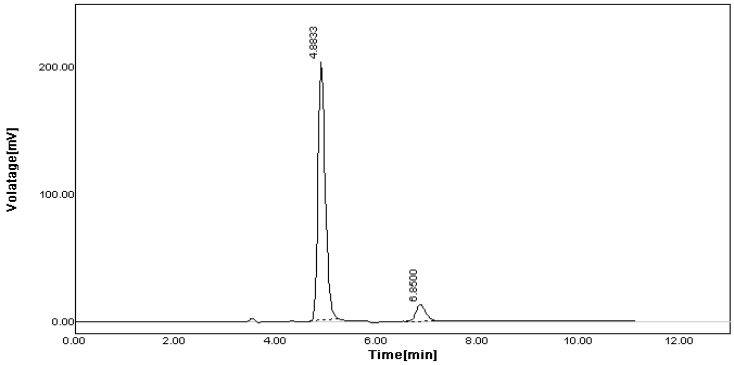

Fig. 27: Chromatogram Inter-day precision.

Table 12: Result of Intra-day and Inter day Precision studies on RP-HPLC and UV method for Paracetamol and Ondansetron.

Method	Drug	Conc^{n} (µg/ml)	Intraday Precision		Inter-day Precision	
			Mean± SD	% Amt. Found	Mean± SD	% Amt. Found
Rp-HPLC Method	PARA	125	1500.5 ±1.15	99.95	1508 ±1.19	99.36
		187.5	2240.5 ±26.5	99.17	2258± 26.8	98.99
		250	2993.3 ±10.6	99.2	2999.2 ±10.8	99.5
	ONDA	2	113.87±1.91	100.4	118± 1.93	100.00
		3	180.21± 2.63	98.86	188.32± 2.61	98.55
		4	257.71± 1.50	102.08	255.01 ±1.24	102.00

***Mean of each 3 reading for RP-HPLC**

1) Flow Rate Change 0.9 ml

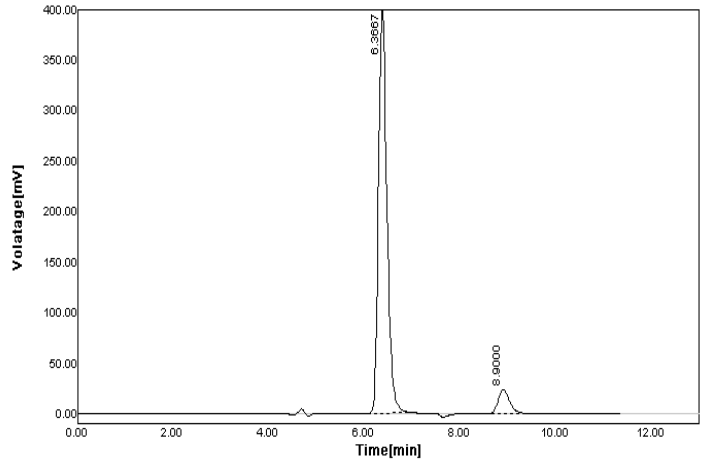

Fig. 28: Chromatogram of Flow rate change 0.8ml.

2) Flow Rate Change 1.0 ml

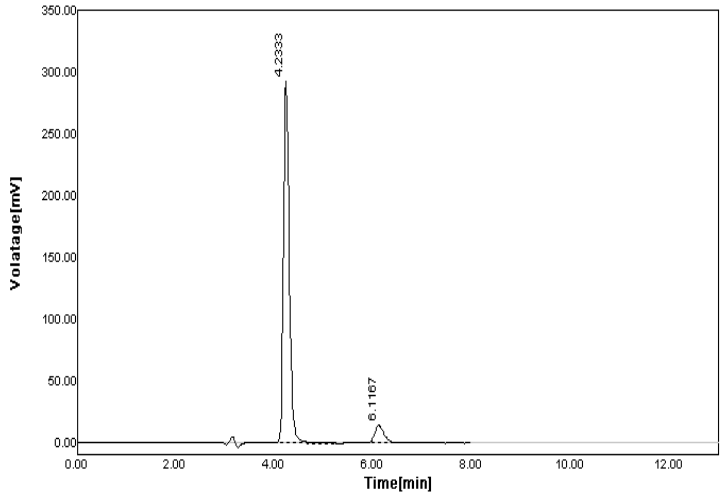

Fig. 29: Chromatogram of Flow rate change 1.0 ml.

3) Mobile phase composition Change : 69ml MeoH + 31ml water

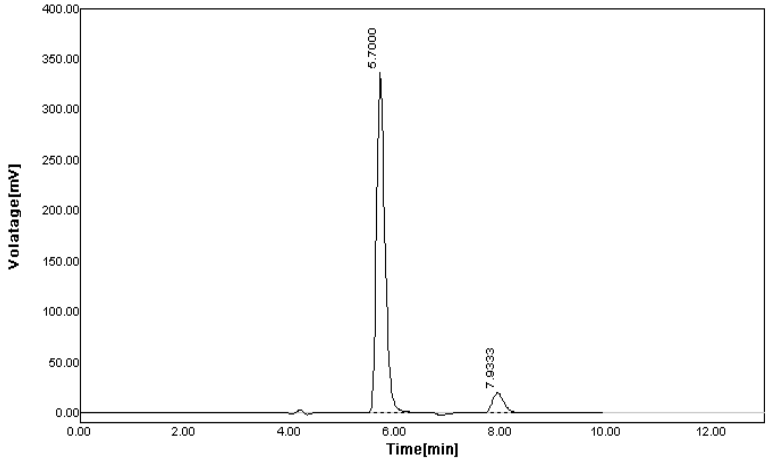

Fig. 30: Chromatogram of Mobile phase composition change 69ml MeoH + 31 ml water.

4) Mobile phase composition Change:=71ml MEOH+29ml water

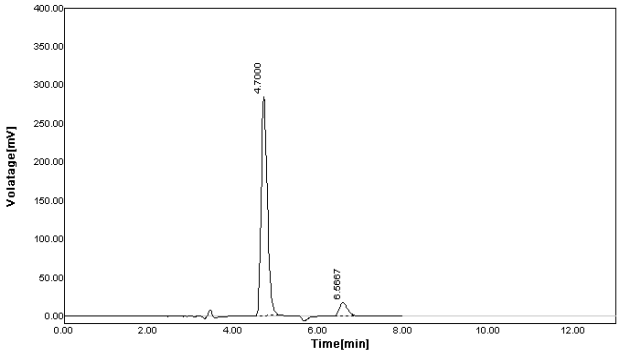

Fig. 31: Chromatogram of Mobile phase composition change 71ml MEOH + 29ml water.

5) Wavelength Change 285nm

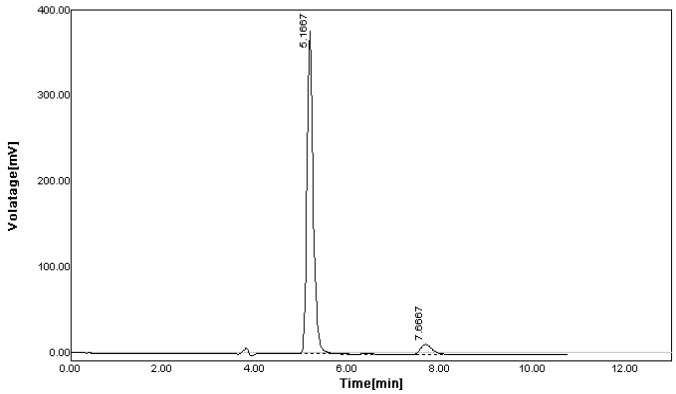

Fig. 32: Chromatogram of comp change wavelength change 285nm.

6) Wavelength Change 287nm

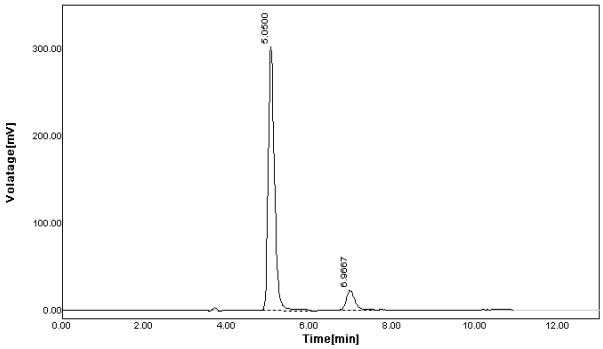

Fig. 33: Chromatogram of comp change wavelength change 287nm

Table 13: Result of Robustness Study of Paracetamol.

Parameters	Conc.(µg/ml)	Amount of detected (mean ±SD)	%RSD
Chromatogram of flow change 0.9ml	312.5	3695.07±29.78	0.62
Chromatogram of flow change 1.1 ml	312.5	3707.08 ±1.60	0.04
Chromatogram of comp change 69ml MEOH +31ml water	312.5	3707.78 ±4.80	0.13
Chromatogram of comp change 71ml MEOH +29 ml water	312.5	3757.54± 2.79	0.07
Chromatogram of comp change wavelength change 285nm	312.5	3631.34± 18.16	1.50
Chromatogram of comp change wavelength change 287nm	312.5	3771.39± 37.70	1.00

Table 14: Result of Robustness Study of Ondansetron.

Parameters	Conc.(µg/ml)	Amount of detected (mean ±SD)	%RSD
Chromatogram of flow change 0.9ml	5	318.88 ±1.39	0.44
Chromatogram of flow change 1.1 ml	5	314.63±1.36	0.43
Chromatogram of comp change 69ml MEOH +31ml water	5	324.29±1.24	0.38
Chromatogram of comp change 71ml MEOH +29 ml water	5	314.48± 1.58	0.50
Chromatogram of comp change wavelength change 285nm	5	323.09 ±3.88	1.20
Chromatogram of comp change wavelength change 287nm	5	325.68± 5.44	1.67

Brand Name: Ondem-p
Take 5ml in 10 ml Methanol sonicate 10 min
i.e. 1200 µgm/ml PARA and 2 µgm/m ----- STOCK -I
Take 5ml in 10ml Methanol= 1 200 µgm/ml PARA and 2 µgm/ml ONDA.

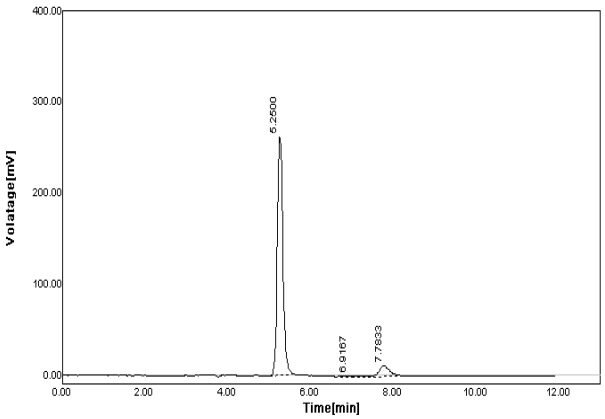

Fig. 34: Chromatogram for Marketed Formulation.

Table 15: Analysis of marketed formulation.

Assay	Drug	Label Claimed	Amt. Found	% Label Claim	SD	% RSD
Rp-HPLC Method	**PARA**	125	1530.88	101.95	8.00	0.52
	ONDA	2	111.28	99.00	2.07	1.81
	PARA	125	1519.57	101.20	0.52	0.16
	ONDA	2	115.21	101.40	0.13	0.14

Analysis of marketed formulation were also % Label Claim was found to be 99-101% Satisfactory are concluded**. (Table No. 15).**

CONCLUSION

Simple, rapid, accurate and precise RP-HPLC as well as spectrophotometric methods have been developed and validated for the routine analysis of Paracetamol and Ondansetron in API and tablet dosage forms. Both methods are suitable for the simultaneous determination of Paracetamol and Ondansetron in multi-component formulations without interference of each other. The developed methods are recommended for routine and quality control analysis of the investigated drugs in two component pharmaceutical preparations. The amount found from the proposed methods was in good agreement with the label claim of the formulation. Also the value of standard deviation and coefficient of variation calculated were satisfactorily low, indicating the suitability of the proposed methods for the routine estimation of tablet dosage forms.

The study is to establish and generate inheriting stability data for Paracetamol and Ondansetron through stress degradation studies under ICH recommended stress conditions (like temperature, light, solvent system, exposure to UV radiations delayed analysis) and develop stability indicating assay.

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