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A VALIDATED RP HPLC METHOD FOR ASPIRIN AND OMEPRAZOLE IN ITS PURE AND CAPSULE DOSAGE FORM

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ABSTRACT

The present study described a new simple accurate and precise development of RP-HPLC method for the simultaneous estimation aspirin and omeprazole in capsule dosage form. The chromatographic method was standardized using a Agilent C_{18} (150mm*4.5mm,5µ), with gradient conditions and mobile phase containing buffer-p^H 3: Methonal (30:70) at flowrate of 1ml/min using UV detection at 240nm. The retention time of Omeprazole and Aspirin was found to be 2.427mins and 4.432mins respectively. The system suitability parameters for Omeprazole and Aspirin such as theoretical plates and tailing factor were found to be 2733, 1.6 and 3500, 1.4. Resolution was 4.6. The % purity Omeprazole and Aspirin in pharmaceutical dosage form was found to be 99.84 and 100.14% respectively. The validation of method carried out utilizing ICH guidelines.

Keywords
Aspirin, omeprazole, simultaneous estimation, HPLC, stability

INTRODUCTION

There are many methods on development and validation for simultaneous estimation of aspirin and omeprazole pharmaceutical dosage forms[1] and bilk drugs by RP-HPLC method. Hence alternative methods for existing products are developed to reduce the cost and time for better precision and ruggedness. Trail runs are conducted, method is optimized and validated. Here the study includes validation parameters and stability studies effecting by temperature. This method can be used for the analysis of formulation development and stability testing to some extent in quality control laboratory use

The proposed work attempts shall be made to

- To develop a new, simple, sensitive, accurate and economical analytical method and validation for the Simultaneous estimation of Omeprazole and Aspirin pure and pharmaceutical Tablet dosage form by using RP-HPLC.
- To validate the developed method in accordance with ICH guidelines for the intended analytical application i.e., to apply the proposed method for analysis of the drug in its dosage form.
- To apply the developed method for the simultaneous estimation of Omeprazole and Aspirin in pure and pharmaceutical capsule dosage form.

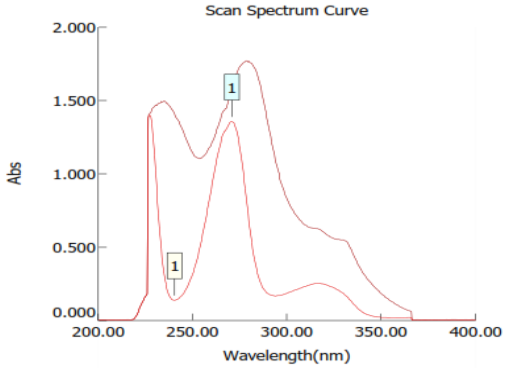
Drug profile:

Omeprazole[2-4] is crystalline powder Soluble in water (10mg/ml), methanol, DMSO (~25mg/ml), DMF (~30mg/ml), and ethanol (~30mg/ml). Melting Point is 140-141 °C and the Pka 9.35 Mechanism of action of Omeprazole is a selective and irreversible proton pump inhibitor. It suppresses stomach acid secretion by specific inhibition of the H^+/K^+-ATPase system found at the secretary surface of gastric parietal cells. Aspirin is crystalline powder soluble in water Melting Point of aspirin is 135°C Mechanism of action of aspirin is much of this is believed to be due to decreased production of prostaglandins and TXA2. Aspirin's ability to suppress the production of prostaglandins and thromboxanes is due to its irreversible inactivation of the Cyclo oxygenase (COX) enzyme. Cyclo oxygenase is required for prostaglandin and thromboxane synthesis.

Method Development[5-6]

The detection wavelength was selected by dissolving the drug in mobile phase to get a concentration of 10µg/ml for individual and mixed standards. The resulting solution was scanned in U.V range from 200-400nm. The overlay spectrum of Omeprazole and Aspirin was obtained and the isobestic point of Omeprazole and Aspirin showed absorbance's maximum at 240 nm.

Spectrum showing overlapping spectrum of Omeprazole and Aspirin

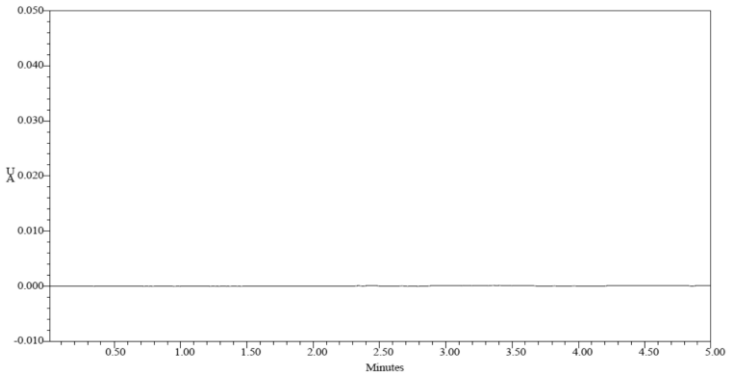


The chromatographic method development for the simultaneous estimation of Omeprazole and Aspirin were optimized by several trials for various parameters as different column, flow rate and mobile phase, finally the following chromatographic[7] method was selected for the separation and quantification of Omeprazole and Aspirin in API and pharmaceutical dosage form by RP-HPLC method.

Optimization

Column	:	Agilent C^{18} (4.5×150 mm) 5.0 µm
Column temperature	:	Ambient
Wavelength	:	240 nm
Mobile phase ratio	:	(70:30) methanol: phosphate buffer
Flow rate	:	1 ml/min
Injection volume	:	10µl
Run time	:	10.0 minutes

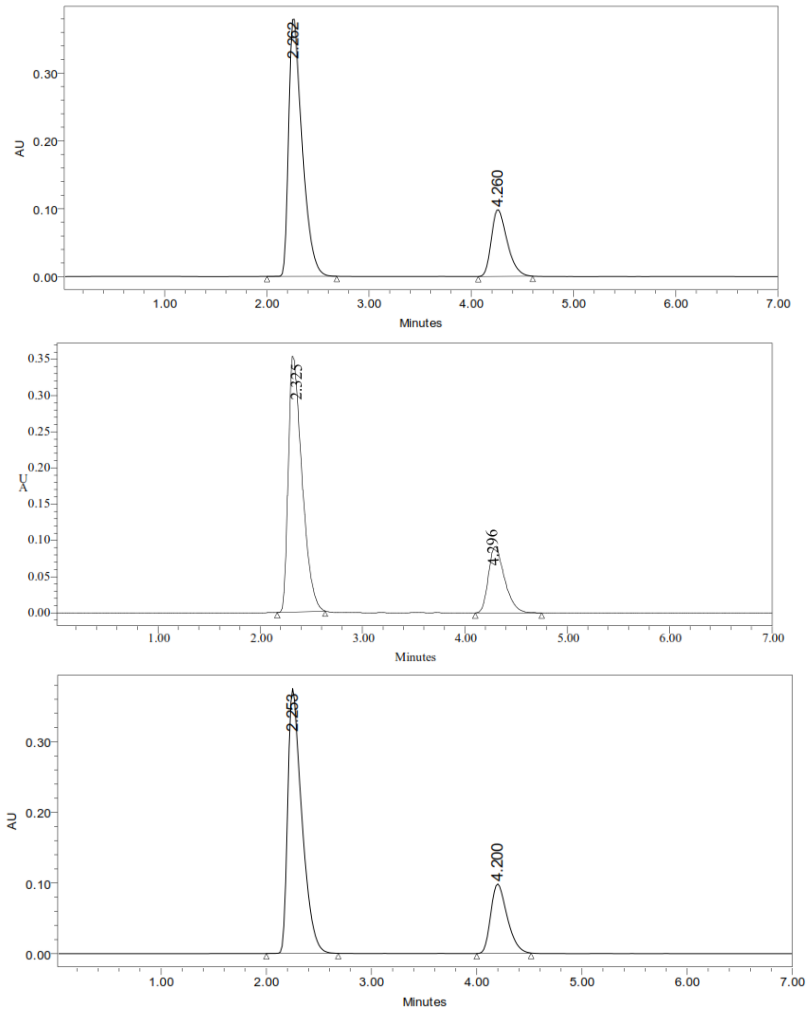
Chromatogram showing blank preparation (mobile phase)



Assay calculation for Omeprazole and Aspirin

The assay[8] study was performed for the Omeprazole and Aspirin. Each three injections of sample and standard were injected into chromatographic system.

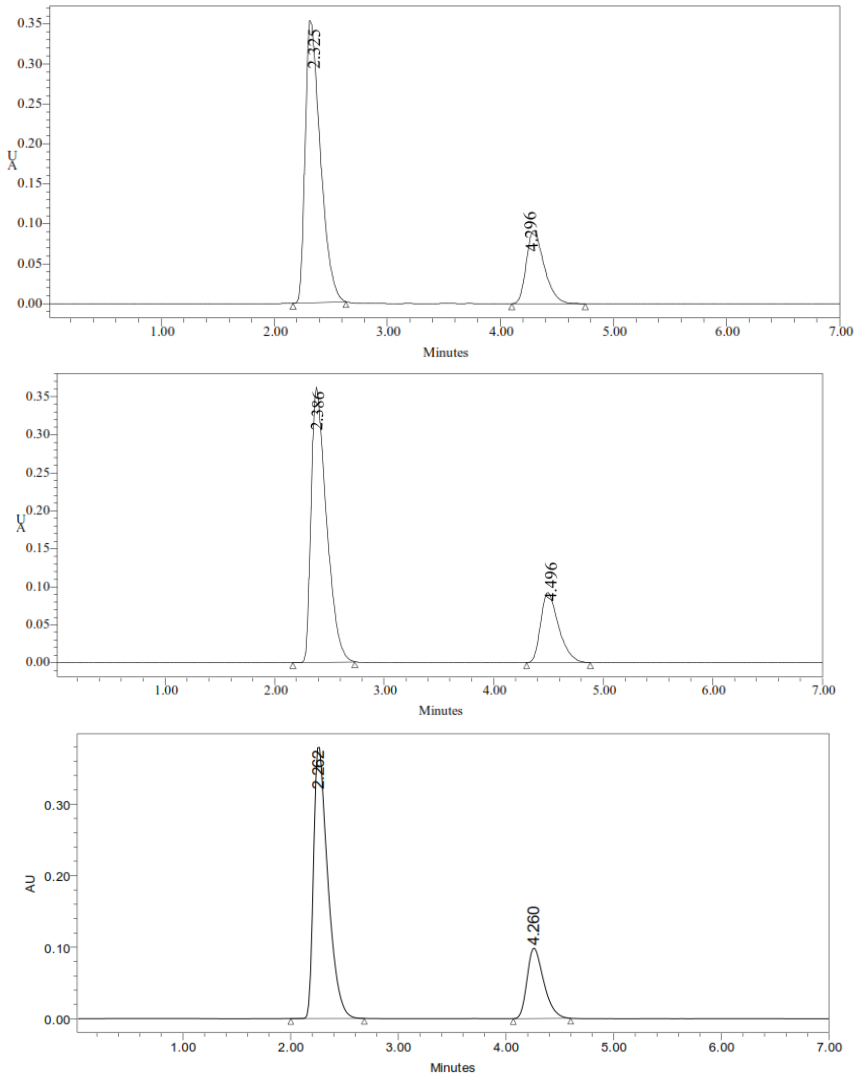
Chromatogram showing assay of sample injection-1, 2, 3



	Name	Rt	Area
1	Omeprazole	2.262	3497318
2	Omeprazole	2.325	3487678
3	Omeprazole	2.253	3494274
Mean			3493093
Std.dev			4927.8
%RSD			0.14

	Name	Rt	Area
1	Aspirin	4.260	1058190
2	Aspirin	4.296	1078378
3	Aspirin	4.200	1065083
Mean			1067217
Std.dev			10261.8
%RSD			1.0

Chromatogram showing standard of sample injection -1,2,3



	Name	Rt	Area
1	Omeprazole	2.325	3488550
2	Omeprazole	2.386	3497133
3	Omeprazole	2.262	3476527
Mean			3487403
Std.dev			10350.7
%RSD			0.3

	Name	Rt	Area
1	Aspirin	4.296	1060075
2	Aspirin	4.496	1064862
3	Aspirin	4.260	1078494
Mean			1067810
Std.dev			9556.9
%RSD			0.9

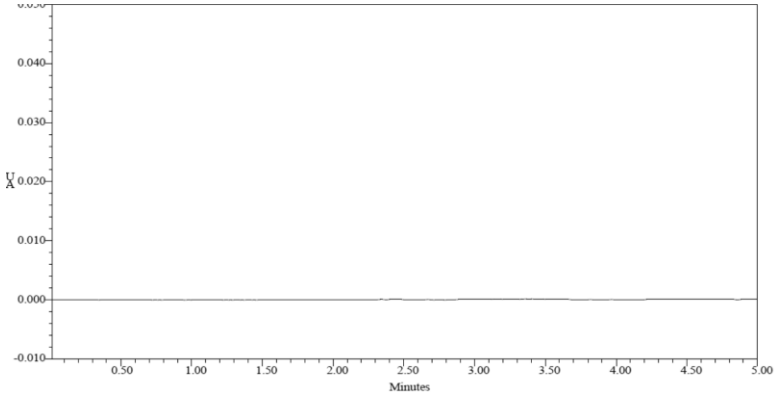
The retention time of Omeprazole and Aspirin was found to be 2.427mins and 4.432mins respectively. The system suitability parameters for Omeprazole and Aspirin such as theoretical plates and tailing factor were found to be 2733, 1.6 and 3500, 1.4. Resolution was 4.6. The % purity Omeprazole and Aspirin in pharmaceutical dosage form was found to be 99.84 and 100.14% respectively.

VALIDATION PARAMETERS

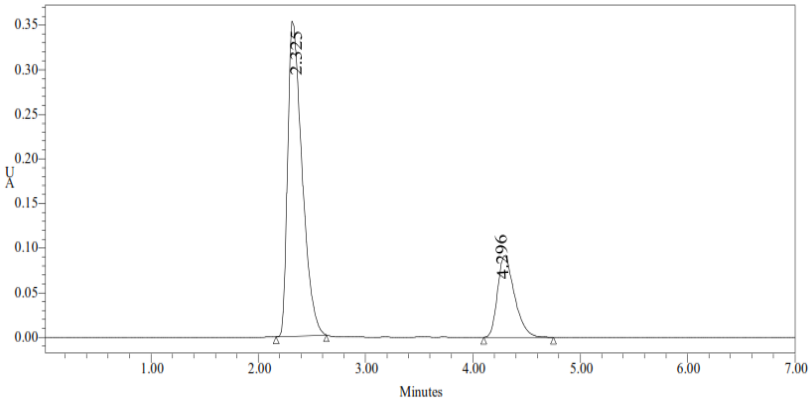
1. Specificity[9]

The system suitability for specificity was carried out to determine whether there is any interference of any impurities in retention time of analytical peak.

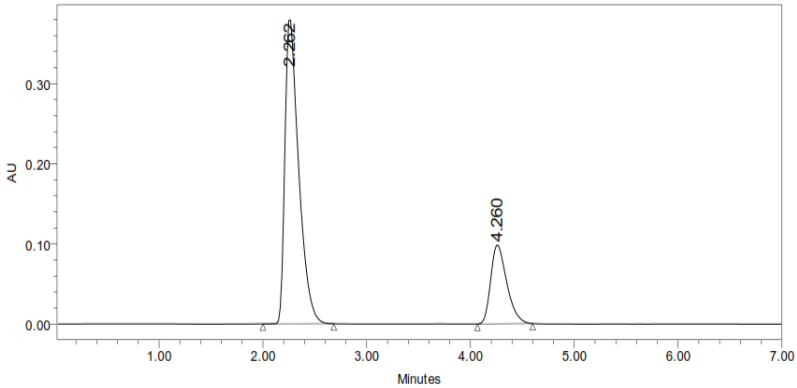
Chromatogram showing blank (mobile phase preparation)



Chromatogram showing standard injection



Chromatogram showing sample injection

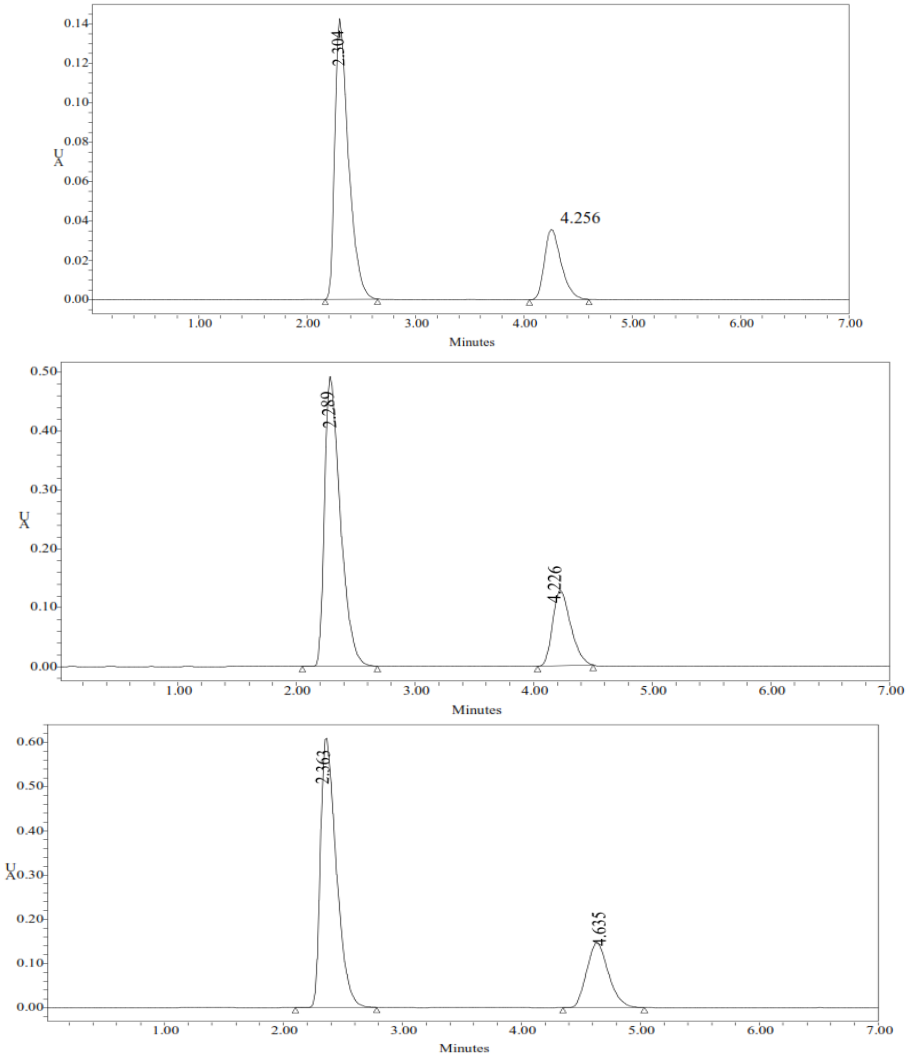


The specificity test was performed for Omeprazole and Aspirin. It was found that there was no interference of impurities in retention time of analytical peak.

2. Linearity [10-12]

The linearity study was performed for the concentration of 25ppm to 125ppm Omeprazole and 15ppm to 75ppm Aspirin level. Each level was injected into chromatographic system. The area of each level was used for calculation of correlation coefficient.

Chromatograms showing linearity level-1 to level 5 (25ppm-125ppm of Omeprazole and 15ppm -75ppm of Aspirin) injections.



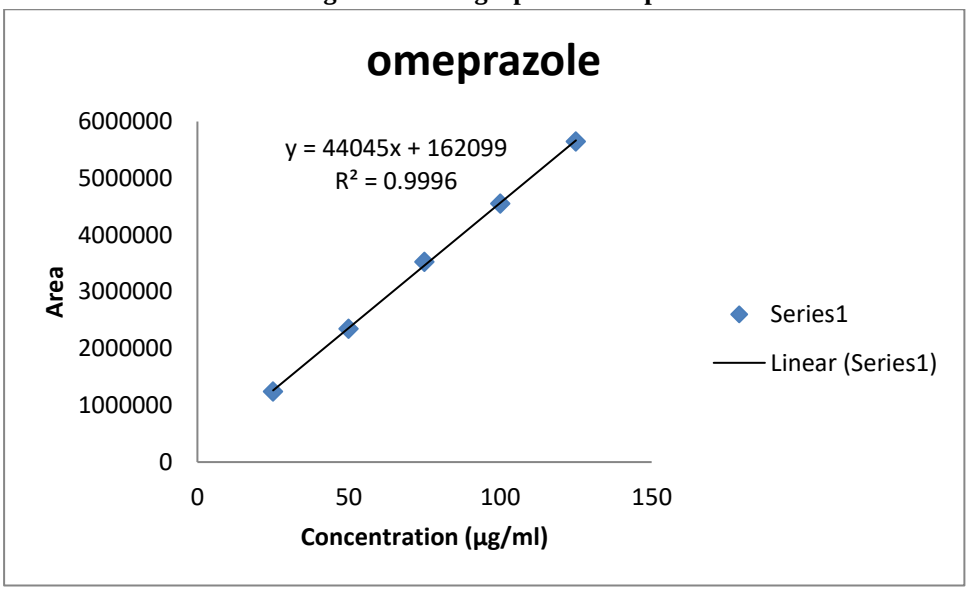
Linearity Results for Omeprazole:

S. No	Linearity Level	Concentration	Area
1	I	25	1246587
2	II	50	2248079
3	III	75	3529801
4	IV	100	4553376
5	V	125	5649583
Correlation Coefficient			0.999

Linearity Results for Aspirin:

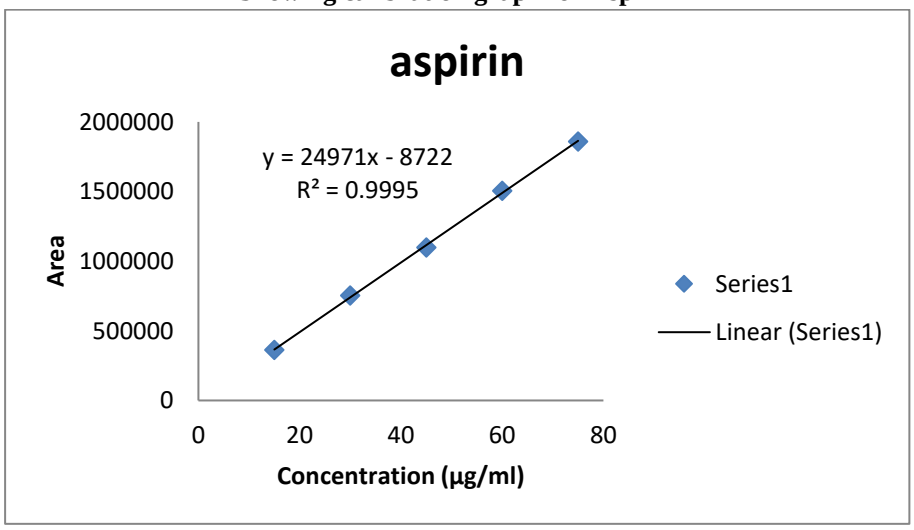
SI.NO	Linearity level	Concentration	Area
1	I	15	361744
2	II	30	751959
3	III	45	1068815
4	IV	60	1503717
5	V	75	1858720
Correlation Coefficient			0.999

Showing calibration graph for Omeprazole



Omeprazole r2 = 0.999

Showing calibration graph for Aspirin



Aspirin r^2 = 0.999

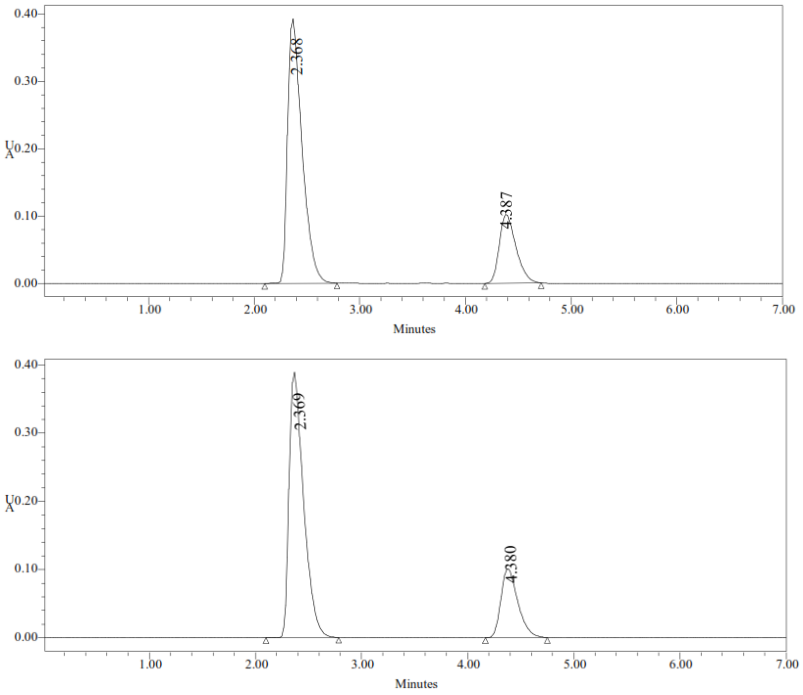
The linearity study was performed for concentration range of 25µg -125µg Omeprazole and 15µg - 75 µg Aspirin and the correlation coefficient was found to be 0.999 and 0.999.(NLT 0.999)respectively.

3. Accuracy[13-15]

The accuracy study was performed for 50%, 100% and 150 % for Omeprazole andAspirin. Each level was injected in triplicate into chro

matographic system. The area of each level was used for calculation of % recovery.

Chromatogram showing accuracy -100% injection-1,2,3.

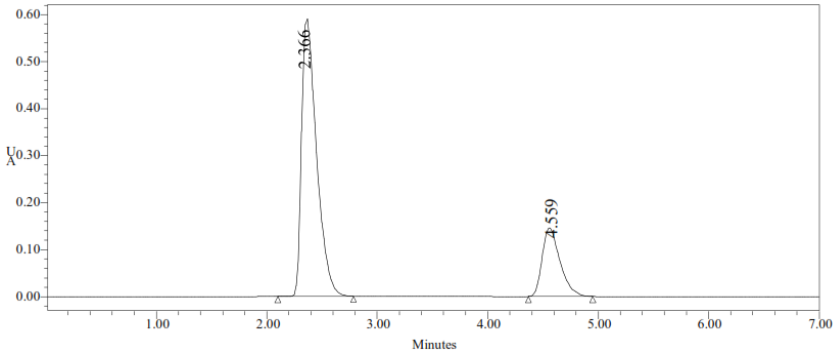


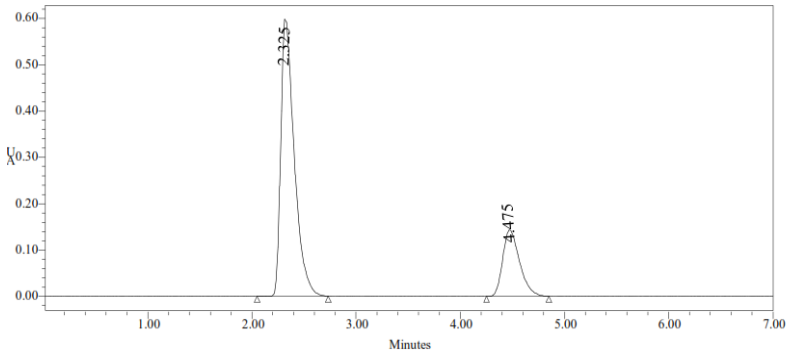
	Name	Rt	Area
1	Omeprazole	2.368	3610125
2	Omeprazole	2.369	3672197
3	Omeprazole	2.510	3644408
Mean			3642243
Std.dev			31092.5
%RSD			0.85

Accuracy 150%

Chromatogram showing accuracy -150 % injection-1,2,3.

	Name	Rt	Area
1	Aspirin	4.387	1118339
2	Aspirin	4.380	1117228
3	Aspirin	4.641	1110769
Mean			1115445
Std.dev			4087.6
%RSD			0.37





	Name	Rt	Area
1	Omeprazole	2.366	5539965
2	Omeprazole	2.325	5523682
3	Omeprazole	2.342	5542462
Mean			5535371
Std.dev			10196.8
%RSD			0.18

Showing accuracy results for Omeprazole

%Concentration (at specification level)	Average area	Amount added (mg)	Amount found (mg)	% Recovery	Mean recovery
50%	1829739	5	5.02	100.1%	100.19%
100%	3642243	10	9.96	99.60%	
150%	5535371	15	15.1	101.30%	

Showing accuracy results for Aspirin

	Name	Rt	Area
1	Aspirin	4.559	1683483
2	Aspirin	4.475	1681034
3	Aspirin	4.527	1684878
Mean			1682465
Std.dev			2101.7
%RSD			0.12

%Concentration (at specification level)	Average area	Amount added (mg)	Amount found (mg)	% Recovery	Mean recovery
50%	564367	5	5.0	101.1%	102.30%
100%	1115445	10	10.5	105.5%	
150%	1682465	15	15.0	100.5%	

The accuracy study was performed for % recovery of Omeprazole and Aspirin. The % recovery was found to be 100.19 % and 102.30 % respectively (NLT 98% and NMT 102%)

4 Precision

- Repeatability
- Intermediate Precision

Repeatability[16]

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

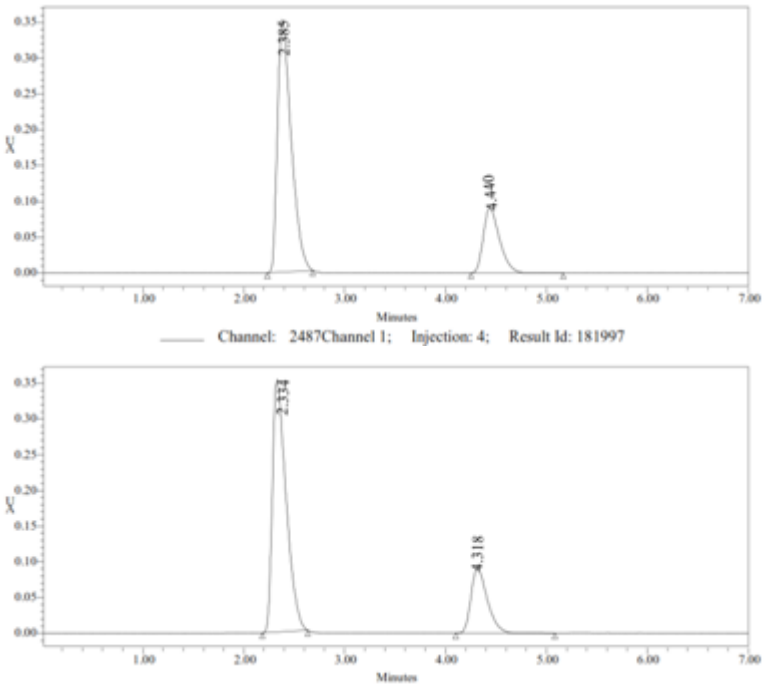
Intermediate precision/Ruggedness

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

Repeatability

The precision study was performed for five injections of Omeprazole and Aspirin. Each standard injection was injected into chromatographic system.

The area of each Standard injection was used for calculati on of % RSD. **Chromatograms showing precision injections -1 to 5**



Showing% RSD results for Omeprazole

s.no	Peak name	RT	Area
1	Omeprazole	2.357	3367917
2	Omeprazole	2.353	3324161

3	Omeprazole	2.388	3390163
4	Omeprazole	2.385	3323428
5	Omeprazole	2.334	3329454
Mean			3347025
Std.dev			30354.9
%RSD			0.9

Showing %RSD results for Aspirin

s.no	Peak name	RT	Area
1	Aspirin	4.373	1025541
2	Aspirin	4.377	1023214
3	Aspirin	4.442	1023881
4	Aspirin	4.440	1020840
5	Aspirin	4.318	1026447
Mean			1023985
Std.dev			2178.2
%RSD			0.21

The Method precision study was performed for the %RSD of Omeprazole and Aspirin was found to be 0.9 and 0.21(NMT 2).

6 Detection limits

LOD's can be calculated based on the standard deviation of the response (SD) and the slope of the calibration curve (S) at levels approximating the LOD according to the formula. The standard deviation of the response can be determined based on the standard deviation of y-intercepts of regression lines.

Formula:

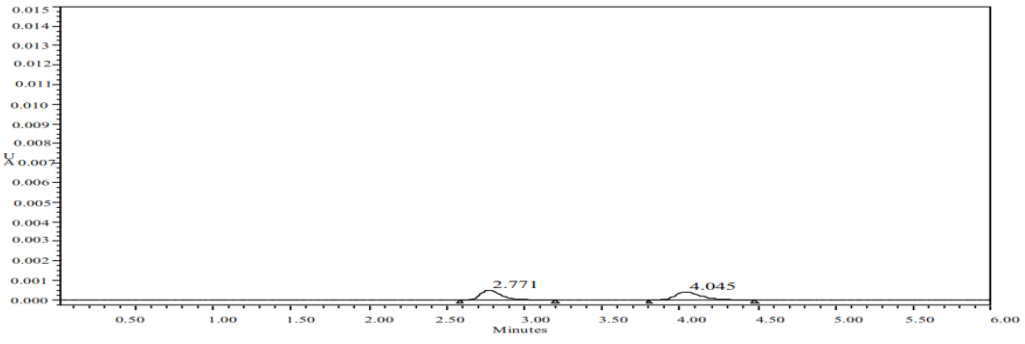
$$\text{LOD} = 3.3 \text{ X } \frac{\sigma}{S}$$

Where

σ - Standard deviation (SD)

S - Slope

Chromatogram for LOD



The LOD was performed for Omeprazole and Aspirin was found to be3.17and 0.1372 respectively

7 Quantitation limit

LOQ's can be calculated based on the standard deviation of the response (SD) and the slope of the calibration curve (S) according to the formula. Again, the standard deviation of the response can be determined based on the standard deviation of y-intercepts of regression lines.

Formula:

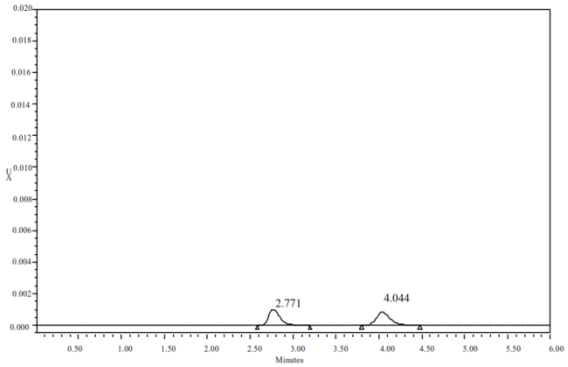
$$LOQ = 10\ X\ \frac{\sigma}{S}$$

Where

σ - Standard deviation

S - Slope

Chromatogram for LOQ

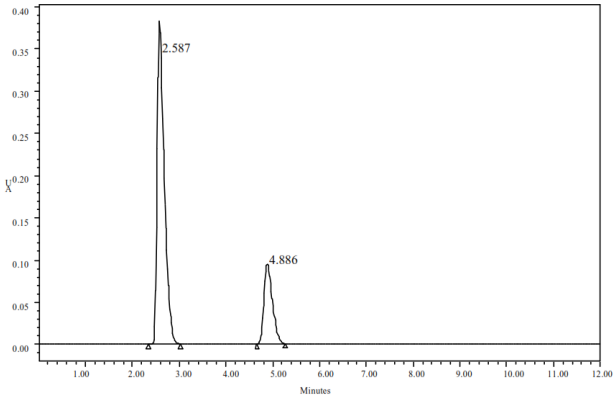


The LOQ was performed for Omeprazole and Aspirin was found to be 5.60 and 0.132 respectively.

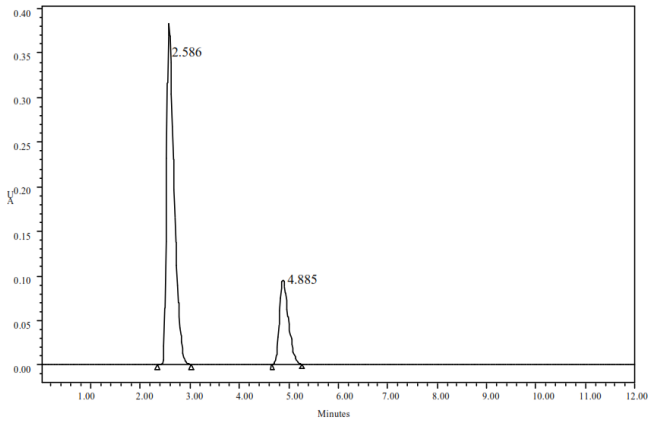
8. Robustness

The robustness was performed for the flow rate variations from 0.8ml/min to 1.0 ml/min and mobile phase ratio variation from more organic phase to less organic phase ratio for Omeprazole and Aspirin . The method is robust only in less flow condition and the method is robust even by change in the Mobile phase ±5%.

Chromatogram showing more flow rate 1.2ml/min



Chromatogram showing less flow rate 0.8 ml/min



The results are summarized on evaluation of the above results, it can be concluded that the variation in flow rate affected the method significantly. Hence it indicates that the method is robust even by change in the flow rate ±0.2ml/min. The method is robust only in less flow condition.

Showing system suitability results for Omeprazole

S. No	Flow rate (ml/min)	System suitability results	
		USP Plate Count	USP Tailing
1	0.8	2231.8	1.3
2 2	**1**	**2784**	**1.3**
3	1.2	2704.0	1.3

Showing system suitability results for Aspirin

s.no	Flow rate (ml/min)	System suitability results	
		USP Plate Count	USP Tailing
1 1	0.8	2953.6	1.3
2 2	**1**	**2996**	**1.3**
3 3	1.2	2071.6	1.3

CONCLUSION

An accurate assay method was developed for the determination of omeprazole and aspirin by using RP-HPLC method the surveillance and outcome obtained from each validation experiment incliuding specifity, linarity ranage, lod, loq, precision ,accuracy ,robustness, ruggedness stability indicating on temperature studies and system suitability lies well inside the acceptances criteria since all the results are within the limit the developed analytical method is considered as validated and suitable for anticipated use and all parameters are subjected as per the ICH guidelines

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