Available online on 15.09.2018 at http://jddtonline.info

Journal of Drug Delivery and Therapeutics

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RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF ASPIRIN AND OMEPRAZOLE IN BULK AND DOSAGE FORM

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ABSTRACT

RP-HPLC method was developed for the determination of Omeprazole (OME) & Aspirin (ASP) in bulk and dosage form. Mobile phase use for the separation of OME & ASP is methanol and 0.05%OPA in water (pH= 3.5) with ratio of 60:40. The Colum used as C_{18} (Cosmosil) 4.6×150mm and flow rate 0.7mL/min. UV detector is used and the detection wavelength is 231nm. Retention time of OME and ASP are 4.61 & 8.03 min, respectively. This method was validated as per ICH guidelines. Linearity was observed at 10-50µg/mL of OME and 20-100µg/mL of ASP. The % RSD is found to be less than 2%.The resolution between OME and ASP is 11.55 and the tailing factors of both are less than 2.0.Therotical plates for OME and ASP are 5060, and 9367, respectively. Total run time is 15min. The developed RP-HPLC method was accurate, precise, selective and rapid for simultaneous estimation of Omeprazole and Aspirin in the pharmaceutical dosage form."

Keyword: Omeprazole, Aspirin, RP-HPLC validation.

Article Info: **Received** 23 June, 2018; Review Completed 10 Aug 2018; Accepted 24 Aug 2018; Available online 15 Sep 2018

Cite this article as:

Sarode TK, Jadhav PB, RP-HPLC method development and validation for simultaneous estimation of aspirin and omeprazole in bulk and dosage form, Journal of Drug Delivery and Therapeutics. 2018; 8(5):322-328

DOI: http://dx.doi.org/10.22270/jddt.v8i5.1825

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INTRODUCTION

YOSPRALA is a combination of aspirin, an anti-platelet agent, and omeprazole, a proton pump inhibitor (PPI), indicated for patients who require aspirin for secondary prevention of cardiovascular and cerebrovascular events and who are at risk of developing aspirin associated gastric ulcers.

Reducing the combined risk of death and nonfatal stroke in patients who have had ischemic stroke or transient ischemia of the brain due to fibrin platelet emboli,

• Reducing the combined risk of death and nonfatal MI in patients with a previous MI or unstable angina pectoris,

• Reducing the combined risk of MI and sudden death in patients with chronic stable angina pectoris,

• Use in patients who have undergone revascularization procedures (Coronary Artery Bypass Graft [CABG] or Percutaneous Transluminal Coronary Angioplasty [PTCA]) when there is a pre-existing condition for which aspirin is already indicated.

The omeprazole component of YOSPRALA is indicated for decreasing the risk of developing aspirin associated gastric ulcers in patients at risk for developing aspirin-associated gastric ulcers due to age (≥ 55) or documented history of gastric ulcers.

The aim of the present work was to develop simple, economic, accurate, specific and precise RP-HPLC

methods for simultaneous estimation of omeprazole and aspirin in bulk drugs and combined pharmaceutical formulation and validation of newly developed analytical method.

Omeprazole is use proton pump inhibitor (Antacid), chemically (4-methoxy-3,5-dimethylpyridyl,5-methoxybbenzimidazol.Litreature survey of omeprazole has been estimated by UV, HPLC, HPTLC, RP-HPLC.

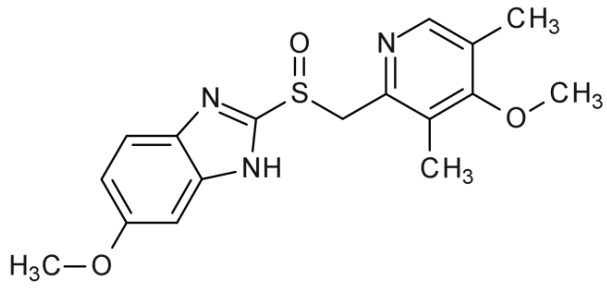


Figure 1: Omeprazole

Aspirin is use as antithrombotic, antianginal, antipyretic and anti-inflammatory agent chemically (2-Acetoxybenzoic acid) aspirin is a use to lower risk to heart attack, artery disease [1, 2, 3, 4]

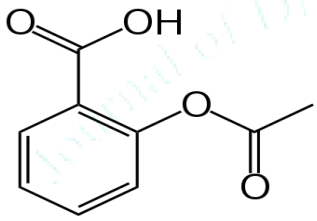


Figure 2: Aspirin

MATERIALS AND METHODS

Instrument and equipment

1. RP-HPLC –Yonglin acme 9000

2. Sonicator- Vensor

3. UV Spectroscopy- Shimatzu 2080

Materials

1. **Aspirin**-Aspirin (AR grade) gifted sample from Sci-tech laboratory Musalgaon, MIDC, Sinner, Nashik .

2. **Omeprazole**-(AR grade) gifted sample from sci-tech laboratory Musalgaon MIDC, Sinner, Nashik.

3. **Methanol**-HPLC grade methanol purchase from Merck ltd, Mumbai.

4. **Ortho phosparic acid water-** 0.1mL OPA in 200mL water.(0.05%)

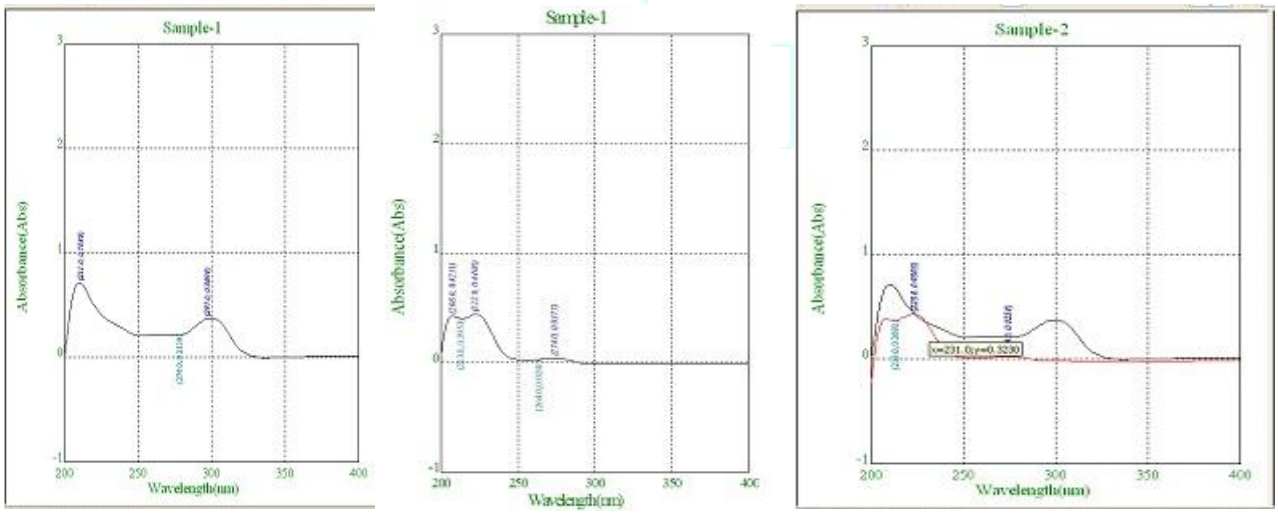
Methods

Identification of Aspirin & Omeprazole [4]

Melting point was determined using digital melting point apparatus with one end open capillary method. The reference melting point of aspirin and omeprazole is 136°C and 156°C respectively.

Determination of wavelength maxima [5, 6]

Both the standard solution was scanned between 400nm to 200nm.The overlain spectrum of both drugs was recorded. From the overlain spectrum, 297nm (λmax of omeprazole) and 222 nm (λmax of Aspirin) were selected for estimation of drugs and the isobestic point was found to be at 231 nm and it is shown in fig 3



-UV of Aspirin -UV of Omeprazole

Figure 3: Isobestic point of Omeprazole & Aspirin

Name of sample	Wavelength	Absorbance
Omeprazole	297nm	0.3698
Aspirin	222nm	0.4408
Isobestic Omeprazole & Aspirin	231nm	0.3230

Selection of chromatographic mode

Proper selection of the method depend upon the nature of the sample (ionic, ionisable and neutral, its molecular weight and solubility. Both the drugs are freely soluble in organic. Hence, reversed phase HPLC was selected for the initial separations because of its simplicity and suitability.

Selection of Mobile phase

Omeprazole and Aspirin are freely soluble in HPLC grade of methanol. Not freely soluble in orthophosphoric acid and used uitrasonication. Both drugs are soluble in methanol and orthophosphoric acid.

Mobile Phase	60+40 (Methanol+(0.05%) OPA)
Selection of column	4.6×150mm
Wavelength	231nm
Flow rate	0.7ml/min
Column temp	Ambient
Sample size	20µL
Retention time	4.61 omeprazol,8.35 aspirin
Conclusion	Satisfactory Resolution, Theoretical Plate, Tailing Factor.

RESULT AND DISCUSSION

Orthophosparic acid water - 0.1mL OPA in 200mL water. (0.05%)

Preparation of stock solution

Accurately weighed of Omeprazole 10mg and 20mg Aspirin was taken in 10mL volumetric flask and dissolved in 10 mL methanol. To get standard stock solution.1000 µg/mL Omeprazole and 2000 µg/mL Aspirin**.** STOCK -I

Preparation of mobile phase

Pipette out 60 ml of concentrated methanol and 40 ml of 0.05% OPA make up the volume up to 100ml and Sonicate 5 min.

1) Take 0.1mL from the stock I and make up the volume with mobile Phase 10 mL = 10 µg/mL Omeprazole and 20 µg/mL Aspirin.

2) Take 0.2 mL from the stock I and make up the volume with mobile Phase 10 mL = 20 µg/mL Omeprazole and 40 µg/mL Aspirin.

3) Take 0.3 mL from the stock I and make up the volume with mobile Phase 10 mL = 30 µg/mL Omeprazole and 60 gm/mL Aspirin.

4) Take 0.4 mL from the stock I and make up the volume with mobile Phase 10 mL = 40 µg/mL Omeprazole and 80 µg/mL Aspirin.

5) Take 0.5 mL from the stock I and make up the volume with mobile Phase 10 mL = 50 µg/mL Omeprazole and 100 µg/mL Aspirin.

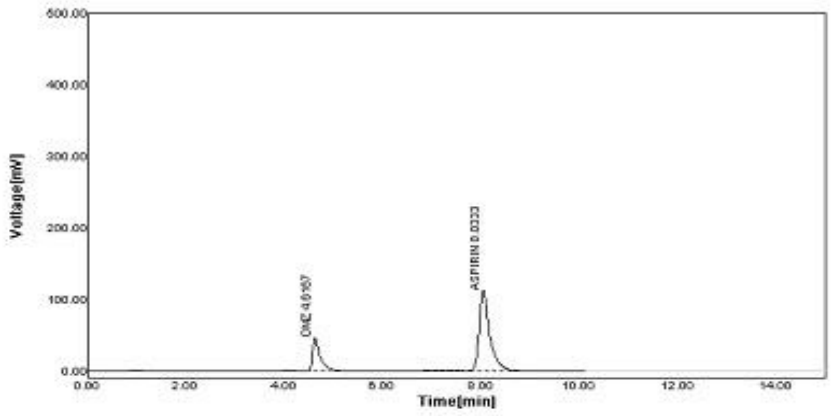


Figure 4: Chromatogram of Ome & Asp

Table 1: Final chromatographic conditions

Peak Name	Retention time	Area	Plate count	T F
Ome	4.6167	507.77	5060.1	1.1297
Asp	8.0333	1753.2	9367.1	1.3266

Tab solution Preparation:

Brand Name: Yosprala

Weigh and finely powder 20 tablets. Accurately weigh and transfer a quantity of powder sample equivalent to 10mg of Omeprazole and 20mg of Aspirin into a 10 ml clean dry volumetric flask, add 10ml methanol and sonicate to dissolve it completely and make volume up to the mark with the diluents(1000 µg/mL Ome and 2000 µg/mL. From these solutions 0.1ml was pipette out and transferred into 10ml volumetric flask and makeup the volume up to the mark with methanol & Orthophosphoric acid (10 µg/mL Omeprazole and 20 µg/mL Aspirin) and measured the absorbance at 231nm. The % purity of the drug was calculated by comparing the absorbance of test solution with standard.

Tab Assay [7,8]

0.4 ml from tab stock and make up 10 ml with mobile phase and produced 40 µg/mL Ome + 80 µg/mL Asp for assay

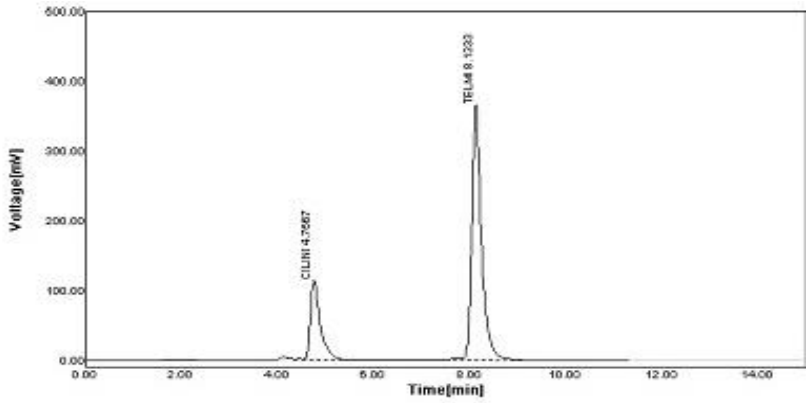


Figure 5: Chromatogram for Assay

Table 2: Assay

Name	RT(min)	TP	TF	Area
Ome	4.7667	3827.0	1.33	1265.54
Asp	8.1333	9876.3	1.37	5033.44

Validation Parameter [9,10,11,12]

Linearity:-The con. of ome and asp solution take in different concentration respect.10-50ug/mL, 20-100ug/mL. At selected wavelength 231nm.

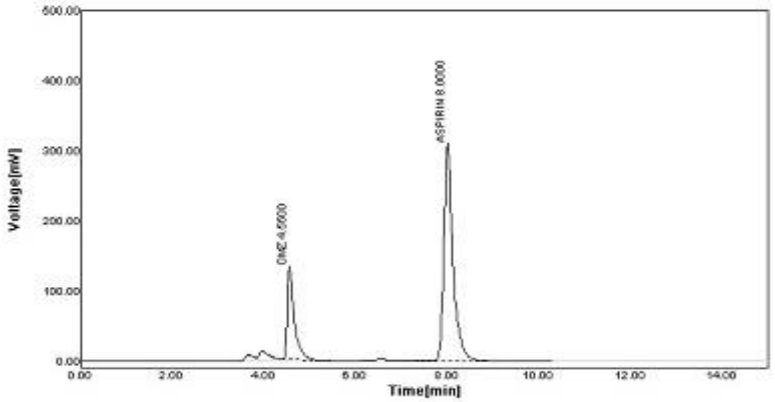


Figure 6: Linearity 50 µg/mL Ome & 100 µg/mL

Peak Name	Retention time	Area	Plate count	T F
Ome	4.6000	1230.7	5503.1	1.1472
Asp	8.0167	5042.1	9537.0	1.1699

Accuracy: The accuracy of an analytical method is the closeness of test results obtained by that method to the true value. The three triplicates of stock solution of Omeprazole and Aspirin equivalent to 10ppm, 20ppm, 30ppm and 20ppm, 40ppm, 60ppm respectively were preparing by using standard solution. From stock solution aliquots of Ome and Aspirin 0.1 ml, 0.2ml, 0.3ml were taken and diluted to 10ml mobile phase with diluted such that the final concentration of Ome-10, 20, 30 µg/mL and Aspirin-20, 40, 60 µg/mL.

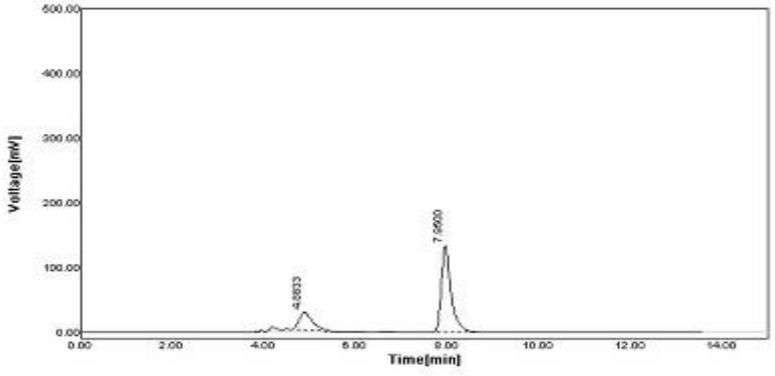


Figure 7: Chromatogram of accuracy Omeprazole 30µg/mL Aspirin 60 µg/mL.

Table 3: Accuracy of Omeprazole

Conc. (µg/ml)	Standard Deviation		%RSD
	Area Mean	SD	%RSD
10	288.91	2.63	0.91
20	525.86	7.71	1.47
30	778.18	3.34	0.43

Table 4: Accuracy of Aspirin

Conc. (µg/ml)	Standard Deviation		%RSD
	Area Mean	SD	RSD
20	706.42	5.03	0.71
40	1741.04	7.25	0.99
60	2863.04	3.78	0.13

Precision:-The precision intraday and inter day Precision system result showed good reproducibility. Intraday precision study was carried out by preparing drug solution from stock solution, aliquots of Omeprazole and Aspirin 0.2, 0.3, 0.4 ml were taken an diluted to 10 ml with diluents such that the final concentration of Ome-20, 30, 40 µgm/mL and Asp-40, 60, 80 µg/mL and analyzed. The same procedure was followed for second day also to determine inter-day precision.

Table 5: Intra-day precision

Conc µg/mL	Area Mean	SD	%Amt Found	% RSD
OME				
20	530.32	6.74	100.42	1.27
30	748.75	6.60	97.98	0.88
40	996.01	5.40	99.84	0.54
ASP				
40	1765.1	10.9	99.87	0.58
60	2880.9	6.19	100.95	0.22
80	3913.4	1.44	98.58	0.21

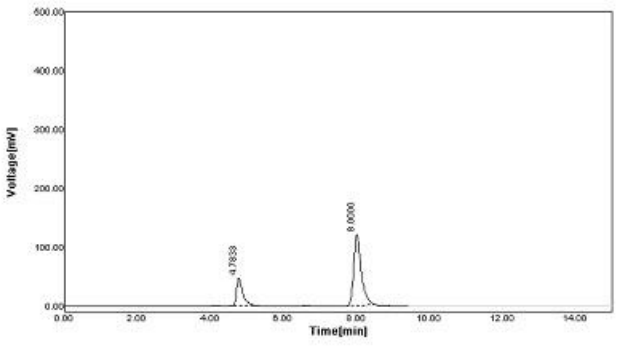


Figure 8: Precision-20ug/ml and 40ug/ml Intra-day

Table 6: Inter day precision

Conc. ug/ml	Area Mean	SD	%Amt Found	% RSD
OME				
20	530.32	6.74	100.42	1.27
30	763.32	4.88	100.15	1.57
40	996.01	5.40	99.84	0.54
ASP				
40	1765.5	10.1	99.87	0.58
60	2880.9	6.19	100.95	0.22
80	3913.4	1.44	99.58	0.04

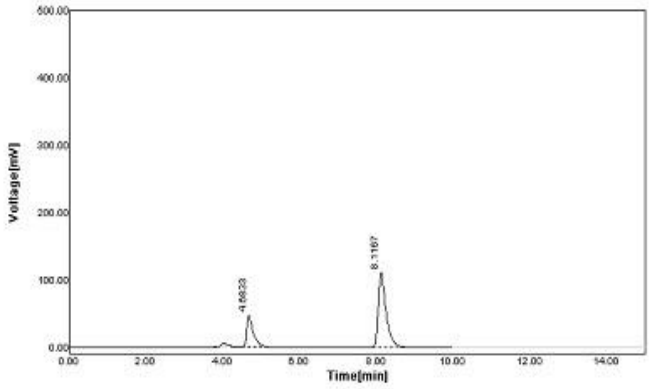


Figure 9: Precision-20ug/mL and 40ug/ml interday

% Recovery [13,14]

The recovery of an analytical method is determined by applying the method to analyzed samples to which known amounts of analyte have been added. The Recovery is calculated from the test results as the percentage of analyte recovered by the assay. Three replicate injections, each of three different test concentrations in the range of 80%, 100% and 120% of labeled claim of tablet under study yielded the result within 98 to 102% of true concentration of each drug. The results indicated that the method is accurate.

Table 7: Statistical Validation of Recovery Studies Ome and Asp

Level of Recovery (%)	Drug	Mean % Recovery	Standard Deviation	% RSD
80%	Ome	98.20	1.70	1.73
	Asp	99.10	0.75	0.64
100%	Ome	101.16	1.78	0.44
	Asp	100.75	1.51	0.27
120%	Ome	101.58	1.07	0.37
	Asp	99.23	0.41	0.11

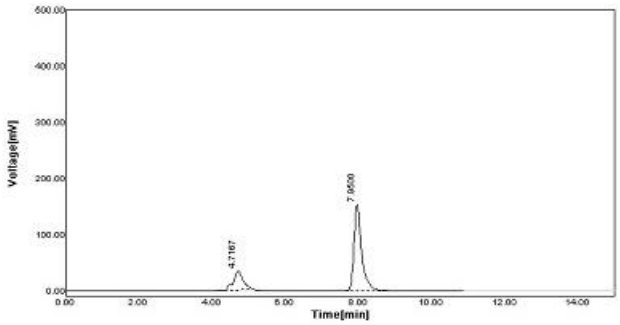


Figure 10: Recovery 120 %

Robustness [16,17]

The robustness of an analytical method is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage. To verify the robustness of the method, the analysis was done under variables flow rate, mobile phase ratio and wavelength.

System suitability parameter

The system was evaluated by analyzing repeatability, retention time, tailing factor and theoretical plats of the column.

Table 8: Robustness study of Ome & Asp

Parameter change	Conc. ug/ml	Amt. Mean±SD	%RSD
Flow rate 0.6ml	32	1217.7±5.69	0.88
0.8ml	32	1812.21±4.68	0.26
69ml+31ml Me+OPA	32	2099.53±24.9	1.19
59ml+41ml Me+OPA	32	1320.56±7.42	0.56
230nm	32	2099.53±5.69	0.47
232m	32	1855.70±32.8	1.77

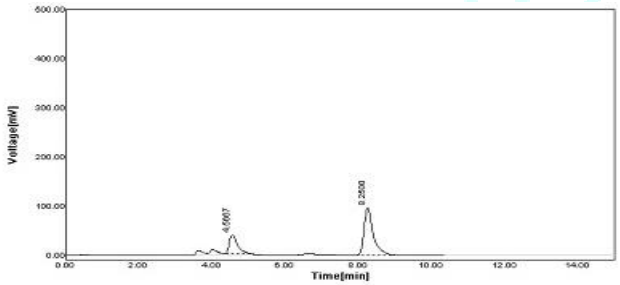


Figure 11: Change in wavelength 230 nm.

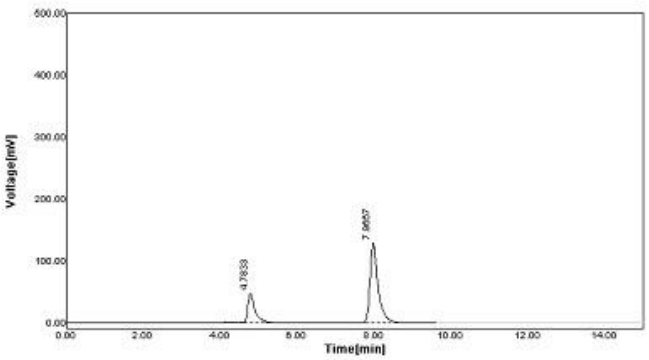


Figure 12: Change in wavelength 232 nm.

Limit of Detection (LOD)

The LOD is the lowest limit that can be detected. Based on the S.D. of the response and the slope. The limit of detection (LOD) may be expressed as:

LOD = 3.3 (SD)/S

Where, SD= Standard deviation of the Y intercept

S = Slope

LOD = 3.3 x 4.65/ 23.46 = **0.5946** (µg/ml) (Omeprazole)

LOD = 3.3 x 8.81/ 54.08 = **0.5375** (µg/ml) (Aspirin)

The LOD of Omeprazole and Aspirin was found to be **0.5946** (µg/ml) and **0.5375** (µg/ml) respectively.

Limit of Quantitation

The LOQ is the lowest concentration that can be quantitatively measured. Based on the S.D. deviation of the response and the slope.

The quantitation limit (LOQ) may be expressed as

LOQ = 10 (SD)/S

Where, SD = standard deviation of Y intercept

S = Slope

LOQ = 10 x 4.65/23.46 = **1.9820** (µg/ml (Omeprazole)

LOQ = 10 x 8.81/54.08 = **1.6290** (µg/ml (Aspirin)

The LOQ of Omeprazole and Aspirin was found to be **1.9820** (µg/mL) and **1.6290** (µg/mL) respectively.

Summary of validation parameter

Parameter (Unit)		Ome	Asp	Accep. Criteria
Linearity range (µg/ ml)		10-50	20-100	-----
Correlation Coefficient		0.9995	0.9987	0.999
% Recovery		98.20-101.58%	99.10- 99.23%	98%- 102%
Precision %RSD	Intraday	1.27%	0.58 %	% RSD NMT 2
	Inter day	1.27%	0.58 %	
Robustness		Robust	Robust	Robust
LOD		0.5946 µg /ml	0.5375 µg /ml	NMT 2
LOQ		1.9820 µg /ml	1.6290 µg/ ml	NMT 2

CONCLUSION

RP-HPLC method has been developed for the simultaneous estimation of Omeprazole and Aspirin. These methods have good resolution both the drugs good and short analysis time. Literature survey revealed that several methods have been reported for determination of Omeprazole and Aspirin individually or in combination with other drugs in pharmaceutical dosage forms. The developed method was validated. It was found to be simple, precise, accurate and robust. The proposed method can be used for routine analysis of omeprazole and Aspirin in combined dosage form.

ACKNOWLEDGMENT

Authors are thankful to sci-tech pharma, sinner, india. Provide gift sample of Omeprazole and Aspirin.

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