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Development and validation of RP-HPLC method for simultaneous estimation of aspirin and omeprazole in dosage

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Abstract
RP-HPLC method was developed for simultaneous estimation of Aspirin and Omeprazole using Phenomenex Luna C8 column (150×4.6mm, 5um) as stationary phase and Acetonitrile: Methanol: Buffer (25:10:65 pH2.5) as mobile phase at a flow rate 1.0ml/min and UV detection at 228nm.Aspirin and Omeprazole eluted at 4.6 &3.2 min respectively and showed linear response in conc. range of 20-100ug/ml and 10-50ug/ml with correlation coefficient for both the drugs (r^2=0.999) ASP &OMP respectively. The developed method was validated with regard to linearity, accuracy, precision, selectivity, specificity, robustness and system suitability. LOD for Aspirin and Omeprazole is 1.23 and 1.25 respectively & LOQ for Aspirin and omeprazole is 4.12 & 4.18 respectively. The developed method was validated as per ICH guidelines. The RSD for precision were found to be less than 2%. The percentage recoveries obtained for Aspirin and Omeprazole 98-102 % respectively.

Keywords: aspirin, omeprazole, RP-HPLC, validation

1. Introduction

Aspirin (ASP) is chemically 2-(acetyloxy)-benzoic acid. It is non-selective cyclo-oxygenase inhibitor used as an antipyretic, analgesic, anti-inflammatory and antithrombotic agent. It reduces non-fatal myocardial infarction. It is official in Indian Pharmacopoeia (IP), British Pharmacopoeia (BP), United States pharmacopoeia (USP) and European Pharmacopoeia (EP). It is estimated by acid-base titration method as per IP, BP, and USP & EP Literature review reveals that HPLC, UV Spectrophotometric methods has been reported for estimation of ASP in pharmaceutical dosage forms [1]. Recently aspirin is a salicylate medicine and used to lower risk of heart attack in patients with chronic coronary artery disease, such as patients with history of heart attack or angina (severe chest pain). It is also used to lower risk of recurrent stroke in patients who had an ischemic stroke or transient ischemic attack [2].

Omeprazole is chemically known as 5-methoxy-2-[[(4-methoxy-3, 5-dimethyl-2-pyridinyl) methyl] sulfinyl] benzimidazole. It is officially listed in BP 2011 and U.S.P.XXXII. It is a proton pump inhibitor, used in treatment of peptic ulcer disease and NSAID-associated ulceration, in gastro-esophageal reflux disease and the Zollinger-Ellison syndrome [3]. It works by decreasing the amount of acid produced by the stomach. In acidic condition of the stomach both (R & S forms) are converted to achiral products which reacts with the cystine group in H+/K+. ATPas,there by destroying the ability of the parietal cells to produce Gastric acid. Omeprazole, a well-studied proton pump inhibitor, inhibits the gastric parietal cell proton pump, dose-dependently reducing basal and stimulated gastric secretion and raising intragastric pH 9 [4]. A survey of the literature revealed that omeprazole has been estimated in pharmaceuticals by UV-Spectrophotometric, spectrofluorimetry, HPLC, HPTLC, capillary electrophoresis and electrochemical methods. Literature survey does not reveal any HPLC method for simultaneous estimation of ASP and OMP in pharmaceutical dosage form. The present developed method is simple, rapid, precise and accurate for simultaneous estimation of both drugs in pharmaceutical dosage form as per ICH guidelines.

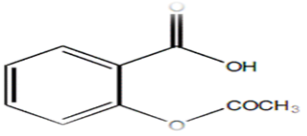

Fig 1: Structure of Aspirin

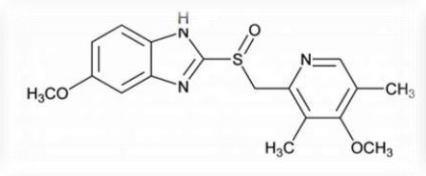

Fig 2: Structure of Omeprazole

2. Material and Methods

2.1 Chemicals and reagents

Pure drug sample of Aspirin and Omeprazole was obtained from Unichem Pharma Ltd. Goa India as a gift sample. Acetonitrile LiChrosolv®, Methanol LiChrosolv®, and Water LiChrosolv® were purchased from Merk specialties Pvt. Ltd Mumbai.

2.2 Instrumentation

The analysis was performed by using Younglins Acme 9000 with Phenomenex Luna C8 (150×4.6, 5um) column quaternary gradient pump SP930D HPLC system used for chromatographic separation. It contains Rhenodyne valve with 20ul fixed loop injector and UV 730D UV-visible

detector, Electronic balance AY 220(Shimadzu Japan) was used for weighing. The solution was filtered through 0.45u syringe filter (Phenomenex), ultra sonicator (microlean-103) was used for degassing the mobile phase.

2.3 Chromatographic condition

The chromatographic separation was performed Analytical Column Phenomenex C8column (150 mm × 4.6 mm, 5µm) using Mobile Phase Acetonitrile: Methanol: Phosphate Buffer pH 2.5 (25:10:65) at a Flow Rate 1ml/min with isocratic elution. The Injection Volume 20 µl and the run time was 10 min. UV detection was carried out at 228nm.

2.4 Preparation of Phosphate Buffer pH 2.5

Accurately weigh 0.136 gm of potassium dihydrogen phosphate transferred in 100ml conical flask and dissolved in HPLC water & adjust the volume up to the mark and then adjust the pH 2.5 by using O-phosphoric acid.

2.5 Preparation of standard stock solution

i) Standard stock solution of Aspirin

Accurately weigh 10 mg of ASP transferred in 10 ml volumetric flask & dilute with methanol and adjust the volume up to the mark to obtain concentration (1000ug/ml) from above solution 4ml of was diluted to 10 ml to get final concentration (200ug/ml).

ii) Stock solution of Omeprazole

Accurately weigh 10 mg of OMP transferred in 10 ml volumetric flask & dilute with methanol and adjust the volume up to the mark to obtain concentration (1000ug/ml) 2ml of above solution was diluted to 10 ml to get final concentration (100ug/ml).

iii) Combined Standard Stock Solution of ASP and OMP

2ml of 'Std Stock ASP' (1000µg/ml) and 1ml of 'Std Stock OMP' (1000µg/ml) mixed to get conc. of 200µg/ml of ASP and 100µg/ml of OMP. The solution was labeled as 'Std Stock MIX AO'.

Selection of wavelength

Standard solution of ASP & OMP were injected separately as well as in combination into HPLC system then scanned over UV range 200-400nm.The max of Aspirin was detected at 228nm and max Omeprazole was detected at 232nm.the detection was carried out at 228nm.

Method validation

Linearity 1, 2, 3, 4, and 5ml of 'Std Stock AO' were transferred in a series of 10ml volumetric flasks. The volume was made up to the mark with mobile phase to obtain the conc. of 20, 40, 60, 80, and 100µg/ml of ASP and 10, 20, 30, 40 and 50µg/ml of OMP. The solutions were filtered through syringe filter and 20µl injected into the HPLC system and their chromatogram were recorded for 10mins. Calibration curves of ASP and OMP were constructed by plotting the peak area of ASP *v/s* conc. of ASP and peak area of OMP *v/s* conc. of OMP, respectively. The correlation coefficient (r^2=0.999) of least square linear regression for ASP and OMP was calculated

Precision

Precision of the method was determined in the terms of intra-day and inter-day variation (%RSD) was assessed by analyzing standard drug solution within the calibration range, three times on the same day. Inter-day precision (%RSD) was assessed by analyzing drug solution within the calibration range on three different day over a period of 7 days.

Intermediate Precision

Intra-day Precision

Intra-day precision was determined by analyzing the standard solution of ASP (20µg/ml) and OMP (10µg/ml) at 8.00am and 4.00pm on same day following the procedure of repeatability.

Range

He range of analytical method was decided from the interval between upper and lower level of calibration curves by plotting the curve. The correlation coefficient (r^2=0.999) of least square linear regression for ASP and OMP was calculated.

Accuracy

20 tablets of ASP were weighed and finely powdered; an accurately weighed powder (15.54mg) equivalent to 10 mg of ASP was dissolved and diluted to 50 ml methanol. 2ml of above solution was transferred in four different 10ml volumetric flask labelled as 0%, 50%, 100%, and 150%. Then 0, 1, 2, 3 ml of 'Std Stock Mix AO' (200ug/ml ASP &100ug/ml OMP), were added and made up to the mark with mobile phase & their chromatogram were obtain under the same chromatographic condition after getting a stable baseline. Peak area were recorded and percent recoveries were calculated.

Sensitivity

The sensitivity of measurement of ASP and OMP by the use of proposed method was estimated in terms of Limit of Detection (LOD) and Limit of Quantitation (LOQ). The LOD and LOQ were calculated by equation. Based on the standard deviation of the response and the slope, LOD and LOQ were estimated using the formulae: LOD= 3.3 σ/S Where, σ = the standard deviation of the response S = the slope of the calibration curve LOQ = 10 σ/S Where, σ = the standard deviation of the response. S = the slope of the calibration curve LOD and LOQ were determined from the standard deviations of the responses for six replicate determinations.

Specificity

The specificity of the HPLC method is illustrated in Fig. 3. Where complete separation of ASP and OMP in presence of tablet excipients.

Repeatability

Repeatability of sample application was assessed by injecting 30µg/ml and 60µg/ml of drug solution of Omeprazole and Aspirin respectively six times.

Robustness

Combined standard solution of ASP (20µg/ml), OMP '= (10µg/ml) was prepared and analyzed at different flow rates (0.9, 1.0, 1.1 ml/min) separately.

System suitability

The suitability of the chromatographic system was tested before each stage of validation. Five replicate injections of standard preparation were injected and resolution, asymmetry, number of theoretical plates and relative standard deviation of peak area were determined as shown in table no.7

3. Result and discussion

Method development

The HPLC method was optimized for simultaneous determination of ASP and OMP. The mobile phase Acetonitrile: Methanol: Phosphate Buffer pH 2.5 (25:10:65 v/v) resulted in good resolution and sharp and symmetrical peaks. Using a reversed-phase C8 column, the retention times for ASP and OMP is 4.5 for ASP and 3.2 for OMP respectively. Total time of analysis was less than 5 min. The maximum absorption of ASP and OMP together as detected at 228 nm and this wavelength was chosen for the analysis.

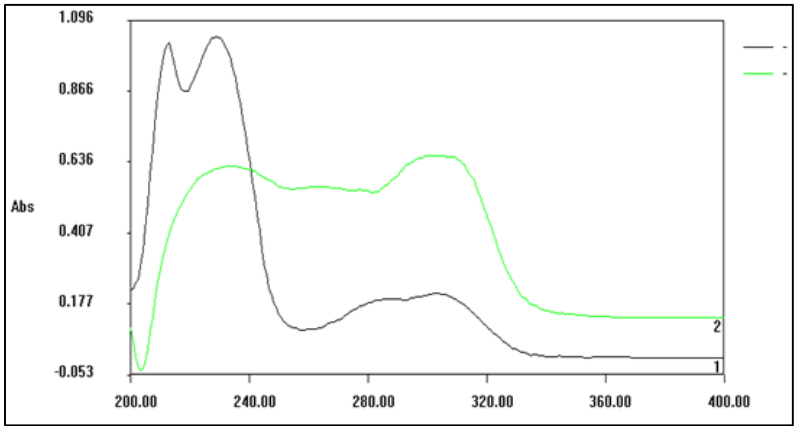

Fig 3: Overlain spectra of ASP and OMP between 200-400nm in mobile phase

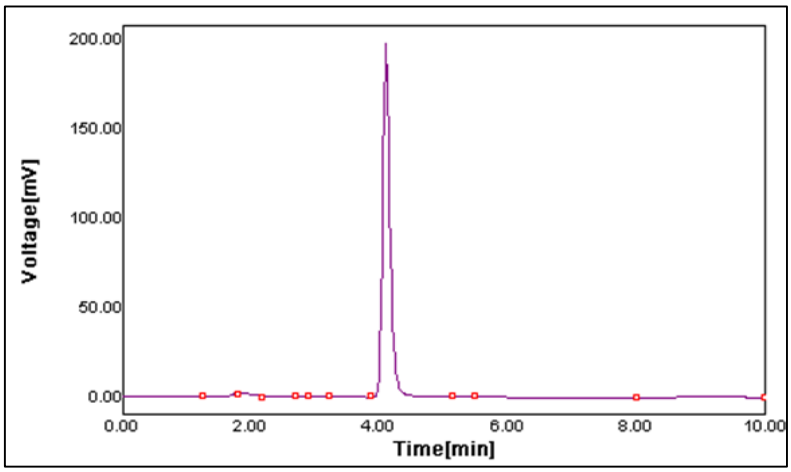

Fig 4: Chromatogram of ASP (20µg/ml) in optimized chromatographic conditions

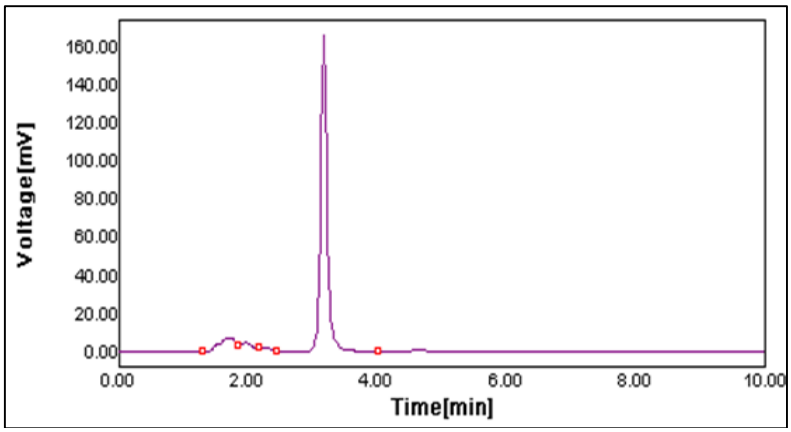

Fig 5: Chromatogram of OMP (10µg/ml) in optimized chromatographic conditions

Table 1: Chromatogram for optimized method

Drug	Retention time	Area	Theoretical plate	Tailig Factor	Resolution
Aspirin	4.5	1150	7440	1.02	
Omeprazole	3.2	510	10203	1.1	5.6
			N > 2000	T < 2	R >2

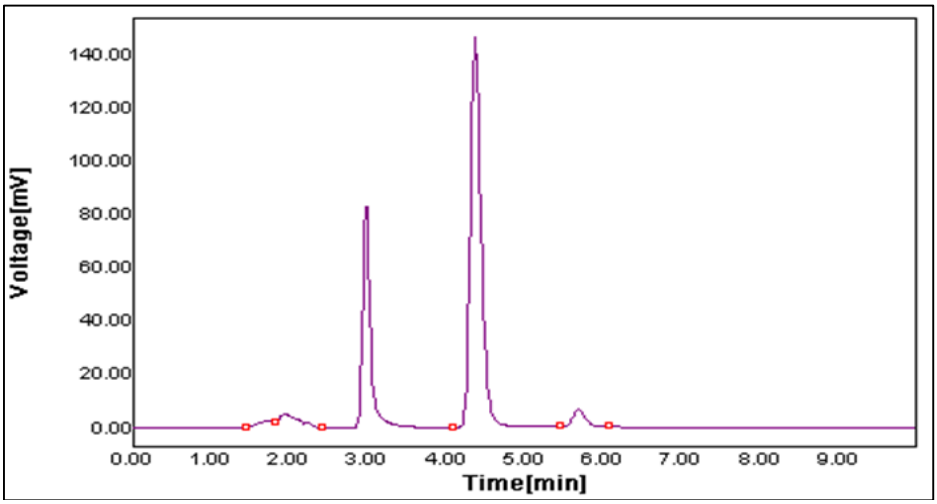

Fig 6: Chromatogram of combination of ASP (20µg/ml) &OMP (10µg/ml) in optimized chromatographic conditions

Specificity

The chromatogram of standard solution of mixture of ASP (40ug/ml) and OMP (20ug/ml) was compared with tablet formulation to observe the interference of excipient.

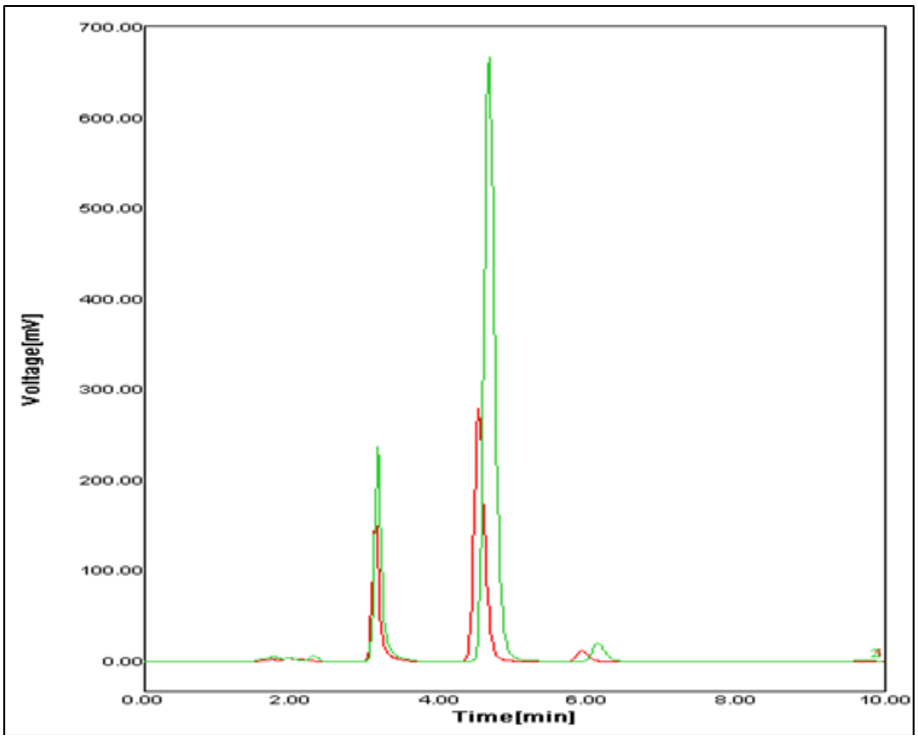

Fig 7: Overlain Chromatograms of sample and standard solution of similar concentrations of drug

Linearity

Linear regression data for the calibration plots revealed good linear relationships between area and concentration over the ranges 20-100 µg/ml for ASP and and 10-50 µg/ml for OMP. The linear equations for the calibration plots were y = 71.67x -84.9 and y =55.26 x – 5.8 with Regression (r^2) is 0.999 for both ASP and OMP respectively.

Table 2: Response of ASP at various linearity levels

Sr. No	Conc. of ASP(µg/ml)	Peak Area (mV)
1	20	1354
2	40	2756
3	60	4227
4	80	5683
5	100	7058

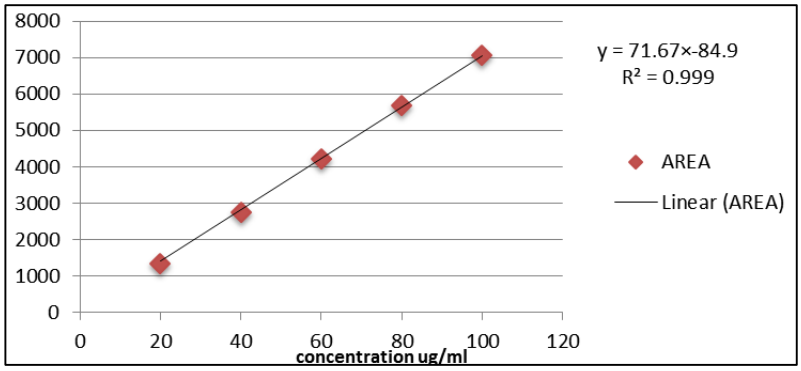

Fig 8: Linear calibration curve of Aspirin

Table 3: Response of OMP at various linearity levels

Sr. No.	Conc. of OMP (µg/ml)	Peak Area (mV)
1.	10	562
2.	20	1100
3.	30	1628
4.	40	2190
5.	50	2780

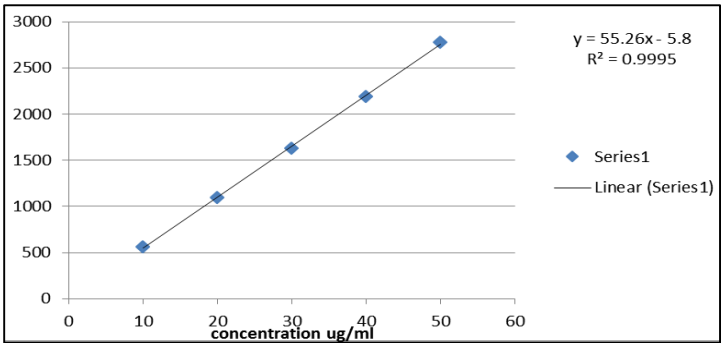

Fig 9: Linear calibration curve for Omeprazole

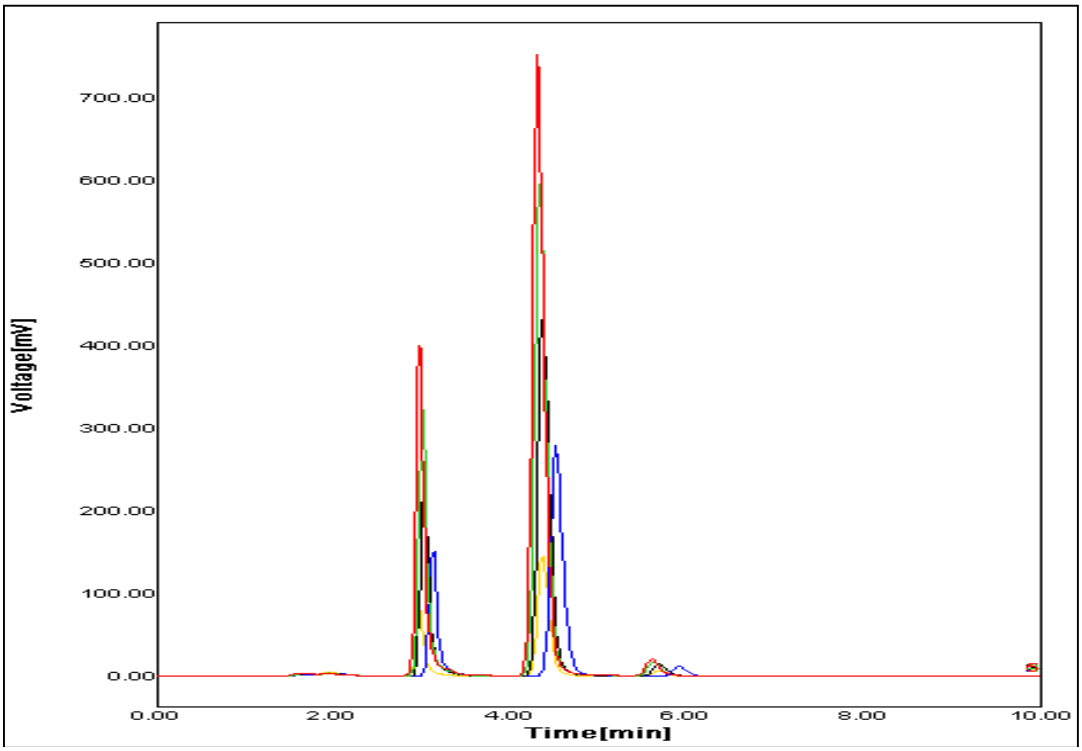

Fig 10: Overlain Chromatograms of serial dilutions of ASP and OMP in optimazed chromatographic conditions

Accuracy

When the method was used for accuracy and subsequent analysis of both the drugs, and spiked with 50, 100, and 150% of additional pure drug, the recovery was found to be 98-102% for ASP and OMP.

Table 2: Accuracy

Sr. No.	Level of % Recovery	Amount of 'Sample Stock-A' (ml)	Amount of Standard Drug Added (µg/ml)		Total Amount Found (µg/ml		Amount Recovered (µg/ml)		% Recovery	
			ASP	OMP	ASP	OMP	ASP	OMP	ASP	OMP
1	0	2	0	0	35.87					
2	50	2	20	10	55.16	10.2	19.6	9.9	98	102
3	100	2	40	20	76.63	20	41.0	20	102	100
4	150	2	60	30	95.76	31.03	60.2	31.03	100	103

Precision

The precision of the method was expressed as relative standard deviation (RSD %). The %R.S.D. values for intra-day precision study and inter-day study listed in (Table 3 and 4)

Table 3: Repeatability Study for ASP and OMP

Inj.	Peak Area(mV)	
	ASP	OMP
1	4227	1628
2	4343	1752
3	4266	1666
4	4234	1684
5	4208	1706
6	4149	1698
SD	29.54	23.11
RSD	1.69	1.89

Intermediate Precision

Table 4: Intra-day Precision of ASP and OMP

Inj.	Peak Area(mV) at 8am		Peak Area(mV) at 4pm	
	ASP	OMP	ASP	OMP
1	4227	1628	4198	1632
2	4343	1706	4336	1710
3	4266	1666	4232	1657
4	4234	1684	4423	1675
5	4208	1698	4220	1695
SD	29.44	23.33	29.56	23.11
RSD	1.69	1.89	1.65	1.86

Detection Limit (LOD)

Table 5: Limit of Detection data of ASP and OMP

	ASP	OMP
LOD (µg/ml)	1.23	1.25

Quantification Limit (LOQ)

Table 6: Limit of Quantitation data of ASP and OMP

	ASP	OMP
LOQ (µg/ml)	4.12	4.18

Robustness

Table 7: Result of Robustness Study: Variation in Flow Rate (ml/min)

Flow Rate (ml/min)	Analyte	Retention Time (min)	Tailing Factor (T)	Theoretical Plates (N)	Resolution (R)
0.9	ASP	4.1	1.3	3786	2.6
	OMP	2.8	1.2	4354	
1.0	ASP	4.6	1.19	3798	1.3
	OMP	3.2	1.6	4567	
1.1	ASP	5.1	1.17	7296	1.6
	OMP	3.5	1.77	7868	

System Suitability Testing

Table 8: Results of System Suitability Parameters

Analytes	Retention Time (min)	Tailing Factor (T)	Theoretical Plates (N)	Resolution (R)
ASP	4.5	1.02	7440	3.6
OMP	3.1	1.1	10203	5.3
Required limits	--	T < 2	N > 2000	R >2

4. Conclusion

The developed HPLC method is simple, precise, accurate and reproducible and can be used for simultaneous determination of ASP and OMP in pharmaceutical dosage forms. The method was validated as per International Conference on Harmonization (ICH) guidelines. The statistical parameters and recovery data reveals the good accuracy and precision of the method. Finally, since no pharmacopoeial method for determination of ASP & OMP in bulk and pharmaceutical formulations have been reported yet. The method could be useful and suitable for the estimation of the ASP & OMP in bulk and pharmaceutical formulations.

5. Acknowledgement

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6. References

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