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Research Article

Analytical method development and validation for the estimation of aspirin and omeprazole using RP-HPLC method

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ABSTRACT

A simple and selective LC method is described for the determination of Aspirin and Omeprazole in tablet dosage forms. Chromatographic separation was achieved on a c_{18} column using mobile phase consisting of a mixture of 30 volumes of ammonium acetate buffer, 40 volumes of acetonitrile and 30 volumes of Methanol with detection of 233 nm. Linearity was observed in the range 18-42 µg/ml for Aspirin (r^2 =0.983) and 6-14 µg /ml for Omeprazole (r^2 =0.970) for the amount of drugs estimated by the proposed methods was in good agreement with the label claim. The proposed methods were validated. The accuracy of the methods was assessed by recovery studies at three different levels. Recovery experiments indicated the absence of interference from commonly encountered pharmaceutical additives. The method was found to be precise as indicated by the repeatability analysis, showing %RSD less than 2. All statistical data proves validity of the methods and can be used for routine analysis of pharmaceutical dosage form.

Keywords: Aspirin and Omeprazole**,** Reverse phase HPLC.

INTRODUCTION

A drug includes all medicines intended for internal or external use for or in the diagnosis, treatment, mitigation or prevention of disease or disorder in human beings or animals, and manufactured exclusively in accordance with the formulae mentioned in authoritative books [1].

Pharmaceutical analysis is a branch of chemistry involving a process of identification, determination, quantification, purification and separation of components in a mixture or determination of chemical structure of compounds. There are two main types of analysis – Qualitative and Quantitative analysis [2].

Qualitative analysis is performed to establish composition of a substance. It is done to determine the presence of a compound or substance in a given sample or not [4-6]. The various qualitative tests

are detection of evolved gas, limit tests [7-10], color change reactions, determination of melting point and boiling point, mass spectroscopy, determination of nuclear half-life etc [3].

AIM AND PLAN OF WORK

Aim

To develop new RP HPLC method for the simultaneous estimation of Aspirin and Omeprazole pharmaceutical dosage form.

Plan of work

Solubility determination of Aspirin and Omeprazole various solvents and buffers.

- Determine the absorption maxima of both the drugs in UV–Visible region in different solvents/buffers and selecting the solvents for HPLC method development.
- Optimize the mobile phase and flow rates for proper resolution and retention times.
- Validate the developed method as per ICH guidelines.

METHODOLOGY

Mobile Phase

A mixture of Ammonium Acetate Buffer+Acetonitrile+Methanol were prepared. The mobile phase was sonicated for 10min to remove gases and filtered through 0.45µ membrane filter for degassing of mobile phase.

Determination of Working Wavelength (λmax)

In estimation of drug wavelength maxima is used.. So this wavelength is used in estimation to estimate drug accurately.

Preparation of standard stock solution of aspirin

10 mg of ASPIRIN was weighed in to 100ml volumetric flask and dissolved in Methanol and then dilute up to the mark with methanol and prepare 10 µg /ml of solution by diluting 1ml to 10ml with methanol, were scanned using UV-Visible spectrophotometer.

Preparation of standard stock solution of omeprazole

10 mg of OMEPRAZOLE was weighed and transferred in to 100ml volumetric flask and dissolved in methanol and then make up to the mark with methanol and prepare 10 µg /ml of solution by diluting 1ml to 10ml with methanol, were scanned using UV-Visible spectrophotometer.

RESULTS AND DISCUSSIONS

Solubility studies

These studies are carried out at 25 ^{0}C

Aspirin

Freely soluble in methanol,water and mixed phosphate buffer.

Omeprazole

Freely soluble in ethanol and methanol, and slightly soluble in acetone and very slightly soluble in water.

Wavelength determination

In simultaneous estimation of two drugs isobestic wavelength is used. Isobestic point is the wavelength where the molar absorptivity is the same for two substances that are interconvertible. So this wavelength is used in simultaneous estimation to estimate both drugs accurately.

Preparation of standard stock solution of aspirin

10 mg of ASPIRIN was weighed in to 100ml volumetric flask and dissolved in Methanol and then dilute up to the mark with methanol and prepare 10 µg /ml of solution by diluting 1ml to 10ml with methanol, wavelength is found to be 238nm.

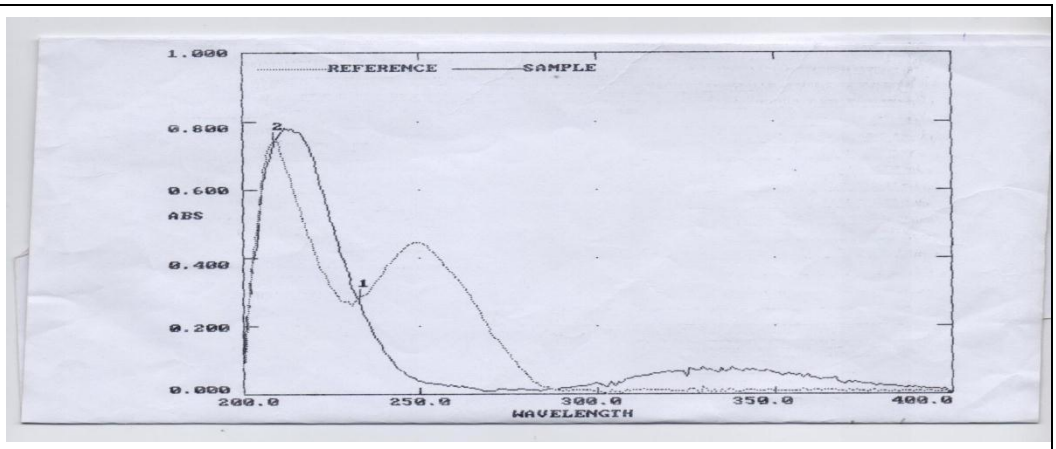
Preparation of standard stock solution of omeprazole

10 mg of OMEPRAZOLE was weighed and transferred in to 100ml volumetric flask and dissolved in methanol and then make up to the mark with methanol and prepare 10 µg /ml of solution by diluting 1ml to 10ml with methanol, wavelength is found to be 236nm.

Results

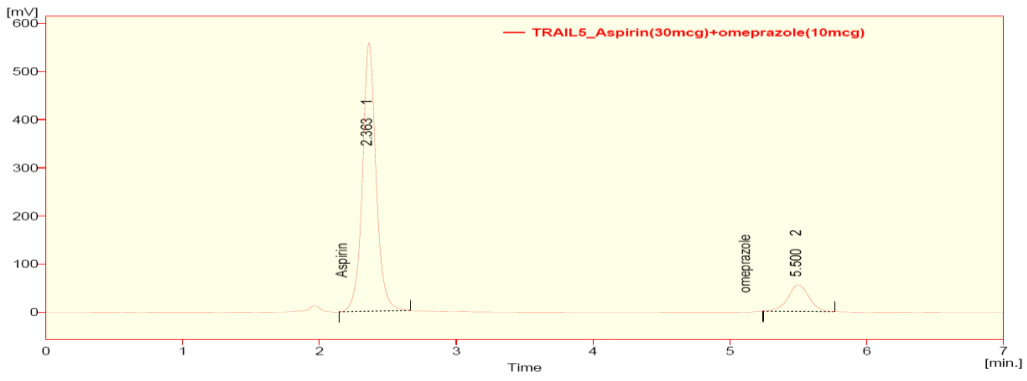
The wavelength of maximum absorption (λ_{max}) of the drug, 10 µg/ml solution of the drugs in methanol were scanned using UV-Visible spectrophotometer within the wavelength region of 200–400 nm against methanol as blank. The resulting spectra and the absorption curve shows the isobestic point was found to be 233 nm for the combination.

METHOD DEVELOPMENT OF ASPIRIN AND OMEPRAZOLE



Preparation of standard solution

Weigh accurately 10mg of OMEPRAZOLE and ASPIRIN in 100 ml of volumetric flask and dissolve in 10ml of mobile phase and make up the volume with mobile phase. From above stock solution 10 µg/ml of OMEPRAZOLE and of ASPIRIN is prepared by diluting 1ml to 10ml with mobile phase. This solution is used for recording chromatogram.



Result Table (Uncal - TRAIL 5 _Aspirin(30mcg)+omeprazole(10mcg))

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]
1	2.363	3862.887	556.867	86.69
2	5.500	593.001	55.259	13.31
	Total	4455.888	612.127	100.00

Column Performance Table (From 50% - TRAIL 5_Aspirin(30mcg)+omeprazole(10mcg))

	Reten. Time	W05 [min]	Asymmetry [-]	Capacity [-]	Efficiency [th.pl]	Eff/l [t.p./m]	Resolution [-]
1	2.363	0.103	1.097	0.00	2898	57957	-
2	5.500	0.167	0.960	0.00	6033	120661	13.672

Fig. 3: Chromatogram of OMEPRAZOLE AND ASPIRIN

Observation

- Peak Asymmetry factor for OMEPRAZOLE and ASPIRIN meet the system suitability requirements.
- The run time is very correct.
- Theoretical plates were more than 2000.

Hence it is taken for optimization.

Table 1: Optimized chromatographic conditions

Mobile phase	Ammonium acetate buffer+ACN+Methanol(30:40:30)
Ph	6.0
Column	Inertsil ODS 3V column,C18(150x4.6 ID) 5µm
Flow rate	1.0 ml/min
Column temperature	Room temperature(20-25^{o}C)
Sample temperature	Room temperature(20-25^{o}C)
Wavelength	233
Injection volume	20 µl
Run time	7 min
Retention time	About 2.363 min for ASPIRIN and 5.500 min for OMEPRAZOLE.

ASSAY

Preparation of samples for Assay

Preparation of standard solution

Preparation of mixed standard solution

Weigh accurately 10mg of ASPIRIN and 10 mg of OMEPRAZOLE in 100 ml of volumetric flask and dissolve in 10ml of mobile phase and make up the volume with mobile phase. From above stock solution 10µg/ml of ASPIRIN and OMEPRAZOLE is prepared by diluting 1ml to 10ml with mobile phase. This solution is used for recording chromatogram.

Tablet sample

10 tablets (each tablet contains OMEPRAZOLE– 40 mg, ASPIRIN -325 mg) were weighed and taken into a mortar and crushed to fine powder and uniformly mixed. Tablet stock solutions of OMEPRAZOLE and ASPIRIN (µg/ml) were prepared by dissolving weight equivalent to 10 mg of OMEPRAZOLE and 20 mg of ASPIRIN and dissolved in sufficient mobile phase. After that filtered the solution using 0.45-micron syringe filter and Sonicated for 5 min and dilute to 10ml with mobile phase. Further dilutions are prepared in 5 replicates of 10µg/ml of OMEPRAZOLE and ASPIRIN was made by adding 1 ml of stock solution to 10 ml of mobile phase.

Calculation

The amount of ASPIRIN and OMEPRAZOLEpresent in the formulation by using the formula given below, and results shown in above table:

$$\%\ \text{Assay} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \frac{AW}{LC} \times 100$$

Where,

AS: Average peak area due to standard preparation

AT: Peak area due to assay preparation

WS: Weight of ASPIRIN /OMEPRAZOLEin mg

WT: Weight of sample in assay preparation

DT: Dilution of assay preparation

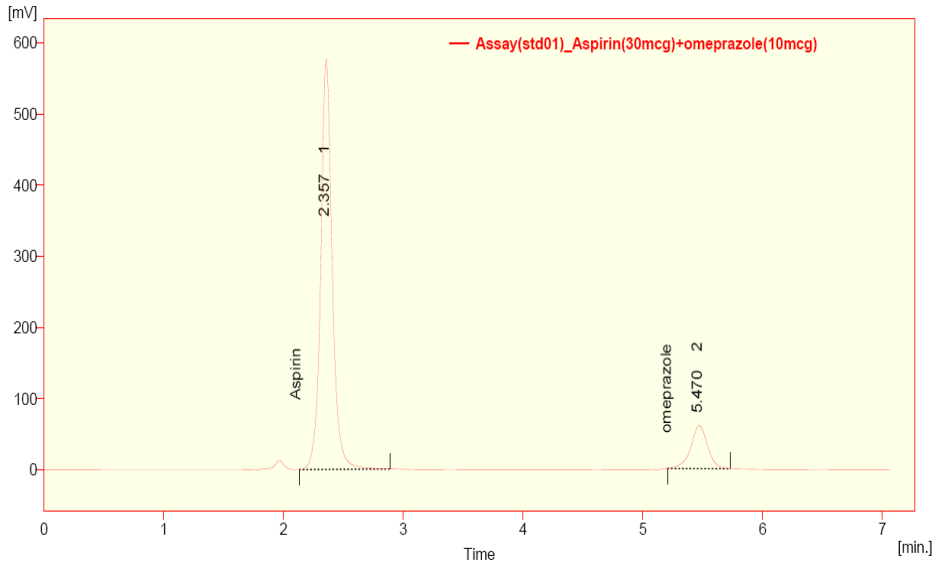


Fig: Chromatogram of Assay standard preparation-1

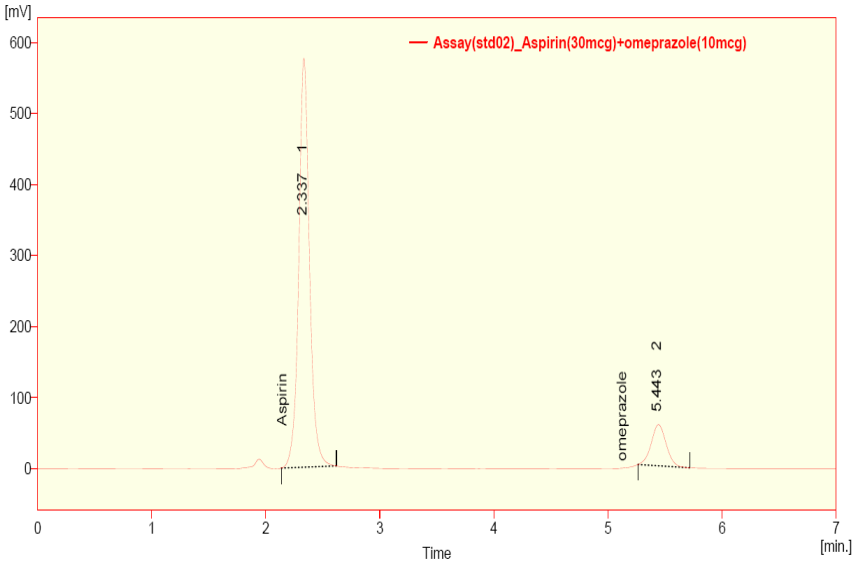


Fig: Chromatogram of Assay standard preparation-2

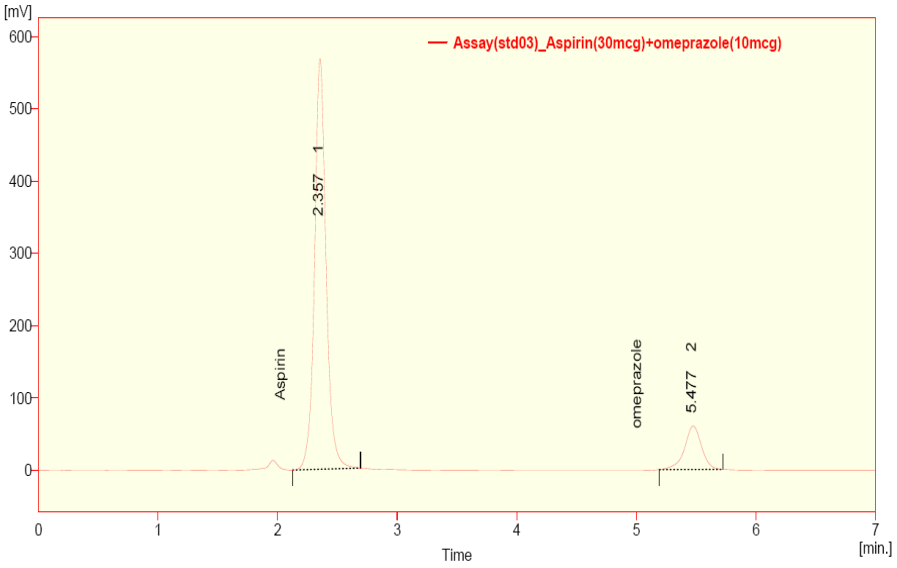


Fig. 8.4.3: Chromatogram of Assay standard preparation-3

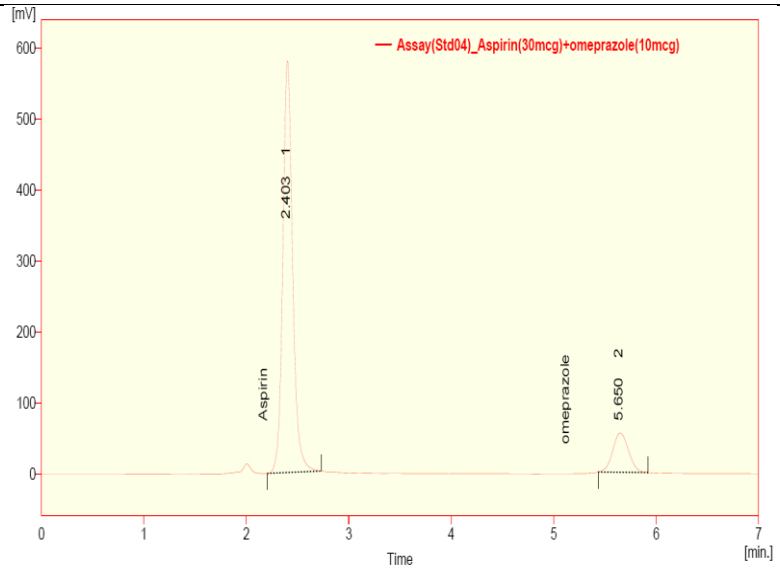


Fig: Chromatogram of Assay standard preparation-4

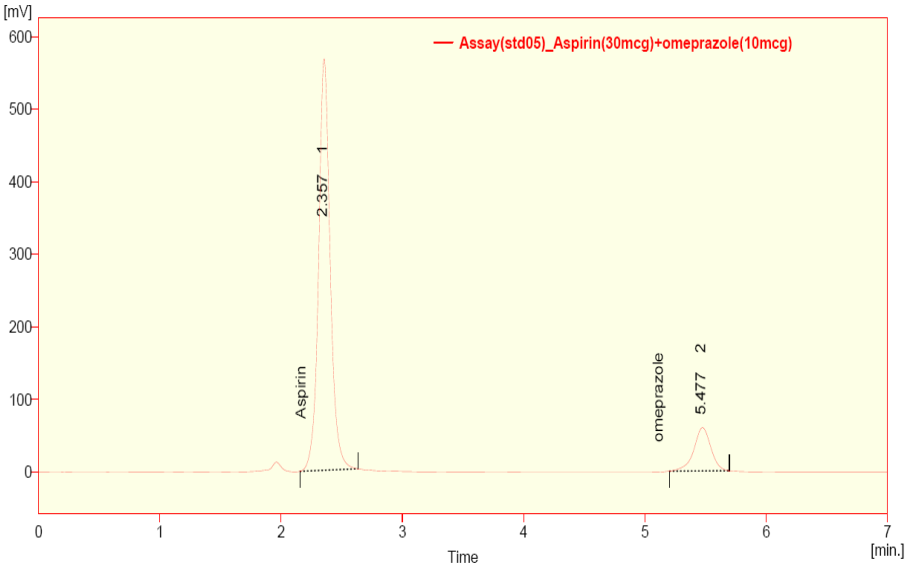


Fig: Chromatogram of Assay standard preparation-5

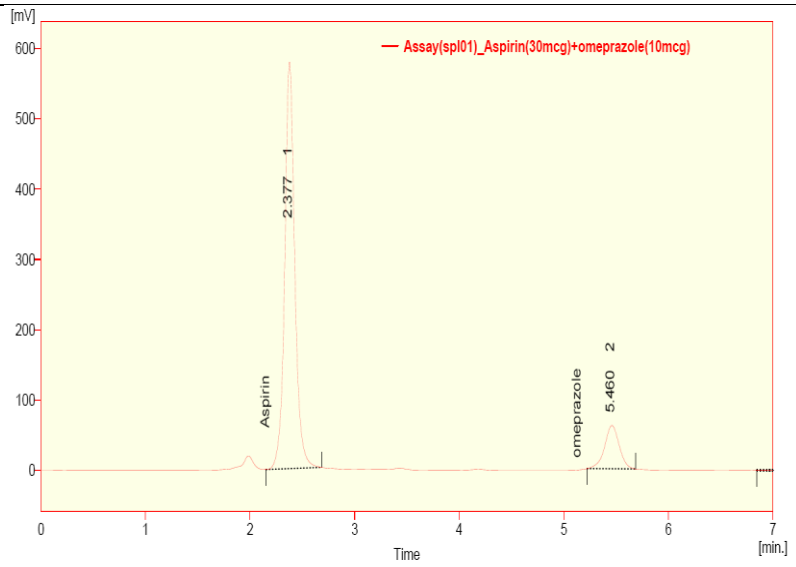


Fig: Chromatogram of Assay sample preparation-1

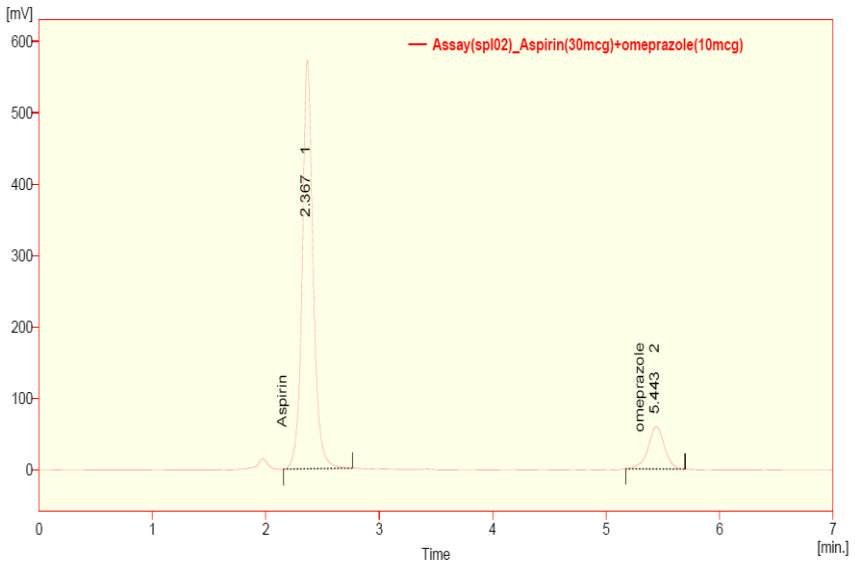


Fig: Chromatogram of Assay sample preparation-2

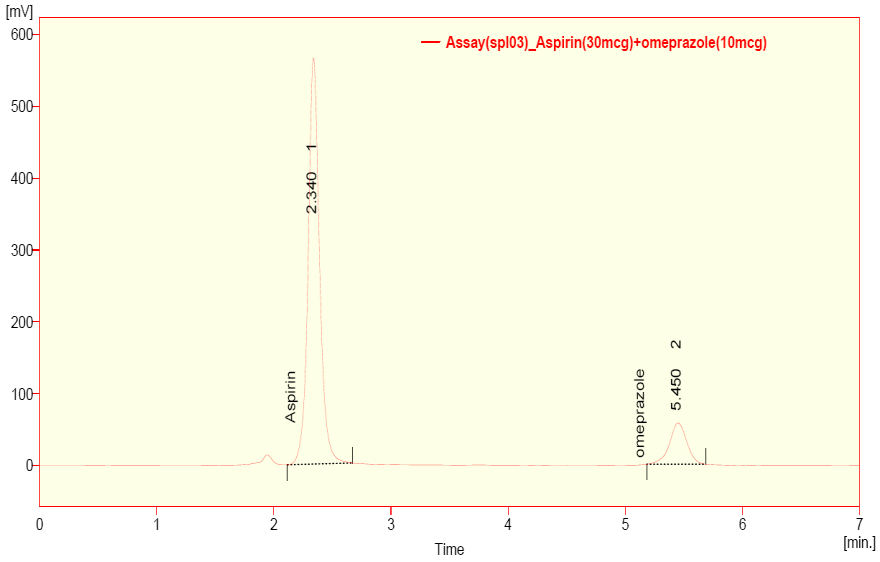


Fig: Chromatogram of Assay sample preparation-3

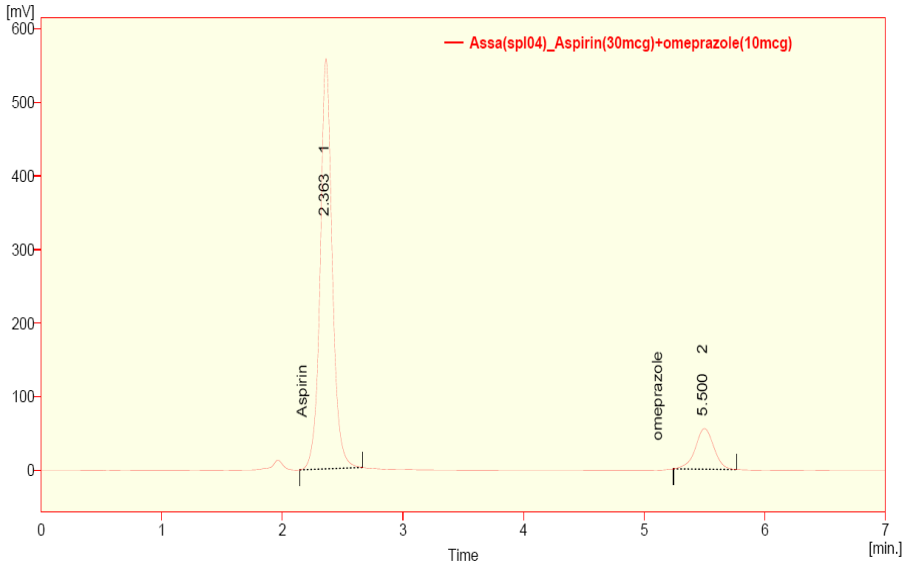


Fig: Chromatogram of Assay sample preparation-4

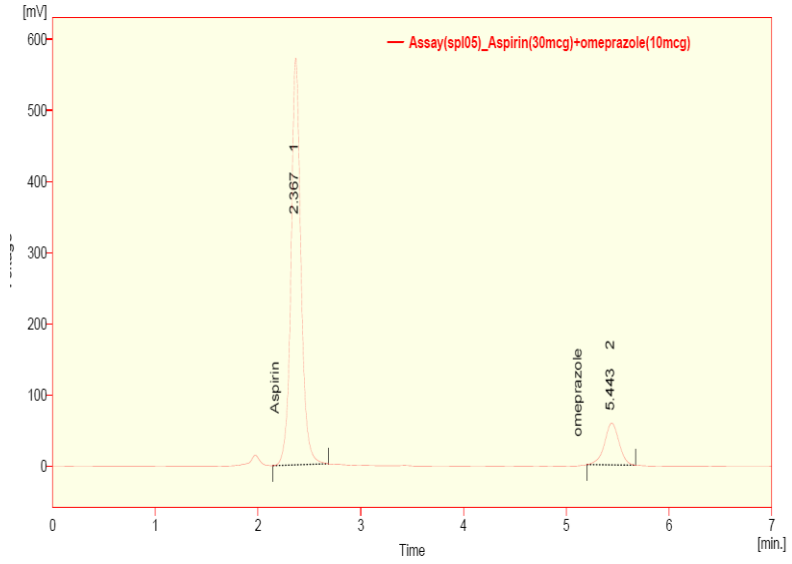


Fig: Chromatogram of Assay sample preparation-5

Table: Assay Results

ASPIRIN			**OMEPRAZOLE**	
	Standard Area	**Sample Area**	**Standard Area**	**Sample Area**
Injection-1	3830.368	3854.231	591.586	599.141
Injection-2	3764.595	3837.468	529.156	597.246
Injection-3	3803.722	3831.450	596.018	591.321
Injection-4	3869.323	3862.887	592.63	593.001
Injection-5	3769.348	3817.148	583.611	579.450
Average Area	3807.471	3840.637	578.6002	592.0318
Assay(%purity)	100.871066		102.321396	

Observation

The amount of ASPIRIN and OMEPRAZOLE present in the taken dosage form was found to be 100.87 % and 102.32 % respectively.

VALIDATION

Specificity by Direct comparison method

There is no interference of mobile phase, solvent and placebo with the analyte peak and also the peak purity of analyte peak which indicate that the method is specific for the analysis of analytes in their dosage form.

Preparation of mixed standard solution

Weigh accurately 10mg of ASPIRIN and 10 mg of OMEPRAZOLE in 100 ml of volumetric flask and dissolve in 10ml of mobile phase and make up the volume with mobile phase. From above stock solution 10µg/ml of ASPIRIN and OMEPRAZOLE is prepared by diluting 1ml to 10ml with mobile phase. This solution is used for recording chromatogram.

Tablet sample

10 tablets (each tablet contains OMEPRAZOLE– 40 mg, ASPIRIN -325 mg) were weighed and taken into a mortar and crushed to fine powder and uniformly mixed. Tablet stock solutions of OMEPRAZOLE and ASPIRIN (µg/ml) were prepared by dissolving weight equivalent to 10 mg of OMEPRAZOLE and 20 mg of ASPIRIN and dissolved in sufficient mobile phase. After that filtered the solution using 0.45-micron syringe filter and Sonicated for 5 min and dilute to 10ml with mobile phase. Further dilutions are prepared in 5 replicates of 10µg/ml of OMEPRAZOLE and ASPIRIN was made by adding 1 ml of stock solution to 10 ml of mobile phase.

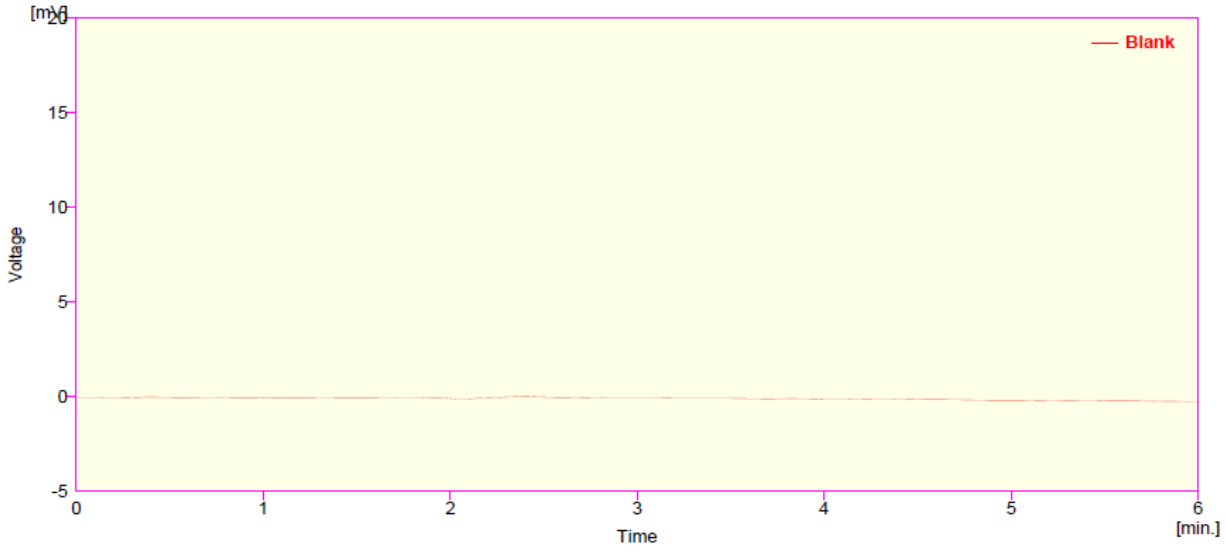


Fig: Blank chromatogram for specificity by using mobile phase

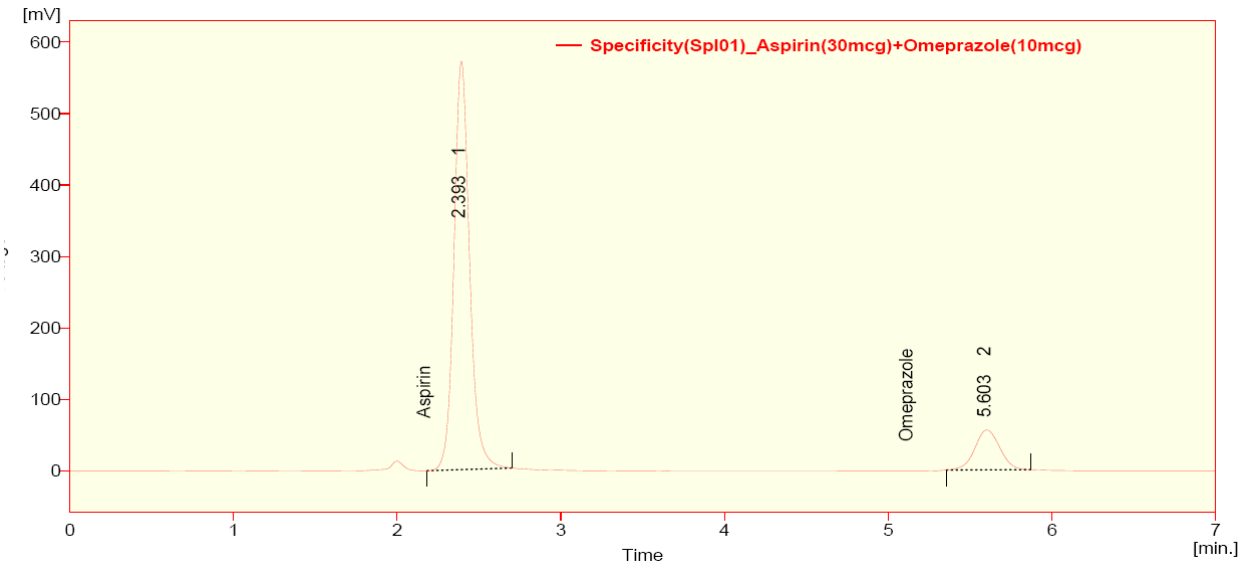


Fig: Chromatogram for specificity of OMEPRAZOLE and ASPIRIN sample

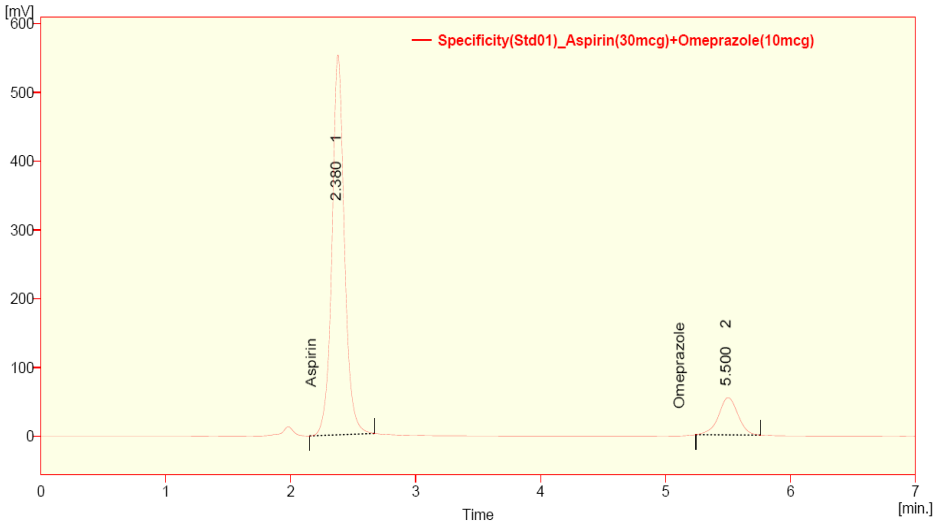


Fig: Chromatogram for Specificity of OMEPRAZOLE and ASPIRIN standard

Observation

It is observed from the above data, diluent or excipient peaks are not interfering with the ASPIRIN and OMEPRAZOLE peaks.

Linearity and range

Preparation of standard stock solution

Standard stock solutions of ASPIRIN and OMEPRAZOLE (microgram/ml) were prepared by dissolving 10 mg of ASPIRIN and OMEPRAZOLE dissolved in sufficient mobile phase and dilute to 100 ml with mobile phase. Further dilutions were given in the table

Table: Linearity Preparations

Preparations	Volume from standard stock transferred in ml		Volume made up in ml (with mobile phase)	Concentration of solution(µg /ml)	
				ASPIRIN	OMEPRAZOLE
Preparation 1	**0.18**	**0.06**	10	18	6
Preparation 2	0.24	0.08	10	24	8
Preparation 3	0.3	0.1	10	30	10
Preparation 4	0.36	0.12	10	36	12
Preparation 5	0.42	0.14	10	42	14

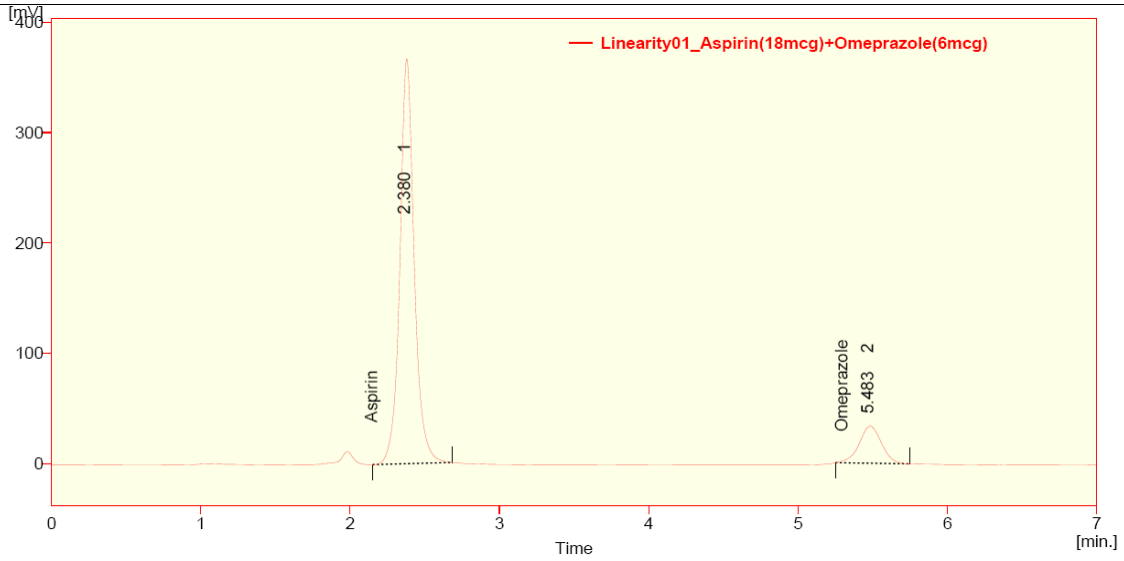


Fig: Chromatogram of OMEPRAZOLE and ASPIRIN preparation-1

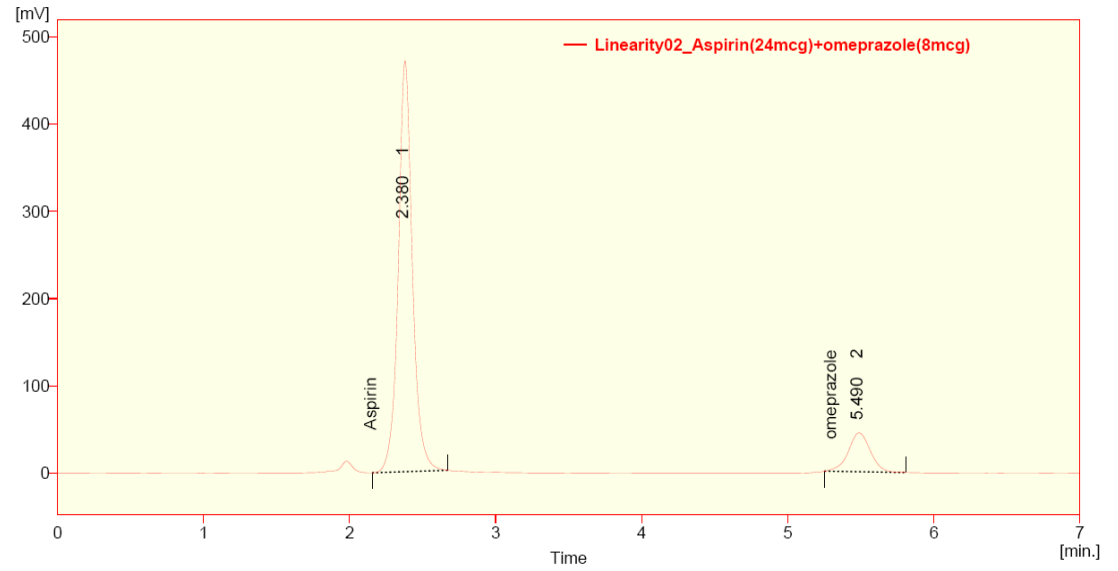


Fig: Chromatogram of OMEPRAZOLE and ASPIRIN preparation-2

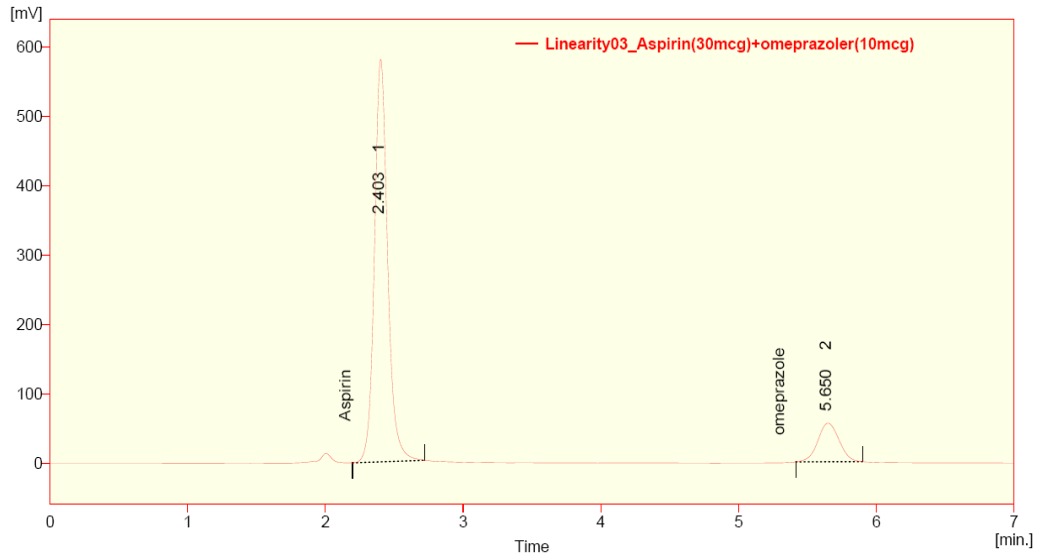


Fig: Chromatogram of OMEPRAZOLE and ASPIRIN preparation-3

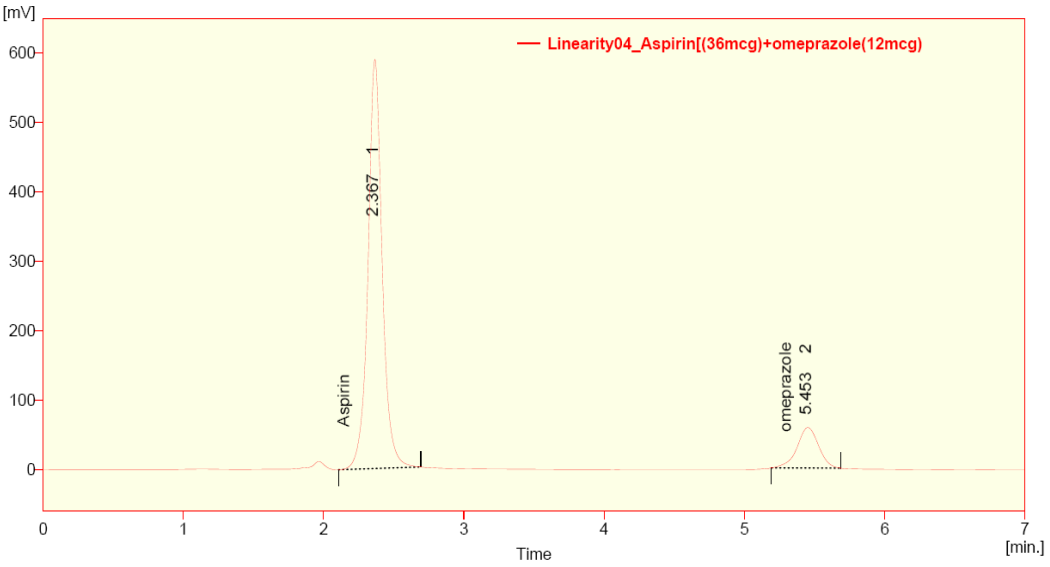


Fig: Chromatogram of OMEPRAZOLE and ASPIRIN preparation-4

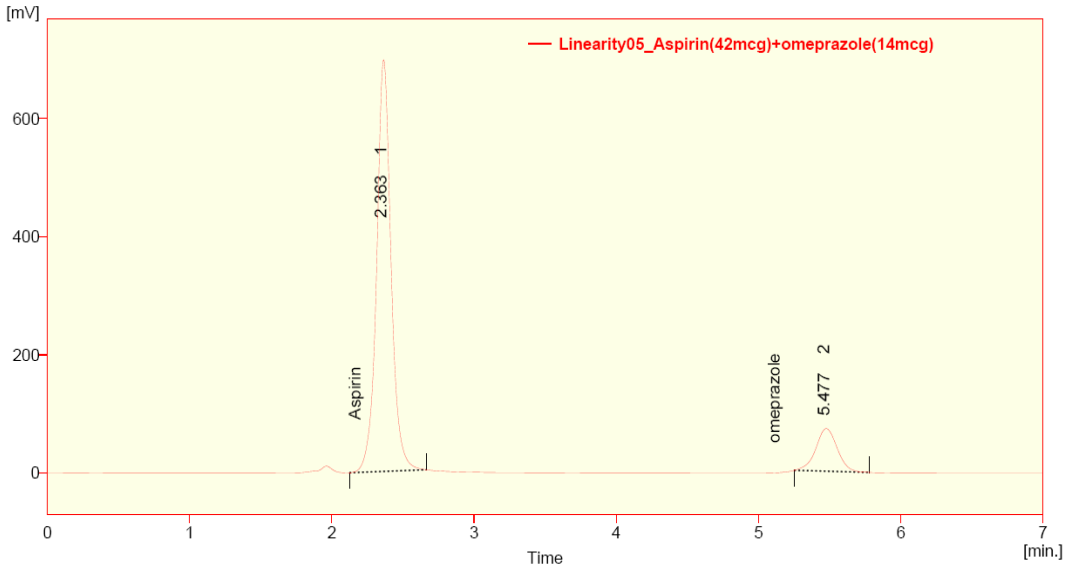


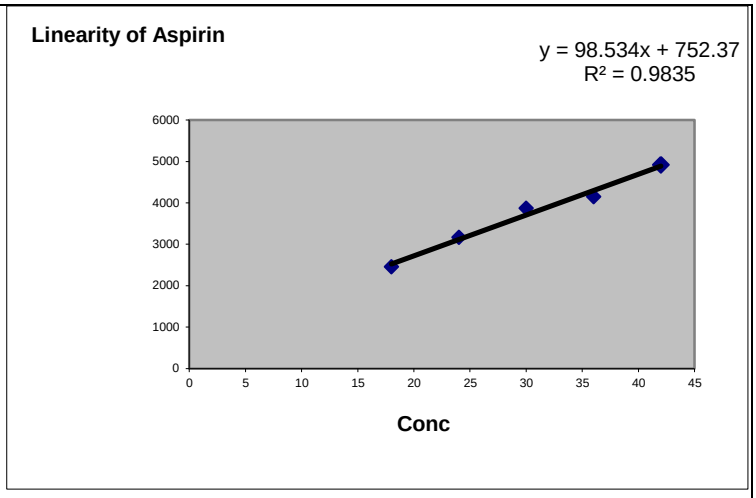
Fig: Chromatogram of OMEPRAZOLE and ASPIRIN for preparation-5

Table: Linearity of ASPIRIN

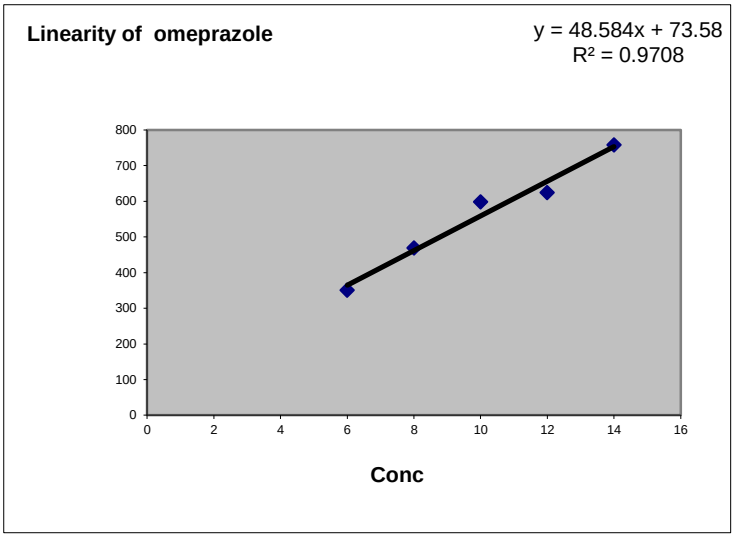
S.No.	Conc.(µg/ml)	Area
1	18	2451.56
2	24	3164.372
3	30	3865.732
4	36	4140.964
5	42	4919.27
6	18	2451.56

Table: Linearity of OMEPRAZOLE

S.No.	Conc.(µg/ml)	Area
1	6	349.568
2	8	468.031
3	10	598.079
4	12	624.026
5	14	757.414



Linearity graph of ASPIRIN



Linearity graph of OMEPRAZOLE

Acceptance criteria

The relationship between the concentration of ASPIRIN and OMEPRAZOLE and area of ASPIRIN and OMEPRAZOLE should be linear in the specified range and the correlation should not be less than 0.99.

Observation

The correlation coefficient for linear curve obtained between concentration vs. Area for standard preparations of ASPIRIN and OMEPRAZOLE is 0.999 and 0.996. The relationship between the concentration of ASPIRIN and OMEPRAZOLE and area of ASPIRIN and OMEPRAZOLE is linear in the range examined since all points lie in a straight line and the correlation coefficient is well within limits.

Accuracy

Accuracy of the method was determined by Recovery studies. To the formulation (pre analyzed sample), the reference standards of the drugs were added at the level of 100%, 120%, 140%. The recovery studies were carried out three times and the percentage recovery and percentage mean recovery were calculated for drug is shown in table. To check the accuracy of the method, recovery studies were carried out by addition of standard drug solution to pre-analyzed sample solution at three different levels 100%, 120%, 140%.

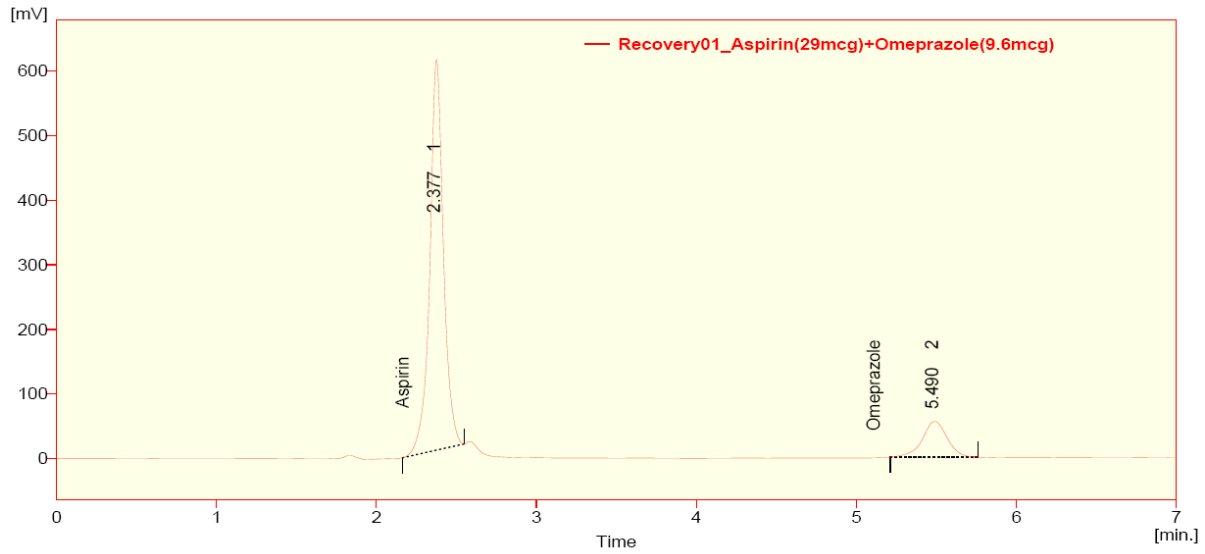


Fig: Chromatogram of 50% recovery (injection 1)

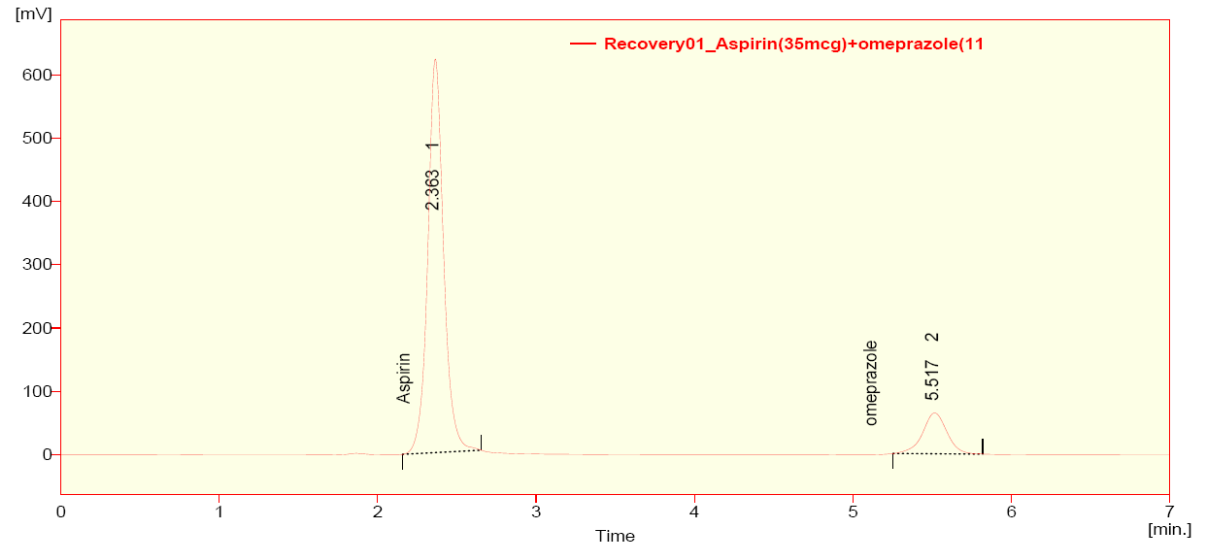


Fig: Chromatogram of 100% recovery (injection 2)

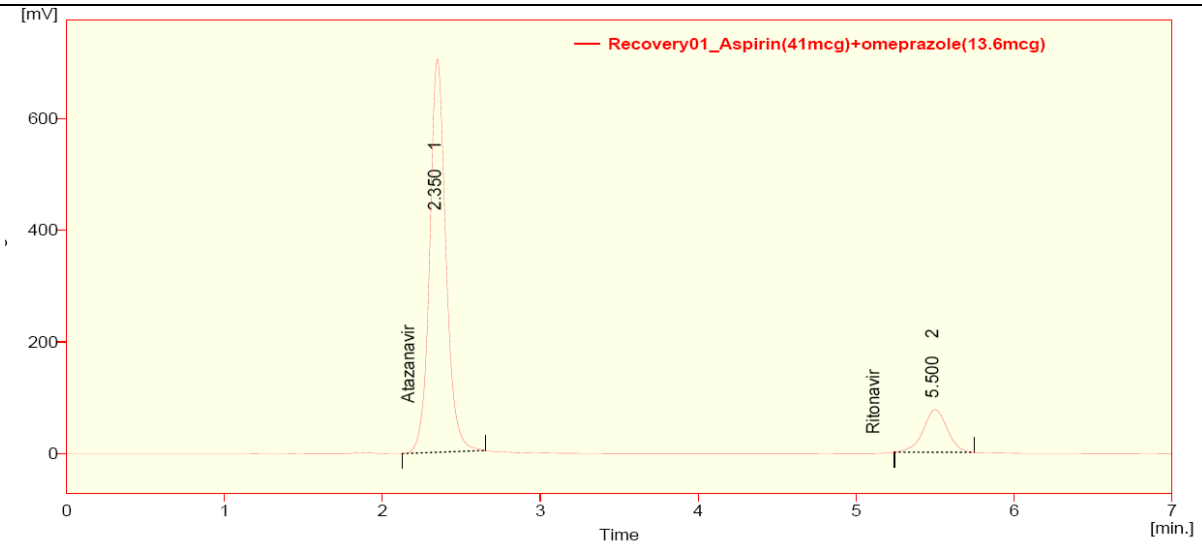


Fig: Chromatogram of 150% recovery (injection 3)

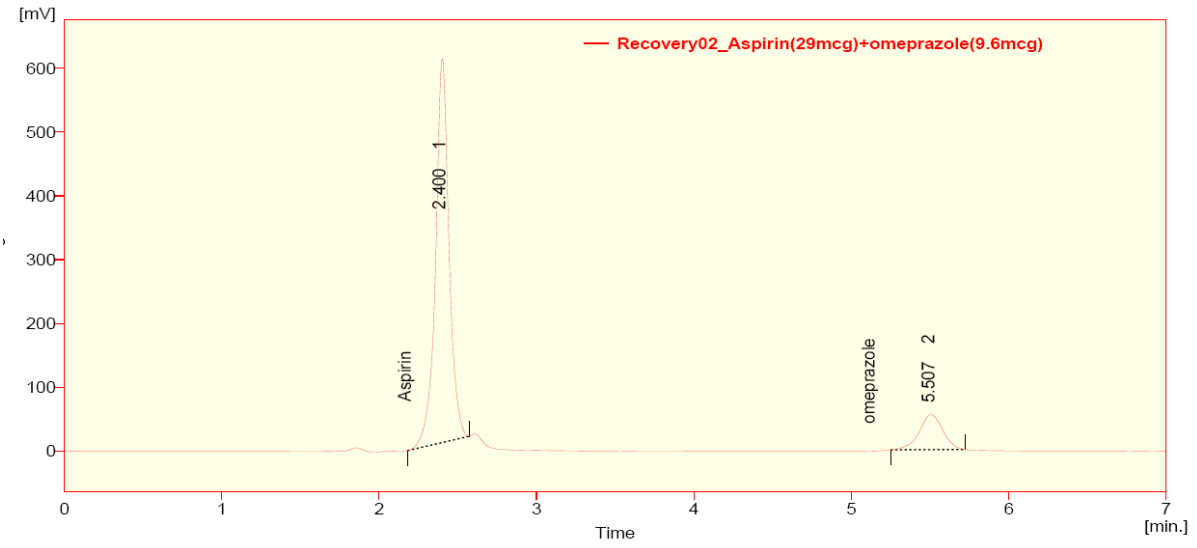


Fig: Chromatogram of 50% recovery (injection 1)

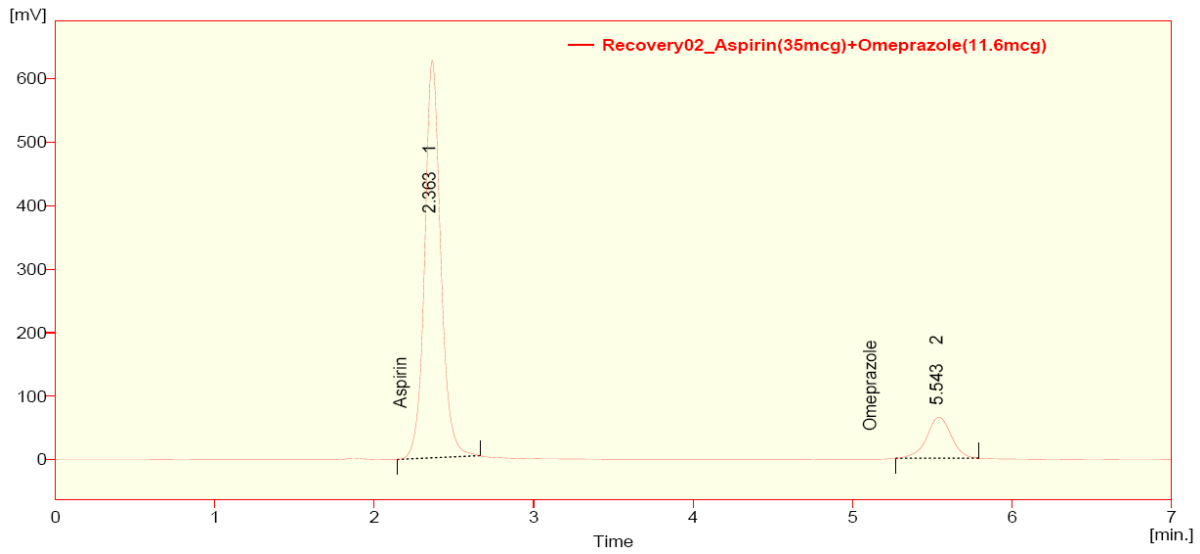


Fig: Chromatogram of 100% recovery (injection 2)

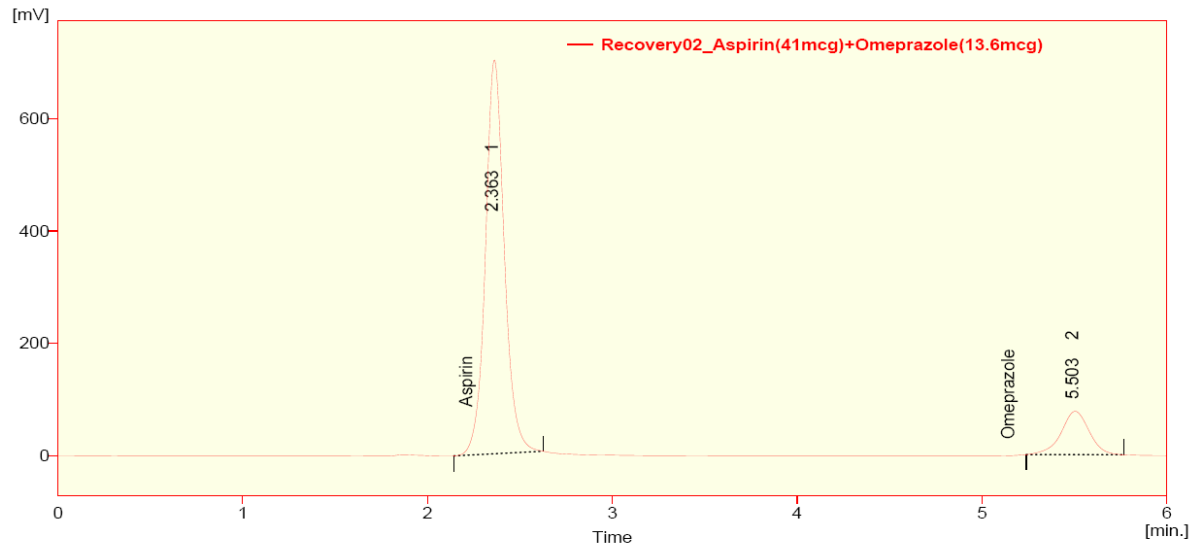


Fig: Chromatogram of 150% recovery (injection 3)

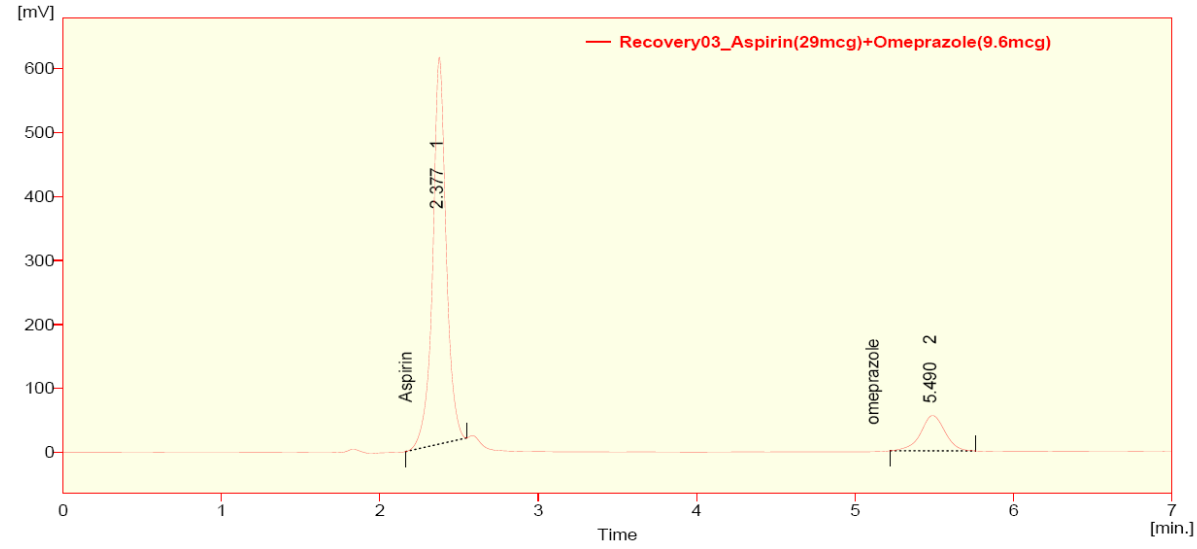


Fig: Chromatogram of 50% recovery (injection 1)

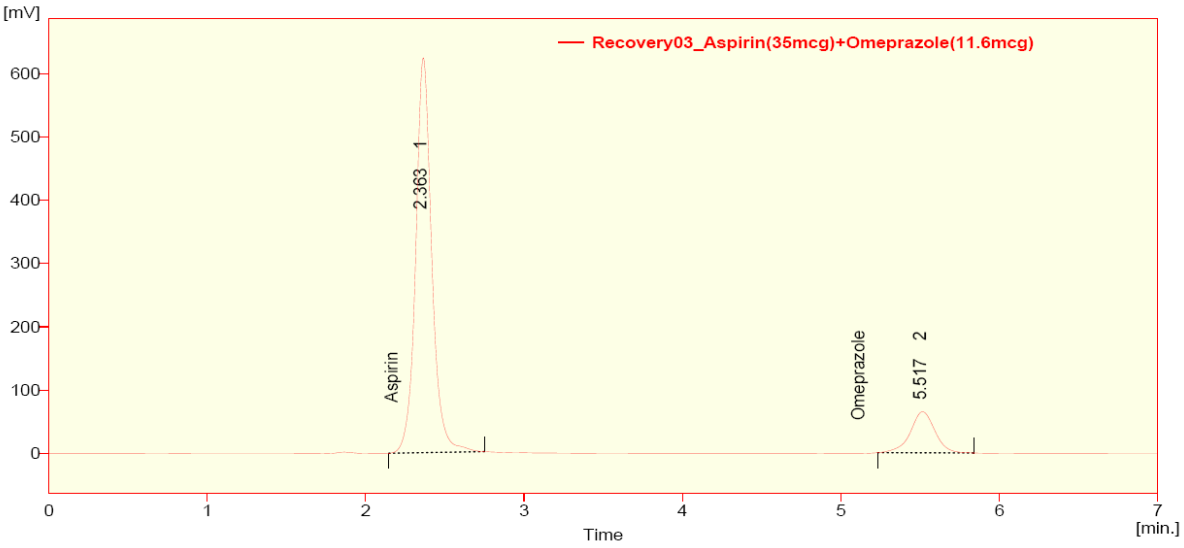


Fig: Chromatogram of 100% recovery (injection 2)

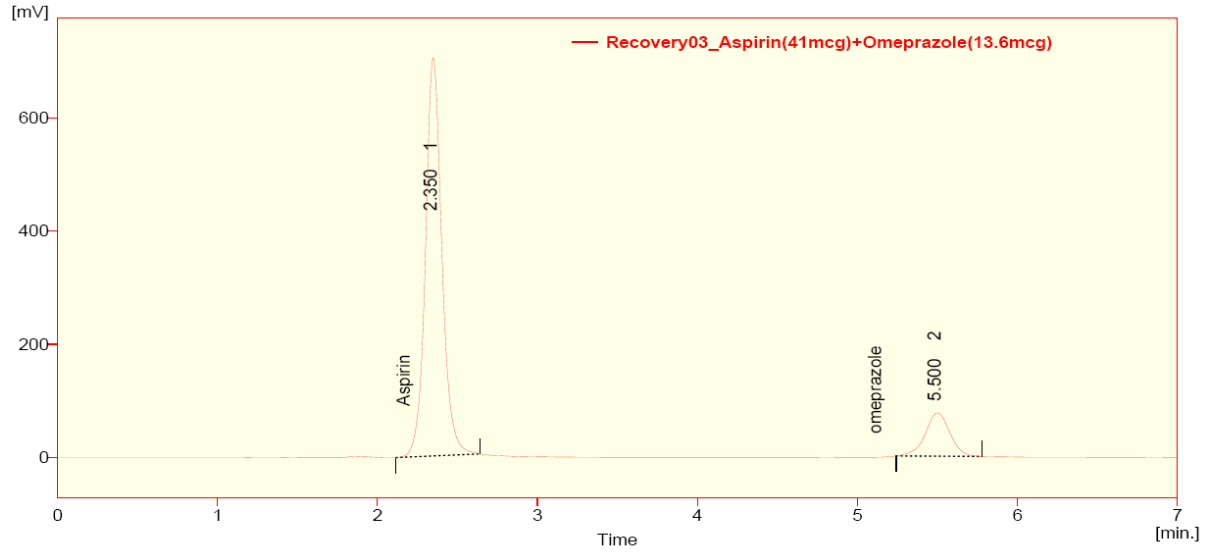


Fig: Chromatogram of 150% recovery (injection 3)

Acceptance criteria

The % recovery of OMEPRAZOLE and ASPIRIN should lie between 98% and 110%.

Table: Recovery results for OMEPRAZOLE

Recovery level	Accuracy OMEPRAZOLE			Average % Recovery
	Amount taken(mcg/ml)	Area	%Recovery	
50	20	**596.773**	**200.256988**	102.83
	20	**573.717**	**192.5201682**	
	20	**592.565**	**198.8449244**	
100	40	**692.39**	**116.1714219**	96.36
	40	**694.362**	**116.5022904**	
	40	**705.19**	118.3190471	99.103
120	60	**811.347**	**81.67824604**	
	60	**818.058**	**82.35384194**	
	60	**819.999**	82.54924228	

Table: Recovery results for ASPIRIN

Recovery level	Accuracy ASPIRIN			Average % Recovery
	Amount taken(mcg/ml)	Area	%Recovery	
50	**20**	**3770.016**	**98.20879979**	
			101.1976169	**100.5075706**
	20	**3884.750**	102.1162951	
	20	**3920.016**		

100	**40**	**4333.839**	**101.6067343**	
	40	**4411.349**	**103.4239541** 103.556793	
	40	**4417.015**		**102.8624939**
150	**60**	**4966.631**	**103.5044667**	
	60	**4956.669**	**103.2968589** 101.2968589	
	60	**4880.549**		**102.8372819**

Observation

The percentage mean recovery of ASPIRIN and OMEPRAZOLE is 99.19 % and 99.89 % respectively.

Precision

Method precision

Prepared sample preparations of OMEPRAZOLE and ASPIRIN as per test method and injected 6 times in to the column.

Acceptance criteria

The % Relative standard deviation of Assay preparations of OMEPRAZOLE and ASPIRIN should be not more than 2.0%.

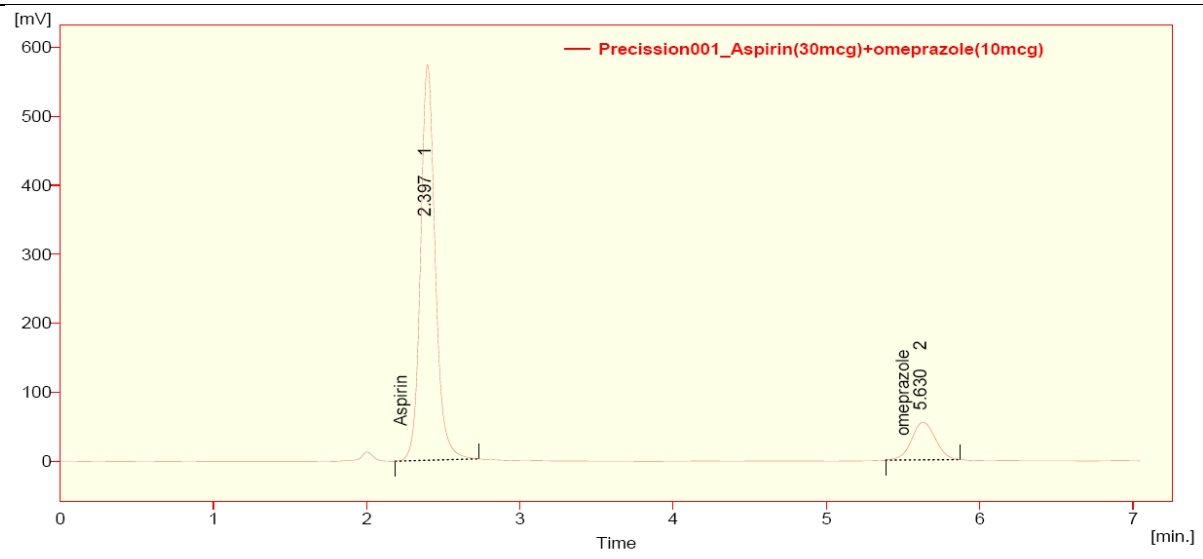


Fig: Chromatogram of precision injection 1

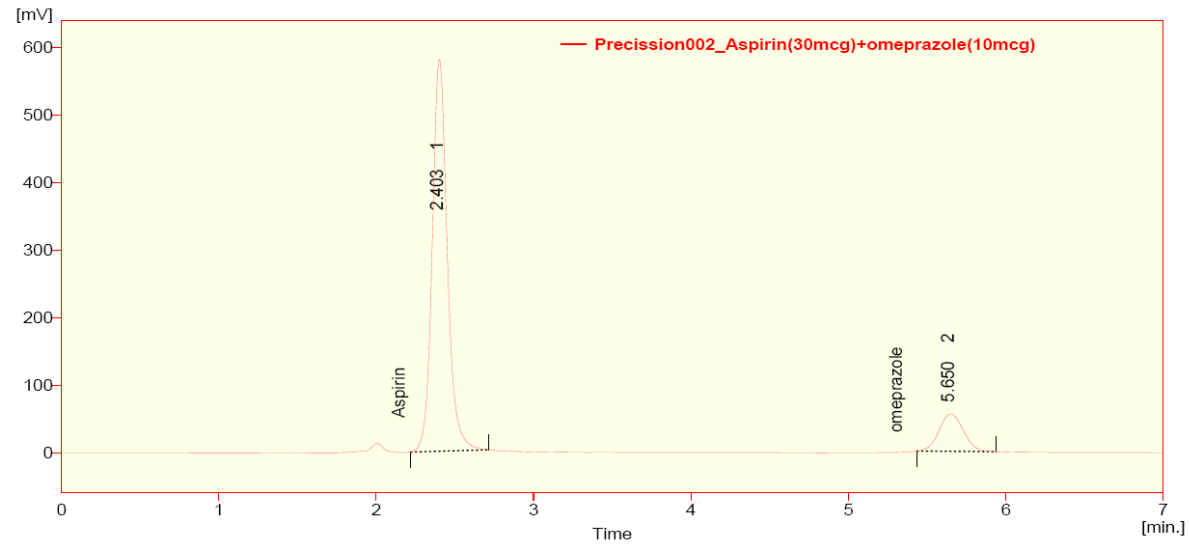


Fig: Chromatogram of precision injection 2

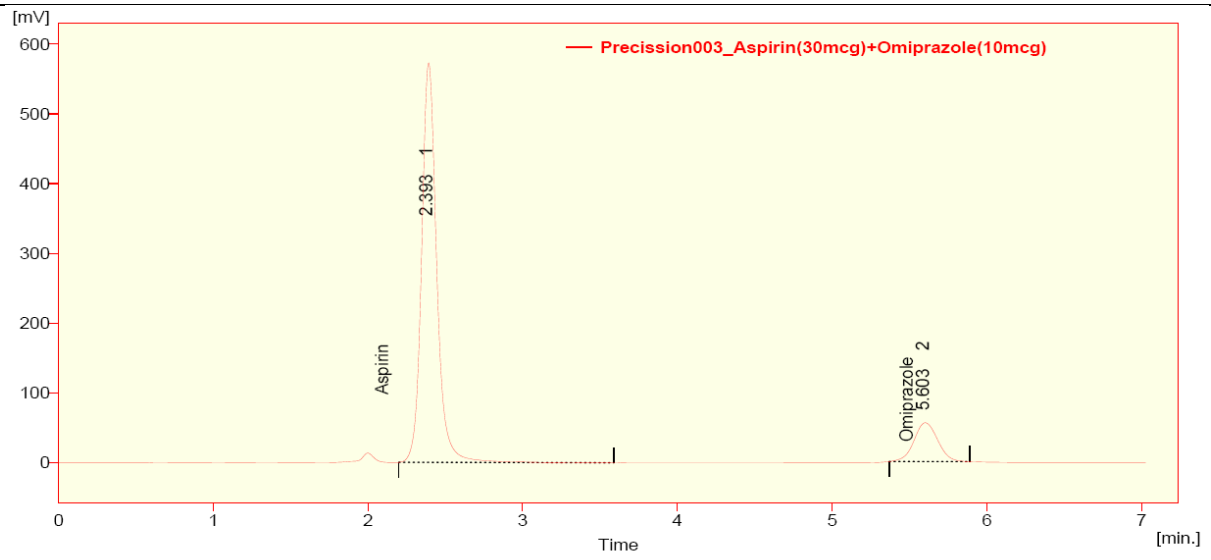


Fig: Chromatogram of precision injection 3

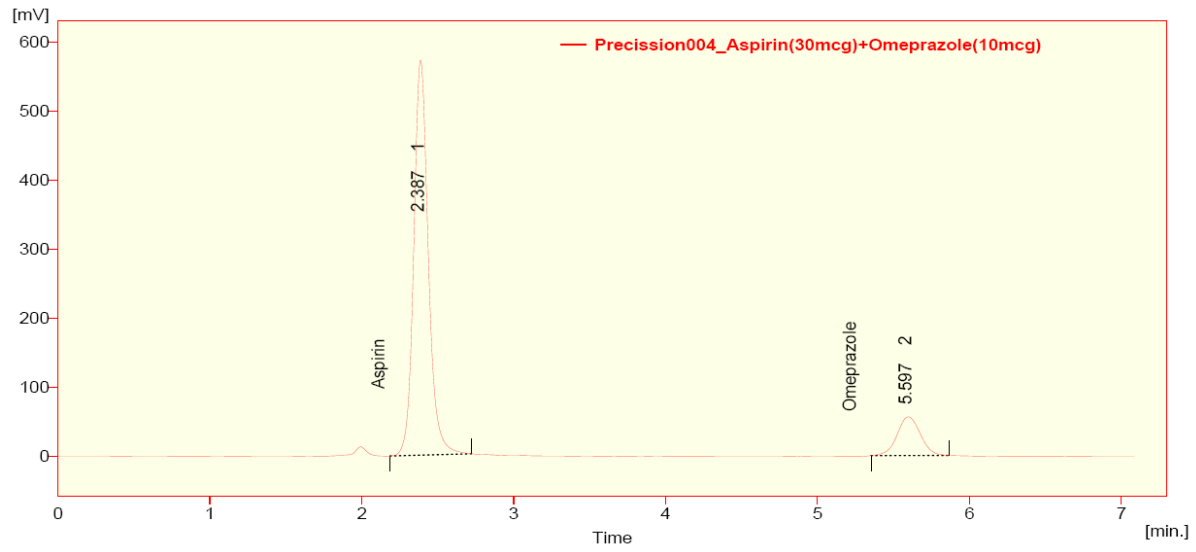


Fig: Chromatogram of precision injection 4

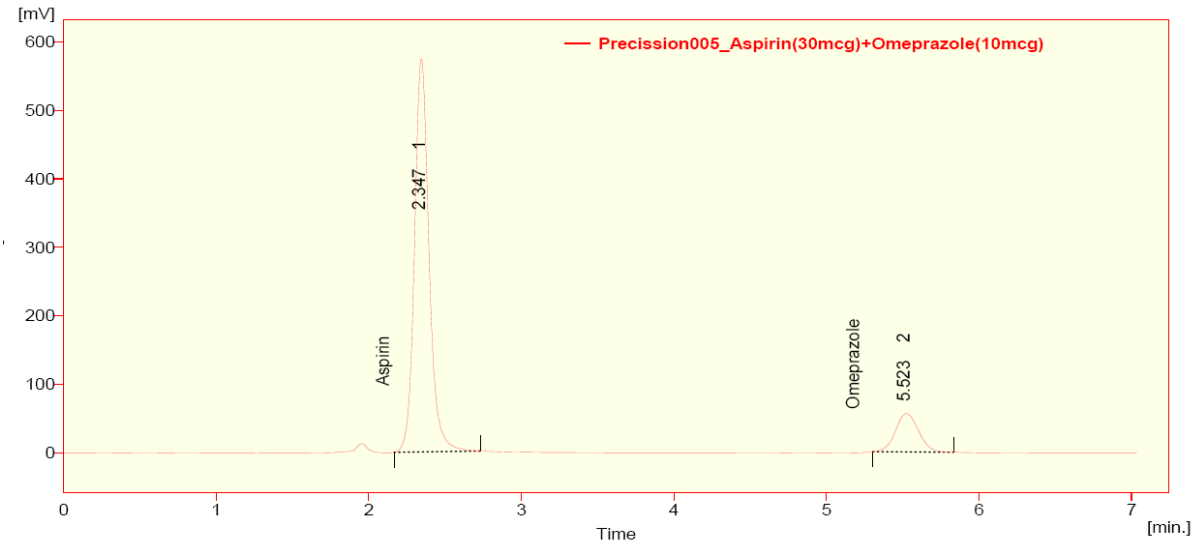


Fig: Chromatogram of precision injection 5

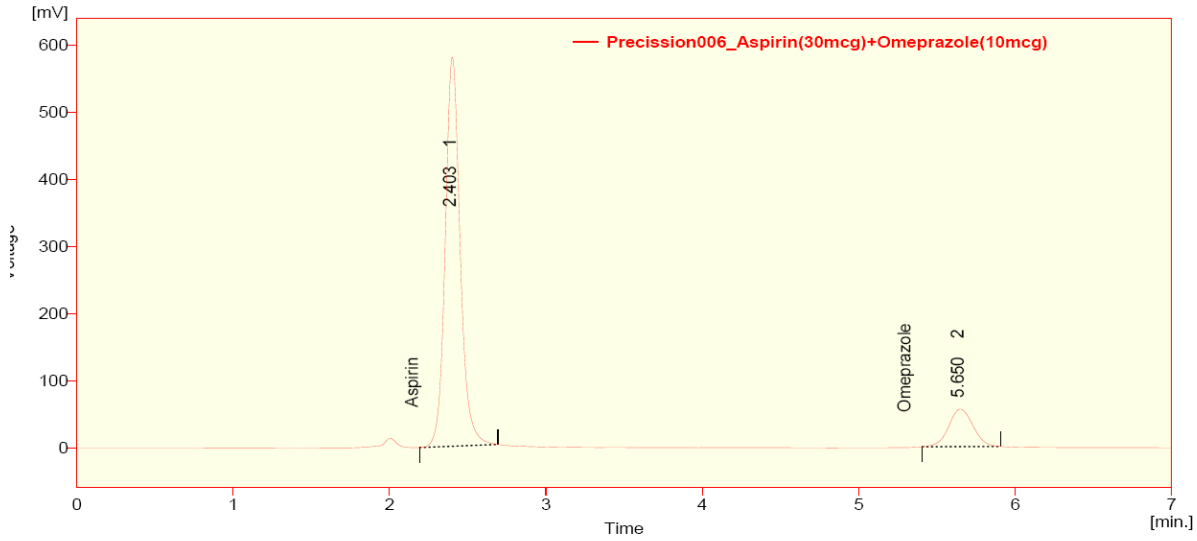


Fig: Chromatogram of precision injection 6

Table: Results for precision of OMEPRAZOLE and ASPIRIN

ASPIRIN			**OMEPRAZOLE**		
S.No.	Rt	Area	S.No.	Rt	Area
1	2.397	3898.811	1	5.630	591.352
2	2.403	3954.691	2	5.650	605.193
3	2.393	3996.327	3	5.603	610.259
4	2.387	3941.128	4	5.597	620.470
5	2.347	3902.778	5	5.523	610.067
6	2.403	3948.922	6	5.650	614.702
Avg	2.3883	3940.443	avg	5.609	608.674
Stdev	0.0212	36.195	stdev	0.048	9.921
%RSD	**0.89**	**0.92**	**%RSD**	**0.85**	**1.63**

Observation

Test results for Aspirin and Omeprazole are showing that the %RSD of Assay results are within limits.

ROBUSTNESS

Chromatographic conditions variation

To demonstrate the robustness of the method, prepared solution as per test method and injected at different variable conditions like using different conditions like flow rate and wavelength. System suitability parameters were compared with that of method precision.

Acceptance criteria

The system suitability should pass as per the test method at variable conditions.

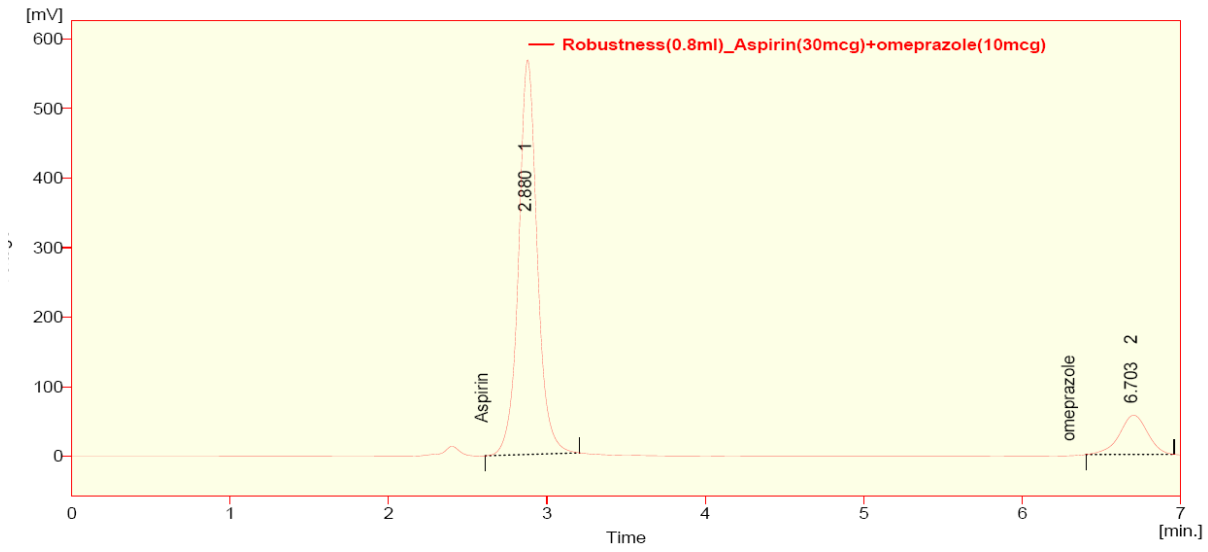


Fig. 9.8.1: Chromatogram of ASPIRIN and OMEPRAZOLE Robustness (0.8 ml/min)

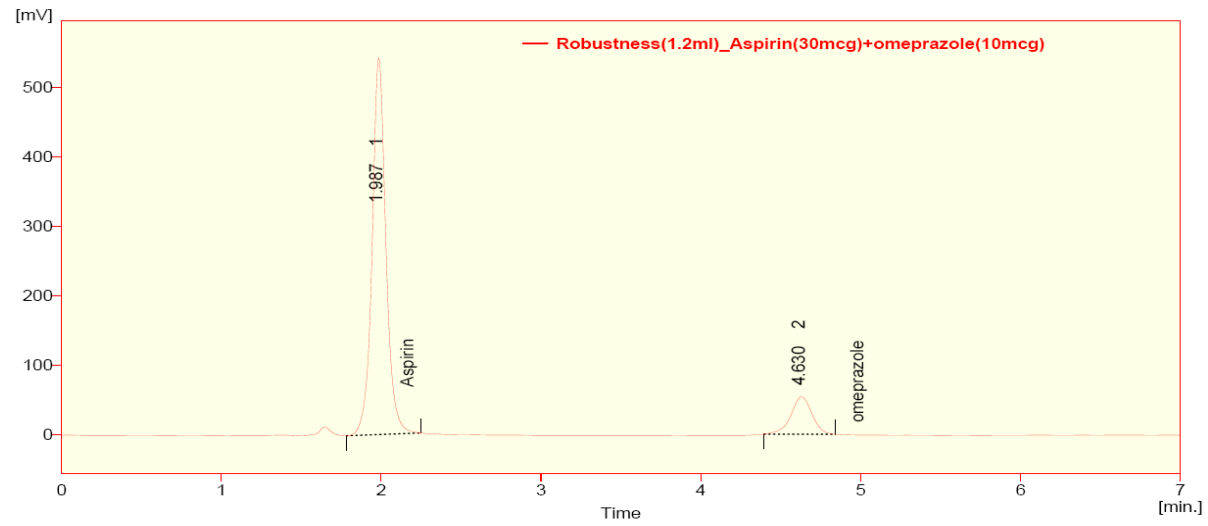


Fig: Chromatogram of OMEPRAZOLE and ASPIRIN Robustness (1.2 ml/min)

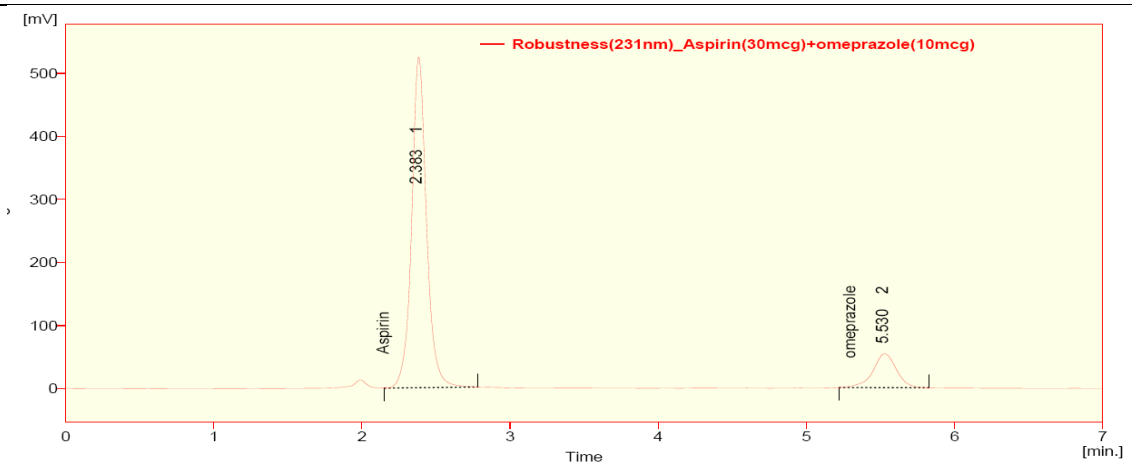


Fig: Chromatogram of OMEPRAZOLE and ASPIRIN for Robustness (231nm)

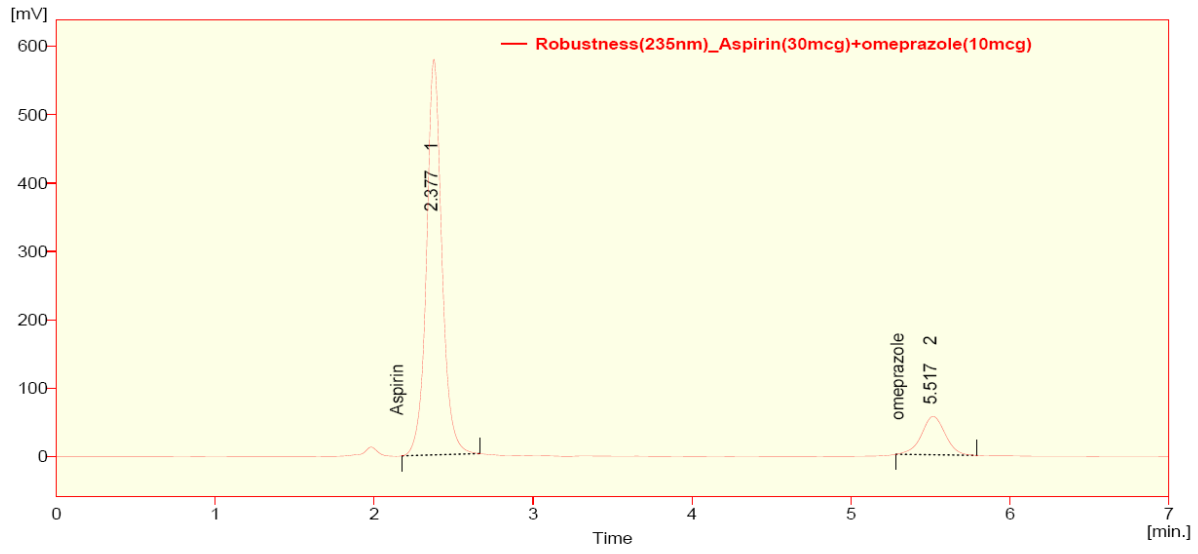


Fig: Chromatogram of OMEPRAZOLE and ASPIRIN for Robustness (235nm)

Table: Result of Robustness study

Parameter	ASPIRIN		OMEPRAZOLE	
	Retention time(min)	Tailing factor	Retention time(min)	Tailing factor
Flow Rate				
0.8 ml/min	2.880	1.054	6.703	1.054
1.2 ml/min	1.987	1.074	4.630	0.932
Wavelength				
231nm	2.383	1.167	5.530	0.907
235nm	2.377	1.100	5.517	1.021

Observation

From the observation it was found that the system suitability parameters were within limit at all variable conditions.

Ruggedness

The ruggedness of the method was studied by the determining the analyst to analyst variation by performing the Assay by two different analysts

Acceptance criteria

The % Relative standard deviation of Assay values between two analysts should be not more than 2.0%.

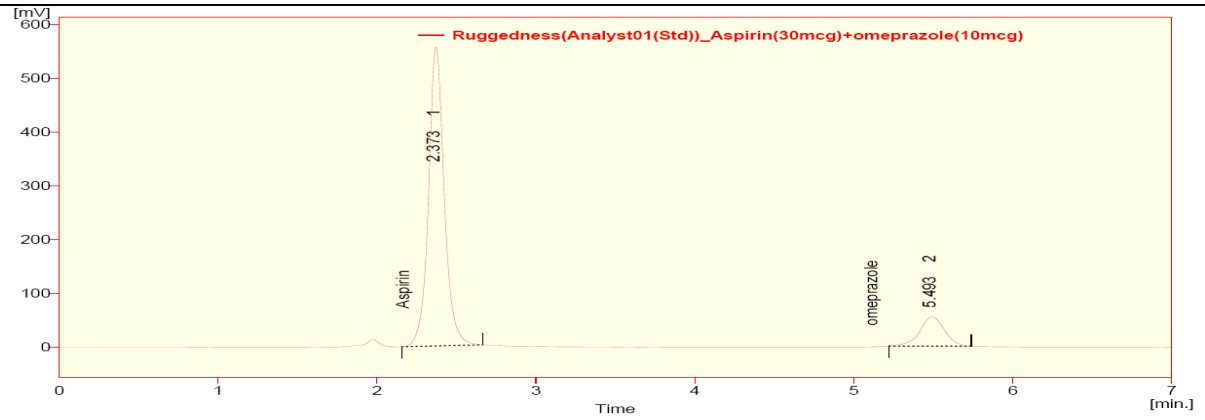


Fig: Chromatogram of Analyst 01 standard preparation

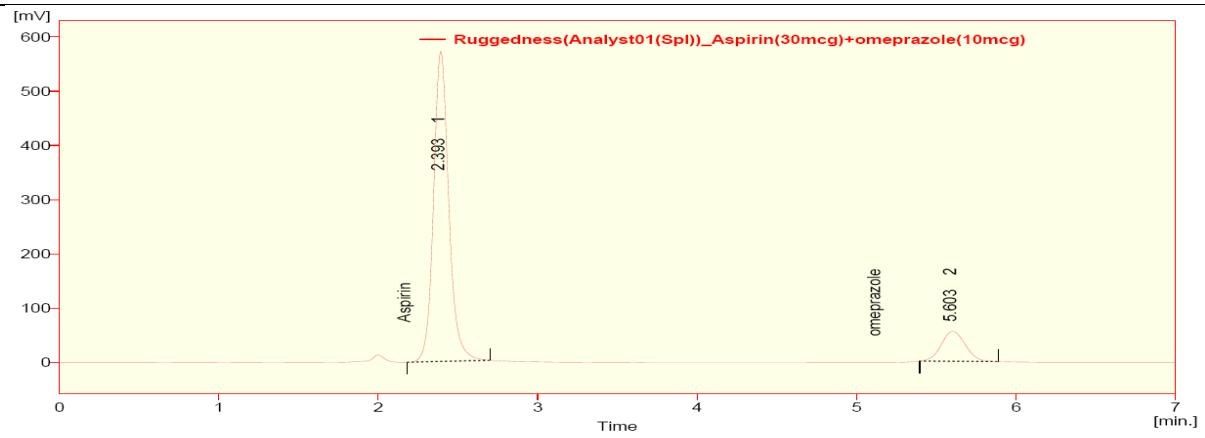


Fig: Chromatogram of Analyst 01 sample preparation

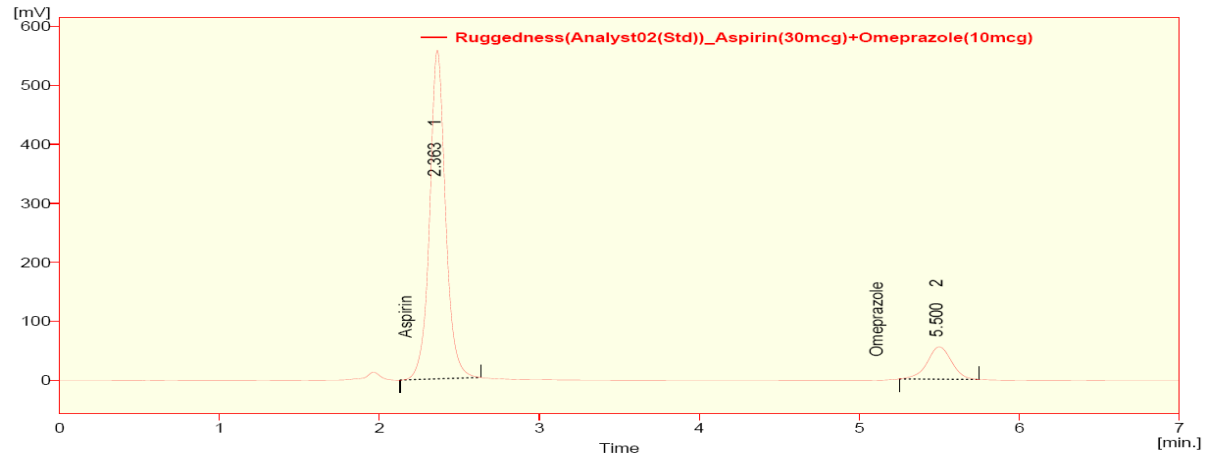


Fig: Chromatogram of Analyst 02 standard preparation

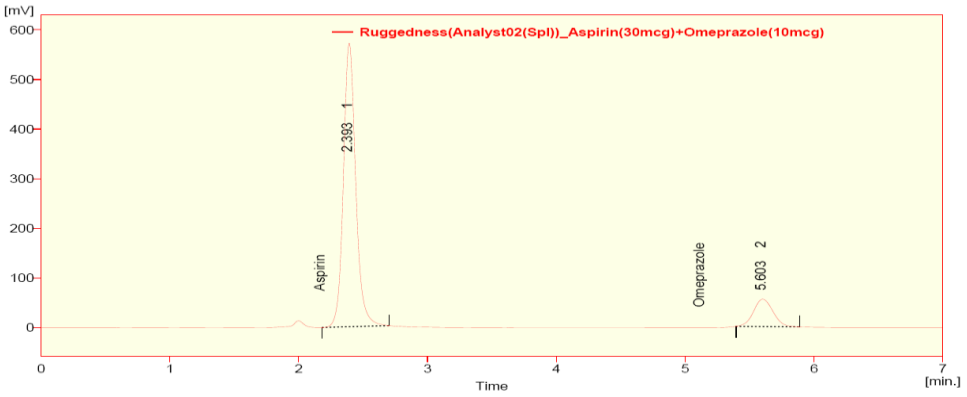


Fig: Chromatogram of Analyst 02 sample preparation

Table: Results for Ruggedness

ASPIRIN	%Assay	OMEPRAZOLE	%Assay
Analyst 01	100.5	**Analyst 01**	98.9
Anaylst 02	99.5	**Anaylst 02**	100.6

Observation

From the observation the between two analysts Assay values not greater than 2.0%, hence the method was rugged.

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