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

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ABSTRACT

A simple, rapid, precise, sensitive and reproducible reverse phase high performance liquid chromatography (RP-HPLC) method has been developed for the quantitative analysis of Aspirin and ethoheptazine citrate in pharmaceutical dosage form. Chromatographic separation of Aspirin and ethoheptazine citrate was achieved on waters e2695, by using Inertsil -BDS C18 column and the mobile phase containing Acetonitirile and Water in the ratio of 55:45 v/v. The flow rate was 1.0 ml/min and detection were carried out by absorption at 256 nm using a photodiode array detector at ambient temperature. The number of theoretical plates and tailing factor for Aspirin and ethoheptazine citrate were NLT 3000 and RSD NMT 2 respectively. The linearity of the method was excellent over the concentration range 26-104 µg/ml and 6-24 µg/ml for Aspirin and ethoheptazine citrate respectively. The correlation coefficient of Aspirin and ethoheptazine citrate were 0.999 and 0.999. The retention time of Aspirin and ethoheptazine citrate were 2.951 min and 4.195 min respectively. The proposed method was validated according to ICH guidelines. The method was found to be simple, economical, suitable, precise, accurate and robust method for quantitative analysis of Aspirin and ethoheptazine citrate in pure and pharmaceutical dosage form.

KEYWORDS: HPLC, Aspirin and ethoheptazine citrate.

INTRODUCTION

Aspirin is also known as acetylsalicylic acid, it is often used as an analgesic to relieve minor aches and pains, as an antipyretic to reduce fever, and as an anti-inflammatory medication. Aspirin is also used long-term, at low doses that helps to prevent heart attacks, strokes, and blood clot formation in people who are at the high risk of developing blood clots. Low doses of aspirin may be given immediately after a heart attack to reduce the risk of another heart attack or of the death of cardiac tissue. It is effective at preventing certain types of cancer, particularly colorectal cancer. Aspirin is chemically known as 2-(acetoxy) benzoic acid, and its Chemical Formula $C_9H_8O_4$, with the molecular weight180.157 g/mol. Aspirin is a weak acid that is only slightly soluble in water.

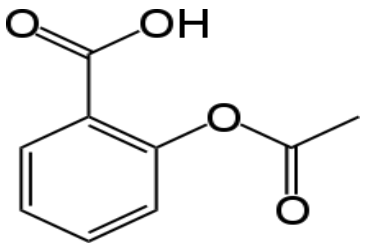

Fig. No 1: structure of Aspirin.

Ethoheptazine is an opioid analgesic from thephenazepine family. Opioids and non-steroidal anti-inflammatory drugs (NSAIDs) are the commonest drugs used to treat pain. Opioids mimic the actions of endogenous opioid peptides by interacting with mu, delta or kappa opioid receptors. The opioid receptors are coupled to G1 proteins and the actions of the opioids are mainly inhibitory. Ethoheptazine is chemically known as Ethyl 1-methyl-4-phenylazepane-4-carboxylate and its chemical formula $C_{16}H_{23}NO_2$ with the molecular weight 261.36 g/mol. It is soluble in 0.575 mg/ml (water).

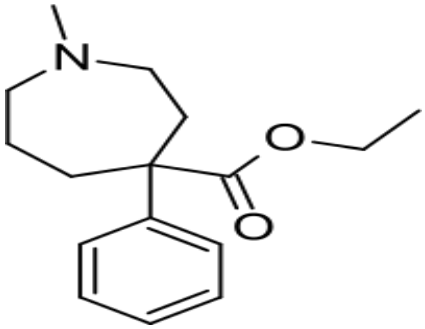

Fig. No 2: structure of Ethoheptazine.

Literature survey revealed that other than official method there is no any available method for determination of Aspirin and Ethoheptazine citratedosage form. Moreover, there is no stability indicated method reported for this dosage form. our present plan is to develop a new, simple, precise and accurate RP-HPLC method for its analysis in formulation.

Experimental

Instrumentation

HPLC is equipped with PDA Detector, Model 2695 WATERS, Auto sampler, with Empower 2 software. A double beam UV-visible spectrophotometer LAB INDIA 3000 series with UV –win software and 1cm quartz cell.

Chemicals and Reagents

The solvents used were of HPLC/AR grade. Double distilled water was used in preparation of mobile phase.

Chromatographic conditions

When several mobile phases were tried, the mobile phase containing a mixture of Acetonitrile: water in the ratio of (55:45) was considered as appropriate and the colum used was Inertsil -BDS C18(250 x 4.6 mm, 5 µ) with ambient temperature. The mobile phase was filtered through 0.45µm membrane filter and then ultrasonicated for 15 minutes. The flow rate was set to 1.0 ml/min and UV detection was carried out at 226nm.

Standard Solution Preparation

Accurately Weighed and transferred 325 mg of Aspirin and 75 mg Ethoheptazine Citrate of working Standards into a 25 ml clean dry volumetric flask, add three fourth volume of diluent, sonicated for 5 min and make up to the final volume with diluents. 1ml from the above two stock solutions was taken into a 10 ml volumetric flask and made up to 10 ml.

Sample Solution Preparation

For analysis of commercial formulation, 20 tablets of Aspirin 325 mg and Ethoheptazine Citrate 75 mg were weighed the average weight was calculated and powdered. A quantity equivalent to 325 mg of Aspirin and 75 mg of Ethoheptazine Citrate was weighed and transferred to a 100 ml volumetric flask which contain mobile phase and then shake it for 10mins and sonicate it for 20 min. The solution was allowed to stand at a room temperature for 20-30mins and filtered it through a Whatman filterpaper.

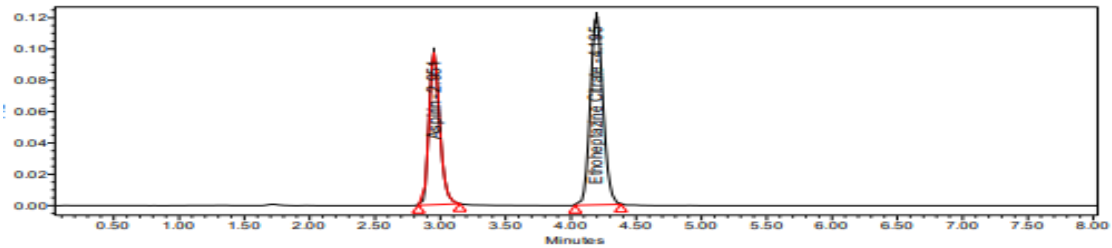

Fig.no 3: Chromatogram of standard.

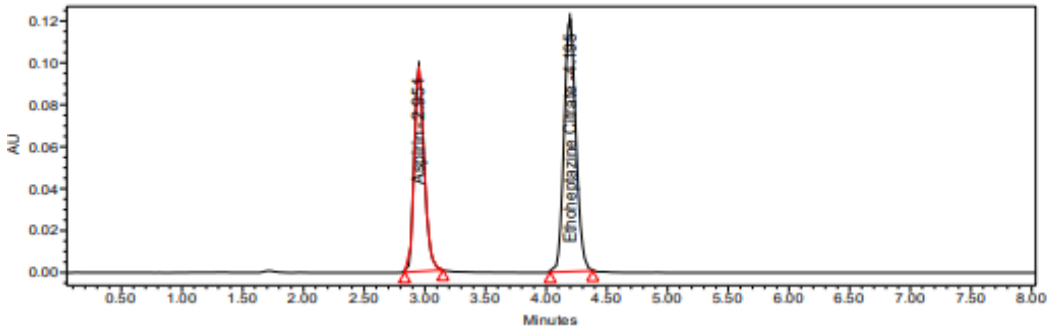

Fig.no 4: Chromatogram of sample.

RESULTS AND DISCUSSION

Assay

Standard preparations are made from the API and Sample Preparations are from Formulation. Both sample and standards are injected six homogeneous samples. Drug in the formulation was estimated by taking the standard as the reference. The Average % Assay was calculated and found to be 99.3 and 98.5 for Aspirin and Ethoheptazine citrate respectively.

Table no. 1: Assay Results.

S.No	Aspirin %Assay	Ethoheptazine citrate %Assay
1	98.55	98.6
2	98.88	99.02
3	99.40	98.12
4	99.30	98.31
5	100.53	98.81
6	98.28	98.36
Avg	99.278	98.48
ST DEV	0.827	0.3526
□ RSD	0.83	0.35

METHOD VALIDATION

1) SYSTEM SUITABILITY

A Standard solution was prepared by using Aspirin and Ethoheptazine Citrate working standards as per test method and was injected Five times into the HPLC system.

The system suitability parameters were evaluated from standard chromatograms by calculating the % RSD from five replicate injections for Aspirin and Ethoheptazine Citrate. The %RSD for retention times and peak areas were found to be within the limit.

Table no. 2: Data of System Suitability for Aspirin.

Injection	RT	Peak Area	USP Plate count	USP Tailing
1	2.951	729374	10953.609752	1.604407
2	2.950	729587	10951.014286	1.604878
3	2.948	729020	10003.278630	1.590957
4	2.949	729174	10986.906427	1.584354
5	2.949	729744	10946.878423	1.566451
Mean	2.9514112	729379.8	10768.34	1.590209
SD	0.004658	294.7104	-------	-------
% RSD	0.131	0.040	------	-------

Table no. 3: Data of System Suitability for Ethoheptazine Citrate.

Injection	RT	Peak Area	USP Plate count	USP Tailing
1	4.195	202274	9478.317159	1.021108
2	4.193	202478	9452.196217	1.080574
3	4.189	201254	9569.928335	1.090824
4	4.190	207894	9619.633847	1.089932
5	4.189	209874	9749.907462	1.108610
Mean	4.192841	2748461	9573.997	1.07821
SD	0.00148	297.998	-------	-------
% RSD	0.250	0.0108	------	-------

2) PRECISION

Repeatability: For System Precision studies, the standard solution was prepared and analysis was carried for five replicated injections. The percentage relative standard deviation (% RSD) was calculated for the peak areas for Aspirin and Ethoheptazine Citrate and it was found to be not more than 2.0%. The acceptance criterion of method precision was found to be RSD NMT 2.0%.

A) System precision

Table no. 4: Data of Repeatability (System precision) for Aspirin.

	Injection	PeakAreas of Aspirin	%Assay
Concentration 52 µl	**1**	734360	98.66
	2	739098	99.30
	3	755696	101.53
	4	748289	100.53
	5	744147	99.98
Statistical Analysis	**Mean**	744318	100.00
	SD	8241.164	1.107678
	%RSD	1.1	1.10

Table no. 5: Data of Repeatability (System precision) for Ethoheptazine Citrate.

	Injection	PeakAreas of Ethoheptazine Citrate	%Assay
Concentration 12 µl	**1**	205625	99.95
	2	206225	100.24
	3	205840	100.06
	4	204283	99.30
	5	205735	100.00
Statistical Analysis	**Mean**	205541.6	99.91
	SD	739.0046	0.35819
	%RSD	0.35	0.35

(b) Method Precision

Table no. 6: Data of Repeatability (Method precision) for Aspirin.

	Injection	Peak Areas of Aspirin	%Assay
Concentration 52 µl	**1**	733495	98.55
	2	735992	98.88
	3	739828	99.40
	4	739098	99.30
	5	748289	100.53
	6	731322	98.28
Statistical Analysis	**Mean**	738004	99.278
	SD	5988.879	0.827236
	%RSD	0.81	0.83

Table no. 7: Data of Repeatability (Method precision) for Ethoheptazine Citrate.

	Injection	Peak Areas of Ethoheptazine Citrate	%Assay
Concentration 12 µl	**1**	202110	98.6
	2	203700	99.02
	3	201851	98.12
	4	202255	98.31
	5	203283	98.81
	6	202349	98.36
Statistical Analysis	**Mean**	738004	98.48
	SD	5988.879	0.352647
	%RSD	0.81	0.35

3) Intermediate precision (analyst to analyst variability): A study was conducted by two analysts as per test method. Individual % assays and % RSD of Assay are within limit.

Table no. 8: Data of Intermediate precision (Analyst 2) for Aspirin.

	Injection	Peak Areas of Aspirin	%Assay
Concentration 52 µl	**1**	736792	99.99
	2	734360	99.66
	3	755696	101.53
	4	744147	99.98
	5	744127	99.97
	6	752525	101.10
Statistical Analysis	**Mean**	744607.8	100.37
	SD	8392.59	0.753536
	%RSD	1.1	0.75

Table no. 9: Data of Intermediate precision (Analyst 2) for Ethoheptazine Citrate.

	Injection	Peak Areas of Ethoheptazine Citrate	%Assay
Concentration 12 µl	**1**	205267	99.78
	2	205625	99.95
	3	205840	100.00

	4	202735	98.55
	5	208991	101.50
	6	208543	101.37
Statistical Analysis	**Mean**	206333.5	100.19
	SD	2572.599	1.100898
	%RSD	1.24	1.09

4) ACCURACY (RECOVERY)

A study of Accuracy was conducted. Drug Assay was performed in triplicate as per test method with equivalent amount of Aspirin and Ethoheptazine Citrate into each volumetric flask for each spike level to get the concentration of Aspirin and Ethoheptazine Citrate equivalent to 50%, 100%, and 150% of the labeled amount as per the test method. The average % recovery of Aspirin and Ethoheptazine Citrate were calculated. The recovery results indicating that the test method has an acceptable level of accuracy.

Table no. 10: Data of Accuracy for Aspirin.

Concentration % of spiked level	**Amount added (ppm)**	**Amount found (ppm)**	**% Recovery**	**Statistical Analysis of % Recovery**	
50% Injection 1	26	26.04	100.1	MEAN	100.06
50% Injection 2	26	26.98	99.92	%RSD	0.18
50% Injection 3	26	26.02	100.08		
100 % Injection 1	52	52.01	100.02	MEAN	100.04
100 % Injection 2	52	52.05	100.14	%RSD	0.091
100 % Injection 3	52	51.98	99.96		
150 % Injection 1	78	78.08	100.1	MEAN	100.02
150 % Injection 2	78	77.97	99.96	%RSD	0.09
150 % Injection 3	78	77.98	99.98		

Table no. 11: Data of Accuracy for Ethoheptazine Citrate.

Concentration % of spiked level	**Amount added (ppm)**	**Amount found (ppm)**	**% Recovery**	**Statistical Analysis of % Recovery**	
50% Injection 1	6	6.05	100.75	MEAN	99.69333
50% Injection 2	6	5.98	99.31	%RSD	0.92
50% Injection 3	6	5.99	99.02		
100 % Injection 1	12	11.99	99.70	MEAN	99.83333
100 % Injection 2	12	12.04	100.30	%RSD	0.41
100 % Injection 3	12	11.98	99.50		
150 % Injection 1	18	18.1	100.21	MEAN	99.97333
150 % Injection 2	18	17.95	99.61	%RSD	0.31
150 % Injection 3	18	18.05	100.20		

5) LINEARITY

The Linearity for the both drugs are done by preparing a Series of solutions of Aspirin and Ethoheptazine Citrate working standards at concentration levels from 26ppm to 104ppm for Aspirin and 6ppm to 24ppm for Ethoheptazine Citrate. The calibration curve constructed by plotting concentration vs. peak area, it was found that there exists a linear relationship with 0.999 and 0.999 as the value of correlation coefficient for the both drugs respectively.

Table no. 12: Data of Linearity (Aspirin)

Concentration (ppm)	**Average Area**	**Statistical Analysis**	
0	0	Slope	14094
26	372546	y-Intercept	10541
39	558296	Correlation Coefficient	0.999
52	744400		
65	930308		
78	1116282		
91	1302046		
104	1462877		

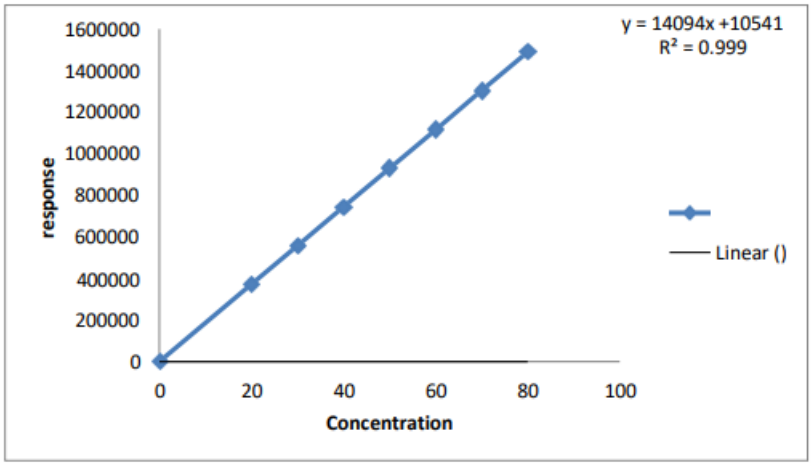

Fig. no 5: (a) Linearity Plot (Concentration Vs Response) of Aspirin.

Table no. 13: Data of Linearity (Ethoheptazine Citrate).

Concentration (ppm)	Average Area	Statistical Analysis	
0	0	Slope	16721
6	102965	y-Intercept	4723
9	154371	Correlation Coefficient	0.999
12	205856		
15	257167		
18	308577		
21	359903		
24	399878		

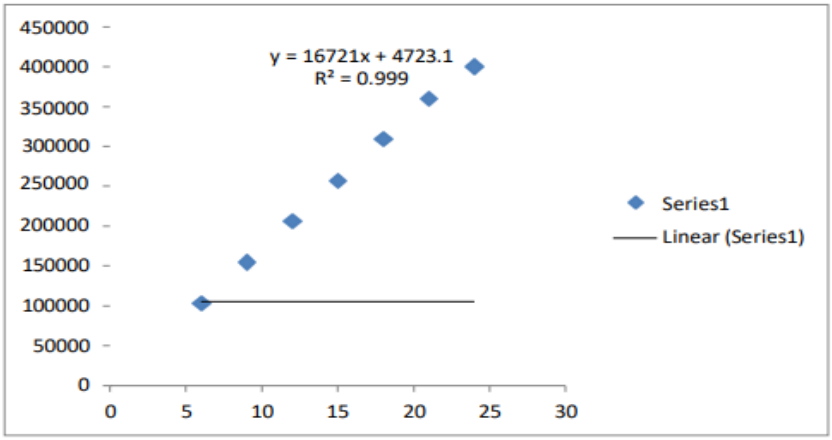

Fig. no 6: (b) Linearity Plot (Concentration Vs Response) of Ethoheptazine Citrate.

6) Ruggedness

a) System to system variability: System to system variability study was conducted on different HPLC systems, under similar conditions at different times. Six samples were prepared and each was analyzed as per test method. Comparison of both the results obtained on two different HPLC systems, shows that the assay test method is rugged for system-to-system variability. The % RSD was found within the limit.

Table no. 14: (i) Data of system to system variability (system 2)

S.NO:	Peak area	Assay % of Aspirin
1	734360	98.65
2	734098	98.63
3	735696	98.86
4	733289	98.52
5	734147	98.63
6	733495	98.55
Mean	734180.8	98.64
%RSD	0.11	0.12

Table no. 15: Data of system-to-system variability (Ethoheptazine Citrate) System-2.

S.NO:	Peak area	Assay % of Ethoheptazine Citrate
1	203625	99.98
2	202225	99.30
3	202840	98.60
4	204283	99.30
5	202735	98.55
6	203110	98.73
Mean	203136.3	99.07667
%RSD	0.35	0.56

7) Robustness

Table no. 16: Data for Effect of variation in flow rate (Aspirin).

	Std Area	Tailing factor		Std Area	Tailing factor		Std Area	Tailing factor
Flow 0.8 ml	1120286	1.32208	**Flow 1.0ml**	734322	1.60487	**Flow 1.2ml**	602077	1.28537
	1119282	1.33192		735792	1.58435		601854	1.31938
	1121337	1.29643		734360	1.54380		602403	1.29205
	1120456	1.31545		735696	1.56859		603421	1.30456
	1120765	1.32655		733147	1.55998		602465	1.29462
Avg	1120425	1.31849	Avg	734663	1.57232	Avg	602444	1.29919
SD	754.0018	0.01372	SD	1100.91	0.02332	SD	599.883	0.01322
%RSD	0.06	1.04	%RSD	0.14	1.48	%RSD	0.09	1.01

Table no. 17: Data for Effect of variation in flow rate (Ethoheptazine Citrate)

	Std Area	Tailing factor		Std Area	Tailing factor		Std Area	Tailing factor
Flow 0.8 ml	273707	1.36208	**Flow 1.0ml**	206349	1.28057	**Flow 1.2ml**	166195	1.28537
	273211	1.35261		205267	1.27993		165885	1.29938
	273948	1.37692		205625	1.26172		166303	1.30806
	273465	1.34575		205840	1.27608		167243	1.27466
	273862	1.37492		205735	1.25064		165762	1.26763
Avg	273638	1.36246	Avg	205763	1.26979	Avg	166277	1.28702
SD	301.36	0.01360	SD	392.16	0.01314	SD	582.975	0.01678
%RSD	0.11	0.99	%RSD	0.19	1.03	%RSD	0.35	1.3

8) LIMIT OF DETECTION AND LIMIT OF QUANTITATION (LOD and LOQ)

Aspirin: From the linearity plot the LOD and LOQ are calculated

LOD = 3.3 σ / S

3.3×1474.027 ÷ 14904 = 0.345

LOQ = 10σ/S

10×1474.027 ÷14904 =0.989

Ethoheptazine Citrate

LOD = 3.3 σ / S

3.3× 531.673 ÷ 6721 = 0.104

LOQ = 10σ/S

10×531.673 ÷ 16721 =0.329

9) FORCED DEGRADATION STUDIES

i) Acid hydrolysis: Acid-induced, forced degradation was performed by adding an aliquot of stock solution (1 mg/ml) of Equagesic to 10 ml each of methanol and 0.1 NHCl and refluxing the mixture at 60°C for approximately six hours. The solution was then left to reach room temperature, neutralized to pH 7 by the addition of 0.1 NNaOH, and diluted to 100 ml with the mobile phase so as to get a final concentration of 10 µg/ml

ii) Alkaline hydrolysis: Forced degradation in alkaline media was performed by adding an aliquot of stock solution (1 mg/ml) of Equagesic to 10 ml each of methanol and 0.1 NNaOH, and refluxing the mixture at 60°C for approximately six hours. The solution was then left to reach room temperature, neutralized to pH 7 by addition of 0.1 NHCl, and diluted to 100 ml with the mobile phase, so as to get a final concentration of 10 µg/ml.

iii) Oxidative degradation: To study the effect of oxidizing conditions, an aliquot of stock solution (1 mg/ml) of Equagesic was added to 10 ml of 3% H2O2 solution and the mixture was refluxed at 60°C for approximately six hours. The solution was left to reach room temperature and diluted to 100 ml with the mobile phase, so as to get a final concentration of 10 µg/ml.

iv) Thermal degradation: To study the effect of temperature, approximately 50 mg Equagesic was stored at 100°C in a hot air oven for 24 hours. It was then dissolved in 10 ml of methanol and the volume

was adjusted to 50 ml with the mobile phase. The above solution was further diluted with the mobile phase, to give a solution of final concentration equivalent to 10 µg/ml of equagesic.

% Degradation = Au-At ÷ Au ×100
Where: Au=Area of Untreated Solution
At= Area of Treated Solution

Table no. 18: Forced Degradation for Aspirin.

Mode of Degradation	Condition	Peak Area	% Degradation as compared with Control
Control sample	No treatment	729374	---
Acid	0.1 N Hcl	698451	4.24
Base	0.1 N NaoH	984521	-34.98
Oxidative	30% H_2O_2	412673	43.42
Thermal	100 °C	708124	2.9

Table no. 19: Forced Degradation for Ethoheptazine Citrate.

Mode of Degradation	Condition	Peak Area	% Degradation as compared with Control
Control sample	No treatment	2748977	---
Acid	0.1 N Hcl	1324508	51.82
Base	0.1 N NaoH	3879545	-41.13
Oxidative	30% H_2O_2	2463912	10.37
Thermal	100 °C	7487914	-174.39

SUMMARY

The analytical method was developed by studying different parameters. Maximum absorbance was found to be at 265 nm for Aspirin and 256 nm for Ethoheptazine Citrate. Common wavelength was 256 nm and the peaks purity was excellent. Injection volume was selected to be 20 µl which gave a good peak area. The column used for study was Inertsil C18, BDS and obtained good peak shape. Ambient temperature was found to be suitable for the nature of drug solution. The flow rate was fixed at 1.0 ml/min because of good peak area, satisfactory retention time and resolution. Different ratios of mobile phases were studied, mobile phase with ratio of 55:45 acetonitrile: water was fixed due to good symmetrical peaks and resolution.

CONCLUSION

The present recovery was found to be 98.0-101.50% and was linear and precise over the same range. Both system and method precision was found to be accurate and well within range. Detection limit was found to be 0.57 µg for Aspirin and 0.56 µg for Ethoheptazine Citrate. Linearity study, correlation coefficient and curve fitting was found to be r2 = 0.999. The analytical method showed linearity over the range of 20-80 µg of the target concentration for both the drugs. The analytical method passed both robustness and ruggedness tests. On both cases, relative standard deviation was well satisfactory.

BIBLIOGRAPHY

1. Gurdeep RC, Sham KA. 2006. Instrumental Methods of Analysis, 4th Ed. ShingHanse, Mumbai, PP134-145.
2. http://www.wiziq.com/hmu
3. Hamilton R j, Swell.1984. Introduction to HPLC, 2nd Ed, 2-9.
4. http://www.chromtech.com/ab/in/tec
5. Willard M, Dean S. 1986. Instrumental Methods of analysis, 7th Ed, CBS Publishers and Distributors, New Delhi, PP 622-628.
6. Skoog DA, West DM, Holler FJ. 2000. Fundamentals of Analytical chemistry, 6th Ed, Saunders college Publishing, New York, PP713.
7. Beckett AH, Stenlake JB. 2011. Practical Pharmaceutical Chemistry 4th Ed, part-II, PP85-157.
8. http://www.altech.web.com
9. Snyder R, Kirkland J, Glajch L. 1997. Practical HPLC Method Development, 2nd Ed, Wiley International Publication, PP4-39.
10. International Conference on Harmonization, Validation of analytical procedures; Methodology, Federal Register, Nov. 1996; 1-8.
11. Shah DA, Bhatt K, Mehta RS. 2007. Development and validation of a RPHPLC method for determination of atorvastatin calcium and aspirin in a capsule dosage form. Indian J Pharm Sci, 69; 4: 546-49.
12. Lodhe SV, Deshmukh RS, Mulgund SV.2011. Development and Validation of a Reversed-phase HPLC Method for Simultaneous Determination of Aspirin, Atorvastatin Calcium and Clopidogrel Bisulphate in Capsules. Indian J Pharm Sci, 73; 1: 23-9.
13. Patel D, Vaisya NP.2013. Development and Validation of RP-HPLC Method for Simultaneous Estimation of Aspirin and Esomeprazole Magnesium in Tablet Dosage Form. Journal of Chemistry, 12; 2: 1-10.
14. Boyka GT, Ivanka PP, PlamenTP. 2012 Development and validation of RPHPLC method for simultaneous determination of metoprolol and aspirin in fixed dose combinations. Der Pharma Chemica, 4; 4: 1512-16.
15. RajputRK, George FM, Deepti B. 2008. RuikarChemometric Simultaneous Estimation of Clopidogrel Bisulphate and Aspirin from Combined Dosage Form. Indian J Pharm Sci, 70; 4: 450-54.

16. BasniwalK, ShrivastavaSK. 2008. Concurrent estimation of clopidogrel bisulfate and aspirin in tablets by validated RP-HPLC method. indian j pharm sci, 70; 5: 667-69.
17. Najma S, Muhammad S, Kiran A. 2011. Simultaneous Determination of Clopidogrel and Aspirin by RP-HPLC from Bulk Material and Dosage Formulations Using Multivariate Calibration Technique. Journal of Chromatographic Science, 5; 49: 12-4.
18. Tejaswi P, Avanthi K, Sudheer D. 2013. Simple rp-hplc method for simultaneous estimation of aspirin and rosuvastatin calcium in capsule dosage form. Indo American Journal of Pharmaceutical Research, 12; 5: 45-9.
19. Ghulam M, Shujaat AK, Arham M. 2011. Development of a UVspectrophotometric method for the simultaneous determination of aspirin and paracetamol in tablets. Scientific Research and Essays, 6; 2: 417-21.
20. https://en.wikipedia.org/wiki/Aspirin
21. http://www.drugs.com/aspirin.html
22. http://www.drugbank.ca/drugs/db00945
23. https://en.wikipedia.org/wiki/Ethoheptazine
24. http://pubchem.ncbi.nlm.nih.gov/compound/ethoheptazine#section=Literature