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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 was 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 .

Key words: HPLC, Aspirin and Ethoheptazine citrate.

INTRODUCTION:

Aspirin (BAN, USAN), also known as acetylsalicylic acid it is a salicylate drug, often used as an analgesic to relieve minor aches and pains, as an antipyretic to reduce fever, and as an anti-inflammatory medication. Aspirin also has an antiplatelet effect by inhibiting the production of thromboxane, which under normal circumstances binds platelet molecules together to create a patch over damaged walls of blood vessels. Because the platelet patch can become too large and also block blood flow, locally and downstream, aspirin is also used long-term, at low doses, to help prevent heart attacks, strokes, and blood clot formation in people at high risk of developing blood clots. Also, 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.[1-4] Aspirin may be effective at preventing certain types of cancer, particularly colorectal cancer. IUPAC name of Aspirin is 2-(acetoxy) benzoic acid. Molecular Formula is $C_9H_8O_4$. Molecular Weight is 180.1. g/mol

Ethoheptazine is an opioid analgesic from the phenazepine 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. They close N-type voltage-operated calcium channels and open calcium- dependent inwardly-rectifying potassium channels. This results in hyperpolarization and a reduction in neuronal excitability. They also decrease intracellular cAMP which modulates the release of nociceptive neurotransmitters (e.g. substance P). Inhibition of prostaglandin synthesis by cyclooxygenase is the principal mode of the analgesic and anti-inflammatory actions of NSAIDs. Cyclo-oxygenase is inhibited irreversibly by aspirin and reversibly by other NSAIDs. IUPAC name of Ethyl 1-methyl-4-phenylazepane-4-carboxylate. Molecular formula is $C_{16}H_{23}NO_2$. Molecular Weight is 261.36 g/mol.

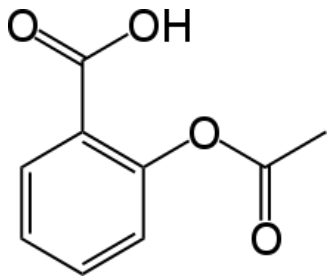

Figure 1: Structure of Aspirin

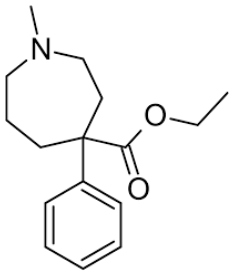

Figure 2: Structure of Ethoheptazine citrate

Literature survey shows that a number of methods have been reported for estimation of Aspirin And Ethoheptazine citrate individually or in combination with other drugs Those are UV ,HPLC.[5-13] However, there is only few HPLC methods are reported for the simultaneous estimation of these drugs in combined dosage forms. I got better results than already published one. The aim of the present study was A New Rp-Hplc Method for Simultaneous Estimation of Aspirin and Ethoheptazine citrate in Its Bulk and Tablet Dosage Form.

MATERIALS AND METHODS:

Chemicals and Reagents: Aspirin and Ethoheptazine citrate were Purchased from Hetero drugs. NaH_2PO_4 was analytical grade supplied by Finerchem limited, Orthophosphoric acid (Merck), and Water and Methanol for HPLC (Lichrosolv (Merck).

Equipment and Chromatographic Conditions: The chromatography was performed on a Waters 2695 HPLC system, equipped with an auto sampler, UV detector and Empower 2 software.

Flow rate	: 1 ml/min
Column	: Inertsil - C18, BDS column
Detector wavelength	: 256 nm
Column temp	: Ambient

Injection volume : 20 µl
Run time : 8 min
Retention time : 2.951 min for Aspirin and 4.195for Ethoheptazine Citrate

Preparation of solutions:

Mobile Phase: Acetonitrile: water in the ratio of (55:45)
Standard 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 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 whatmann filter paper.

Procedure:
Inject 20 µL of the standard, sample into the chromatographic system and measure the areas for Aspirin and Ethoheptazine citrate peaks and calculate the %Assay by using the formulae**.**

RESULTS AND DISCUSSION
METHOD:
The developed chromatographic method was validated for system suitability, linearity accuracy, precision, ruggedness and robustness as per ICH guidelines.
System suitability parameters: To evaluate system suitability parameters such as retention time, tailing factor and USP theoretical plate count, the mobile phase was allowed to flow through the column at a flow rate of 1.0 ml/min to equilibrate the column at ambient temperature. Chromatographic separation was achieved by injecting a volume of 20 µL of standard into Inertsil -BDS C_{18}(250 x 4.6 mm, 5 µ), the mobile phase of composition Mixed buffer: Acetonitrile: water (55:45) was allowed to flow through the column at a flow rate of 1.0 ml per minute. Retention time, tailing factor and USP theoretical plate count of the developed method are shown in table 1,2.

Table 1: System suitability parameters of 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 2: Results for system suitability of 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	-------	-------

Assay of pharmaceutical formulation: The proposed validated method was successfully applied to determine Aspirin and Ethoheptazine citrate in their tablet dosage form. The result obtained for was comparable with the corresponding labeled amounts and they were shown in Table-3.

Table 3: Assay results for Aspirin and Ethoheptazine citrate

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
STDEV	0.827	0.3526
% RSD	0.83	0.35

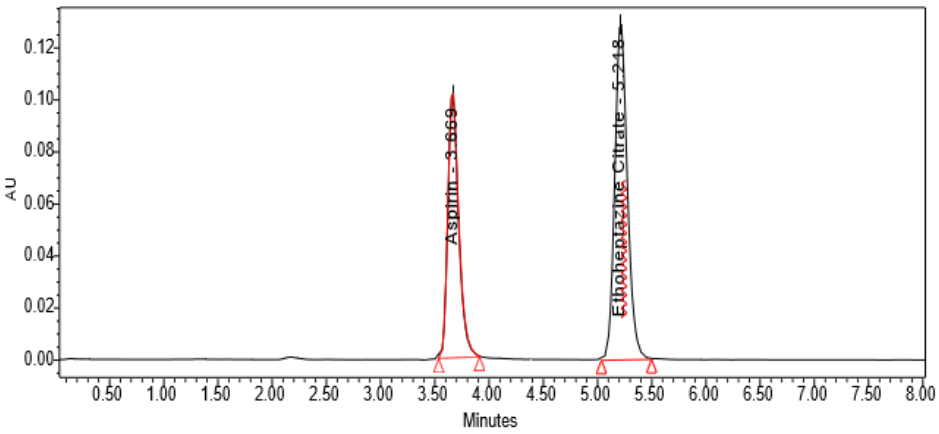

Figure 3: Standard chromatogram

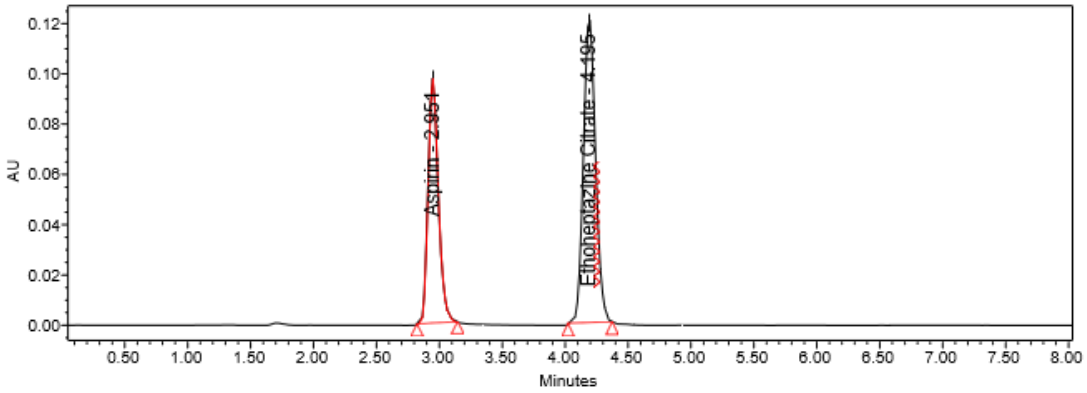

Figure 4: Sample chromatogram

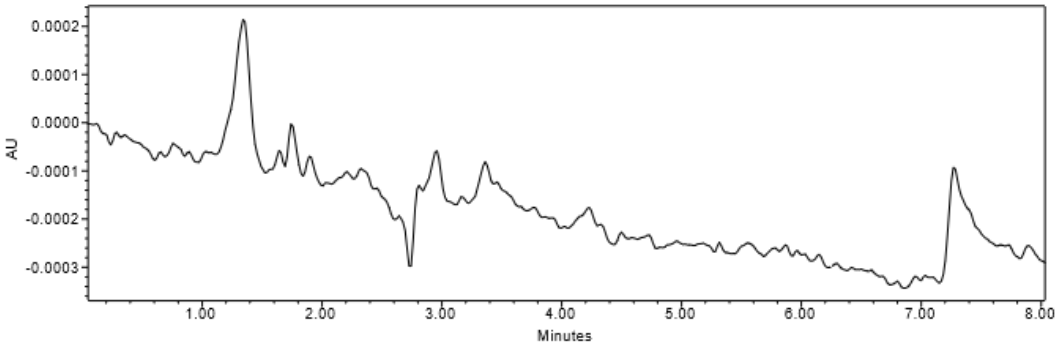

Figure 5: Blank chromatogram

Validation of Analytical method:

Linearity: The linearity study was performed for the concentration of 58.8 µg/ml to 137.2 µg/ml and 61 µg/ml to 142 µg/ml level. Each level was injected into chromatographic system. The area of each level was used for calculation of correlation coefficient. Inject each level into the chromatographic system and measure the peak area. Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient. The resulte are shown in table 4,5.

Table 4: Linearity results of 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		

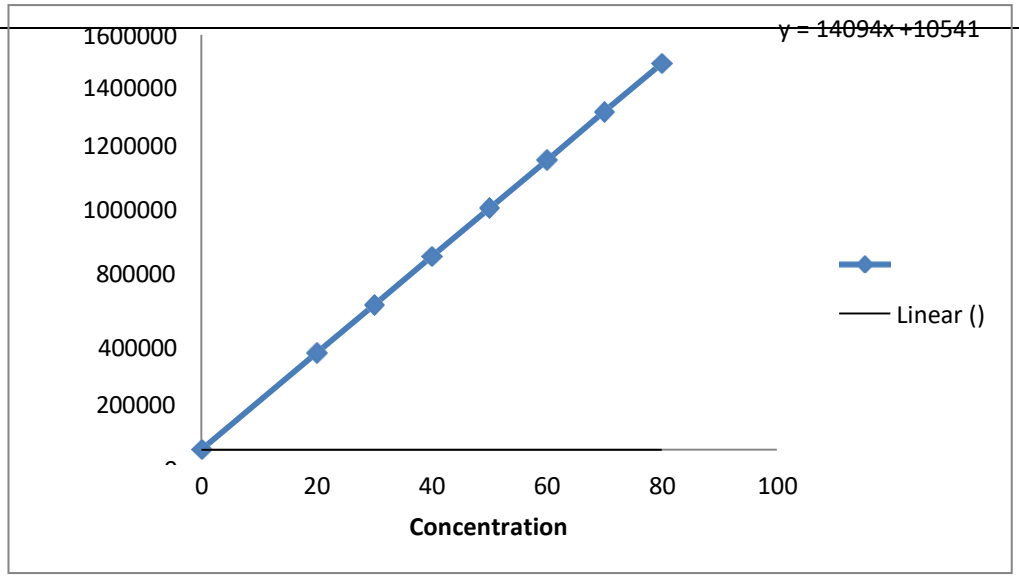

Figure 6: Linearity graph for Aspirin

Table 5: Linearity results of 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		

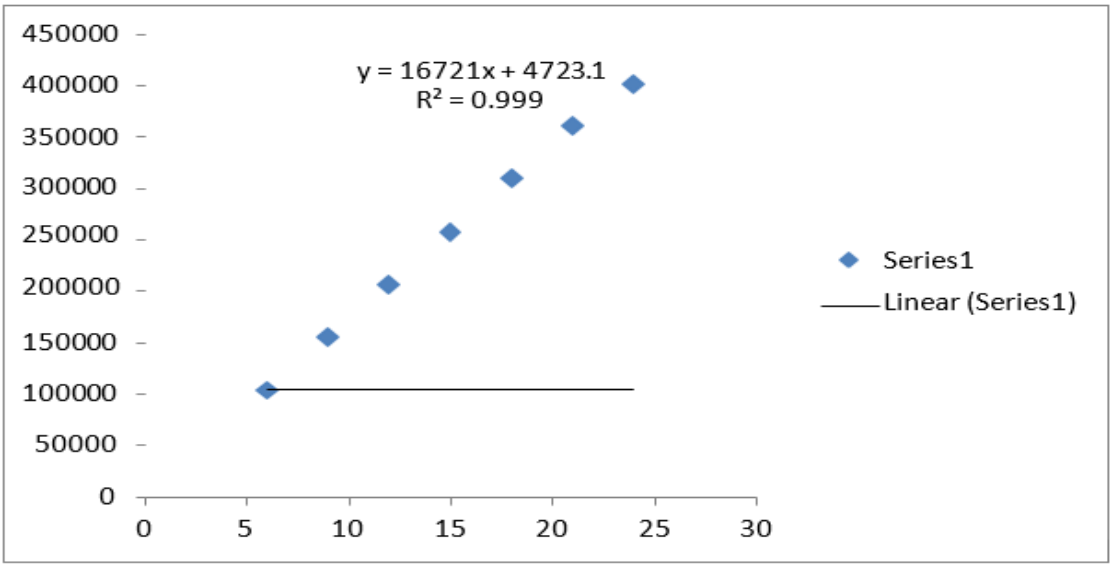

Figure 7: Linearity graph for Ethoheptazine citrate

Accuracy studies: The accuracy was determined by help of recovery study. The recovery method carried out at three level 100%, 120%, 140% and 100%, 120%, 140% Inject the standard solutions into chromatographic system. Calculate the Amount found and Amount added for Aspirin and Ethoheptazine citrate and calculate the individual recovery and mean recovery values. The results are shown in table 6,7.

Table 6: Showing accuracy results for Aspirin

Concentration % of spiked level	Amount added (ppm)	Amount found (ppm)	% Recovery	Statistical Analysis of % Recovery	
50% Injection	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	MEAN	100.02
150% Injection 1	78	78.08	100.1		
150% Injection 2	78	77.97	99.96	%RSD	0.09
150% Injection 3	78	77.98	99.98		

Table 7: Showing accuracy results 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		

Precision Studies: precision was calculated from Coefficient of variance for six replicate injections of the standard. The standard solution was injected for six times and measured the area for all six Injections in HPLC. The %RSD for the area of six replicate injections was found. The results are shown in table 8.

Table 8: Precision results for Aspirin and Ethoheptazine citrate

Injection	Area for Aspirin	Area for Ethoheptazine citrate
Injection-1	733495	202110
Injection-2	735992	203700
Injection-3	739828	201851
Injection-4	739098	202255
Injection-5	748289	203283
Injection-6	731322	202349
Average	738004	202687.6
Standard Deviation	5988.879	771.5483
%RSD	0.81	0.38

Ruggedness: To evaluate the intermediate precision of the method, Precision was performed on Analyst. The standard solution was injected for six times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found. The results are shown in table 9.

Table 9: Ruggedness results of Aspirin and Ethoheptazine citrate

Injection	**Area for Aspirin**	**Area for Ethoheptazine citrate**
Injection-1	98.65	99.98
Injection-2	98.63	99.30
Injection-3	98.86	98.60
Injection-4	98.52	99.30
Injection-5	98.63	98.55
Injection-6	98.55	98.73
Average	98.64	99.07667
Standard Deviation	0.12	0.56
%RSD	98.65	99.98

Robustness: As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition, Temperature Variation was made to evaluate the impact on the method. The flow rate was varied at 0.8 ml/min to 1.2 ml/min. The results are shown in table 10.

Table 10: Flow variation results for Aspirin

	Std Area	Tailing factor		Std Area	Tailing factor		Std Area	Tailing factor
Flow 0.8 ml	1120286	1.32208	Flow 1.0 ml	734322	1.60487	Flow 1.2 ml	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

Flow variation results for Ethoheptazine Citrate

	Std Area	Tailing factor		Std Area	Tailing factor		Std Area	Tailing factor
Flow 0.8 ml	273707	1.36208	Flow 1.0 ml	206349	1.28057	Flow 1.2 ml	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

LOD and LOQ: The sensitivity of RP-HPLC was determined from LOD and LOQ. Which were calculated from the calibration curve using the following equations as per ICH guidelines. The results are shown in table 11.

LOD = 3.3σ/S and

LOQ = 10 σ/S, where

σ= Standard deviation of y intercept of regression line,

S = Slope of the calibration curve

Table 11: LOD, LOQ of Aspirin and Ethoheptazine citrate

Drug	LOD	LOQ
Aspirin	0.345	0.98
Ethoheptazine citrate	0.104	0.329

CONCLUSION:

A simple and selective LC method is described for the determination of Aspirin and Ethoheptazine citrate in tablet dosage forms. 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.

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