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SIMULTANEOUS ESTIMATION OF ACECLOFENAC AND PARACETAMOL BY USING RP-HPLC

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ABSTRACT

A simple, Accurate, precise method was developed for the simultaneous estimation of the Aceclofenac & Paracetmol in solid dosage form. Instrument used is Waters HPLC with auto sampler and UV detector. Chromatogram was run through Symmetry (4.6 x 150mm, 5µm) .Mobile phase containing Methanol: Acetonitrile: 0.1% OPA (40:60) was pumped through column at a flow rate of 1 ml per min. Buffer used in this method was 0.1% OPA at Ambient Temperature. Optimized wavelength for Aceclofenac & Paracetmol was 276nm. Retention time of Aceclofenac & Paracetamol Were found to be 2.360 min, 5.119min And %RSD of method precision for Aceclofenac & Paracetamol were and found to be 1.0, 0.2.% recovery was obtained as 100.13%, 100.04 for Aceclofenac & Paracetamol respectively.

Keywords: Aceclofenac, Paracetmol, Chromatogram, Methanol and Acetonitrile.

INTRODUCTION

High performance liquid chromatography is a very sensitive analytical technique most widely used for quantitative and qualitative analysis of pharmaceuticals. The principle advantage of HPLC compared to classical column chromatography is improved resolution of the separated substance, faster separation times and the increased accuracy, precision and sensitivity. Non-polar stationary phase and polar mobile phase is used here. The majority of the HPLC separation are done with Reversed phase separation, probably over 90%. Aceclofenac is an oral non-steroidal anti-inflammatory drug (NSAID) with marked anti-inflammatory and analgesic properties used to treat osteoarthritis, rheumatoid arthritis and ankylosing spondylitis. Aceclofenac potently inhibits the cyclo-oxygenase enzyme (COX) that is involved in the synthesis of prostaglandins, which are inflammatory mediators that cause pain, swelling, inflammation, and fever. Aceclofenac belongs to BCS Class II as it possesses poor aqueous solubility. It is reported to be highly protein-bound (>99%). Acetaminophen (Paracetamol) is an analgesic drug used alone or in combination with opioids for pain management, and as an antipyretic agent. its ability to inhibit the cyclooxygenase (COX) pathways. Acetaminophen has 88% oral bioavailability and reaches its highest plasma concentration 90 minutes after ingestion.

EXPERIMENTAL METHODOLOGY

MATERIALS AND REAGENTS

Paracetamol and aceclofenac used as working standard. Acetonitrile, orthophsporic acid, Water for HPLC Methanol for HPLC, and sodium hydroxide. other chemicals were analytical and HPLC grade. Tablets were purchased from the Indian market, containing: paracetamol, 500mg; aceclofenac, 100mg.

CHROMATOGRAPHIC CONDITIONS

Instrument used is Waters HPLC with auto sampler and UV detector at a Ambient Temperature Column is Symmetry (4.6 x 150mm, 5µm). The Buffer used is 0.1% OPA at a pH 3.0.The Mobile phase used in the

experimentation with a ratio is Methanol: Acetonitrile: 0.1% OPA (40:60) with a Flow rate of 1 ml/min at a Wavelength of 276 nm. The Injection volume is 20 µl. The Run time for conducting the experimentation is 10 min. The peak purity was checked with the photo- diode array detector. Mobile phase consisted of acetonitrile and buffer (40:60,v/v),buffer containing 0.1% orthophosporic acid. The mobile phases was premixed and filtered and degassed. **(Fig. 1: Chromatogram for Blank, Fig. 2: Chromatogram for system suitability and Table 1: Results of system suitability parameters)**

Assay

Standard and sample solution injected as described under experimental work. The corresponding chromatograms and results are shown below.

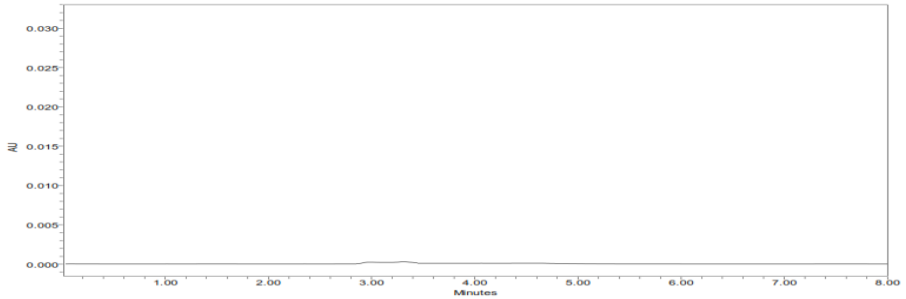

Fig. 1: Chromatogram for Blank

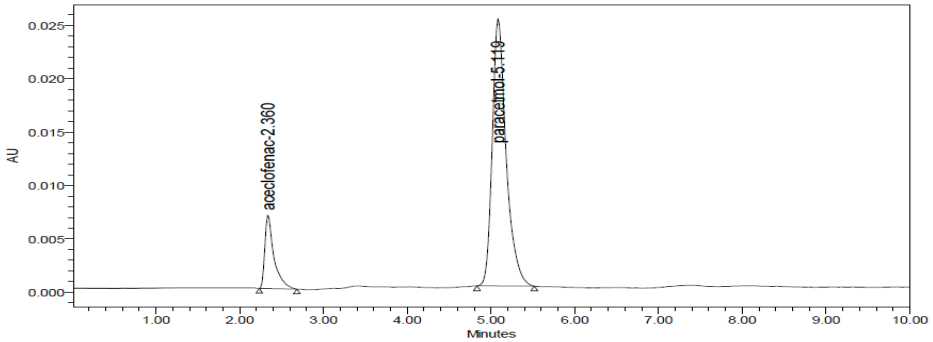

Fig. 2: Chromatogram for system suitability

Table 1: Results of system suitability parameters

S. No.	Name	RT(min)	Area (µV sec)	Height (µV)	USP resolution	USP tailing	USP plate count
1	Aceclofenac	2.360	185937	6819	**NA**	1.24	4264.69
2	Paracetamol	5.119	857262	25306	3.65	1.31	3988.44

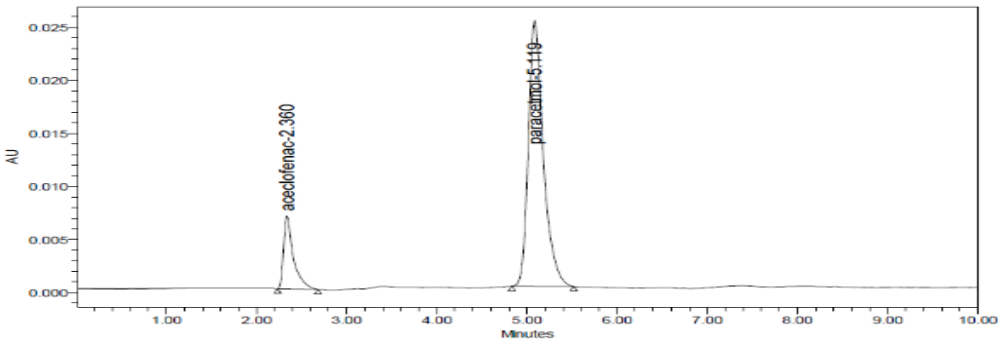

Fig. 3: Chromatogram for standard

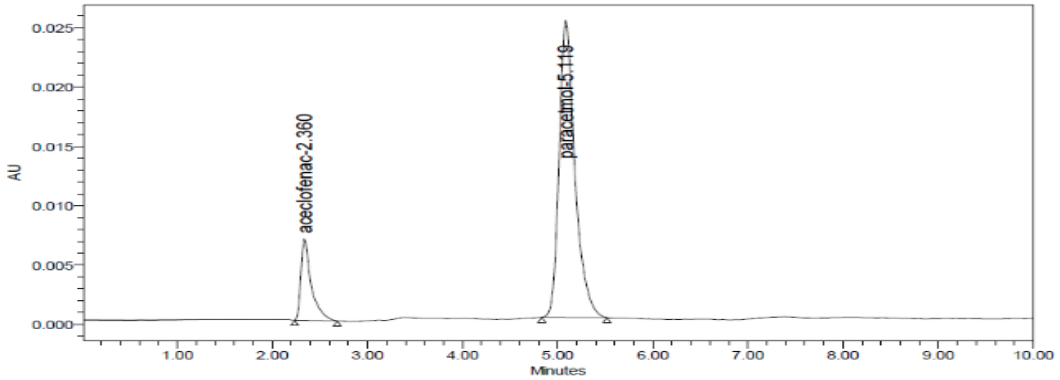

Fig. 4: Chromatogram for sample

Table 2: Results of Assay for Aceclofenac and Paracetamol

	Label Claim (mg)	% Assay
Aceclofenac	100	100.32
Paracetamol	500	99.74

Assay Calculation (Aceclofenac):

$$\%\ \boldsymbol{Assay} = \frac{\mathbf{188581}}{\mathbf{187596.3}} * \frac{\mathbf{30}}{\mathbf{30}} * \frac{\mathbf{99.8}}{\mathbf{100}} * \mathbf{100} = \mathbf{100.32}$$

Assay Calculation (Paracetamol):

$$\%\ \boldsymbol{Assay} = \frac{\mathbf{862916.3}}{\mathbf{863395}} * \frac{\mathbf{150}}{\mathbf{150}} * \frac{\mathbf{99.8}}{\mathbf{100}} * \mathbf{100} = \mathbf{99.74}$$

Preparation of sample solutions

Accurately weigh 10 tablets crush in motor and pestle and transfer equivalent to 100 mg of Aceclofenac and 500 mg of Paracetamol (690 mg of tablet power) sample into a 100 ml clean dry volumetric flask add about 50 mL of diluent and sonicate it up to 30 mins to dissolve it completely and make volume up to the mark with the same solvent. Then it is filtered through 0.45 micron injection filter. (Stock solution) Further pipette 2.5 ml of the above stock solutions into a 25 ml volumetric flask and dilute up to the mark with diluent. Further pipette 3 ml of the above stock solutions into a 10 ml volumetric flask and dilute up to the mark with diluent (30 ppm Aceclofenac and 150 ppm Paracetamol).

Standard solutions

Standard solutions were prepared by dissolving the drugs in the diluents, and they were diluted to the desired concentration. Diluents used for the standard and sample preparations areas follow: diluent A: acetonitrile; diluent B: acetonitrile and buffer.

Accuracy (Recovery test)

Inject the standard solution, Accuracy -50%, Accuracy -100% and Accuracy -150% solutions. Calculate the Amount found and Amount added for Aceclofenac and Paracetamol and calculate the individual recovery and mean recovery values. The % Recovery for each level should be between 98.0 to 102.0%.

Preparations of standard solutions

Paracetamol

125mg of paracetamol was accurately weighed, transferred into 50mL volumetric flask, dissolved with 10mL diluent A, and diluted up to the mark with diluents B (2500 µg/mL).

Aceclofenac:

25 mg of aceclofenac was accurately weighed, transferred into a 50-mL volumetric flask, dissolved with 10mL diluent A, and diluted upto mark with diluents B (500µg/mL).

METHOD VALIDATION SUMMARY

Specificity

For Specificity Blank and Standard are injected into system. There is no any interference of any peak in blank with the retention time of the analytical peaks

Linearity

Accurately weigh and transfer 100 mg of Aceclofenac and 500 mg of Paracetamol working standard into a 100 ml clean dry volumetric flask add about 50 ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.(Stock solution) Further pipette 2.5 ml of the above stock solutions into a 25 ml volumetric flask and dilute up to the mark with diluent.

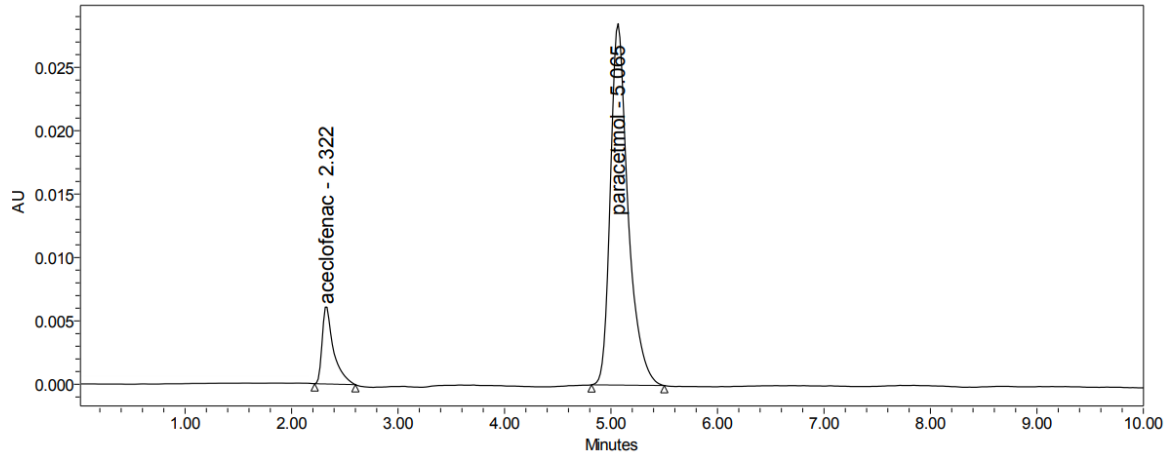

Fig. 5: Chromatogram for linearity

Table 3: Analytical performance parameters of Aceclofenac and Paracetamol

Parameters	Aceclofenac	Paracetamol
Slope (m)	6199.4	5914.4
Intercept (c)	518.29	12000
Correlation coefficient (R^2)	0.999	0.999

Table 4:

Name of the drug	%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
Paracetamol	50%	435103.7	250	251.47	100.59	100.04
	100%	860280	500	497.20	99.44	
	150%	1299030	750	750.78	100.10	
Aceclofenac	50%	93917.7	50	49.96	99.93	100.13
	100%	187375.0	100	99.68	99.68	
	150%	284140.7	150	151.16	100.77	

Table 5: Variation in flow

S. No	Drug	Flow Rate (ml/min)	System Suitability Results	
			USP Tailing	USP Plate Count
1	Aceclofenac	0.9	1.25	3988.49
2	Aceclofenac	1.0	1.24	4264.69
3	Aceclofenac	1.1	1.22	4633.54
4	Paracetamol	0.9	1.31	3874.20
5	Paracetamol	1.0	1.31	3988.44
6	Paracetamol	1.1	1.31	3553.22

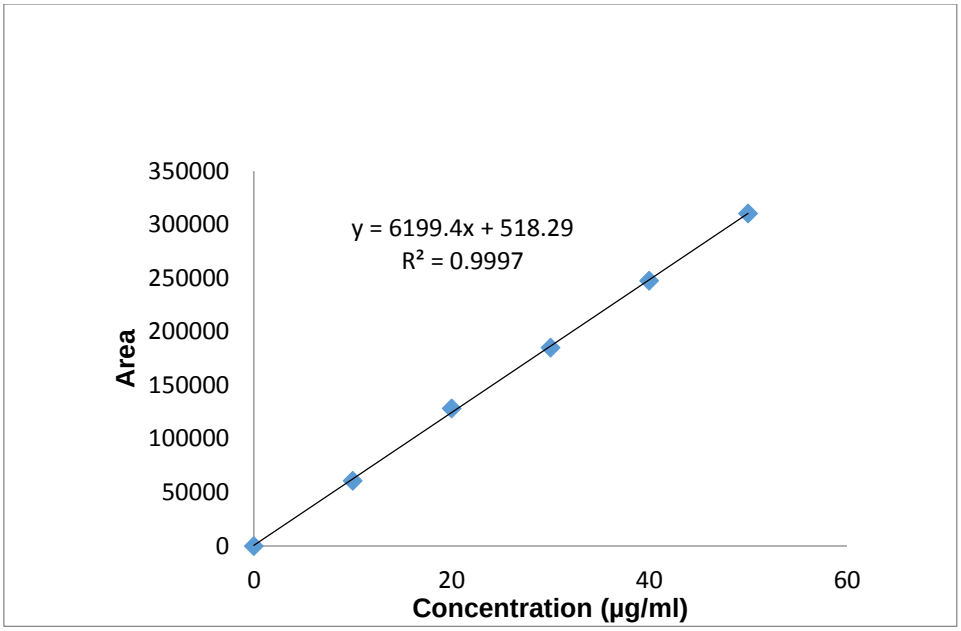

Fig. 6: Calibration graph for Aceclofenac

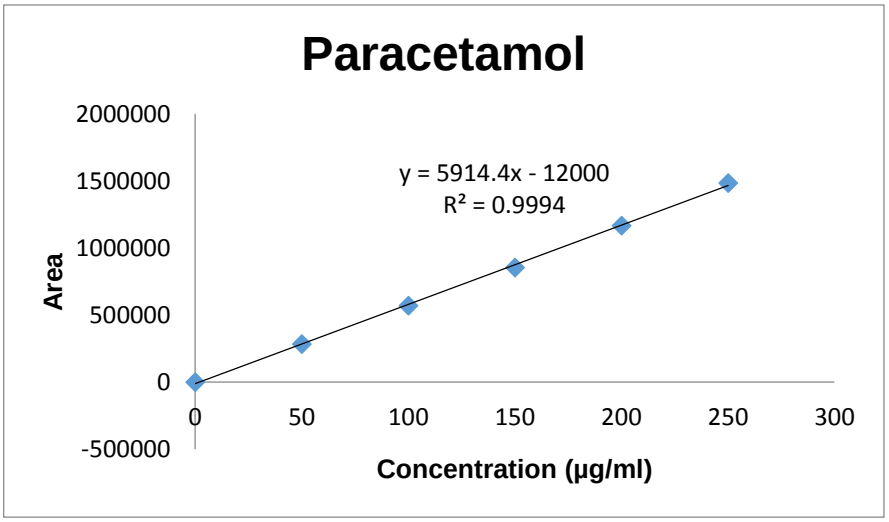

Fig. 7: Calibration graph for Paracetamol

Determination of the limit of detection and quantitation

For determining the limit of detection (LOD) and limit of quantitation(LOQ),the method based on the residual standard deviation of a regression line and slope(12).To determine the LOD and LOQ, a specific calibration curve was studied using samples containing the analytes in the range of LOD and LOQ. The LOD for paracetamol & aceclofenac, were 0.027 µg/mL &0.074 µg/mL and LOQ were 0.081 µg/mL & 0.222 µg/mL respectively.

SUMMARY AND CONCLUSION

The estimation of Aceclofenac and Paracetamol was done by RP-HPLC. The assay of Aceclofenac and Paracetamol was performed with tablets and the % assay was found to be 100.32 and 99.74 which shows that the method is useful for routine analysis. The linearity of Aceclofenac and Paracetamol was found to be linear with a correlation coefficient of 0.999 and 0.999, which shows that the method is capable of producing good sensitivity. This method can be used for the simultaneous Estimation of paracetamol & aceclofenac in pharmaceutical dosage form by using RP-HPLC. The method was validated and shown to be accurate and precise. It can be used in quality control department for the assay and dissolution of tablets containing paracetamol & Aceclofenac.

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