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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF TRAMADOL AND ACECLOFENAC IN COMBINED TABLET DOSAGE FORM

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ABSTRACT

The development and validation of a Reverse-Phase High-Performance Liquid Chromatography (RP-HPLC) method for the simultaneous estimation of tramadol and aceclofenac in combined tablet dosage forms are presented. This method addresses the need for a reliable and efficient analytical technique to quantify both drugs in a single run. The RP-HPLC analysis was conducted using a Waters HPLC system equipped with an auto sampler and a PDA Detector 996 model. The chromatographic separation was achieved using a Symmetry ODS C18 column (4.6 mm × 150 mm, 5 µm particle size) with a mobile phase consisting of methanol and water in a 40:60 (v/v) ratio. The flow rate was set at 1 mL/min, and the detection was performed at a wavelength of 240 nm. The injection volume was 10 µL, and the total run time for each analysis was 7 minutes.

The method was meticulously validated according to ICH guidelines, evaluating parameters such as specificity, linearity, precision, accuracy, and robustness. The developed RP-HPLC method demonstrated excellent resolution and peak symmetry for both tramadol and aceclofenac, with a high degree of accuracy and precision. This method provides a reliable tool for the simultaneous quantification of tramadol and aceclofenac in combined tablet formulations, ensuring quality control and compliance with pharmaceutical standards.

Keywords: RP-HPLC, TRAMADOL, ACECLOFENAC.

1. INTRODUCTION

Tramadol is a antibiotic belonging to the chemical class of pencillins derivatives Chemically it is (2S,5R,6R)-6- [3-(2,6-dichlorophenyl)-5-methyl-1,2-oxazole-4-amido]- 3,3- dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane2-carboxylic acid.1-3 Tramadol exerts a bactericidal action against penicillin-susceptible microorganisms during the state of active multiplication. All penicillins inhibit the biosynthesis of the bacterial cell wall. By binding to specific penicillin-binding proteins (PBPs) located inside the bacterial cell wall, Tramadol inhibits the third and last stage of bacterial cell wall synthesis. Cell lysis is then mediated by bacterial cell wall autolytic enzymes such as autolysins; it is possible that Tramadol interferes with an autolysin inhibitor. Tramadol and Aceclofenac are formulated together in the form of a tablet. Literature survey revealed no method reported for simultaneous determination of the two drugs. The present RP-HPLC method uses simple mobile phase ratio, higher sensitivity and analysis will complete before 7 min. Therefore the present study was to determine both drugs concurrently by sensitive, accurate, rapid and precise RP-HPLC4-9 method for routine analysis.

2. METHODOLOGY

Preparation of standard solution:

Accurately weigh and transfer 10 mg of Tramadol and Aceclofenac working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol.

Further pipette 1.0ml of the above Tramadol and 0.5ml of the Aceclofenac stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

2.1. Procedure:

Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

2.2. Mobile Phase Optimization:

Initially the mobile phase tried was Methanol: Water and Water: Acetonitrile and Methanol: TEA Buffer: ACN with varying proportions. Finally, the mobile phase was optimized to Acetonitrile: Water in proportion 40:60v/v respectively.

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2.3. Optimization of Column:

The method was performed with various columns like C18 column, Symmetry and Zodiac column. Symmetry ODS C18 (4.6mm×150mm, 5µ Particle Size) was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

2.4. METHOD VALIDATION PARAMETERS

2.4.1. SYSTEM SUITABILITY

Accurately weigh and transfer 10 mg of Tramadol and 10mg of Aceclofenac working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 1.0ml of the above Tramadol and 0.5ml of the Aceclofenac stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

2.4.2. SPECIFICITY STUDY OF DRUG:

Preparation of Standard Solution:

Accurately weigh and transfer 10mg of Tramadol and 10mg of Aceclofenac working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 1.0ml of the above Tramadol and 0.5ml of the Aceclofenac stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

Preparation of Sample Solution:

Take average weight of one Tablet and crush in a mortar by using pestle and weight 10 mg equivalent weight of Tramadol and Aceclofenac sample into a 10mL clean dry volumetric flask and add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Further pipette 1.0ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

Inject the three replicate injections of standard and sample solutions and calculate the assay by using formula:

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

2.4.3. PREPARATION OF DRUG SOLUTIONS FOR LINEARITY:

Accurately weigh and transfer 10 mg of Tramadol and 10mg of Aceclofenac working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Preparation of Level – I (20 ppm of Tramadol & 30 ppm of Aceclofenac):

Pipette out 0.6ml of Tramadol and 0.3ml of Aceclofenac stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – II (40 ppm of Tramadol & 40 ppm of Aceclofenac):

Pipette out 0.8ml of Tramadol and 0.4ml of Aceclofenac stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – III (60 ppm of Tramadol & 50 ppm of Aceclofenac):

Pipette out 1.0ml of Tramadol and 0.5ml of Aceclofenac stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – IV (80 ppm of Tramadol & 60 ppm of Aceclofenac):

Pipette out 1.20ml of Tramadol and 0.6ml of Aceclofenac stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – V (100 ppm of Tramadol & 70ppm of Aceclofenac):

Pipette out 1.40ml of Tramadol and 0.7ml of Aceclofenac stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

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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.

2.4.4. PRECISION REPEATABILITY

Accurately weigh and transfer 10 mg of Tramadol and 10mg of Aceclofenac working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 1.0ml of the above Tramadol and 0.5ml of the Aceclofenac stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

2.4.5. INTERMEDIATE PRECISION:

To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different days by maintaining same conditions.

Procedure:

DAY 1:

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 to be within the specified limits.

DAY 2:

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 to be within the specified limits.

Accuracy:

For preparation of 50% Standard stock solution:

Accurately weigh and transfer 10 mg of Tramadol and 10mg of Aceclofenac working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.5ml of the above Tramadol and 0.25ml of the Aceclofenac stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

2.4.6.ROBUSTNESS:

Accurately weigh and transfer 10 mg of Tramadol and 10mg of Aceclofenac working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)**.** Further pipette 1.0ml of the above Tramadol and 0.5ml of the Aceclofenac stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

Effect of Variation of flow conditions:

The sample was analyzed at 0.9 ml/min and 1.1 ml/min instead of 1ml/min, remaining conditions are same. 10µl of the above sample was injected and chromatograms were recorded.

Effect of Variation of mobile phase organic composition:

The sample was analyzed by variation of mobile phase i.e. Methanol: Water was taken in the ratio and 47:53, 37:63 instead (40:60), remaining conditions are same. 10µl of the above sample was injected and chromatograms were recorded.

3. RESULTS AND DISCUSSION

3.1. System suitability for Tramadol

Table 1. system suitability for Tramadol

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Tramadol	2.117	658658	67854	6895	1.06
2	Tramadol	2.118	657893	67582	6847	1.07
3	Tramadol	2.116	658985	67895	6875	1.06
4	Tramadol	2.109	659863	67852	6845	1.06
5	Tramadol	2.102	658784	67456	6865	1.07

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Mean			658836.6			
Std. Dev			707.2067			
% RSD			0.107342			

Acceptance criteria:

- %RSD of five different sample solutions should not more than 2.
- The %RSD obtained is within the limit, hence the method is suitable.

Table 2. system suitability for Aceclofenac

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Aceclofenac	3.547	8658485	845250	8542	1.18	4.65
2	Aceclofenac	3.539	8695847	847584	8574	1.19	4.66
3	Aceclofenac	3.547	8657474	847612	8569	1.18	4.65
4	Aceclofenac	3.565	8625698	846985	8532	1.18	4.65
5	Aceclofenac	3.537	8675842	847526	8541	1.19	4.66
Mean			8662669				
Std. Dev			25911.66				
% RSD			0.299119				

Acceptance Criteria:

- %RSD for sample should be NMT 2.
- The %RSD for the standard solution is below 1, which is within the limits hence method is precise.

3.2. SPECIFICITY

The ICH documents define specificity as the ability to assess unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products, and matrix components. Analytical method was tested for specificity to measure accurately quantitate Tramadol and Aceclofenac in drug product.

Table 3. Peak Results for Assay Standard

S.No.	Name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count	Injection
1	Tramadol	2.102	658985	67854		1.06	6859	1
2	Aceclofenac	3.537	8659852	845798	4.68	1.18	8643	1
3	Tramadol	2.105	657542	67259		1.07	6874	2
4	Aceclofenac	3.552	8652874	846354	4.69	1.19	8596	2
5	Tramadol	2.112	658935	67823		1.06	6982	3
6	Aceclofenac	3.560	8659875	849653	4.68	1.18	8569	3

3.3. LINEARITY

Table 4: Linearity study of Tramadol:

Concentration µg/ml	Average Peak Area
20	395687
40	523568
60	659748
80	791286
100	927987

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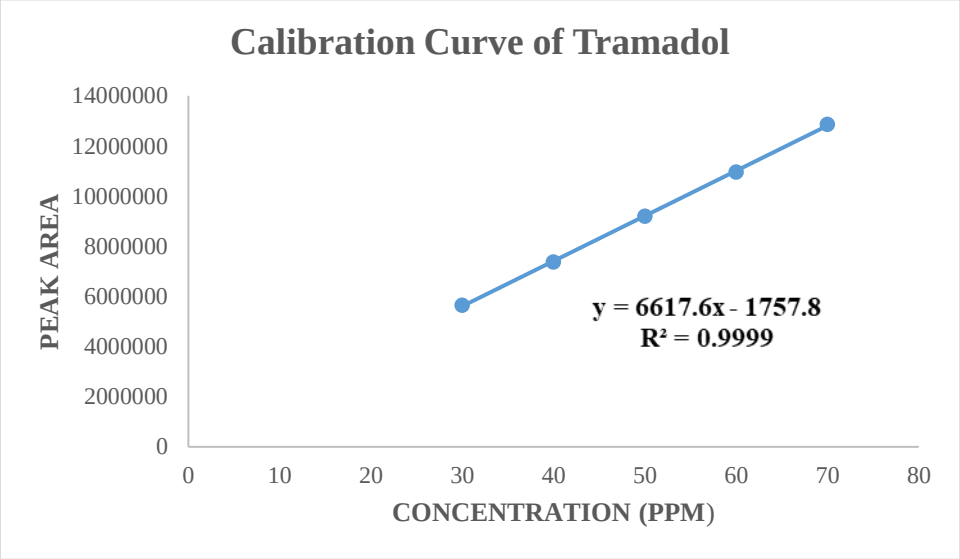

Figure1: Calibration graph for Tramadol

Table 4: Linearity study of Aceclofenac

Concentration µg/ml	Average Peak Area
30	5648983
40	7379854
50	9195825
60	10965984
70	12858656

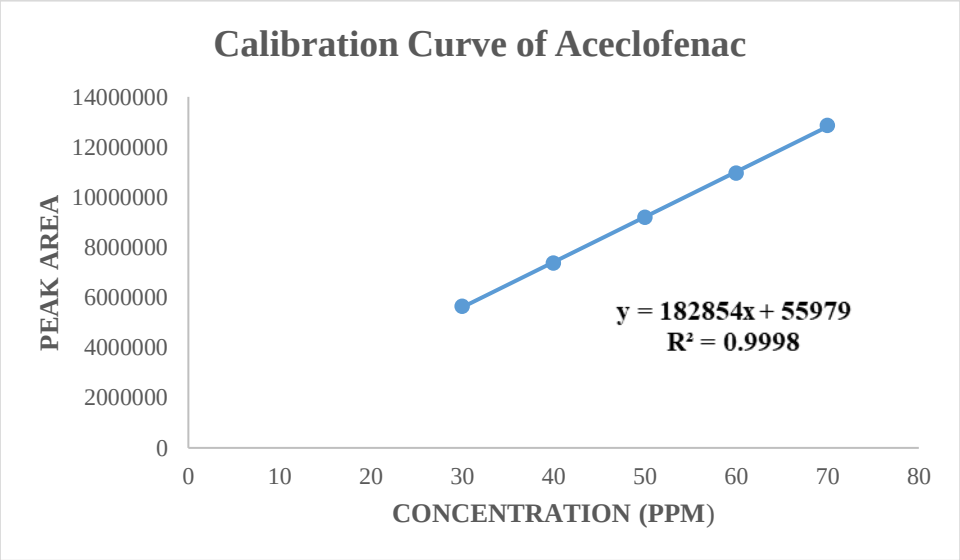

Figure2: Calibration graph for Aceclofenac

Table 5: Results of repeatability for Tramadol

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Tramadol	2.108	654879	67598	6825	1.06
2	Tramadol	2.105	658498	67259	6849	1.06
3	Tramadol	2.113	653593	67254	6826	1.07
4	Tramadol	2.109	654854	67369	6879	1.06
5	Tramadol	2.109	659852	67458	6845	1.07

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Mean			656335.2			
Std. Dev			2686.993			
% RSD			0.409393			

Table-6: Results of method precision for Aceclofenac:

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Aceclofenac	3.552	8659854	845865	8569	1.19
2	Aceclofenac	3.550	8645985	845798	8575	1.18
3	Aceclofenac	3.564	8657494	847584	8597	1.19
4	Aceclofenac	3.564	8659873	847592	8549	1.18
5	Aceclofenac	3.565	8659874	845685	8543	1.19
Mean			8656616			
Std. Dev			6031.092			
% RSD			0.06967			

3.4. ACCURACY:

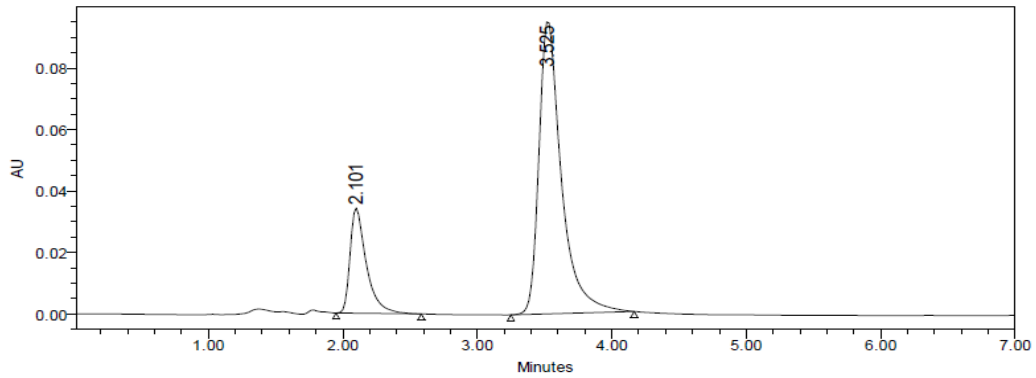

Fig-3: Chromatogram showing accuracy-50% injection

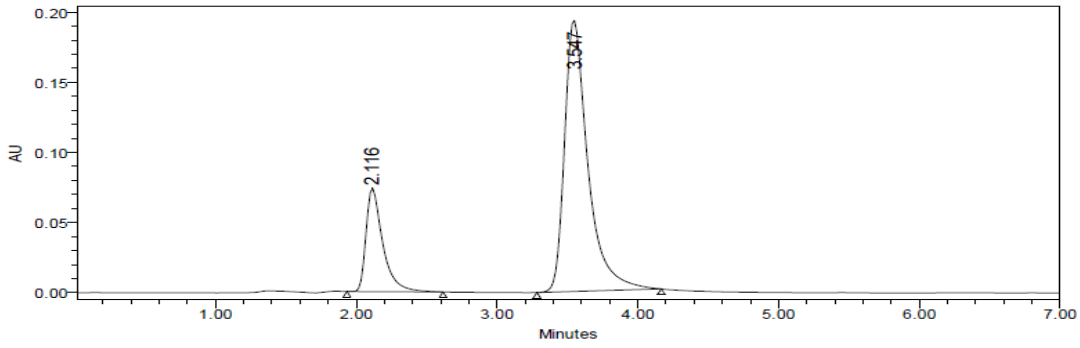

Fig 4: Chromatogram showing accuracy-100% injection

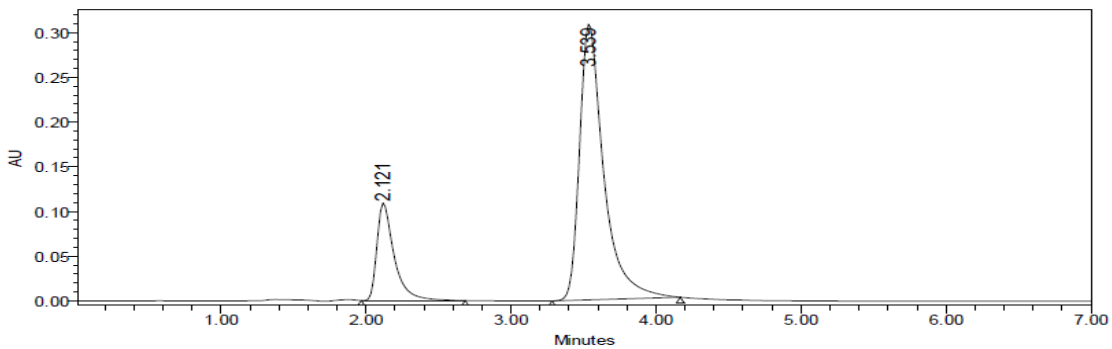

Fig-5: Chromatogram showing accuracy-150% injection

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3.5. LIMIT OF DETECTION

Tramadol:=0.96µg/ml

Aceclofenac:=2.5µg/ml

3.6. LIMIT OF QUANTITATION

Tramadol:= 2.88µg/ml

Aceclofenac= 7.5µg/ml

3.7. ROBUSTNESS

Table7-: Results for tramadol Robustness

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	658748	2.118	6852	1.06
Less Flow rate of 0.9 mL/min	725416	2.330	6985	1.05
More Flow rate of 1.1 mL/min	648514	1.950	6548	1.02
Less organic phase	635254	2.290	6354	1.03
More organic phase	625098	1.998	6487	1.04

Table 8-: Results for Acceclofenac Robustness

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	8695825	3.539	8548	1.18
Less Flow rate of 0.9 mL/min	9145487	3.885	8785	1.17
More Flow rate of 1.1 mL/min	8524583	3.263	8256	1.16
Less organic phase	8245147	4.435	8461	1.14
More organic phase	8365876	3.009	8199	1.15

4. CONCLUSION

The RP-HPLC method developed and validated in this study offers a robust and precise analytical solution for the simultaneous estimation of tramadol and aceclofenac in combined tablet dosage forms. By employing a Symmetry ODS C18 column and a well-defined mobile phase composition, the method achieves effective separation and accurate quantification of both drugs within a concise analysis time of 7 minutes. The thorough validation, according to ICH guidelines, ensures the method's reliability, accuracy, and precision. This method is suitable for routine quality control in pharmaceutical manufacturing and quality assurance processes, facilitating compliance with industry standards and ensuring the efficacy and safety of combined tramadol and aceclofenac formulations.

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