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RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR OF PARACETAMOL & ZALTOPROFEN IN PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

In this investigation, we tried developing a precise and reliable stability assay method for paracetamol and Zaltoprofen simultaneous measurement in API and pharmaceutical dosage forms using RP-HPLC. To obtain adequate separation, an Agilent TC C18 column was used, and a PDA detector was used for the study. 0.2% Triethylamine: Acetonitrile (50:50 v/v) mixture, flowing at 1 milliliter per minute. Using a diode array detector, the eluent at 243 nm was observed. The retention time of paracetamol is 3.0 while that of Zaltoprofen is 5.2. With a correlation coefficient better than 0.99, the measured signal was demonstrated to be precise, accurate, and linear over the concentration range evaluated. The current method's accuracy was assessed at 80%, 100%, and 120%. Recovery rates for both paracetamol and Zaltoprofen is 100.2% and 98.93%, respectively, the HPL.C. Method for determining the assay of Paracetamol and Zaltoprofen is correct. There was determined to be a precision RSD within range. The RP-HPLC approach may be successfully employed for the simultaneous measurement of Paracetamol and Zaltoprofen in their formulation, as demonstrated by the results from the preceding data.

Keywords: RP-HPLC, Zaltoprofen, Paracetamol, PDA detectors

INTRODUCTION

A crucial step in guaranteeing the safety and efficacy of pharmaceuticals is the development and validation of dosage forms. The analytical method's suitability for its intended use is guaranteed by validation. Typically, it includes the following elements: Accuracy that indicates how closely the measured value matches the actual value. Measurements of precision were made under various circumstances, including different days and analysts. The method's specificity refers to its capacity to quantify the analyte in the presence of other substances. The capacity of the procedure to yield findings that are exactly proportionate to the analyte concentration is referred to as linearity. Range interval: the range of analyte concentrations that the technology can reliably measure, from the highest to the lowest. Robustness is the ability of the method to withstand changes by making gradual, intentional adjustments to the method's parameters, such as the flow rate and stability, which yield reliable findings over time. Each of these factors guarantees the precision, accuracy, and dependability of the pharmaceutical dosage analytical method that has been developed and validated **[1-5]**.

A non-steroidal anti-inflammatory medicine (NSAID) called Zaltoprofen is used to treat inflammation and pain. It is especially well-known for helping to control rheumatoid arthritis and osteoarthritis symptoms. It functions by blocking inflammation-causing enzymes, just as other NSAIDs, which helps lessen pain and swelling. Propionic acid, a COX-2 prefential inhibitor, is a member of the NSAID medication class. It inhibits the action of prostaglandin production by blocking COX in the arachidonic acid metabolic pathway **[6-9]**.

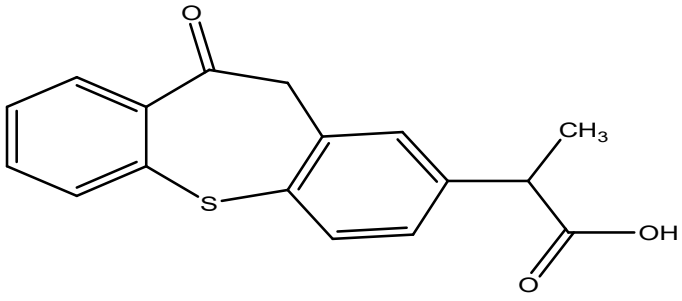

Figure 1: Structure of Zaltoprofen

Paracetamol is also known as acetaminophen. It's a common over-the-counter medication used to relieve pain and reduce fever. It's often used for headaches, muscle aches, arthritis, backaches, toothaches, colds, and fevers. Paracetamol predominantly act by inhibiting the prostaglandin synthesis in the CNS **[10-14]**.

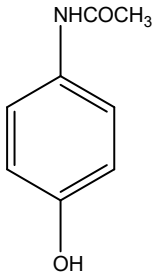

Figure 2: Structure of Paracetamol

The principal objective of this endeavour is to develop and authenticate a Reverse Phase-High Performance Liquid Chromatography technique that will enable adherence to the official requirements for ascertaining the solid dosage form of Paracetamol and Zaltoprofen **[15-16]**.

METHOD & DEVLOPMENT

Analytical & Software:-

An Agilent 1260 Infinity II with an Agilent 5 TC-C18 (2) 250 × 4.6mm column was used for the chromatography. PDA detector was used. The mobile phase contained 0.2% triethylamine: Acetonitrile (50:50 v/v) and had a flow rate increased to 1 milliliter per minute. For the detection, a 50 µl injection volume and a wavelength of 243 nm were selected.

Reagent & Chemicals:-

For this investigation, HPLC grade solvents from Research Lab, including triethylamine, o-phosphoric acid, acetonitrile, and isopropyl alcohol, were employed. Paracetamol and Zaltoprofen active pharmaceutical ingredient (API) was procured from Aeon Formulation in Chennai.

Preparation of Mobile Phase:-

In order to prepare the mobile phase, 1 ml of triethylamine was combined with 500 ml of HPLC grade water. Orthophospharic acid is added to adjust pH 5 and acetonitrile were added in a 50:50 v/v ratio, and the mixture was well mixed. The mobile phase was then filtered using what's man filter paper no. 41 and degassed using sonication for approximately 10 minutes.

Chromatographic Condition:-

For chromatographic resolution and wavelength detection at 243 nm, an Agilent 5 TC-C18 (2) 250 × 4.6mm column was used with an Agilent 1260 Infinity II.0.2% triethylamine: Acetonitrile (50:50) v/v was employed in the mobile phase. By injecting 50 µl and adjusting the flow rate to 1 ml/min, the elution was measured.

Preparation of Standered Solution:-

Weigh 20 mg of Paracetamol and 5 mg of Zaltoprofen transferred it into different 100 ml volumetric flask. Then add the diluent 60 ml and sonicated it for 10 min. After sonication adjust the volume up to the mark of volumetric flask. Then pipette out 10 ml from each flask and transferred into same 100 ml volumetric flask and adjust the volume up to the mark of volumetric flask.

RESULT & DISCUSSION

Optimization of Chromatographic Condition:-

To maximize chromatographic conditions, the effects of chromatographic parameters including mobile phase ratio, mobile phase composition, and flow rate were investigated. Following the recording of the resulting chromatograms, chromatographic properties such as tailing factor, capacity factor, and theoretical plate count were calculated. Finally, 0.2% Triethylamine: Acetonitrile (50:50) was used to generate a simple and affordable method. A list of optimal chromatographic conditions can be found in **Table 1**.

Method Validation:-

Linearity & Range:-

The calibration curve was constructed by plotting concentrations versus peak areas, and the regression equations were calculated **(Figure 3, 4, 5)**.

The findings demonstrate that every system suitability parameter satisfies the requirements for acceptance. The reaction is determined to be linear, according to the results. Since the correlation coefficient is more than 0.99, the procedure is linear in the tested range, which is 12.5 PPM-27.5 PPM for Paracetamol and 3 PPM-7 PPM for Zaltoprofen.

Precision:-

The precision of an analytical method is defined as the degree of agreement between a set of measurements obtained by serial sampling of the same homogenous sample under the necessary conditions. A sample was injected six times in duplicate. The relative standard deviation, or RSD, as a percentage of the results was calculated **(Table 2)**.

The RP-HPLC method for determining paracetamol and Zaltoprofen is exact because its percentage RSD is 1.44% for Zaltoprofen and 1.62% for paracetamol, respectively.

Accuracy:-

The correctness of an analytical method expresses the degree of agreement between True value and Found value. The results of the accuracy test indicate that the procedure's accuracy is within allowable limitations. **Tables 3 and 4** demonstrate the accuracy of the HPLC method in determining the assay of Paracetamol and Zaltoprofen since the percentage RSD of paracetamol 0.01and recovery 100.2 and for Zaltoprofen the percentage RSD 0.02 and recovery these two medications are 0.02 and 0.01 and & mean recovery 98 respectively.

Robustness:-

It is the ability to stay constant despite slight but intentional changes to the parameters of the procedure. The method's robustness was confirmed by purposefully changing the instrumental condition by a flow rate of (+) 0.4. Robustness testing was done for flow

rate changes. Below is a report on the analysis **(Table 5)**.

Every adjusted condition's difference from the original condition stays within the allowed range. Consequently, the approach is reliable.

Stability of Analytical Solutions:

After the study of stability study it found that both Standard and sample solutions are stable for 24 hrs at room temperature **(Table 6)**.

Absolute variation in the assay of the active components in the sample solution should not exceed 2.0% relative to the initial assay result, according to the acceptance criterion. Thus, at room temperature, we may state that the standard and sample solutions are stable for a 24 hrs.

Assay Analysis:-

The % Assay of Paracetamol & Zaltoprofen from the tablet was 102.50 and 99.32 respectively. As per IP, NLT 97% and NMT 103%. The % Assay of paracetamol & Zaltoprofen from the tablet was 102.50 and 99.32 respectively **(Table 7)**.

Table 1: Chromatographic condition

Sr. No	Parameters	Condition
1	HPLC system	Agilent 5 TC
2	Column	C18
3	Mobile phase	0.2%Tea (PH- 5): Acetonitrile (50:50)
4	Flow rate	1.0ml/min
5	Injection volume	10
6	Detection wavelength	243 nm
7	Detector	PDA
8	Column temp	Room temp
9	Sample size	50 µL

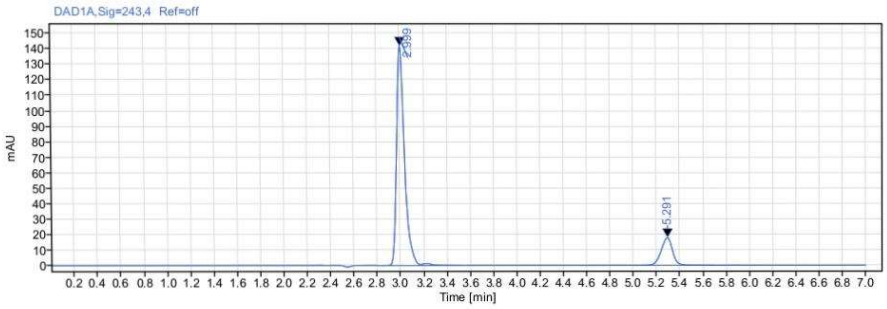

Figure 3: Chromatogram of Paracetamol & Zaltoprofen

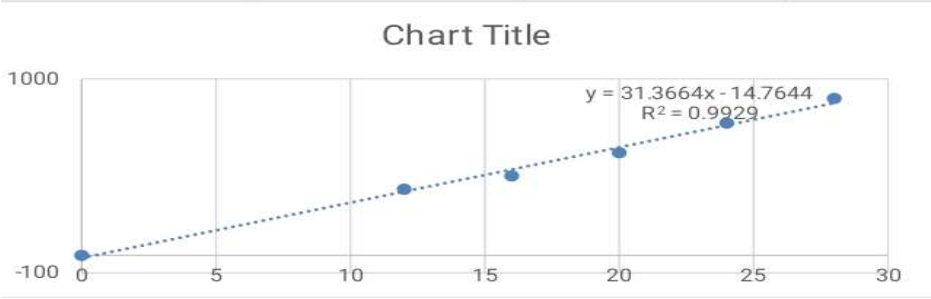

Figure 4: Linearity graph of Paracetamol

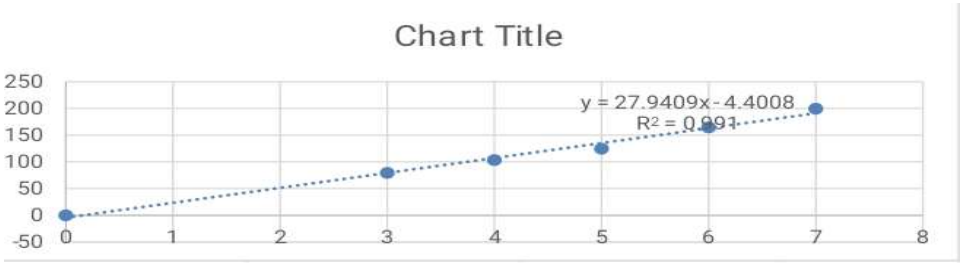

Figure 5: Linearity graph of Zaltoprofen

Table 2: Data sheet for Precision

	Sample area			% Assay	
Sr. No.	Para	Zalto		Para	Zalto
1.	751.53	133.33		101.65	99.23
2.	702.74	130.09		101.34	99.94
3.	734.08	136.95		97.41	101.08
4.	646.24	120.65		100.03	102.93
5.	715.09	125.59		98.87	100.91
6.	694.57	127.537		98.99	102.65
		Average		99.714	101.125
		SD		1.62	1.46
		% RSD		1.62	1.44

Table 3: Data Sheet for Accuracy of Paracetamol

%Level	Set	Area	Amount added mg	Amount recovery	% recovery	Mean	SD	%RSD
80%	1	577.94	16.5	16.92	102.56	98.75	3.30	0.03
	2	515.88	15.6	15.10	96.82			
	3	522.74	15.8	15.31	96.87			
100%	1	647.53	19.5	18.96	97.23	100.70	3.00	0.03
	2	725.19	20.7	21.23	102.58			
	3	723.04	20.7	21.17	102.28			
120%	1	814.22	24	23.84	99.34	101.26	1.67	0.01
	2	854.74	24.5	25.03	102.15			
	3	855.92	24.5	25.06	102.29			

Table 4: Data Sheet for Accuracy of Zaltoprofen

% Level	Set	Area	Amount added mg	Amount recovery	%recovery	Mean	SD	%RSD
80%	1	100.39	4	4.02	100.6	99.04	2.22	0.02
	2	108.38	4.5	4.34	96.49			
	3	94.92	3.8	3.80	100.08			
100%	1	119.75	4.9	4.80	97.91	97.42	0.85	0.01
	2	114.86	4.7	4.60	97.91			
	3	113.13	4.7	4.53	96.43			
120%	1	151.96	6	6.08	101.47	100.35	2.20	0.02
	2	153.80	6.3	6.16	97.81			
	3	154.92	6.1	6.20	101.79			

Table 5: Data sheet for Robustness (Paracetamol & Zaltoprofen)

	0.8 ml / min		1.2 ml / min	
Sr. No.	Area of Paracetamol	Area of Zaltoprofen	Area of Paracetamol	Area of Zaltoprofen
1	1107.606	203.178	687.674	120.433
2	1059.266	196.479	686.594	121.143
3	1070.781	195.323	677.477	120.051
4	1082.231	195.333	699.911	124.54
5	1097.126	197.347	677.788	120.109
Avg	1083.402	195.532	685.888	121.255
SD	19.476	3.268	9.174	1.886
%RSD	1.79	1.65	1.34	1.56

Table 6: Solution stability data for Paracetamol and Zaltoprofen

Sr. No	Standard solution in hrs.	Area		Assay		% relative change		%absolute Change	
		Para	Zalto	Para	Zalto	Para	Zalto	Para	Zalto
1	0 hrs	764.874	141.891	NA	NA	0.3	1.07		
2	24 hrs	762.132	143.437	NA	NA				
3	0 hrs Sample	715.088	125.588	101.65	99.23			2.08	1.77
4	24 hrs Sample	729.697	127.829	103.73	101.00				

Table 7: Assay Analysis

Parameter	Paracetamol	Zaltoprofen
Sample area	760.181	132.517
% Assay	102.505	99.327

DISCUSSION

RP-HPLC was employed to examine the formulation including Paracetamol and Zaltoprofen. These procedures are validated by their precision, accuracy, robustness, linearity, and scope. These methods yield results that are exact and accurate. Because these procedures necessitate calculation on the part of the analyst, they are less time consuming, stable, sensitive, robust, accurate, and exact. Methods are validated based on their accuracy, precision, specificity, robustness, linearity, and range. The results of this process are exact and reliable. This approach is more accurate, precise, and takes less time.

CONCLUSION

Due to its ease of use, sensitivity, and ability to analyze complex samples, RP-HPLC has gained significant traction in the analysis community. In the current study, this technique was used for the simultaneous estimation of paracetamol and Zaltoprofen in tablet dosage form using Agilent HPLC with open lab CDS software and an Agilent 5TC C18 column. The study used a DAD detector. 0.2%TEA: Acetonitrile (50%:50 v/v) was the mobile phase that was determined to be appropriate, and 243 nm was the chosen wavelength. With a correlation coefficient better than 0.99, the measured signal was demonstrated to be precise, accurate, and linear over the concentration range evaluated (60-140 ug/ml). Furthermore, the reduced solvent usage results in a chromatographic process that is both economical and environmentally sustainable. The precision mean recovery is within the bounds of the per cent RSD. The current method's accuracy was assessed at 80%, 100%, and 120%. Since the recoveries for Zaltoprofen and Paracetamol are 100.2% and 98.93%, respectively, the HPL.C. Method for determining the assay of Zaltoprofen and Paracetamol injection is accurate. There was determined to be a precision RSD within range. The RP-HPLC approach may be successfully employed for the simultaneous measurement of Zaltoprofen and Paracetamol in their formulation, as demonstrated by the results from the preceding data. We thus come to

the conclusion that the devised RP-HPLC validation method for the simultaneous estimation of Zaltoprofen and Paracetamol in combination bulk (API) and dosage form is sound, efficient, straightforward, specific, linear, accurate, exact, and robust.

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