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ANALYTICAL METHOD DEVELOPMENT FOR SIMULTANEOUS DETERMINATION OF DICLOFENAC SODIUM AND ACETAMINOPHEN IN A FORMULATION

Poornima D. Potphode[1] and Dr. Nutan Rao*

[1]Oriental College of Pharmacy, Sanpada. (Affiliated to University of Mumbai).

*Associate Professor, Department of Pharmaceutical Chemistry and Quality Assurance, Oriental College of Pharmacy, Sanpada.

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***Corresponding Author**
Dr. Nutan Rao
Associate Professor, Department of Pharmaceutical Chemistry and Quality Assurance, Oriental College of Pharmacy, Sanpada.

ABSTRACT

A simple, specific, accurate and precise reverse phase high performance liquid chromatographic method was developed and validated for the estimation of Diclofenac Sodium and Acetaminophen in a formulation. An Hypersil, Octadecylsilane, 5µm column having 250 x 4.6mm internal diameter in isocratic mode with mobile phase containing Methanol: Water: Acetonitrile: Glacial Acetic Acid in the ratio of 600:250:150:10 (v/v/v) was used. The flow rate was 1.0ml/min and effluents were monitored at 281nm. The retention time for Diclofenac Sodium and Acetaminophen was 9.461min and 2.782min simultaneously. The method was validated for limit of detection, limit of quantification, robustness, linearity, accuracy, precision, and specificity. Limit of detection and limit of quantification were found to be 7.05 to 13.09 mcg/ml and 45.37 to 84.2 mcg/ml respectively and recovery of Diclofenac Sodium and Acetaminophen from tablet formulation was found to be 96.8% and 100.5% respectively. The proposed method was successfully applied for the quantitative determination of Diclofenac Sodium and Acetaminophen in tablet formulation.

KEYWORDS: Diclofenac Sodium and Acetaminophen, HPLC, Linearity, Validation, Robustness, RP-HPLC, Simultaneous estimation, Method validation, Force degradation.

INTRODUCTION

Acetaminophen or N-(4-hydroxyphenyl) acetamide (Fig. 1a), is one of the most popular analgesics and antipyretic drugs. Different analytical methods for determination of Acetaminophen in its pharmaceutical formulations have been reported like: spectrophotometry, spectro-fluorimetry, voltammetry, TLC, UPLC, HPLC and capillary electrophoresis.

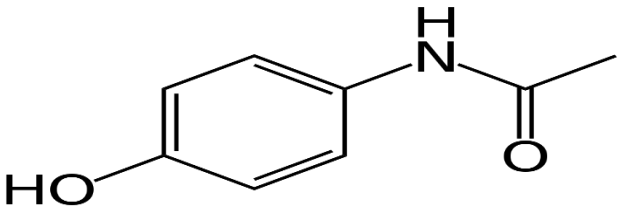

Figure 1.a: Structure of Acetaminophen.

Diclofenac (DCF) is 2-[(2,6- Dichlorophenyl) amino] benzene acetic acid (Fig. 1b). It is a NSAID used in relief of pain and inflammation in various conditions like musculoskeletal and joint disorders such as rheumatoid arthritis and osteoarthritis and other painful conditions such as renal colic, acute gout, migraine and after some surgical conditions. Diclofenac has been determined in its pharmaceutical formulations by different methods such as: spectrophotometry, spectro-fluorimetry, TLC, HPLC and capillary electrophoresis. Simultaneous determination of such binary has been determined previously by TLC method. In comparison with other analytical techniques, HPLC greatly reduces the analysis time and allows for the determination of many individual components in a mixture using one single procedure. Current research focuses on a simple, sensitive and rapid RPLC method for simultaneous determination of Acetaminophen and diclofenac as API's and combination formulation.[1][2]

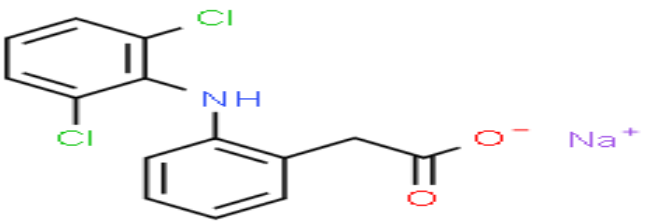

Figure 1.b: Structure of Diclofenac Sodium.

Experimental

Apparatus

Chromatographic separation was performed on a Agilent 1200 Infinity series ® liquid chromatographic system equipped with pump, injector and UV Detector. Mobile phases were filtered using Millipore filter and degassed.

Octadecylsilane, 250mm x 4.6mm, 5µm (Preferably: Peerless Basis C18) was used for the separation, mobile phase of a mixture of Methanol: Water: Acetonitrile: Glacial Acetic Acid in the ratio of 600:250:150:10 filter and degas was delivered at a flow rate of 1.0mL/min with detection at 281 nm. The mobile phase was filtered through a 0.45µ PVDF membrane filter and degassed. The injection volume was 20 mL; Analysis was performed at ambient temperature.

MATERIALS AND REAGENTS

The solvents were of HPLC grade, and all chemicals used were of Analytical Reagent grade. Pure drug sample of Acetaminophen and Diclofenac Sodium, % purity 99.56% and 99.69% was used without further purification. Acetaminophen, Diclofenac Sodium and Serratiopeptidase tablets 325mg+50mg+15mg formulation from Alkem Laboratories Pvt. Ltd (R&D Taloja), containing Acetaminophen and Diclofenac Sodium were used for analysis.

Acetonitrile and Methanol (HPLC grade) was procured from E. Merck (India) Ltd, Mumbai. Glacial acetic acid (GR grade) were procured from E. Merck (India) Ltd, Mumbai. Water (HPLC grade) was obtained from a Milli-Q water purification system. Reference standards of acetaminophen and diclofenac sodium were procured from ALKEM laboratories Ltd, Taloja. Placebo of tablet were made at Lab scale only.

Preparation of standard solutions

Weigh accurately about 325mg of Acetaminophen working standard and 50mg of Diclofenac Sodium working standard into a 250ml volumetric flask. Add 150ml of Methanol and sonicate to dissolve and dilute to 250ml with Methanol. Further dilute 5ml of this solution to 100ml with mobile phase.

Note: *The standard solution is stable upto 26 hours at 25ºC.

Preparation of sample solution

Weigh and powder 20 tablets, weigh and take accurately a quantity of powder containing about 325mg of Acetaminophen and about 50 mg of Diclofenac Sodium into a 250 ml

volumetric flask, add 150ml of Methanol sonicate for 15 minutes, with intermittent shaking, dilute to volume with Methanol. Filter through 0.45µ PVDF membrane filter, and further dilute 5ml to 100ml with mobile phase.

Note: *The sample solution is stable upto 26 hours at 25ºC.

Assay method

With the optimized chromatographic conditions, a steady baseline was recorded, the mixed standard solution was injected and the chromatogram was registered. The retention times of Acetaminophen and diclofenac sodium were found to be 2.782 and 9.461 min, respectively. This procedure was repeated for the sample solution. The response factor (peak area ratio of standard peak area and internal standard peak area) of the standard and sample solution were calculated respectively. From the peak responses, calculate the content of Acetaminophen and Diclofenac Sodium in the sample.

Calculation

$$\text{Content of Acetaminophen (As \% LA)} = \frac{AT}{AS} \times \frac{Wstd}{250} \times \frac{5}{100} \times \frac{250}{Wtest} \times \frac{100}{5} \times \frac{Aw}{L.C} \times \frac{P}{100} \times 100$$

Where,

AT = Average of the area counts of the Acetaminophen peak obtained from the Chromatograms of the Assay Preparation.

AS = Average of the area counts of the Acetaminophen peak obtained from the chromatograms of the standard Preparation.

Wstd = Weight of Acetaminophen working standard in mg.

Wtest = Weight of sample in mg.

L. C = Label claim of Acetaminophen in mg/tablet.

P = Potency of Acetaminophen working standard (% on as is basis).

$$\text{Content of Diclofenac Sodium (As \% LA)} = \frac{AT}{AS} \times \frac{Wstd}{250} \times \frac{5}{100} \times \frac{250}{Wtest} \times \frac{100}{5} \times \frac{Aw}{L.C.} \times \frac{P}{100} \times 100$$

Where,

AT = Average of the area counts of the Diclofenac peak obtained from the Chromatograms of the Assay Preparation.

AS = Average of the area counts of the Diclofenac peak obtained from the chromatograms of the standard Preparation.

Wstd = Weight of Diclofenac Sodium working standard in mg.

Wtest = Weight of sample in mg.

L.C. = Label claim of Diclofenac Sodium in mg/tablet.

P = Potency of Diclofenac Sodium working standard (% on as is basis)

RESULTS AND DISCUSSION

Estimation of Acetaminophen and diclofenac sodium in dosage forms: The HPLC procedure was optimized with a view to develop precise and stable assay method. Both the pure drugs Acetaminophen and diclofenac sodium were run in different mobile phase compositions with different columns Hypersil BDS C8 (250mm*4.6mm, 5 µ), Hypersil Gold (Octadecylsilane column, 250mm*4.6mm, 5 µ), Preferably: Peerless Basis C18 (Octadecylsilane, 250mm x 4.6mm, 5µm). The flow rate was same 1.0mL/min throughout the trials. Finally, Peerless Basis C18 (Octadecylsilane, 250mm x 4.6mm, 5µm), with a mobile phase of a mixture of Methanol: Water: Acetonitrile: Glacial Acetic Acid in the ratio of 600:250:150:10 at a flow rate of 1.0 mL/min with a detection at 281nm gave sharp and symmetrical peaks with retention time 2.782 and 9.461 min for Acetaminophen and diclofenac sodium respectively. The typical chromatogram of sample solution is shown in Fig.2. The peak area ratio of sample and standard solutions was calculated respectively. The assay procedures were repeated for six times and mean peak area and mean weight of standard drugs was calculated. The percentage of individual drugs found in formulations, mean, standard deviation in formulations was calculated and presented in Table 1. The results of analysis show that the amounts of drugs were in good agreement with the label claim of the formulation.

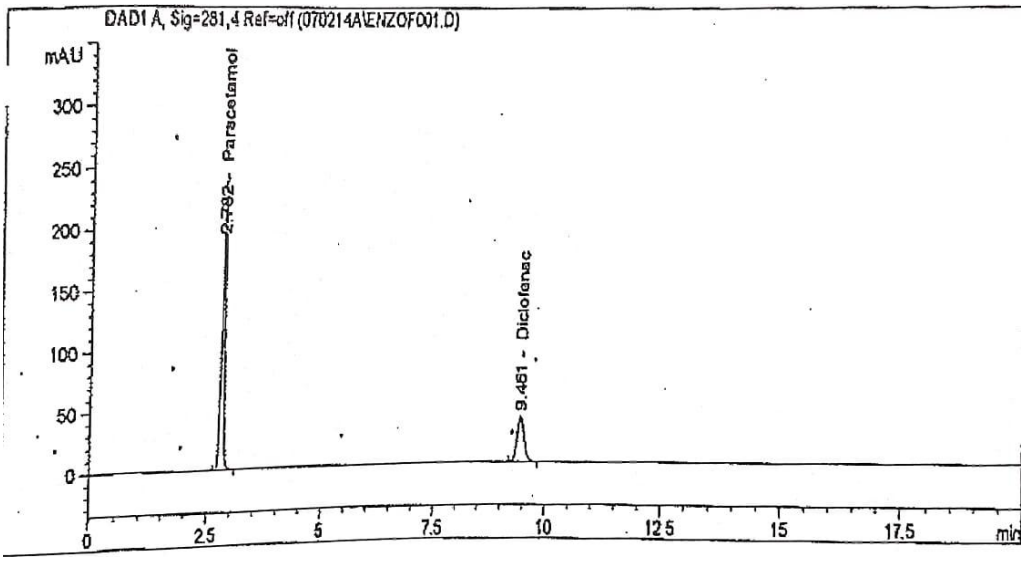

Figure 2: chromatogram of sample solution for method development.

Table 1: The percentage of individual drugs found in formulations, mean, standard deviation in formulations was calculated.

Sample No.	% Assay of Acetaminophen	% Assay of Diclofenac sodium
1	100.48	100.13
2	99.19	99.19
3	100.07	100.05
4	100.33	100.41
5	100.98	100.95
6	99.65	99.76
Mean	100.12	100.08
SD	0.633	0.594
%RSD	0.63	0.59
Over all Mean	100.20	100.47
Over all SD	0.743	0.831
Over all %RSD	0.74	0.83

Analytical Method validation

(1) Linearity

Linearity of Acetaminophen and Diclofenac Sodium was performed using the standard solution in the range of 45.37mcg/ml to 84.26mcg/ml of Acetaminophen (i.e. 70% to 130% of test concentration) and 7.05mcg/ml to 13.09mcg/ml of Diclofenac Sodium (i.e. 70% to130% of test concentration). A graph was plotted with concentration on X axis and mean peak areas on Y-axis for Acetaminophen and Diclofenac and correlation coefficient was determined. The results are tabulated in Table – 2a, and Table – 2b.

(2) System precision

The standard solution was injected into HPLC system in six replicates. The mean, SD and % RSD for peak areas of Acetaminophen and Diclofenac Sodium were calculated. The results are tabulated in Table-3a.

Method precision

As per test method, six samples of a single batch were analysed. The % assay for Acetaminophen and Diclofenac Sodium in six samples was calculated and the results are tabulated in Table-3b.

(3) Intermediate precision (Ruggedness)

Ruggedness of the method has been verified by analyzing the six samples of the same batch used for method precision as per test method by different analyst using different instrument and different column on different day. The percentage assay of Acetaminophen and Diclofenac Sodium in Acetaminophen, Diclofenac sodium and Serratiopeptidase Tablets was determined. Calculated % RSD for assay of Acetaminophen and Diclofenac sodium in six samples and overall %RSD for ruggedness results with the method precision results. The results are tabulated in table-4a and 4b.

(4) Reproducibility

The areas were measured using same instrument by two analysts for sample solution and the values obtained were evaluated using t-test to verify their reproducibility.

(5) Accuracy as recovery

Placebo was spiked with the known amount of Acetaminophen and Diclofenac Sodium at 70%, 100% and 130% of test concentration as Acetaminophen and Diclofenac Sodium. The amount of Acetaminophen and Diclofenac Sodium was quantified as per the test method. The percentage recovery was calculated from the amount found and then actual amount added. The results are tabulated in Table – 5a and 5b.

(6) Specificity

Specificity of the method was evaluated by injecting the blank (Mobile phase), placebo and control sample solution prepared as per the proposed method and injected into HPLC system to check for the interference if any at the retention time of Acetaminophen peak and Diclofenac Sodium peak. There was no interference from the blank and placebo at the

retention time of Acetaminophen and Diclofenac Sodium peak and peak purity for Acetaminophen and Diclofenac Sodium was passing. The peak purity data are compiled in Table-6.

Robustness

To evaluate the robustness, the following small deliberate variations made in the method and analyzed the sample in triplicate.

1. Flow rate (± 0.1ml)
2. Wavelength (± 2 nm)
3. Column Oven Temperature. (± 5°C)
4. Mobile phase (± 2% organic content)
5. Mobile phase (± 0.2 unit pH)

The system suitability was evaluated in each condition and compared the results with precision results. The results are tabulated in table-7a and 7b.

Summary of system suitability

System suitability was evaluated by injecting standard solution during different days of validation. The tailing factor and column efficiency for the first standard injection and % relative standard deviation for the peak areas of Acetaminophen and Diclofenac from five replicate injections of standard solution were verified at every stage. The results are tabulated in table – 8a and 8b.[10-16]

Table 2a: Linearity of Acetaminophen.

Spike level in %	Concentration of Paracetamol in mcg/ml	Mean peak areas of the Paracetamol peaks
70	45.37	741725
80	51.85	845564
90	58.33	951826
100	64.81	1064940
110	71.29	1161302
120	77.77	1278558
130	84.26	1377281
Slope	16433	
Intercept	-4887	
‘r’	0.99985	
RSS	94202733	

Table 2b - Linearity of Diclofenac Sodium.

Spike level in %	Concentration of Diclofenac Sodium in mcg/ml	Mean peak areas of the Diclofenac peaks
70	7.05	295629
80	8.06	337055
90	9.07	379567
100	10.07	425169
110	11.08	464002
120	12.09	511579
130	13.09	551449
Slope	42601.600	
Intercept	-5627	
'r'	0.99984	
RSS	16700384	

Table 3a: System Precision.

Sr. No.	Peak areas of the Acetaminophen	Peak areas of Diclofenac Sodium
1	1038.87891	409.48743
2	1034.43250	409.34534
3	1035.88770	410.62708
4	1037.50171	411.40118
5	1037.28479	411.12244
6	1037.55945	410.23935
Mean	1036.92418	410.37047
SD	1.547	0.842
%RSD	0.15	0.21

Table 3b: Method Precision.

Sample No.	% Assay of Acetaminophen	% Assay of Diclofenac Sodium
1	100.0	96.3
2	100.0	96.3
3	100.3	96.5
4	100.1	96.5
5	100.8	97.2
6	101.8	98.2
Mean	100.5	96.8
SD	0.704	0.747
%RSD	0.70	0.77

Table 4a: Ruggedness.

Sample No.	% Assay of Acetaminophen	
	Analyst-I	Analyst-II
1	100.48	100.78
2	99.19	101.55
3	100.07	100.55
4	100.33	99.01
5	100.98	99.62
6	99.65	100.24
Mean	100.12	100.29
SD	0.633	0.893
%RSD	0.63	0.89
Over all Mean	100.20	
Over all SD	0.743	
Over all %RSD	0.74	

Table 4b: Ruggedness.

Sr. No.	% Assay of Diclofenac sodium	
	Analyst-I	Analyst-II
1	100.13	101.26
2	99.19	101.96
3	100.05	101.53
4	100.41	99.59
5	100.95	100.10
6	99.76	100.71
Mean	100.08	100.86
SD	0.594	0.898
%RSD	0.59	0.89
Over all Mean	100.47	
Over all SD	0.831	
Over all %RSD	0.83	

Table 5a: Results of the recovery analysis of Acetaminophen.

Level no/Spike level in %	Actual Amount added in mg of Acetaminophen	Amount found in mg of Acetamionphen	%Recovery	Mean	SD	%RSD
Level – 1 (70%)	226.50	229.04	101.12	101.16	0.506	0.50
	226.50	228.03	100.68			
	226.50	230.32	101.69			
Level – 2 (100%)	323.57	326.10	100.78	100.63	0.150	0.15
	323.57	325.65	100.64			
	324.37	325.93	100.48			
Level – 3 (130%)	420.64	420.79	100.04	100.04	0.020	0.02
	420.64	420.89	100.06			
	420.64	420.73	100.02			
	Over all Mean		100.61			
	Over all SD		0.554			
	Over all % RSD		0.55			

Table 5b: Results of the recovery analysis of Diclofenac Sodium.

Level no/Spike level in %	Actual Amount added in mg of Diclofenac Sodium	Amount found in mg of Diclofenac Sodium	%Recovery	Mean	SD	%RSD
Level – 1 (70%)	34.91	35.34	101.23	100.27	0.845	0.84
	35.19	35.17	99.94			
	35.65	35.52	99.64			
Level – 2 (100%)	50.41	50.44	100.05	100.20	0.254	0.25
	50.13	50.38	100.49			
	50.35	50.38	100.05			
Level – 3 (130%)	65.00	65.23	100.36	100.24	0.375	0.37
	64.93	65.28	100.54			
	65.36	65.24	99.82			
	Over all Mean		100.24			
	Over all SD		0.480			
	Over all % RSD		0.48			

Table 6: Specificity.

Sample type	Peak name	Retention Time	Match Factor
Standard	Acetaminophen	2.782	-
	Diclofenac	9.461	-
Control sample	Acetaminophen	2.783	999.958
	Diclofenac	9.462	999.776

Table 7a: Summary of System suitability – Acetaminophen.

Sr. No.	*Name of Experiment*	Tailing factor	Theoretical plates	%RSD
1	System precision, Method precision and Specificity	0.78	10325	0.16
2	Linearity, Recovery	1.27	8863	0.08
3	Solution stability[#]	1.2	5786	0.11
4	Robustness (Minus Wavelength)[#]	1.2	6751	0.07
5	Robustness (Plus Wavelength) [#]	1.2	6329	0.09
6	Robustness (Minus flow) [#]	1.2	6560	0.24
7	Robustness (Plus flow) [#]	1.2	5901	0.11
8	Robustness (Minus temp.) [#]	1.2	6162	0.52
9	Robustness (Plus temp.) [#]	1.3	6522	0.24
10	Forced degradation[#]	1.3	4086	0.27
11	Ruggedness[#]	1.2	6086	0.17
12	Robustness (Minus organic)[#]	1.2	5874	0.41
13	Robustness (Plus organic) [#]	1.2	5993	0.43
14	Filter paper selection study	0.77	10120	0.85

Table 7b: Summary of System suitability – Diclofenac Sodium.

Sr. No.	*Name of Experiment*	Tailing factor	Theoretical plates	%RSD
1	System precision, Method precision and Specificity	0.9	18214	0.23
2	Linearity, Recovery	1.1	15539	0.95
3	Solution stability[#]	1.1	11855	0.08
4	Robustness (Minus Wavelength)[#]	1.0	13056	0.09
5	Robustness (Plus Wavelength)[#]	1.0	12996	0.12
6	Robustness (Minus flow)[#]	1.0	13800	0.16
7	Robustness (Plus flow)[#]	1.0	12105	0.06
8	Robustness (Minus temp.)[#]	1.0	12511	0.22
9	Robustness (Plus temp.)[#]	1.0	13842	0.14
10	Forced degradation[#]	1.1	11864	0.25
11	Ruggedness[#]	1.1	11292	0.1
12	Robustness (Minus organic)[#]	1.1	11552	0.24
13	Robustness (Plus organic)[#]	1.1	11210	0.29
14	Filter paper selection study	0.93	17936	0.15

Forced degradation

The Acetaminophen, Diclofenac sodium and Serratiopeptidase Tablets Sample was subjected to forced degradation under the following stress conditions.

a) Acid degradation

The Acetaminophen, Diclofenac sodium and Serratiopeptidase Tablets sample was treated separately with 5.0ml of 0.5N Hydrochloric acid and Kept on bench top for 20 hours. The treated sample was analysed as per the proposed method and results are given in Table 8.13a & Table 8.13b.

b) Base degradation

The Acetaminophen, Diclofenac sodium and Serratiopeptidase Tablets sample was treated separately with 5.0ml of 0.05N Sodium Hydroxide and kept on bench top for 18 hours. The treated sample was analysed as per the proposed method and results are given in Table 8.13a & Table 8.123b**.**

c) Oxidative degradation

The Acetaminophen, Diclofenac sodium and Serratiopeptidase Tablets sample was treated separately with 5.0ml of 1.0%v/v solution of hydrogen peroxide and kept on bench top for 20 hours. The treated sample was analysed as per the proposed method and results are given in Table 8.13a & Table 8.123b.

d) Thermal Degradation

The Acetaminophen, Diclofenac sodium and Serratiopeptidase Tablets sample was subjected to thermal degradation by keeping the sample at 105oC for 22 hours and the treated sample was analyzed as per the proposed method. The results are given in Table 8.13a & Table 8.13b.

e) Photolytic Degradation

The Acetaminophen, Diclofenac sodium and Serratiopeptidase Tablets sample was exposed to UV light at 254 nm for about 22 hours and the treated sample was analysed as per the proposed method. The results are given in Table 8.13a & Table 8.13b.

The results of forced degradation studies are summarized in Table 8.13a and Table 8.13b. At every stage of forced degradation study, purity Angle and purity threshold of the Acetaminophen and Diclofenac sodium peak were evaluated. The peak purity data given in Table 8.13a & Table 8.13b. Indicates that peaks are pure with no coeluting peaks. This illustrates that the method proposed is stability indicating.

Refer fig. 8.8a, 8.8b, 8.9a, 8.9b, 8.10a, 8.10b, 8.11a, 8.11b, 8.12a and 8.12b for chromatograms and purity plot of treated sample solutions.

Acceptance criteria

The peak purity for Acetaminophen and Diclofenac peak shall be passed.

Table 8a - Forced degradation (Acetaminophen).

Condition	% Assay	% Degradation w.r.t. Control	Peak Purity data		
			Purity Angle	Purity Threshold	Purity Flag
Untreated sample	*100.12	-	-	-	-
3.0% v/v H_2O_2	97.70	2.42	0.124	1.008	No
0.5N HCl	96.89	3.23	0.115	1.009	No
0.05N NaOH	97.27	2.85	0.103	1.007	No
105^0C	98.38	1.74	0.112	1.008	No
UV-light at 254 nm	97.58	2.54	0.110	1.007	No

*- Mean of method precision result

Table 8b: Forced degradation (Diclofenac sodium).

Condition	% Assay	% Degradation w.r.t. Control	Peak Purity data		
			Purity Angle	Purity Threshold	Purity Flag
Untreated sample	*100.08	-	-	-	-
3.0% v/v H_2O_2	94.82	5.26	0.443	1.296	No
0.5N HCl	93.12	6.95	0.336	1.294	No
0.05N NaOH	92.56	7.51	0.307	1.281	No
105^0C	95.31	4.77	0.324	1.287	No
UV-light at 254 nm	93.67	6.40	0.311	1.292	No

*- Mean of method precision result

CONCLUSION

The proposed HPLC methods are simple, accurate, and precise for Acetaminophen and Diclofenac Sodium. Six replicate samples of Acetaminophen and Diclofenac Sodium were determined for UV and HPLC methods and the results were correlated.

A simple, specific, linear, precise and accurate HPLC method has been developed and validated for quantitative determination of Acetaminophen, and Diclofenac Sodium Tablet formulation. The method is very simple and specific as peak of Acetaminophen and Diclofenac Sodium is well separated and there is no interference by excipient with total run time of 4 min and simple mobile phase which makes it especially suitable for routine quality control analysis work.

The HPLC method for identification of Acetaminophen, and Diclofenac Sodium Tablet formulation is simple, accurate, and precise and requires a very small amount of mobile phase, compared to UPLC method.

The peak purity Acetaminophen and Diclofenac is passing in all storage condition. The method is stability indicating for estimation of Acetaminophen and Diclofenac sodium in Acetaminophen, Diclofenac Sodium and Serratiopeptidase Tablets 325mg+50mg+15mg.

REFERENCES

1. Sethi, N. et al. "Development And Validation Of A Stability-Indicating RP-UPLC Method For The Quantitative Analysis Of Nabumetone In Tablet Dosage Form". *Journal Of Chromatographic Science*, 2012; 50(2): 85-90. *Oxford University Press (OUP)*, doi:10.1093/chromsci/bmr001. Accessed 28 Feb 2020.

2. El-Ashry, S. M. et al. "Spectrophotometric And Spectrofluorimetric Determination Of Indacaterol Maleate In Pure Form And Pharmaceutical Preparations: Application To Content Uniformity". *Luminescence*, 2015; 30(6): 891-897. *Wiley*, doi:10.1002/bio.2838.
3. Meyyanathan, SN et al. "A RP-HPLC Method For Simultaneous Estimation Of Paracetamol And Aceclofenac In Tablets". *Indian Journal Of Pharmaceutical Sciences*, 2007; 69(1): 137. *OMICS Publishing Group*, doi:10.4103/0250-474x.32130. Accessed 28 Feb 2020.
4. Shukla, Ameesh. "METHOD DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF PARACETAMOL, PHENYLEPHRINE HYDROCHLORIDE AND TRIPROLIDINE HYDROCHLORIDE IN BULK AND COMBINED TABLETS DOSAGE FORMS". *World Journal Of Pharmaceutical Research*, 2017; 483-492. *World Journal Of Pharmacy And Pharmaceutical Sciences*, doi:10.20959/wjpr20179-9097.
5. "RP-HPLC Method Development And Validation For The Analysis Of Pharmaceutical Drugs", 2016; 5(3): 2093-2095. *International Journal Of Science And Research*, doi:10.21275/v5i3.nov162367.
6. Srividya, P. et al. "SIMULTANEOUS ANALYSIS OF LEVOCETIRIZINE DIHYDROCHLORIDE, AMBROXOL HYDROCHLORIDE, AND MONTELUKAST SODIUM BY RP-HPLC - PDA METHOD". *Journal Of Liquid Chromatography & Related Technologies*, 2013; 36(20): 2871-2881. *Informa UK Limited*, doi:10.1080/10826076.2012.725701.
7. Agrawal, Uttam Kumar, and Jeyabalan Govindasamy. "Development And Validation Of RP-HPLC Method For Simultaneous Estimation Of Metformin And Pioglitazone". *Indian Journal Of Pharmaceutical And Biological Research*, 2018; 5(01): *IJPBR*, doi:10.30750/ijpbr.5.1.7.
8. Reddy, B. Prasanna et al. "Validation And Stability Indicating RP-HPLC Method For The Determination Of Tadalafil API In Pharmaceutical Formulations". *Research In Pharmaceutical Biotechnology*, 2010; 21: Accessed 28 Feb 2020.
9. Alexandar, S et al. "A RP-HPLC Method For Simultaneous Estimation Of Metformin And Pioglitazone In Pharmaceutical Formulation". *Research Journal Of Pharmaceutical, Biological And Chemical Sciences*, 2010. *RJPBCS*, https://www.researchgate.net/publication/279600500_A_RP-HPLC_method_for_simultaneous_estimation_of_metformin_and_pioglitazone_in_pharmaceutical_formulation. Accessed 28 Feb 2020.

10. "A VALIDATED RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF PARACETAMOL AND DICLOFENAC POTASSIUM IN PHARMACEUTICAL FORMULATION". Vol 2, 2010. *IJCRGG*, Accessed 28 Feb 2020.
11. "DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR AMIODARONE TABLETS IN PHARMACEUTICAL DOSAGE FORMS". 2013. *AJPAMC*, Accessed 28 Feb 2020.
12. 2020, https://shodhganga.inflibnet.ac.in/bitstream/10603/71149/13/13_chapter%205.pdf. Accessed 28 Feb 2020.
13. Mohite, M. T. et al. "Validation Of Stability Indicating RP-HPLC, Method Of Analysis For Assay Of Ivabradine Hcl In SR Tablet". *Asian Journal Of Pharmaceutical Analysis*, 2019; 9(3): 133. *Diva Enterprises Private Limited*, doi:10.5958/2231-5675.2019.00025.5. Accessed 28 Feb 2020.
14. "A STABILITY-INDICATING HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD FOR THE DETERMINATION OF COBICISTAT", 2015; 3(4): *IJPDA*, Accessed 28 Feb 2020.
15. "Analytical Method Development And Validationfor Residual Solvent Of Diltiazem Hydrochloride Extended Release Capsule By Gas Chromatography", 2020; 2(4). *Ijpar*, Accessed 28 Feb 2020.
16. Dhaneshwar, Sunil R., and Vaijanath N. Jagtap. "Development and Validation of RP-HPLC-PDA Method for Simultaneous Determination of Dexketoprofen and Thiocolchicoside in Pharmaceutical Dosage Form." *Journal of Pharmacy Research*, 2013; 6(6): 604–608., doi:10.1016/j.jopr.2013.04.053.