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Research Article

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A NOVEL RP – HPLC METHODOLOGY FOR METHOD DEVELOPMENT AND VALIDATION OF ACECLOFENAC AND TIZANIDINE PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT
A simple and selective LC technique is chosen for the determination of Aceclofenac and Tizanidine in pill indefinite quantity forms. Chromatographic process separation was achieved on a c18 column victimization mobile part consisting of a combination of fifty volumes of Triethylamine buffer, fifty volumes of acetonitrile with detection of 230nm. Dimensionality was discovered within the vary 5-15 µg/ml for aceclofenac (r2 =0.999) and 1-3 µg /ml for tizanidine (r2 =0.998) for the number of medicine calculable by the planned strategies was in smart agreement with the label claim. The planned strategies have a sound procedure. At three completely different levels the accuracy of the strategies was assessed by recovery studies. The recovery experiments indicated the absence of interference from unremarkably encountered pharmaceutical additives showing %RSD below a pair of this technique was found to be precise as indicated by the repeatability analysis. All applied mathematics information proves all ways have valid procedure and might be used for routine analysis of pharmaceutical dose kind

INTRODUCTION

Pharmaceutical analysis just means examination of pharmaceuticals. Webster' word reference portrays a pharmaceutical is a medication. An additionally fitting term for a pharmaceutical is dynamic pharmaceutical fixing to remember it from a planned thing or prescription thing is set up by figuring a solution substance with idle concentration (excipients) to set up a drug thing that is sensible for association to patients [1].

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Reverse Phase High Performance Liquid Chromatography (RP-HPLC)

Reverse phase chromatography uses hydrophobic bonded packing, usually with an octadecyl or octyl functional group and a polar aqueous mobile phase. Polar substances prefer the mobile phase and eluted first. Because the hydrophobic character of the solutes will increase, and followed retention will also increase. Generally, the lower the polarity of the mobile section, the upper is its eluent strength. The elution order of the classes of compounds in table is reversed (thus the name reverse-phase chromatography) [2].

Drug Profile

Aceclofenac is a non-steroidal calming drug (NSAID) with stamped mitigating and pain relieving properties. It is accounted for to have a higher mitigating activity or if nothing else tantamount impacts than ordinary NSAIDs in twofold visually impaired investigations. Aceclofenac intensely hinders the cyclo-oxygenase protein (COX) that is engaged with the combination of prostaglandins, which are fiery middle people that reason torment, swelling, irritation, and fever [3]. It is orally regulated for the alleviation of agony and aggravation in osteoarthritis, rheumatoid joint pain and ankylosing spondylitis. Aceclofenac has a place with BCS Class II as it has poor watery dissolvability. It shows high porousness to infiltrate into synovial joints where in patients with osteoarthritis and related conditions, the loss of articular ligament in the territory causes joint torment, delicacy, solidness, crepitus, and nearby aggravation [4]. Aceclofenac is likewise answered to be compelling in other excruciating conditions, for example, dental and gynecological conditions. In 1991, aceclofenac was produced as a simple of a usually recommended NSAID, Diclofenac, by means of substance adjustment in push to enhance the gastrointestinal mediocrity of the medication. It is an all the more generally recommended medicate in Europe [5]. Through COX-2 hindrance, aceclofenac downregulates the generation of different provocative go betweens including prostaglandin E2 (PGE2), IL-1β, and TNF from the arachidonic corrosive (AA) pathway. Restraint of IL-6 is believed to be interceded by diclofenac changed over from aceclofenac [6]. Stifled activity of incendiary cytokines diminishes the creation of receptive oxygen species. Aceclofenac is appeared to diminished generation of N_2O in person articular chondrocytes. Also, aceclofenac meddles with neutrophil bond to endothelium by diminishing the statement of L-selectin (CD62L), which is a cell grip atom communicated on lymphocytes. Aceclofenac is proposed to fortify the amalgamation of glycosaminoglycan in human osteoarthritic ligament which might be interceded through its inhibitory activity on IL-1 creation and movement. The chrondroprotective impacts are created by 4'-hydroxyaceclofenac which stifles IL-1 interceded generation of promatrix metalloproteinase-1 and metalloproteinase-3 and meddles with the arrival of proteoglycan from chrondrocytes [7]. Tizanidine is a short-acting medication for the administration of spasticity. Tizanidine is an agonist at a2-adrenergic receptor, and it is very close to clonidine and apparently lessens spasticity by expanding presynaptic hindrance of engine neurons. In creature models, tizanidine has no immediate impact on skeletal muscle filaments or the neuromuscular intersection, and no significant impact on monosynaptic spinal reflexes. The impacts of tizanidine are most prominent on polysynaptic pathways. The general impact of these activities is thought to decrease assistance of spinal engine neurons [8]. Tizanidine reduces spasticity by causing presynaptic inhibition of motor neurons via agonist acts at Alpha-2 adrenergic receptor sites. This drug is centrally acting and leads to a reduction in the release of excitatory amino acids like glutamate and aspartate, which cause neuronal firing that leads to muscle spasm. The above reduction and excitatory neurotransmitter release results in presynaptic inhibition of motor neurons. The strongest effect of tizanidine has been shown to occur on spinal polysynaptic pathways. The anti-nociceptive and anticonvulsant activities of tizanidine may also be attributed to agonist action on Alpha-2 receptors. Tizanidine also binds with weaker affinity to the Alpha-1 receptors, explaining its slight and temporary effect on the cardiovascular system [9, 10]. In young nursing rats, abnormal results were obtained in tests indicative of central nervous system function. Various developmental changes that may have been attributable to the drug were observed. It is unknown whether tizanidine is excreted in human milk. It is a lipid-soluble drug, however, and likely to be excreted into breast milk [11].

Quality investigation plays a very important role in quality specification establishment of chemical drugs. The numbers of drugs launch into the retail every year .very often there's a time lag from the time of launch of a drug into the market to the date of its insertion in pharmacopoeias. Hence, standards and analytical procedures for these drugs may not be available in the pharmacopoeias. It becomes necessary, so to develop newer analytical strategies for such medication [12].

To develop new RP HPLC method, of pharmaceutical dosage form. The plan of work includes solubility determination of aceclofenac and tizanidine various solvents and buffers. Establish the maximum absorbance of each of the medication in UV-Visible region in numerous solvents/buffers and choosing the dissolving agents for HPLC methodology development. Optimize the mobile phase and flow rates for proper resolution and retention times. Validate the developed method as per ICH guidelines.

MATERIAL AND METHOD

Instruments Used

The instruments which were used for this work were, UV Visible apparatus which was manufactured by Nicolet evolution 100, UV-Visible Software that was developed by Vision Pro, HPLC software that was developed by Spin chrome (LC solutions), HPLC and Electronic balance manufactured by Shimadzu(LC 20 AT VP), Ultra sonicator which was manufactured Citizen, Digital Ultrasonic Cleaner, pH meter manufactured by Global digital, Syringe was used for injection which was manufactured by Hamilton, HPLC Column was obtained from Inertsil ODS 3V(250x4.6mm) 5µm

Reagents Used

Water, Methanol, was used according to HPLC Grade; Ammonium acetate was used as per AR Grade.

Drugs Used

Aceclofenac and Tizanidine drugs are obtained as gift Samples obtained from Chandra labs; Hyd. ACENT (100/2mg) was obtained from local pharmacy

Preparation of Mobile Phase

A concoction of fifty volumes of triethylamine buffer maintaining a pH of 3.0 and same volume of acetonitrile were created. The mobile section was sonicated for 10min to get rid of gases and filtered through 0.45µ membrane for degassing from the mobile section

Determination of Wavelength using UV Visible Spectroscopy [13]

Preparation of stock solution of aceclofenac

Accurately weighed 10 mg of aceclofenac was pass on in to a 100 ml flask and aceclofenac was dissolved in methanol, make up to the 100ml with same solvent to prepare 100 µg /ml. From this solution pipette out 1ml and prepare up to 10ml with methanol to preprare 10 µg /ml of drug solution.

Preparation of stock solution of tizanidine

Weighed 10 mg of drug was shifted in to a 100ml volumetric flask and tizanidine was dissolved in methanol, make up to the 100ml with same solvent to prepare 100µg /ml. From this suspension pipette out 1ml and prepare up to 10ml with methanol to preprare 10 µg /ml of drug solution.

Method Development

Trial – 1

The Mobile phase that is used are Phosphate buffer: Acetonitrile: Methanol maintaining a pH of 5.0 in an ratio, 40:30:30 weigh accurately 5 mg of aceclofenac and 5 mg of tizanidine in 100 ml of meter flask and add 10ml of mobile phase and dissolve, and ,make up the volume with mobile phase. From above stock solution, to prepare, 10µg/ml of aceclofenac and 20 µg/ml of tizanidine, it is prepared by diluting 2.4ml of stock solution in 10ml of mobile phase. This liquid is better used for chromatogram recording [14].

Trial- 2

The mobile phase that is used are Triethylamine: Acetonitrile maintaining a pH of 3.0 in an ratio, 50:50. Weigh accurately 5 mg of aceclofenac and 5 mg of tizanidine in 100 ml of volumetric flask and add 10ml of mobile phase and dissolve and make up the capacity with mobile phase. From above stock solution, to prepare, 10µg/ml of aceclofenac and 20 µg/ml of tizanidine, it is prepared by diluting 2.4ml in 10ml of mobile phase. This liquid is better used for chromatogram recording [15].

Assay [16]

Preparation of mixed standard solution

Weigh accurately 5 mg of aceclofenac and 5 mg of tizanidine in 100 ml of volumetric flask and dissolve in 10ml of mobile phase and make up the volume with mobile phase. From above stock solution 10µg/ml of aceclofenac and 20µg/ml of tizanidine is prepared by diluting 2.4ml in 10ml of mobile phase. This liquid is better used for chromatogram recording.

Tablet sample

10 tablets (each tablet contains aceclofenac -100 mg and tizanidine – 100 mg) were weighed and taken into a mortar and crushed to fine powder and uniformly mixed. Stock solutions of tizanidine and aceclofenac tablets (µg/ml) were made by solubilizing aceclofenac and tizanidine drugs equivalent to 5 mg in sufficient mobile phase. After that filter the solution using 0.45-micron wheel filter and sonicate for five minutes and mix it up to 10 ml with mobile phase. Further dilutions are prepared

in 5 replicates of 20 µg/ml of tizanidine and 10µg/ml of aceclofenac was made by adding 2.4 ml of stock solution upto 10 ml of mobile phase.

Validation [17].

Linearity and range

Standard stock solutions of aceclofenac and tizanidine (µg/ml) were prepared by dissolving 10 mg of aceclofenac and tizanidine in 10 ml of mobile phase and dilute and make up the volume to 100 ml with mobile phase.

Accuracy

Accuracy of the method is determined by recovery studies. To the formulation (pre analyzed sample), the reference standards of the drugs were added at the level of 50%, 100%, 150%. The recovery studies were dole out three times and also the percentage recovery and percentage mean recovery were calculated. To check the accuracy of the maneuver, recovery studies were disbursed by addition of ordinary drug resolution to pre- analyzed sample solution at 3 totally different levels 50%, 100%, 150%.

Precision

Method precisionPrepared sample preparations of tizanidine and aceclofenac as per test method and injected 6 times in to the column. The percentage recovery of tizanidine and aceclofenac should not be more than 2.0 %.

Robustness

To demonstrate the strength of the method, prepared solution as per test method and injected at completely different variable conditions like flow and wavelength. System suitableness parameters were compared there upon of technique preciseness.

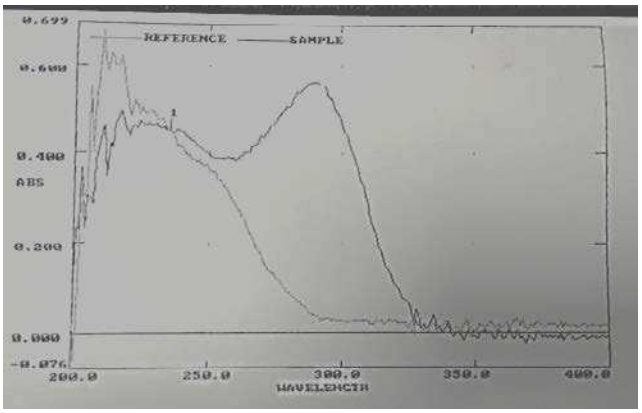
Ruggedness

The strength of the tactic was studied by the determining the analyst to analyst variation by performing the Assay by 2 totally different analysts. The percentage relative standard deviation should be not more than 2.0 %

RESULTS

Determination of Wavelength of Aspirin and Clopidogrel using UV Visible Spectroscopy

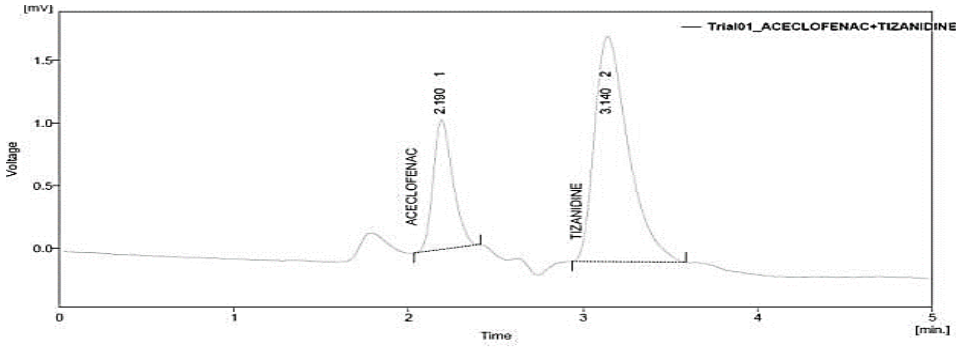
(X – Axis – Wavelength and Y – axis – Absorbance)



The wavelength (λmax) at where maximum absorbance of the drug of 10 µg/ml concentration using methanol as solvent was scanned using UV-Visible spectrophotometer in the range of 200–400 nm comparing methanol as blank. and the isobestic value was observed to be 230 nm for mixture of the medications.

Method Development of Aceclofenac and Tizanidine

Trial – 01 (X – Axis – Time and Y – axis – Voltage)



Result Table (Uncal - Trial01_ACECLOFENAC+TIZANIDINE

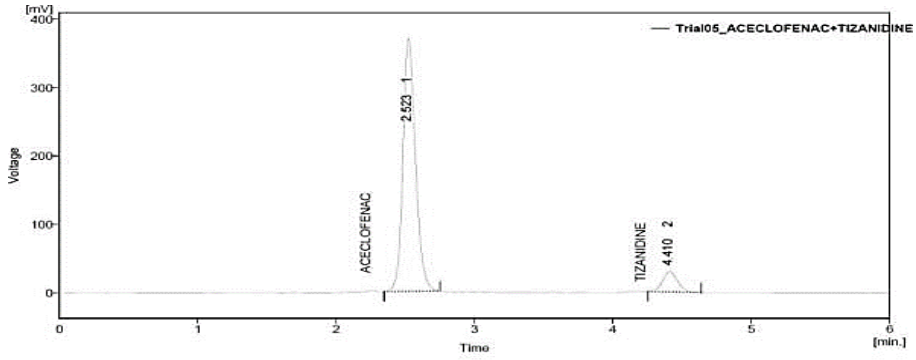
	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	2.190	8.048	1.032	24.9	36.5	0.12
2	3.140	24.246	1.798	75.1	63.5	0.20
	Total	32.294	2.830	100.0	100.0	

Column Performance Table (From 50% - Trial01_ACECLOFENAC+TIZANIDINE

	Reten. Time	W05 [min]	Asymmetry [-]	Capacity [-]	Efficiency [th.pl]	Eff.l [t.p./m]	Resolution [-]
1	2.190	0.123	1.500	0.00	1747	17468	-
2	3.140	0.203	1.952	0.00	1321	13211	3.423

Trial – 01 - The Efficiency was not satisfactory for aceclofenac and the baseline was not proper. Hence it was not taken for optimization.

Trial – 02 (X – Axis – Time and Y – axis – Voltage)



Result Table (Uncal - Trial05_ACECLOFENAC+TIZANIDINE

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	2.523	2325.117	369.999	91.2	92.6	0.10
2	4.410	224.128	29.732	8.8	7.4	0.12
	Total	2549.245	399.731	100.0	100.0	

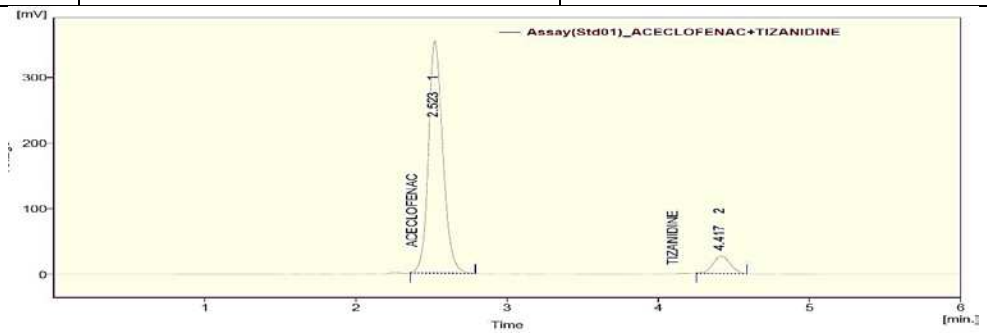
Column Performance Table (From 50% - Trial05_ACECLOFENAC+TIZANIDINE

	Reten. Time	W05 [min]	Asymmetry [-]	Capacity [-]	Efficiency [th.pl]	Eff/l [t.p./m]	Resolution [-]
1	2.523	0.097	1.375	0.00	3775	75498	-
2	4.410	0.120	1.161	0.00	7482	149642	10.218

Trial – 02 – In this trial, all the system suitability requirements were met. The peak asymmetry factor was below 2 for both tizanidine and aceclofenac. The efficiency was more than 2000 tizanidine and aceclofenac. Resolution between two peaks is >1.5. Hence, the method is taken for optimization

Assay Results

	ACECLOFENAC		**TIZANIDINE**	
	Standard Area	**Sample Area**	**Standard Area**	**Sample Area**
Injection-1	2334.362	2344.463	207.967	212.684
Injection-2	2323.199	2351.614	199.968	209.655
Injection-3	2337.863	2337.863	207.039	207.039
Injection-4	2331.502	2334.732	207.632	210.092
Injection-5	2328.483	2341.801	198.197	210.080
Average Area	2331.082	2342.095	204.1606	209.91
Standard deviation	6.489006		2.004044	
%RSD	0.276506		0.952806	
Assay(%purity)	100.27		102.61	

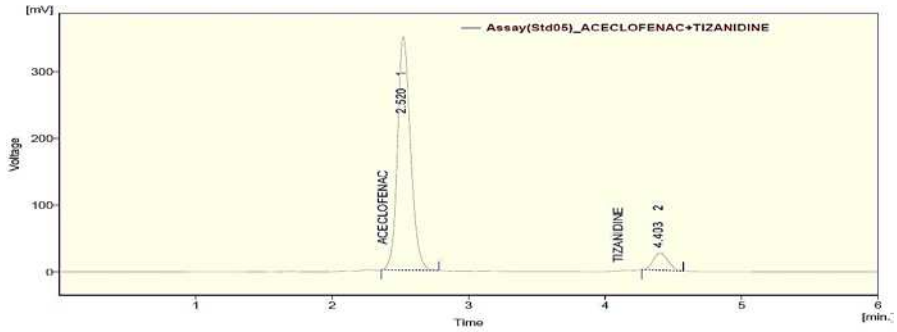


Result Table (Uncal - Assay(Std01)_ACECLOFENAC+TIZANIDINE

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]
1	2.523	2334.362	353.400	91.82
2	4.417	207.967	26.796	8.18
	Total	2542.329	380.196	100.00

Column Performance Table (From 50% - Assay(Std01)_ACECLOFENAC+TIZANIDINE

	Reten. Time	W05 [min]	Asymmetry [-]	Efficiency [th.pl]	Eff/l [t.p./m]	Resolution [-]
1	2.523	0.103	1.308	3304	66071	-
2	4.417	0.120	1.121	7505	150095	9.977

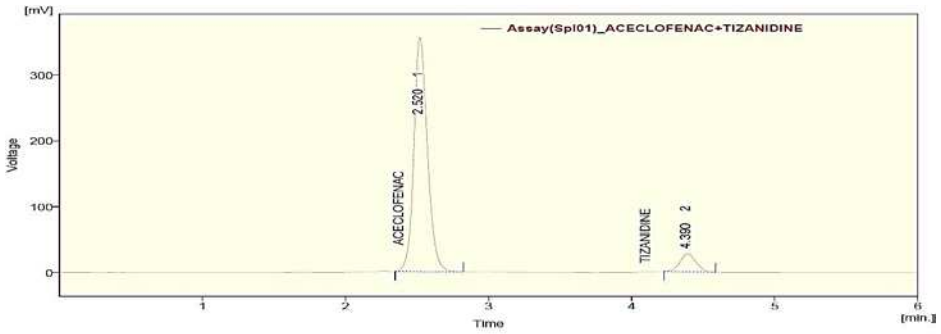


Result Table (Uncal - Assay(Std05)_ACECLOFENAC+TIZANIDINE

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]
1	2.520	2328.483	349.718	92.16
2	4.403	198.197	26.112	7.84
	Total	2526.679	375.830	100.00

Column Performance Table (From 50% - Assay(Std05)_ACECLOFENAC+TIZANIDINE

	Reten. Time	W05 [min]	Asymmetry [-]	Efficiency [th.pl]	Eff/l [t.p./m]	Resolution [-]
1	2.520	0.103	1.400	3295	65896	-
2	4.403	0.120	1.194	7460	149190	9.924

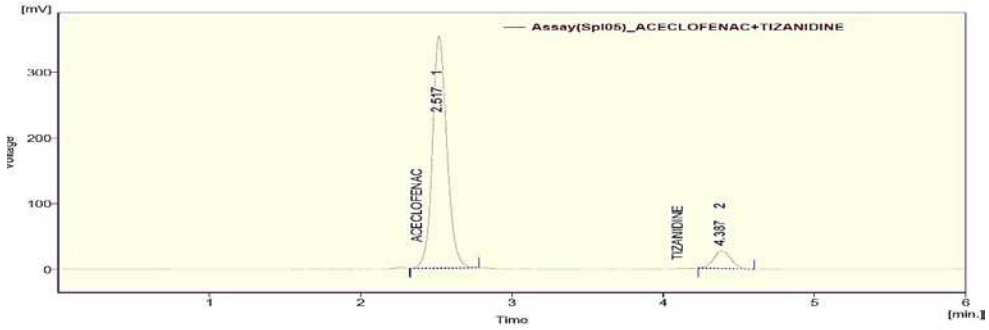


Result Table (Uncal - Assay(Spl01)_ACECLOFENAC+TIZANIDINE

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]
1	2.520	2344.463	355.494	91.68
2	4.390	212.684	27.195	8.32
	Total	2557.147	382.689	100.00

Column Performance Table (From 50% - Assay(Spl01)_ACECLOFENAC+TIZANIDINE

	Reten. Time	W05 [min]	Asymmetry [-]	Efficiency [th.pl]	Eff/l [t.p./m]	Resolution [-]
1	2.520	0.103	1.308	3295	65896	-
2	4.390	0.123	1.152	7019	140381	9.709



Result Table (Uncal - Assay(Spl05)_ACECLOFENAC+TIZANIDINE

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]
1	2.517	2341.801	353.112	91.77
2	4.387	210.080	26.856	8.23
	Total	2551.882	379.969	100.00

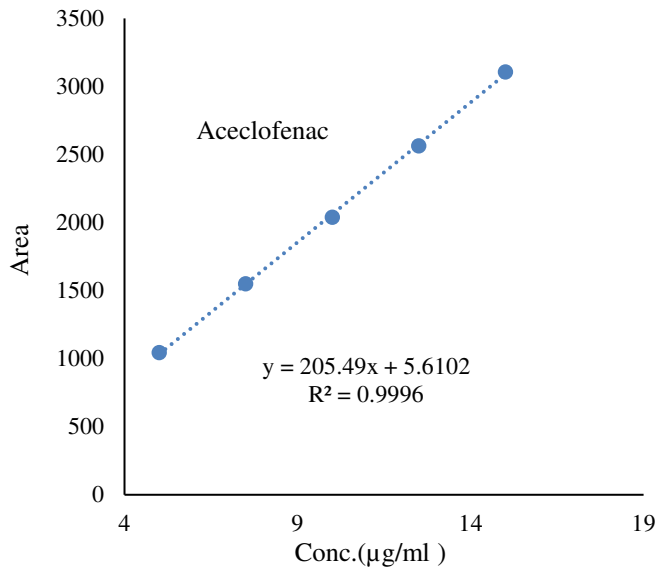
Column Performance Table (From 50% - Assay(Spl05)_ACECLOFENAC+TIZANIDINE

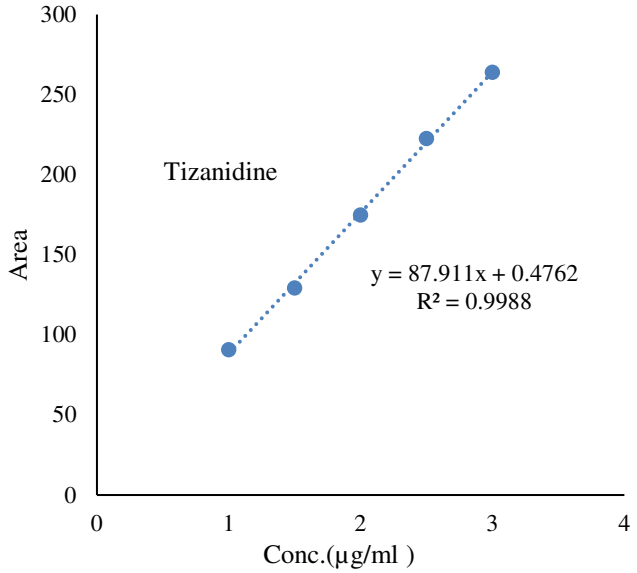
	Reten. Time	W05 [min]	Asymmetry [-]	Efficiency [th.pl]	Eff/l [t.p./m]	Resolution [-]
1	2.517	0.103	1.308	3280	65722	-
2	4.387	0.123	1.121	7008	140168	9.709

The amount of Aceclofenac and Tizanidine present in the taken dosage form was found to be 100.27% and 102.61 % respectively.

Linearity of Aceclofenac and Tizanidine

S. No.	Aceclofenac		Tizanidine	
	Conc.(µg/ml)	**Area**	**Conc.(µg/ml)**	**Area**
1	5	1044.606	1	90.783
2	7.5	1549.853	1.5	129.306
3	10	2038.421	2	174.849
4	12.5	2563.186	2.5	222.682
5	15	3106.591	3	263.873

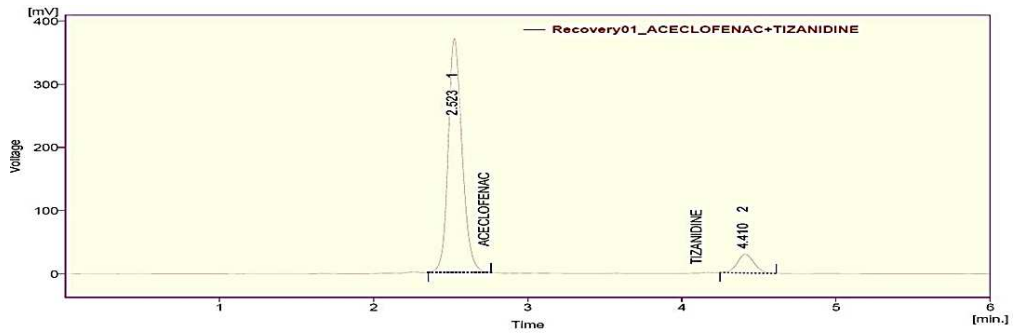




The correlation coefficient for linear curve obtained between concentration vs. Area for standard preparations of aceclofenac and tizanidine is 0.99 and 0.98. The relationship between the concentration of aceclofenac and tizanidine and area of aceclofenac and tizanidine is linear in the range examined since all points lie in a straight line and hence the correlation coefficient is well within limits.

Accuracy results for Aceclofenac and Tizanidine

Recovery level	**Accuracy ACECLOFENAC**				**Accuracy TIZANIDINE**			
	Amount taken (mcg/ml)	**Area**	**% Recovery**	**Average % Recovery**	**Amount taken (mcg/ml)**	**Area**	**% Recovery**	**Average % Recovery**
50%	5	2326.313	96.27	100.28	1	224.953	103.20	102.28
	5	2211.866			1	280.286		
	5	2194.643			1	280.051		
100%	10	2608.241	101.86		2	236.388	97.60	
	10	2609.160			2	234.158		
	10	2605.517			2	242.445		
150%	15	3112.744	102.72		3	3112.744	102.72	
	15	3109.681			3	267.053		
	15	3106.682			3	269.916		

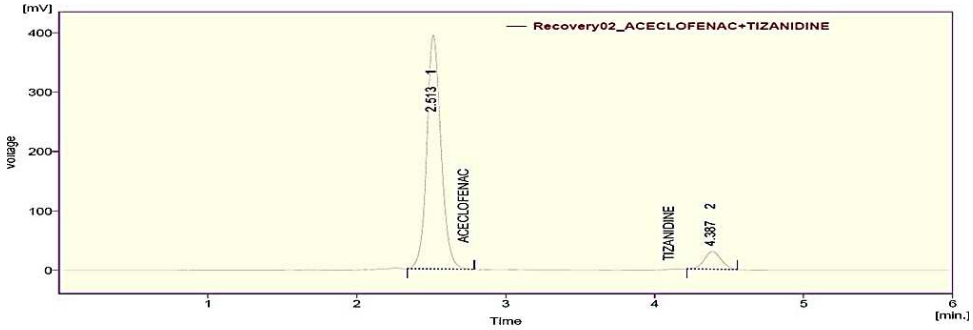


Result Table (Uncal - Recovery01_ACECLOFENAC+TIZANIDINE

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]
1	2.523	2326.313	369.993	91.18
2	4.410	224.953	29.782	8.82
	Total	2551.266	399.775	100.00

Column Performance Table (From 50% - Recovery01_ACECLOFENAC+TIZANIDINE

	Reten. Time	W05 [min]	Asymmetry [-]	Efficiency [th.pl]	Eff/l [t.p./m]	Resolution [-]
1	2.523	0.097	1.375	3775	75498	-
2	4.410	0.120	1.125	7482	149642	10.248

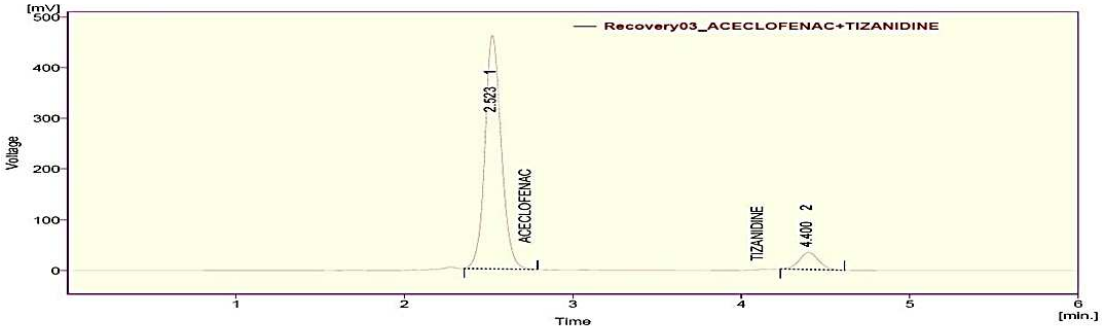


Result Table (Uncal - Recovery02_ACECLOFENAC+TIZANIDINE

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]
1	2.513	2609.160	393.667	91.76
2	4.387	234.158	29.908	8.24
	Total	2843.318	423.574	100.00

Column Performance Table (From 50% - Recovery02_ACECLOFENAC+TIZANIDINE

	Reten. Time	W05 [min]	Asymmetry [-]	Efficiency [th.pl]	Eff/l [t.p./m]	Resolution [-]
1	2.513	0.103	1.222	3277	65548	-
2	4.387	0.123	1.088	7008	140168	9.726



Result Table (Uncal - Recovery03_ACECLOFENAC+TIZANIDINE

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]
1	2.523	3106.682	460.815	92.02
2	4.400	269.243	33.705	7.98
	Total	3375.925	494.519	100.00

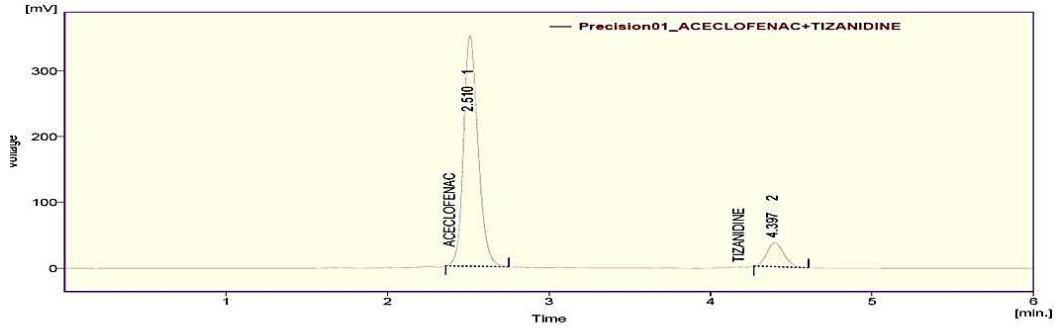
Column Performance Table (From 50% - Recovery03_ACECLOFENAC+TIZANIDINE

	Reten. Time	W05 [min]	Asymmetry [-]	Efficiency [th.pl]	Eff/l [t.p./m]	Resolution [-]
1	2.523	0.107	1.179	3100	62006	-
2	4.400	0.123	1.057	7051	141021	9.602

The percentage mean recovery should lie between 98 to 103 % and the percentage mean recovery of aceclofenac and tizanidine is 100.28 % and 102.25 % respectively

Results for Method Precision of Aceclofenac and Tizanidine

S. No.	ACECLOFENAC		TIZANIDINE	
	Rt	Area	Rt	Area
1	2.510	2192.147	4.397	267.545
2	2.523	2322.573	4.410	211.442
3	2.523	2321.138	4.413	202.102
4	2.523	2333.196	4.413	200.853
5	2.507	2350.119	4.397	202.888
6	2.497	2341.355	4.390	198.551
Avg	2.513833	2310.088	4.403333	213.8968
stdev	0.010926	58.82541	0.009893	21.512
%RSD	0.433746	1.021	0.224216	0.0213

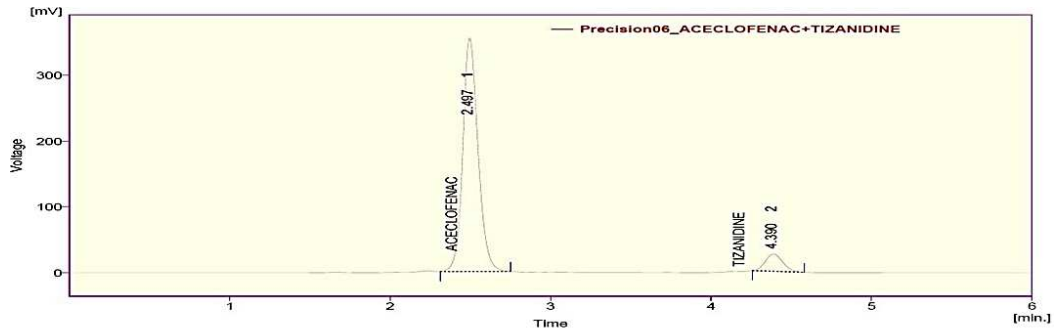


Result Table (Uncal - Precision01_ACECLOFENAC+TIZANIDINE

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]
1	2.510	2192.147	350.818	89.12
2	4.397	267.545	36.508	10.88
	Total	2459.691	387.325	100.00

Column Performance Table (From 50% - Precision01_ACECLOFENAC+TIZANIDINE

	Reten. Time	W05 [min]	Asymmetry [-]	Efficiency [th.pl]	Eff/l [t.p./m]	Resolution [-]
1	2.510	0.097	1.375	3735	74702	-
2	4.397	0.117	1.241	7868	157360	10.408



Result Table (Uncal - Precision06_ACECLOFENAC+TIZANIDINE

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]
1	2.497	2341.355	354.527	92.18
2	4.390	198.551	26.362	7.82
	Total	2539.905	380.888	100.00

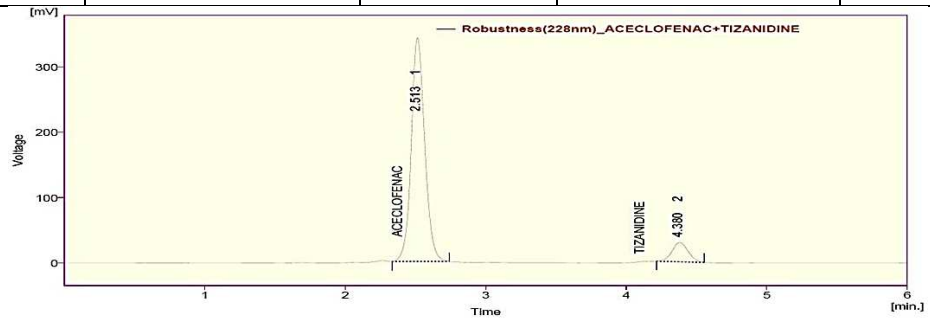
Column Performance Table (From 50% - Precision06_ACECLOFENAC+TIZANIDINE

	Reten. Time	W05 [min]	Asymmetry [-]	Efficiency [th.pl]	Eff/l [t.p./m]	Resolution [-]
1	2.497	0.103	1.269	3234	64681	-
2	4.390	0.123	1.200	7019	140381	9.830

The test results for Aceclofenac and tizanidine are showing that the %RSD within the acceptable limits.

Result of Robustness study

Parameter	ACECLOFENAC		TIZANIDINE	
	Retention time (min)	Tailing factor	Retention time (min)	Tailing factor
Flow Rate				
0.8 ml/min	3.130	1.258	5.443	1.167
1.2 ml/min	2.090	1.036	3.663	0.943
Wavelength				
233nm	2.513	1.222	4.380	1.088
237nm	2.517	1.179	4.380	1.125

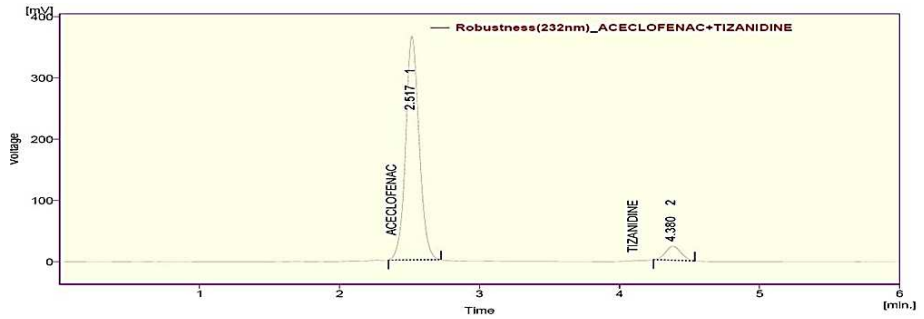


Result Table (Uncal - Robustness(228nm)_ACECLOFENAC+TIZANIDINE

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]
1	2.513	2284.209	342.721	90.61
2	4.380	236.634	29.883	9.39
	Total	2520.843	372.604	100.00

Column Performance Table (From 50% - Robustness(228nm)_ACECLOFENAC+TIZANIDINE

	Reten. Time	W05 [min]	Asymmetry [-]	Efficiency [th.pl]	Eff/l [t.p./m]	Resolution [-]
1	2.513	0.103	1.222	3277	65548	-
2	4.380	0.127	1.088	6624	132484	9.551



Result Table (Uncal - Robustness(232nm)_ACECLOFENAC+TIZANIDINE

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]
1	2.517	2454.896	364.747	93.35
2	4.380	174.802	22.773	6.65
	Total	2629.697	387.519	100.00

Column Performance Table (From 50% - Robustness(232nm)_ACECLOFENAC+TIZANIDINE

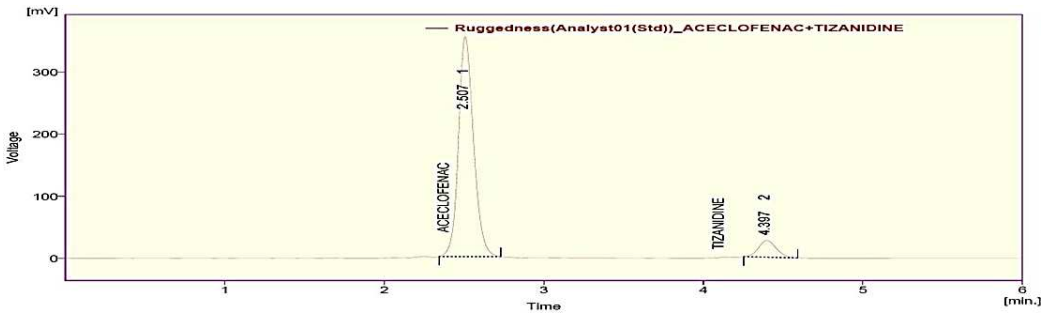
	Reten. Time	W05 [min]	Asymmetry [-]	Efficiency [th.pl]	Eff/l [t.p./m]	Resolution [-]
1	2.517	0.103	1.179	3286	65722	-
2	4.380	0.123	1.125	6987	139742	9.674

From the observation it was found that the system suitability parameters were within the acceptable limits at all variable conditions.

Results for Ruggedness

ACECLOFENAC	% Assay	**TIZANIDINE**	% Assay
Analyst 01	99.36%	Analyst 01	96.28%
Anaylst 02	99.30%	Anaylst 02	95.97%

From the observation between the two analysts, it has been found out that, the percentage relative standard deviation should not greater than 2.0%, hence the method is considered as rugged.

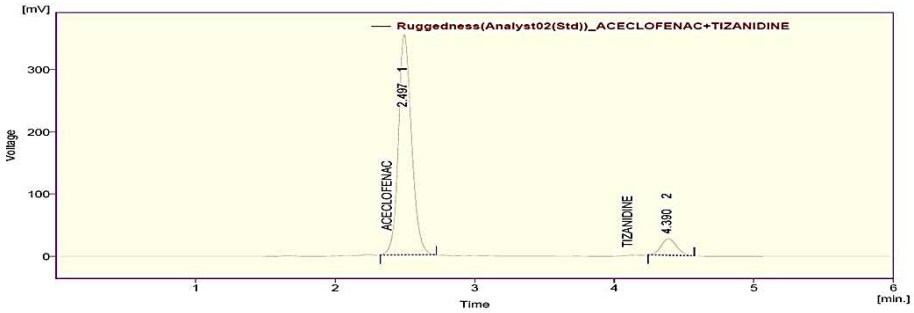


Result Table (Uncal - Ruggedness(Analyst01(Std))_ACECLOFENAC+TIZANIDINE

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]
1	2.507	2319.879	353.740	91.75
2	4.397	208.704	27.066	8.25
	Total	2528.583	380.806	100.00

Column Performance Table (From 50% - Ruggedness(Analyst01(Std))_ACECLOFENAC+TIZANIDINE

	Reten. Time	W05 [min]	Asymmetry [-]	Efficiency [th.pl]	Eff/l [t.p./m]	Resolution [-]
1	2.507	0.103	1.360	3260	65201	-
2	4.397	0.123	1.226	7040	140808	9.813



Result Table (Uncal - Ruggedness(Analyst02(Std))_ACECLOFENAC+TIZANIDINE

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]
1	2.497	2327.917	354.012	91.88
2	4.390	205.718	26.797	8.12
	Total	2533.635	380.809	100.00

Column Performance Table (From 50% - Ruggedness(Analyst02(Std))_ACECLOFENAC+TIZANIDINE

	Reten. Time	W05 [min]	Asymmetry [-]	Efficiency [th.pl]	Eff/l [t.p./m]	Resolution [-]
1	2.497	0.100	1.269	3453	69065	-
2	4.390	0.123	1.125	7019	140381	9.977

The HPLC Technique is a very powerful separation tool in the determination of aceclofenac and tizanidine in pharmaceutical dosage forms. The analysis has been performed with various mobile phases which include, phosphate buffer: Acetonitrile: Methanol, and Triethylamine: Acetonitrile, which have provided optimization.

Post optimization, validation parameters including, linearity and range, accuracy, precision, robustness, ruggedness, have been carried out according to ICH guidelines and indicated the procedure to be valid, specific, accurate and more easily reproducible within the limits, agreeable with the label claim. The low values of %RSD for method precision suggested that the method is precise. Linearity evaluated for the analyte peak showed a good linear response over a wide range of concentration

CONCLUSION

From the above experimental results and parameters it was concluded that, this newly developed method for the simultaneous estimation of Aceclofenac and Tizanidine was found to be simple, precise, accurate and the higher resolution and shorter retention time makes this method more acceptable and cost effective. As the advanced research is being continued followed up by developing drug combinations and multiple dosage forms, the method which has been performed, it can be effectively applied for routine analysis in research institutions, quality control department in meant in industries, approved testing laboratories studies in near future.

FINANCIAL ASSISTANCE

Nil

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTION

Nenavath Adilakshmi and Nellore Dharani Sai Sreekanth conceived and planned the research experiment. Nenavath Adilakshmi did detailed literature review for the plan of the experiment. Nellore Dharani Sai Sreekanth had carried out the method development procedure trials until it reached optimization. Based on the trials, Nenavath Adilakshmi and Nellore Dharani Sai Sreekanth performed the validation parameters respectively, further contributing to the interpretation of final results. Nellore Dharani Sai Sreekanth took the lead in writing the manuscript with discussions with Nenavath Adilakshmi.

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