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Research Article

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Validation and Development of RP-HPLC Assay Method for Estimation of Paracetamol, Aceclofenac and Chlorzoxazone in Combined Tablet Dosage Form

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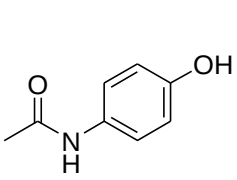
Abstract A simple, rapid, and precise reversed-phase liquid chromatographic method is developed for determination of paracetamol, aceclofenac, and chlorzoxazone in their ternary mixtures of commercial pharmaceutical preparation. This method uses C_{18} (Phenomenex) (150mm×4.6mm), 5 µm analytical column. Mobile phase is Methanol: Disodiumhydrogen orthophosphate (ph-3, 0.05M) 65:35 v/v. The instrumental settings are at a flow rate of 1.5 ml/min; the column temperature is 20°C, and detector wavelength is 271 nm. The sample concentrations are measured on weight basis to avoid the internal standard. The method is validated and shown to be linear. The correlation coefficients for paracetamol, aceclofenac, and chlorzoxazone are 0.9993, 0.9994, and 0.999 respectively. The recovery values for paracetamol, aceclofenac, and chlorzoxazone found 98.86%, 99.67%, and 99.51%, respectively. The relative standard deviation for six replicates is always less than 2%. This HPLC method is successfully applied to the quantitative analysis of the title drugs in tablets.

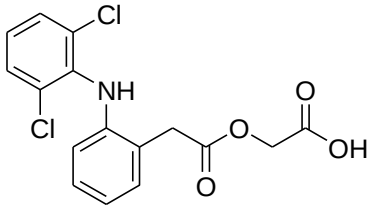
Keywords RP-HPLC, Paracetamol, Chlorzoxazone, Aceclofenac

Introduction

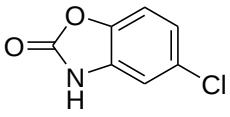
Multicomponent formulation have gained lot of importance now a days due to greater patient acceptability, increased potency, multiple action, fewer side effect.



Paracetamol



Aceclofenac



Chlorzoxazone

Aceclofenac (ACF) inhibits synthesis of the inflammatory cytokines interleukins and tumor necrosis factor and inhibits prostaglandin E_2 production. It increases glycosaminoglycans (GAG) synthesis, the principle macromolecule of the extracellular matrix which aids in repair and regeneration of articular cartilage. Thus, has positive effect on cartilage anabolism combined with modulating effect of matrix catabolism. Chlorzoxazone (CLZ) primarily acts at the level of the spinal cord and subcortical area of the brain where it inhibits multisynaptic areas

resulting in reduction of skeletal muscle spasm with relief of pain and increased mobility of the involved muscles. Paracetamol (PCM) has analgesic and antipyretic action with weak anti-inflammatory activity. These effects are related to inhibition of prostaglandins synthesis.

Materials and methods

Chromatographic separation was performed with schimadzoo high performance liquid chromatography having C18, Phenomenex, (150mm × 4mm), 5µm analytical column with photodiode array detector. Chromatographic data were recorded by LC Solution software.

Standard preparation of Aceclofenac

20 mg of Aceclofenac WS was weighed and transferred to100 ml of volumetric flask, dissolved it and volume made with methanol, mixed well. Again diluted 2 ml of it to 50 ml with mobile phase.

Standard preparation of Paracetamol

100 mg of paracetamol WS was weighed and transferred to 100 ml of Volumetric Flask, dissolved and volume made with methanol, mixed and further diluted 2 ml to 50 ml with mobile phase.

Standard preparation of Chlorzoxazone

75 mg of chlorzoxazone WS WS was weighed and transferred to 100 ml of volumetric flask, dissolved and volume made with methanol, mixed well and further diluted 2 ml to 50 ml with mobile phase.

Sample preparation

Sample equivalent to 50 mg of Paracetamol, 75 mg of chlorzoxazone and 20 mg of Aceclofenac was weighed and transferred to 100 ml of volumetric flask. Dissolve & make it up to volume with methanol, mix well and further dilute 2 ml to 50 ml with mobile phase.

Table 1: Optimised Condition

Column	C18 (150*4.6), 5mm (Brava BDS)
Mobile Phase	Methanol: Disodiumhydrogen orthophosphate (50:50 v/v)
Flow rate	1.5 ml/min
Column Temperature	25 ^{o}C
Detection	271
Injection vol.	20µl
Run time	5 min

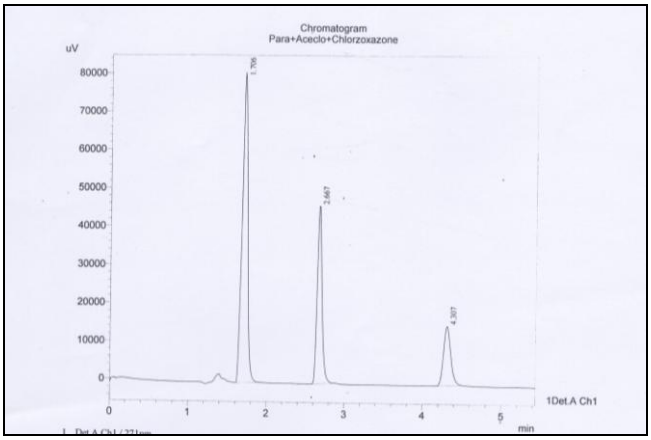


Figure 1: Chromatogram of Aceclofenac, Chlorzoxazone, and Paracetamol

Chromatographic conditions: For HPLC a number of preliminary trials were conducted with combinations of different organic solvents, compositions, and flow rate to check the retention time, shape, resolution, and other chromatographic parameters. Among all tried experiments, the mobile phase combination of Mobile phase is methanol: Disodiumhydrogen orthophosphate (pH 3, 0.05 M) 65:35 v/v. The instrumental settings are at a flow rate of 1.5 ml/min; the column temperature is 20 °C, and detector wavelength is 271 nm found to be most suitable. Best resolution and sensitivity of the method were obtained for ACF, PCM and CLZ. Typical chromatogram with optimized condition gives sharp and symmetric peak with retention time of 7 min.

Results and Discussion

System suitability:

The standard solution was analyzed 6 times as per chromatographic conditions and inject at the start of study and acceptance criteria are as follows:

Table 2: Acceptance criteria

Name of compound	Mean peak area	%RSD of peak area	Mean theoretical plates of peak	Mean tailing factor of peak
Aceclofenac	212477	1.05	2217	1.19
Paracetamol	839586.2	1.06	7334	1.1
Chlorzoxazone	417749	1.08	9550	1.09
Limit	NA	NMT 2.0	NLT 2000	NMT 2.0

Linearity

For Paracetamol

The linearity was determined at 4 levels over the range of 70% to 130 % of the sample concentration. The graph of mean area versus concentration in µg/ml was plotted and regression equation was determined.

Table 3: Linearity data for Paracetamol

S. No.	Concentration (µg/ml)	Mean Peak Area
1	20	394788
2	40	821311
3	60	1216269
4	80	1598203
Slope		19996
Y-intercept		7342
R^2		0.9993

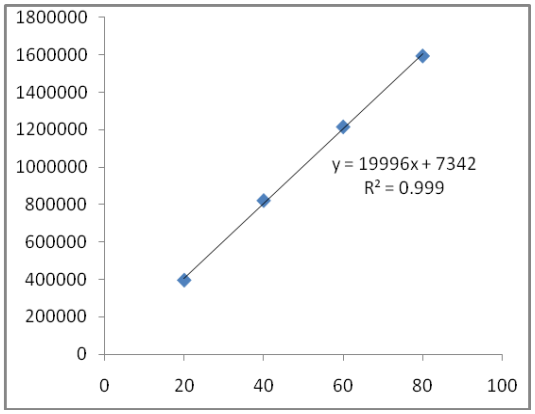


Figure 2: *Linear graph of Paracetamol*

Table 4: Linearity data for Aceclofenac

S. No.	Concentration (µg/ml)	Mean Peak Area
1	4	96840
2	8	206203
3	12	303118
4	16	403804
Slope		25445
Y-intercept		-1960.5
R^2		0.9994

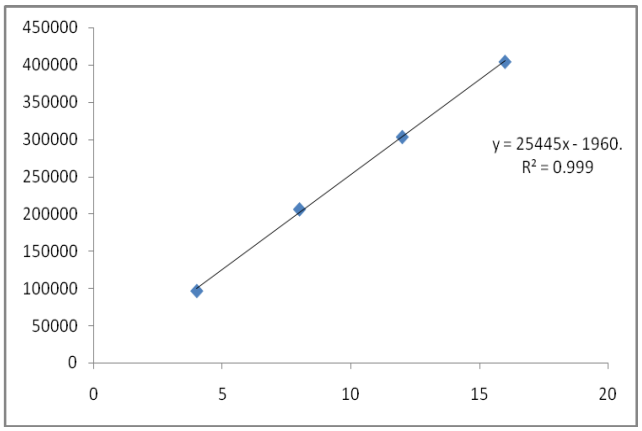


Figure 3: *Linear graph of Aceclofenac*

Table 5: Linearity data of Chlorzoxazone

Sr. No.	Concentration (µg/ml)	Mean Peak Area
1	15	199073
2	30	417732
3	45	615880
4	60	804452
Slope		13415
Y-intercept		5714
R^2		0.999

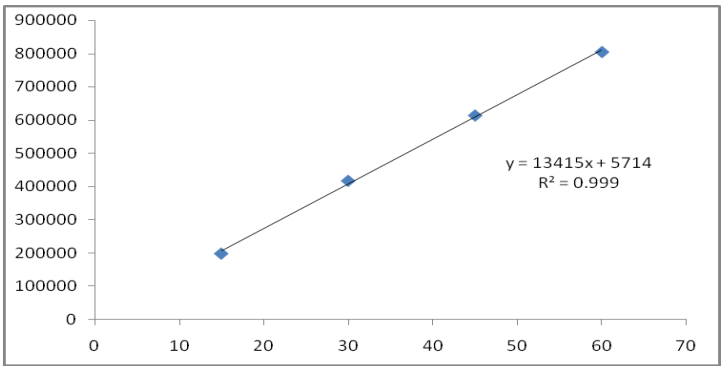


Figure 4: *Linear graph of chlorzoxazone*

Acceptance criteria

- The correlation coefficient should not be less than 0.999.
- %RSD of response factor should not be more than 2.00.

Results:

- The correlation coefficient was found to be well within limit.
- % RSD of response factor was found to be well within limit.

Accuracy

The accuracy of method was checked by recovery of Aceclofenac, Paracetamol and Chlorzoxazone tablet from 3 placebo preparation accurately spiked with three concentration of active ingredient. The result is reported in table. Result indicate that there no significant difference between the calculated percentage recovery and actual percentage value.

Table 6: Accuracy data for Aceclofenac

Obs. No.	Concentration selected	Concentration of Std added	Area of Spike solution				%Recovery
			I	II	III	Avg.	
1.	4 µg/ml	2 µg/ml	158063	157682	161012	158919	100.56%
2.	4 µg/ml	4 µg/ml	212542	211226	205848	209872	98.75%
3.	4 µg/ml	6 µg/ml	268012	265672	259071	264251.67	99.47%
Mean							99.59
SD							0.9113
RSD (Limit: NMT 2%)							0.92%

Table 7: Accuracy data for Paracetamol

Obs. No.	Concentration selected	Concentration of Std added	Area of Spike solution				%Recovery
			I	II	III	Avg.	
1.	20 µg/ml	10 µg/ml	631026	628097	621824	626982.3	99.60%
2.	20 µg/ml	20 µg/ml	829298	838124	840882	836101.3	99.62%
3.	20 µg/ml	30 µg/ml	1049121	1024568	1036874	1036854	98.30%
Mean							98.84%
SD							0.6919
RSD (Limit: NMT 2%)							0.70%

Table 8: Accuracy data for Chlorzoxazone

Obs. No.	Concentration selected	Concentration of Std added	Area of Spike solution				%Recovery
			I	II	III	Avg.	
1.	15 µg/ml	7.5 µg/ml	314478	313256	312048	3132607	99.97%
2.	15 µg/ml	15 µg/ml	465648	416472	418380	416813.3	98.76%
3.	15 µg/ml	22.5 µg/ml	522023	517892	519080	519665	99.51%
Mean							99.41%
SD							0.6107
RSD (Limit: NMT 2%)							0.61%

Acceptance criteria

- Recovery of Drugs should be between 97.0% and 103.0%.
- %Relative standard deviation for recovery at each level should not be more than 3.00.
- Overall %relative standard deviation for all the levels should not be more than 3.00.

Results:

- Recovery of Drugs for all the levels was found to bewithin the limit.
- %Relative standard deviation for %recovery at each level and overall %relative standard deviation for all the levels was found to be within the limit.
- Results indicated that calculated percentage recovery was found well within the acceptance criteria.

Conclusion

The proposed HPLC method was sufficiently sensitive and reproducible for the analysis of Paracetamol, Aceclofenac and chlorzoxazone Tablet formulation dosage forms within a short analysis time. The method was proved to be superior to most of the reported methods. The mobile phases was simple to prepare and economical. The sample recoveries in the formulation were in good agreement with their respective label claims and they suggested non-interference of formulation excipients in the estimation. Hence the proposed method was found to be rapid, accurate, precise, specific, robust and economical.

Acknowledge

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