



# Simultaneous Determination of Paracetamol, Chlorzoxazone and Diclofenac Sodium in Tablet Dosage Form by High Performance Liquid Chromatography

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**Abstract:** A simple, precise and rapid isocratic reverse phase high performance liquid chromatographic method was developed for the simultaneous determination of paracetamol, chlorzoxazone and diclofenac sodium from tablet dosage form. The chromatographic separation was performed on an inertsil C<sub>18</sub> column (250 mm × 4.6 mm i.d 5 μm particle size). Mobile phase consisted of a mixture of phosphate buffer (0.02 M KH<sub>2</sub>PO<sub>4</sub>, pH adjusted to 3.7 using orthophosphoric acid), acetonitrile and methanol in the ratio of (25: 25: 50) at a flow rate of 1.0 mL/min. The wavelength was set at 220 nm. The proposed method was validated for linearity, accuracy, precision, LOD and LOQ. The calibration was linear over the range of 50-150 μg/mL for paracetamol, 50-150 μg/mL for chlorzoxazone and 5-15 μg/mL for diclofenac sodium. The retention times were found as 2.8 min for paracetamol, 4.2 min for chlorzoxazone and 6.4 min for diclofenac sodium. The method can be easily adopted for quality control analysis.

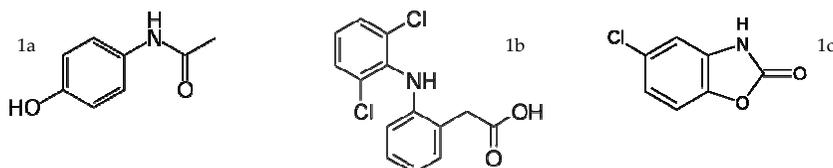
**Keywords:** Paracetamol, Chlorzoxazone, Diclofenac, RP-HPLC, Validation

## Introduction

Paracetamol (*p*-hydroxy acetanilide) is a compound (Figure 1a) with analgesic and antipyretic properties. It is much safer than aspirin in terms of gastric irritation, ulceration and bleeding<sup>1,2</sup>. Diclofenac sodium [2-[(2,6-dichlorophenyl)amino]benzene acetic acid monosodium salt] is a compound (Figure 1b) with potent anti-inflammatory property. It affords quick relief of pain and wound edema<sup>3,4</sup>. Chlorzoxazone (5-chloro-2(3*H*)-benzoxazolone) is a compound (Figure 1c) with skeletal muscle relaxant property. It is used to decrease muscle tone and tension and thus to relieve spasm and pain associated with musculoskeletal

disorders<sup>5,6</sup>. Rao *et al.* developed a method for simultaneous estimation of paracetamol and diclofenac sodium in pharmaceutical preparations<sup>7</sup>. Zarapkar *et al.* developed a reverse phase HPLC method for the simultaneous estimation of paracetamol and chlorzoxazone<sup>8</sup>.

Literature survey reveals that various analytical techniques *viz.*, UV spectrophotometry<sup>9-11</sup> and supercritical fluid chromatographic method (SFC)<sup>12</sup>. Few HPLC methods<sup>13-17</sup> have been reported for the simultaneous determination of paracetamol, chlorzoxazone and diclofenac sodium. The aim of the present work was to develop and validate the rapid and sensitive high performance liquid chromatography (HPLC) method for simultaneous determination of paracetamol (PCM), chlorzoxazone (CHZ) and diclofenac (DCF) sodium in tablets.



**Figure 1.** Chemical structures of (1a) paracetamol (1b) diclofenac (1c) chlorzoxazone

## Experimental

All chemicals were of analytical grade. Paracetamol, chlorzoxazone and diclofenac sodium working standard were obtained from JENBURKT Pharmaceutical India limited with the certificates of analysis. Methanol (HPLC grade), acetonitrile (HPLC grade), potassium dihydrogen phosphate buffer (analytical grade) and orthophosphoric acid were purchased from Merck (Mumbai, India)

### Standard stock preparation

50 mg of Paracetamol and 50 mg of chlorzoxazone and 5 mg of diclofenac sodium were accurately weighed and transferred to a 100 cm<sup>3</sup> volumetric flask. It was dissolved in a minimum quantity of methanol and then diluted up to the mark with methanol. The concentration of the solution obtained was 500 µg/mL for Paracetamol, 500 µg/mL for chlorzoxazone and 50 µg/mL for diclofenac sodium (Solution A). 2 cm<sup>3</sup> of this solution A was diluted to 10 cm<sup>3</sup> in a volumetric flask with mobile phase. The concentration of the solution obtained was 100 µg/mL for paracetamol, 100 µg/mL for chlorzoxazone and 10 µg/mL for diclofenac sodium.

### Preparation of Sample solution

Twenty tablets were weighed and their average weight was calculated. These tablets were powdered and weight equivalent to one tablet containing 500 mg of paracetamol and 500 mg of chlorzoxazone and 50 mg of diclofenac sodium was taken in a 100 mL dilution flask. Then about 50 mL of diluent was added to it. Then sonicated for 20-25 min at an ambient temperature with intermittent swirling, cooled and diluted up to the mark with diluent, mixed well. Then solution from the flask was filtered through syringe filter. This solution was used for further analysis.

### Chromatographic conditions

The chromatography was performed using waters HPLC system having Waters 501 isocratic pump equipped with Waters<sup>TM</sup> 717 plus autosampler and a Waters 486 tunable absorbance UV-detector. The data was recorded using millenium<sup>32</sup> chromatographic software. Separation was performed on a 250 mm × 4.6 mm i.d., 5 µ particle size Inertsil C18 column. Mobile phase consisted of a mixture of buffer: acetonitrile: methanol (25: 25: 50), pH 3.7 adjusted with orthophosphoric acid. Flow rate was kept at 1.0 mL/min. Wavelength was set at 220 nm.

### *Method validation*

The method was validated as per ICH guidelines for specificity, linearity, quantification limit, precision, accuracy, recovery and stability. Specificity was investigated by analyzing the blank diluents and samples of 100% level for any interference of the excipients at the retention times of PCM, CHZ and DCF. The accuracy of the method was determined by recovery experiments. The precision of the method was demonstrated by interday and intraday variation studies, six repeated injections of standard and sample were made and percentage RSD was calculated. In the intraday variation studies six repeated injections of standard and sample solution was carried out by injecting on the same day at different intervals and percentage RSD was calculated. In the inter day variation studies six repeated injections of standard and sample solution were made for three consecutive days and percentage RSD was calculated. The linearity of the method was demonstrated at seven concentration levels of the mixed standards of PCM, CHZ and DCF.

## **Results and Discussion**

### *Optimization of the chromatographic conditions*

In order to develop an isocratic reverse phase HPLC method for the determination of PCM, CHZ and DCF in combined dosage form the chromatographic conditions were optimized. For better separation and resolution the different buffers were tried. It has been found that potassium dihydrogen phosphate buffer, pH 3.7 adjusted with orthophosphoric acid gave better peak shape than other buffers. The different compositions of mobile phase were changed for getting better separation of analytes. Thus the mobile phase composed of the mixture of buffer (0.02 M  $\text{KH}_2\text{PO}_4$ , pH 3.7 adjusted with orthophosphoric acid) acetonitrile, methanol in the ratio of (25: 25: 50 v/v) was finalized. The better separation, peak symmetry and reproducibility were obtained with Inertsil C18, 250 mm x 4.6 mm, 5  $\mu\text{m}$  column compared to thermo BDS hypersil C8, 150 mm x 4.6 mm, 5  $\mu\text{m}$  column. Both the analytes were gave better response at 265 nm wavelength using UV detector. The flow rate kept was 1.0 mL/min. There was no peak tailing observed under these optimized chromatographic conditions. The retention times of PCM, CHZ and DCF were found to be 2.8 min, 4.2 and 6.4 min respectively.

### *Validation*

The proposed method was showed short elution time and good separation between PCM, CHZ and DCF. The system suitability test was performed as per the USP and international conference of harmonization (ICH) guidelines to confirm the suitability and the reproducibility of the method. Six consecutive injections of the standard solution were performed and evaluated for repeatability, tailing factor, theoretical plates and resolution. %RSD values were found to be 0.52, 0.40 and 0.50 for PCM, CHZ and DCF respectively. The tailing factor and theoretical plates were found to be perfectly within the limits.

The method was linear over the range 50-150  $\mu\text{g/mL}$  for paracetamol, 50-150  $\mu\text{g/mL}$  for chlorzoxazone and 5-15  $\mu\text{g/mL}$  for diclofenac sodium. The calibration curve was constructed by plotting response factor against concentration of drugs. The slope and intercept value for calibration curve was  $Y = 28594x + 34014$  ( $r^2 = 0.9995$ ) for paracetamol,  $Y = 43904x + 3130$  ( $r^2 = 0.9993$ ) for chlorzoxazone and  $Y = 69489x + 30886$  ( $r^2 = 0.9994$ ) for diclofenac sodium the results shows that an excellent correlation between response factor and concentration of drugs.

The limit of detection (LOD) and limit of quantification (LOQ) of the developed method were determined by injecting progressively low concentrations of the standard solutions using the developed RP-HPLC method. The LOD is the smallest concentration of

the analyte that gives a measurable response (signal to noise ratio of 3). The limit of quantification (LOQ) and limit of detection (LOD) was established at a signal-to-noise ratio. The LOQ and LOD of PCM, CHZ and DCF were experimentally determined. The LOD of PCM, CHZ and DCF was found to be 0.0020 µg/mL, 0.040 µg/mL and 0.025 µg/mL respectively. The LOQ of PCM, CHZ and DCF was found to be 0.0065 µg/mL, 0.085 µg/mL and 0.070 µg/mL respectively.

The system precision study was performed to determine the repeatability of the method. Six samples of standard were prepared at 100% level and assayed according to the procedure. The method precision study was performed to determine the reproducibility of the method. Six samples of tablets were prepared at 100% level and assayed according to the procedure. The accuracy of the method was determined by the standard addition method at three different levels. The sample solution of 100% level was considered as a zero level and 10, 20 and 30% of the standard drug of analytes were added respectively. Each determination was performed in triplicates. The accuracy was then calculated as the percentage of the standard drug recovered by the recovery study. Mean recoveries for paracetamol, chlorzoxazone and diclofenac sodium from the combination formulation are shown in Table 1. The results are well within the acceptance limit and hence the method is accurate. RSD values were found to be 0.52, 0.40 and 0.50 for PCM, CHZ and DCF respectively. The tailing factor and theoretical plates were found to be perfectly within the limits.

**Table 1.** % Recovery of paracetamol, chlorzoxazone and diclofenac sodium

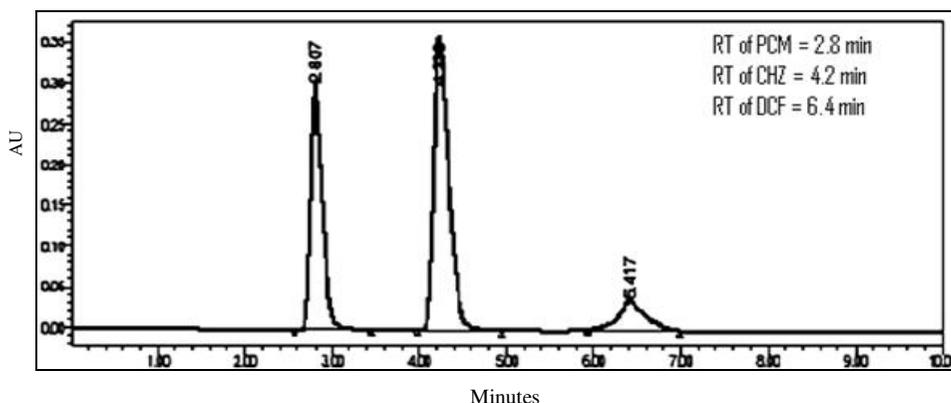
Amount of paracetamol, mg								
S. No	% Added	Original amount	Added amount	Total amount	Mean (n = 5)	% Recovery	S.D	%RSD
1	0	100	0	100	100.25	100.25	0.452	0.451
2	10	100	10.33	110.33	110.14	99.82	0.302	0.303
3	20	100	20.33	120.33	119.85	99.6	0.275	0.276
4	30	100	30.43	130.43	130.76	100.25	0.347	0.346
Amount of chlorzoxazone, mg								
1	0	100	0	100	99.59	99.59	0.376	0.378
2	10	100	10.47	110.47	110.94	100.43	0.409	0.407
3	20	100	20.34	120.34	120.1	99.80	0.26	0.261
4	30	100	30.42	130.42	131.05	100.48	0.334	0.332
Amount of diclofenac sodium, mg								
1	0	10	0	10	9.96	99.56	0.532	0.534
2	10	10	1.067	11.067	10.92	98.67	0.543	0.550
3	20	10	2.062	12.062	11.88	98.49	0.621	0.631
4	30	10	3.133	13.133	12.94	98.53	0.524	0.532

The stability of both the standard and the sample was determined by monitoring the peak area responses of the standard solution and the sample solution of PCM, CHZ and DCF at 6, 12 and 24 hours at room temperature. The results showed that there were no significant differences. The results are shown in Table 2.

The specificity of the method was determined by exposing 100% sample solution of PCM, CHZ and DCF. The Chromatogram of the sample solution shows that there should not be any interference of the placebo at the retention times of the analytes. It is shown in Figure 2.

**Table 2.** Solution stability of paracetamol, chlorzoxazone and diclofenac sample solution

Conditions	Level in %	Peak area	% Assay in mg/tab	% Label claim	% Relative Deviation
Paracetamol					
Initial-0 h	100	2894498	503.79	100.76	-
Initial-2 h	100	2898223	504.43	100.89	0.13
Initial-12 h	100	2890124	503.02	100.60	-0.28
Initial-24 h	100	2841879	494.63	98.93	-1.67
	Mean	2881181.0	501.47	100.29	
	S.D.	22871.4	3.98	0.8	
	% RSD	0.79	0.79	0.79	
Chlorzoxazone					
Initial-0 h	100	4405874	502.00	100.40	-
Initial-2 h	100	4282241	499.31	99.86	-0.54
Initial-12 h	100	4387587	499.92	99.98	0.12
Initial-24 h	100	4356781	496.41	99.28	-0.70
	Mean	4383120.8	499.41	99.88	
	S.D.	17551.5	2.00	0.4	
	% RSD	0.40	0.40	0.40	
Diclofenac					
Initial-0 h	100	710152	49.61	99.23	-
Initial-2 h	100	712564	49.78	99.57	0.34
Initial-12 h	100	709863	49.59	99.19	-0.38
Initial-24 h	100	709214	49.55	99.10	-0.09
	Mean	710448.3	49.64	99.27	
	S.D.	1267.9	0.09	0.18	
	% RSD	0.18	0.18	0.18	



**Figure 2.** Chromatogram of paracetamol, chlorzoxazone and diclofenac sodium in 100% sample solution

### Method applications

The validated HPLC method was applied to the simultaneous determination of PCM, CHZ and ACF in tablet dosage form. The samples were analysed and the assay results are as per the label claim shown in Table 3.

**Table 3.** Results of assay experiments

Drug	Label claim, mg	Amount found (n=7), mg	S.D	%RSD	% Assay
Paracetamol	500	500.38	2.03	0.4	100.08
Chlorzoxazone	500	500.74	2.2	0.44	100.15
Diclofenac	50	49.96	0.32	0.64	99.92

### Conclusion

The isocratic RP- HPLC method has proved to be simple, specific, precise and accurate and is suitable for simultaneous quantification of paracetamol, chlorzoxazone and diclofenac sodium. The proposed method gives a good resolution among the analytes. The method is very simple, rapid and no complicated sample preparation is needed. High percent of recovery shows the method is free from interference of excipients present in the formulations and the method is accurate.

### Acknowledgment

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