Simultaneous Determination of Aceclofenac, Paracetamol, and Chlorzoxazone by RP-HPLC in Pharmaceutical Dosage Form

K.A. Shaikh* and **A.B. Devkhile**

Department of Chemistry, Yeshwant Mahavidyalaya, Swami Ramanad Thirth Marathwada University, Nanded- 431602, (MS), India

Abstract

A simple, rapid, and precise reversed-phase liquid chromatographic method is developed for simultaneous determination of paracetamol, aceclofenac, and chlorzoxazone in their ternary mixtures of commercial pharmaceutical preparation. This method uses a Zorbax SB C18, 250 x 4.6 mm, 5 µm analytical column. Mobile phase is acetonitrile and buffer (40:60, v/v), buffer containing 50mM orthophosporic acid; pH of the buffer is adjusted to 6 with 10% w/v sodium hydroxide solution. The instrumental settings are at a flow rate of 1 mL/min; the column temperature is 25°C, and detector wavelength is 270 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 0.9981, 0.9990, and 0.9986, respectively. The recovery values for paracetamol, aceclofenac, and chlorzoxazone ranged from 100.7–101.4%, 100.4–101.0%, and 100.5–101.3%, respectively. The relative standard deviation for six replicates is always less than 2%. This HPLC method is successfully applied to the simultaneous quantitative analysis of the title drugs in tablets and can be applied for assay and dissolution test of tablets for the estimation of paracetamol, aceclofenac, and chlorozoxazone in their commercial samples.

Introduction

Paracetamol is chemically Acetamide, *N*-(4-hyroxyphenyl)-4'-Hydroxyacetanilide, aceclofenac is chemically [[[2-[(2,6-Dichlorophenyl)amino]phenyl]acetyl]oxy]acetic acid, and chlorozaoxzone is chemically 2(3H) benzoxazolinone, 5-chloro-2- benzoxazolinone (1–3). The tablets commercially marketed as "HIFENAC MR" by Intas Pharmaceuticals Ltd. (Matoda, Ahmedabad, India). The ingredients of the tablet contain: paracetamol, 500 mg; acelofenac, 100 mg; and chlorzoxazone, 500 mg in combination. The adequate retention and better resolution of paracetamol, aceclofenac, and chlorzoxazone peaks are achieved by using an appropriate mobile phase. Literature survey shows that there are some analytical methods available for few other ingredients in combination with paracetamol and chlorzoxazone (4–6) and (10). The methods reported for estimation of paracetamol and aceclofenac as well as paracetamol and chlorzoxazone in tablet dosage form (8,9), in spiked human plasma (7), and in bulk drug formulation (11). Yet there is not any analytical method reported in the literature for the simultaneous determination of paracetamol, aceclofenac, and chlorzoxazone in pharmaceutical dosage form. In the present research work, a reverse-phase HPLC method has been developed for simultaneous determination of paracetamol, aceclofenac, and chlorzoxazone. Though there is a difference in label claim of paracetamol, aceclofenac, and chlorzoxazone per tablet, a precise HPLC method has been developed for the estimation of title drugs.

Experimental

Materials and reagents

Paracetamol, aceclofenac, and chlorzoxazone used as working standard, were obtained from Bharati Vidhypeeth, Pune, (MS), India. Acetonitrile, orthophsporic acid, and sodium hydroxide were obtained from E. Merck (India) Ltd. Worli, Mumbai, India. The 0.45-µm nylon filters were obtained from Advanced

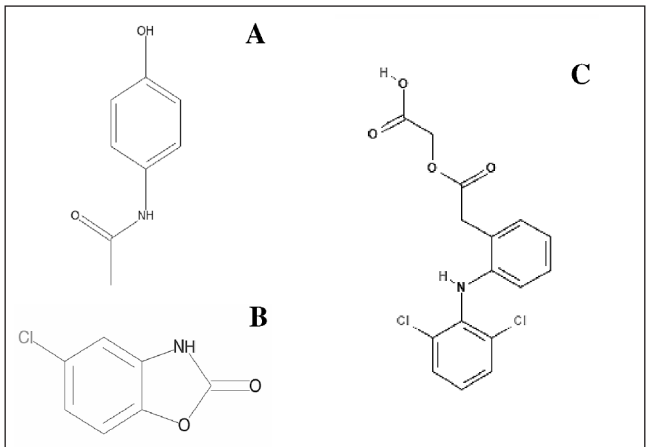

Figure 1. Chemical structures of paracetamol (A), chlorzoxazone (B), and aceclofenac (C).

* Author to whom correspondence should be addressed: email shaikh_kabeerahmed@rediffmail.com.

Microdevices Pvt. Ltd. (Chandigad, India). Double distilled water were used throughout the experiment; other chemicals were analytical and HPLC grade. Tablets were purchased from the Indian market, containing: paracetamol, 500 mg; aceclofenac, 100 mg; and chlorzoxazone, 500 mg.

Chromatographic conditions

A Jasco HPLC was utilized for experimental work, which consist of the following components: 3500 pump, AS 3000 auto sampler, and PD 1000 detector. A Zorbax SB C18 250 × 4.6 mm, 5 µm analytical column (Agilent, Palo Alto, CA) was used for the experiment. The instrumental settings are: flow rate, 1 mL/min; column oven temperature, 25°C; detector wavelength, 270 nm; and injection volume, 10 µL. Data acquisition was made with the Borwin software. The peak purity was checked with the photodiode array detector. Mobile phase consisted of acetonitrile and buffer (40:60, v/v), buffer containing 50mM orthophosporic acid with pH of buffer adjusted to 6 with 10% w/v sodium hydroxide solution. The buffer used in the mobile phase consisted of 50mM orthophoporic acid in double distilled water. The mobile phases was premixed and filtered through a 0.45-µm nylon filter and degassed.

Standard solutions

Standard solutions were prepared by dissolving the drugs in the diluents, and they were diluted to the desired concentration. Diluents used for the standard and sample preparations are as follow: diluent A: acetonitrile; diluent B: acetonitrile and buffer (40:60, v/v), buffer containing 50mM orthophosporic acid, pH of buffer is adjusted to 6 with 10% w/v sodium hydroxide solution.

Preparations of standard solutions

Paracetamol: 125 mg of paracetamol was accurately weighed, transferred into 50 mL volumetric flask, dissolved with 10 mL diluent A, and diluted up to the mark with diluent B (2500 µg/mL).

Aceclofenac: 25 mg of aceclofenac was accurately weighed, transferred into a 50-mL volumetric flask, dissolved with 10 mL diluent A, and diluted up to mark with diluent B (500 µg/ mL).

Chlorzoxazone: 125 mg of chlorzoxazone was accurately weighed, transferred into a 50-mL volumetric flask, dissolved with 10 mL diluent A, and diluted up to the mark with diluent B (2500 µg/mL).

A mixed standard solution was prepared from the listed stock solutions by transferring 1 mL of aceclofenac, 1 mL of paracetamol, and 1 mL of chlorzoxazone into a 50-mL volumetric flask; they were diluted with diluent B. This solution contains 50 µg/mL of paracetamol, 10 µg/mL of aceclofenac, and 50 µg/mL of chlorzoxazone. From the listed stock solutions of paracetamol, aceclofenac, and chlorzoxazone, solutions were prepared for the calibration curve, containing the concentration range between 2.5–15 µg/mL of aceclofenac, 12.5–75 µg/mL of paracetamol, and 12.5–75 µg/mL of chlorzoxazone in each calibration level.

Preparation of sample solutions

Ten tablets were weighed and finely powdered. A quantity equivalent to one tablet containing 500 mg paracetamol, 100 mg aceclofenac, and 500 mg chlorzoxazone was transferred in to 100-mL volumetric flask. To this flask, 20 mL diluent A was added and sonicated for 10 min with continuous shaking; the solution was cooled to ambient temperature and diluted up to the mark with diluent B. This solution was centrifuged at 10000 rpm for 5 min. From the centrifuged solution, 1 mL clear solution was transferred into 100-mL volumetric flask and diluted up to mark with diluent B.

Table I. Results of the Linearity and Precision Study*

Ingredient	Precision (%RSD†)	Linearity (µg/mL)	Linear regression equation with coefficient of correlation
Paracetamol	1.21	12.5–100.0	$Y = 193152x + 44549 = 0.9981$
Aceclofenac	1.59	2.5–20.0	$Y = 34294x + 1763 = 0.9990$
Chlorzoxazone	1.46	12.5–100.0	$Y = 104532x + 1858.2 = 0.9986$

* $n = 6$.
% RSD = percent relative standard deviation.

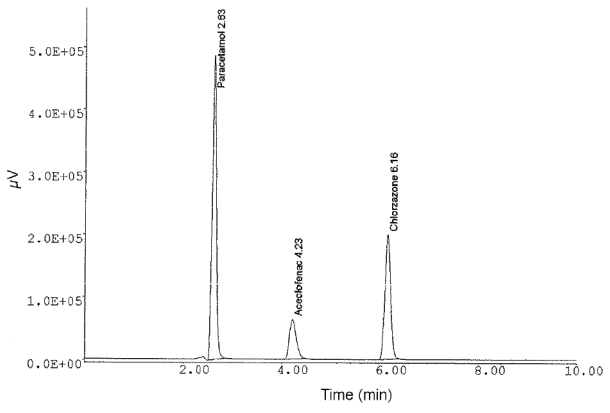

Figure 2. A typical HPLC chromatogram of the tablets containing paracetamol, aceclofenac, and chlorzoxazone.

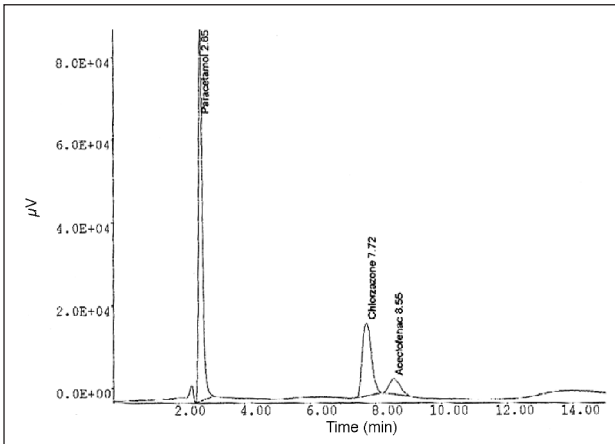

Figure 3. A typical HPLC chromatogram of the tablets with a mobile phase containing buffer at pH 5 showing a change in elution order (paracetamol at 2.05 min, chlorzoxazone at 7.72 min, and aceclofanac at 8.56 min).

Results and Discussion

Optimization of the chromatographic conditions

The chromatographic conditions were optimized by changing the pH of the buffer in the mobile phase. As the mobile phase used throughout the experiment, containing acetonitrile and buffer (40:60, v/v), buffer containing 50mM orthophosporic acid, pH of buffer is adjusted to 6 with 10% w/v sodium hydroxide solution. The sequence of peak elution was observed at the retention times: for paractamol, 2.83 min; aceclofenac, 4.23 min; and chlorzoxazon, 6.16 min (Figure 2), using the described mobile phase. Experiments were performed by changing the pH of the buffer used in the mobile phase. The buffer is adjusted at pH 7 with 10% sodium hydroxide; it is observed that, there is no change in retention order of the all ingredients. When the buffer is adjusted to pH 5 with 10% sodium hydroxide, the elution order of analytes changed. The retention times were as follows: paracetamol, 2.85 min; chlorzoxazone, 7.72 min; and aceclofenac, 8.56 min. Means the elution order can affect aceclofenac and chlorzoxazone at pH 5; the chromatogram is shown in Figure 3. From this study, it has been observed that at pH 5 buffer used in mobile phase, ionic strength concentration of the mobile phase can significantly affect the retention order of the analytes. At pH 5, COO- of aceclofenac become protonated and the compound is less polar, giving it a longer retention in the reverse phase. The pka value of aceclofenac is higher than the other molecule; at pH 5, it will retain with stationary phase and required more time for elution than other analytes. As the pka value of aceclofenac is 4.157, parcetamol is 0.339, and chlorzoxazone is 2.190. But when pH 7 buffer is used in mobile phase, ionic strength concentrations cannot affect on elution order of the aceclofenac and chlorzoxazone. At pH 6 buffer mobile phase, the peaks of paracetamol, aceclofenac, and chlorzoxazone gave adequate retentions and better resolution. The chromatographic runtime is less than 10 min. Resolutions between the peaks are checked, at higher concentration of paracetamol (100 µg/mL) and chlorzoxazone (100 µg/mL) with very low concentration of aceclofenac (2 µg/mL). In such condition, resolution between paracetamol and aceclofenac was 7.70 and between aceclofenac and chlorzoxazone was 8.45. It indicates that there is a better resolution between the peaks.

Validation of the method

Specificity

The specificity of the method was checked by peak purity test of the sample solution by photodiode array detector. The peak purity for paracetamol, aceclofenac, and chlorzoxazone are observed to be 999, 996, and 999, respectively. The result of the peak purity analysis shows that the peak of analytes was pure and excipients in the formulation are not interfering with the analyte peaks.

Calibration and linearity

Linearity of the method for paracetamol, aceclofenac, and chlorzoxazone was tested from 25–150% of the targeted level of the assay concentration for all analytes. The mixed standard solutions containing 12.5–75 µg/mL of paracetamol, 2.5–15 µg/mL of aceclofenac, and 12.5–75 µg/mL chlorzoxazone in each linearity level. Linearity solutions were injected in triplicate. In the simultaneous determination, the calibration graphs were found to be linear for all the analytes in the mentioned concentrations; the correlation coefficients are shown in Table I.

Precision (Reproducibility)

The precision of the method was studied by determining the concentrations of each ingredient in the tablets for six times. The results of the precision study (Table I) indicate that the method is reliable and reproducible, with a relative standard deviation less than 2.0%.

Accuracy (Recovery test)

Accuracy of the method was studied by recovery experiments by adding known amounts of the drugs in the placebo. The recoveries of the method were performed for three levels, at 80%, 100%, and 120% of the label claim per tablet: paracetamol, 500 mg; aceclofenac, 100 mg; and chlorzoxazone, 500 mg. Three samples were prepared for each recovery level. The recovery values for paracetamol, aceclofenac, and chlorzoxazone ranged from 100.7–101.4%, 100.4–101.0%, and 100.5–101.3%, respec-

Table II. Results of the Recovery Tests for the Drugs*

Level of addition (%)	Ingredient	Amount added (mg)	(%) Recovery	(%) Average recovery‡
80	Paracetamol	400	101.5, 101.2, 101.7	101.4
	Aceclofenac	80	100.8, 101.1, 100.6	100.8
	Chlorzoxazone	400	100.7, 101.1, 100.6	100.8
100	Paracetamol	500	101.5, 101.1, 100.1	100.9
	Aceclofenac	100	99.9, 100.4, 100.9	100.4
	Chlorzoxazone	500	101.2, 100.7, 101.9	101.3
120	Paracetamol	600	100.1, 100.9, 101.1	100.7
	Aceclofenac	120	100.4, 100.9, 101.6	101.0
	Chlorzoxazone	600	100.7, 101.2, 99.7	100.5

* $n = 3$.
‡ Average recovery = the average of three levels, nine determinations.

Table III. Assay Results of Active Ingredients in Tablets

Set	Ingredient	Label value (mg)	Found (mg) *	% Label claim
Precision	Paracetamol	500	507.4	101.5
	Aceclofenac	100	101.2	101.2
	Chlorzoxazone	500	502.7	100.5
Intermediate Precision	Paracetamol	500	504.7	100.9
	Aceclofenac	100	99.7	99.7
	Chlorzoxazone	500	500.7	100.1

* Average of 6 analyses.

Table IV. System Suitability Parameters

Parameters	Paracetamol	Aceclofenac	Chlorzoxazone
Theoretical plates*	5069	6426	10798
Resolution	0	9.10	9.96
Tailing factor	1.50	0.95	1.14
% RSD	1.21	1.59	1.46

* per column length.

tively, as shown in Table II. The average recovery of three levels (nine determinations) for paracetamol, aceclofenac, and chlorzoxazone were 101.0%, 100.7%, and 100.8%, respectively.

Intermediate precision

Intermediate precision of the method was done by analyzing the samples six times on different days, by a different chemist, using a different analytical column of the same make and different HPLC systems. The assay results are shown in Table III.

Determination of the limit of detection and quantitation

For determining the limit of detection (LOD) and limit of quantitation (LOQ), the method based on the residual standard deviation of a regression line and slope (12). To determine the LOD and LOQ, a specific calibration curve was studied using samples containing the analytes in the range of LOD and LOQ. The LOD for paracetamol, aceclofenac, and chlorzoxazone were 0.027 µg/mL, 0.074 µg/mL, and 0.067µg/mL and LOQ were 0.081 µg/mL, 0.222 µg/mL, and 0.201 µg/mL, respectively.

Solution stability

The stability of the standard solutions and the sample solutions were performed at intervals of 12 h, 24 h, and 48 h. The stability of the solutions was determined in terms of the assay of the drugs in standard solutions and sample solutions against the freshly prepared standard solutions. The relative standard deviation for the assay values determined up to 48 h for paracetamol, aceclofenac, and chlorzoxazone in samples were 0.94%, 0.77%, and 0.47%, respectively. The relative standard deviation for the assay values were within ± 2% after 48 h. The results indicate that the solutions were stable for 48 h at ambient temperature.

System suitability

For system suitability studies, five replicate injections of mixed standard solutions were injected, and the suitability parameters like relative standard deviation of peak area, column efficiency, resolution, and tailing factor of the peaks were calculated. Results are shown in Table IV.

Determination of active ingredients in tablets

The contents of three drugs in tablets were determined by the proposed method using a calibration curve. The determinations were carried out in two sets; one for precision and the second for intermediate precision and six samples were prepared for each set. The results are shown in Table III.

Conclusion

This method can be used for the simultaneous determination of paracetamol, aceclofenac, and chlorzoxazone in pharmaceutical dosage form. The method was validated and shown to be accurate and precise. It can be used in quality control department for the assay and dissolution of tablets containing paracetamol, aceclofenac, and chlorzoxazone in combination.

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