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SPECTROPHOTOMETRIC SIMULTANEOUS DETERMINATION OF CHLORZOXAZONE AND DICLOFENAC SODIUM IN TABLET DOSAGE FORM

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Abstract

The aim of the study to describe a simple, precise sensitive, rapid, accurate and economical simultaneous equation method for the determination of chlorzoxazone and diclofenac sodium in tablet dosage form. The method involved solving simultaneous equations based on measurement of absorbance at two wavelengths 244nm and 280nm. The proposed method was validated for linearity, accuracy and precision. The linearity was obtained in the concentration range 5- 20 µg/mL for Chlorzoxazone and 1- 4µg/mL for Diclofenac sodium. The percentage recovery was found to be 98 - 101% for Chlorzoxazoneand 98 – 100% for Diclofenac sodium indicates that the method was accurate and precise for simultaneous estimation of Chlorzoxazone and Diclofenac sodium in tablets.
Key Words: Simultaneous equation method, Paracetamol, Diclofenac sodium

Intrduction

Chlorzoxazone (5-chloro-2(3H)-benzoxazolone)[Fig1] is a compoundwith skeletal muscle relaxant property. It is centrally acting agent for painful musculoskeletal conditions. Chlorzoxazone inhibits degranulation of mast cells, subsequently preventing the release of histamine and slow-reacting substance of anaphylaxis (SRS-A), mediators of type I allergic reactions. Chlorzoxazone also may reduce the release of inflammatory leukotrienes.Chlorzoxazone is rapidly metabolized and is excreted in the urine, primarily in a conjugated form as the glucuronide.Diclofenac sodium [2-[(2,6- dichlorophenyl)] amino] benzene acetic acid monosodium salt] [Fig 2] is a compound with potent anti-inflammatory property. Diclofenac is a phenylacetic acid derivative and non-steroidal anti-inflammatory drug (NSAID).Label NSAIDs inhibit cyclooxygenase (COX)-1 and-2 which are the enzyme responsible for producing prostaglandins (PGs). PGs contribute to inflammation and pain signaling. Diclofenac, like other NSAIDs, is often used as first line therapy for acute and chronic pain and inflammation from a variety of causes.Literature survey reveals that various analyticaltechniques viz,UV spectrophotometry, High performance liquid chromatography (HPLC), HPTLC methodswere reported for the analysis ofCHZ and DCF in pharmaceuticals. Few analytical methods have been reported[1-9] for the simultaneous determination ofCHZ and DCF.Aim of present work wasto develop simple, economical, rapid, precise and accuratemethod for simultaneous determination of CHZ and DCF.

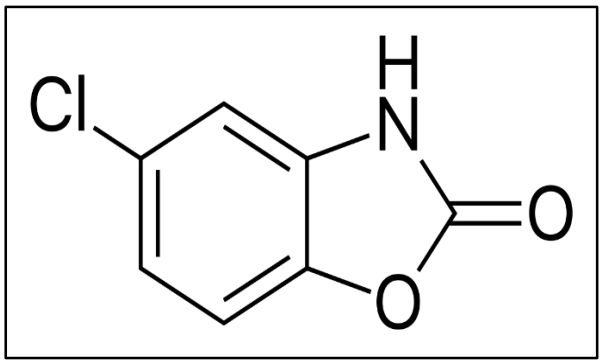

Figure-1 Structure of Chlorzoxazone

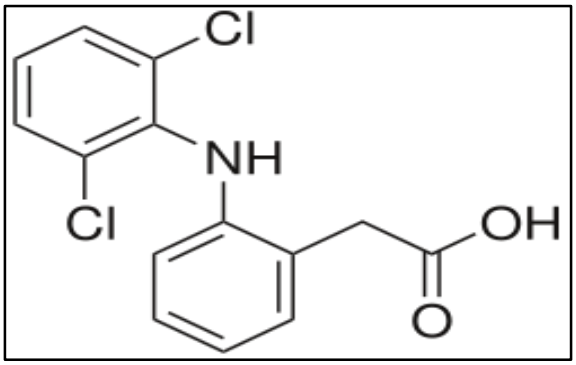

Figure -2 Structure of Diclofenac

Experimental

Chemicals and reagents

CHZ and DCF working standard were obtained from Glenmark (Mumbai, India), Tablets containing CHZ (250mg) and DCF(50mg)were obtained fromUnichem(Mumbai, India), AR grade methanol and acetonitrile were purchased from Baker (Mumbai, India).

Preparation of standard drug solution

50mg of Chlorzoxazoneand 10 mg of Diclofenac sodium was separately weighed and transferred to a 50cm^3 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 1000µg/mL for CHZ and 200 µg/mL for DCF. 10cm^3 of each of this solutionwas diluted to 100 cm^3 in a volumetric flask with methanol. The concentration of the solution obtained was 100µg/mL for CHZ and 20µg/mL for DCF.

Preparation of Sample solution

Twenty tabletswere weighed and average weight was calculated. These tablets were powdered and weight equivalent to one tablet was taken in a 100 mL volumetric flask, 10mL of methanol was added and sonicated for 20minutes and shaken by mechanical means for 20minutes at 250rpm. Then the solution was diluted with methanol. The solution was mixed and allows settled for 5 minutes. The solution was filtered through Whatman filter paper No 41. Then 4.0mL of the filtrate was diluted to 100mL with diluents and mixed. The concentrations obtained were 100 µg/mL of Chlorzoxazone and 20 µg/mL of Diclofenac sodium. This sample solution was used for further determination.

Methodin Brief

The present work describes an ultraviolet spectrophotometric method for the quantitative simultaneous determination of Chlorzoxazone and Diclofenac sodium from its bulk drug and pharmaceutical preparation. Chlorzoxazone & Diclofenac sodiumabsorb the electromagentic radiation in the ultraviolet region. The Proposed ultraviolet spectrophotometric method is based on the measurement of absorbed ultraviolet radiations by both the analytes. The absorbance measurement was carried out at the λ_{max} of Chlorzoxazone and Diclofenac sodium. Chlorzoxazoneshows maximum absorbance at 244nm wavelength while Diclofenac sodium shows at 280nm. The molar absorptivities of both the analytes were found at their

respective λmax value. Using the molar absorptivities of both the analytes simultaneous equation was constructed and the concentration of analytes was determined. The proposed ultraviolet spectrophotometric method was subjected to statistical validation to determine its accuracy and precision.

Optimization of Experimental Conditions

The instrument used for the analysis of the samples is LAMBDA 25 UV/Visible Spectrophotometer, Range: 190 nm - 1100 nm, Bandwidth: 1 nm.The solvents that are commonly used in spectrophotometric analysis are water, dilute bases and organic solvents. Most of the drugs are soluble in organic solvents like methanol, acetonitrile etc. In the present study the drugs used are Chlorzoxazone and Diclofenac sodium. Both the drugs are highly soluble in methanol. Other solvents were also tried but methanol gives higher $E_{1\%}$ Value.

Table- 1: $E_{1\%}$ Value for Chlorzoxazone and Diclofenac sodium

Solvent	**Chlorzoxazone**		**Diclofenac sodium**	
	Conc. in µg/mL	**$E_{1\%}$**	Conc. in µg/mL	**$E_{1\%}$**
Methanol	12.5	**954**	2.5	**450**

From the above $E_{1\%}$ value data it has been found that methanol was chosen as a solvent for the preparation of solution as it gives higher $E_{1\%}$ value.

Spectral Characteristics

To find out the wavelength for maximum absorbance, standard solutions of CHZ and DCF in methanol was prepared in the given range of concentration. CHZ and DCF having the concentration from 5 µg/mL to 20 µg/mL and 1 µg/mL to 4.5 µg/mL respectively. The standard solutions of these analytes were scanned on spectrophotometer from 200nm to 400nm against methanol as the regent blank. CHZ shows the maximum absorbance at 244nm wavelength and DCF shows at 280nm wavelength. The spectra of CHZ and DCF are as shown below. Chlorzoxazone Showing λmax at 244nm

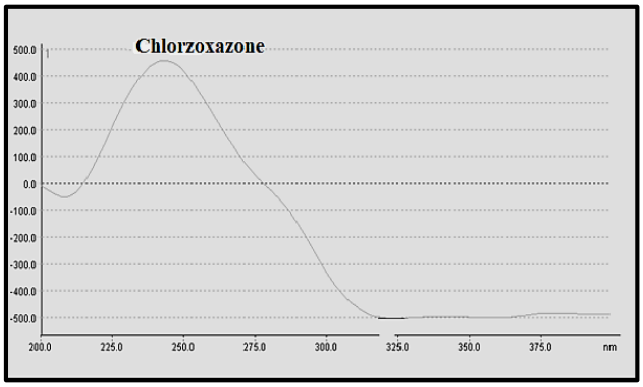

Figure -3: Absorption Spectra of CHZ showing λmax at 244nm

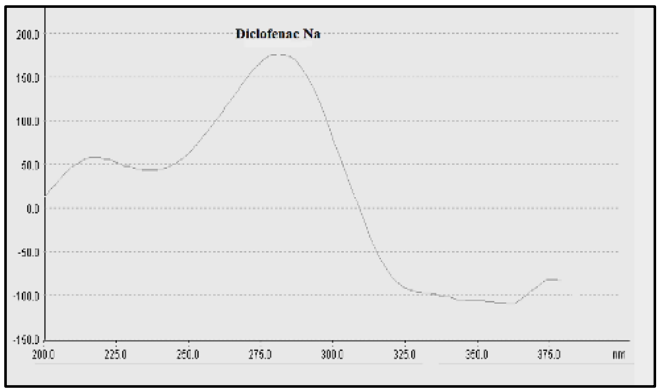

Figure -4: Absorption Spectra of DCF showing λmax at 280nm

The method was validated as per ICH guidelines [10] forspecificity, linearity, precision, accuracy, recovery and ruggedness. Specificity was investigated by analyzing the blank diluents and samples of 100% level for any interference of the endogenous material at the

absorbance of CHZ and DCF. The linearity of the method was tested by taking several aliquots of standard solutions of CHZ and DCFin 50mL volumetric flask and diluted upto the mark with solvent. The final concentration of CHZ and DCFwas 5-20 µg/mL and 1-4 µg/mL respectively.

The accuracy of the method was determined by recovery experiments.A standard addition method was employed for this experiment. A known quantity of each standard drug substance (CHZ and DCF) corresponding to 0%, 10%, 20% and 30% of the label claim of each drug was added. The accuracy was expressed as a percentage of analytes recovered by the assay. In the present research work 12.5 ppm &2.5ppm of sample solution was considered as 100% (0 level).

As a part of method validation, Intermediate precision (ruggedness) was performed by carrying out the same assay procedure on a different instrument on a different day under similar experimental conditions.

D. Results and Discussions

To develop rapid, low cost and sensitive UV method for simultaneous determination of CHZ&DCF the optimized conditions were necessary. A study of absorption spectra of CHZ and DCF in methanol showsthat at 244nm Chlorzoxazone shows maximum absorbance whereas Diclofenac sodiumshows at 280nm. Hence it was possible toconstruct simultaneous equation.

$$Cx = \frac{A_2 ay_1 - A_1 ay_2}{ax2\ ay1 - ax1\ ay2}, \qquad Cx = \frac{A_2 0.0189 - A_1 0.0490}{0.0208 \times 0.0189 - 0.0962 \times 0.0490}$$

$$\text{and} \quad Cy = \frac{A_1 ax_2 - A_2 ax_1}{ax2\ ay1 - ax1\ ay2}, \qquad Cy = \frac{A_1 \times 0.0208 - A_2 \times 0.0962}{0.0208 \times 0.0189 - 0.0962 \times 0.0480}$$

Where ax_1& $ax_2$are the absorptivity of the Chlorzoxazone at the wavelength 244nm & 280nm respectively and ay_1& ay_2 are the absorptivity's of the Diclofenac Na at the wavelength 244nm & 280nm respectively.

Linearity:

Linearity of the method was tested from 40% to 160% of the targeted level of theassay concentration (12.5 μg/mL Chlorzoxazone and 2.5 μg/mL Diclofenac Na) for thetwo analytes. The standard solutions containing 5 -12.5 μg/mL CHZ and1.0 - 4.0 μg/mL DCF were prepared from the standard stock solutions ofCHZ and DCF. Linearity test solutions were injected and analyzedin triplicate. The calibration graphs were plotted by using absorbance of theanalytes against the concentration of the drug (in micrograms per milliliter). Inthe simultaneous determination, the calibration graphs were found to be linearfor both the analytes in the mentioned concentration ranges. The regressionequations for CHZ and DCF were found to bey = 0.0792X + 0.156 andy = 0.0403X + 0.0155, and the correlation coefficientsfor the regression lines were 0.9980 and 0.9990, respectively.

Sensitivity:

Sandell's sensitivity of CHZ and DCF was found to be sufficientlylow.Table 2 shows that very less amount of both the drugs can beeffectively detected by this method.

Table-2:Sensitivity Parameters for Chlorzoxazone and Diclofenac Na

Analyte	λ max (nm)	Molar absorptivity	Sandell's Sensitivity
Chlorzoxazone	244	1.342×10^4 lit.mol^{-1} cm^{-1}	0.01262 µg/cm^3/cm^2
Diclofenac	280	1.281×10^4 lit.mol^{-1} cm^{-1}	0.02482 µg/cm^3/cm^2

Accuracy (%Recovery):

The accuracy of the method was determined by the standard addition method atthree different levels. The sample solution of 100% level was considered as a zerolevel and 10%, 20% and 30% of the standard drug of analytes were addedrespectively. Each determination was performed in triplicates. The accuracy wasthen calculated as the percentage of the standard drug recovered by therecovery study. The recovery of CHZ and DCF from the standardmixture solution was found to be 99.84% - 100.94% and 99.50 - 100.39%respectively. The recovery results shows that CHZ and DCF couldbe quantified by this procedure simultaneously. The results are well within theacceptance limit and hence the method is accurate. Table 3 shows the % recoveries of CHZ and DCF.

Table -3: % Recovery of Chlorzoxazone and Diclofenac sodium

Sr.No	% Added	Original amount	Added amount	Total amount	Mean (n =5)	% Recovery	S.D	% R.S.D
Amount of Chlorzoxazone in mg								
1	0	12.5	0	12.5	12.48	99.84	0.53	0.52
2	10	12.5	1.25	13.75	13.65	99.27	0.51	0.46
3	20	12.5	2.50	14.75	14.89	100.94	0.44	0.42
4	30	12.5	3.80	16.30	16.20	99.38	0.47	0.48
Amount of Diclofenac Sodium in mg								
1	0	2.5	0	2.5	2.495	99.80	0.42	0.43
2	10	2.5	0.26	2.76	2.771	100.39	0.51	0.50
3	20	2.5	0.51	3.01	2.995	99.50	0.44	0.43
4	30	2.5	0.76	3.26	3.245	99.53	0.46	0.45

Ruggedness (Intermediate precision):

Intermediate precision was examined by carrying out the same assay procedure on a different instrument on a different day. The experimental conditions kept same but the UV system was changed. On different day six sample preparation of CHZ and DCF tablets were analyzed as per the methodology. The cumulative % relative standard deviation was found to be 0.0036&0.0176 for CHZ and DCF respectively. Low value of cumulative % RSD of assay of method precision study and Intermediate precision study showed that method is rugged (Table 4).

Table -5: Cumulative % RSD of Chlorzoxazone &Diclofenac in Method precision and Intermediate precision

Obs No	Chlorzoxazone mg/tab	% LC CHZ	Diclofenac mg/tab	% LC DCF
M.P 1	250.05	100.02	50.05	100.1
M.P 2	249.55	99.82	51.01	102.02
M.P 3	248.95	99.58	49.55	99.10
M.P 4	250.55	100.22	49.01	98.02
M.P 5	250.98	100.39	49.10	98.20
M.P 6	251.05	100.42	50.54	101.08
I.P 1	248.55	99.806	51.26	102.52
I.P 2	249.65	99.624	50.23	100.46
I.P 3	249.25	99.676	49.63	99.26
I.P 4	250.85	100.62	49.15	98.30
I.P 5	250.98	100.33	49.84	99.68
I.P 6	251.05	100.46	51.65	103.30
Mean	250.121	100.048	50.085	100.17
S.D.	0.907	0.363	0.877	1.754
Cumulative %	0.0036	0.0036	0.0175	0.0176
Limits	NMT 2.00%		NMT 2.00%	

Conclusion:

The UV method has proved to be simple, specific, precise and accurate and issuitable for simultaneous determination of Chlorzoxazone and Diclofenac Sodium. Theproposed method gives a good result among these analytes. High percentage of recoveryshows that the method is accurate.

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