Development and Validation of RP-HPLC Method for the Estimation of Chlorzoxazone in bulk drug and pharmaceutical Formulations

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ABSTRACT

A simple, precise, accurate and rapid isocratic reverse phase high performance Liquid chromatographic method has been developed for the estimation of Chlorzoxazone (CHZ) in bulk and pharmaceutical formulations. The chromatographic Colum was used the Hypersil BDS (C_{18}, 150 X 4.6, 5µ analytical Colum) UV-Vis Detector with PDA .The mobile phase comprising Potassium dihydrogen orthophosphate and acetonitrile in the ratio (60: 40 v/v) adjusted pH 4.0 with dilute orthophosphoric acid solution. At a flow rate of 1.0 mL/min the wavelength was set at 287 nm at the retention time is 4.290 min. The correlation coefficient for Chlorzoxazone is 1 the recovery values of Chlorzoxazone ranged from 98–102%; the relative standard deviation for six replicates is always less than 2%**.** The proposed method was validated for linearity, accuracy, precision, LOD is 0.1511 µg/mL and LOQ is 0.458 µg/mL. The calibration was linear over the range of 25-150 µg/mL. The method can be easily adopted for quality control analysis.

Keywords: Chlorzoxazone, RP-HPLC, Validation

INTRODUCTION

Chlorzoxazone (CHL) is chemically 5-chloro-3Hbenzooxazol-2-one [1] it is a colorless or white to creamy white crystalline powder, very slightly soluble in water Chlorzoxazone (CHL) is a skeletal muscle relaxant. It acts by inhibiting multi synaptic reflexes involved in producing and maintaining skeletal muscle spasm of varied etiology [2]. It acts on the spinal cord by depressing reflexes. CHL, a synthetic compound, inhibits antigen-induced broncho spasms and hence, is used to treat asthma and allergic rhinitis. CHL 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. CHL also may reduce the release of inflammatory leukotrienes Chlorzoxazone is used to relieve pain and stiffness caused by muscle strains and sprains. Chlorzoxazone (CHL) is official in United States Pharmacopeia (USP) [3]. USP describes liquid chromatography method for its estimation. Literature survey reveals Fluorimetry [4], Electrochemical [5], HPLC [6-8], GC-MS [9] methods for determination of CHL alone. Literature survey also reveals UV [10-12], HPLC [13-21] and HPTLC [22] methods for the determination of CHL with other drugs combination. Hence properly developed and validated analytical method is necessary for quality control of the drugs in market. The available methods are either poorly validated or uneconomical. In fact a properly validated and economical method is needed. Therefore the present research work aim to develop a simple, accurate, precise, sensitive and reproducible method for determination of CHL in single dosage forms by RP-HPLC method. The chemical structure of Chlorzoxazone is shown below (Figure-1).

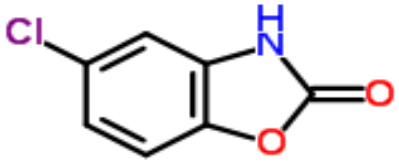

Figure: 1. Chemical structure of Chlorzoxazone

Drugs and chemicals used

Chlorzoxazone pure sample of CHL 250 mg were obtained as a gift sample from Hetero drugs limited, Hyderabad, Andhra Pradesh India. The solvent Acetonitrile (HPLC grade) purchased from SR Scientifics Private Limited (Tirupati, India). Other chemicals and reagents such as potassium dihydrogen orthophosphate, dipotassium hydrogen orthophosphate and phosphoric acid, were of AR grade obtained from Bros Scientifics. Tirupati, Andhra Pradesh ,India. Purified HPLC grade water prepared by using 0.45 Millipore Milli-Q water Purification system was used throughout the experiment.

PREPARATION OF SOLUTIONS AND REAGENTS

Mobile phase

Based on the solubility and chemical properties of the drugs, a mobile phase consists of Phosphate buffer, acetonitrile in the ratio of 60:40 v/v. Phosphate buffer was prepared by

dissolving accurately weighed 2.72 g Potassium dihydrogen orthophosphate in 1000ml water, adjusted to p^H 4.0 with dilute orthophosphoric acid solution of HPLC grade water. The mixture was filtered through 0.4 µm membrane filter and sonicated for about 15 min.

Standard stock preparation

100 mg of chlorzoxazone were accurately weighed and transferred to a 100ml volumetric flask. It was dissolved in 30ml of diluent and make up to the mark with diluent. The concentration of the solution obtained was 100 µg/mL for chlorzoxazone (Solution A) 10 ml of the solution was transferred in to 100 ml volumetric flask and dilute to volume with mobile phase. The concentration of the solution obtained was 1000 µg/mL of chlorzoxazone.

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 100 mg of chlorzoxazone was taken in a 100 mL dilution flask. Then about 50 mL of diluent was added to it. Then the solution was sonicated for 10 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 0.45 µm membrane filter. This solution was used for further analysis.

SPECIFICITY

Standard Solution Preparation: Weigh and transfer 20 mg of Chlorzoxazone working standard into 100 mL volumetric flask add 100 mL of diluent and sonicated to dissolve and

dilute to volume with diluent. Further transfer 10 mL of the above solution into 100 mL volumetric flask and dilute to volume with diluent.

Sample Solution: Use assay solution as sample preparation.

Blank Preparation: Use diluent as Blank solution.

Procedure: Inject Blank, Individual standards, mixed standard and sample Solution.

PRECISION

System Precision

Preparation of solution: Dilute 10 ml of standard stock solution with 100 mL of diluent. Inject the above solution six times.

Method Precision:

Preparation of solution**:** Dilute 10 ml of standard stock solution, with 100 mL of diluent. Prepare six solutions and inject each solution.

Acceptance criteria: The % of RSD for Area and RT from Repeated injections should not be more than 2.0**%**

Linearity

The Linear detector response for Chlorzoxazone drug is demonstrated by concentration versus Area obtained by linear sample preparations. Over the range of 25 to 150% with respect to the target concentration (Dosage).

Accuracy

The accuracy of the test method is demonstrated by % of recovery. The sample preparations are spiked with known amount of standard at three concentration levels and each concentration is injected three times (Like 50% 100% and 150%). **Acceptance criteria:** The % of recovery should be between 98 to 102%.

Ruggedness: The ruggedness of test method is demonstrated by carrying out precision studies with different analysts and on different days. **Acceptance criteria:** The % of RSD of areas from six injections should not be more than 2.0**%**

Robustness: The robustness of test method is demonstrated by carrying out intentional method variations like mobile phase flow changes, mobile phase compositions and column oven temperature variations etc…

The results should show some variation from standard results. **Acceptance criteria:** The % of RSD of areas & RTs from repeated injections should not be more than 2.0 **%**

Assay

Standard préparation : Transfer 10 ml of standard stock solution in to 100 mL volumetric flask and make up to volume with diluent.

Sample Preparation: Transfer samples 20 mg of chlorzoxazone in to 100 mL volumetric flask add 100 mL of diluent, sonicate to dissolve for 10 minutes and dilute to volume with diluent. Further filter the solution through filter paper. Dilute 10 ml of filtrate to 100 ml with mobile phase.

Procedure: Inject 20 µL of blank solution, standard solution, and sample solution record the chromatogram. And calculate percentage of assay.

Chromatographic conditions

Waters HPLC 2 2695 series consisting 4 pump. Auto sampler with 5 racks, each has 24 vials holding capacity with temperature control. Auto injector has capacity to inject 5µL to 500µL. UV-Vis Detector with PDA. Thermostat column compartment connected it has a capacity to maintain 5°C to 60°C column temperature .The data was recorded using Waters (alliance) HPLC System is equipped with Empower software-2 software. Separation was performed on a 150 × 4.6, 5 µ particle size Hypersil C_{18} column. Mobile phase consisting of a mixture of buffer: acetonitrile (60:40), pH 4.0 adjusted with orthophosphoric acid. Flow rate was kept at 1.0 mL/min. Wavelength was set at 287 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 CHL. The accuracy of the method was determined by recovery experiment. 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 six concentration levels of the mixed standards of CHL.

RESULTS AND DISCUSSION

Optimization of the Chromatographic Conditions

In order to develop an isocratic reverse phase HPLC method for the determination of CHL in single dosage form the chromatographic conditions were optimized. For better separation and resolution the different buffers were tried. It has been found that potassium dihydrogen orthophosphate buffer, pH 4.0 adjusted with orthophosphoric acid give better peak shape than other buffers. Hypersil C_{18}, 150 mm x 4.6 mm, 5 µm column. The analyte gave better response at 287 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 CHL were found to be 4.290 min.

VALIDATION

The proposed method was showed short elution time. 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. 0.23%RSD value was found to be CHL. The tailing factor and theoretical plates were found to be perfectly within the limits. The method was linear over the range 25-150 µg/mL of chlorzoxazone the calibration curve was constructed by plotting response factor against concentration of drug. The slope and intercept value for calibration curve was Y= 90366x+17765 (r^2=1) shows that an excellent correlation between response factor and concentration of drug. The limit of detection (LOD) and limit of quantification (LOQ) of the developed method were determined by injecting progressively low concentrations of the standard solution using the developed RP-HPLC method the LOD and LOQ of CHZ were experimentally determined. The LOD was found to be 0.1511 µg/mL and LOQ is 0.458 µg/mL.

The system precision study was performed to determine the repeatability of the method. Six samples of standard were prepared at 50%, 100% & 150% levels 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 50%, 100% &150% levels 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, 10 and 20 of the standard drug of analyte was 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 chlorzoxazone from the combination formulation are shown in Table 4. The results are well within the acceptance limit and hence the method is accurate. The tailing factor and theoretical plates were found to be perfectly within the limits. The system precision and method precision results are shown in tables -2 & 3.Ruggedness, Assay and Robustness results are shown in tables-5, 6, 7, 8 & 9.

Conclusion

The isocratic RP- HPLC method has proved to be simple, specific, precise and accurate and suitable for quantification of chlorzoxazone. The proposed method gives good resolution among the analyte. 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 formulation and the method is accurate.

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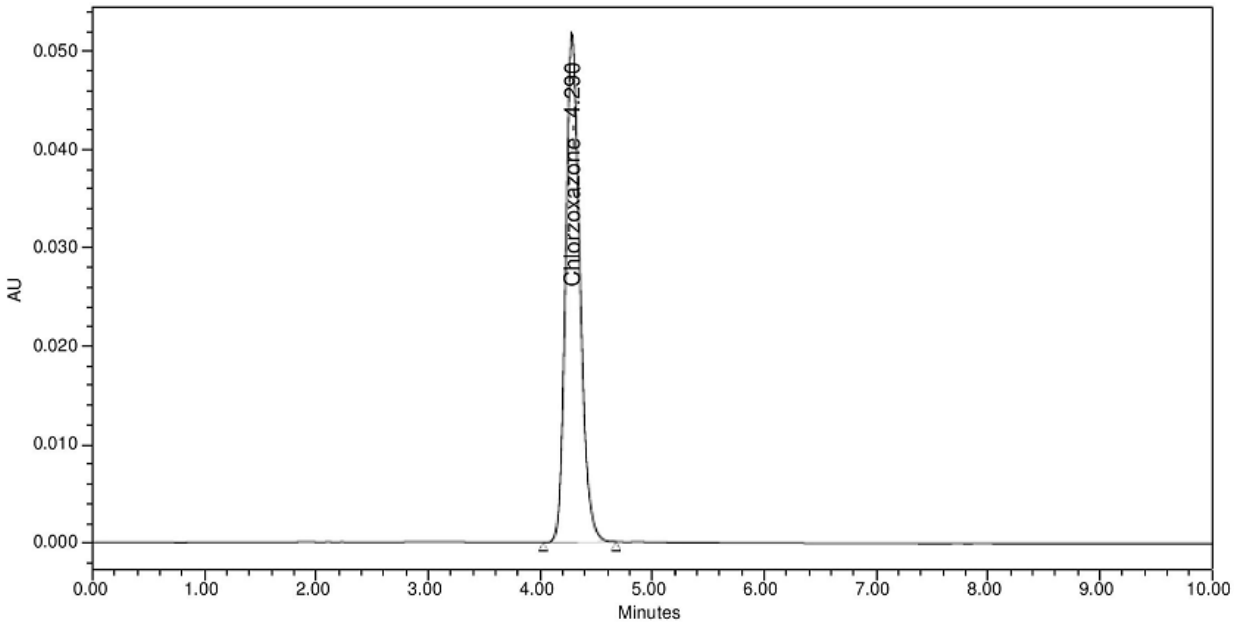

	Peak Name	RT	Area	% Area	Height	USP Plate Count	USP Tailing
1	Chlorzoxazone	4.290	464550	100.00	52061	5370	1.21

Figure: 2. Typical chromatogram of Chlorzoxazone

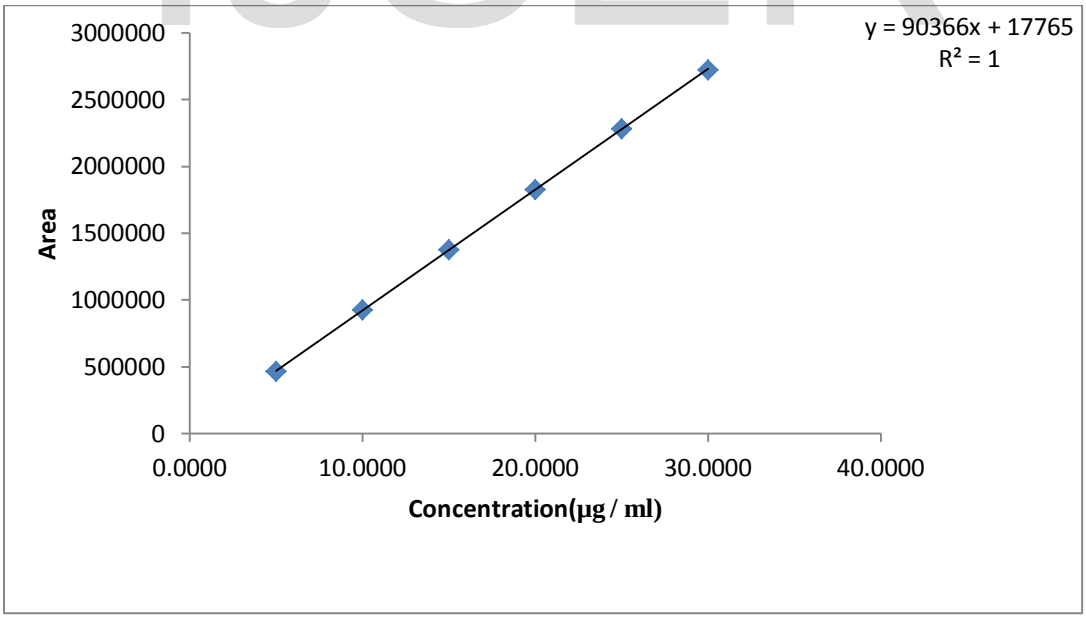

Figure: 3. Linearity of Chlorzoxazone

Table: 1. Linearity of Chlorzoxazone

%	Conc(mcg)	Area
25	5.0000	464550
50	10.0000	923403
75	15.0000	1377350
100	20.0000	1824865
125	25.0000	2282418
150	30.0000	2722456

Table: 2. System Precession of Chlorzoxazone

S.No	Name	RT	Area
1	Injection-1	4.303	1828423
2	Injection-2	4.303	1823633
3	Injection-3	4.312	1821539
4	Injection-4	4.308	1830954
5	Injection-5	4.309	1832189
6	Injection-6	4.309	1826549
Avg		4.307	1827215
Std Dev(±)		0.004	4139.4
%RSD		0.084	0.23

Table: 3. Method precession of Chlorzoxazone

S.No	Name	RT	Area
1	Injection-1	4.301	1820565
2	Injection-2	4.305	1824535
3	Injection-3	4.307	1833218
4	Injection-4	4.309	1835426
5	Injection-5	4.309	1831310
6	Injection-6	4.312	1829047
Avg		4.307	1829017
Std Dev(±)		0.004	5576.9
%RSD		0.089	0.30

Table: 4. Accuracy of Chlorzoxazone

Accuracy -50%		**Accuracy-100%**		**Accuracy-150%**	
S.No	**Area**	**S.No**	**Area**	**S.No**	**Area**
Injection-1	920132	Injection-1	1821078	Injection-1	2721090
Injection-2	918932	Injection-2	1820895	Injection-2	2720589
Injection-3	916540	Injection-3	1820133	Injection-3	2719564
Avg	918535	Avg	1820702	Avg	2720414.333
Amt Recovered	50.3	Amt Recovered	99.71	Amt Recovered	149.02
%Recovery	100.64	%Recovery	99.71	%Recovery	99.35

Table: 5. Ruggedness of Chlorzoxazone Day-1

S.No	Name	RT	Area
1	Injection-1	4.301	1820565
2	Injection-2	4.305	1824535
3	Injection-3	4.307	1833218
4	Injection-4	4.309	1835426
5	Injection-5	4.309	1831310
6	Injection-6	4.312	1829047
Avg		4.307	1829017
Std Dev(±)		0.004	5576.9
%RSD		0.089	0.30

Table: 6. Ruggedness of Chlorzoxazone Day-2

S.No	Name	RT	Area
1	Injection-1	4.311	1823127
2	Injection-2	4.313	1824904
3	Injection-3	4.315	1825635
4	Injection-4	4.310	1822908
5	Injection-5	4.308	1821096
6	Injection-6	4.309	1822366
Avg		4.311	1823339
Std Dev(±)		0.003	1669.1
%RSD		0.060	0.092

Table: 7. Ruggedness of Chlorzoxazone Day-1&Day-2

S.No	Name	RT	Area
1	Injection-1	4.301	1820565
2	Injection-2	4.305	1824535
3	Injection-3	4.307	1833218
4	Injection-4	4.309	1835426
5	Injection-5	4.309	1831310
6	Injection-6	4.312	1829047
7	Injection-7	4.311	1823127
8	Injection-8	4.313	1824904
9	Injection-9	4.315	1825635
10	Injection-10	4.310	1822908
11	Injection-11	4.308	1821096
12	Injection-12	4.309	1822366
AVG		4.309	1826178.08
Std Dev(±)		0.00370	4918.780
%RSD		0.09	0.27

Table: 8. Assay Results of Chlorzoxazone

1826561	20.02	10	100	100	99.75	100	1.000	Result
1828603	100	100	20.03	10	100			99.84

Table: 9. Robustness of Chlorzoxazone

S.N	Peak Name	RT	Area	% Area	Height	USP Plate Count	USP Tailing
1	**Chlorzoxazone F1&F2**	5.971	2532379	100.00	215470	5934	1.28
2		3.372	1420608	100.00	173801	3950	1.24
1	**Chlorzoxazone** T1&T2	4.375	1825793	100.00	185825	4578	1.29
2		4.229	1803527	100.00	191488	4702	1.25

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