Development and validation of UV-spectrophotometric methods for simultaneous estimation of chlorzoxazone and tramadol in laboratory mixture

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ABSTRACT

The present manuscript describes simple, sensitive, rapid, accurate, precise and economical simultaneous equation method and first order derivative spectrophotometry method for the simultaneous determination of Chlorzoxazone and Tramadol in laboratory mixture. The absorbance values at 243.3 nm and 271 nm for simultaneous equation method and 236.6 nm and 213.3 nm for first derivative spectrum was used for the estimation of Chlorzoxazone and Tramadol. This method obeyed Beer’s law in the concentration range of 2-10 μg/ml for Chlorzoxazone and 10-100 μg/ml for Tramadol. The solvents used for UV-spectrophotometric methods was 0.1 N NaOH. The % RSD of accuracy was found to be 0.2086 for Chlorzoxazone and 0.4717 for Tramadol. The method was successfully applied to laboratory prepared mixture because no interference from the mixture excipients was found. The suitability of this method for the quantitative determination of Chlorzoxazone and Tramadol was proved by validation. The results of analysis have been validated statistically and by recovery studies.

Keywords—— Chlorzoxazone, Tramadol, UV-spectrophotometry

1. INTRODUCTION

Chlorzoxazone is chemically 5-chloro-2, 3-dihydro-1, 3 -benzoxazol-2-one. It is having skeletal muscle relaxant property. It is used to decrease muscle tone and tension and thus to relieve spasm and pain associated with musculoskeletal disorders. It can also be administered for acute pain in general and for tension headache (muscle contraction headache). It acts on the spinal cord by depressing reflexes.

![Fig. 1: Structure of Chlorzoxazone](image1.png)

Tramadol is chemically cis-2-[((dimethylamino) methyl]-1-(3-methoxyphenyl) cyclohexanol). It is centrally acting analgesic, having agonist action at the μ-opioid receptor and affects reuptake at the nor-adrenergic and serotonergic system.

![Fig. 2: Structure of Tramad](image2.png)
Tramadol is a very weak μ-opioid receptor agonist, induces serotonin release, and inhibits the reuptake of nor epinephrine. Tramadol is converted to O-desmethyl tramadol, a significantly more potent μ-opioid agonist. The opioid agonistic effect of tramadol and its major metabolite(s) is almost exclusively mediated by such μ-opioid receptors. Literature survey shows that Chlorzoxazone is analysed either separately and in combination with other drug by UV Spectrophotometry and HPLC and Tramadol is analysed either separately and in combination with other drugs by UV spectrophotometry and HPLC.

2. EXPERIMENTAL MATERIALS

2.1 Chemicals

All chemicals used during the project work were AR grade. The reagents and chemicals used during experimental work are Water, Methanol, 0.1N NaOH Solution, Acetonitrile.

2.2 Instruments


2.3 Reagent and material

The drug Chlorzoxazone and Tramadol was kindly gifted by flamingo pharmaceutical pvt. Ltd.

2.4 Preparation of standard stock solutions:

Accurately about 10 mg of pure drug of Chlorzoxazone and Tramadol were weighed accurately and transferred in the two different 100 ml calibrated volumetric flasks. Both the drugs were dissolved separately in 0.1N NaOH solution to give stock solutions of concentration 100 µg/ml respectively.

3. METHODOLOGY

3.1 Simultaneous Equation Method

Appropriate dilutions were prepared for each drug from the standard stock solution and scanned in the spectrum mode from 400 nm to 200 nm. Chlorzoxazone and Tramadol showed absorbance maxima at 243.4 nm and at 271.0 nm respectively.

3.1.1 Validation of proposed method: The proposed method was validated according to ICH guidelines.

Linearity: Appropriate aliquots were pipetted out into 10 ml calibrated volumetric flasks and dilutions were made with 0.1 N NaOH solution to obtain working standard solutions of concentrations 2, 4, 6, 8, 10 µg/ml for Chlorzoxazone and 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 µg/ml for Tramadol. Absorbance for these solutions were measured at 243.4 and 271.0 nm.

Precision: Precision of the method was verified by using stock solutions in the ratio of 1:4 containing 5µg/ml CXZ and 20 µg/ml of TRM. System repeatability was done by repeating the assay three times of three replicate dilutions of the same concentration after every two hours on the same day for intraday precision. Inter day precision was carried out by performing the assay of three sample sets after 24 hours and 48 hours,72 hours. In Intraday study the % RSD was found to be 0.31174 for Chlorzoxazone and 0.32899 for Tramadol. In Interday study the % RSD was found to be 0.36087 for Chlorzoxazone and 0.58534 for Tramadol.

Accuracy: Accuracy of method was determined by calculating the recovery of Chlorzoxazone and Tramadol by standard addition solution. Known amount of standard solution of Chlorzoxazone and Tramadol were dded at 80%, 100%, 120%. The % RSD was found to be 0.2086 for Chlorzoxazone and 0.4717 for Tramadol.

Ruggedness: It is determined by analysis of aliquots from homogenous slot by two analyst using same operational and environmental conditions. The % RSD was found to be 0.2059 for Chlorzoxazone and 0.0907 for Tramadol.

Robustness: It expresses the precision within laboratories, Variation like different solvent, 0.11N NaOH solution was taken for analysis. The % RSD was found to be 0.2052 for Chlorzoxazone and 0.0906 for Tramadol.

Fig. 3: Overlay Spectrum of CXZ & TRM

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Table 1: Summary of Validation Parameter of CXZ and TRM

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Chlorzoxazone</th>
<th>Tramadol</th>
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</thead>
<tbody>
<tr>
<td>Linearity range [µg/ml]</td>
<td>2 – 10</td>
<td>10– 100</td>
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<tr>
<td>Regression equation [Y = mX +C]</td>
<td>Y = 0.049X + 0.003</td>
<td>Y = 0.005X + 0.005</td>
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<td>Accuracy [% RSD, n = 3]</td>
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<td>Precision [% RSD]</td>
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<tr>
<td>Intra-day [n = 3]</td>
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<td>0.32899</td>
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<tr>
<td>Inter-day [n = 3]</td>
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<td>0.58534</td>
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3.2 First Order Derivative Spectroscopy

Appropriate dilutions were prepared for each drug from the standard stock solution and scanned in the spectrum mode from 400 nm to 200 nm. Chlorzoxazone and Tramadol showed absorbance maxima at 236.6 nm and at 213.3 nm for and the overlain spectra of both the drug is represented in Figure 4.

**Fig. 4: Overlay Spectrum of First order Derivative of CXZ and TRM**

### 3.2.1 Validation of proposed method:

**Linearity:** The proposed method was validated according to ICH guidelines. Appropriate aliquots were pipetted out into 10 ml calibrated volumetric flasks and dilutions were made with 0.1 N NaOH solution to obtain working standard solutions of concentrations 2 , 4 , 6 , 8 , 10 µg/ml for Chlorzoxazone and 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 µg/ml for Tramadol. Absorbance for these solutions were measured at 236.6 and 213.3 nm.

**Precision:** Precision of the method was verified by using stock solutions in the ratio of 1:4 containing 5µg/ml CXZ and 20 µg/ml of TRM. System repeatability was done by repeating the assay three times of three replicate dilutions of the same concentration after every two hours on the same day for intraday precision. Inter day precision was carried out by performing the assay of three sample sets after 24 hours and 48 hours, 72 hours. In Intraday study the % RSD was found to be 0.3114 for Chlorzoxazone and 0.3299 for Tramadol. In Interday study the % RSD was found to be 0.3687 for Chlorzoxazone and 0.5853 for Tramadol.

**Accuracy:** Accuracy of method was determined by calculating the recovery of Chlorzoxazone and Tramadol by standard addition solution. Known amount of standard solution of Chlorzoxazone and Tramadol were added at 80%, 100%, 120%. The % RSD was found to be 0.2086 for Chlorzoxazone and 0.4717 for Tramadol.

**Ruggedness:** It is determined by analysis of aliquots from homogenous slot by two analyst using same operational and environmental conditions. The % RSD was found to be 0.2051 for Chlorzoxazone and 0.0904 for Tramadol.

**Robustness:** It expresses the precision within laboratories, Variation like different solvent, 0.11N NaOH solution was taken for analysis. The % RSD was found to be 0.2057 for Chlorzoxazone and 0.0905 for Tramadol.

Table 2: Summary of Validation Parameter of Chlorzoxazone and Tramadol.

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<th>Parameters</th>
<th>Chlorzoxazone</th>
<th>Tramadol</th>
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<tr>
<td>Linearity range [µg/ml]</td>
<td>2 – 10</td>
<td>10– 100</td>
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<tr>
<td>Regression equation [Y = mX +C]</td>
<td>Y = 0.002X + 0.000</td>
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4. RESULTS

The working standard solution of chlorzoxazone (5µg/ml) and Tramadol (20 µg/ml) were prepared separately in 0.1 N NaOH. They were scanned in the wavelength range of 200–400 nm. The overlain spectra of Chlorzoxazone and Tramadol were shown in
the figure 3 and 4. From the overlay derivatised spectra of two drugs, it is evident that show a zero crossing point at 243.4 nm and 271 nm. These two wavelenghts were employed for the determination of Chlorzoxazone and Tramadol. Linear correlation was obtained between absorbance and concentrations of Chlorzoxazone and Tramadol in the concentration ranges of 2-10 μg/ml and 10-100 μg/ml for both the drugs. The linearity of the calibration curve was validated by the high values of correlation coefficient of regression. The RSD values of Chlorzoxazone were found to be 2.0 % at 243.3 nm, respectively. The RSD value of Tramadol was found to be 1.99 % at 271 nm, respectively. Relative standard deviation was less than 2 %, which indicates that proposed method is repeatable. No interference of the excipients with the absorbance of interest appeared; hence the proposed method is applicable for the routine simultaneous estimation of Chlorzoxazone and Tramadol.

5. CONCLUSION
The proposed spectrophotometric method was found to be simple, sensitive, accurate and precise for determination of Chlorzoxazone and Tramadol in Laboratory mixture. The method utilizes easily available and cheap solvent for analysis of Chlorzoxazone and Tramadol hence the method was also economic for estimation of Chlorzoxazone and Tramadol from Laboratory mixture. The common excipients and additives are usually present in the synthetic mixture do not interfere in the analysis of Chlorzoxazone and Tramadol in method, hence it can be conveniently adopted for routine quality control analysis of the drugs in mixture.

6. ACKNOWLEDGEMENT
I take immense pleasure to express my sincere and deep sense of gratitude to my supervising guide Dr. Zameeruddin Mohhamad (M.pharm, Ph.D) Department of Quality Assurance, Indira College of Pharmacy, I am fortunate to have Dr. Prakash Katakam, Principal, SSS Indira College Of Pharmacy, Nanded, and Mr. Bharkad V. B. Principal, SSS College Of Pharmacy, Nanded. Their Dynamic Approach Boosted My Moral, Which Helped Me to Great Extent In Completion Of My Dissertation.

7. REFERENCES
