A novel, safe, economic and sensitive method of spectrophotometric estimation has been developed using Azeotropic mixture (water:methanol: 60:40, v/v) for the quantitative determination of Lornoxicam, a practically water-insoluble drug. Hence, Lornoxicam stock solution was prepared in Azeotropic mixture. Lornoxicam showed maximum absorbance at 383 nm. Beer’s law was obeyed in the concentration range 4–24 μg/mL with regression coefficient of 0.999. The method was validated in terms of linearity ($R^2 = 0.999$), precision (CV for intra-day and inter-day was 0.28–0.68 and 0.12–0.92, respectively), accuracy (98.03–100.59% w/w) and specificity. This method is simple, precise, accurate, sensitive and reproducible and can be used for the routine quality control testing of the marketed formulations.

1. Introduction

All liquid mixtures have forces of intermolecular attraction. That is why they form liquids and not gases. The molecular interactions when two or more components are mixed may cause the mixture to form certain “inseparable” compositions where the vapor and liquid compositions at equilibrium are equal within a given pressure and temperature range. These specific mixture compositions are called azeotropes [1,2].

These azeotropes are forming nonideal solutions but due to this it will increase the solubility power of the other solvent. So we are using the same principle for spectrophotometric study of Lornoxicam.
Lornoxicam (chlortenoxicam) is a non-steroidal anti-inflammatory drug (NSAID) of the oxicam class with analgesic, anti-inflammatory and antipyretic properties, is available in oral and parenteral formulations [3]. Lornoxicam (LOR) differs from other oxicam compounds in its potent inhibition of prostaglandin biosynthesis, a property that explains the particularly pronounced efficacy of the drug.

Extensive literature survey reveals there are various methods reported for the estimation of LOR in pharmaceutical formulations in combination with other drugs by HPLC and other spectroscopic methods [4–9] but there were no any reported methods that use azeotropic mixture as solvent. Though LOR is not practically soluble in water all the methods used organic solvents for estimation of LOR which was not economic as well as environment-friendly. So we have applied concept of azeotropes and combine organic solvent and water to make method economic and environment-friendly (Fig. 1).

2. Materials and methods

2.1. Materials

UV1700 series (Shimadzu, Japan) and Beckman UV–visible double beam spectrophotometer Lambda 19 (Perkin Elmer, USA) using a 1 cm quartz cells were used for absorbance measurements. Standard of Lornoxicam was gift sample from Macleods Pharmaceuticals Ltd. Vapi, Gujarat and tablets were procured from the local market (Lorox, Marketed by Dyota). Whatmann filter paper no. 42 was used to filter the solutions.

2.2. Methods

2.2.1. Preparation of standard stock solution

A 10 mg of LOR (Lornoxicam) standard was weighed and transferred to a 25 mL volumetric flask. 10 mL mixture of methanol and water (40:60, v/v) was transferred to volumetric flask and sonicated for 5 min. The flask was shaken and the volume was made up to the mark with the mixture of methanol and water (40:60, v/v) to give a solution containing 400 mg/mL LOR. From this solution 5 mL was transferred to a 25 mL volumetric flask. The volume was adjusted to the mark with the mixture of methanol and water (40:60, v/v) to give a solution containing 80 mg/mL LOR.

2.2.2. Selection of analytical wavelength

The solution of LOR at concentrations of 4–24 µg/mL was prepared in the mixture of methanol and water (40:60, v/v) and spectra were recorded between 200 and 600 nm. LOR showed $\lambda_{\text{max}}$ at wavelength 383 nm. The overlain spectra of LOR at different concentrations were recorded.

2.2.3. Calibration curve for LOR

Appropriate volume of aliquots from standard LOR stock solution was transferred to different volumetric flasks of 10 mL capacity. The volume was adjusted to the mark with mixture of methanol and water (40:60, v/v) to obtain concentrations of 4,
2.3. Determination of Lornoxicam in tablet dosage form

2.3.1. Sample preparation
Twenty tablets were weighed and finely powered. Powder equivalent to 10 mg LOR was accurately weighed and transferred to volumetric flask of 25 mL capacity. 15 mL of the mixture of methanol and water (40:60, v/v) was transferred to volumetric flask and sonicated for 5 min. The flask was shaken and volume was made up to the mark with the mixture of methanol and water (40:60, v/v). The above solution was filtered through Whatman filter paper (0.45 μm). From this solution 5 mL was transferred to volumetric flask of 25 mL capacity. The volume was made up to the mark to give a solution containing 80 μg/mL LOR (Solution A).

From the solution A, 1.5 mL was transferred to volumetric flask of 10 mL capacity. The volume was made up to the mark with the mixture of methanol and water (40:60, v/v) to give a solution containing 12 μg/mL LOR (Solution 1). This solution was used for the estimation of LOR.

2.3.2. Estimation of LOR in tablet dosage form
The solution 1 was measured at 383 nm for quantification of LOR. The amount of LOR present in the sample solution was determined. From the absorbance obtained in the spectrum, the amount of drug was calculated.

3. Results and discussion

3.1. Method validation
As per the ICH guidelines [10,11], spectra for concentration range 4–24 μg/mL were obtained with n = 6 (Fig. 2, Table 1). Absorbance at 383 nm was measured and the plot of absorbance vs. concentration was plotted. The straight-line equation was determined (Fig. 3). The proposed method obeys Beer’s law in the concentration range 4–24 μg/mL. In this method, the correlation coefficient (R²) was found to be 0.999, the slope was 0.0399 and the intercept was –0.0217.

3.1.1. Accuracy
To investigate the accuracy in sample preparation (i.e., extraction efficiency), we prepare a spiked solution by adding known amounts of related substances into a sample matrix. Thereafter responses of the spiked solutions and the neat standard solutions were taken to assess the recovery from the sample preparation. In this stage, 20 tablets were taken, analysis of the sample was carried out and the results of analysis are shown in Table 2. Recovery studies were carried out by addition of standard drug to the sample at three different concentration levels taking into consideration percentage purity of added bulk drug samples. The method was found to be accurate with % recovery 98.03–100.59% for LOR (Table 3).

3.1.2. Precision
The experiment was repeated three times in a day to determine intra-day precision and on three different days to determine inter-day precision. The coefficient of variance (CV) was calculated at each concentration level. As shown in Table 4, CV for intra-day and inter-day was 0.28–0.68 and 0.12–0.92, respectively. The reproducibility was confirmed by repeating the methods and robustness study was also carried out at variable conditions, then the percent relative standard deviation (% RSD) was calculated.

| Concentration (μg/mL) | Absorbance at 383 nm (Mean ± SD) | CV |
|-----------------------|----------------------------------|----|
| 4                     | 0.1488 ± 0.0018                  | 1.190 |
| 8                     | 0.2938 ± 0.0008                  | 0.276 |
| 12                    | 0.4456 ± 0.0015                  | 0.343 |
| 16                    | 0.6094 ± 0.0021                  | 0.342 |
| 20                    | 0.7866 ± 0.0023                  | 0.294 |
| 24                    | 0.9377 ± 0.0011                  | 0.121 |

*Six replicate samples.

Figure 3 Calibration curve of LOR at 383 nm.

| Amount of drug in tablet powder taken (mg) | Amount found (mg) | Percentage estimated |
|-------------------------------------------|-------------------|----------------------|
|                                           | Formulation 1     | Formulation 2        | Formulation 1 | Formulation 2 |
| 8                                         | 7.92              | 7.91                 | 99.02         | 98.87         |
| 8                                         | 7.89              | 7.93                 | 98.71         | 99.10         |
| 8                                         | 7.99              | 7.84                 | 99.95         | 98.03         |

Formulation 1 is Lornoxi 8 mg; marketed by Hetero HC.
Formulation 2 is Lorox 8 mg; marketed by Dyota.
method is simple, rapid and precise. They do not suffer from any interference due to common excipients of tablets. Therefore, the developed method may be useful for routine analysis of Lornoxicam in bulk drug and pharmaceutical dosage form as shown in Table 2.

4. Conclusion

The spectrophotometric method described in this paper achieves the established pharmacopoeias requirements to be used as a routine method for the quality control of pharmaceutical formulations. The proposed validated method has advantage as it used cheaper solvents in comparison with other standard methods and having accuracy, precision and sensitivity as good as the methods used very expensive solvents.

Acknowledgments

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3.1.3. Other statistical and validation parameter

The summary of method validation is given in Table 5. The method was also found to be specific as no interference observed when the drug was estimated in the presence of excipients. The method was also rugged as there was no change in absorbance up to 48 h of preparation of solution in the proposed solvent.

Validation of the proposed method of analysis is confirmed statistically by low values of standard deviation, percent coefficient of variation and standard error. The proposed method is simple, rapid and precise. They do not suffer from any interference due to common excipients of tablets. Therefore, the developed method may be useful for routine analysis of Lornoxicam in bulk drug and pharmaceutical dosage form as shown in Table 2.

4. Conclusion

The spectrophotometric method described in this paper achieves the established pharmacopoeias requirements to be used as a routine method for the quality control of pharmaceutical formulations. The proposed validated method has advantage as it used cheaper solvents in comparison with other standard methods and having accuracy, precision and sensitivity as good as the methods used very expensive solvents.

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