Research Article

Method Development and Validation for Estimation of Eperisone Hydrochloride as API and in Tablet Dosage Form by Two Spectroscopic Methods

Joytosh Banerjee, Renu Solanki, and Badri Prakash Nagori

Department of Quality Assurance, Lachoo Memorial College of Science & Technology (Autonomous), Jodhpur, Rajasthan 342003, India

Correspondence should be addressed to Joytosh Banerjee; joytoshb@gmail.com

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Two simple and sensitive spectrophotometric methods have been developed for the determination of eperisone hydrochloride based on its ability to be detected in UV region (Normal UV) and its oxidation using potassium permanganate in alkaline medium (kinetic spectroscopic). The detection was done at 261.40 nm and 603.5 nm. The different experimental parameters affecting the method development were studied and optimized. The initial rate and fixed time method were utilized to construct calibration graph, and 5 minutes and 3 minutes, respectively, were found suitable for the determination of the concentration of drug. Linearity was found over the concentration range of 2–20 μg/mL, 15–30 μg/mL, and 15–35 μg/mL by UV, initial rate, and fixed time methods, respectively. The results were validated as per the ICH guidelines. RSD values were found to be less than 2%. The methods were applied for estimation of eperisone hydrochloride in RAPISONE (Abbott, Maharashtra). The assay results were found to be 100.4% ± 0.08, 99.93% ± 0.05, and 99.41% ± 0.04 by UV, initial rate, and fixed time method, respectively. Statistical comparison of the proposed methods showed a good agreement indicating no significant difference in accuracy and precision, thus confirming the suitability of UV and kinetic method for the estimation of eperisone hydrochloride in bulk as well as in tablet dosage forms.

1. Introduction

Eperisone hydrochloride is an antispasmodic drug sold in Japan, India, Philippines, and Bangladesh [1]. It belongs to piperidinopropiophenone analogues, and its structure is shown in Figure 1. It acts by relaxing both skeletal muscles and vascular smooth muscles and demonstrates reduction of myotonia, improvement of circulation, and suppression of pain reflex [2]. Kinetic spectrophotometric methods have regained interest in the recent past due to its specific advantages as well as improved capabilities of the modern instrument [3–5]. LC-ESI-MS [6], GC-MS [7, 8], and HPLC [9, 10] methods were proposed for the determination of eperisone hydrochloride in biological fluids. Various methods were proposed for the estimation of degradation products [11]. Japanese Pharmacopoeia reports potentiometric method for its estimation [12]. Kinetic spectrophotometric method has not been reported so far in any literature. The objective of the research is to develop and validate UV and kinetic spectrophotometric method for the estimation of eperisone hydrochloride for routine analysis in bulk and tablet dosage forms. The methods would be statistically compared for their suitability for estimation of the drug.

2. Material and Method

Double distilled water was used as solvent to prepare solutions. Potassium permanganate (KMnO₄) (LOBA CHEMIE, India) was accurately weighed and dissolved in distilled water to obtain a solution of 6 × 10⁻³ M as given in the Indian Pharmacopoeia (I.P.). Sodium hydroxide (NaOH) (LOBA CHEMIE, India) was also accurately weighed and dissolved in distilled water to obtain a solution of 1.0 M in distilled water. Shimadzu UV-1800 model with spectral band width one nm and wavelength accuracy of 0.3 nm was used for spectral measurements. One cm matched quartz cells were
used for carrying the analyte solution. Weighing was done on electronic balance (GR-200, A & D Company). Glassware used in each procedure were washed and dried at the end of each day’s work. Pure drug of eperisone hydrochloride was obtained as gift sample from Sun Pharma Industries, Mumbai. Tablets of brand name RAPISONE (Batch no. AME-008B), marketed by Abbott, Maharashtra, were procured from local pharmacy.

2.1. Preparation of Standard Stock Solution

2.1.1. For Normal UV Spectrophotometric Method. Standard stock solution of 1000 μg/mL was prepared by dissolving 100 mg of pure active pharmaceutical ingredient in 100 mL of double distilled water in a volumetric flask.

2.1.2. For Kinetic Spectrophotometric Method. Stock solution A (for method development): 100 mg of eperisone hydrochloride was accurately weighed and transferred to 100 mL volumetric flask. It was dissolved in 25 mL of doubly distilled water, and the volume was made up to 100 mL with the same. The final solution contained 1000 μg/mL of eperisone hydrochloride solution.

Stock solution B (for determination of stoichiometry): 1775 mg of eperisone hydrochloride was accurately weighed and transferred to 10 mL volumetric flask. It was dissolved in 25 mL of doubly distilled water, and the volume was made up to 10 mL with the same. The final solution contained 17750 μg/mL of eperisone hydrochloride solution.

2.2. Preparation of Sample Solution of Pharmaceutical Tablets

2.2.1. For Normal UV Method. Twenty tablets were taken, and the I.P. method was followed to determine the average weight. Weighed tablets were triturated well. A quantity of powder equivalent to 10 mg of drug was transferred to 100 mL volumetric flask and mixed with 25 mL of doubly distilled water. The solution was sonicated for 10 minutes, and the volume was made up to the mark with doubly distilled water solvent. The solution was then filtered through Whatman filter paper no. 42.

2.2.2. For Kinetic Spectrophotometric Method. 20 tablets were weighed and triturated well. A quantity of powder equivalent to 50 mg of drug was transferred to 100 mL volumetric flask and mixed with 25 mL of double distilled water, and the solution was sonicated for 15 minutes. The volume was finally made up to 100 mL with double distilled water. The solution was filtered through Whatman filter paper no. 42. The filtrate was diluted with water to obtain working concentration in the range of 15–25 μg/mL for the drug.

2.3. Procedure

2.3.1. Normal UV Method. Ten mL of stock solution A was taken and further diluted to 100 mL to obtain working stock solution of 100 μg/mL. From the solution, 2 mL was transferred to a ten mL volumetric flask, and the volume was made up to ten mL with double distilled water to get the final solution of 20 μg/mL. The solution was scanned between 200 and 400 nm. Wavelength of maximum absorbance (λmax) was found to be 261.40 nm. Different aliquots (0.2, 0.4, ...2.0 mL) of working stock solution were accurately measured and diluted in a series of ten mL volumetric flask with doubly distilled water to obtain concentrations of 1, 2, 3, 4, ... , and 20 μg/mL.

2.3.2. Initial Rate Method. A series of dilutions (150–350 μg/mL) was prepared by pipetting out different aliquots of stock solution A into a series of 10 mL volumetric flask. At the time of observation, 1.6 mL of sodium hydroxide solution (1M) was added, followed by 2.0 mL of potassium permanganate solution (0.006M) to each flask and then diluted to the volume with double distilled water. The content of mixture of each flask was mixed well, and the increase in absorbance was recorded (in Kinetics mode) at 603.5 nm as a function of time for 15 minutes against reagent blank treated similarly. The initial rate of reaction at different concentrations was obtained from the slope of tangent to the absorbance-time curve (at 5 minutes).

2.3.3. Fixed Time Method. In this method, the absorbance was recorded accurately at preselected time of 3, 6, 9, 12, and 15 minutes against reagent blank treated similarly. The calibration graphs were constructed by plotting the absorbances against various concentrations of the series of drug solution. The concentration of the drug was determined from either calibration curve or regression equation.

3. Results and Discussion

3.1. For Normal UV Method. Calibration curve was plotted at λmax of 261.40 nm as shown in Figure 2. Linearity was found in the range of 2–22 μg/mL as shown in Figure 3. The various optical parameters are reported in Table 1.
Table 1: Optical parameters and regression characteristic for eperisone hydrochloride.

| Sr. no. | Parameter                      | Result                  |
|---------|--------------------------------|-------------------------|
| (1)     | Absorption maxima (nm)         | 261.40                  |
| (2)     | Linearity range (µg/mL)        | 2–20                    |
| (3)     | Standard regression equation   | $Y = 0.04867X - 0.00058$|
| (4)     | Correlation coefficient ($r^2$)| 0.99936                 |
| (5)     | Molar absorptivity (L/mol/cm)  | 14718.93                |
| (6)     | A (1%, A cm)                   | 497.5                   |
| (7)     | Sandell’s sensitivity (µg/cm²) | 0.02                    |
| (8)     | Intraday precision (% RSD)     | 0.14                    |
| (9)     | Interday precision (% RSD)     | 0.28                    |
| (10)    | LOD (µg/mL)                    | 0.33                    |
| (11)    | LOQ (µg/mL)                    | 1.0                     |

Figure 3: Calibration curve of eperisone hydrochloride in doubly distilled water (2–22 µg/mL).

3.2. Optimization of Parameters for Kinetic Spectrophotometric Method. Potassium permanganate, as strong oxidizing agent, has been used in oximetric analytical method for the determination of many compounds. The valency of manganese changes during the course of the reaction. The heptavalent manganese ion changes to the green color (Mn VI), while, in neutral and acidic medium, the permanganate is further reduced to colorless (Mn II). The behavior of permanganate was the basis for its use in the development of spectrophotometric method. The absorption spectrum of aqueous potassium permanganate solution in alkaline medium exhibited an absorption band at 526 nm. The addition of eperisone hydrochloride to this solution produces a new characteristic band at 603.5 nm. This is shown in Figure 4. The band is due to the formation of manganate ion, which resulted from the oxidation of eperisone hydrochloride by potassium permanganate in alkaline medium. The intensity of the color increases with time; therefore, a kinetically based method was successfully developed for the determination of eperisone hydrochloride in their pharmaceutical dosage formulations.

Figure 4: Absorption spectra of (A) eperisone hydrochloride (20 µg/mL); (B) alkaline potassium permanganate (0.006 M), and (C) the reaction product.

Figure 5: Effect of potassium permanganate on the reaction between the investigated eperisone hydrochloride (20 µg/mL) and alkaline potassium permanganate.

3.3. Effect of Potassium Permanganate Concentration. The absorbance increases substantially with increasing the concentration of potassium permanganate as shown in Figure 5. Maximum absorbance was obtained when 2.0 mL of 0.006 M potassium permanganate was used. Thus, the adoption of 2 mL of potassium permanganate in the final solution proved to be adequate for the determination of eperisone hydrochloride (final concentration was 0.0012 M).

3.3.1. Effect of Sodium Hydroxide Concentration. Maximum absorption was obtained when 1.6 mL of 1 M NaOH was used. Over this volume, no deliberate change in absorbance
could be detected. So, 1.6 mL of 1 M of NaOH was used as an optimum value as shown in Figure 6.

3.3.2. Effect of Temperature. At room temperature, the reaction rate increases substantially with time. Moreover, heating the solution was found to increase the rate of the reaction but caused precipitation of MnO$_2$ also. Therefore, room temperature was selected as the optimum temperature.

3.3.3. Stoichiometry and Reaction Mechanism [13, 14]. The stoichiometric ratio between potassium permanganate and eperisone hydrochloride was determined by Job’s method and was found to be 4 : 1. Corrected Job’s plot is shown in Figure 7. Eperisone hydrochloride was found to be susceptible for oxidation with alkaline potassium permanganate producing a green color at $\lambda_{\text{max}}$ of 603.5 nm.

3.3.4. Kinetics of the Reaction. Under the optimum conditions, the absorbance time curves of eperisone hydrochloride with potassium permanganate reagent were constructed (as shown in Figures 8 and 9). The initial rate of the reaction was determined from the slope of tangents of the absorption time curves. The order of the reaction with respect to permanganate was determined by studying the reaction at different concentrations of permanganate with fixed concentration of investigated eperisone hydrochloride. The plot of initial rate ($\Delta A/\Delta t$) against initial absorbance was linear passing through origin indicating that the initial order of the reaction with respect to permanganate was 1 as shown in Figure 10.

The order with respect to investigated eperisone hydrochloride was evaluated by measuring the rate of the reaction at several concentrations of eperisone hydrochloride at a fixed concentration of permanganate reagent as shown
in Figure 11. This was done by plotting the logarithm of initial rate of the reaction versus logarithm of molar concentration of investigated eperisone hydrochloride and was found to be approximately 1 as shown in Figure 12. However, under the optimized experimental conditions, the concentrations of eperisone hydrochloride were determined using relative excess amount of potassium permanganate and sodium hydroxide solutions. Therefore, pseudo-zero-order conditions were obtained with respect to their concentrations.

3.3.5. Quantitation by Kinetic Methods

(1) Initial Rate Method. Initial rate of reaction would follow pseudo-first-order and were found to obey (1):

\[ V = \frac{dA}{dt} = K' C^n, \]  

where \( V \) is the reaction rate, \( A \) is absorbance, \( t \) is the measuring time, \( K' \) is the pseudo-first-order rate constant, \( C \) is the molar concentration, and \( n \) is the order of the reaction. The logarithmic form of the reaction is shown in (2):

\[ \log V = \log \frac{\Delta A}{\Delta t} = \log K' + n \log C. \]  

Regression analysis was performed using the method of least square to evaluate the slopes, intercepts, and correlation coefficients. The analytical parameters and results of regression analysis are given in Table 2. The value of \( n (=1) \) in the regression equation confirmed that the reaction of eperisone hydrochloride with the potassium permanganate was a pseudo-first-order with respect to eperisone hydrochloride concentration. The limits of detection (LOD) were calculated, and results obtained confirmed good sensitivity.
of the proposed method and consequently their capabilities
to determine low amount of eperisone hydrochloride.

(2) Fixed Time Method. In this method, the absorbance of the
reaction solution containing varying amounts of eperisone
HCl was measured at preselected fixed time. Calibration
curve of absorbance versus the concentration of the drug at
fixed time was plotted (shown in Figure 13). The regression
equation, correlation coefficients, and detection limits were
calculated and reported in Table 3. The lowest detection limit
was obtained at fixed time of 6 minutes. However the fixed
time of 3 minutes showed a wider concentration range for
quantification.

3.4. Validation of Proposed Methods [15, 16]

3.4.1. Specificity. Specificity of the UV method was evaluated
by addition of common tablet excipients available in the
laboratory and recording the UV scan. No interference was
detected from the added excipients at 261.40 nm, and the
variations in absorbance were negligible.

In case of kinetic spectroscopic method, both the pro-
posed methods were performed in visible range away from
the UV-absorption region. A very minor change in the
absorbance values was seen during the time duration.

3.4.2. Linearity. Linearity data are studied for both methods
and reported in Tables 1, 2, and 3.

3.4.3. Accuracy. Accuracy studies in terms of percentage
recovery (percentage) were studied for normal UV and
kinetic spectroscopic method and reported in Tables 4, 5, and
6. The results of mean % recovery were within the range of 98–
102%, and % RSD was less than 2% so the proposed method
was found to be accurate.

3.4.4. Precision. The developed methods were subjected to
precision studies. The analytical techniques demonstrated
good precision, and the data is given in Tables 7, 8, and 9.

3.4.5. Detection and Quantification Limits. The values are
reported in Tables 1, 2, and 3.

4. Application of the Proposed Method

The developed methods were applied for the determination
of eperisone HCl on pharmaceutical tablet dosage form.
The tablet content was computed from its corresponding
regression equations. The obtained mean recovery values of
the labeled amounts were 100.4% ± 0.07, 99.93% ± 0.05,
and 99.41% ± 0.04 as reported in Table 10.

5. Statistical Treatment of Data [17, 18]

The accuracy and precision of the kinetic methods (initial
rate and fixed time) were statistically compared with that
of UV spectrophotometric method. Statistical comparison
was done by applying Student’s t-test and ANOVA. There
was no significant difference found between the calculated
and theoretical values at 95% confidence level as reported in
Tables 11, 12, and 13. This indicated the similarities between
the proposed and the reference method for the determination
of eperisone HCl in the tablet dosage form.

6. Conclusion

In the present study, two spectroscopic methods (UV and
kinetic spectroscopy) were developed, validated, and statis-
tically compared for determining eperisone HCl in API as
well as in tablet dosage forms. Normal UV method proved
Table 8: Data showing intraday precision analysis for initial rate method and fixed time method.

| Conc. (µg/mL) | Initial rate method |  | Fixed time method |  |
|---------------|---------------------|----|-------------------|----|
|               | Mean ± S.D.         | % RSD | Mean ± S.D.       | % RSD |
| 20            | 20.02 ± 0.04        | 0.211 | 19.89 ± 0.18      | 0.92  |
| 25            | 25.0 ± 0            | 0    | 24.78 ± 0.19      | 0.76  |
| 30            | 30.01 ± 0.01        | 0.033 | 29.89 ± 0.19      | 0.64  |
| Mean % RSD   | 0.081               | 0.777 |

Table 9: Data showing interday precision analysis for initial rate method and fixed time method.

| Conc. (µg/mL) | Initial rate method |  | Fixed time method |  |
|---------------|---------------------|----|-------------------|----|
|               | Mean ± S.D.         | % RSD | Mean ± S.D.       | % RSD |
| 20            | 20.02 ± 0.15        | 0.076 | 19.83 ± 0.16      | 0.805 |
| 25            | 25.06 ± 0.06        | 0.246 | 24.76 ± 0.20      | 0.829 |
| 30            | 30.07 ± 0.06        | 0.220 | 29.65 ± 0.35      | 1.180 |
| Mean % RSD   | 0.180               | 0.938 |

Table 10: Estimation of eperisone hydrochloride in tablet dosage form.

| Brand name | Label claim (mg) | Conc. found (mg) | Conc. found (mg) (initial rate) | Conc. found (mg) (fixed time) |
|------------|------------------|------------------|---------------------------------|------------------------------|
| RAPISONE   | 50 mg            | 10.04            | 20.03                           | 19.94                        |
|            |                  | 9.94             | 19.93                           | 19.84                        |
|            |                  | 10.14            | 19.98                           | 19.87                        |
| Mean       |                  | 10.04            | 19.98                           | 19.88                        |
| % assay    |                  | 100.4            | 99.93                           | 99.42                        |

Table 11: Results of t-test applied for recovery of drugs to compare initial rate method and fixed time method with normal UV method.

| S. no. | Tablet name | Initial rate method | UV method | Fixed time method | UV method |
|--------|-------------|---------------------|-----------|-------------------|-----------|
| (1)    | RAPISONE    | 99.93               | 101.8     | 99.42             | 101.8     |
| (2)    | RAPISONE    | 100.29              | 99.95     | 99.12             | 99.95     |
| (3)    | RAPISONE    | 101.41              | 100.11    | 99.15             | 100.11    |
| Mean   | RAPISONE    | 100.54              | 100.62    | 99.22             | 100.62    |
| Df     | 2           | 2                  |           |                   |           |
| t Stat | 0.081       | 2.79               |           |                   |           |
| t Critical two-tail | 4.302         | 4.302               |           |                   |           |

Table 12: Results of t-test applied for precision data of drugs to compare both the methods.

| S. no. | Tablet name | Initial rate method | UV method | Fixed time method | UV method |
|--------|-------------|---------------------|-----------|-------------------|-----------|
| (1)    | RAPISONE    | 100.08              | 101.2     | 100               | 101.2     |
| (2)    | RAPISONE    | 100.22              | 100.3     | 98.41             | 100.3     |
| (3)    | RAPISONE    | 100.23              | 98.33     | 100               | 98.33     |
| Mean   | RAPISONE    | 100.18              | 99.94     | 99.47             | 99.94     |
| Df     | 2           | 2                  |           |                   |           |
| t Stat | −0.268      | 0.434              |           |                   |           |
| t Critical two-tail | 4.302         | 4.302               |           |                   |           |

to be a simple and cost-effective method for the estimation of the drug. Kinetic spectroscopic method has been used in the past for the elemental analysis and detection of biomolecules in biological fluids (like enzymes). The applicability of this method has been successfully studied and extended in this research. The developed method involves measurements in the visible region and is more selective than the reported UV based methods. Further, the developed methods utilized doubly distilled water as solvent which is easily available, safe, and economical as well. The initial rate and fixed time
methods can be easily applied as they do not require elaborate samples and/or tedious procedures for the analysis. Besides, both methods are sensitive enough for the analysis of lower amount of the drug. Furthermore, the developed methods do not require expensive instruments and/or critical analytical reagents. Statistical comparison of the two developed methods proved that there was no significant difference in the accuracy, precision, and quantitative capabilities. These advantages give the proposed methods a great value to the spectroscopic analysis of eperisone HCl in quality control laboratories.

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