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Research Article

Development and Validation of a UV Spectroscopic Method for Quantification of Eperisone Hydrochloride

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A B S T R A C T

A simple, efficient, rapid, sensitive, precise and economical UV Spectrophotometric method has been developed for estimation of eperisone hydrochloride from bulk and pharmaceutical formulation. The method was developed and validated according to International Conference on Harmonization (ICH Q2 R1) guidelines. The λmax of agomelatine in acetonitrile was found to be 259 nm. The analytical method validation parameters linearity, precision, accuracy, robustness were studied according to International Conference on Harmonization guidelines. Pure drug concentration was prepared in the range of 1-5 µg/ ml and the linear regression analysis data showed good linear relationship with correlation coefficient value 0.9997. The precision of the method was studied as an intra- day, inter-day variations with value less than 2 % RSD. The limit of detection and limit of quantitation were found to be 0.554 and 1.467 µg/ mL, respectively. Recoveries were found to be in the range of 99.26 to 100.35% and % RSD was less than 2%. This proposed UV spectroscopic method is simple and suitable for routine analysis.

Keywords: Eperisone Hydrochloride, Validation, UV Spectrophotometric Method

Introduction

Eperisone hydrochloride (EPS) is chemically 1-(4-Ethylphenyl)-2 -methyl-3-(1-piperidyl) propan-1-one; hydrochloride (Figure 1). Its molecular formula is C17H26ClNO and its molecular weight is 295.85 g/ mol. Eperisone is used in treatment of muscle spasm. It relieves painful spasms of the skeletal muscles. Eperisone is a muscle relaxant. It works on the centres in the brain and spinal cord to relieve muscle stiffness or spasm without reduction in strength. This improves pain and movement of muscles. It is soluble in organic solvents such as DMSO and dimethyl formamide.[1,2,3]

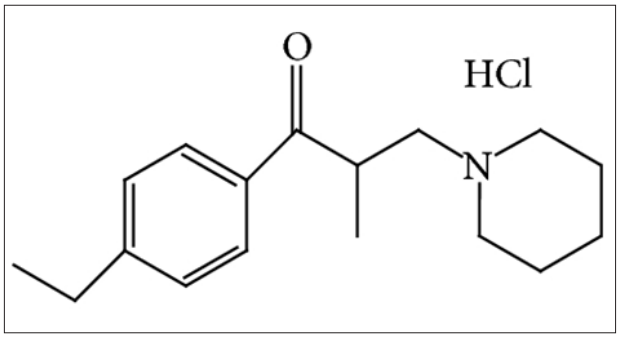

Figure 1.Chemical structure of eperisone hydrochloride (EPS)

The literature reports some analytical assays applied to EPS in different matrices. Among them, we highlighted HPLC chromatographic methods for quantitation in pharmaceuticals,[4-7] HPTLC method8, UV spectrophotometric methods[9,10,11] with other drugs in combinations. In the present study we aimed to develop an UV specrophotometric method for quantitative analysis of agomelatine in commercial sample, applying validation protocols.

There is a need for a simple, rapid, cost effective and reproducible method for assay of EPS in its dosage forms. Therefore, it was thought of interest to develop simple, rapid, accurate, specific and precise UV method for the analysis of EPS in its tablet formulation. The objective of the current work is, therefore, to develop a simple UV method for analysis of EPS in tablet formulations.

Instrumentation

A double beam Shimadzu UV/ Vis spectrophotometer, model 1800 (Japan) having a spectral bandwidth of 1 nm, wavelength accuracy of ±0.5 nm and a pair of 1 cm quartz cells was used.

Selection of Solvents

The UV spectrum of EPS was recorded in various solvents. The spectral pattern and absorbance maxima of EPS were thoroughly analysed. It was found that significant spectra of EPS appeared in methanol and this solvent was selected for determining EPS content in formulation by UV spectroscopic method. Stock solution of EPS was prepared by dissolving 50 mg of drug in 50 mL of methanol to obtain the concentration of 1000 µg/mL. It was further diluted to obtain concentration ranging from 1-5 µg/mL.

Determination of Wavelength of Maximum Absorption

The stock solution was suitably diluted with methanol, so as to contain 5 µg/mL of EPS. This solution was scanned in the UV region and found that EPS exhibited maximum absorbance at about 259 nm. Hence 259 nm was selected for the proposed study.

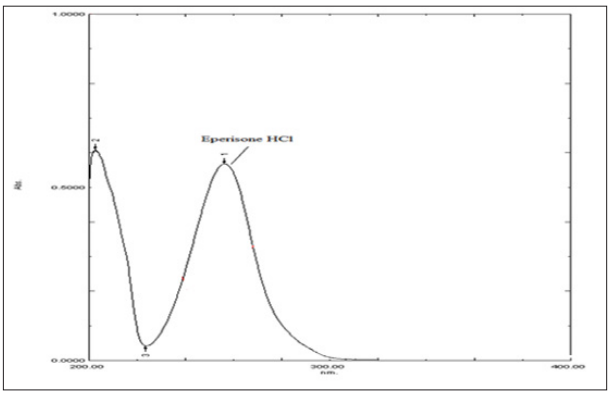

Figure 2.UV spectrum of eperisone hydrochloride

Standard Preparation

Accurately weighed quantity of 50 mg of EPS was transferred into 50 mL volumetric flask containing 25 mL methanol. The volume was made up with methanol with strength of 1000 µg/mL (stock solution). It was further diluted to obtain the stock solution with a concentration 50 µg/ mL. From this solution, 5 mL was taken and diluted to 50 mL to get a concentration of 5 µg/mL. The resultant solution was scanned in the wavelength range of 200 - 400 nm and the absorbance was measured.

Study of Beer- Lambert's Law

Adequate dilutions were made from stock solution to get a concentration ranging from 1-5 µg/mL for EPS using methanol. Absorbance of these solutions were measured at 259 nm (Table 1). The measured absorbance was plotted against concentration. From the graph it was found that the Beer's law concentration for EPS lies between 1-5 µg/ mL. The spectrum is shown in Figure 2.

Table 1.Absorbance of eperisone hydrochloride

Concentration (µg/mL)	Absorbance
1	0.113
2	0.167
3	0.220
4	0.274
5	0.332

Method Validation

The analytical method was validated as per ICH guidelines.

Specificity

The specificity was assessed by testing analytical interferences from excipients. This analytical parameter was determined by comparing ultraviolet absorption spectra obtained from EPS standard solution, sample solution and placebo. The spectra were obtained in the range of 200 to 400 nm, and the overlap of absorption bands was evaluated.

Linearity

A calibration graph was obtained by plotting EPS concentrations against their corresponding absorbance values. The linearity of standard solution was found to be in the range of 1-5 µg/mL with linear correlation coefficient 0.9997. Beer-Lambert's law was obeyed in the range.

Accuracy

Accuracy of the method was checked by the recovery studies at three different levels, i.e., 50, 100 and 150 %.

Precision

The precision of the assay method was evaluated in terms

of repeatability by carrying out six independent assays of EPS test sample preparation and calculated the % RSD of assay (intraday). Intermediate precision of the method was checked by performing same procedure on the different day (inter day) by another person under experimental condition.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

ICH defines the limit of detection of an analytical method as the lowest amount of analyte in a sample, which can be detected but not necessarily quantitated as an exact value whereas limit of quantitation of an analytical procedure is the lowest amount of analyte in a sample, which can be determined quantitively with suitable precision and accuracy. LOD and LOQ were calculated by using following formula: LOD=3.3×σ/S and LOQ=10×σ/S, where, σ is the standard deviation of y-intercepts of regression line, S is slope of the calibration curve.

Robustness

The study of robustness was carried out to evaluate the influence of slightly changed conditions in the Spectrophotometric method. The factors chosen for this study were the diluents change and analyst change.

Solution Stability

The stability study of solution for test preparation was carried out. The solution was preserved at ambient temperature and 2-5˚C and tested at interval of 12, 24, 36 and 48 hr. The responses for the aged solution were evaluated using a freshly prepared standard solution.

Analysis of Eperisone Hydrochloride in Formulation

The average weight of twenty tablets of EPS (Myosone tablets 50 mg EPS) was weighed. An aliquot quantity equivalent to 50 mg of EPS was weighed and transfered to 50 mL volumetric flask. The contents were shaken with methanol and sonicated for a minimum 30 min. The sample was filtered through 0.45µm Whatman filter paper. Then it was made upto the volume. It was further diluted to obtain the stock solution with a concentration 50 µg/mL. From this solution, 5 mL was taken and diluted to 50 mL to get a concentration of 5 µg/mL. The resultant solution was scanned in the wavelength range of 200 - 400 nm and the absorbance was measured. The concentration of drug was determined by single point standardization method.

Result and Discussion

Method Development and Optimization

The standard solution of EPS was prepared in methanol during development and optimization phase, as EPS is freely soluble in organic solvents like methanol. And further dilutions were made using methanl. The λ_{max} in methanol was found to be 259 nm.

Method Validation

The analytical method was validated as per ICH guidelines.

Linearity

A calibration graph was obtained by plotting EPS concentrations against their corresponding absorbance values. Linearity was good in concentration range 1 to 5 µg/mL. The response of drug was found to be linear regression equation y=0.0545x + 0.0577 with correlation coefficient 0.9997 (Figure 3). All the quantitative parameters were estimated is listed in Table 2.

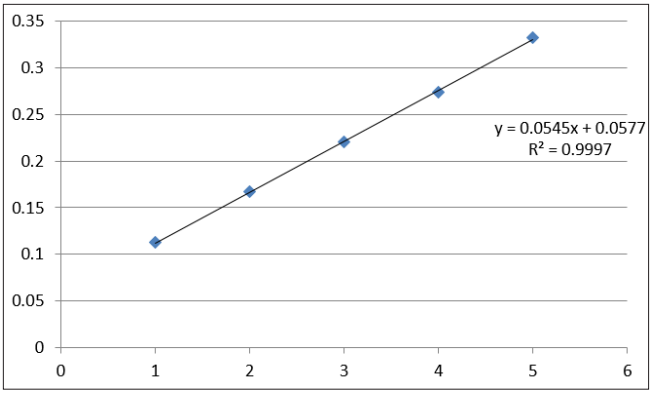

Figure 3.Calibration curve of pure eperisone hydrochloride

Table 2.Quantitative parameters of UV spectrophotometric method

Parameter	Result
λ_{max} (nm)	259
Beer's law limits (µg/mL)	1-5
Regression equation	Y= 0.0545x + 0.0577
Slope	0.0545
Intercept	0.0577
Correlation coefficient (R2)	0. 9997

Precision

Here, number of measurements made [n] are 6. The % RSD was found to be in the range of 0.594- 1.038 for intra-day precision and 0.810- 1.217 for inter-day precision (Table 3).

Table 3.Result of intra-day and inter-day precision

Concentration (µg/mL)	Intra-day precision		Inter-day precision	
	SD	%RSD	SD	%RSD
1	0.021	0.594	0.031	0.810
3	0.059	1.038	0.068	1.217
5	0.040	0.607	0.135	0.863

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The sensitivity of the method was assessed by determining the LOD and LOQ. The LOD and LOQ for EPS were found to be 0.554 and 1.467 µg/mL, respectively.

Accuracy

Accuracy of the method was checked by the recovery studies at three different levels, i.e., 50, 100 and 150 %. The mean of the recovery for EPS was found to be 99.72 % (Table 4).

Table 4.Results of recovery studies

Amount of sample (µg/mL)	Amount of drug added (µg/mL)	Percent of spiked sample	Amount recovered (µg/mL)	Percent recovery
2	1	50 %	2.978	99.26
2	2	100 %	4.014	100.35
2	3	150 %	4.973	99.55

Robustness

The test is performed by making deliberately change in the procedure, to check either the method is stable with slight variations or not. In this procedure test is performed by preparing samples of 100% label claim. Samples kept on 4°C, 25°C and on 35°C for 4hrs and assayed according to the test procedure (Table 4).

Table 4.Assay in different storage conditions

Storage Condition	Assay (mg/ tablet)	Assay (% of label claim)
4°C	49.97	99.94
25°C (ambient temperature)	50.04	100.08
35°C	50.07	100.14

Table 5.Analysis of eperisone hydrochloride formulation

Tablet	Amount of drug mg/ tab	Amount of drug estimated mg/ tab	% Label claim
Myosone	50	50.5	101
		50.7	101.4
		50.87	101.74
		50.46	100.92
		50.24	100.48
		50.82	101.64
		Mean±SD	101.20±0.48
		% RSD	0.005

Analysis of the Commercial Formulation

The concentration of drug was determined by single point standardization method and results are shown in (Table 5).

Conclusion

Based on the satisfactory results of all performance parameters, the method is validated and found accurate, fast and precise for the estimation of eperisone hydrochloride in bulk and tablet formulation by UV Spectrophotometry. Therefore it can be used to determine eperisone hydrochloride in routine quality control.

Conflict of Interest: None

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