Research Article
AMERICAN JOURNAL OF PHARMACY AND HEALTH RESEARCH
www.ajphr.com
2013, Volume 1, Issue 1
ISSN : 2321–3647(online)

Quantitative Estimation of Single Component Eperisone as API and in Tablet Dosage form using U.V. Spectrophotometry

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ABSTRACT

U.V. spectrophotometric method for estimation of Eperisone hydrochloride as individual and in tablet formulation using graphical extrapolation method has been developed. Methanol was selected as solvent for estimation at 258 nm. Linearity was followed in the range of 2.5-17.5 μg/ml with correlation coefficient of 0.997. Detection limit and quantitation limit were found to be 0.082 μg/ml and 0.22 μg/ml .Developed method has followed all the criteria for validation as per ICH norms and found to be accurate, precise, reproducible and sensitive with negligible excipients interference. Accuracy of the developed was assessed using spiking technique on available tablet dosage forms. Results obtained greater than 98% meant good accuracy of method for analysis in any dosage form.

Keywords: Eperisone hydrochloride, Graphical method, Methanol, Recovery, Validation.

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Received 21 January 2013, Accepted 26 February 2013

Please cite this article in press as: Jhanwar B. *et al.*, Quantitative Estimation of Single Component Eperisone as API and in Tablet Dosage form using U.V. Spectrophotometry. American Journal of Pharmacy & Health Reserach 2013.

INTRODUCTION

Eperisone hydrochloride (Figure.1), chemically called 4-ethyl 2-methyl piperidinopropiophenone (EMPP) hydrochloride, is an antispasmodic agent.[1] It has a relatively low incidence of central depression when compared with other anti-spasmodic drugs, which makes it widely used for the therapeutic treatment of spastic patients to relieve skeletal muscle stiffness and back pain. It is useful in Diabetical angiopathy and thromboangitis obliterans, raynauds syndrome.[2, 3] Eperisone Hydrochloride is official in Japanese pharmacopoeia. However, literature did not reveal any reported methods for the estimation of Eperisone HCl in any dosage form. Hence, aim of present work is to develop and validate an analytical technique for quantification of Eperisone Hydrochloride individually as API and in tablet dosage form.

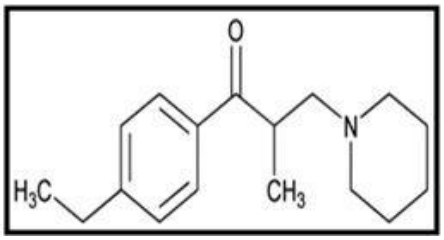

Figure 1:- Eperisone hydrochloride

MATERIAL AND METHODS

Shimadzu UV-1800, connected to computer having UV- Probe software was employed with matching pair of 1 cm quartz cuvette (Shimadzu Corporation, Kyoto, Japan). The spectral bandwidth is 0.5 nm. All weights were measured on Digital balance GR-100 (A&D comp Ltd.).

Chemicals

Eperisone hydrochloride was kindly provided by Sun Pharma, Silvassa. The purity of reference standards were more than 98.5%w/w. Starch, lactose, magnesium stearate and talc were obtained from Merck Ltd, Maharashtra. Distilled water was obtained from in house laboratory. Chemicals and solvents used were of A.R. grade.

Preparation of Standard Stock Solution

Accurately weighed Eperisone (50 mg) were separately transferred to 100 ml volumetric flask and dissolved in methanol. Volume was made up to the mark with methanol. Further 10 ml of each resulted solution was transferred to a 100 ml volumetric flask separately and volume was made up to the mark to produce final working standard stock solutions containing 50µg/ml of EPR.

Simultaneous Equation Method

Working standard solutions having 15 µg/ml of EPR were scanned individually in range of 200-

400 nm to determine there λ_{max}. It was found to be 261.4 nm (Figure 4).

Standard solutions having concentration 2.5 to 17.5 µg/ml for EPR were obtained by transferring 0.5, 1.0, 1.5, 2.0, 2.5, 3.0 and 3.5 ml of standard stock solution to 10 ml volumetric flasks. The volume was made up with methanol. The absorbance of these dilutions were measured at λ_{max} using methanol at reference side Standard curve was plotted at 258 nm (Figure 2). Linear equation generated by calibration curves is as follows EPR at 258.0 nm, **Y = 0.06028x - 0.00911**

Effect of solvent on absorbance maxima

Different solvents have been used to determine the shifting in maximum absorption wavelength of the Eperisone HCl as shown in figure 3 to 7

Identification of Standard API

Identification of API sample was done using ATR spectroscopy and was compared with the standard official IR graph form Japanese Pharmacopoeia as shown in figure 8 and 9

Method Validation

The developed methods has been validated in terms of linearity, range, specificity, accuracy, precision, assay, LOD and LOQ as per ICH Q2 (R1), 2005.[13]

Linearity

Appropriate aliquots of EPR standard stock solution was transferred to volumetric flask of 10 ml capacity. The volume was adjusted to the mark with methanol. All absorbance were measured at 258 nm. Calibration curve was constructed by plotting average absorbance (n=6) versus concentrations using software. Straight line equation was obtained from calibration curve. Optical and regression parameters are given in Table 3.

Range

Range of an analytical method is defined as the interval between upper and lower levels. **Working range:** It begins from limit of quantitation to the maximum concentration used for the development of the analytical method.

Linearity range: It is the interval in which the response is directly proportional to the concentration between the upper and lower levels.

Target concentration: It is defined as the concentration, which is equal to the midpoint of linearity range.

Target range: It is that concentration which is 80%, 100% and 120% of the target concentration. The various ranges have been reported in table 4.

Specificity

Commonly used excipients (lactose, starch, magnesium stearate and talc) were added into a pre-

weighed quantity of standard drug to prepare synthetic mixture and then absorbance was measured before and after addition of excipients. Calculations were done to determine the quantity of the drugs and the interference of additives on absorbance as shown in table

Accuracy

Accuracy was determined from recovery of the method by spiking of standard drug mixture of EPR to the pre-analyzed tablet mixture preparation at 3 different concentration levels 80%, 100% and 120%. Each concentration was analyzed 9 times and average recoveries are shown in table 6.

Precision in mixture

Repeatability

It indicates the precision under the same operating conditions over a short interval of time and inter-assay precision. Repeatability was performed for six times with single target concentration (100%) in tablet mixture.

Intermediate Precision

In intra-day study concentration of drugs were calculated on the same day at an interval of two hour. In inter day study the drug contents were calculated on three different days at 80%, 100% and 120% of target concentration in tablet mixture. The results of statistical analysis as are given in table 7.

Limit of Detection and Quantitation

LOD and LOQ were determined from the linearity data. This helps to determine sensitivity of the method.

Assay

Twenty tablets of each (Trade name: Skelact and Rapisone), containing 50 mg EPR were weighed and finely triturated. A quantity of powder equivalent to 50 mg of drugs were transferred to 100 ml volumetric flask, and 50 ml of analytical grade methanol was added and solution was sonicated for 10 minutes, there after volume was made up to mark with same solvent. From this stock solution 0.2 ml was transferred to six different 10 ml volumetric flasks and volume was made up to mark. The resultant solution was scanned under UV range for unknown concentration. Amount of drug present per tablet was estimated from the respective standard curve using in build UV software. The results of analysis shown in given in table 8.

RESULTS AND DISCUSSION

Linearity

Beer law was obeyed in the range of 2.5-17.5 µg/ml for Eperisone hydrochloride at 258 nm with

coefficient of correlation 0.998. The data are shown in table 1 and 2.

Table 1: Standard curve data

S. no	Concentration (µg/ml)	Mean Absorbance*	Molar absorbtivity	S.D.	% RSD
1	2.5	0.1386	55.44	0.00098	0.7070
2	5.0	0.2860	57.2	0.00000	0.0000
3	7.5	0.4486	59.81	0.00098	0.2180
4	10.0	0.5920	59.2	0.00091	0.1537
5	12.5	0.7386	59.0	0.00098	0.1326
6	15.0	0.8813	58.75	0.00091	0.1032
7	17.5	1.0713	61.22	0.00091	0.0849

** Average of three determinations*

Table 2: Optical and regression characteristics

Parameters		Eperisone HCl (258.40nm)
Beer's law limit		2.5 - 17.5
Absorptivity coefficient		58.66
Molar absorptivity		17354
Sandell's sensitivity		0.01704
Regression	slope (m)	0.06028
Equation	intercept (c)	-0.00911

Table 3: Value of different type of range

S. No	Range	Eperisone HCl	
1	Linearity	2.5	17.5
2	Working	0.498	17.5
3	Target concentration	10.0	
4	Target range	8.0, 10.0, 12.0	

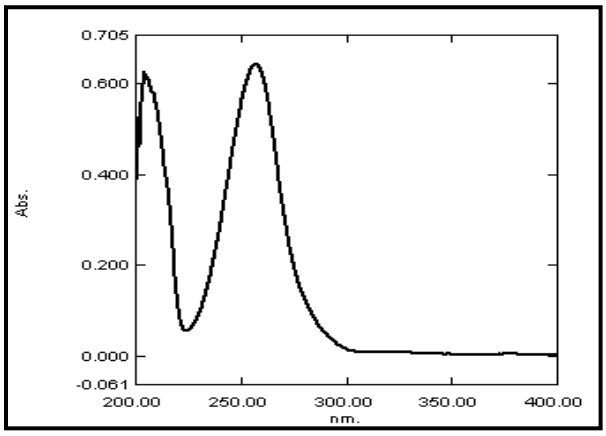

Figure 2:- Spectrum scan of Eperisone HCl (λ_{max}258.0)

Specificity

Developed method has negligible excipients interference of less than 0.5%. The data are shown in table 4.

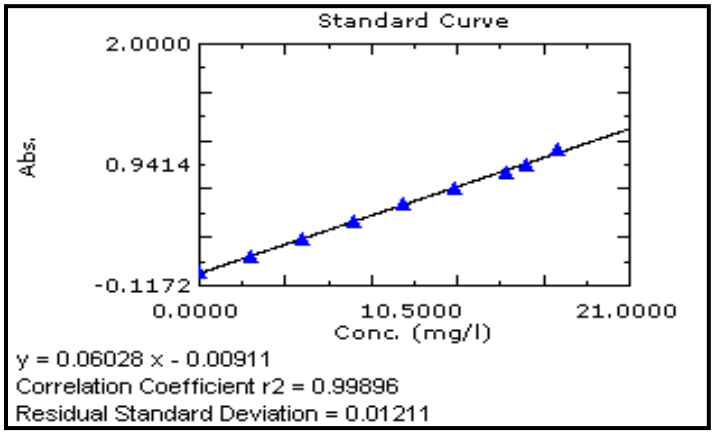

Figure 3:- Standard curve of Eperisone HCl at 258 nm.

Table 4: Specificity study for the synthetic tablet mixture

Concentrations (µg/ml)	Absorbance Before excipients	After excipients	% Interference
2.5	0.139	0.143	0.28
5.0	0.286	0.294	0.6
7.5	0.448	0.454	0.4
10.0	0.592	0.611	0.8
12.5	0.738	0.747	0.08
15.0	0.881	0.895	0.533
17.5	1.072	1.101	0.057
Mean			**0.458**

Accuracy

Accuracy was determined using recovery studies on tablet dosage form. Recovery was found to be greater than 98% which showed good accuracy of method for estimation as show in table 5.

Table 5: Data for accuracy or recovery studies

Preanalyzed tablet mixture	Spiking level 80, 100. 120 %	Mean percent recovery	%RSD or %CV
Eperisone HCl (EPR)	8, 10.0 and 12.0 µg/ml	100.63 ± 0.3489	0.3467

EPR: - Eperisone

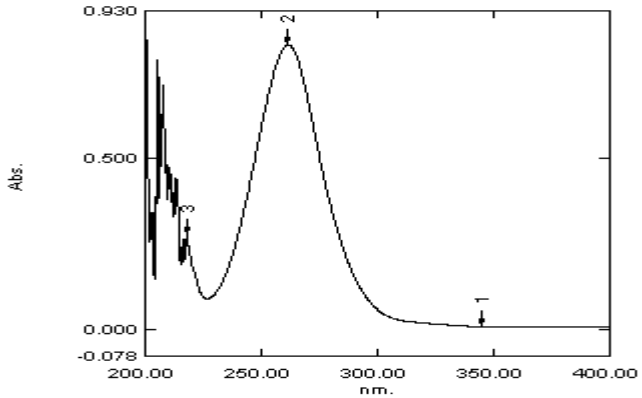

Figure 4:- Spectrum scan of Eperisone HCl 20 µg/ml in water (λ_{max}261.4)

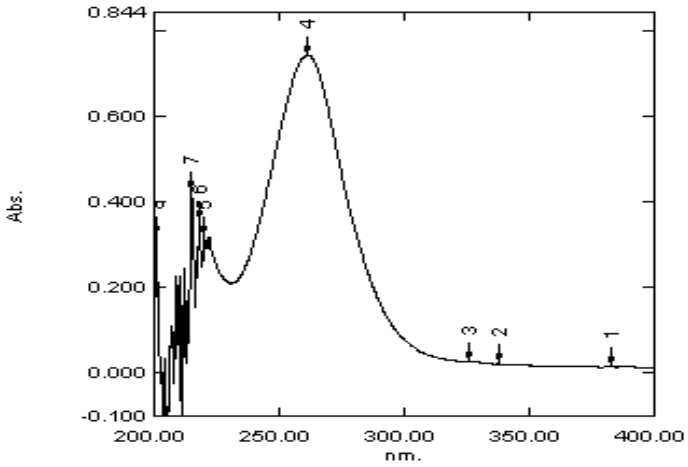

Figure 5:- Spectrum scan of Eperisone HCl 15 µg/ml in phosphate buffer(λ_{max} 261)

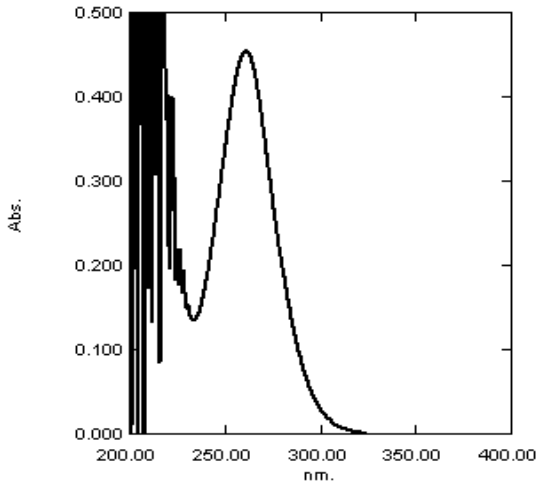

Figure 6:- Spectrum scan of Eperisone HCl 10 µg/ml in 0.1 N HCl (λ_{max} 261.2)

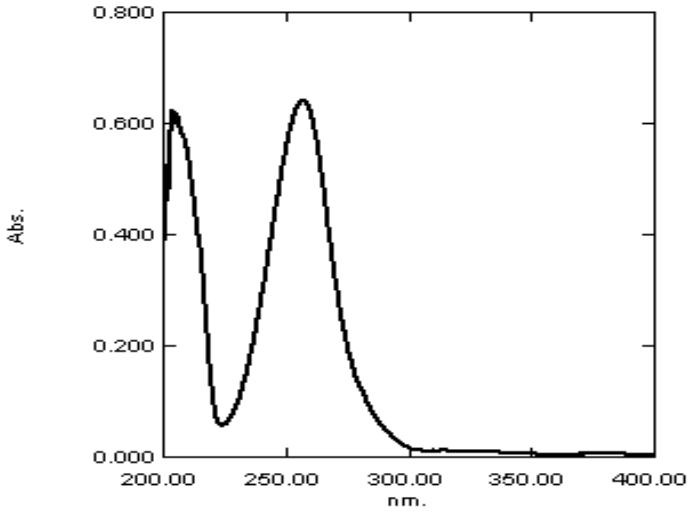

Figure 7:- Spectrum scan of Eperisone HCl 10 µg/ml in ethanol (λ_{max} 257)

Precision

Precision was determined by studying the repeatability and intermediate precision. Results indicate good repeatability and intermediate precision. Percentage RSD was found to be less than 2 as shown in table 6.

Table 6: Precision studies values and results

Concentration levels	Repeatability of target concentration (10.0 µg/ml)	Reproducibility Intra-day	Inter-day
8.0(80%)	----------	8.02 ±0.0219	8.068±0.0538
10.0(100%)	10.04±0.05	10.05±0.0551	10.046±0.0429
12.0(120%)	----------	12.06±0.0283	12.07±0.0387
Mean% RSD	0.498	0.352	0.471

EPR: - Eperisone, RSD: - Relative standard deviation

Sensitivity

The LOD value was found 0.0820 µg/ml whereas LOQ value was found to be 0.331 µg/ml. Low values of LOD and LOQ indicated good sensitivity of developed method.

Assay

Percentage assay found in formulation is 98.76% for EPR with standard deviation less than 2 respectively. The data are shown in table 7.

Table 7: Result of assay using developed method

Brand name	Actual Claim weight (mg)/tab	Mean Concentration found(µg/ml) *	Mean Amt. found per tab (mg) *	% amount found per tab
Skelact (Sun Pharma)	50	10.03 ±0.060	50.16 ± 0.3011	100.33 ±0.6022
Rapisone (Piramal)	50	9.88±0.234	49.4±0.241	98.8±0.208

** Average of six determination, µg: - microgram, mg: - milligrams, tab: - tablet*

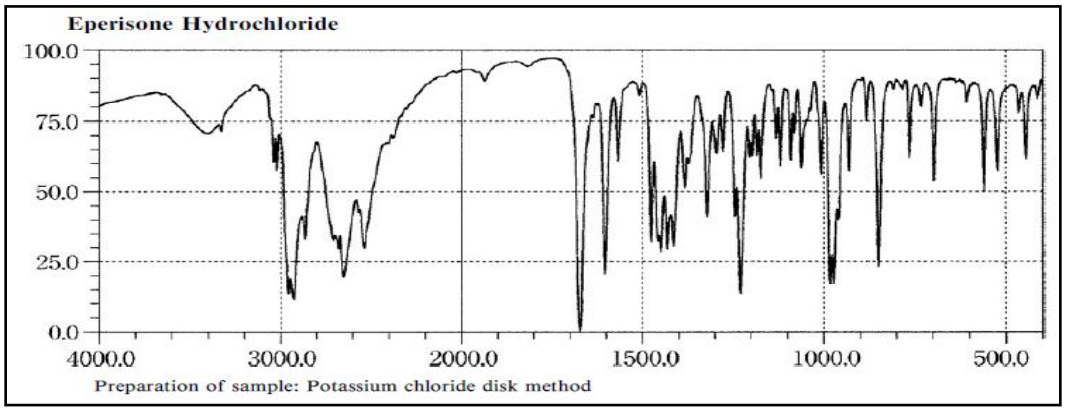

Figure 8:- Standard IR of Eperisone HCl

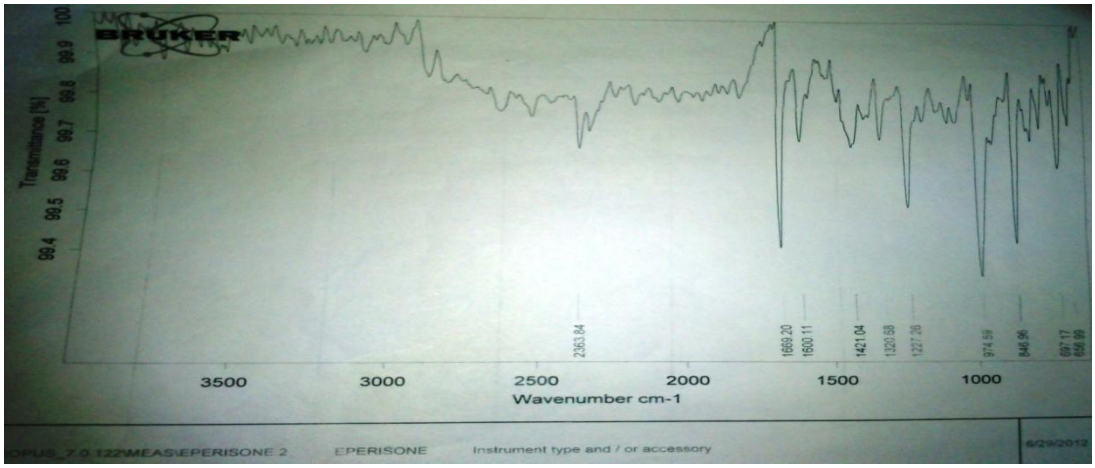

Figure 9 Observed ATR spectra of sample Eperisone HCl

CONCLUSIONS

A simple and economic U.V. spectrophotometric method has been successfully developed using graphical extrapolation technique. It followed all the parameters for validation as per ICH (Q2R1) guidelines. It is specific, accurate and precise as well as having good reproducibility. Hence, it can be used as routine method for estimation of Eperisone hydrochloride in any marketed dosage form.

ACKNOWLEDGMENTS

The authors are grateful to M/s Macleods Pharmaceuticals Ltd, Solan (H.P.) for standard drug. Also thankful to M/s Janta medical store for free market sample. The author is highly grateful to Prof. (Dr) Javed Akhtar (Department of QA, Parul Group of Institutions, Vadodara), for his constant guidance and support.

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