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Spectrophotometric Determination Of Eperisone Hydrochloride And Lornoxicam In Synthetic Mixture

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Abstract: A Simple, accurate and precise UV-Spectrophotometric methods have been developed for simultaneous estimation of Eperisone Hydrochloride (EPE) and Lornoxicam (LOR) in synthetic mixture. In Method (Dual Wavelength Method) LOR shows 363.20 nm as the wavelength having maximum absorbance at which EPE shows zero absorbance and EPE shows 262 nm as the wavelength at which LOR shows absorbance same as it give absorbance at 363.20 nm. So in the mixture at 363.20 nm absorbance is only due to LOR and absorbance at 262 nm is due to EPE and LOR. The linearity was obtained in the concentration range of 4-20 µg/mL for EPE and 4-28 µg/mL for LOR. LOD values were found to be 0.014 µg/mL and 0.33 µg/mL for EPE and LOR. LOQ values were found to be 0.045 µg/mL and 0.100 µg/mL for EPE and LOR respectively. The optimized methods showed good reproducibility with relative standard deviation less than 2.0%.
Keywords: Eperisone Hydrochloride, Lornoxicam, Dual wavelength Method.

Introduction

Eperisone hydrochloride (EPE) {2-methyl-1-(4-methylphenyl)-3-(1-piperidyl) propan-1-one} is a centrally acting muscle relaxant [(1)]. It is not official in IP, BP and USP. It is official in JP [(2)]. Various analytical methods, such as liquid chromatography with – ESI Tandem Mass spectroscopy [(3)], RP-HPLC [(4)], GC/MS [(5)] have been reported. Lornoxicam (LOR) (3E)-6-chloro-3-[hydroxy(pyridin-2-ylamino)methylene]-2-methyl-2,3-dihydro-4H-thieno[2,3-e][1,2]thiazin-4-one 1,1-dioxide is a novel non-steroidal anti-inflammatory drug (NSAID) with marked analgesic activity [(6)].Various analytical methods, such as RPHPLC [(7)], Extractionless HPLC [(8)], UV spectroscopy [(9)], HPTLC [(10)], LC-MS-MS [(11)] determination of Lornoxicam in dosage forms and human plasma. Lornoxicam in combination with other drug like Diacerin [(12)] and Thiocolchicoside [(13)] have been also detected. Present study involves development and validation of dual wavelength spectrophotometry method for the estimation of EPE and LOR in synthetic mixture. The proposed method was optimized and validated as per International Conference on Harmonization (ICH) guidelines[(14,15)]. The chemical structure of Eperisone hydrochloride and Lornoxicam has shown in figure 1 and 2.

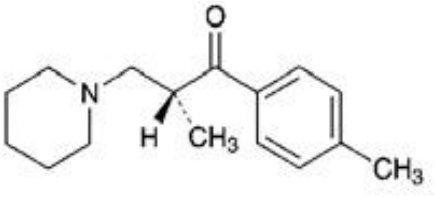

Fig. 1: Chemical structure of Eperisone hydrochloride

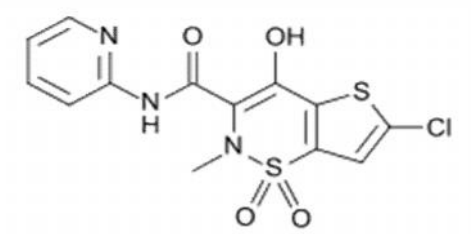

Fig. 2: Chemical structure of Lornoxicam

Experimental

Instrument

A shimadzu model 1700 (Japan) double beam UV/Visible spectrophotometer with spectral width of 2 nm, wavelength accuracy of 0.5 nm and a pair of 10 mm matched quartz cell was used to measure absorbance of all the solutions. Spectra were automatically obtained by UV-Probe 2.0 system software.

Reagents and materials

Eperisone hydrochloride (EPE) and Lornoxicam (LOR) were kindly supplied as a gift samples from Sun Pharma Ltd, Vadodara, Gujarat, India and Acme Pharmaceuticals Ltd, Ahmedabad, Gujarat, India. Methanol (AR Grade, S. D. Fine Chemicals Ltd., Mumbai, India) and Whatman filter paper no. 41 (Milli pore, USA) were used in the study.

Standard stock solutions

Standard stock solutions of EPE and LOR, each of 1 mg/ml concentration in solvent methanol were prepared.

Preparation of sample solution

Marketed formulation of LOR and EPE is not available and the dose ratio is 4:50 (LOR: EPE). So first prepared the 100 and 200 µg/ml conc. of EPE in two 100 ml volumetric flask separately and 100 µg/ml conc. of LOR in other 100 ml volumetric flask. Take 0.8 ml of LOR (100µg/ml) in 10 ml vol. flask and make up the volume with methanol to make 8 µg/ml of LOR.

I. Take 0.8 ml from LOR (100 µg/ml) and 5 ml from EPE (200 µg/ml) in one 10 ml vol. flask and mixed it than make up the volume with methanol.
II. Take 0.8 ml of LOR (8 µg/ml) and 1 ml EPE (100 µg/ml) in 10 ml vol. flask and mixed it than make up the volume with methanol.

Dual wavelength method

The utility of dual wavelength data processing programme is to calculate the unknown concentration of a component of interest present in a mixture containing both the components of interest and an unwanted interfering component by the mechanism of the absorbance difference between two points on the mixture spectra. This is directly proportional to the concentration of the components of interest, independent of the interfering components. The principle for dual wavelength method is “the absorbance difference between two points on the mixture spectra is directly proportional to the concentration of the component of interest”. The method based on determination of EPE at 262 nm and LOR at 363.20 nm. The two drugs follow Beer-Lambert’s law over the concentration range of 4-20 µg/mL for EPE and 4-28 µg/mL for LOR.

Method validation

All the methods were validated as per ICH guidelines for parameters like Linearity, Accuracy, LOD, LOQ and Precision. The accuracy studies were carried out at different concentrations by spiking a known concentration of standard drug to the pre-analyzed sample and contents were reanalyzed by the developed method. Precision was studied by analyzing six replicates of sample solutions. Intermediate precision was determined in a similar manner on the next day using a different instrument.

Stability

Stability was observed by scanning the drug solutions in selected solvent system in time scan mode of UV spectrophotometer for 12 hours.

Limit of detection and quantification

The limit of detection (LOD) and the limit of quantification (LOQ) of the drug were derived by calculating the signal-to-noise ratio (S/N, i.e., 3.3 for LOD and 10 for LOQ) using the following equations designated by International Conference on Harmonization (ICH) guidelines.

$LOD = 3.3 \times \sigma/S$

$LOQ = 10 \times \sigma/S$

Where, σ = the standard deviation of the response and S = slope of the calibration curve

Analysis of synthetic mixture

The absorbance of final sample solution was measured against methanol as blank at 363.20 and 262 nm for quantitation of EPE and LOR respectively. The amount of EPE and LOR present in the sample solution was determined by solving the line regression equation of EPE and LOR.

Results And Discussion

In the present work, dual wavelength method was developed for the simultaneous spectroscopic estimation of EPE and LOR in Synthetic mixture. Methanol was used as the solvent since both the drugs exhibit good solubility in it.

Dual wavelength method

The overlain spectrum of the drugs suggested that a dual wavelength spectrophotometric method was the most suitable method for simultaneous determination of EPE and LOR. In Dual wavelength method the diluted solutions were scanned over the wavelength range of 200 - 400 nm. From the overlain spectra, wavelengths 262 λmax of EPE and 262 and 363.20 λmax of LOR were selected for quantitation by proposed method. For studying Beer's law, two series of different concentrations in range of 4-20 µg/mL for EPE and 4-28 µg/mL for LOR were prepared from stock solutions. The calibration curves were constructed at 262 nm and 363.20nm for Eperisone hydrochloride and Lornoxicam respectively. The absorptivities (A1%, 1 cm) of both the drugs at both the selected wavelengths were determined.

The proposed method was found to be linear in the range of 4-20 µg/mL for EPE and 4-28 µg/mL for LOR with correlation coefficient (r^2) 0.999 for EPE and 0.998 for LOR, a shown in table 1. The method was validated in terms of accuracy, precision, reproducibility and the results are recorded in tables 2 and 3. The accuracy of the method was determined by performing recovery studies by standard addition method in which preanalyzed samples were taken and standard drug was added at three different levels. The precision of the proposed method was estimated in terms of inter-day precision and intraday precision wherein the method was repeated on three different days and repeated for three different time periods in the same day respectively. The results shown in table 3 indicating % RSD of less than 2% at each level clearly indicate that the proposed method is precise enough for the analysis of drug.

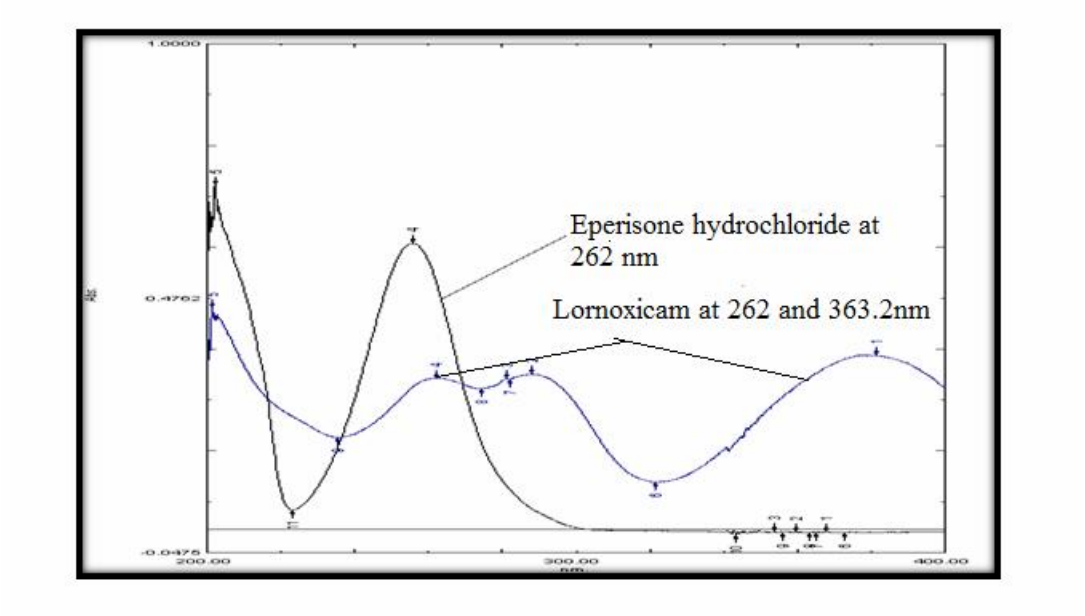

Fig 3: Overlain absorption spectra of the EPE and LOR

Table 1: Data showing linearity of the developed method

Sr. no.	Conc. of EPE	Conc. of LOR	Absorbance at 363.2 nm for LOR	Absorbance at 262 nm for LOR	Absorbance difference at 262 nm and 363.2 nm for EPE
1	4	4	0.132	0.132	0.212
2	8	8	0.263	0.264	0.437
3	12	12	0.383	0.382	0.637
4	16	16	0.503	0.504	0.831
5	20	20	0.640	0.641	1.037
6	24	24	0.776	0.776	_
7	28	28	0.921	0.922	_
Correlation coefficient (r^2)			0.998	0.998	0.999
Slope of regression line			0.0326	0.0326	0.0511

Table 2: Data showing precision of the developed method

Methods	**Dual wavelength method**	
Parameters	**EPE**	**LOR**
Repeatability (RSD, n=6)	0.26	0.30
Intraday(n=3) Precision (RSD)	0.301-0.75%	0.18-0.47%
Interday(n=3) Precision (RSD)	0.47-0.68%	0.39-0.43%
LOD (µg/ml)	0.014	0.033
LOQ (µg/ml)	0.045	0.100

Table 3: Data showing recovery of the developed method

Drug	Amount present in formulation (µg/ml)	Amount added (%)	% Recovery ± S. D. (n = 3)
EPE	100	75	100.07 ± 1.5
	100	100	101.27 ± 1.14
	100	125	100.47 ± 1.58
LOR	8	75	100.13 ± 1.41
	8	100	98.03 ± 0.20
	8	125	99.80 ± 1.2

Table 4: Results of analysis of synthetic mixture containing EPE and LOR

Sample No.	LOR (µg/ml)	EPE (µg/ml)	Amount found		% Lable claim	
			LOR(µg/ml)	EPE(µg/ml)	LOR	EPE
1	8	100	8	—	100	—
2	8	100	7.9	—	98.82	—
3	8	100	7.87	—	98.43	—
4	0.8	10	—	10.17	—	101.76
5	0.8	10	—	10.37	—	103.7
6	0.8	10	—	10.25	—	102.54
Mean			7.92	10.26	99.08	102.66
SD			0.068	0.100	0.81	0.97
RSD			0.85	0.98	0.82	0.95

Conclusion

The proposed dual wavelength method gives accurate and precise results for determination of EPE and LOR in synthetic mixture and is easily applied for routine analysis. The most striking feature of the dual wavelength method is its simplicity and rapidity. Method validation has been demonstrated by variety of tests for linearity, accuracy, precision. The developed method has several advantages, as it is simple, accurate, precise. The proposed method was successfully applied for determination of these drugs in synthetic mixture.

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