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RP-HPLC METHOD DEVELOPMENT AND VALIDATION OF LORNOXICAM AND EPERISONE HYDROCHLORIDE

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Keywords:

RP-HPLC, Lornoxicam, Eperisone hydrochloride, Methanol, Ammonium hydroxide, Validation

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ABSTRACT: Several spectrophotometric and HPLC methods for the determination of lornoxicam and eperisone hydrochloride have been documented individually or in combination with other drugs in pharmaceutical dosage forms. Therefore, in the present study, a reasonable and practical reverse phase HPLC method was developed and validated for lornoxicam and eperisone hydrochloride in bulk formulation. In RP-HPLC method, the analyte was resolved by using an isocratic system, Methanol: Water (55:45) with 0.1% v/v Ammonium Hydroxide, pH 7.4 was used as mobile phase, at a flow rate of 1.0 ml/min, on HPLC system containing C18 analytical column (25 × 0.46 cm, 5 µm). The detection was carried out at 274 nm. The retention time was found to be 7.4 min and 9.2 min for lornoxicam and eperisone hydrochloride, respectively. Good resolution and retention time were observed. The results of the analysis in the method were validated with respect to following parameters linearity, precision, specificity and system suitability, limit of detection and limit of quantification.

INTRODUCTION: Lornoxicam [1] (chlortenoxicam) is a non-steroidal anti-inflammatory drug (NSAID) of the oxicam class with analgesic, anti-inflammatory and antipyretic properties. Lornoxicam differs from other oxicam compounds in its potent inhibition of prostaglandin biosynthesis, a property that explains the particularly pronounced efficacy of the drug. Lornoxicam is absorbed rapidly and almost completely from the GI tract (90-100%). It is 99% bound to plasma proteins. Lornoxicam is metabolized completely by cyp 2C92, with the principal metabolite being 5'-hydroxy-lornoxicam, and only negligible amounts of intact lornoxicam are excreted unchanged in the urine. Approximately 2/3 of the drug is eliminated via the liver and 1/3 *via* the kidneys in the active form.

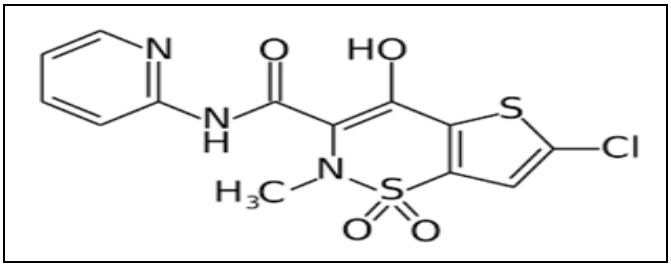

FIG. 1: CHEMICAL STRUCTURE OF LORNOXICAM

Eperisone [3] is an antispasmodic drug that relaxes both skeletal muscles and vascular smooth muscles and demonstrates a variety of effects such as reduction of myotonia, improvement of circulation, and suppression of the pain reflex [4].

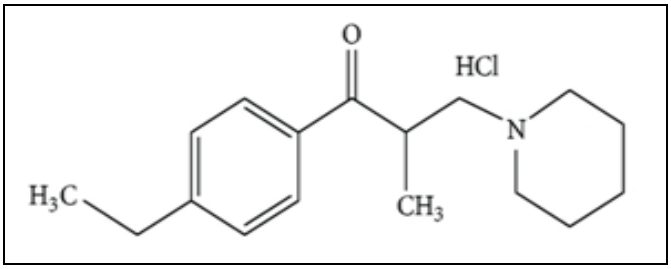

FIG. 2: CHEMICAL STRUCTURE OF EPERISONE HYDROCHLORIDE

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Literature review reveals that Different methods like UV spectrophotometry, HPLC, HPTLC for the estimation of Lornoxicam as single component and combinations with other drugs excluding Eperisone have been reported. Very few methods have been reported for estimation of Eperisone hydrochloride as single component like HPLC, GCMS. Very few methods have been reported so far in literature for simultaneous estimation of Lornoxicam and Eperisone hydrochloride as a combination in any dosage forms or bulk formulation [5-23].

MATERIALS AND METHODS:

Instruments and Apparatus: Separation was achieved using Phenomenex C_{18} Analytical Column (25 × 0.46 cm, 5 µm) on HPLC Shimadzu LC-20AD. Digital weighing balance (A & D company, GR (200)), pH meter (Lab India digital pH meter), Enertech ultrasonicator and pipettes and volumetric flasks (Borosilicate) used during the study.

Reagents and Chemicals: Lornoxicam and Eperisone were procured from Innova captab and Macleod's, respectively. Methanol HPLC grade, HPLC grade water, LR grade ammonium hydroxide was obtained from Merck Ltd. India.

Chromatographic Conditions: Stationary phase: Phenomenex C_{18} Analytical Column (25×0.46 cm, 5 µm).

Mobile Phase: Methanol: Water (55:45) with 0.1% v/v Ammonium Hydroxide.

Flow Rate: 1.0ml/min

Injection Volume: 20µl

Wavelength used: 260nm

Run Time: 15 min.

Preparation of Mobile Phase (Methanol and Water (55:45) and 0.1% Ammonium Hydroxide solution): Mobile phase prepared by mixing Methanol (HPLC grade) and water (HPLC grade) in selected proportion. Prepared Mobile phase taken separately filtered through membrane nylon filters of size 4.5 µ, to the filtered solution 1.5 ml of Ammonium Hydroxide Solution added and the mixed solution then sonicated for 15 min and filtered through membrane nylon filters of size 4.5 µ.

Preparation of Working Standard Mixture:

Standard Stock Solution of Lornoxicam (1000 µg/ml): Weigh accurately 10 mg of Lornoxicam into a 10ml volumetric flask. Add a sufficient amount of methanol, sonicate to dissolve, cool, and dilute up to the mark with methanol.

Standard Stock Solution of Eperisone (1000 µg/ml): Weigh accurately 10 mg of Eperisone into a 10ml volumetric flask. Add a sufficient amount of methanol, sonicate to dissolve, cool, and dilute up to the mark with methanol.

Standard Working Solution of Lornoxicam and Eperisone: The above stock solutions were further diluted 1 ml to 10 ml with methanol.

Method Validation: [24-33]

Specificity and System Suitability: Specificity can be defined as the ability to measure the concentration of an analyte in the presence of all other sample materials accurately. Blank and standard samples were injected to perform the specificity. Specificity of the procedure was established by proving that the excipients did not interfere. System suitability was determined by calculating parameters like theoretical plates, resolution, tailing factor, and % RSD.

Linearity: The linearity of the analytical technique is defined as the ability to obtain variable data test results, which is directly proportional to the analyte concentration in the sample. A series of standard solutions 25-100µg/ml was prepared for both drugs. Mix the equal volumes of lornoxicam and eperisone of each sample respectively, then injected into HPLC and recorded it. The plot of average peak area versus concentration is plotted, and from this the coefficient of correlation and the equation of regression are developed.

Precision: The closeness of agreement (degree of scatter) between a series of measurements obtained under the prescribed conditions from multiple sampling of the same homogenous sample. Method precision was calculated by repeatability test where %RSD should not be more than 2.0%.

Limit of Detection: The lowest analyte concentration the analytical technique can reliably discern from the background noise called the limit of detection. The limit of detection (LOD) was calculated using the following formulae:

$$LOD = 3.3(SD)/S$$

Where, SD = the standard deviation of response and S = Slope of the calibration curve.

Limit of Quantification: LOQ is defined as the lowest analyte concentration in a sample, which can be calculated with reasonable accuracy and precision. The Limit of Quantification was calculated using the following formulae:

$$LOQ = 10 (SD)/S$$

Where, SD = the standard deviation of response and S = Slope of the calibration curve.

RESULTS AND DISCUSSION:

Method Development and Optimization: Method development work was started with the use of UV-spectra in the range 200-400 nm of Lornoxicam and Eperisone (10µg/ml) standard solution. Then samples were scanned on a UV-Visible spectro-photometer between wavelength ranges of 200 nm to 400 nm. Two isosbestic points found in overlain spectra of Lornoxicam and Eperisone are 243 nm and 274 nm. From which, 274 nm was selected as detection wavelength.

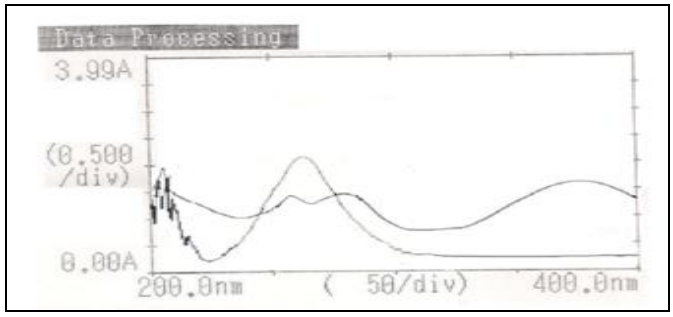

FIG. 3: OVERLAIN UV SPECTRA OF LORNOXICAM & EPERISONE

The retention time for Lornoxicam was found to be 7.4 min and for Eperisone 9.2 min as shown in **Fig. 4.**

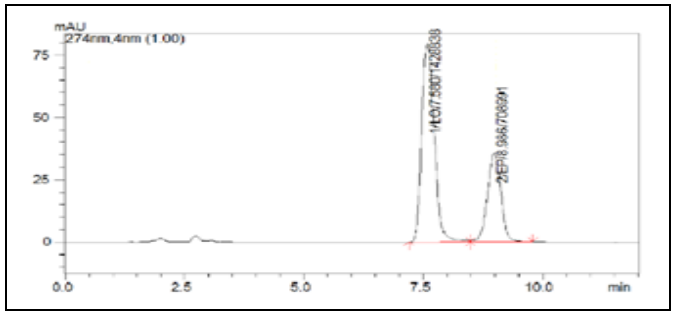

FIG. 4: HPLC CHROMATOGRAM OF MIXTURE OF WORKING STANDARDS

Method Validation:

TABLE 1: DATA FOR SPECIFICITY TEST OF LORNOXICAM

Sample Name	Area µAU*sec Lornoxicam	Retention Time Lornoxicam	Similarity factor for Lornoxicam
STD 1	1428838	7.580	
STD 2	1427258	7.572	
STD 3	1428844	7.580	1.00
%RSD	0.064	0.061	

TABLE 2: DATA FOR SPECIFICITY TEST OF EPERISONE

Sample Name	Area µAU*sec Eperisone	Retention Time Eperisone	Similarity factor for Eperisone
STD 1	708991	8.986	
STD 2	707950	8.988	
STD 3	702786	9.004	0.996
%RSD	0.470	0.110	

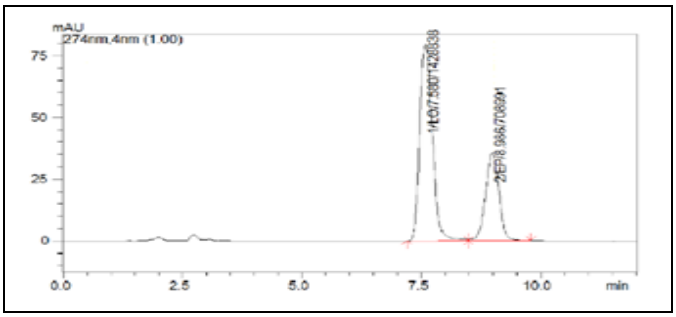

FIG. 5: HPLC CHROMATOGRAM OF SPECIFICITY OF LORNOXICAM

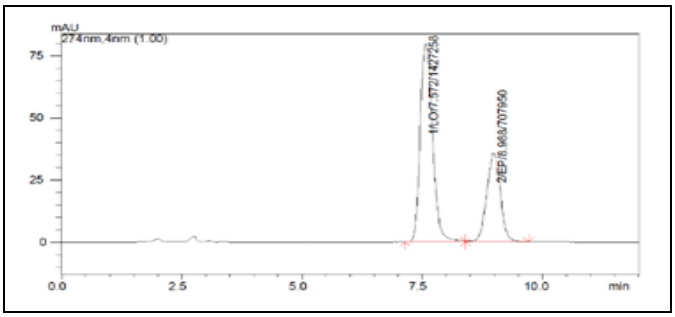

FIG. 6: HPLC CHROMATOGRAM OF SPECIFICITY OF EPERISONE

TABLE 3: DATA FOR SYSTEM SUITABILITY

Parameter	Acceptance Criteria	Lornoxicam	Eperisone
Tailing Factor	NMT 2	1.253	1.002
Capacity Factor	NLT 2	2.03	2.52
Similarity Factor	0.98 to 1.02	1.0	0.996
%RSD of STD A for Area	NMT 2	0.064%	0.470%
%RSD of STD A for Retention time	NMT 2	0.061%	0.110%

Linearity: The linearity for Lornoxicam and Eperisone was found between 25-100µg/ml. The result is shown in **Table 4.**

TABLE 4: LINEARITY OF STANDARDS FOR LORNOXICAM

Sample Name	Area µAU*min Lornoxicam	Concentration Lornoxicam
Sample 1	379544	25
Sample 2	744941	50
Sample 3	1062460	75
Sample 4	1428838	100

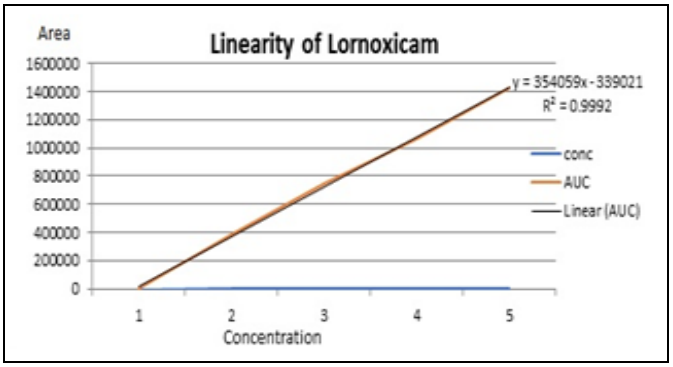

FIG. 7: CALIBRATION CURVE FOR LORNOXICAM

TABLE 5: LINEARITY OF STANDARDS FOR EPERISONE

Sample Name	Area µAU*min Eperisone	Concentration Eperisone
Sample 1	195435	25
Sample 2	344940	50
Sample 3	531714	75
Sample 4	708991	100

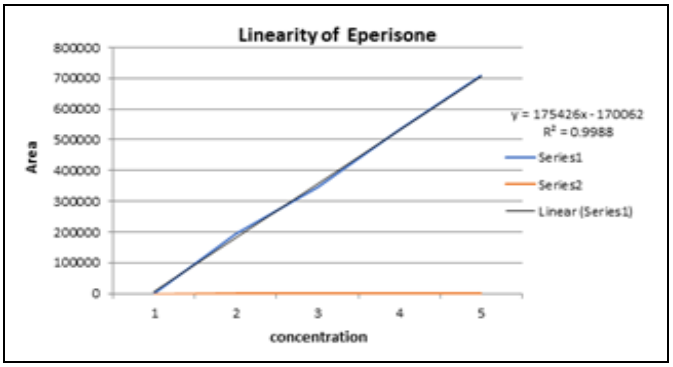

FIG. 8: CALIBRATION CURVE FOR EPERISONE

Precision: The % RSD for repeatability study for Lornoxicam and Eperisone was found to be 0.064 and 0.47, respectively. The results are shown in **Table 5**.

TABLE 6: DATA OF REPEATABILITY TEST FOR LORNOXICAM

Sample Name	Retention time	Area µAU*sec Lornoxicam	Area %
Sample 1	7.580	1428838	66.836
Sample 2	7.572	1427258	66.844
Sample 3	7.580	1428844	67.031
Mean	7.577	1428313	66.904
% RSD	0.061	0.064	0.165

TABLE 7: DATA OF REPEATABILITY TEST FOR EPERISONE

Sample Name	Retention time	Area µAU*sec Eperisone	Area %
Sample 1	8.986	708991	33.164
Sample 2	8.988	707950	33.156
Sample 3	9.004	702786	32.969
Mean	8.993	706576	33.096
% RSD	0.110	0.470	0.333

TABLE 8: SUMMARY OF REPEATABILITY

Parameter	Acceptance Criteria	Lornoxicam	Eperisone
%RSD of Area	NMT 2	0.064	0.47
Similarity Factor	0.98 to 1.02	0.98	1.00

TABLE 9: LOD FOR LORNOXICAM AND EPERISONE

Sample Name	LOD
Lornoxicam	0.025 µg/ml
Eperisone	0.03 µg/ml

TABLE 10: LOQ FOR LORNOXICAM AND EPERISONE

Sample Name	LOQ
Lornoxicam	0.07 µg/ml
Eperisone	0.09 µg/ml

CONCLUSION: The method developed is specific, linear, precise, and there was no interference in the chromatogram. Thus, it can be concluded that this newly developed approach can be used for the routine study of combined dosage forms due to easily accessible and cost-effective reagents.

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CONFLICTS OF INTEREST: The authors declare there is no conflict of interest.

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