### 3.1 Drug profile

Lornoxicam is a non-steroidal anti-inflammatory drug of the oxicam class with analgesic, anti-inflammatory and antipyretic properties. It is available in oral and parenteral formulations. Lornoxicam differs from other oxicam compounds in its potent inhibition of prostaglandin biosynthesis, a property that particularly explains the pronounced efficacy of the drug. Lornoxicam is used for the treatment of various types of pain, especially resulting from inflammatory diseases of the joints, osteoarthritis, surgery, sciatica, and other inflammations [(1)].

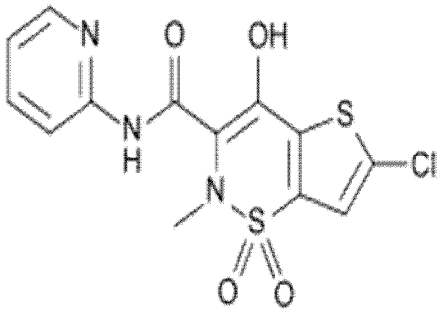



**Figure 3.a: Structure of Lornoxicam**

| | | |
|---|---|---|
| IUPAC name | : | (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 |
| Molecular formula | : | $C_{13}H_{10}ClN_3O_4S_2$ |
| Molecular mass | : | 371.8192 g/mol |
| Drug Bank accession number | : | DB06725 |
| CAS number | : | 70374-39-9 |
| Half life | : | 3 – 4 hrs |
| Therapeutic category | : | Non steroidal anti inflammatory drug (NSAID) |
| Route | : | Oral, Parenteral |
| Solubility | : | Poorly soluble in water, Soluble in 0.1N NaOH solution |

**Mechanism of action:**

Lornoxicam's anti-inflammatory and analgesic activity is related to its inhibitory action on prostaglandin and thromboxane synthesis through the inhibition of both COX-1 and COX-2. This leads to the reduction of inflammation, pain, fever, and swelling, which are mediated by prostaglandins. However, the exact mechanism of lornoxicam, like that of the other Non steroidal anti-inflammatory drugs (NSAIDs), has not been fully determined.

**Dosage:** The adult dosage of Lornoxicam for pain relief is 8-16mg daily and maximum of 24mg/day. The daily dosage for Osteoarthritis is 12 mg daily in 2-3 divided doses, up to 16 mg daily.

**Adverse effects:**

The most common side effects reported with the regular use of the tablet form of Lornoxicam include dizziness, headache, stomach pain, upset stomach, diarrhea, nausea, vomiting and indigestion. As an injection, users most commonly report headache, flushing, insomnia and redness and irritation at the injection spot.

The market available formulations, dosage form and manufacture details of Lornoxicam[(2)] are shown in table 3.1

| S.NO | Brand name | Available form | Labile claim | Manufacture |
|---|---|---|---|---|
| 1 | Camri | tablet | 4mg | Zydus |
| 2 | Lornoxi | tablet | 4mg | Hetero |
| 3 | Lornofan | Tablet | 4mg | Emar |
| 4 | Lorasid | vial | 8mg | piramal |
| 5 | Xilor | tablet | 8mg | Sanify(syntonic) |
| 6 | Zelorn | tablet | 4mg | Anthus |

**Table 3.1: List of Brand names of Lornoxicam**

## 3.2 Review of literature

Very few analytical methods have been reported previously for the estimation of Lornoxicam in bulk, pharmaceutical dosage forms and biological samples using different analytical techniques. The summary results of the previously reported methods were discussed below.

***Aher K et al*** [3] developed a simple, rapid, and precise method for quantitative analysis of Lornoxicam in pharmaceutical dosage forms. Chromatographic separation of Lornoxicam was achieved on a C18 analytical column with potassium dihydrogen phosphate buffer : acetonitrile, 70:30 (v/v), as mobile phase at ambient temperature. The flow rate was 1.0 ml/min and detection was carried out by absorption at 291 nm using a photodiode-array detector. The number of theoretical plates and tailing factor for Lornoxicam were 6,577 and 1.03, respectively. The linearity of the method was excellent over the range 10–100 µg/ ml Lornoxicam. The correlation coefficient was 0.9999. Relative standard deviations of peak areas from six measurements were always less than 2%. The proposed method was found to be suitable and accurate for quantitative analysis of Lornoxicam.

***Mahesh Attimarad et al*** [4] developed a simple RP-HPLC method for the simultaneous determination of paracetamol and lornoxicam without prior separation. In this method, Kromasil C8 (250 mm, 4.6 mm, 5 µm) column was used. The mobile phase used was methanol:phosphate buffer (60:40, v/v, pH 6.4), at flow rate of 1 ml $min^{-1}$ . UV detection was monitored at 302 nm. Calibration graphs were established in the range of 1-150 µg $ml^{-1}$ and 0.5-100 µg $ml^{-1}$ for paracetamol and lornoxicam, respectively. The average retention time for paracetamol and lornoxicam was found to be 3.15 ± 0.03 min and 5.25 ± 0.06 min, respectively. The detection limit and quantification limit for paracetamol were 0.19 µg $ml^{-1}$ and 0.59 µg $ml^{-1}$ and for lornoxicam 0.10 µg $ml^{-1}$ and 0.31 µg $ml^{-1}$ , respectively. The intraday and interday precisions expressed as percent relative standard deviation were below 2%. The mean recovery of paracetamol and lornoxicam was found to be in the range of 99.03-101.2%.

***B.S. Kuchekar et al*** [5] developed a simple, selective, rapid, and precise RP-HPLC-PDA method for the simultaneous estimation of Lornoxicam (LOR) and Thiocolchicoside (THIO) in pharmaceutical dosage form by reverse phase liquid chromatography using Waters Symmetry C18 (250 mm × 4.6 mm, 5.0 µ) column. The

mobile phase consisting of methanol: THF: acetate buffer (60: 10: 30 v/v); pH adjusted to 5.5 with glacial acetic acid at a flow rate of 0.75 ml min-1and column was maintained at $50^0$C with detection at 382 nm. The retention time of Thiocolchicoside and Lornoxicam was 3.36 and 4.08 minutes, respectively. The method was validated according to the ICH guidelines with respect to specificity, linearity, accuracy, precision and robustness, limit of detection and limit of quantification. Linearity of Lornoxicam and Thiocolchicoside were in the range of 0.2 to 80 µg/ml and 0.1 to 40 µg/ml, respectively and its percentage recovery were found to be 100.37 % and 100.51 %, respectively. The proposed method was suitable for simultaneous determination of Lornoxicam and Thiocolchicoside in pharmaceutical dosage form. Method was successfully applied for dissolution study of tablet formulation.

***Patel a et al*** [(6)] developed and subsequently validated a simple reverse phase liquid chromatographic method for simultaneous determination of paracetamol and lornoxicam in combination. The separation was carried out using a mobile phase consisting of potassium dihydrogen phosphate, pH adjusted to 7.3 with triethyl amine and acetonitrile 70:30(%v/v).The column was used phenomex C18, 5 µm, (250x 4.6 mm) with flow rate 1.5ml/min using UV detection was at 257nm. The described method was linear over concentration range 20 to 60 µg/ml & 0.2 to1.8µg/ml for assay of paracetamol & lornoxicam respectively. The retention time of paracetamol & lornoxicam were found to be 2.33 & 7.61 min, respectively. Result of analysis was validated statistically. The method show good reproducibility & recovery with % less than 1, all the tests of above mentioned studies were found to be in acceptance criteria. The method was found to be rapid, specific, precise & accurate and can be successfully applied for routine analysis of paracetamol & lornoxicam in bulk & combined dosage forms.

***G.devala rao et al*** [(7)] described two simple and sensitive visible spectrophotometric methods (A & B) for the determination of lornoxicam (loc) in bulk and pharmaceutical dosage forms. Method-A, was based on oxidation of drug with ferric chloride and subsequent complexation of Fe(II) with 2, 2' bipyridine toform a blood red colored species ( λmax:520nm). Method-B, was based on oxidation of lornoxicam with ferric chloride and chelation of Fe(II) with bathophenanthroline to produce a blue colored chromogen (λmax:610 nm). These methods were extended to the analysis of pharmaceutical formulations and results were compared with the reference method.

## 3.3 Materials and Methods

### 3.3.1 Instrumentation:

Chromatographic separation was performed on a PEAK chromatographic system equipped with LC-P7000 isocratic pump; Rheodyne injector with 20µl fixed volume loop, variable wavelength programmable UV detector UV7000 and the output signal was monitored and integrated by PEAK Chromatographic Software version 1.06. Teccomp UV-2301 double beam UV-Visible spectrophotometer was used to carry out spectral analysis and the data was recorded by Hitachi software. Sonicator (1.5L), Ultrasonicator was used to sonicating the mobile phase and samples. Standard and sample drugs were weighed by using Denver electronic analytical balance (SI-234) and pH of the mobile phase was adjusted by using Systronics digital pH meter.

### 3.3.2 Chemicals and Solvents:

The drug samples, Lornoxicam working standard was obtained as gift sample by Hetero Pharmaceuticals, Hyderabad, AP, India. The pharmaceutical formulations were procured from local market. Methanol, Ethanol, Acetonitrile and water used were HPLC grade and were purchased from Merck Specialties Private Limited, Mumbai, India. Buffer solutions used were AR Grade and purchased from Merck Specialties Private Limited, Mumbai, India.

### 3.3.3 Preparation of standard stock solution:

Standard stock solution of Lornoxicam pure drug was prepared by accurately weighing about 100 mg of the drug and transfering the drug in to 100 ml volumetric flask. The drugs were dissolved with 25ml of methanol, and sonicated to dissolve it completely and made up to the mark with the same solvent; results 1000µg/ml solution was obtained. The contents were mixed well and filtered through Ultipor $N_{66}$ Nylon 6, 6 membrane sample filter paper. From this 10ml was taken and further diluted to 100ml, results 100µg/ml solution was obtained. That solution was used as a secondary stock solution and required concentrations were prepared from this secondary stock solution by selected dilutions.

### 3.3.4 Preparation of sample solution:

Lornoxicam tablets (CAMRI -4mg) were purchased from local pharmacy. Ten tablets were weighed and average weight was calculated. The tablet powder equivalent to 10mg of Lornoxicam was transferred in to a 10 ml volumetric flask. 5ml of methanol was added and sonicated for complete solubility. The volume was made up to the mark with methanol. Then the solution was filtered through 0.45µ membrane filter. From this standard concentration of 1mg/ml, required concentration of 10µg/ml was prepared by proper dilution.

## 3.4 Method Development

Method development includes the optimization of the analytical conditions for the analysis of Lornoxicam. In the development, one of the parameter will be changed by keeping remaining all parameters constant. During the preliminary method development stage, all individual components should be investigated before the final method optimization.

### 3.4.1 Selection of Mobile phase:

Depends up on the order of solubility, nature of the drug and its response, different ratios of solvents like Water, Methanol, and Acetonitrile were changed in order to get a symmetric peak within the acceptance criteria. In the composition different ratios of solvents with or without buffer solutions were used and the final suitable mobile phase was selected. In every mobile phase change instrument was stabilizes at least 20min prior to the injection of the sample.

### 3.4.2 Selection of Wavelength:

Wavelength of the drug was measured using UV spectrophotometer and then the wavelength was changed on changing the mobile phase. The wavelength where the drug absorb maximum by comparing the area values obtained in the result was considered as the suitable wavelength for the analysis of Lornoxicam.

### 3.4.3 Selection of Stationary Phases:

Preliminary development trials have performed with octa decyl columns with different types, configurations and from different manufacturers to get symmetric, sharp peak and obey system suitability parameters.

### 3.4.4 Selection of Flow Rate:

The flow of the mobile phase was changed in between 0.5-1.2ml/min in the selected mobile phase, wavelength and stationary phase conditions. The flow rate conditions where best result obtained was considered as a suitable flow rate for the analysis of Lornoxicam.

By comparatively change in the mobile phase ratio, wavelength and flow rate for the development of suitable analytical conditions for the estimation of Lornoxicam in pharmaceutical dosage forms, single sharp, symmetric peak was observed at a mobile phase composition of Methanol, Acetonitrile in the ratio of 80:20 (v/v), 260nm

wavelength at a flow rate of 1 ml/min. Hence these conditions were found to be the most suitable conditions for the analysis of Lornoxicam in pharmaceutical dosage form. The optimized chromatographic conditions were shown in the table 3.1 and standard chromatogram was shown in figure 3.C.

| Parameter | Condition |
|---|---|
| **Mobile Phase** | Methanol: Acetonitrile 80:20 (v/v) |
| **Wavelength** | 260nm |
| **Stationary phase** | Zodiac C18 column (250 X 4.6 mm, 5µ) |
| **Pump Mode** | Isocratic |
| **Flow Rate** | 1ml/min |
| **pH of Mobile Phase** | 5.1 |
| **Injection volume** | 20µl |
| **Pump Pressure** | 5.4±5MPa |
| **Standard concentration** | 9µg/ml |
| **Retention Time** | 3.28min |
| **Run Time** | 7min |
| **Peak Area Response** | 988105 |
| **Theoretical plates** | 6186 |
| **Tailing Factor** | 1.58 |

**Table 3.2: Optimized chromatographic conditions for the estimation of Lornoxicam**

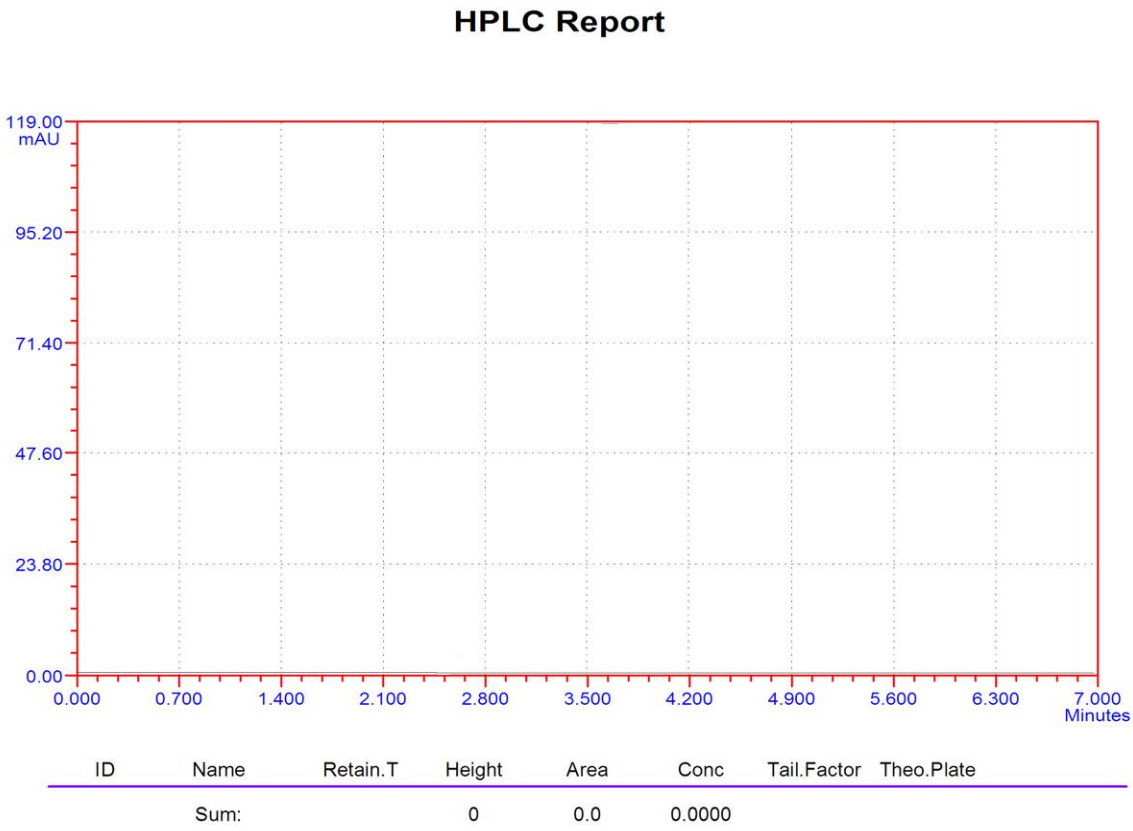
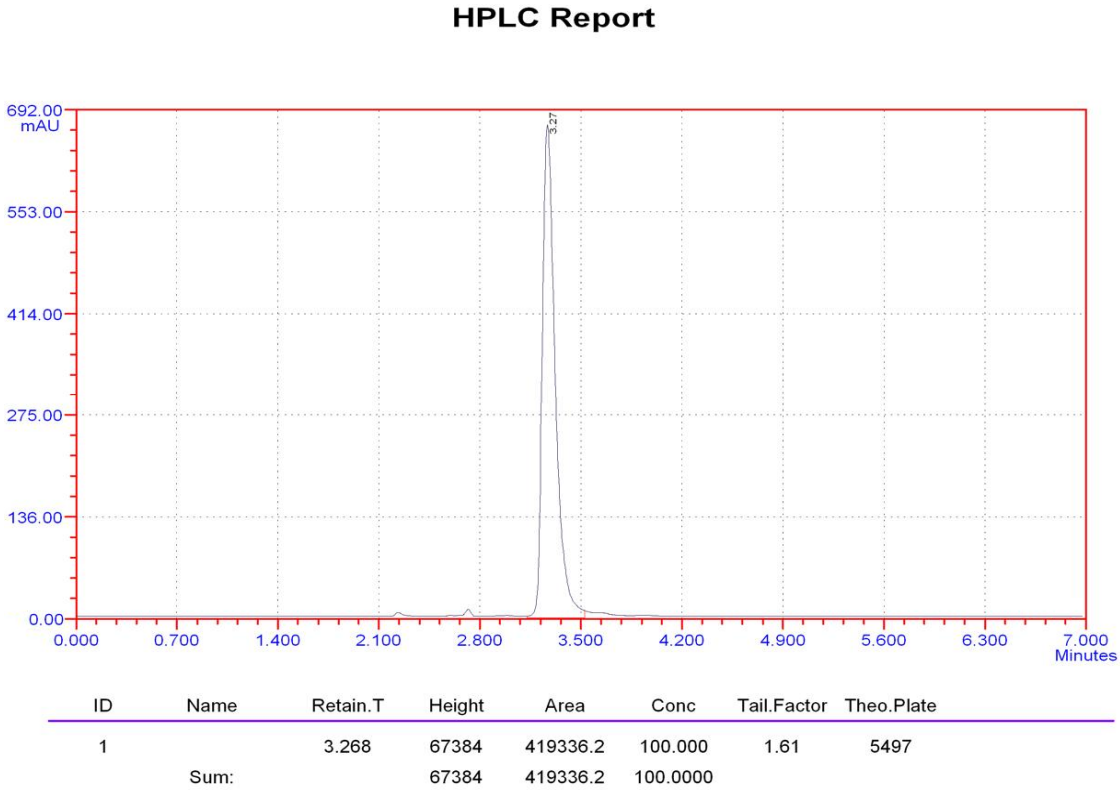



**Figure 3.b: Chromatogram of blank**




| ID | Name | Retain.T | Height | Area | Conc | Tail.Factor | Theo.Plate |
|---|---|---|---|---|---|---|---|
| 1 | | 3.268 | 67384 | 419336.2 | 100.000 | 1.61 | 5497 |
| | Sum: | | 67384 | 419336.2 | 100.0000 | | |

**Figure 3.c: Chromatogram of Lornoxicam - Standard (**9µg/ml)

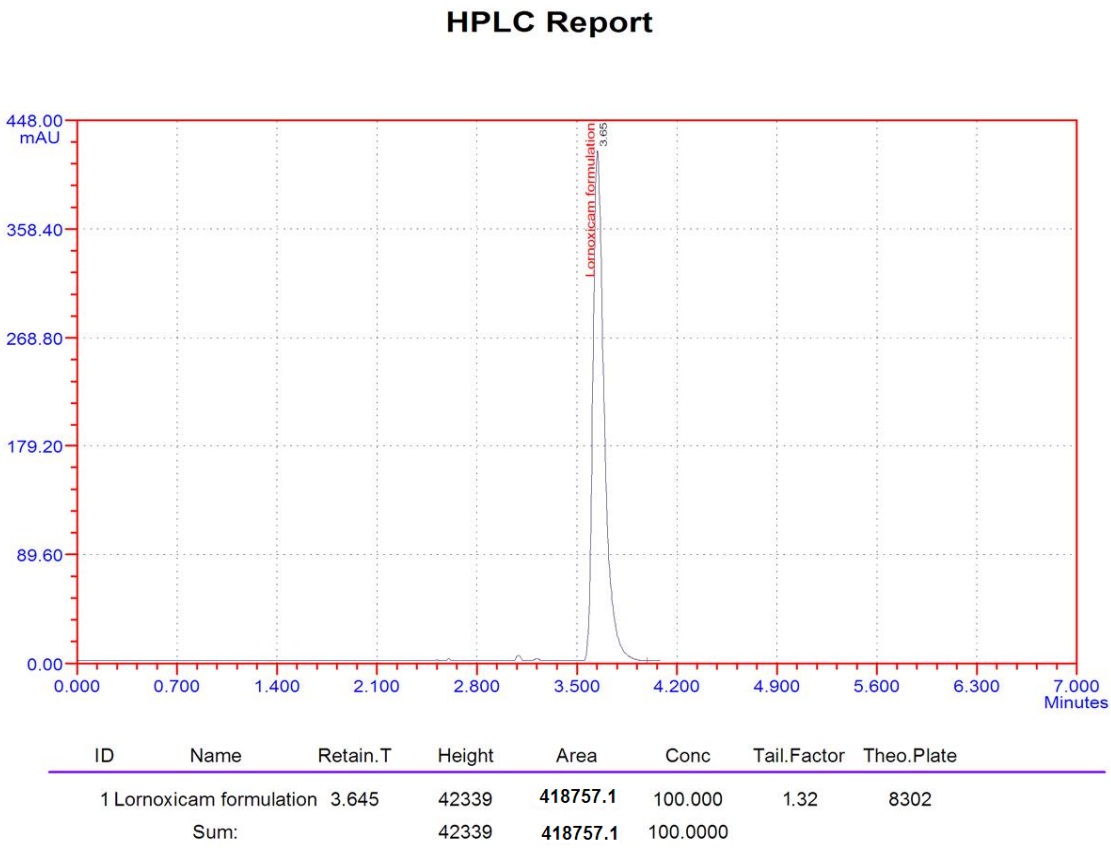



**Figure 3.d: Chromatogram of Lornoxicam Formulation (10**µg/ml)

By applying these optimized chromatographic conditions, standard solution of Lornoxicam and blank solutions were prepared and injected. It was found that standard solution show symmetrical peak with high theoretical plates and low tailing factor. The peak obey the system suitability conditions of <2 tailing factor and >2000 theoretical plates. Blank and placebo solutions don't show any peaks and a clean base line was observed. This indicates that the method is free of impurities and formulation excipients are not detected in the proposed method. Hence the proposed method is specific and obeys system suitability. Results were shown in the table 3.3, chromatogram of blank, Standard, Formulation were shown in figure 3.b and 3.c, 3.d respectively.

After the optimization of the conditions, the acceptance of the optimized conditions was tested by performing the validation procedure.

## 3.5 Validation of the Proposed Method

The validation of an analytical method is the process by which it is established the performance characteristics of the method, such as Precision, Accuracy, Specificity, Linearity, Limit of Detection (LOD), Limit of Quantification (LOQ) and Robustness meet the requirements for the intended applications. The developed method was validated as per ICH guidelines.

### 3.5.1 System suitability:

To measure the system suitability of the developed method, standard drug solution at standard concentration of 9µg/ml was prepared and injected. Result of the peak obtained was used to express the system suitability.

### 3.5.2 Specificity:

By comparing the results obtained by injecting standard, blank and placebo specific of the developed method was established.

| Condition | Result | |
|---|---|---|
| | **Standard** | **Blank** |
| **Retention Time** | 3.29 min | No peak was observed |
| **Theoretical plates** | 6186 | 0 |
| **Tailing factor** | 1.58 | 0 |

**Table 3.3: System suitability and Specificity results for Lornoxicam**

### 3.5.3 Linearity:

From the standard stock solution six different concentrations of 6-12µg/ml were prepared. From this 20µl of the sample was injected in to the HPLC system. Peak area versus concentration was measured for ascertaining the linear relationship. Linearity range was found to be 6 - 12µg/ml with $r^2$ value of 0.999. Regression equation was found to be y = 43079x + 1494. Results of the linearity were shown in table 3.4 and calibration curve was shown in figure 3.e

| Linearity Level | Concentration in µg/ml | Peak Area response |
|---|---|---|
| Level – 1 | 6 | 268673 |
| Level – 2 | 7 | 303596 |
| Level – 3 | 8 | 339505 |
| Level – 4 | 9 | 388105 |
| Level – 5 | 10 | 425134 |
| Level – 6 | 11 | 482433 |
| Level – 7 | 12 | 518493 |
| Range: 6 to 12µg/ml | **Correlation coefficient $r^2$ : 0.999**<br>**Slope: 43079**<br>**Intercept : 1494** | |

**Table 3.4: Linearity results for Lornoxicam**

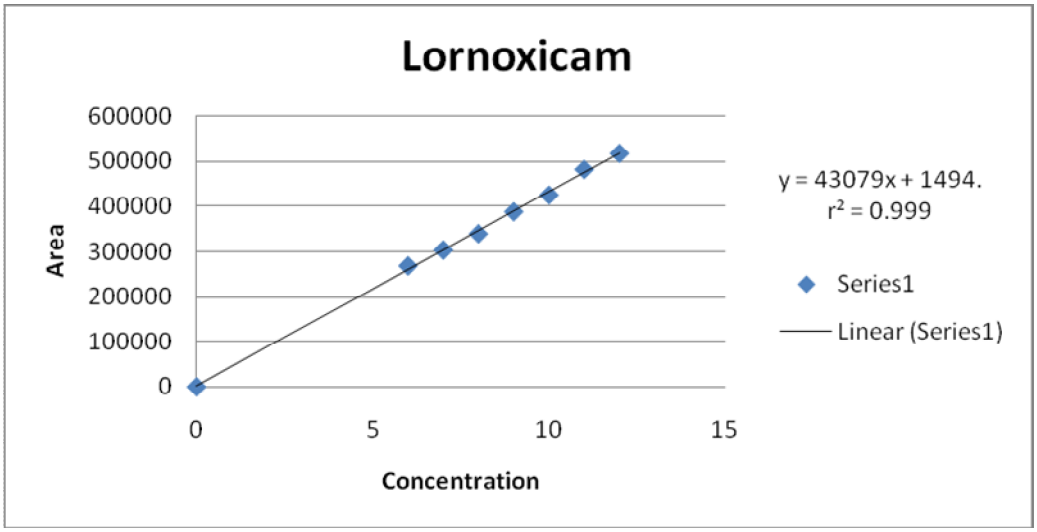



**Figure 3.e: Calibration curve for Lornoxicam.**

### 3.5.4 Precision:

Precision is the degree of argument among the individual test results. Standard Lornoxicam solution at system suitability concentration i.e 9µg/ml was used to express the developed method was precise or not. Precision was carried out by two tests.

### 3.5.4.I. Intraday test:

This can be performed by establishing repeatability of the developed method. Standard drug solution at standard concentration i.e 9µg/ml was prepared in 6

replicates in different time intervals in a single day and the area obtained in 6 replicates was noted and %RSD was calculated.

| Sample | Concentration | Injection No. | Peak Areas | Results |
|---|---|---|---|---|
| Lornoxicam | 9µg/ml | 1 | 372036 | **SD: 3340.897**<br>**Mean: 373231**<br>**% RSD :0.89** |
| | | 2 | 372531 | |
| | | 3 | 374757 | |
| | | 4 | 379163 | |
| | | 5 | 371120 | |
| | | 6 | 369779 | |

**Table 3.5: Results of Intraday precision for Lornoxicam.**

### 3.5.4.II. Interday precision:

Interday variation of the developed method was performed at standard concentration of µg/ml. six replicates of the standard was prepared in three successive days and an aliquots of the sample was injected. The area obtained in 6 replicates was noted and %RSD was calculated and it was found to be 0.89 intraday and 0.45 for interday precision. This is found to be well within the acceptance criteria of > 2. This indicates that the proposed method is precise.

| Sample | Concentration | Injection No. | Peak Areas | Results |
|---|---|---|---|---|
| Lornoxicam | 9µg/ml | 1 | 380251 | **SD :1712.732**<br>**Mean:382342.8**<br>**% RSD :0.45** |
| | | 2 | 382541 | |
| | | 3 | 385251 | |
| | | 4 | 381454 | |
| | | 5 | 381574 | |
| | | 6 | 382986 | |

**Table 3.6: Results of Interday precision for Lornoxicam**

### 3.5.5 Accuracy:

Accuracy is the closeness of the analytical results obtained by the analyses to the true values, and usually presented as a percent of nominal. Accuracy was performed by standard addition method. 50%, 100% and 150% level of the sample was added to a standard fixed sample. The test results were compared with the standard values and % recovery was calculated. Accuracy of the method was confirmed by recovery studies. On standard addition of 50%, 75% and 100% levels of target 6µg/ml was compared with the standard values and the % recovery was found to be 98.11 to 101.75 which was under the acceptance criteria of 98-102%, this indicates that the proposed method was accurate. Results of the recovery were shown in table 3.7.

| % Recovery | Target (µg/ml) | Spiked (µg/ml) | Total (µg/ml) | Amount found | % Recovery | Mean | %RSD |
|---|---|---|---|---|---|---|---|
| 50% | 6 | 3 | 9 | 8.89 | 98.78 | 98.44 | 0.34 |
| | 6 | 3 | 9 | 8.83 | 98.11 | | |
| | 6 | 3 | 9 | 8.86 | 98.44 | | |
| 75% | 6 | 4.5 | 10.5 | 10.41 | 99.14 | 99.62 | 1.08 |
| | 6 | 4.5 | 10.5 | 10.38 | 98.86 | | |
| | 6 | 4.5 | 10.5 | 10.59 | 100.86 | | |
| 100% | 6 | 6 | 12 | 12.21 | 101.75 | 101.42 | 0.43 |
| | 6 | 6 | 12 | 12.11 | 100.92 | | |
| | 6 | 6 | 12 | 12.19 | 101.58 | | |

**Table 3.7: Results of Recovery for Lornoxicam**

### 3.5.6 Ruggedness:

Interday precision at person to person variation was measured to express the ruggedness of the developed method. Standard drug solution at a standard concentration of 9µg/ml was prepared by 6 different persons and the solution was injected in to HPLC system. Peak area response was noted and % RSD was calculated. and it was found to be 0.89, which is well within the acceptance criteria of < 2. This indicates that the proposed method is rugged. Results of the Ruggedness were shown in the table 3.8.

| Sample | Concentration | Injection No. | Peak Areas | Results |
|---|---|---|---|---|
| Lornoxicam | 9µg/ml | 1 | 384652 | SD: 3411.096<br>Mean: 384124.8<br>% RSD :0.89 |
| | | 2 | 380256 | |
| | | 3 | 386451 | |
| | | 4 | 384826 | |
| | | 5 | 388582 | |
| | | 6 | 379982 | |

**Table 3.8: Results of Ruggedness for Lornoxicam**

### 3.5.7 Robustness:

Robustness is the reliability of an analysis with respect to deliberate variations in method parameters. Robustness was performed by change in mobile phase ratio, wavelength, flow rate of the mobile phase at a concentration equal to standard i.e 10µg/ml and % change when change in the condition when compared to standard and it was found to be < 2 indicate that the proposed method was Robust. Results of the robustness were shown in table 3.9.

| S.NO | PARAMETER | CONDITION | AREA | % OF CHANGE |
|---|---|---|---|---|
| 1 | Standard | Standard | 425134 | 0 |
| 2 | MP – 1 | Methanol: ACN 75:25 | 426389 | 0.30 |
| 3 | MP – 2 | Methanol: ACN 85:15 | 427958 | 0.66 |
| 4 | WL – 1 | 258 nm | 427148 | 0.47 |
| 5 | WL – 2 | 262nm | 429325 | 0.99 |
| 6 | pH change - 1 | 5.2 | 420573 | 1.09 |
| 7 | pH change - 2 | 5.0 | 427581 | 0.58 |

**Table 3.9: Results of Robustness for Lornoxicam**

### 3.5.8 Limit of Detection (LOD):

LOD is the known low concentrations of Lornoxicam with those of blank samples and establishing the minimum concentration at which the

Lornoxicam can be reliably detected. A signal-to-noise ratio 2:1 is generally considered acceptable for estimating the limit of detection.

### 3.5.9 Limit of Quantification (LOQ):

The limit of quantification is the known concentrations of Lornoxicam at which it can be quantified with acceptable accuracy and precision. A signal-to-noise ratio between 10:1is generally considered acceptable for estimating the limit of quantification.

LOD and LOQ values were found to be 0.06µg/ml and 0.2µg/ml respectively. This indicates that the proposed method was sensitive and can be applicable at lowest concentration of up to 0.2µg/ml.

| Parameter | Concentration (µg/ml) |
|---|---|
| Limit of Detection | 0.06 |
| Limit of Quantification | 0.2 |

**Table 3.10: LOD and LOQ results**

### 3.5.10 Formulation Analysis:

From the prepared formulation solution, 20µl of the sample was injected into HPLC system. Peak area response was compared with the standard values and the % assay was calculated. Formulation estimation was carried out at market available tablet of Lornoxicam (CAMRI-4) a formulation result was found to be 98.56%. This indicates that the proposed method can be successfully applicable for the estimation of Lornoxicam in formulations up to 98.56%. Results were shown in table 3.11 and formulation chromatogram was shown in figure 3.d

| S.NO | Formulation | Available form | Label claim | Sample concentration | Sample estimated | % Assay |
|---|---|---|---|---|---|---|
| 1 | CAMRI -4mg | Tablet | 4mg | 10 µg/ml | 9.85±3 µg/ml | 98.56% |

**Table 3.11: Formulation results of Lornoxicam**

## 3.6 Results & Discussions

The development of an analytical method for the determination of drug by RP-HPLC has received considerable attention in recent years because of their importance in quality control of drugs and drug products. Hence we develop a suitable analytical method for the estimation of Lornoxicam in pharmaceutical dosage forms.

To develop the method, all the physiochemical properties of the drug was studied and found that RP-HPLC was suitable for the estimation of Lornoxicam, hence used different polar solvents like methanol, water, acetonitrile, ethanol etc as mobile phases in different ratios with and without different buffer solutions. Different non polar columns like C18, C8, and Phenyl etc were used as stationary phases. The suitability of the mobile phase was decided on the basis of the good separation, suitability, time required for the analysis, ease of preparation and use of a readily available cost-effective solvents. UV detection was carried out at 260nm as Lornoxicam showed good absorbance at this wavelength. It was found that a mixture of solvents Methanol and Acetonitrile in the ratio of 80:20 (v/v) and Zodiac C18 column (250 X 4.6 mm, 5µ) as stationary phase with UV detection of 260nm was found to be most suitable conditions.

With the optimized chromatographic conditions, a steady baseline was recorded. The peak shape was symmetrical and asymmetry factor was less than 2 and theoretical plates found to be more than 2000. Thus, the system meets suitable criteria. The calibration curve was obtained for a series of concentration in the range of 6-12µg/ml and it was found to be linear. The data of regression analysis of the calibration curves were shown in Table 3.4. Selectivity and specificity were studied for the examination of various excipients generally present in the tablet dosage form of Lornoxicam. The results indicated that they did not interfere in the assay. The proposed method was validated as per the ICH guidelines.

The precision was measured in terms of repeatability, which was determined by sufficient number of aliquots of a homogenous sample at a concentration of 9µg/ml. The % RSD was found to be 0.89 for intraday precision and 0.45 for interday precision. Results obtained were lying within the range of ±2. Results were shown in table 3.5 for intraday and 3.6 for interday precision. This showed that the precision of the methods were satisfactory.

The recovery technique was performed to study the accuracy and reproducibility of the proposed method. For this, known quantities of the 6µg/ml solution were mixed with definite amounts of pre-analyzed formulations and the mixtures were analyzed. The total amount of Lornoxicam was determined by using the proposed methods and the amount of added drug was calculated by the difference. The % recovery was found to be 98.11 to 101.75%; this was found to be acceptance criteria of 98-102%. Results were shown in table 3.7. This showed that the recoveries of Lornoxicam by the proposed methods were satisfactory.

Robustness of the method was confirmed by change in the optimized chromatographic conditions and % change in each changed condition was calculated. % changes in all changed conditions were found to be well acceptance criteria of less than 2. This indicates that the proposed method was Robust. Results were shown in table 3.9

Ruggedness of the method is ascertained by change in the analysts in the laboratory conditions. Standard concentration was prepared and injected in 6 replicates and % RSD was calculated. % RSD was found to be 0.89, which is within the acceptance criteria of <2. Results were shown in table 3.8.

The Limit of Detection (LOD) and Limit of Quantification (LOQ) of the developed method were determined by injecting progressively low concentrations of the standard solutions using the developed RP-HPLC method. The LOD is the smallest concentration of the analyte that gives a measurable response (signal-to-noise ratio of 3). The LOQ is the smallest concentration of the analyte, which gives response that can be accurately quantified (signal-to-noise ratio of 10). LOD and LOQ for Lornoxicam were found to be 0.06 and 0.2µg/ml respectively.

Formulation study was carried out by using marketed formulation tablet (CAMRI -4mg) of Lornoxicam standard concentration was prepared and the area of the peak response was used for the calculation of the % assay. It was found that up to 98.56% accurately estimate Lornoxicam in pharmaceutical dosage forms.

Thus the method developed in the present investigation is simple, sensitive, accurate, rugged, robust, rapid and precise. Hence, the above said method can be successfully applied for the estimation of Lornoxicam in tablet dosage forms.

## 3.7 References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