CHAPTER 5

ANALYTICAL METHODS (LORNOXICAM)

5. Analytical Methods (Lornoxicam)

The analytical methods developed for testing of Lornoxicam in bulk powder and extended release formulation of are employed in this section. Table 1 - 5 and 2 - 5 show list of material and equipment used respectively. Method for Assay, content uniformity, dissolution studies and determination of drug during in-vivo studies are discussed in this section.

Sr. No.	Material	Source
1.	Lornoxicam	Cadila Healthcare Ltd., Moraiya, Ahmedabad, Gujarat, India
2.	Dichloromethane	Merck Limited, Mumbai, India
3.	Methanol	Merck Limited, Mumbai, India
4.	Acetone	Merck Limited, Mumbai, India
5.	Isopropyl alcohol	Merck Limited, Mumbai, India
6.	Glacial acetic acid	S. D. Finechem Limited, Mumbai, India
7.	Hydrochloric acid	S. D. Finechem Limited, Mumbai, India
8.	Potassium dihydrogen phosphate	S. D. Finechem Limited, Mumbai, India
9.	Sodium hydroxide	S. D. Finechem Limited, Mumbai, India
10.	Sodium lauryl sulphate	S. D. Finechem Limited, Mumbai, India
11.	HPLC grade Methanol, Acetonitrile, Acetic acid	S. D. Finechem Limited, Mumbai, India
12.	Ethanol (99.5%V/V)	Baroda Chem. Ind. Ltd., Baroda, India
13.	Water (distilled)	Prepared in laboratory by distillation

Table 1 - 5 List of materials

Sr. No.	Equipments	Source/Make
1.	Digital weighing balance	AG-64, Mettler Toledo, Switzerland
2.	pH meter	Mettler Toledo, Switzerland
3.	Friability tester	EF-2, Electrolab, Mumbai, India
4.	Bath sonicator	DTC 503, Ultra Sonics
5.	Dissolution apparatus	Electrolab, Mumbai, India
6.	HPLC system	LC 20-AT prominence, Shimadzu Corp., Japan
7.	UV-Visible Spectrophotometer	Shimadzu UV-1601, Japan
8.	Calibrated pipettes of 1.0 ml, 5.0 ml and 10.0 ml.	Schott & Corning (India) Ltd., Mumbai
9.	volumetric flasks of 10 ml, 25 ml, 50 ml and 100 ml capacity.	Schott & Corning (India) Ltd., Mumbai
10.	Funnels (i.d. 5.0 cm)	Schott & Corning (India) Ltd., Mumbai
11.	Beakers (250 ml) and other requisite glass wares	Schott & Corning (India) Ltd., Mumbai
12.	Nuclepore Polycarbonate membrane 2 µm 25mm	Whatman, USA

Table 2 - 5 List of Equipments

5.1 Preparation of reagents and buffers

5.1.1 Preparation of Acetate Buffer pH 4.5

2.99 gm of sodium acetate trihydrate and 200 ml of distilled water was placed in a 1000 ml volumetric flask. 14 ml of acetic acid solution was added and the volume was adjusted with distilled water upto 1000 ml. (USP 30, 2007).

5.1.2 Preparation of Phosphate Buffer pH 7.5

Dissolve 6.8 g of monobasic potassium phosphate and 1.6 g of Sodium Hydroxide in 1000 ml of water. Adjust the pH to 7.5 with 2 N Sodium Hydroxide. (USP 30, 2007)

5.2 Estimation of Lornoxicam

5.2.1 Estimation of Lornoxicam in acetate buffer 4.5, Phosphate Buffer 7.5 and PBS 7.5 : Methanol (50: 50)

Lornoxicam shows strong absorbance in UV-Visible region. Hence, the estimation of Lornoxicam was performed by UV-visible spectrophotometry.. For determination of Lornoxicam for assay and content uniformity 50:50 mixture of PBS and Methanol was used and UV spectrophotometric method for estimation of *in-vitro* drug release was developed in acetate buffer 4.5 and Phosphate Buffer 7.5. An analytical method for determination of lornoxicam in plasma during *in-vivo* studies was developed using HPLC.

5.2.1.1 Preparation of standard stock solutions of Lornoxicam in PBS pH 7.5

50 mg of Lornoxicam was accurately weighed using single pan electronic balance and transferred to 50 ml volumetric flask. 25 ml of PBS 7.5 was accurately measured and transferred to the above volumetric flask, the drug was dissolved properly and then the final volume of the flask was made up to 50 ml with PBS pH 7.5 to produce 1000 µg per ml of Lornoxicam.

25 ml of the above solution was accurately measured by calibrated graduated pipette and transferred to the 100 ml volumetric flask. The final volume was made up to 100 ml with PBS 7.5 to prepare stock solution of 250 µg per ml of Lornoxicam.

5.2.1.2 Calibration curve of Lornoxicam in PBS pH 7.5

Suitable aliquots of standard stock solution were accurately measured and transferred to the 10 ml of volumetric flasks. The final volume was made up to 10 ml with PBS 7.5 to give final concentrations of 5,10, 15, 20, 25, 30 µg/ml and analyzed by UV spectrophotometry at 278 nm. No interference due to excipients used in the formulation was observed. The above procedure was repeated three times. The data was recorded in Table along with standard deviation.

Concentration (µg/ ml)	Average*	SD	RSD
5	0.0910	0.0013	0.9428
10	0.2500	0.0021	0.8556
15	0.4190	0.0040	0.9633
20	0.5870	0.0036	0.6193
25	0.7710	0.0034	0.4381
30	0.9340	0.0029	0.3954

Table 3 - 5 Calibration for Lornoxicam in PBS 7.5

Regression equation** Y= 0.034X - 0.0859; Correlation coefficient = 0.9996

*Mean of 3 values

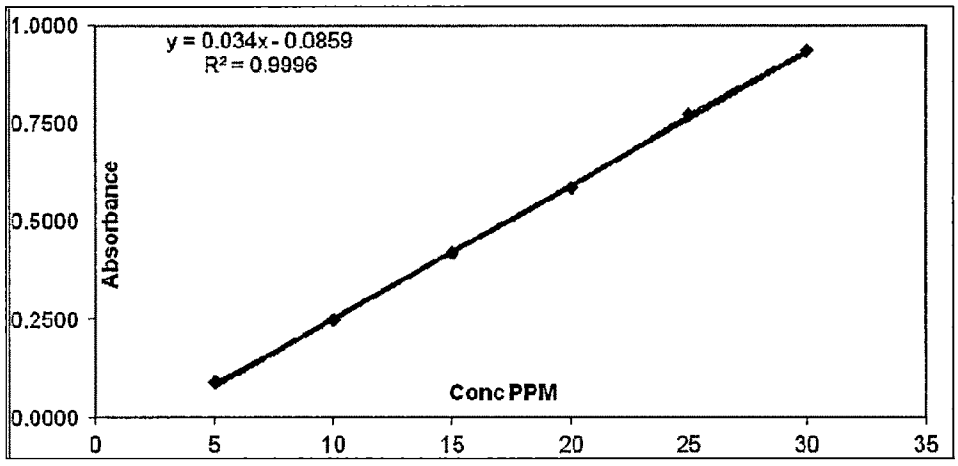

Figure 1 - 5 Regressed calibration curve for estimation of Lornoxicam in PBS 7.5

Figure 1-5 shows Regressed calibration curve for estimation of Lornoxicam in PBS 7.5, values as mentioned in table 4 - 5.

Accuracy and repeatability

Accuracy of an analytical method is the closeness of test results obtained by that method to true value (USP30-NF25, 2007). Accuracy is calculated from the test results as the percentage of analyte recovered by assay. Accuracy was calculated by analysis of three replicate samples by the above described methods. The observed concentrations of the drug were then back calculated using the equation of standard calibration curve and compared with actual concentrations. 1 ml of 50 µg/ml solution of drug was spiked to 4 ml of sample to achieve minimum quantifiable concentration using the discussed analytical method. 1 ml of 50 µg/ml solution of drug was spiked to 4 ml of sample to achieve minimum quantifiable

concentration using the discussed analytical method. After measuring concentration through discussed method spiked 50 µg amount was deducted and amount available in 4 ml of sample was calculated.

Accuracy of method for analysis of Lornoxiam in PBS 7.5 was show in 5 -5.

In order to determine the accuracy of the developed method, known amounts of Lornoxicam (5 µg/mL, 10 µg/mL and 30 µg/mL) were subjected to recovery studies as per the procedure described above. To determine the repeatability of the analytical method Intraday result for three different concentrations (5, 10, 30 µg/ml) determined 5 times at two different days and RSD for results were compared. The results obtained are tabulated in table 5 -5.

Conc. of LOR (µg/ml) Std.	AVG Recovery (µg/ml)	% Recovery	SD	RME	Confidence	RSD .Intra Day	RSD Inter Day
5	5.1428	102.9	0.0607	0.0272	5.1428 + 0.1693	1.18	1.57
10	9.8828	98.8	0.2898	0.1296	9.8828 + 0.8079	2.93	1.76
30	29.9802	99.9	0.1155	0.0516	19.9802 + 0.3219	0.39	0.34

Table 4- 5 Evaluation of accuracy and repeatability of the estimation method of LOR in PBS pH 7.5

** At 95% Confidence level; t_{tab} = 3.18 for 4 degrees of freedom (n=5)*

5.2.1.3 Preparation of standard stock solutions of Lornoxicam in Acetate Buffer pH 4.5

50 mg of Lornoxicam was accurately weighed using single pan electronic balance and transferred to 50 ml volumetric flask. 25 ml of Acetate Buffer pH 4.5 was accurately measured and transferred to the above volumetric flask, the drug was dissolved properly and then the final volume of the flask was made up to 50 ml with Acetate Buffer pH 4.5 to produce 1000 µg per ml of Lornoxicam.

25 ml of the above solution was accurately measured by calibrated graduated pipette and transferred to the 100 ml volumetric flask. The final volume was made up to 100 ml with Acetate Buffer pH 4.5 to prepare stock solution of 250 µg per ml of Lornoxicam.

5.2.1.4 Calibration curve of Lornoxicam in Acetate Buffer pH 4.5

Suitable aliquots of standard stock solution were accurately measured and transferred to the 10 ml of volumetric flasks. The final volume was made up to 10 ml with Acetate Buffer pH 4.5 to

give final concentrations of 10, 20, 40, 50,60 µg/ml and analyzed by UV spectrophotometry at 282 nm. No interference due to excipients used in the formulation was observed. The above procedure was repeated three times. The data was recorded in Table along with standard deviation. Figure 2-5 shows Regressed calibration curve for estimation of Lornoxicam in acetate buffer pH 4.5, values as mentioned in table 6 - 5.

Concentration (µg/ ml)	Average*	SD	RSD
10	0.224	0.0030	1.3367
20	0.370	0.0026	0.7027
40	0.680	0.0022	0.3235
50	0.825	0.0034	0.3312
60	0.965	0.0031	0.3253

Table 5 - 5 Calibration for Lornoxicam in Acetate Buffer pH 4.5

Regression equation** Y= 0.0149X + 0.0752; Correlation coefficient = 0.9997

*Mean of 3 values

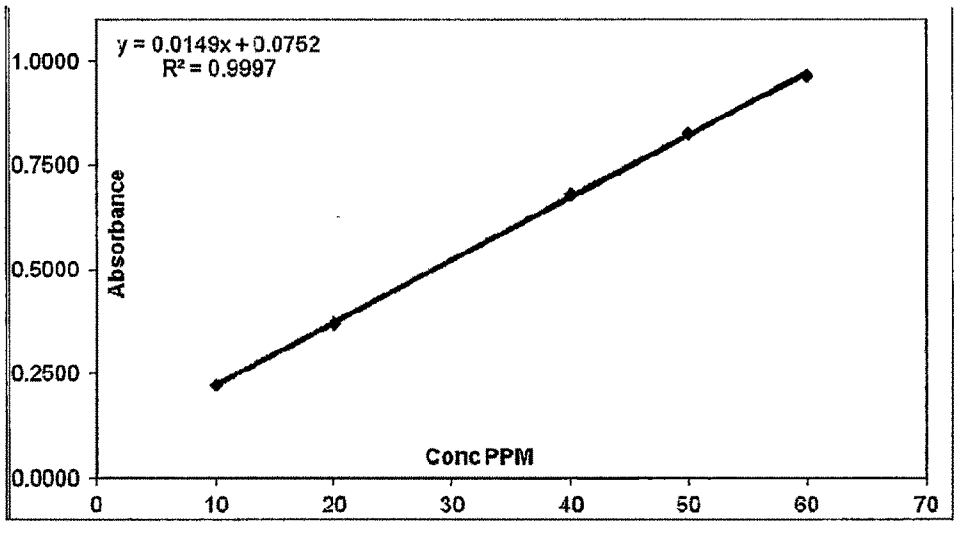

Figure 2- 5 Regressed calibration curve for estimation of Lornoxicam in Acetate Buffer pH 4.5

Accuracy and Repeatability

Accuracy of an analytical method is the closeness of test results obtained by that method to true value (USP30-NF25, 2007). Accuracy is calculated from the test results as the percentage of analyte recovered by assay. Accuracy was calculated by analysis of three

replicate samples for the above described methods. The observed concentrations of the drug were then back calculated using the equation of standard calibration curve and compared with actual concentrations. Accuracy of method for analysis of Lornoxiam in acetate buffer 4.5 is shown in Table 6 - 5.

1 ml of 50 µg/ml solution of drug was spiked to 4 ml of sample to achieve minimum quantifiable concentration using the discussed analytical method. 1 ml of 50 µg/ml solution of drug was spiked to 4 ml of sample to achieve minimum quantifiable concentration using the discussed analytical method. After measuring concentration through discussed method spiked 50 µg amount was deducted and amount available in 4 ml of sample was calculated.

In order to determine the accuracy of the developed method, known amounts of Lornoxicam (10 µg/mL, 20 µg/mL and 60 µg/mL) were subjected to recovery studies as per the procedure described above. To determine the repeatability of the analytical method Intraday result for three different concentrations (10, 20, 60 µg/ml) determined 5 times at two different days and RSD for results were compared. The results obtained are tabulated in table 6 - 5.

Conc. of LOR (µg/ml) Std.	AVG Recovery (µg/ml)	% Recovery	SD	RME	Confidence	RSD Intra Day	RSD Inter Day
10	10.1325	101.3	0.1784	0.0798	10.1325 ± 0.4972	1.76	2.93
20	19.9802	99.9	0.1155	0.0516	19.9802 ± 0.3219	0.58	0.52
60	59.9379	99.9	0.0842	0.0376	59.9379 ± 0.2346	0.14	0.17

Table 6 - 5 Evaluation of accuracy and precision of the estimation method of lor in Acetate Buffer 4.5

** At 95% Confidence level; t_{tab} = 3.18 for 4 degrees of freedom (n=5)*

5.2.1.5 Preparation of standard stock solutions of Lornoxicam in PBS 7.5 : Methanol (50 : 50)

50 mg of Lornoxicam was accurately weighed using single pan electronic balance and transferred to 50 ml volumetric flask. 25 ml of PBS 7.5 : Methanol (50 : 50) was accurately measured and transferred to the above volumetric flask, the drug was dissolved properly and then the final volume of the flask was made up to 50 ml with PBS 7.5 : Methanol (50 : 50) to produce 1000 µg per ml of Lornoxicam.

25 ml of the above solution was accurately measured by calibrated graduated pipette and transferred to the 100 ml volumetric flask. The final volume was made up to 100 ml with PBS: Methanol (50 : 50) to prepare stock solution of 250 µg per ml of Lornoxicam.

5.2.1.6 Calibration curve of Lornoxicam in PBS 7.5 : Methanol

Suitable aliquots of standard stock solution were accurately measured and transferred to the 10 ml of volumetric flasks. The final volume was made up to 10 ml with PBS 7.5 : Methanol (50 : 50) to give final concentrations of 10, 12, 14, 18, 20, 25 µg/ml and analyzed by UV spectrophotometry at 278 nm. No interference due to excipients used in the formulation was observed. The above procedure was repeated three times. The data was recorded in Table along with standard deviation. Figure 3-5 shows Regressed calibration curve for estimation of Lornoxicam in PBS 7.5 : Methanol, values as mentioned in table 7 - 5.

Concentration (µg/ ml)	Average*	SD	RSD
10	0.3007	0.0026	0.8714
12	0.3540	0.0029	0.8166
14	0.4007	0.0037	0.9244
18	0.5040	0.0039	0.7741
20	0.5490	0.0038	0.6878
25	0.6667	0.0048	0.7140

Table 7 - 5 Calibration for Lornoxicam in PBS : Methanol

Regression equation** Y= 0.0244X - 0.0598; Correlation coefficient = 0.9995

*Mean of 3 values

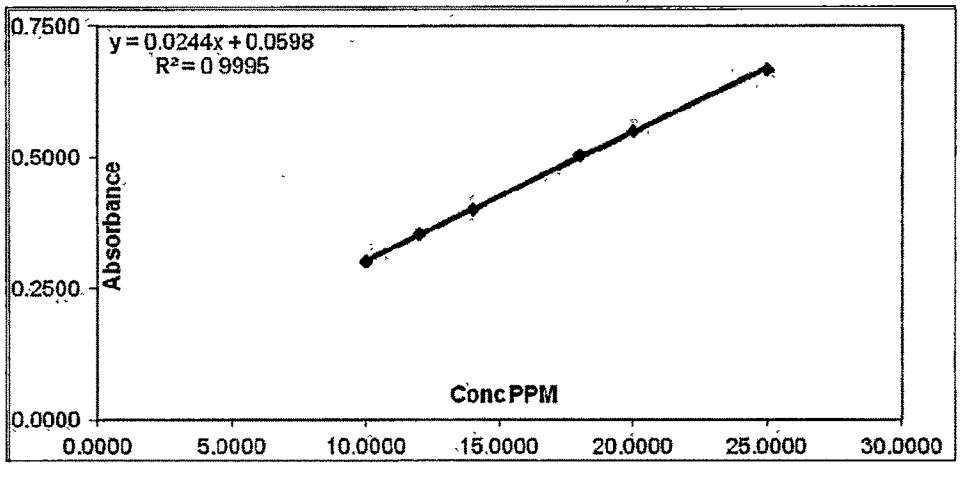

Figure 3 - 5 Regressed calibration curve for estimation of Lornoxicam in PBS 7.5 : Methanol

Accuracy and repeatability

Accuracy of an analytical method is the closeness of test results obtained by that method to true value (USP30-NF25, 2007). Accuracy is calculated from the test results as the percentage of analyte recovered by assay. Accuracy was calculated by analysis of three replicate samples for the above described methods. The observed concentrations of the drug were then back calculated using the equation of standard calibration curve and compared with actual concentrations. Accuracy of method for analysis of Lornoxiam in Medium was show in Table 8 - 5.

1 ml of 50 µg/ml solution of drug was spiked to 4 ml of sample to achieve minimum quantifiable concentration using the discussed analytical method. 1 ml of 50 µg/ml solution of drug was spiked to 4 ml of sample to achieve minimum quantifiable concentration using the discussed analytical method. After measuring concentration through discussed method spiked 50 µg amount was deducted and amount available in 4 ml of sample was calculated.

In order to determine the accuracy of the developed method, known amounts of Lornoxicam (10µg/mL, 14µg/mL and 25µg/mL) were subjected to recovery studies as per the procedure described above. To determine the repeatability of the analytical method Intraday result for three different concentrations (10, 14, 25 µg/ml) determined 5 times at two different days and RSD for results were compared. The results obtained are tabulated in table 8 -5.

Theoretical Conc of BT (µg/ml)	AVG Recovery (µg/ml)	% Recovery	SD	RME	Confidence	RSD Intra Day	RSD Inter Day
10	10.0428	100.4	0.1493	0.0668	10.0428 ± 0.4162	1.49	0.87
14	14.0084	100.1	0.0685	0.0306	14.0084 ± 0.1910	0.49	0.74
25	25.0270	100.1	0.0601	0.0269	25.0270 ± 0.1676	0.24	1.43

Table 8 - 5 Evaluation of accuracy and repeatability of the estimation method of LOR in PBS 7.5 : Methanol

** At 95% Confidence level; t_{tab} = 3.18 for 4 degrees of freedom (n=5)*

5.3 Estimation of Lornoxicam in Formulation for Assay

To determine the amount of Lornoxicam in the tablet, 20 tablets were crushed and added to 10 ml mixture of PBS 7.5 : Methanol (50:50) and subjected to shaking at room temperature for 5 mins for complete disintegration of excipients & extraction of the drug from the formulation. The filtered solution was further diluted with PBS 7.5 : Methanol (50:50) to get detectable concentration and estimated at 278 nm.

5.4 Estimation of Lornoxicam in formulation for content uniformity

To determine the amount of Lornoxicam in the Tablet , 10 tablets individually were crushed and added to 100 ml of PBS 7.5 : Methanol (50 : 50) and subjected to shaking at room temperature for 5 mins for complete disintegration of excipients and extraction of the drug from the formulation. The filtered supernant was further diluted with PBS 7.5 : Methanol (50 : 50) to get detectable concentration and estimated at 278 nm.

5.5 Estimation of LOR for in-vitro release

The release studies for Lornoxicam formulation in different release media. One tablet containing 16 mg drug was placed in dissolution vessel containing 900 ml of release medium maintained using paddle at 50 RPM at 37 ± 2 °C. 5 ml aliquots were taken out at different time and replace with same quantity of release media. The dissolved drug in release medium analyzed as per the method above. The amount of the drug released and cumulative percentage release was calculated.

The spectrophotometeric determination of placebo formulation consisting of all ingredients except drug showed no any absorbance under discussed methods. The capacity of the method to separate Lornoxicam the non-interference with Lornoxicam indicates the specificity of the methods. Stability of the Lornoxicam in the solution was demonstrated to be stable in solvent during the period of 24 h since the change in the λ_{max} was not significant with the RSD value.

5.6 HPLC method for estimation of Lornoxicam

For determination of linearity 100 µg of Lornoxicam was accurately weighed using single pan electronic balance and transferred to aconitrile to produce primary standard of Lornoxicam, which was used to make working standard solutions of lornoxicam by diluting primary standard solution with acetonitrile. Rat plasma calibration standards (0.50, 1.00, 2.00, 3.00, 4.00, 5.00 µg/ml) of lornoxicam were prepared by spiking the working standard in to the drug free rat plasma. The aliquot samples were (200 µL) stored at -20° C into polypropylene tubes until analysis.

200 µL of blank plasma, calibration standards and QC samples were mixed with 200 µL of 0.5 M HCl. The samples were extracted with 900 µL of ethyl acetate in 2.0 ml polypropylene tubes by vortex-mixing for 5 min at high speed and centrifuged at 5000 g for 5 min at room temperature. 850 µL of the organic layer was transferred and evaporated to dryness using reduced pressure. The residues were dissolved in 40 µL of 0.1 N methanolic NaOH by vortex-mixing for 2 min, centrifuged at 5000 g for 5 min, transferred to injection vials. To 40 µl of above solution 10 µl of 50 µg/ml solution of drug in 0.1 N methanolic sodium hydroxide was spiked to achieve minimum quantifiable concentration and 10 µL were injected into the HPLC column using the discussed analytical method. 1 ml of 50 µg/ml solution of drug was spiked to 4 ml of sample to achieve minimum quantifiable concentration using the discussed analytical method. After measuring concentration through discussed method spiked 50 µg amount was deducted and amount available in 4 ml of sample was calculated.

Method Type : Isocratic System Method

Buffer Solution pH 7.3: Dissolve 2.88 mg Ammonium Dihydrogen Phosphate in 1000ml of water, and adjust pH to 7.3 $\pm$ 0.05 with triethylamine.

Mobile Phase : sodium acetate (pH 7.3) and methanol (45:55) mix and sonicate for 5 min and filter through a 0.45 µm Poly Tetra Fluoro Ethylene (PTFE) membrane filter.

Column : Phenomenex - Luna C 18 100 A 250 x 4.60 mm x 5 µ

Sample Injection Volume: 10 µl

Flow Rate : 1.5 ml/min

λ_{max} : 275 nm

Thermostat : 30°C

Retention time: 8.5 min

Run Time : 15 min

Column was equilibrated with mobile phase until stable baseline is obtained.

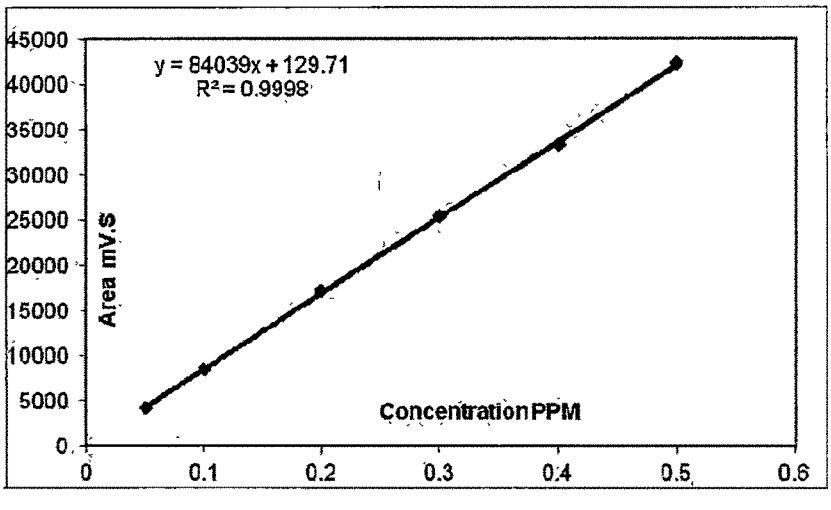

Figure 4 - 5 regressed calibration curve of Lornoxicam (HPLC)

Calibration curve of Lornoxicam

Concentration (µg/ml)	Area mV .S	SD	RSD
0.050	4250	39	0.9176
0.100	8500	72	0.8471
0.200	17142	139	0.8109
0.300	25468	234	0.9188
0.400	33381	281	0.8418
0.500	42298	319	0.7542

Table 9 - 5 Calibration curve of lornoxim Using HPLC

Figures 4 - 5 calibration curve of Lornoxicam tartrate in using HPLC, the values are tabulated in table 9 -5.

Accuracy and repeatability

Accuracy of an analytical method is the closeness of test results obtained by that method to true value (USP30-NF25, 2007). Accuracy is calculated from the test results as the

percentage of analyte recovered by assay. Accuracy was calculated by analysis of three replicate samples for the above described methods. The observed concentrations of the drug were then back calculated using the equation of standard calibration curve and compared with actual concentrations. Accuracy of method for analysis of Lornoxiam in Medium was show in Table 10 - 5.

In order to determine the accuracy of the developed method, known amounts of Lornoxicam (0.05 µg/mL, 0.30 µg/mL and 0.50 µg/mL) were subjected to recovery studies as per the procedure described above. To determine the repeatability of the analytical method Intraday result for three different concentrations (0.05, 0.30, 0.50 µg/ml) determined 5 times at two different days and RSD for results were compared. The results obtained are tabulated in table 10-5.

Stability of the Lornoxicam in solution was verified and found to be stable in solvents during the period of 24 h since the change in the λ_{max} was not significant with the maximum RSD value of 2.90% as mentioned in table 10-5.

It was also confirmed that retention time of Lornoxicam not shifted with the adjustment of the proportion of methanol and the flow rate. But the final result did not show significant change. Considering the stability in the system suitability parameters, the method conditions would be concluded to be robust.

Theoretical Conc of LOR (µg/ml)	AVG Recovery (µg/ml)	% Recovery	SD	RME	Confidence	RSD Intra Day	RSD Inter Day
0.0500	0.0497	99.5	0.0008	0.0004	0.0497 ± 0.0022	1.62	2.90
0.3000	0.2984	99.5	0.0056	0.0025	0.0497 ± 0.0156	1.87	2.75
0.5000	0.5032	100.6	0.0107	0.0048	0.5032 ± 0.0299	2.13	2.68

Table 10 - 5 Evaluation of accuracy and precision of the estimation method of LOR using HPLC

** At 95% Confidence level; t_{tab} = 3.18 for 4 degrees of freedom (n=5)*

System Suitability verified by following parameters

- % RSD for three replicate was not more then 3.0.
- The tailing factor is not more than 2.0.

The developed isocratic high performance liquid chromatographic method was rapid and suitable for the estimation of Lornoxicam in rat plasma. Linearity, repeatability, accuracy and

robustness were verified. The stability of analytical solutions was sufficient for the whole analytical process. Using the established method, the amount of Lornoxicam in plasma was determined.

The chromatographic determination of placebo formulation consisting of all ingredients except drug showed no any absorbance under discussed methods. The capacity of the method to separate Lornoxicam is verified. The non-interference with Lornoxicam indicates the specificity of the methods.

5.7 Discussion

The UV spectroscopic method was developed for the LOR estimation in PBS pH 7.5, Acetate Buffer pH 4.5 and PBS : Methanol (50 : 50), measurement was done at λ_{max} 278 nm, 282 nm and 278 nm respectively for solvents. There was no interference observed with any excipient used. The method was validated for linearity, accuracy and repeatability. The validation parameters were found to meet the "readily pass criteria" and % RSD were found less than 2%.

The absorbance for BT in PBS pH 7.5 was found to be linear in the range of 5 - 30 µg /ml with r^2 value of 0.9996. The recovery was found to be more than 90%, indicating the reliability accuracy to estimate LOR in the mentioned range. The repeatability of the measurement was expressed in terms of % RSD and the % RSD for intra-day and inter-day of BT at 3 different concentration levels do not show significant difference.

The absorbance for LOR in acetate buffer pH 4.5 was found to be linear in the range of 10 - 60 µg /ml with r^2 value of 0.9997. The recovery was found to be more than 90%, indicating the reliability accuracy to estimate LOR in the mentioned range. The repeatability of the measurement was expressed in terms of % RSD and the % RSD for intra-day and inter-day of BT at 3 different concentration levels were shown do not show significant difference.

The absorbance for LOR in PBS : Methanol (50 :50) was found to be linear in the range of 10 - 25 µg /ml with r^2 value of 0.9995. The recovery was found to be more than 90%, indicating the reliability accuracy to estimate BT in the mentioned range. The repeatability of the measurement was expressed in terms of % RSD and the % RSD for intra-day and inter-day of BT at 3 different concentration levels do not show significant difference.

The invitro release study was performed using type II dissolution apparatus using 900 ml release medium. At different time intervals, the samples were removed, replaced with same medium and analysed for the drug. The cumulative percentage drug released was calculated.

For Lornoxicam, the calibration curve was established using HPLC for estimation of drug in plasma with sodium acetate (pH 7.3) and methanol (45:55) mix as mobile phase and detection at 275nm. The linearity of LOR was found to be 0.050 – 0.500 µg/ml (R^2=0.9998). The recovery studies for accuracy and precision were carried out at 0.050, 0.300 and 0.500 µg/ml and the recovery was found to be more than 90%, indicating the reliability accuracy to estimate LOR in the mentioned range.

5.8 References

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