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Development of RP-HPLC Method for Simultaneous Estimation of Lornoxicam and Paracetamol in Combined Dosage Forms

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Abstract:

A simple, rapid reverse phase high-performance liquid chromatographic method was developed and validated for the simultaneous estimation of paracetamol and lornoxicam in bulk and pharmaceutical dosage forms. Determination of the different analytical parameters such as linearity, precision, accuracy, and specificity, limit of detection (LOD) and limit of quantification (LOQ) was done. The calibration curve was found to be linear for each analyte in the desired concentration range. The proposed method is highly sensitive, precise and accurate for paracetamol and lornoxicam respectively and hence the present method can be applied successfully for the quantification of active pharmaceutical ingredient (API) content in the combined formulations of paracetamol and lornoxicam.

INTRODUCTION

Paracetamol (PARA), chemically 4-hydroxy acetanilide, is a centrally and peripherally acting non-opioid analgesic and antipyretic1-3. Literature survey reveals, there are UV and HPLC methods reported for the estimation of PARA in Pharmaceutical formulations. Lornoxicam (LOX) is 6-chloro-4-hydroxy-2-methyl-N-2-pyridinyl-2H-thieno-[2,3-e]-1,2-thiazine-3-carboxamide 1,1- dioxide; is a novel non-steroidal anti-inflammatory drug (NSAID) with marked analgesic properties. LOX belongs to the chemical class oxicams, which includes piroxicam, tenoxicam and meloxicam. LOX, which is commercially available as an 8- mg tablet, is used to treat inflammatory diseases of the joints, osteoarthritis, and pain after surgery. It works by blocking the action of cyclooxygenase, an enzyme involved in the production of chemicals, including some prostaglandins in the body [1-5] . Extensive literature survey reveals, none of the method is available that is based on estimation of Paracetamol and Lornoxicam by HPLC. Aim of present work was to develop simple, precise, accurate and economical RP-HPLC methods for simultaneous determination of binary drug formulation. [6-9]

MATERIALS AND METHOD

I) Preparation of standard solution:

i) Lornoxicam (LOR) standard stock solution:

A precise measurement of approximately 20 milligrammes of LOR was added to a 100.0 millilitre volumetric flask, dissolved in an adequate amount of mobile phase, and then filled to the mark with mobile phase. Fill the 25.0 mL volumetric flask with mobile phase until the mark is reached after transferring 1.0 mL of the aforementioned solution. A concentration of 8 micrograms per millilitre.

ii) Paracetamol (PARA) standard stock solution:

A precise measurement of approximately 625 milligrammes of PARA was added to a 50.0 millilitre volumetric flask, dissolved in an adequate amount of mobile phase, and then filled to the mark with mobile phase. Fill the 25.0 mL volumetric flask with mobile phase until the mark is reached after transferring 1.0 mL of the aforementioned solution. The concentration is 500 micrograms per millilitre.

iii) Mixed standard solution of Lornoxicam (LOR) and Paracetamol (PARA):

The components LOR and PARA, each with an exact mass of 10 milligrammes, were added to a volumetric flask with a capacity of 50.0 millilitres, dissolved in an adequate amount of mobile phase, and then filled up to the mark with mobile phase. (L) (Concentration: 12500 µg/mL PARA and 200 µg/mL LOR)

The mobile phase was used to dilute 1.0 mL of the aforementioned mixed standard solution to a final volume of 25.0 mL. (L1) A solution containing 8 µg/mL of LOR and 500 µg/mL of PARA.

II) Selection of mobile phase:

Using the following chromatographic parameters, various mobile phases were tried to select a suitable one.

Column-Phenomenex ODS 5 µ C18 column (250 X 4.6mm)

Detector-UV-visible detector

Detection wavelength-310.0 nm.

Flow rate-1.0 ml/min

Temperature-28-30^0 C

pH-7.3 with triethylamine

Injection volume - 20µl

Preparation of buffer solution:

Dissolved 2.88 g. ammonium Dihydrogen Phosphate in 1000 ml of double distilled water and mix.

Preparation of mobile phase:

Adjusted the pH to 7.3 using triethlyamine after mixing methanol and buffer in several amounts. Afterwards, sonicate the mixture for 30 minutes after passing each mobile phase through a 0.45 µ membrane filter paper.

The mobile phases used to prepare the mixed standard solutions of LOR and PARA (L1) are detailed in Table No. 1.1.

Procedure:

After combining various volumes of methanol and buffer, the pH was adjusted to 7.3 using triethlyamine. After each mobile phase has been passed through a 0.45 µ membrane filter paper, sonicate the mixture for 30 minutes.

The mobile phases that were utilised to create the LOR and PARA (L1) mixed standard solutions are listed in Table No. 1.1.

Table No. 1.1: Results of selection of mobile phase

Sr.No.	Mobile phase	Retention time (min)			
		PARA	LOR	PARA	LOR
1	Methanol : Buffer (50:50)	2.875	4.312	Fronting	Sharp
2	Methanol : Buffer (60:40)	2.886	3.812	Broad	Tailing
3	Methanol : Buffer (55:45)	3.142	4.162	Sharp	Tailing
4	**Methanol : Buffer (70:30)**	**2.873**	**3.827**	**Sharp**	**Sharp**
5	Methanol : Buffer (65:35)	2.984	3.729	Fronting	Sharp

A recorded chromatogram in selected mobile phase is shown in **Fig. 1.1**

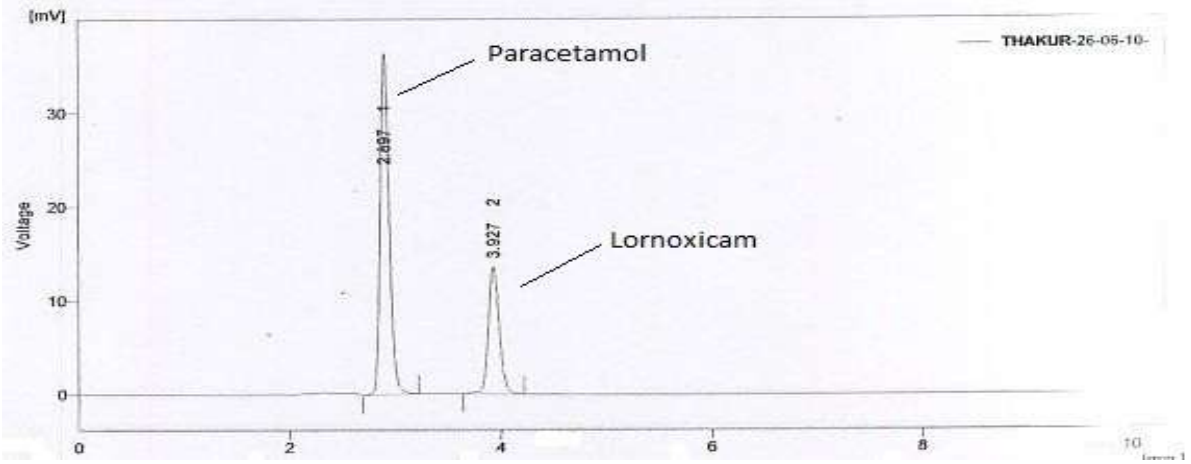


Fig. 1.1: Standard Chromatogram of LOR and PARA

III) Study of system suitability parameters:

Procedure:

Five identical injections of 20 µL solution (L1) were administered after the column was equilibrated with the mobile phase. We recorded the chromatograms and measured the peak area, which is the peak response. Table 1.2 displays the outcomes.

Table No.1.2: Results of system suitability parameters

Sr. No.	Standard weight waken		A.U.C of PARA (mV)	A.U.C. of LOR (mV)
	PARA	LOR		
1	625.0 mg	10.0 mg	215.271	96.588
2			217.039	97.599
3			218.053	98.378
4			217.44	97.839
5			219.201	98.649
Mean			**215.271**	**96.588**
%RSD			**1.47**	**0.85**
Theoretical plate/column			**112935**	**151950**
Retention time			**2.8**	**3.9**
Resolution			**--**	**5.819**
Asymmetry			**1.3**	**1.2**

IV) Study of Beer-Lamberts law:

To achieve concentrations ranging from 250.0 - 1250.0 µg/mL for PARA and 4 - 20 µg/mL for LOR, aliquots of the mixed standard stock solution (L) were diluted in 25.0 mL volumetric flasks containing mobile phase, with the volume being filled up to the mark.

Procedure:

After the mobile phase and stationary phase had equilibrated, a stable base line could be formed. Next, chromatograms were acquired after injecting solutions ranging from 4-20.0 µg/mL for LOR and 250-1250 µg/mL for PARA. For each medicine, we plotted the drug concentration against the area under the curve.

In Figures 6.g and 6.h, we can see that the correlation coefficient for PARA was 0.999 and for LOR it was 0.999.

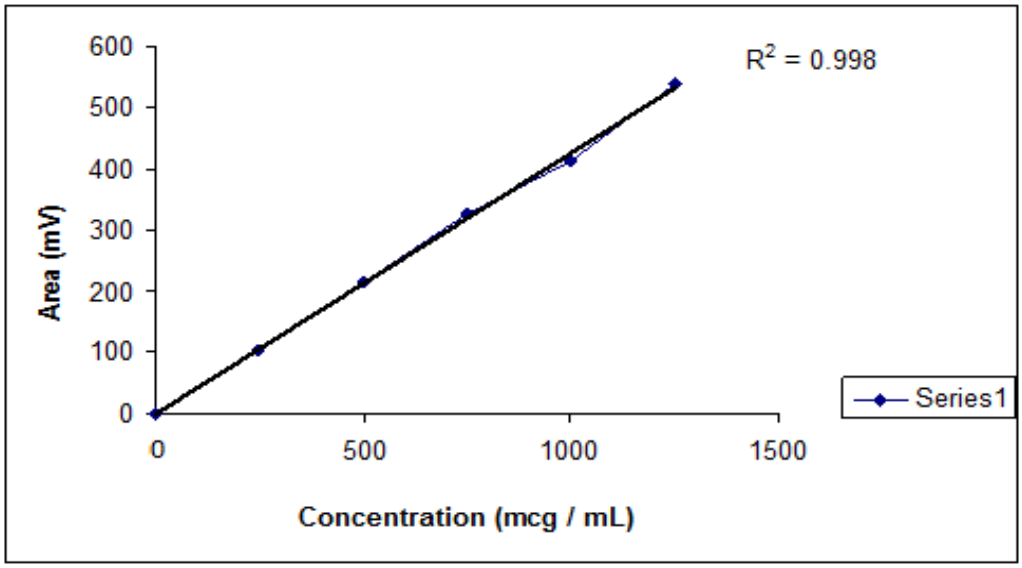


Fig 1.2: Study of Beer-Lamberts law of PARA

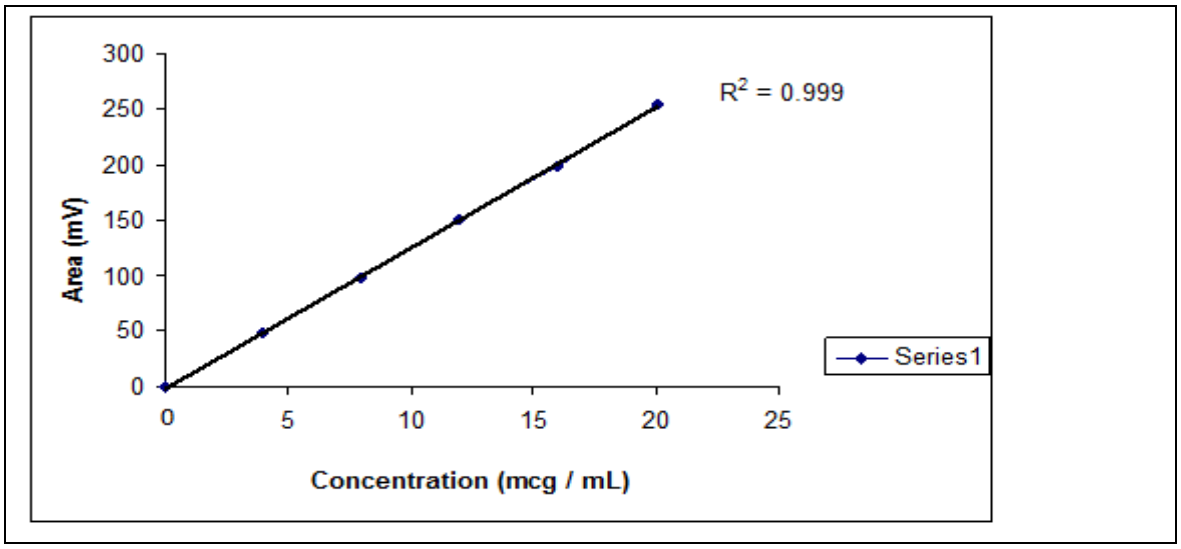


Fig 1.3: Study of Beer-Lamberts law of LOR

V) Application of the proposed method for estimation of LOR and PARA in marketed formulation:

Preparation of sample:

The average weight was calculated by weighing twenty pills. The following steps were taken in a series of 50.0 ml volumetric flasks: 10 mg of LOR and approximately 625 mg of PARA in tablet powder were transferred, 30 mL of mobile phase was added, the mixture was sonicated, and then diluted to volume with mobile phase. Using a 0.45µ membrane filter, the solution was filtered. The filtrate was further diluted with mobile phase to a volume of 25.0 mL from 1.0 mL. After the stationary phase was equilibrated, the chromatograms were recorded and each sample solution was injected independently. To determine the LOR and PARA concentrations, we compared the sample's peak area to the standard's using the following formula:

$$\% \text{ Label Claim} = \frac{Au \times W_{std} \times Avg.\ wt}{As \times W\ tab.\ powder \times L.C.} \times 100 \quad \textbf{(6.11)}$$

Where,

Au = Peak area of sample

As = Peak area of Standard

W std = Wt (mg) of PARA or LOR in std. stock

W tab. Powder = Wt of tablet powder

Avg. wt = Average weight of tablet in mg

L.C. =Label Claim in mg/ tablet

The chromatogram of LOR and PARA in marketed formulation depicted in **Fig. 1.4** and results are shown in **Table No. 1.2**

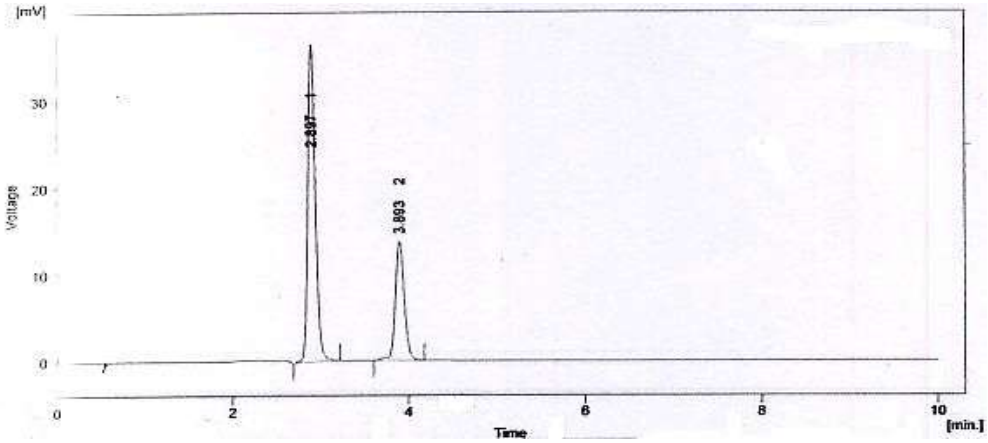


Fig. 1.4: Chromatogram of LOR and PARA in marketed formulation

Table No.1.2: Results of estimation of LOR and PARA in Marketed formulation

LORAX-P ®				PARA -500 mg + LOR-8 mg			
Sr. No.	**Wt. of tablet powder taken (mg)**	**Peak area(AUC) Of Standard (mV)**		**Peak area(AUC) Of Sample (mV)**		**% Labeled claim**	
		LOR	**PARA**	**LOR**	**PARA**	**LOR**	**PARA**
1	884.2	215.271	96.588	98.618	218.13	102.77	101.99
2	884.2			96.692	215.67	100.87	100.84
3	884.1			96.663	217.74	100.74	101.82
4	884.9			97.363	216.62	101.38	101.21
5	884.6			97.059	216.13	101.10	101.01
					Mean	**100.746**	**100.84**
					±SD	**0.7756**	**0.501**
					%RSD	**0.769**	**0.497**
Avg. wt. of tablet - 712 mg							

VI) Recovery study:

It was carried out by standard addition method.

Preparation of standard solution of LOR:

A precisely measured amount of LOR (~ 25 mg) was added to a 25.0 mL volumetric flask along with an adequate amount of mobile phase and water until the mark was reached.(Molecular weight: mg/mL)

Preparation of sample:

The preanalyzed tablet powder, which contained 312.5 mg of PARA and approximately 5.0 mg of LOR, was measured and transferred to a set of five volumetric flasks measuring 50.0 mL. Standard amounts of PARA and LOR were added to the flasks at five different levels, and the mixture was shaken for 30 minutes with enough mobile phase and volume to reach the mark. The Whatmann filter paper (no. 41) was used to filter the contents. The filtrate was further diluted with mobile phase to a volume of 25.0 mL from 1.0 mL.

Procedure

We followed the procedure outlined for estimating LOR and PARA in the marketed formulation. Each drug's amount and percentage of label claim were determined from the standard and sample detector responses using the same formula as given under marketed formulation. Once the amount of each drug included in the pre-analyzed powder was subtracted from the total estimated amount of that drug, the remaining amounts could be reliably recovered from the pure drug that was added.

The percentage of recovery and the actual amount of medication were determined using formulas 1.3 and 1.4, respectively. Table displays the results of the LOR and PARA estimations.

Table 1.3: Results of Recovery study

Sr. No.	Wt. of tablet powder taken (mg)	Peak area of sample (AUC) (mV)		Amt. of pure drug added (mg)		Total amt. of drug estimated (mg)		% Labeled claim	
		LOR	PARA	LOR	PARA	LOR	PARA	LOR	PARA
1	445.2	58.0	129.3	1.0	62.5	6.0	375.0		99.77
2	445.9	68.0	151.0	2.0	125.0	7.0	438.0	99.49	99.89
3	445.5	77.0	172.3	3.0	187.3	8.0	500.0	99.81	99.91
4	445.3	87.0	194.7	4.0	250.0	9.0	563.0	99.92	100.1
5	445.1	97.0	215.3	5.0	312.5	10.0	625.0	99.97	99.97
							Mean	**99.49**	**99.77**
							±SD	**0.207**	**0.129**
							%RSD	**0.002**	**0.001**

VII) Validation of proposed method:
Validation of the proposed method was carried out as per ICH guidelines.

1) Accuracy:

Recovery trials conducted using the usual addition approach confirmed the accuracy of the suggested procedure. Table No. 6.18 displays the results.

2) Precision:

Standards and deviations (SD and RSD) of measurement series are a way to express the precision of an analytical procedure. By doing replicate analyses on uniform samples of tablet powder, we were able to confirm that the suggested approach accurately estimates PARA and LOR.

3) Specificity:

Precisely measured amounts of pre-analyzed tablet powder, corresponding to approximately 625.0 mg of PARA and approximately 10 mg of LOR, were transferred to a set of 50.0 mL volumetric flasks and maintained for 24 hours at the mentioned conditions.

1. With 10.0 mL of 0.1 N NaOH (Alkali) added and the temperature up to 500 C 2. After adding 10.0 mL of 0.1 N HCl (Acid), the temperature is set at 500 C. Step3, with 10.0 mL of 6% H2O2 (Oxide) added, at 500 C

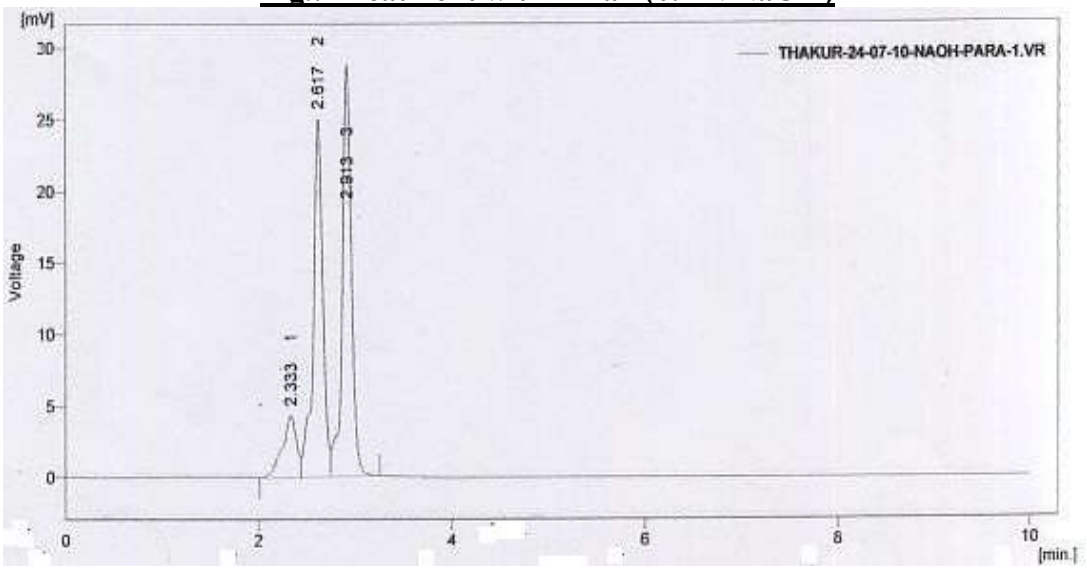
4. 10.0 mL of distilled water is added at 500 C. while being exposed to ultraviolet light

6. In a dry chamber The contents were filtered through whatmann filter paper (no. 41) after 24 hours, after which the appropriate amount of mobile phase was added and the mixture was agitated for 30 minutes. The volume was then increased to the mark. The mobile phase was used to further dilute 1.0 mL of the filtrate until it reached a volume of 25.0 mL. The chromatograms were recorded after the stationary phase was equilibrated and the sample solutions were injected independently. By comparing the peak area of the sample with that of the standard, the content of PARA and LOR under different stress situations could be estimated. Table displays the findings of the specificity investigations, while Figures display the recorded chromatograms.

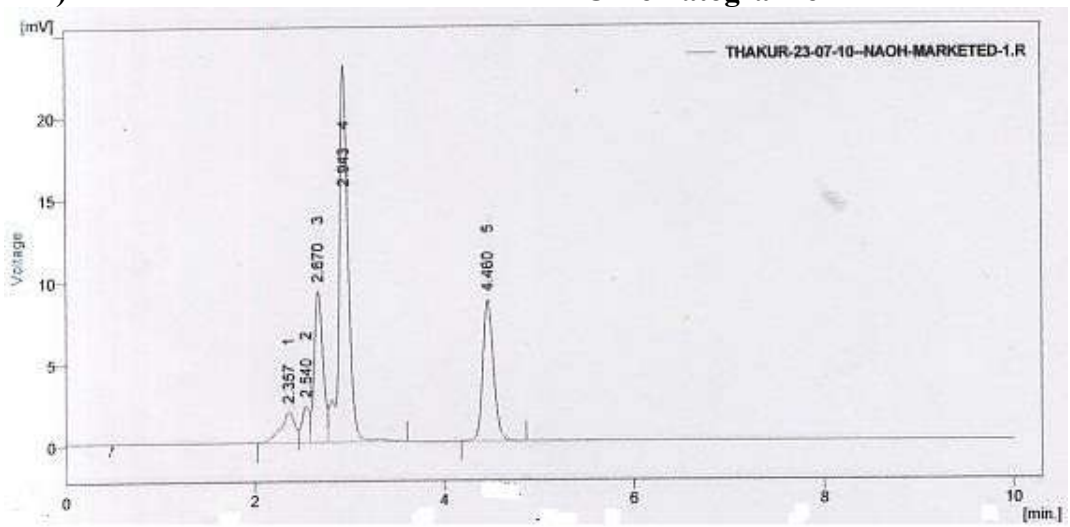
Table 1.4: Results of specificity study

Sample (Treated)	Wt. of tablet powder taken (mg)	A.U.C. (mV)		% Labeled Claim	
		LOR	PARA	LOR	PARA
Alkali	884.8	90.983	153.955	94.75	71.94
Acid	884.6	95.932	1230.54	99.93	575.11
Oxide	884.4	96.238	219.25	100.27	102.49
Aqueous	884.5	96.247	218.258	100.27	102.02
UV	884.6	94.768	217.124	98.71	101.48
Humidity	884.4	96.546	219.185	94.75	102.46

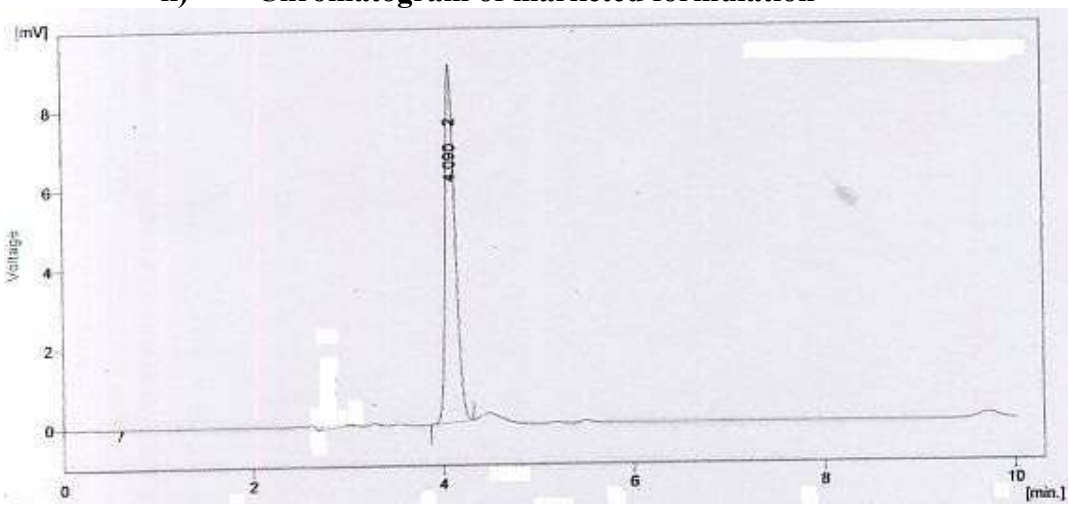
Fig.: Treatment with Alkali (0.1 N NaOH)



i) Chromatogram of PARA

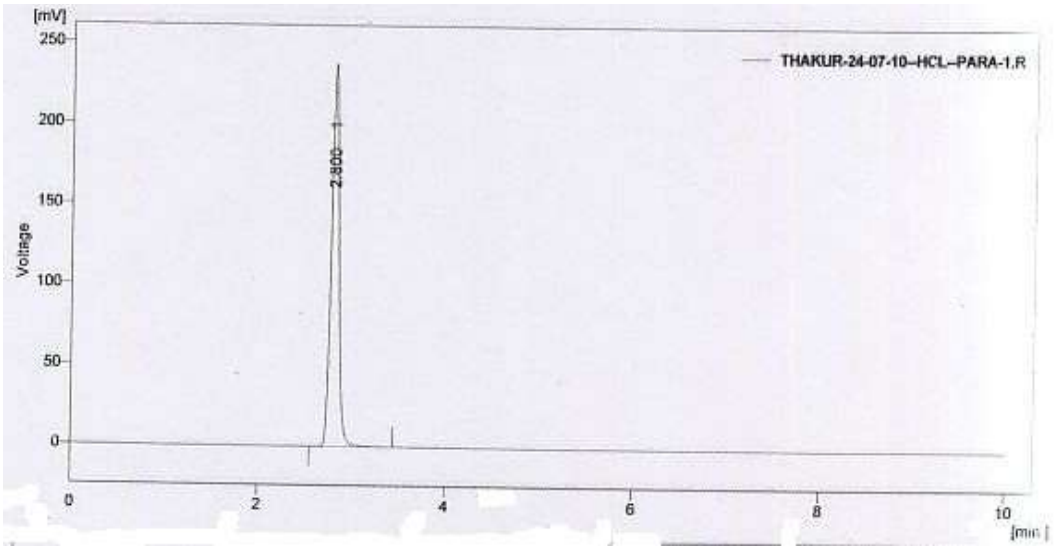


ii) Chromatogram of marketed formulation

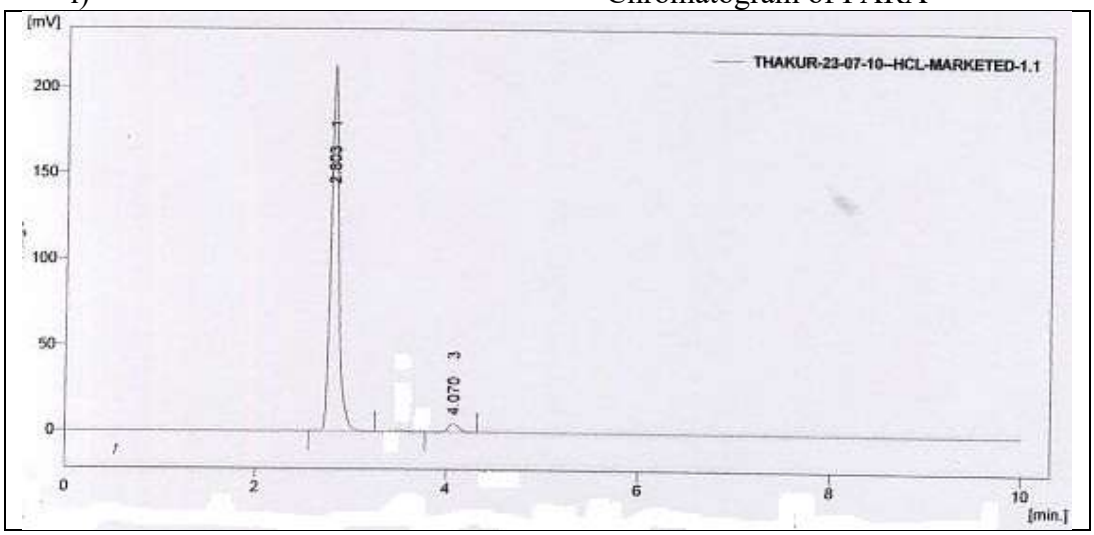


iii) Chromatogram of LOR

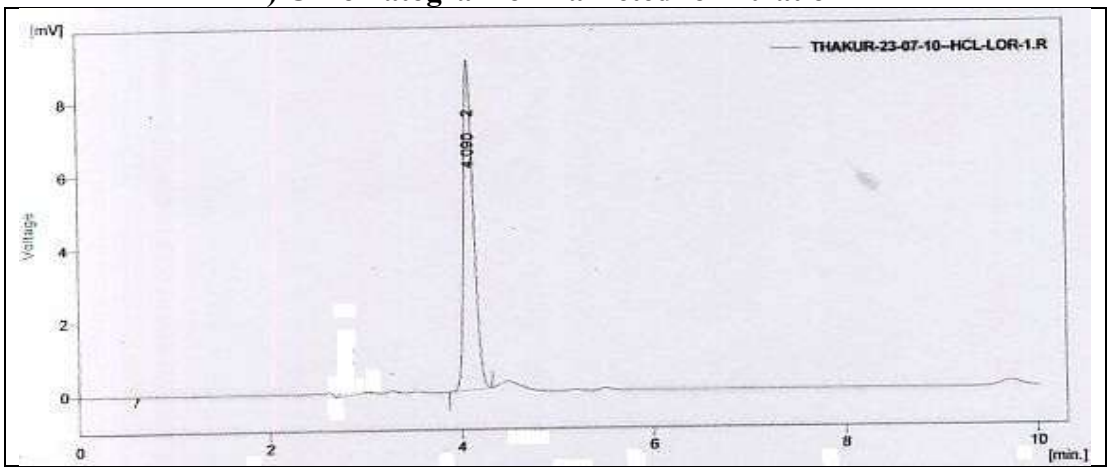
Fig.: Treatment with Acid (0.1 N HCL)



i) Chromatogram of PARA

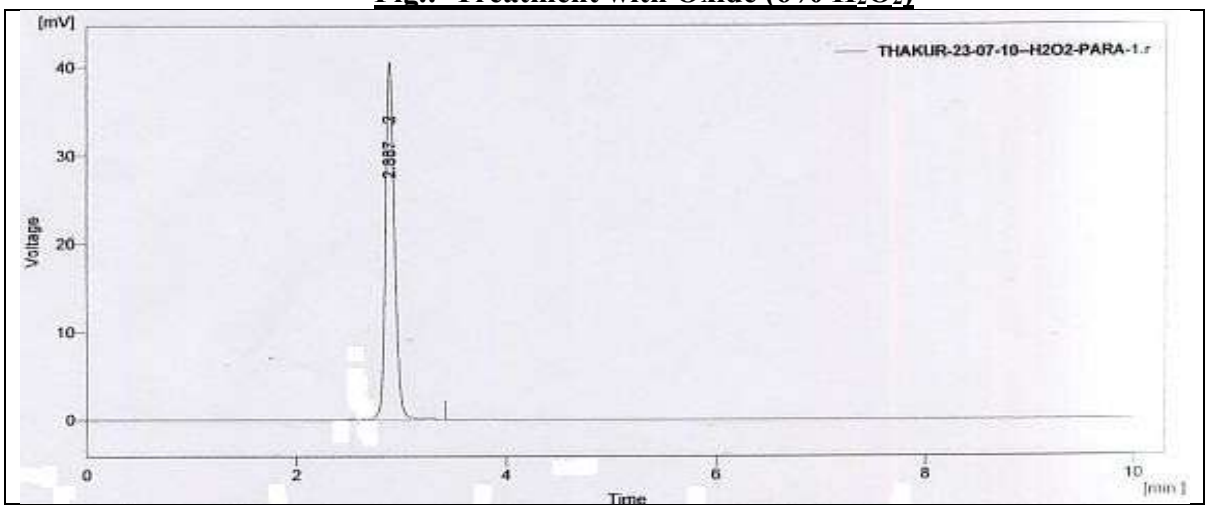


ii) Chromatogram of marketed formulation

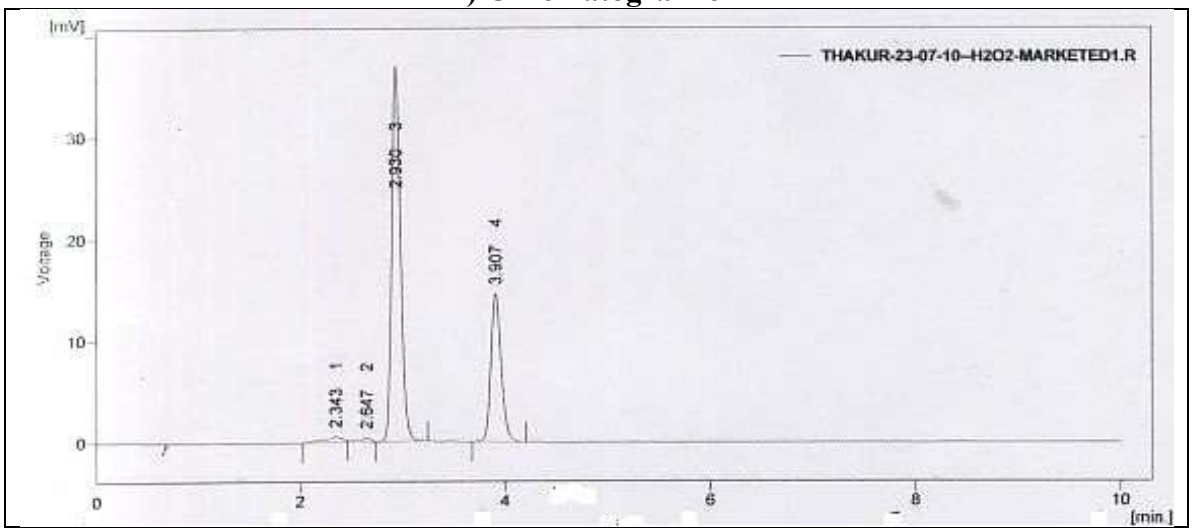


iii) Chromatogram of LOR

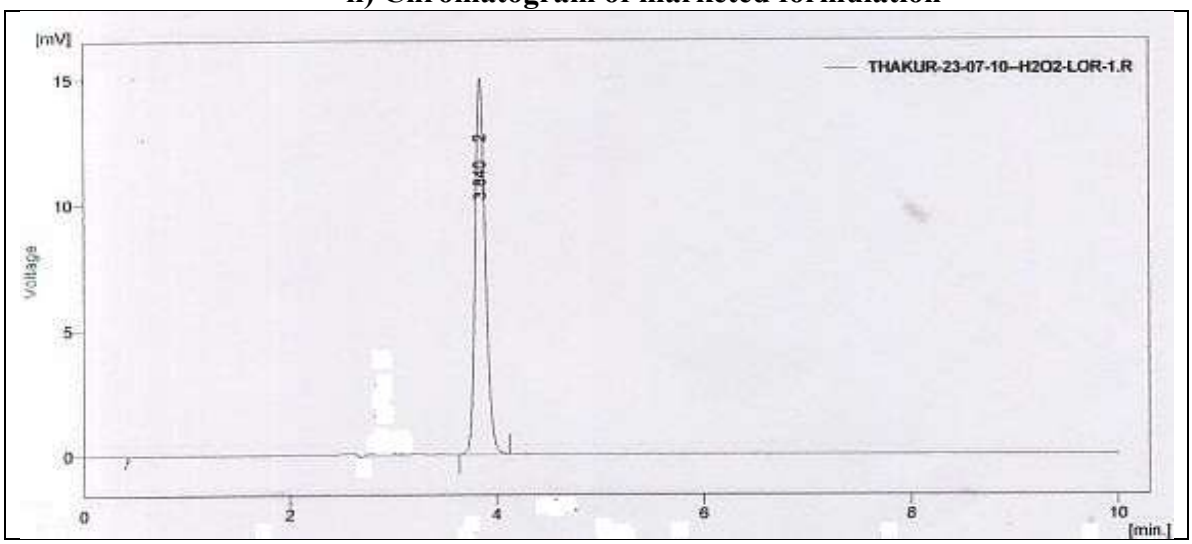
Fig.: Treatment with Oxide (6% H_2O_2)



i) Chromatogram of PARA

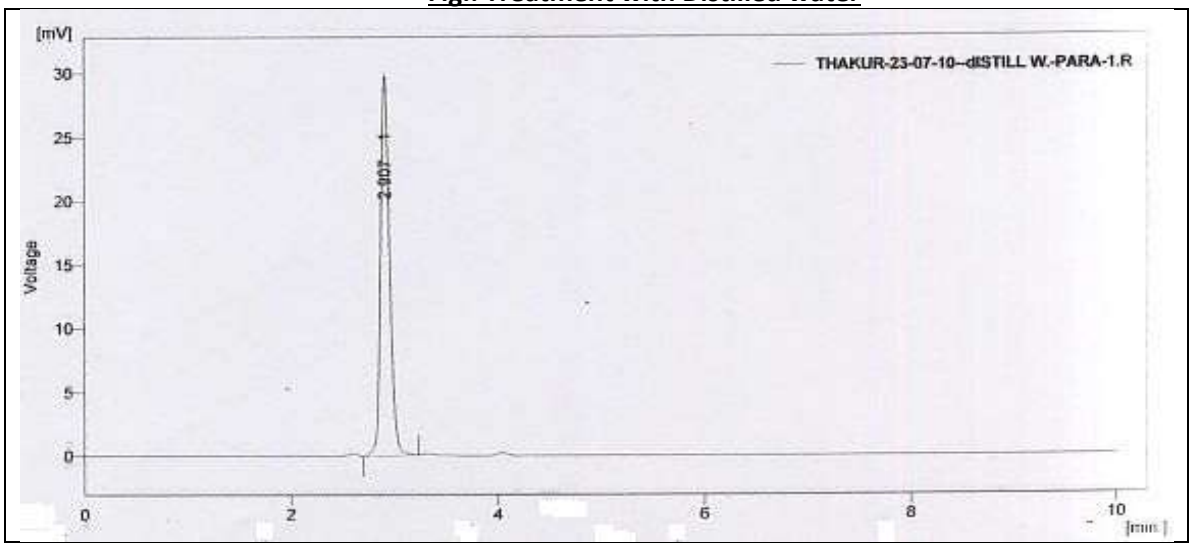


ii) Chromatogram of marketed formulation

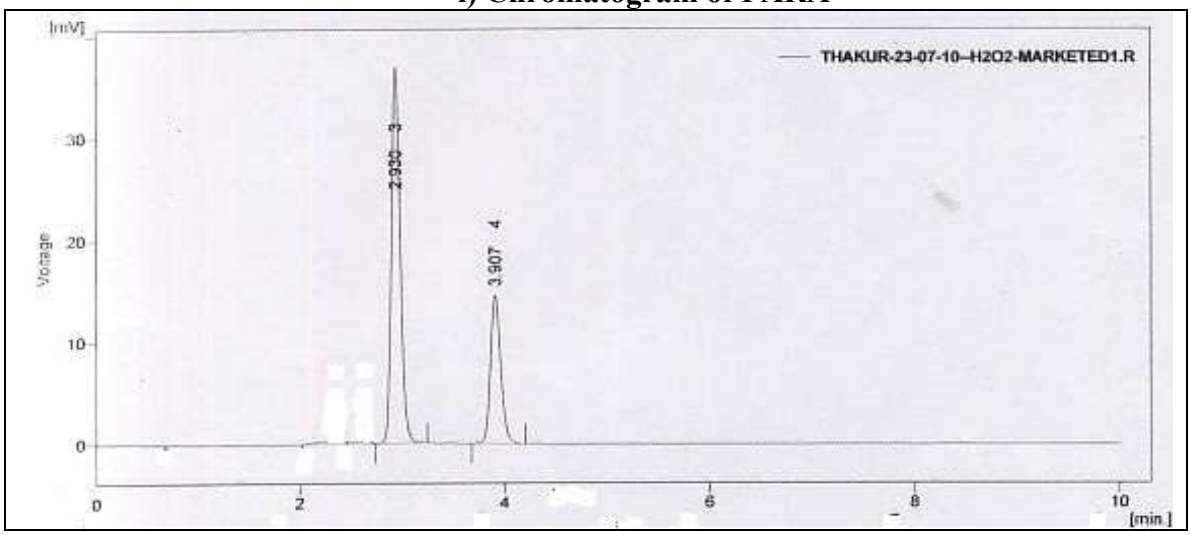


iii) Chromatogram of LOR

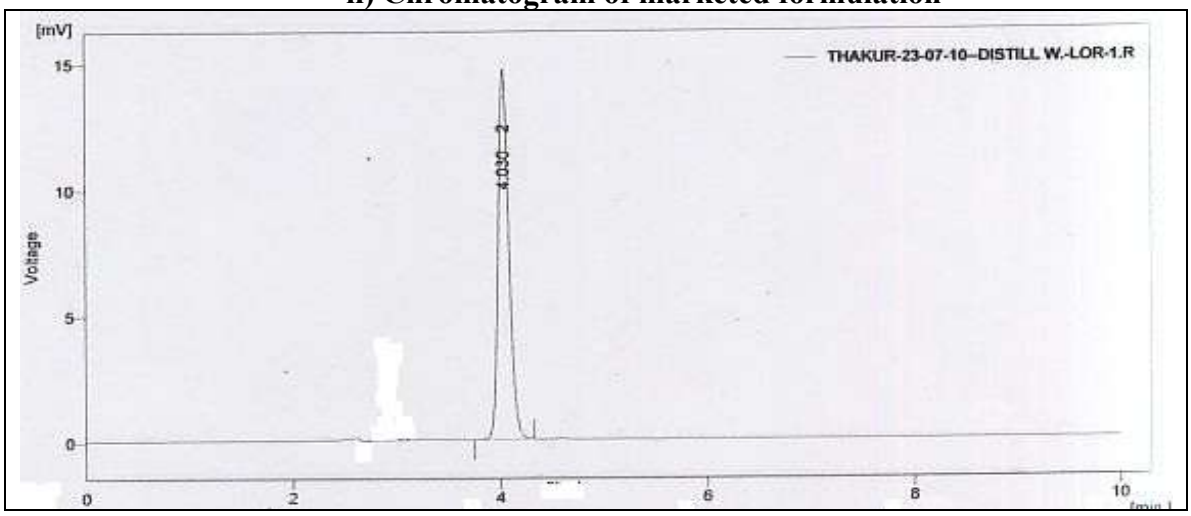
Fig.: Treatment with Distilled water



i) Chromatogram of PARA

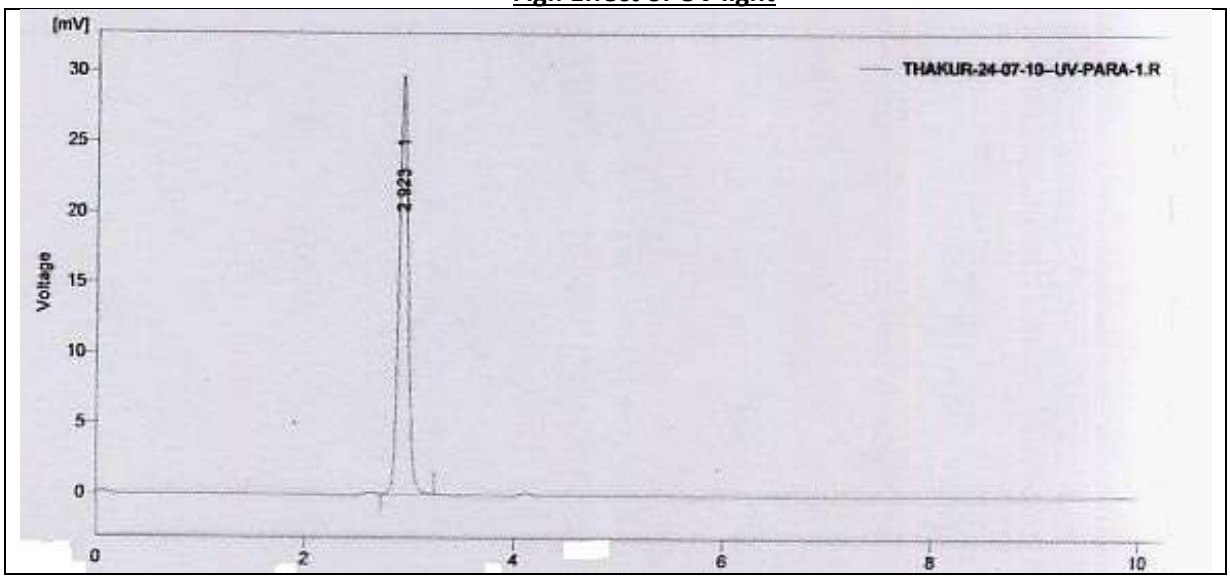


ii) Chromatogram of marketed formulation

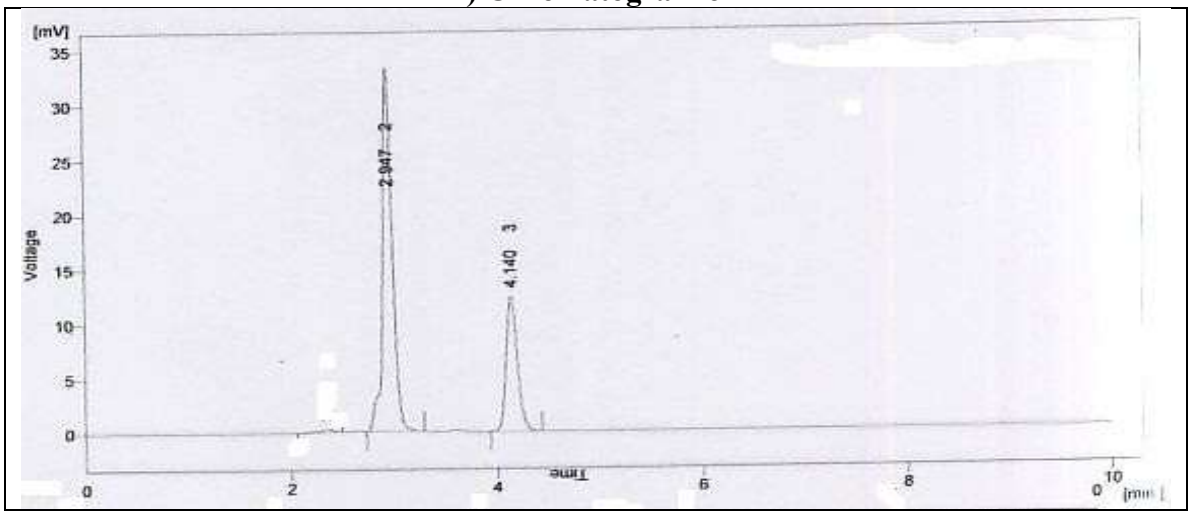


iii) Chromatogram of LOR

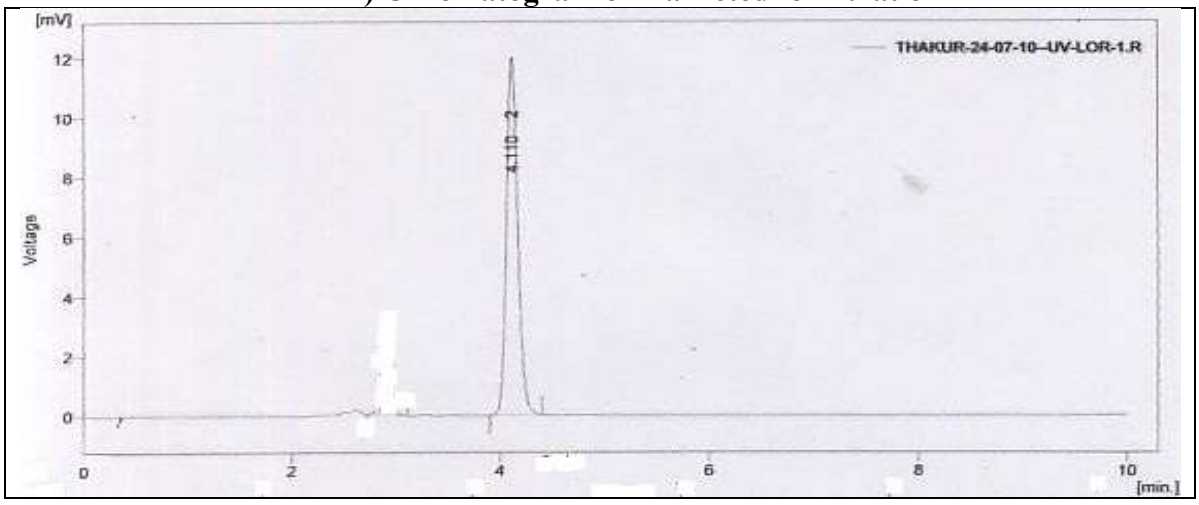
Fig.: Effect of UV light



i) Chromatogram of PARA

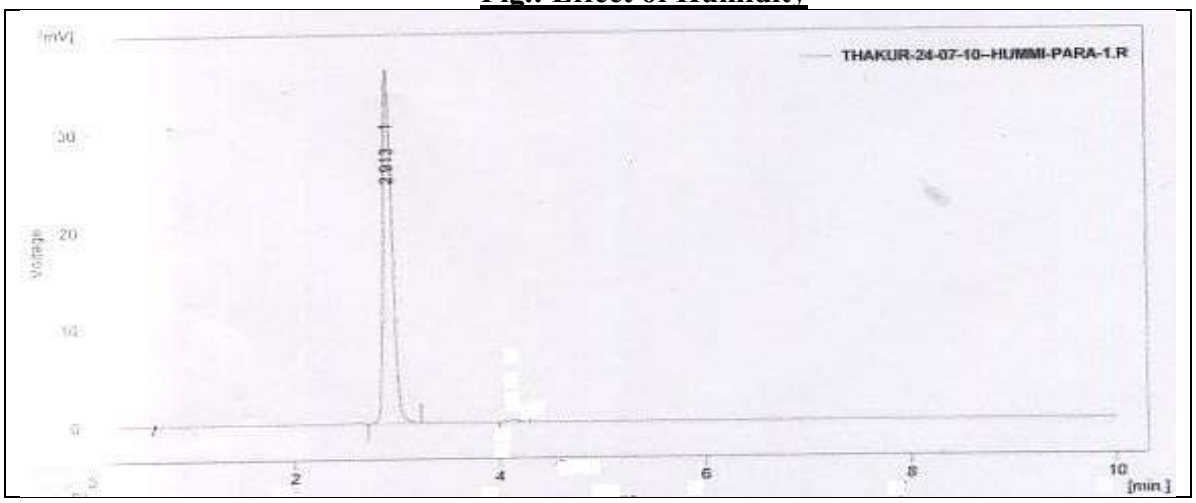


ii) Chromatogram of marketed formulation

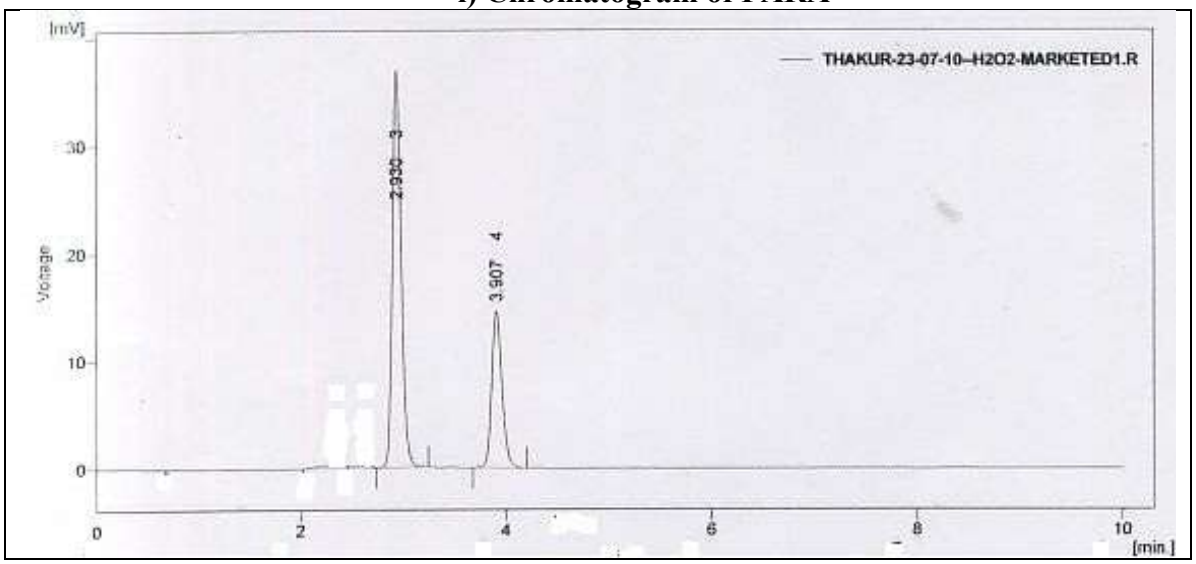


iii) Chromatogram of LOR

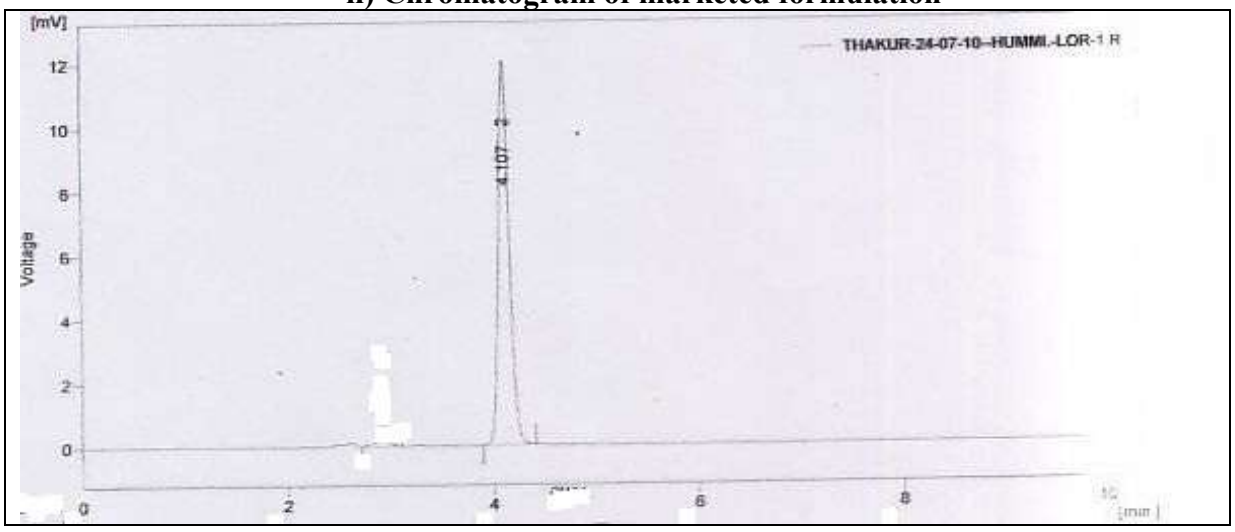
Fig.: Effect of Humidity



i) Chromatogram of PARA



ii) Chromatogram of marketed formulation



iii) Chromatogram of LOR

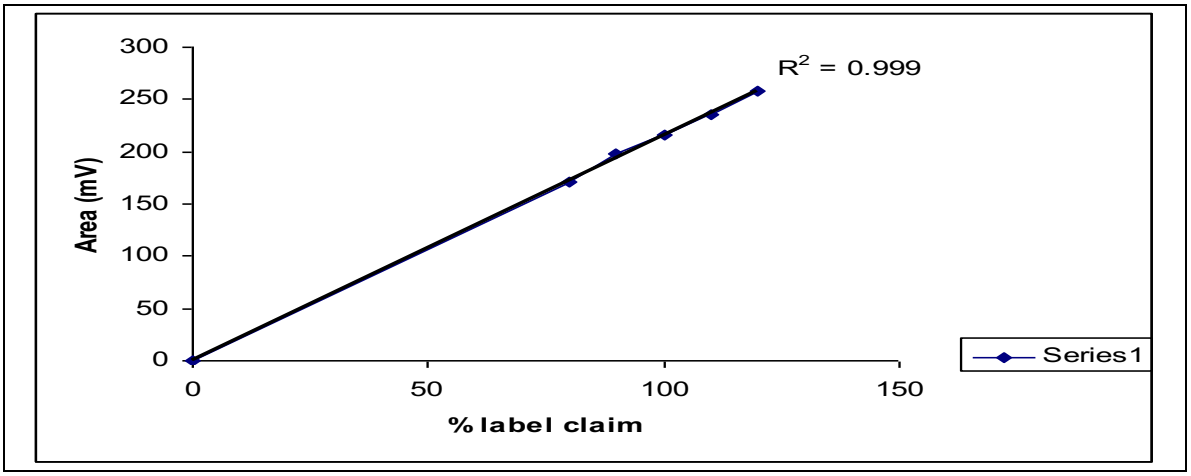
4) Linearity and range:

The amounts of pre-analyzed tablet powder needed to achieve 80, 90, 100, 110, and 120% of the PARA and LOR label claims were measured and diluted according to the instructions provided for the marketed formulation. Chromatograms were then recorded after injecting each solution. Both drugs were analysed by plotting their concentrations against the area under the curve. The PARA and LOR correlation coefficients were determined to be 0.999 and 0.999, respectively.

Table: Observations of Linearity and Range study

Sr.No.	% Label claim	A.U.C. (mV)	
		PARA	LOR
1	80	171.71	77.775
2	90	198.341	86.932
3	100	215.675	97.323
4	110	235.356	106.617
5	120	257.417	118.364
Coefficient of Correlation		0.999	0.999

Fig.: Study of Linearity and Range of PARA



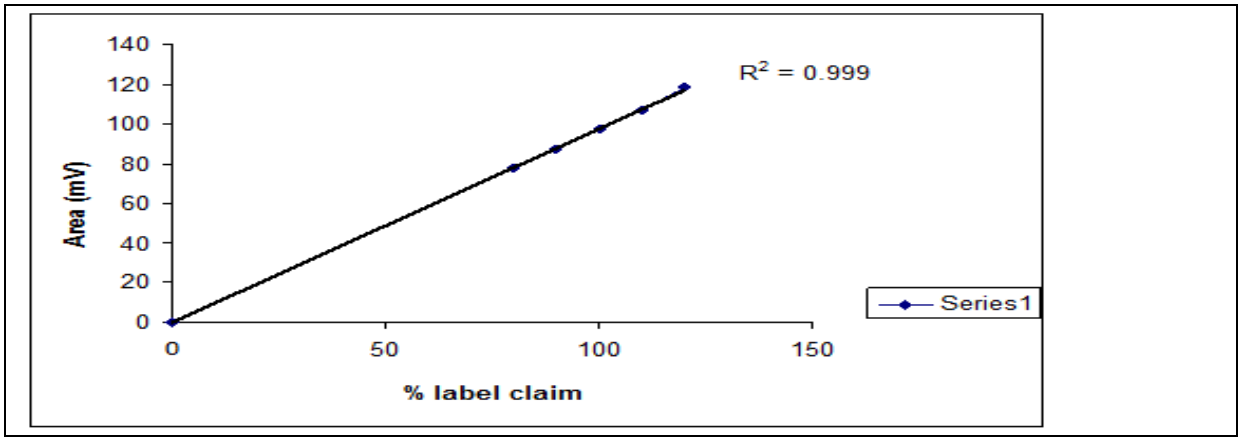


Fig.: Study of Linearity and Range of LOR

5) Ruggedness:

Different analyst:

The percentage of medicines labelled in tablet powder was calculated by three separate analysts using the recommended method. the study examined the variation amongst analysts.

Table: Results of estimation of drugs by Analyst to Analyst variation study

Analyst	Wt. of tablet powder taken (mg.)	A.U.C.(mV)		% Labeled Claim	
		PARA	LOR	PARA	LOR
I	884.6	217.78	97.25	101.78	101.30
II	884.5	218.11	96.74	101.94	100.78
III	884.3	215.89	96.90	100.93	100.97
			Mean	**100.93**	**100.78**
			±SD	**0.542**	**0.245**
			%RSD	**0.537**	**0.243**

Intraday and Interday variation:

The preanalyzed tablet powder, which contained 625 mg of PARA and approximately 10 mg of LOR, was measured and transferred to a 50.0 mL volumetric flask. The flask was then shaken for 10 minutes with an adequate amount of mobile phase. The volume was then filled up to the mark with mobile phase, and the mixture was filtered using Whatmann filter paper (no. 41). The filtrate was further diluted with mobile phase until it reached a volume of 25.0 mL, using 1.0 mL as an example.

Chromatograms were recorded after the stationary phase was equilibrated and sample solutions were injected independently at 0, 3, and 6 hours. On the first, third, and fifth days, the identical solutions were injected. To determine the PARA and LOR concentrations, we compared the sample's peak area to the standard's using the formula provided in the product's marketing materials.

Table: Results of estimation of drugs in Intraday study

Time	Wt. of tablet powder taken (mg)	A.U.C.(mV)		% Labeled Claim	
		PARA	LOR	PARA	LOR
0 hr.	884.6	218.58	97.32	102.16	101.37
3 hr.	884.6	218.49	97.26	102.11	101.31
6 hr.	884.6	216.68	96.77	101.27	100.80
			Mean	**101.27**	**100.80**
			±SD	**0.499**	**0.308**
			%RSD	**0.493**	**0.305**

Table: Results of estimation of drugs in Interday study

Day	Wt. of tablet powder taken (mg)	A.U.C.(mV)		% Labeled Claim	
		PARA	LOR	PARA	LOR
1	884.6	217.47	97.210	101.638	101.26
3	884.6	217.62	97.101	101.708	101.15
5	884.6	217.23	96.212	101.526	100.22

	Mean	101.55	100.22
	±SD	0.089	0.569
	%RSD	0.088	0.568

6) Robustness:

The optimised chromatographic parameters were subject to the following deliberate changes: 1) The detection wavelength was adjusted from 310.0 nm to 305.0 nm and 315.0 mn.

2) The initially set flow rate was reduced to 0.8 and 1.5 mL/min. Finally, the ammonium dihydrogen phosphate buffer's pH was adjusted from 7.3 to 7.2 and 7.4. Separate chromatograms were obtained by injecting equal quantities of sample solution into the stationary phase after it had been equilibrated. By comparing the peak areas of the sample and standard, the content of LOR and PARA were determined. Table 6.24 displays the outcoms.

Table : Result of Robustness study

Sr.No.	Deliberate changes	Wt. of tablet powder taken (mg)	A. U. C.		%Labeled Claim	
			PARA	LOR	PARA	LOR
1	Change in Wavelength (315 nm)	884.6	217.23	101.76	96.60	105.99
2	Change in Wavelength (305 nm)	884.6	215.67	95.63	110.85	99.61
3	Flow rate (0.8 mL/min)	884.6	217.74	96.32	101.76	100.33
4	Flow rate (1.2 mL/min)	884.6	216.61	96.11	101.24	100.11
5	pH 7.4	884.6	215.67	95.92	100.79	99.92
6	pH 7.2	884.6	217.23	96.22	101.52	100.22

Conclusion

The developed assay method was found to be simple, accurate, sensitive, précised, and rapid. This method can be applied for routine quantitative analysis of Paracetamol and Lornoxicam in pharmaceutical formulations.

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