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Research Article

Development and Validation of Stability Indicating Assay for Simultaneous Determination of Bupivacaine and Meloxicam in Bulk and Pharmaceutical Formulations by Using Rp-Hplc Method

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Abstract:A simple, rapid, precise, sensitive, and reproducible reverse phase high-performance liquid chromatography (RP-HPLC) method has been developed for the quantitative estimation of Bupivacaine and Meloxicam in pharmaceutical dosage form. Aim and objective of our study is to determine the amount of bupivacaine and meloxicam by using RP-HPLC using Waters alliance HPLC system, Quaternary gradient pump of e2695 series equipped with an auto sampler injector with 10µl is injected, and eluted with the mobile phase containing Acetonitrile and Water in the ratio of 60:40 v/v which is pumped at a flow rate of 1 ml/min and detected by UV detector at 225 nm. The peak of Bupivacaine and Meloxicam was eluted at retention times of 2.646 min and 3.136 min, respectively. Chromatographic separation of Bupivacaine and Meloxicam was achieved on Waters Alliance-e2695by using Chiralcel ODH 150x4.6mm, 5µcolumn and the mobile phase containing Acetonitrile and water in the ratio of 60:40% **v/v**. The flow rate was 1.0 ml/min; detection was carried out by absorption at 225 nm using a photodiode array detector at ambient temperature. The number of theoretical plates and tailing factor for Bupivacaine and Meloxicamwere NLT 2000 and kept not more than two respectively. Peak areas, Percentage relative standard deviation of all measurements always less than 2.0**.** This method was validated according to ICH guidelines. The method was a simple, economical, suitable, precise, accurate & robust method for quantitative analysis of Bupivacaine and Meloxicam study of its stability.

Key words: HPLC,Bupivacaine And Meloxicam , Method Development, Validation, Stability Studies,

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1. INTRODUCTION

Analytical chemistry is a branch of chemistry in which compounds and mixtures are identified (qualitative analysis), or proportions of constituents are determined (quantitative analysis). Commonly used techniques are precipitation, titration, chromatography, spectroscopy etc. High Performance Liquid Chromatography (HPLC) is a widely used technique because of its several advantages like accuracy, precision, rapidity, specificity in case of automation, and elimination of tedious extraction and isolation procedures[1]. HPLC method is a well-known technique for its analysis speed, greater sensitivity, improved resolution, ideal for substances of low volatility, and excellent reproducibility. HPLC detectors consist of high sensitivity, higher linear dynamic range, application to most of the solutes, no band broadening contribution, non-destructive, faster response[2]. The present aim of the study was to validate stability indicating RP- HPLC method for simultaneous estimation of Bupivacaine & Meloxicam in bulk and pharmaceutical formulations.

1.1 Normal Phase High Performance Liquid Chromatography (NP-HPLC)

Normal-phase liquid-liquid chromatography uses a polar stationary phase and less polar mobile phase.[1-3]. Inorder to select an optimum mobile phase, it is best to start with a pure hydrocarbon mobile phase such as heptane. If the sample is strongly retained, the polarity of the mobile phase should be increased, perhaps by adding small amounts of methanol or dioxin. In the normal phase mode, separations of oil-soluble vitamins, essential oils, nitro phenols, or more polar homologous series have been performed using alcohol/heptane as the mobile phase. Column used in normal phase chromatography for chiral separation: Chiracel OJ and Chiracel OD[4].

1.2 Reverse Phase High Performance Liquid Chromatography (RP-HPLC)

Reverse phase chromatography uses hydrophobic bonded packing, usually with an octadecyl or octyl functional group and a polar mobile phase, often a partially or fully aqueous mobile phase [5]. Polar substances prefer the mobile phase and eluted first. As the hydrophobic character of the solutes increases, retention increases. Generally, the lower the mobile phase's polarity, the higher its eluent strength is. As a result, the elution order of the class of compounds in the table is reversed (thus reverse-phase chromatography).

1.3 The basic principle of HPLC

High performance liquid chromatography (HPLC) is a separation technique utilizing differences in the distribution of compounds to two phases; called stationary phase and mobile phase. The stationary phase designates a thin layer on the surface of fine particles, and the mobile phase establishes the liquid flowing over the particles. Under certain dynamic conditions, each sample component has a different distribution equilibrium depending on solubility in the phases and molecular size[10]. As a result, the components move at different speeds over the stationary phase and are separated. The column is a stainless steel (or resin) tube packed with spherical solid particles[11]. Mobile phase is constantly fed into the column inlet at a constant rate by a liquid pump. A sample is injected from a sample injector located near the column inlet. The injected sample enters the column with the mobile phase, and the components in the sample migrate through it, passing between the stationary and mobile phases. Compound move in the column only when it is in the mobile phase.Compounds that tend to be distributed in the mobile phase therefore migrate faster through the column while compounds that tend to be distributed in the stationary phase migrate slower. In this way, each component is separated on the column and sequentially elutes from the outlet. A detector connected to the outlet of the column detects each compound eluting from the column. basic principle of HPLC. High-performance liquid chromatography (HPLC) is a separation technique utilizing differences in the distribution of compounds to two phases; called stationary phase and mobile phase. Compound move in the column only when it is in the mobile phase. Compounds that tend to be distributed in the mobile phase, therefore, migrate faster through the column while compounds that tend to be distributed in the stationary phase migrate slower. In this way, each component is separated on the column and sequentially elutes from the outlet. A detector connected to the outlet of the column detects each compound eluting from the column.A simple, rapid, precise, sensitive and reproducible reverse phase high performance liquid chromatography (RP-HPLC) method has been developed for quantitatively analysis of Bupivacaine and Meloxicam in pharmaceutical dosage form.

1.4 Bupivacaine

Bupivacaine is a local anesthetic that is given as an epidural injection into the spinal column to produce numbness during surgery, and labor and also used in the dental procedures.[10]

1.5 Structure

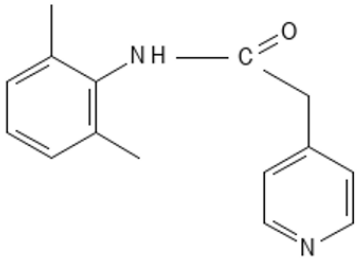


Fig no. 1 Chemical structure of Bupivacaine

1.6 Chemical formula

C18H28N2O.

1.7 Molecular weight

288.435g/mol

1.8 IUPAC Name

(RS)-1-butyl-N-(2,6-dimethyl phenyl)-piperidine-2-carboxamide

1.9 Meloxicam

Meloxicam is used as non-steroidal anti-inflammatory drug which relieves the symptoms of arthritis (rheumatoid arthritis, juvenile rheumatoid arthritis,osteoarthritis) like swelling, joint pain, inflammation, stiffness[23].

1.10 Structure:

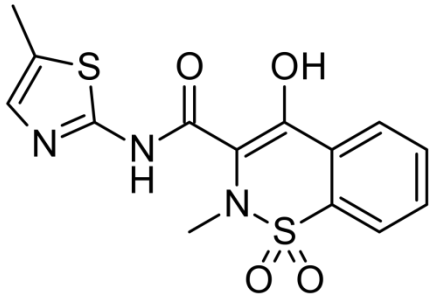


Fig no. 2 Molecular structure of meloxicam

1.11 Chemical formula

C14H13N3O4S2

1.12 Molecular weight

351.40g/mol

1.13 IUPACName

4-hydroxy-2-methyl-N-(5-methyl-2-thiazolyl-2H-1,2-benzothiazine-3-carboxamide-1,1-dioxide

2. MATERIALS AND METHODS

Materials and instruments: Equipment used are HPLC, pH meter, weighing balance, UV/Visible spectrophotometer[14], ultrasonicator, isocratic pump. Chemicals used are acetonitrile and water (milli Q) of HPLC grade. The detector used is photodiode array detector.

2.1 General procedure

2.1.1 Preparation of Mobile Phase

Mobile phase was prepared by mixing Acetonitrile and water taken in the ratio 60:40 and was filtered to remove impurities through 0.45µ filter membrane which may interfere in the final chromatogram[5].

2.2 Preparation of Diluent

Mobile Phase used as a diluent.

2.3 Preparation of standard stock solution

Accurately weighed and transferred 330 mg of Bupivacaine, 10 mg of Meloxicam working standard into a 100 ml clean dry volumetric flask having diluent and sonicated to dissolve completely and by same solvent (stock solution) the volume is made upto the mark. Centrifuged (14000 rpm) for 30 min, made upto the mark with same solvent (stock solution). Pipetted out of about 5ml of the above-prepared solution into a 50 ml volumetric flask and made up to the mark with diluents[6]. (330ppm of Bupivacaine, 10ppm of Meloxicam).

2.4 Sample Solution Preparation

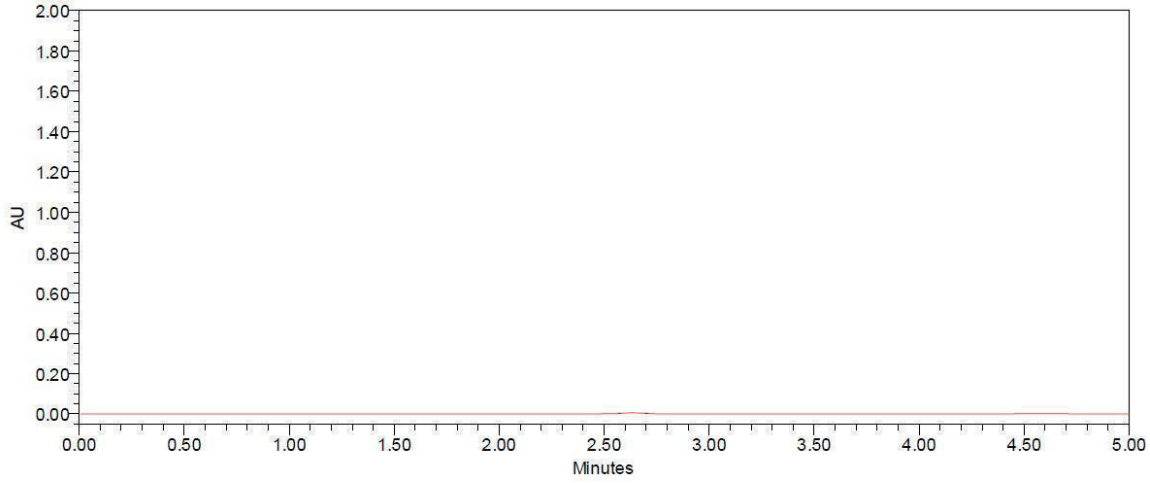
Sample (1.13mg) is transferred into a 10mL clean, dry volumetric flask having diluent , sonicated up to 30 mins to dissolve. Centrifuged (14000 rpm) for 30 min, made upto the mark with the same solvent (stock solution). Further pipetted 1 ml of the above stock solution into a 10ml volumetric flask and diluted up to the mark with diluent. (330ppm of Bupivacaine, 10ppm of Meloxicam).

3. RESULTS AND DISCUSSION

Six trials were conducted in the estimation of two drugs by using different solvent systems, columns, and experimental conditions[24]. The optimized chromatographic conditions used in the solvent system were acetonitrile: water (60:40) and other conditions are represented in Table 1. Chromatograms of blank and placebo were shown in Fig no. 3 and 4.

Table 1. Optimized Chromatographic conditions		
High Performance Liquid Chromatographic equipped with PDA detector.		
Column	:	X-bridge phenyl 250x4.6mm, 5µ
Mobile phase ratio	:	Acetonitrile : Water (60:40)
Detection wavelength	:	225nm
Flow rate	:	1ml/min
Injection volume	:	10µl
Run time	:	5 min

The optimized conditions are HPLCx-bridge phenyl 250x4.6mm , the mobile phase is acetonitrile and water, detector wavelength 225 nm, flow rate is 1ml/min, injection volume is10µl, run time is 5minutes[14].In thisHPLC ,the column used was x- bridge phenyl 250x4.6mm, 5µ and solvents used are acetonitrile: water , the detecting wavelength used was 225nm,, the injection volume was 10µl and the runtime was 5minutes , the temperature is ambient, mode of separation is isocratic detector used is PDA. By adopting the above conditions, sharp peaks were observed.



There were no peaks in the blank, and it is the chromatogram without any impurities peaks.

Fig no. 3 Chromatogram of blank

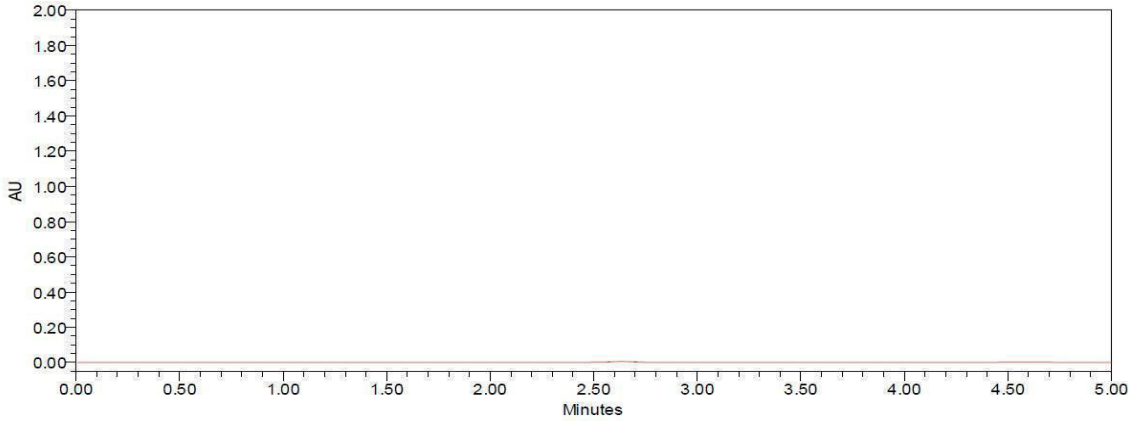


Fig 4 represents the chromatogram of placebo (without the sample), showing no other peaks

Fig no. 4 Chromatogram of placebo

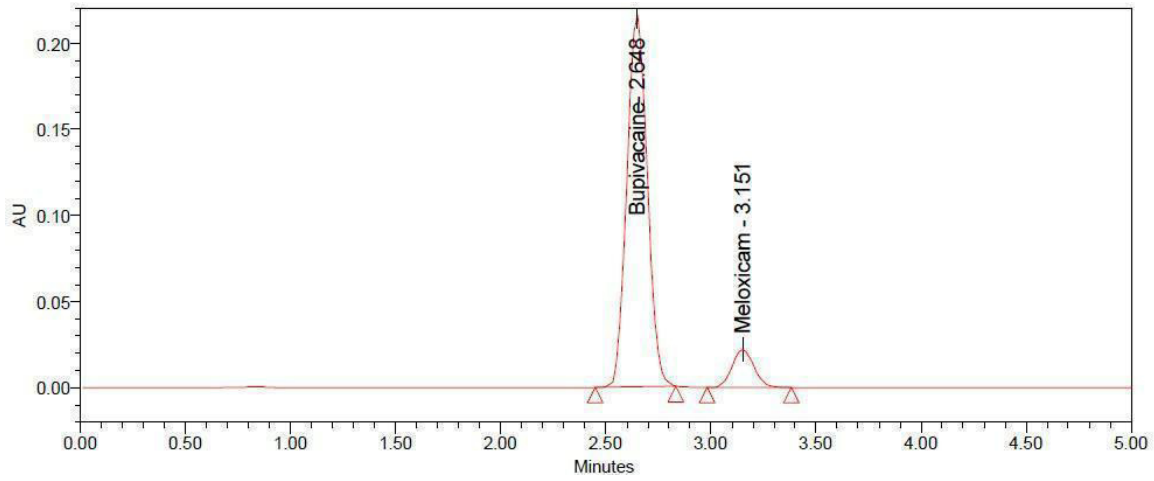


Fig no. 5 Optimized Chromatogram

The Bupivacaine peak was observed at 2.648 min with peak area 2460981, tailing factor 1.10, Meloxicam peak was observed at 3.151 min, with peak area 157486, tailing factor 1.07 and resolution 2.54. This method was optimized. The representative chromatograms of optimized process or trail were depicted in Fig no. 5.

3.1 Validation

3.1.1 System suitability

The system suitability parameters were determined by preparing standard solutions of bupivacaine and meloxicam where, six times, the solutions were injected. Determining parameters like plate count, peak tailing, and resolution were done[20]. For the area of 6 standard injections the %RSD is less than 2. Plate count of bupivacaine was 3312 and meloxicam was 3997. Tailing factor of bupivacaine was 1.10 and meloxicam was 1.07. The resolution of the meloxicam was 2.54.Plate count should be more than 2000, tailing factor should be less than 2 and resolution must be more than 2 according to ICH guidelines[21]. Therefore, all the system suitability parameters were within the limits and were passed. (Table 2 andFig no. 6).

Table 2. System suitability parameters for Bupivacaine and Meloxicam:

S No.	Parameter	Bupivacaine	Meloxicam
1.	Retention time	2.648	3.151
2.	Plate count	3312	3997
3.	Tailing factor	1.10	1.07
4.	Resolution	-----	2.54
5.	%RSD	0.95	1.12

The system suitability parameters are retention time, plate count, tailing factor, resolution, %RSD. The resolution is 2.54 , tailing factor is 1.10,1.07, plate count is 3312,3997 tailing factor is 1.10, 1.07,%RSD 0.95,1.12 these values are within the standard ICH limits.

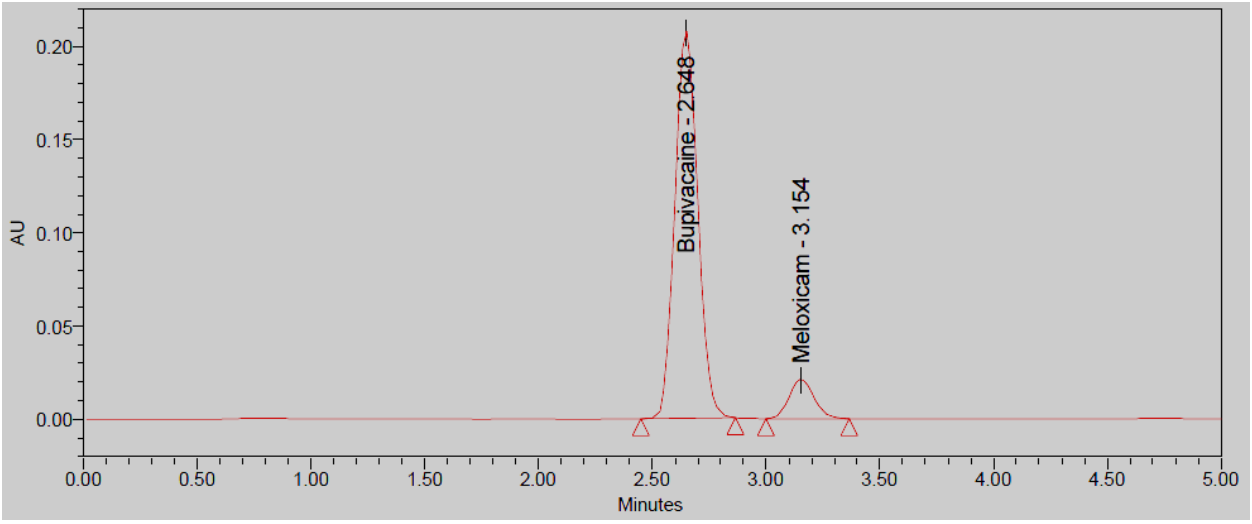


Fig no. 6 Chromatogram of standard which are having the retention times as per the ICH guidelines.

3.2 Linearity

3.2.1 Preparation of stock solution

Accurately weighed and transferred 330 mg of Bupivacaine, 10 mg of Meloxicam working standard into a 100 ml clean dry volumetric flask, with diluent and sonicated to dissolve completely. With the help of same diluent made up the volume [22].

3.3 Preparation of Level – I

About 0.25ml of stoke solution was pipetted out to 10ml of volumetric flasks and diluted up to the mark with diluent.

3.4 Preparation of Level – II

About 0.5ml of stock solution was pipetted into 10ml of volumetric flask and diluted upto the mark with diluent.

3.5 Preparation of Level – III

About 0.75ml of stock solution was pipetted out into 10ml of volumetric flask and diluted upto the mark with diluent.

3.6 Preparation of Level – IV

About 1ml of stock solution was taken in 10ml of volumetric flask and diluted upto the mark with diluent

3.7 Preparation of Level – V

About 1.25ml of stock solution was pippeted in to 10ml of volumetric flask and diluted upto the mark with diluent.

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3.8 Preparation of Level – VI

About 0.75ml of stock solution was pipeted in to 10ml of volumetric flask and diluted upto the mark with the diluant

.Table 3. Linearity for Bupivacaine and Meloxicam:

S no.	Bupivacaine		Meloxicam	
	Conc(µg/ml)	Peak area	Conc(µg/ml)	Peak area
1.	82.50	615842	2.50	35695
2.	165.00	1265845	5.00	72658
3.	247.50	1852631	7.50	110524
4.	330.00	2485714	10.00	153628
5.	412.50	3069587	12.50	182564
6.	495.00	3696956	15.00	216532
Regression equation	y=7453.78x+10414.96		y=14632.91x+481.86	
Slope	7453.78		14632.91	
Intercept	10414.96		481.86	
R^{22}	0.9999		0.9991	

Six linear concentrations of bupivacaine (82- 495µg/ml) and meloxicam (2- 15µg/ml) were injected as shown in Table 3[25]. The average of areas were mentioned above, and linearity equations obtained for Bupivacaine was y=7453.78x+10414.96 (Fig no.7) and Meloxicam was y=14632.91x+481.86 (Fig no. 8). The correlation coefficient of bupivacaine was 0.9999 and meloxicam was 0.9991.

Precision

Six injections were given from a single volumetric flask of working standard solution and areas obtained were presented in Table 4 and Fig no. 9. Average area, standard deviation and % RSD were calculated for Bupivacaine and Meloxicam. % RSD obtained was 0.95 for Bupivacaine and 1.12 for Meloxicam. Precision was observed in this method as the precision limit was less than 2.

Table 4. Precision results:

S no.	Area of Bupivacaine	Area of Meloxicam
1.	2464274	154265
2.	2424315	152417
3.	2448752	152634
4.	2462348	157462
5.	2420421	154524
6.	2436528	155280
Average	2442773	154430.3
Standard deviation	18764.497	1856.307
%RSD	0.77	1.2

Taking the six dilutions plotting the graph area under the curve was calculated and the graph is a straight line passing through origin. Average deviation, percent RSD are within the ICH Guidelines

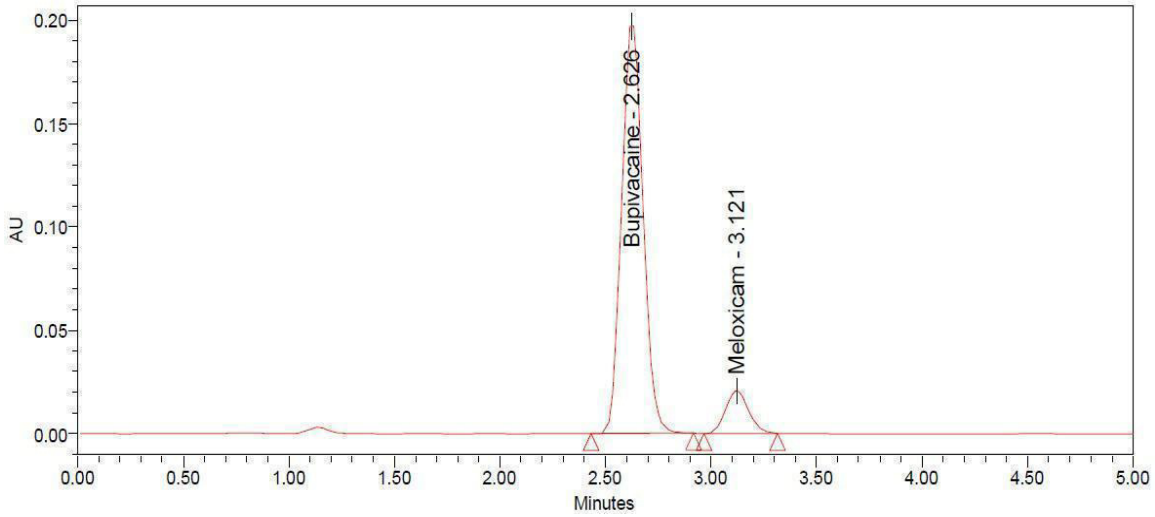

Fig no. 7 Precision chromatogram

3.9 Accuracy

3.9.1 Solution preparation (50%) (With respect to target Assay concentration)

About 1.13 of the sample was transferred into 10mL clean, dry volumetric flask, having diluent, and sonicated to 30 mins, for complete dissolution and with the help of the same solvent (stock solution) to make the volume upto the mark and centrifuged (14000 rpm) for 30min. Then, about 0.5 ml of the above stock solution was pippeted into a 10ml volumetric flask and diluted up to the mark with diluent. (165ppm of Bupivacaine, 5ppm of Meloxicam).

3.9.2 Solution preparation (100%) (With respect to target Assay concentration)

About 1.13 of sample was transferred into a 10mL clean dry volumetric flask, having diluent and sonicated to 30 mins to dissolve. and, centrifuged for 30 min(14000 rpm) and made to the volume same solvent (stock solution) Further pipetted 1 ml of the above stock solution into a 10ml volumetric flask and diluted up to the mark with diluent. (330ppm of Bupivacaine, 10ppm of Meloxicam)

3.9.3 Solution preparation (150%)(With respect to target Assay concentration)

Transferred about 1.13 of sample into a 10mL volumetric flask, diluent and sonicated to 30 mins to dissolve centrifuged for 30 min and made the volume upto the mark with same solvent (stock solution). Then, into a 10ml volumetric flask , pipetted of about 1.5ml of above stock solution and with the help of diluent diluted upto the mark. (495ppm of Bupivacaine, 15ppm of Meloxicam).For each level the % Recovery the limit was between 98.0 to 102.0%.

Table 5. Accuracy of Bupivacaine:

% of Concentration (at specification level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	1206544	165	164.91	98.9	99.3
100%	2451203	330	330.14	100.1	
150%	3635812	495	494.89	99.2	

Taking the concentration of solutions 50%,100% and 150%and the recovery is 98.1, 100.1, 99.2%.

Table 6. Accuracy of Meloxicam:

% of Concentration (at specification level)	Area	Amount added (mg)	Amount found (mg)	%Recovery	Mean Recovery
50%	74851	5	4.491	99.4	99.8
100%	154526	10	9.95	99.8	
150%	234613	15	15.24	100.3	

Taking the concentrations of 50%, 100%, 150% and the recovery is 99.4%, 99.8%, 100% and the mean recovery is 99.8%

By standard addition method, three levels of accuracy samples were prepared. Then, Triplicate injections were given for each level of accuracy and mean %Recovery was obtained as 99.3% for Bupivacaine and for Meloxicam 99.8% respectively which are shown in Table 5 and 6.

3.10 Robustness

3.10.1 Variation of flow rate at 0.8 ml/min to 1.2ml/min.

Standard solution 330 ppm of Bupivacaine, 10ppm of Meloxicam was prepared and analyzed using the varied flow

rates and method flow rates. Comparing the above results, it can be concluded that the flow rate variation affected the method significantly. Hence it indicates that the method is robust even by the change in the flow rate ±20%.

3.10.2 *The variation of Organic Phase ratio.*

Standard solution of 330ppm of Bupivacaine, 10ppm of Meloxicam was prepared and analyzed using the varied in mobile phase ratio[30].

Table 7. Robustness of Bupivacaine:

Parameter	Bupivacaine				
	Condition	Retention time(min)	Peak area	Tailing	Plate count
Flow rate Change (mL/min)	Less flow (0.8ml)	3.030	2357648	1.11	3868
	More flow (1.2ml)	2.330	2646605	1.05	2640
Organic Phase change	Less org (54:46)	3.453	2076683	1.13	3639
	More org (66:34)	2.119	2754810	1.08	2223

Table 8. Robustness of Meloxicam:

Parameter	Meloxicam					
	Condition	Retention time (min)	Peak area	Resolution	Tailing	Plate count
Flow rate change (mL/min)	Less flow (0.8ml)	3.602	142825	2.85	1.06	4984
	More flow (1.2ml)	2.765	172923	2.34	1.05	3547
Organic phase change	Less org (54:46)	4.042	125642	2.80	1.08	4895
	More org (66:34)	2.531	185817	2.28	1.03	3674

Robustness of the meloxicam and bupivacaine was estimated and heywere within the ICH Guidelines.

Robustness conditions like flow minus (0.8ml/min), flow plus (1.2ml/min), organic phase minus (54:46), organic phase plus (66:34) and samples were injected. Thus, all the parameters were passed.

3.11 Sensitivity

3.11.1 LOD and LOQ

As per international conference harmonization (ICH) guidelines, the limit of detection (LOD) and limit of quantification (LOQ) of the drug was calculated by using the following equations[31]

$LOD = 3.3 \times \sigma / S$
$LOQ = 10 \times \sigma / S$

LOD for Bupivacaine was found to be 9.9µg/mL and LOQ for Bupivacaine, was found to be 3.3µg/ml, LOD for Meloxicam was found to be 0.3µg/ml and LOQ for Meloxicam was found to be 1.0µg/ml shown in Table 9 and Fig no. 9, 10.

Table 9. Sensitivity parameters (LOD, LOQ):

Name of the drug	LOD(µg/ml)	LOQ(µg/ml)
Bupivacaine	9.9	3.3
Meloxicam	0.3	1

LOD and LOQ values are within the ICHguidelines

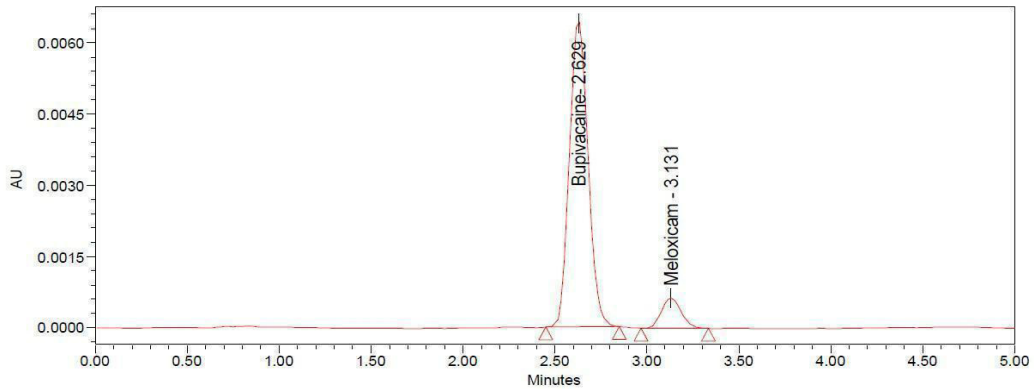

Fig no. 8 Chromatogram for LOD

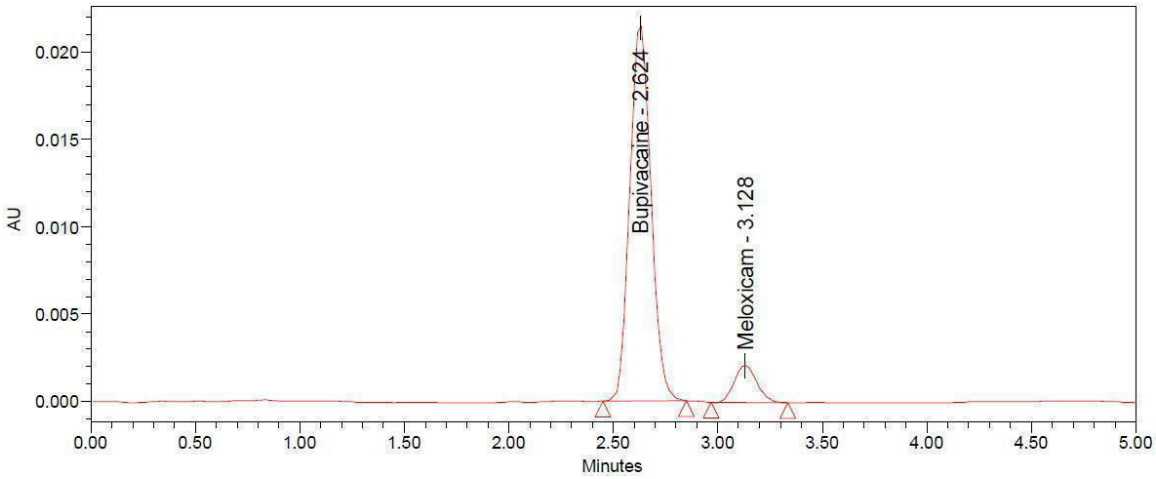

Fig no. 9 Chromatogram for LOQ

3.12 Assay

Percentage Assay for Bupivacaine and Meloxicam was found to be 100.2 and 99.64 (Table 10 and Fig no**. 11.**%) Assay was calculated by the following formula:

3.13 Formula for Assay

$$\%\ Assay = \frac{AT}{AS} * \frac{WS}{DS} * \frac{DT}{WT} * \frac{AW}{LC} * \frac{P}{100} * 100$$

Where: AT = average area counts of test (sample) preparation.
AS = average area counts of standard preparation.
WS = Weight of working standard taken in mg.
DS = Dilution of working standard in ml.
DT = Dilution of test (sample) in ml.
WT = Weight of test (sample) taken in mg.
P = Percentage purity of working standard
LC = Label Claim mg/ml.
AW= Average Weight

Table 10. Assay of Bupivacaine and Meloxicam:

Brand	Drug	Avg sample area (n=2)	Std. wt	Sample in ml	Label amount (mg)	Std. purity	Amount found (µg/ml)	%Assay
-------	Bupivacaine	2451874	330	1.13	29.25	99.9	330.07	100.2
	Meloxicam	153264	10		0.88	99.9	9.81	99.64

Assay of meloxicam and bupivacaine was estimated and the % of assay is 100.2 and 99.64

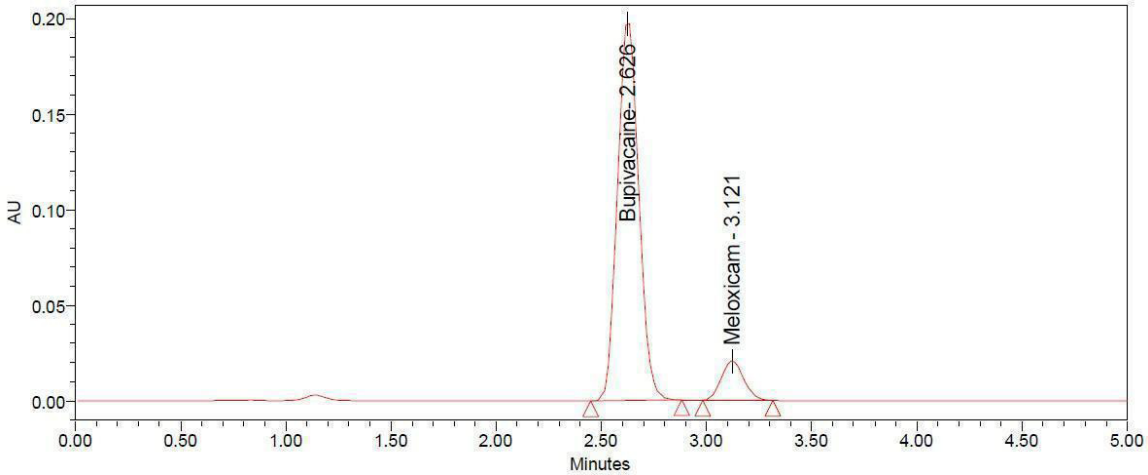

Fig no. 10Chromatogram of assay

Table 10A results of linearity for Bupivacaine and Meloxicam				
S.No.	Bupivacaine		Meloxicam	
	Conc.(µg/ml)	Peak area	Conc.(µg/ml)	Peak area
1	82.50	615842	2.50	35695
2	165.00	1265845	5.00	72658
3	247.50	1852631	7.50	110524
4	330.00	2485714	10.00	153628
5	412.50	3069587	12.50	182564
6	495.00	3696956	15.00	216532
Regression equation	y = 7453.78 x+10414.96		y = 14632.91 x+481.86	
Slope	7453.78		14632.91	
Intercept	10414.96		481.86	
R^2	0.9999		0.9991	

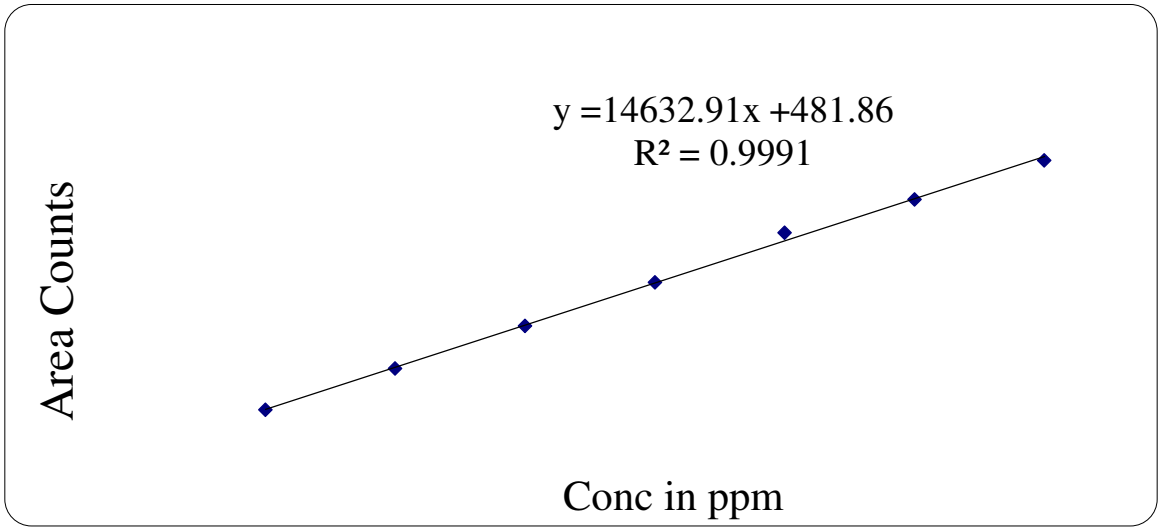

Fig no 11 Calibration curve for Meloxicam at 225 nm

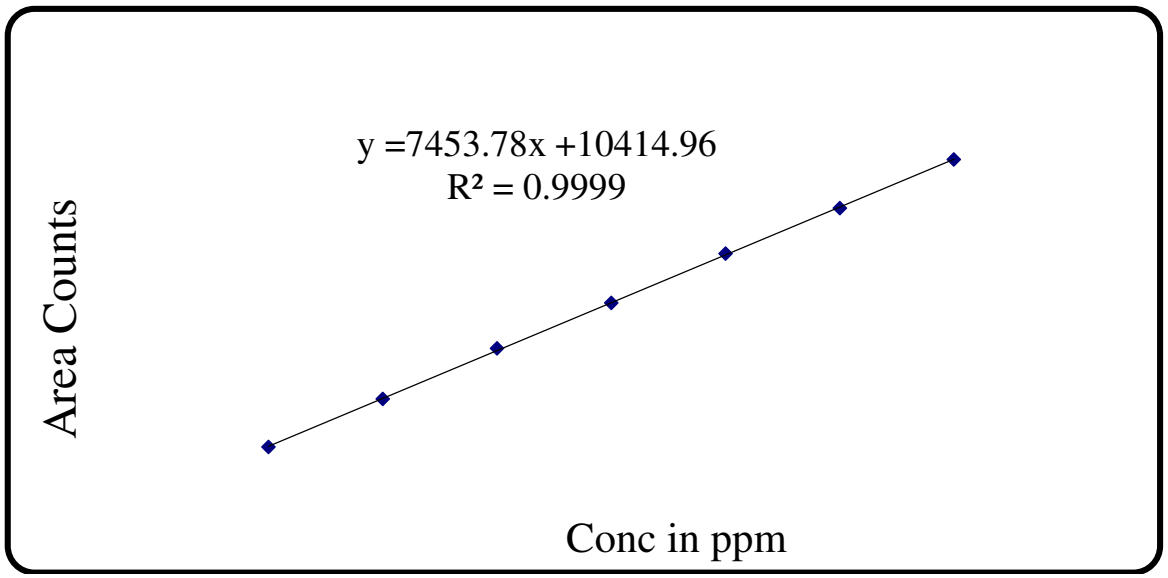

Fig no 12Calibration curve for Bupivacaine at 225 nm

Table 10 B System suitability parameters for Bupivacaine and Meloxicam			
S.No	Parameter	Bupivacaine	Meloxicam
1	Retention time	2.648	3.151
2	Plate count	3312	3997
3	Tailing factor	1.10	1.07
4	Resolution	----	2.54
5	%RSD	0.95	1.12

3.14 Degradation studies

3.14.1 Preparation of stock:

About 1.13 of sample was transferred into a 10mL volumetric flask, having diluent , sonicated to 30 mins to dissolve the contents , centrifuged for 30 mins and with the help of same solvent (stock solution) and was made to the volume upto the mark.

3.14.2 Acid degradation

About 1 ml of the above stock solution was pipetted into a 10ml volumetric flask, and 3 ml of 1N HCl was added and kept at 60°C for 6 hours, followed by neutralization with 1 N NaOH. Then made up to 10ml with diluent. With the help of 0.45micron syringe filters, the solution was filtered and transferred to vials[32].

3.14.3 Alkali degradation

About 1 ml of the above stock solution was pipetted into a 10ml volumetric flask, and 3ml of 1N NaOH was added and kept at 60°C for 6 hours followed by neutralization with 1N HCl. Made up to 10ml with diluent. Then, with the help of 0.45micron syringe filters, the solution was filtered and transferred to vials.

3.14.4 Peroxide degradation

About 1 ml above stock solution was pipetted into a 10ml volumetric flask having 1 ml of 30% w/v of hydrogen peroxide and made up to the mark with diluent. At room temperature ,the volumetric flask was kept for 15min. With the help of 0.45micron syringe filters, solution was filtered and transferred to vials.

3.14.5 Reduction degradation

About 1ml of Stock solution was transferred into a 10ml volumetric flask having 1ml of 30% Sodium Bisulphate and made up to the mark with diluent. At room temperature, the volumetric flask was kept for 15min and with the help of 0.45 micron syringe filters, the solution was filtered and transferred to vials[18].

3.14.6 Hydrolysis degradation

About 1ml of the above stock solution was transferred to 10ml volumetric flask, having 1 ml of water and made up to the mark with diluent. With the help of 0.45micron syringe filters, the solution was filtered and transferred to vials.The obtained results were shown in Table 11 and Fig 12,13,14,15,16,17.

Table 11. Forced degradation results:				
Degradation condition	Bupivacaine		Meloxicam	
	Area	%Degradation	Area	%Degradation
Control	2450599	0	155097	0.1
Acid degradation	2151773	12.2	135446	12.7
Alkali degradation	2174356	11.3	137645	11.3
Peroxide degradation	2125287	13.3	132808	14.4
Reduction degradation	2226620	9.2	142413	8.2
Hydrolysis degradation	2236057	8.8	151880	2.1

The degradation studies are performed and the %degradation is within the ICH guidelines

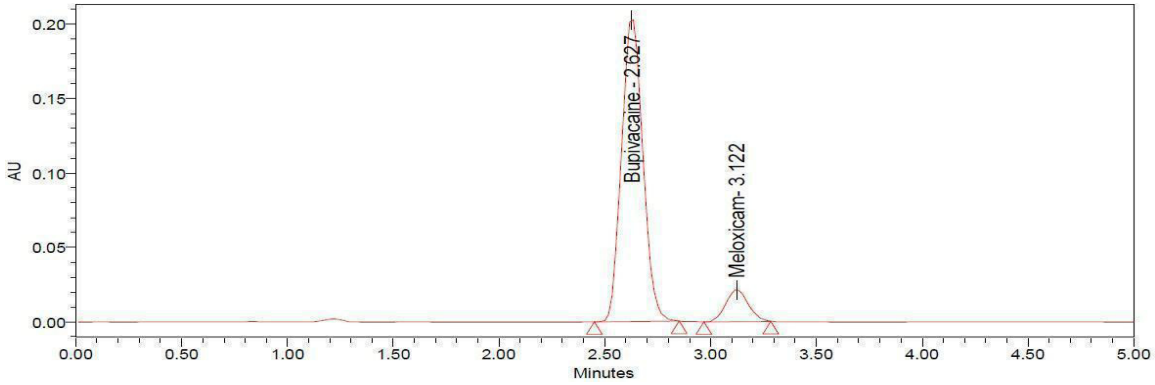

Fig no. 13. Chromatogram of control

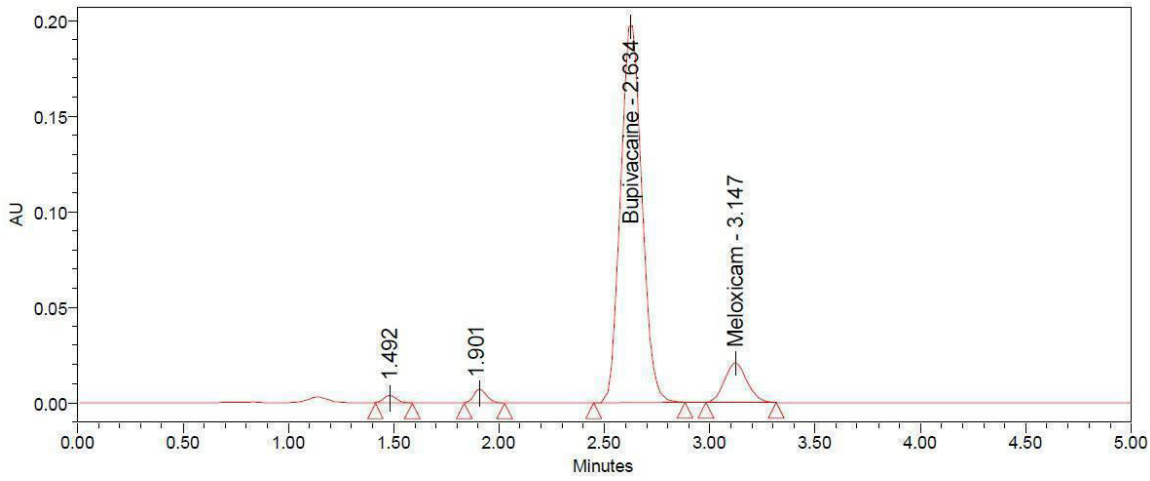

Fig no. 14. Chromatogram of acid degradation

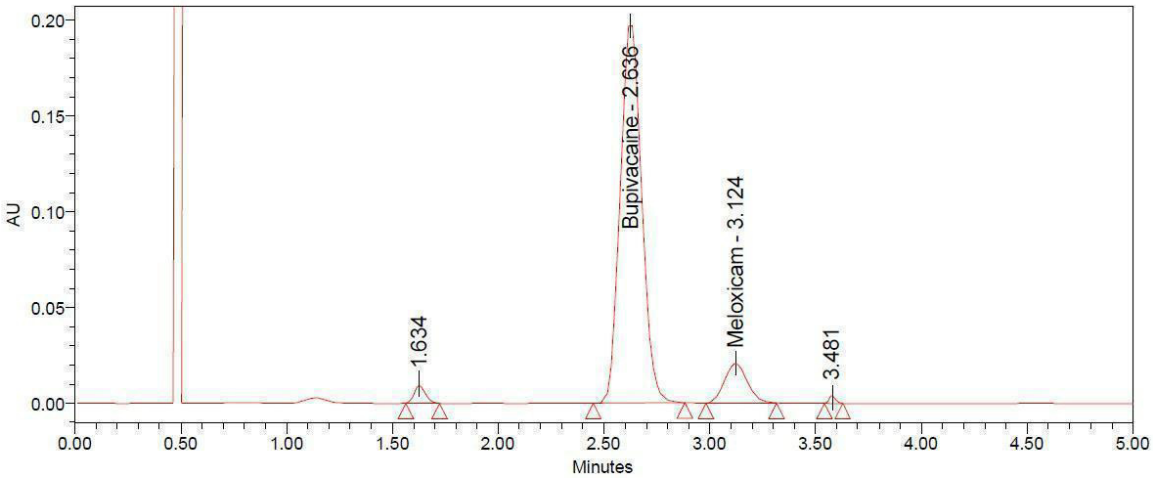

Fig no. 15. Chromatogram of peroxide degradation

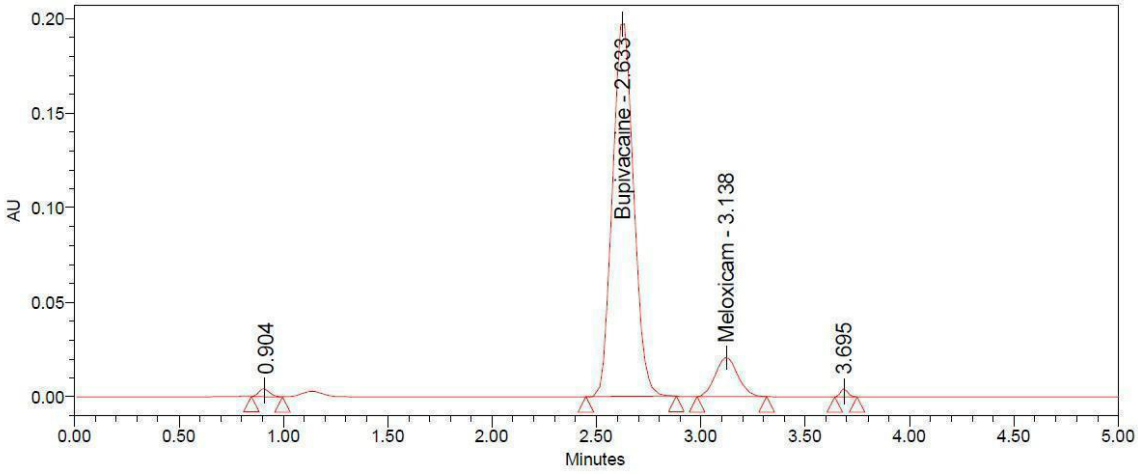

Fig no. 16. Chromatogram of reduction degradation

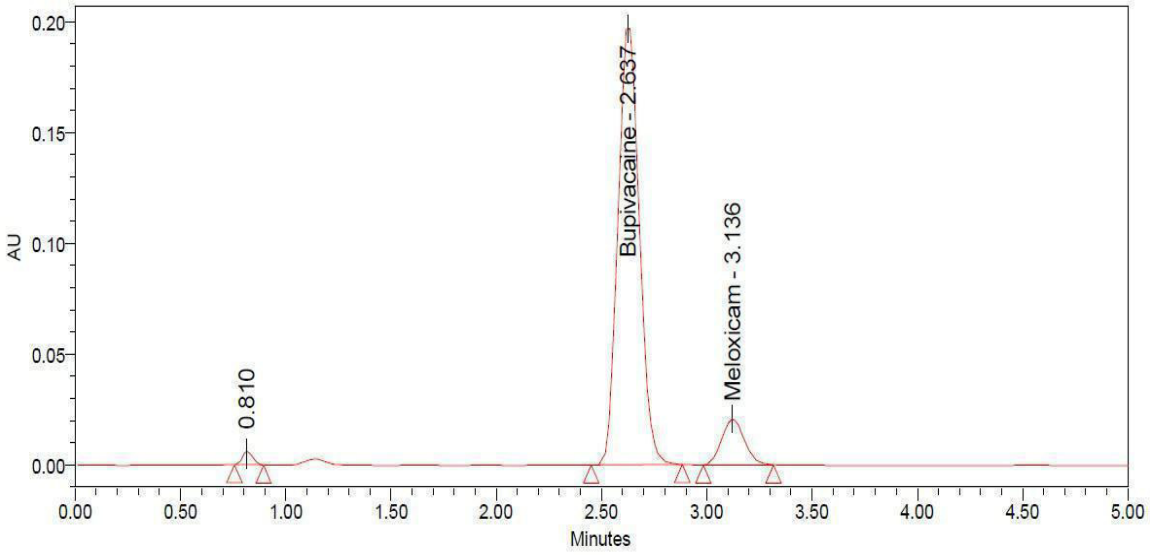

Fig no. 17. Chromatogram of hydrolysis degradation

3.15 Stability

The solution stability of Bupivacaine and Meloxicam, in diluents was determined by storing the sample solution in a tightly capped volumetric flask at room temperature and 2-8°C for 24hrs[19]. The amount of Bupivacaine and Meloxicam was measured at different time intervals like 6,12,18 and 24 hrs and results were compared with freshly prepared Bupivacaine and Meloxicam solution. The results were shown in Table 12,13

Table 12. Results of stability RT:

S no.	Stability	Bupivacaine		Meloxicam	
		Area	%Deviation	Area	%Deviation
1.	Initial	2455784	0.00	154448	0.00
2.	6hrs RT	2436512	-0.80	153257	-0.80
3.	12hrs RT	2425174	-1.20	152974	-0.90
4.	18hrs RT	2403152	-2.10	150219	-2.70
5.	24hrs RT	2388564	-2.70	149956	-2.90

Degradation studies were carried out 6 hours,12 hours,18 hours 24hrs and the 5 Deviation is within the ICHguidelines.

Table 13. Results of stability 2-8°C:

S no.	Stability	Bupivacaine		Meloxicam	
		Area	%Deviation	Area	%Deviation
1.	Initial	2455784	0.00	154448	0.00
2.	6hrs 2-8^{0}°C	2445102	-0.40	152514	-1.20
3.	12hrs 2-8^{0}°C	2412574	-1.70	151748	-1.70
4.	18hrs 2-8^{0}°C	2406357	-2.00	150074	-2.80
5.	24hrs 2-8^{0}°C	2387541	-2.80	149025	-3.50

Using Waters alliance HPLC system, Quaternary gradient pump of e2695 series equipped with an autosampler injector with 10µl is injected eluted with the mobile phase containing Acetonitrile and Water in the ratio of 60:40 v/v which is pumped at a flow rate of 1ml/min and detected by UV detector at 225 nm. The peak of Bupivacaine and Meloxicam was eluted at retention times of 2.646 min and 3.136 min respectively. In this proposed HPLC method for the selected drugs , it showed good linearity. Results for the recoveries of selected drugs were found to be within limits (98 – 102 %). These imply that the present method was accurate for analysis.For the estimation of selected drugs, the developed HPLC method is simple, rapid, accurate, precise, robust and economical. Preparation of mobile phase and solvents are simple, reliable, economical, sensitive and less time consuming. The sample recoveries were in good agreement with their respective label claims and they suggested non interference of formulation recipients in the estimation and can be used in laboratories for the routine analysis of selected drugs.Since the system validation parameters of HPLC method used for estimation of selected drugs are pure and have shown satisfactory, accurate and reproducible results (without any interference of recipients) as well, it is deduced that the simple and short proposed methods are most useful for analysis purposes.

4. CONCLUSION

The present work stability-indicating assay by RP-HPLC method was simple, accurate, precise, and specific and has no interference with the placebo and degradation products. Hence this study can be used for routine analysis of Bupivacaine and Meloxicam. This method was accurate, simple, and precise and was used for routine analysis. The retention time, accuracy, precision, and assay are within the limits of ICH guidelines.

5. ACKNOWLEDGMENT

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6. AUTHOR CONTRIBUTION STATEMENT

Stefi seles have done the research , collected the data and written the manuscript. Dr.S.Padmavathi, did the analysis of the data and guided the manuscript writing according to the journal guidelines. The remaining authors equally contributed their work in performing the data editing and manuscript preparation

7. CONFLICT OF INTEREST

Conflict of interest declared none.

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