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Research Article

A STABILITY INDICATING HPLC METHOD FOR THE DETERMINATION OF MEPIVACAINE HYDROCHLORIDE

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ABSTRACT

Stability indicating HPLC method has been developed for specific determination of Mepivacaine hydrochloride during degradation under acid, alkali, thermal and oxidative stress conditions. Methanol: Orthophosphoric acid solution (2.25 gm/L) adjusted to pH 7.6 with strong sodium hydroxide solution (75:25), was used as the mobile phase, at a flow rate of 1 ml/min. A Gracesmart C_{18} column (5µ), 250 mm, 4.6 mm i.d. was used as stationary phase. Detection was affected spectrophotometrically at 215 nm. The retention time was approximately 4.7 min. Linearity range was 20-100 µg/ml. The minimum detection and Quantitation limits were 1.146 and 3.465 µg/ml respectively. Proposed method was validated for precision, accuracy, ruggedness, robustness and specificity.

Keywords: Mepivacaine, HPLC, Stability, Forced degradation, Stress

INTRODUCTION

Mepivacaine hydrochloride, (RS)-N-(2,6 Dimethyl Phenyl)-1-Methyl Piperidine-2-Carboxamide Hydrochloride, is a local anesthetic . The chemical structure is shown in fig: 1. Mepivacaine blocks the nerve conduction by decreasing the entry of Na^+ ions during the upstroke of Action Potential (AP)[1]

Knowledge from the stability studies is used in the development of manufacturing process, selection of proper packaging and storage conditions, and determination of product shelf life. Literature survey reveals few analytical methods for the determination of Mepivacaine in combination by HPLC[2], separation from related substances[3], determination from Plasma[4], from CSF[5]. However, no Stability indicating assay method was reported for the determination of Mepivacaine Hydrochloride. The objective of this work was to develop a validated Stability indicating assay method for the determination of Mepivacaine hydrochloride in presence of its degradation products generated by subjecting the drug to forced degradation conditions under acid, alkali, thermal and oxidative stress as per the guidelines[6] and to establish the inherent stability of Mepivacaine hydrochloride.

MATERIALS AND METHOD

Materials and Reagents

Mepivacaine hydrochloride was supplied by Wockhardt Pharmaceuticals, Aurangabad as gift sample. Its identity and purity was confirmed by recording the FTIR spectra and the DSC curve. Solvents used were HPLC grade Methanol (Merck) and water from the water treatment plant (SG) of the Institute. The reagents used were Orthophosphoric acid & hydrochloric acid (Qualigens), Sodium hydroxide & Hydrogen peroxide solution (S.D. fine chemicals, Mumbai).

Apparatus

Dionex Liquid Chromatograph, equipped with Dionex P 680 ALPG-4 quadrant pump system, ASI 100 autosampler injection system, UVD 170U multi wavelength quadrant detector and Chromeleon chromatography management software was used. The column used was Gracesmart C_{18} (5µ), 250 mm, 4.6 mm i.d., pH meter (Equiptronics EQ-621) and analytical balance (Citizen)

Methods [7,8,9]

Chromatographic conditions

Chromatographic analysis was carried out at ambient temperature. The mobile phase consists of methanol: orthophosphoric acid solution (2.25 gm/L) adjusted to pH 7.6 with strong sodium hydroxide solution (75:25) at a flow rate 1 ml/min. The detection wavelength was 215 nm. All the solvents were filtered through 0.45µ nylon membrane filter and degassed offline by ultrasonication. The retention time was approximately 4.7 min.

Preparation of Stock and Standard solutions

The stock solution (1 mg/ml) was prepared by dissolving 100 mg of Mepivacaine hydrochloride in up to 100 ml HPLC grade water. It was filtered and ultrasonicated. The standard drug solutions in the concentration range 20-100 µg/ml was prepared by the dilution of suitable stock solution with HPLC grade water.

Preparation of calibration curve

The calibration curve was prepared in the concentration range 20-100 µg/ml by injecting 20 µl of each solution in triplicate and plotting the area under peak (mAU*min) against concentration (µg/ml). The correlation coefficient and equation of the line was determined. The Chromatogram of fresh drug solution (20µg/ml, 20µl) is shown in fig 2 and the calibration curve in fig 3. The data is shown in table 1

Forced degradation studies

The forced degradation of Mepivacaine was done in each of the stress condition at a concentration of 1 mg/ml. The degradation was confirmed in each case by recording the changes in the ultraviolet spectra of each stressed sample comparing it with that of fresh drug solution.

A. Acidic stress

100 mg of Mepivacaine hydrochloride was refluxed in 1 N Hydrochloric acid for 4 hours in a water bath at 70^0C.

B. Alkaline stress

100 mg of Mepivacaine hydrochloride was refluxed in 1 N sodium hydroxide for 4 hours in a water bath at 70^0C.

C. Thermal stress

100 mg of Mepivacaine hydrochloride was kept in solid state at 70^0C for 4 hours in an oven equipped with temperature control probe.

D. Oxidative stress

100 mg of Mepivacaine hydrochloride was dissolved in 1%, 3% and 10% Hydrogen peroxide separately and kept at room temperature for 24 hours in amber colored stoppered vials.

Recording of chromatograms of stressed samples

Suitable aliquots from each stressed sample were diluted with HPLC grade water to obtain concentration of 20 µg/ml. It was

chromatographed employing the conditions mentioned above in the range 20-100 µl injecting each solution in triplicate. The chromatograms of 20 µl injection volume of 20 µg/ml of each stressed sample are shown in fig 4-9.

Method validation[10]

A. Linearity and range

The linearity of the method was established by preparing a calibration curve in water in the range 20-100 µg/ml. Triplicates of each of the solution was injected and chromatograms recorded. The mean (n=3) area under peak (mAU*min) was plotted against concentration (µg/ml). The correlation coefficient and equation of line were determined. The results are shown in fig2-3 and table 1.

B. Precision

The Interday and intraday precision were determined by calculation of the % RSD values on injection of triplicates of each concentration. The mean (n=3) area under peak (mAU*min) of each concentration was compared with that of second run on the same day (intraday) and with that on the next day (interday) and the percent relative deviation calculated. The results are shown in table 2.

C. Accuracy

Accuracy of the method was evaluated by spiking the drug (40µg/ml) at three concentration levels (20, 40 & 60µg/ml). The percent recovery of the added drug was calculated from the linearity plots. The results are shown in table 3.

D. Specificity

The Specificity of the method was established through the determination of the drug in the presence of its degradation products with high degree of precision. The peak homogeneity was confirmed by analyzing the ratio chromatograms at the wavelengths 215 nm and 220 nm[11].

E. Ruggedness

Mean peak area (n=3) was measured for the 20µg/ml solution (20µl) analyzed by two different analysts on different days and the percent relative deviation between the runs was calculated. The results are shown in table 4.

F. Robustness

Deliberate changes in the mobile phase flow rate (+/- 0.1 units) and composition (=/- 5%) were made. Three replicates of each deviation were injected and the %RSD between the mean(n=3) area under peak (mAU*min) and that obtained under optimized chromatographic conditions were determined. The results are shown in table 5.

G. Limit of detection and limit of Quantitation

The limit of detection and limit of Quantitation were calculated based on standard deviation (σ) of responses for triplicate blank injections and the slope (S) of the calibration plot, using the formulae LOD= 3.3σ/s and LOQ=10σ/S as defined by ICH.

H. System suitability

The system suitability parameters Theoretical plates, Asymmetry factor, Linearity and range, accuracy, precision and specificity were determined and are shown in table 6.

RESULTS AND DISCUSSION

Degradation behavior of Mepivacaine hydrochloride

The amount (percent) of Mepivacaine hydrochloride remaining undegraded under each stress condition was determined from the area under peak (mAU*min) of the fresh drug sample relative to that of stressed drug sample. The results are shown in table 7.

Method validation

A. Linearity and range

The data from the linearity curve shows that the response of the drug was strictly linear in the studied concentration range (20-100µg/ml). The correlation coefficient between concentration (µg/ml) and area under peak (mAU*min) was 0.997 and the equation of line was y=0.996 x + 0.188.

B. Precision

The results of intraday and interday precision as shown in table2 reveals that the developed method is highly precise since the %RSD values are extremely low (limit < 2%) i.e. 0.28 % for intraday and 0.3 % for interday runs.

C. Accuracy

Good recoveries were obtained in the range of 101 to 102 %, which is indicative of high accuracy.

D. Specificity

Specificity of the method was revealed by its ability to estimate the analyte accurately and precisely in the presence of its degradation products without interference from the latter. The asymmetry factor ranges from 1.2-1.24 while the number of theoretical plates ranges from 9000-11000. The ratio chromatograms corresponding to the drug's peak were flat in each case of forced degradation which indicates the homogeneity of these peaks.

E. Ruggedness

The ruggedness of the method is revealed by the low % RSD obtained between runs by two different analysts on different days as shown in table 4.

F. Robustness

The robustness of the developed method is revealed by the low % RSD obtained in the readings between runs done employing the optimized chromatographic conditions and that employing the deliberately alter4ed conditions as shown in table 5.

G. Limit of detection and limit of Quantitation

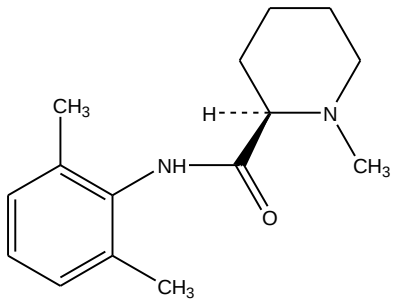
Limit of detection and limit of Quantitation were found to be 1.146 and 3.465 µg/ml respectively.

H. System suitability

The method meets the system suitability criteria as shown in table 6.

Analysis of physical lab mixture

A 3% solution of Mepivacaine hydrochloride was made incorporating 1% sodium chloride. It was diluted to obtain 20µg/ml solution. Five replicates were injected and the amount determined from linearity curve. The results are shown in table 8.



HCl

Fig. 1: Chemical structure of Mepivacaine hydrochloride

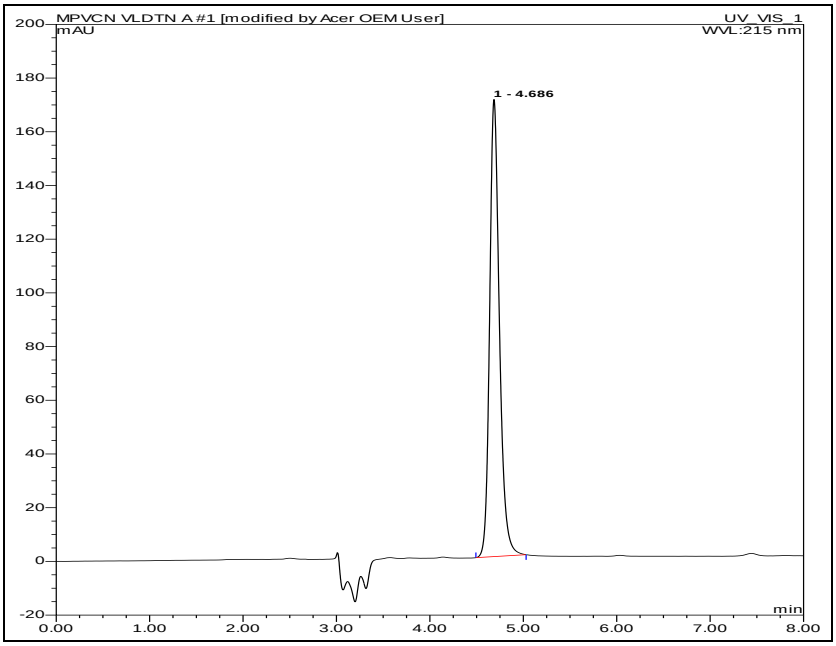


Fig. 2: HPLC Chromatogram of Fresh MPV (20µg/ml,20µl) at 215 nm

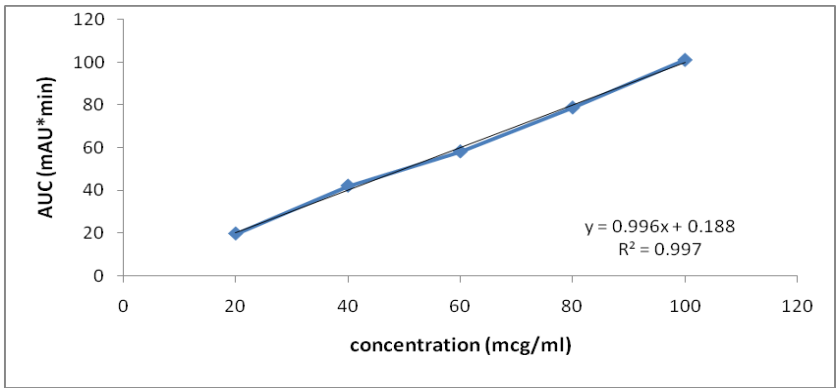


Fig. 3: Calibration curve of MPV at 20 µl Injection Volume & varying Concentration

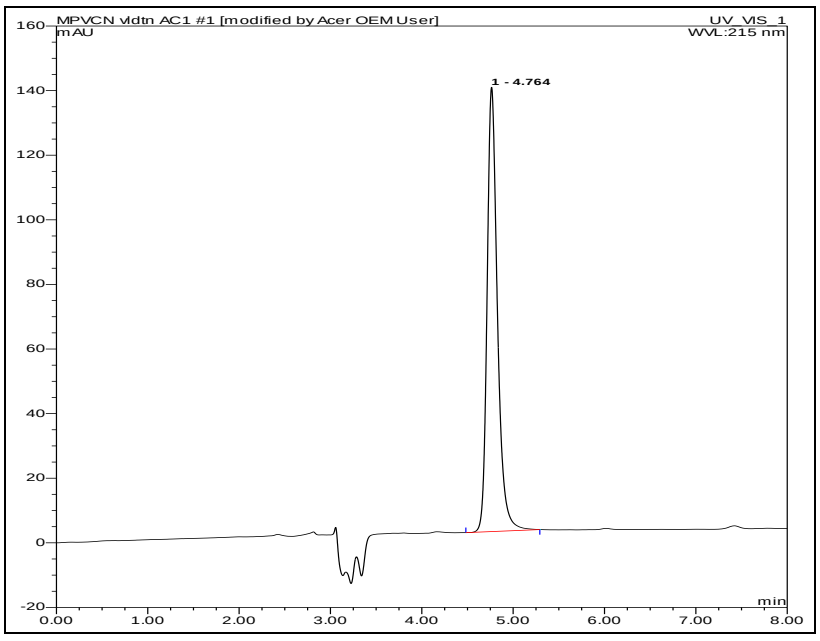


Fig. 4: HPLC Chromatogram of Acid Degraded MPV (20µg/ml, 20µl)

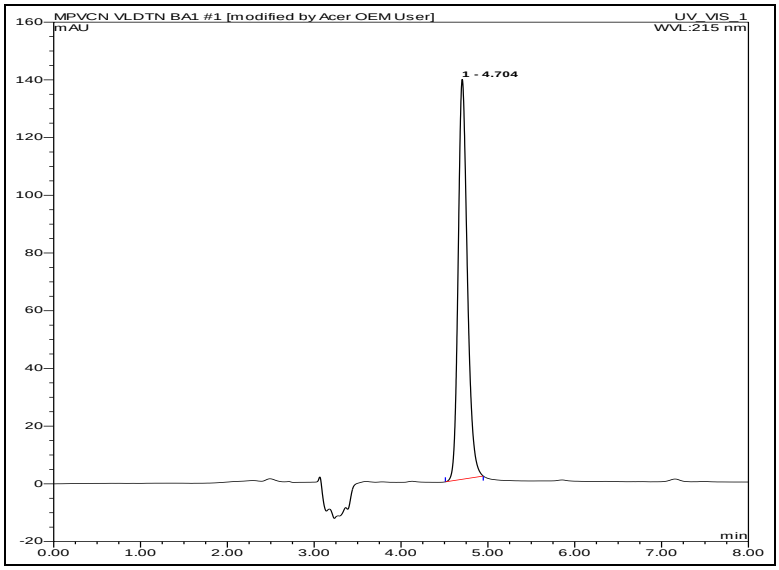


Fig. 5: HPLC Chromatogram of Alkali Degraded MPV (20µg/ml, 20µl)

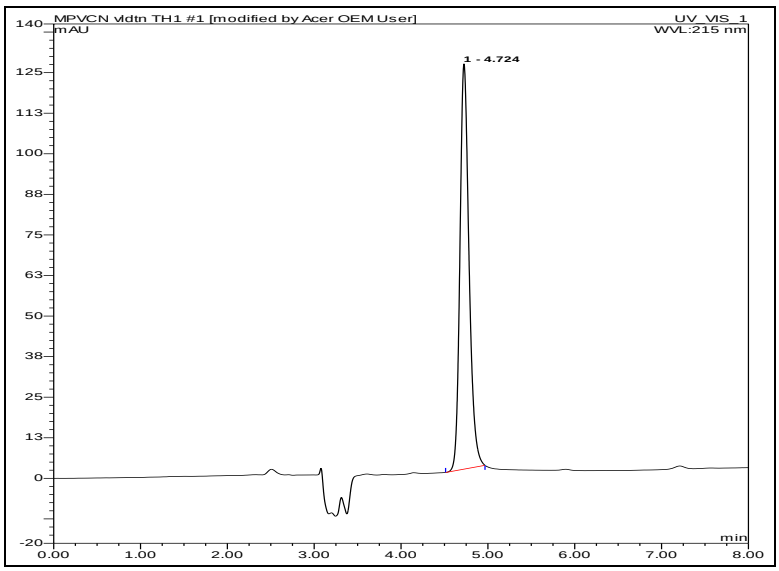


Fig. 6: HPLC Chromatogram of Thermally Degraded MPV (20µg/ml, 20µl)

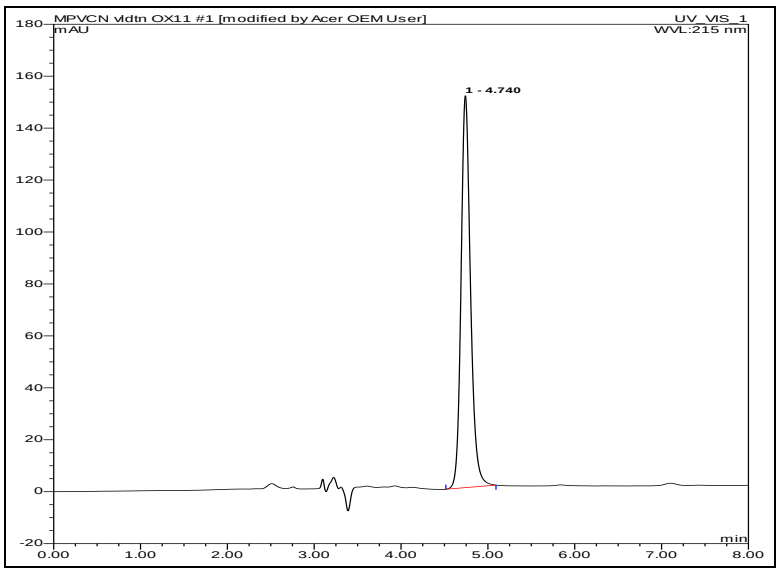


Fig. 7: HPLC Chromatogram of Oxidatively (1% H_2O_2) Degraded MPV (20µg/ml, 20µl)

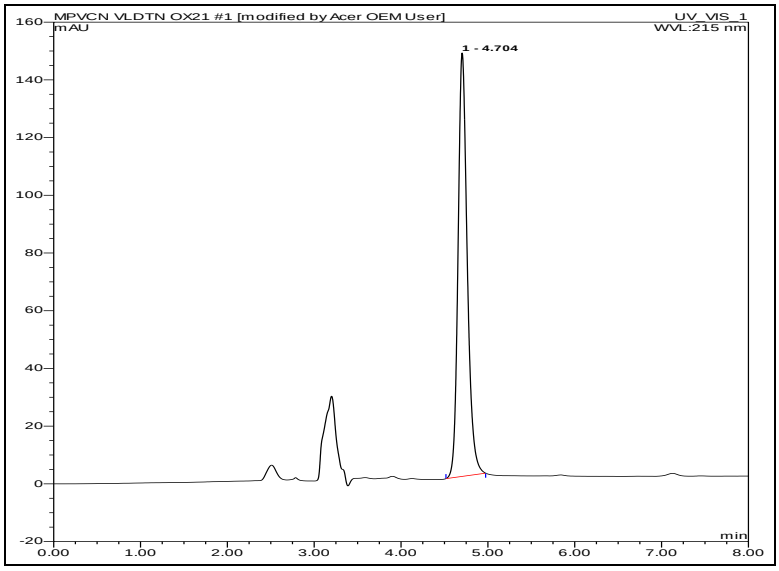


Fig. 8: HPLC Chromatogram of Oxidatively (3% H_2O_2) Degraded MPV (20µg/ml, 20µl)

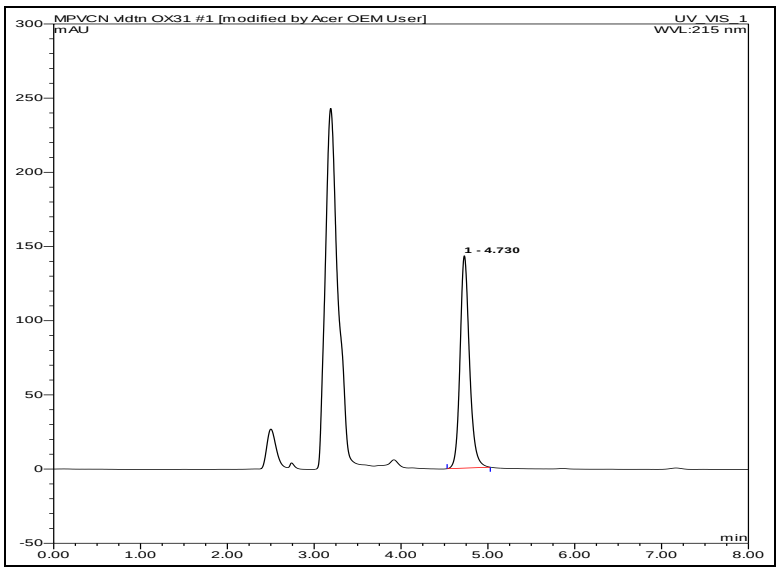


Fig. 9: HPLC Chromatogram of Oxidatively (10% H_2O_2) Degraded MPV (20µg/ml, 20µl)

Table 1: Linearity Study of MPV at 215 nm varying Concentration at 20 µl

Sr. No.	Concentration of MPV in [µg/ml]	Peak area Mean ± S.D. [n = 3]	% R.S.D.
1	20	19.71 ± 0.0757	0.384
2	40	42.29 ±0.1389	0.328
3	60	58.17 ± 0.23	0.396
4	80	78.71 ± 0.555	0.705
5	100	101.02 ± 0.527	0.522
		MEAN % R.S.D.→	0.467%

Table 2: Results of Intra-day and Inter-day Precision studies

Conc.[µg/ml]	Intra-day Precision		Inter-day Precision	
	Mean ± S.D [*n* =3*2]	**% R.S.D.**	**Mean ± S.D. [*n* =3*2]**	**% R.S.D.**
20	20.55 ± 0.035	0.172	20.6 ± 0.1	0.515
40	40.95 ± 0.169	0.414	40.89 ± 0.1	0.224
60	61.24 ± 0.08	0.138	61.13 ± 0.24	0.39
80	81.35 ±0.5	0.625	81.55 ± 0.23	0.277
100	101.37 ±0.05	0.048	101.44 ± 0.5	0.05
	Mean % RSD	**0.28%**	**Mean % RSD**	**0.3%**

Table 3: Results of Recovery Studies

Pre-analyzed sample solution [µg/ml]	Excess drug added[µg/ml] [n = 3]	Amount recovered [µg/ml]	%Recovery	%R.S.D.
MPV 40	0	40.83	101.1	0.312
	20	61.24	101.67	0.316
	40	81.66	102.03	0.124
	60	102.07	102.07	0.217

Table 4: Results of Ruggedness studies

Amount taken [µg/ml] [n = 3]	Analyst I	%R.S.D.	Analyst II	%R.S.D.
20	100.66	0.139	100.32	0.228

Table 5: Result of Robustness studies

	RT	Peak area	%RSD
Mobile phase composition(v/v)	4.8	20.53	0.175
70:30	4.76	20.58	
75:25	4.72	20.6	
80:20			
Mobile phase flow rate (ml min^{-1})	4.82	20.54	0.122
0.9	4.75	20.57	
1.0	4.71	20.59	
1.1			

Table 6: System suitability parameters

Parameter	Value
Linearity range (µg/ml)	20-100
Regression equation[Y = m X + C]	Y = 1.013 x + 0.364
LOD(µg/ml)	1.146
LOQ(µg/ml)	3.465
Recovery (% R.S.D.)	0.242
Intra- day (n = 3) Precision (% R.S.D.)	0.28
Inter- day (n = 3) Precision (% R.S.D.)	0.29
Analyst I (n = 3) Ruggedness (% R.S.D.)	0.139
Analyst II (n = 3) Ruggedness (% R.S.D.)	0.228

Table 7: % Drug Degraded under each Stress Condition

Sr. No.	Condition	% Drug Remaining	% Drug Degraded
1	Normal Fresh	100	0
2	Acid Degraded	88.41	11.59
3	Base Degraded	83.41	16.59
4	Thermally Degraded	75.04	24.95
5	Oxidative Degraded (1%H_2O_2)	92.45	7.54
6	Oxidative Degraded (3%H_2O_2)	86.52	13.47
7	Oxidative Degraded (10%H_2O_2)	85.75	14.25

Table 8: Analysis of MPV in Laboratory Mixture by HPLC

Amount taken [µg/ml]	Amount found [µg/ml]	Amount found [%]
20	20.03	100.06
20	20.01	100.02
20	20.05	100.1
20	19.99	99.98
20	19.98	99.96
Mean ± S.D.	20.012 ± 0.0286	100.024 ± 0.0572
%R.S.D.	0.143	0.057

CONCLUSION

A validated stability indicating assay method has been developed for the determination of Mepivacaine hydrochloride in bulk and in dosage form. A new mobile phase was found during the method development process. The results show that the developed method was accurate, precise, simple, economic, fast and specific. Mepivacaine is most prone to degradation under thermal stress, followed in order by the stress alkali, oxidative (10%), oxidative (3%), acid, oxidative (1%). The method can be considered for routine quality control and stability studies on Mepivacaine hydrochloride.

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