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RESEARCH ARTICLE

A COMPARATIVE STUDY FOR FORCE DEGRADATION OF THREE LOCAL ANESTHETIC DRUGS BUPIVACAINE, ROPIVACAINE, MEPIVACAINE AND QUANTITATIVE ANALYSIS OF THEIR DEGRADED PRODUCTS BY NEWLY DEVELOPED AND VALIDATION HPLC-UV AND LC-MS METHOD

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ABSTRACT

Reversed phase isocratic method has been developed for the quantitative analysis of local anesthetics drugs bupivacaine, ropivacaine and mepivacaine with very good separation for determination in bulk drug and dosage form of pharmaceutical. A Hypersil BDS C18 250×4.6mm,5µm column, mobile phase mix buffer and acetonitrile (34:66v/v) in 1000 ml and adjust pH 7.5 with diluted KOH solution, isocratic flow rate 0.9 mL/min and system detection is performed at 220 nm has been used for HPLC and LC-MS. Forced degradation study is perform in different stress condition such as by keeping it in sunlight, UV light, thermal, humidity, acidic, basic, and oxidative for extended time. Degradation products separation on HPLC chromatography. Degradation products separation on HPLC chromatography. Degradation study experiments are also analyzed on newly developed HPLC method.

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INTRODUCTION

LA is an unpredictable nonattendance of pain sensation and loss of Muscle power to very good decrease feeling pain in a specific area, LA is apply by injection to inject in to pain the area. LA is used two type like short and long acting for procedures. LA are anesthetics belongs two classes amino amide and amino ester. Here we select amino amide like classes of procaine to Bupivacaine, Ropivacaine and Mepivacaine (1-8). MEP and ROP was synthesized in 1891 and 1930, BUP was synthesized in 1957(9-11). LA was used in human during section to prevent spinal anesthesia induced hypotension and side effects (12-13). IUPAC name of Bupivacaine, Ropivacaine and Mepivacaine are (RS)-1-Butyl-N-(2,6-dimethylphenyl) piperidine-2-carboxamide,(S)-N-(2,6-dimethylphenyl)-1-propylpiperidine-2-carboxa-mide and (RS)-N-(2,6-dimethylphenyl)-1-methyl-piperidine-2-carboxamide respectively of structure (Figure 1). BUP, ROP and MEP are used local infiltration, peripheral, nerve block, retro bulbar block, and as well as in postoperative pain area (1-14). Few of TLC, HPLC, GC, LC-MS and GC-MS in human plasma methods have been reported for the determination of BUP, ROP and MEP (14-16). The validation with forced degradation study is performed in different stress condition such as sunlight, UV light, acidic, basic, thermal, water and oxidative (H_2O_2) as per procedure followed the ICH guidelines Q2A and Q2B(17-18). Now a day HPLC is well known, easy and widely used analytical technique for the analysis of bulk drug substance and pharmaceutical products.

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MATERIALS AND METHODS

Instrumentation and software

To develop quantitative analysis on high performance liquid chromatographic method for determination of BUP, ROP and MEP. The HPLC System of Agilent HPLC 1100 Series with variable wavelength detector (VWD) The diode array detector (DAD) microprocessor, quaternary pump, Agilent technologies international sarl,1100 series, auto sample, micro auto sample, preparative auto sample, thermostatic column compartment, used for this entire study. Chromatographic separation and determination was achieved on Hypersil BDS C18 250×4.6mm,5µm column, UV chamber, analytical balance, digital pH meter, 0.45µ membrane filters.

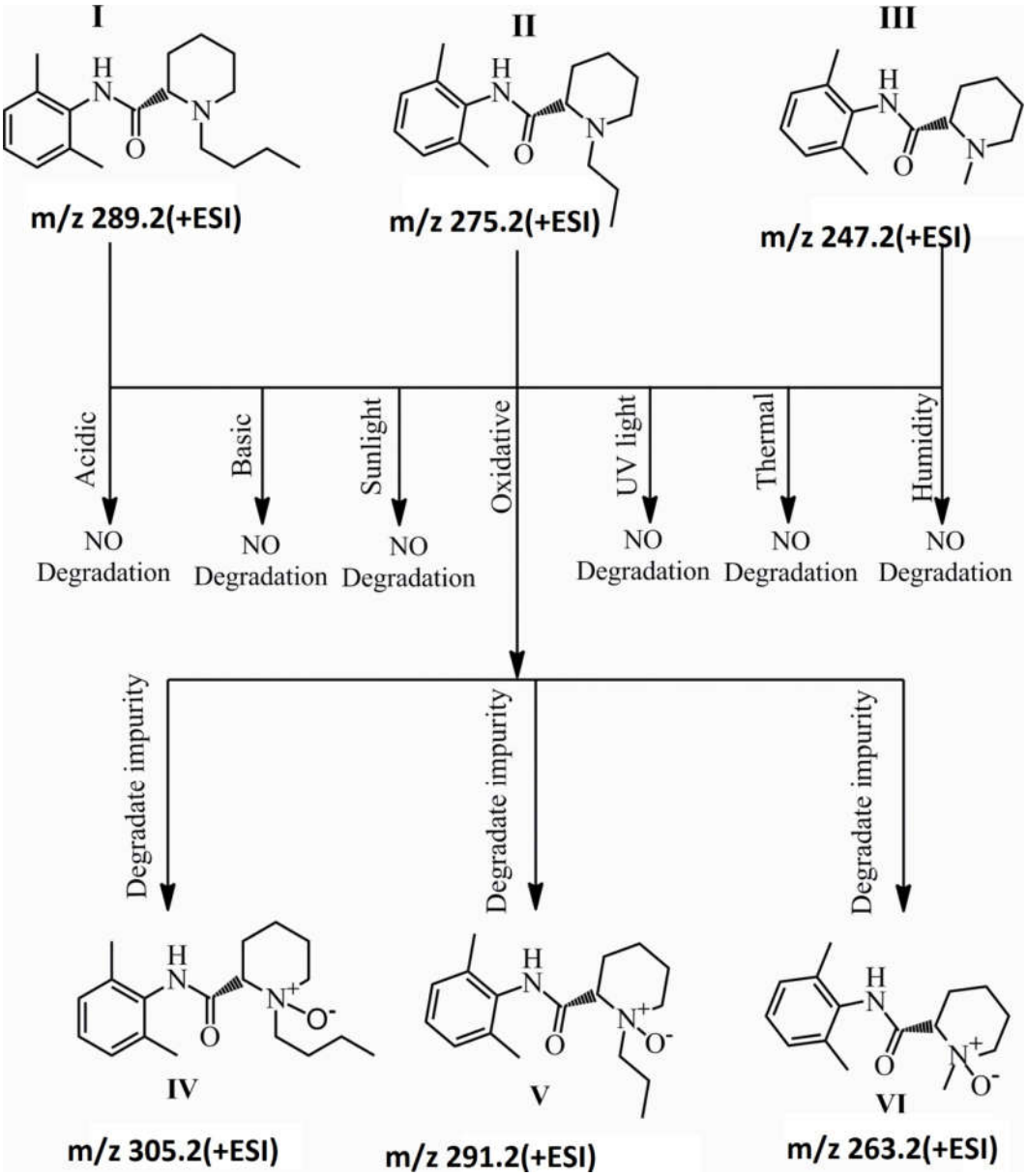

Figure 1. Structure of BUP,ROP,MEP and degradation products with stress condition. I : structure of the BUP, chemical name: (*RS*)-1-Butyl-*N*-(2,6-dimethylphenyl)piperidine-2-carboxamide, II : structure of the ROP, chemical Name: (*S*)-*N*-(2,6-dimethylphenyl)-1-propylpiperidine-2-carboxamide, III: structure of the MEP, chemical name: (*RS*)-*N*-(2,6-dimethylphenyl)- 1-methyl-piperidine-2-carboxamide, IV: structure of the N-Oxide of BUP, (degradation of oxidative) chemical name: (2S)-1-butyl-2-(2,6-dimethylphenylcarbamoyl) piperidine 1-oxide, V: structure of the N-Oxide of ROP, (degradation of oxidative) chemical name: (2S)-2-(2,6-dimethylphenylcarbamoyl)-1-propylpiperidine 1-oxide, VI: structure of the N-oxide of MEP, (degradation of oxidative) chemical name: (2S)-2-(2,6-dimethyl phenylcarbamoyl)-1-methylpiperidine 1-oxide,

Chemicals and reagents

The pharmaceutical API of BUP, ROP and MEP (99.9% pure) each 1000mg were purchased from market. HPLC grade acetonitrile SD fine limited. Analytical grade K2HPO4, K2HPO4, KOH, hydrochloric acid, sodium hydroxide flakes, hydrogen peroxide. Milli-Q water purchased from market.

Details of method chromatographic conditions

The chromatographic quantitative analysis has been performed on a Hypersil BDS C18 250×4.6mm,5µm column, mobile phase mix buffer(dissolved 2.0gm of KH2PO4 and 2.5gm K2HPO4 in 1000ml milli-Q water and adjust pH 6.5 with 10%v/v KOH or 15%v/v ortho phosphoric acid. Filter it through 0.45µm membrane filter) and acetonitrile (34:66v/v) in 1000 ml and adjust pH 7.5 with diluted 10%v/v KOH solution, isocratic flow rate 0.9 mL/min, injection volume 10µL, mobile phase for diluent and system detection is performed at 220nm has been used for HPLC analysis of with forced degradation study like sunlight, UV, thermal, humidity, acidic, basic, and oxidative.

Preparation of solutions

Specificity solution for retention time confirmation and system suitability solution

Accurately weighted 10mg of each BUP,ROP and MEP and transfer into a three different 100mL volumetric flask, add about 50 mL of diluent and sonication to dissolve the content. Make the volume up to the mark with diluent and mix. Conc. of each BUP,ROP and MEP(100µg/mL, 100ppm).

Table 1. Degradation of analyte applying forced degradation (stress condition) and results for proposed method validation (% Purity) of BUP, ROP, MEP

Stress condition	Condition media	Temperature	Time	Degradation Results		
				BUP on (LC-UV /LC-MS m/z)	ROP on (LC-UV /LC-MS m/z)	MEP on (LC-UV /LC-MS m/z)
Acidic	5N HCl	60°C	1 hr	ND/289.2	ND/275.2	ND/247.2
Alkali	5N NaOH	60°C	1 hr	ND/289.2	ND/275.2	ND/247.2
Oxidative	10 % H_2O_2	60°C	1 hr	Deg./305.2	Deg./291.2	Deg./263.2
Sunlight	Solid form	-	72 Hrs	ND/289.2	ND/275.2	ND/247.2
UV-light	UV chamber	200 watts-hours	72 Hrs	ND/289.2	ND/275.2	ND/247.2
Humidity	65% RH	RT	72 Hrs	ND/289.2	ND/275.2	ND/247.2
Thermal	Oven	105°C	72 Hrs	ND/289.2	ND/275.2	ND/247.2

ND= Not degradation ,Deg.= degradation

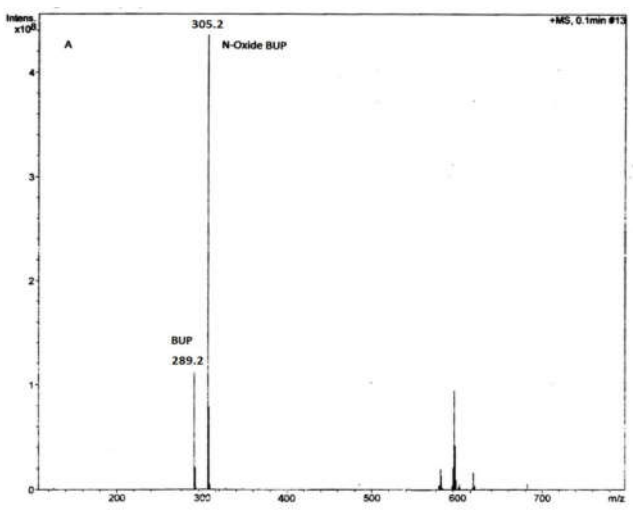

Figure 2A.

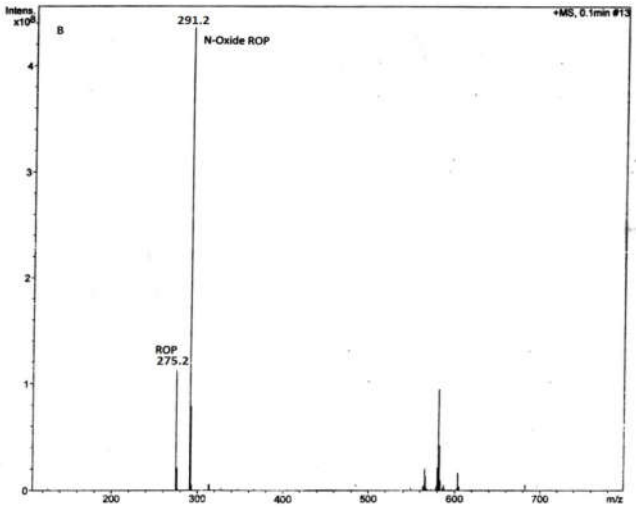

Figure 2B.

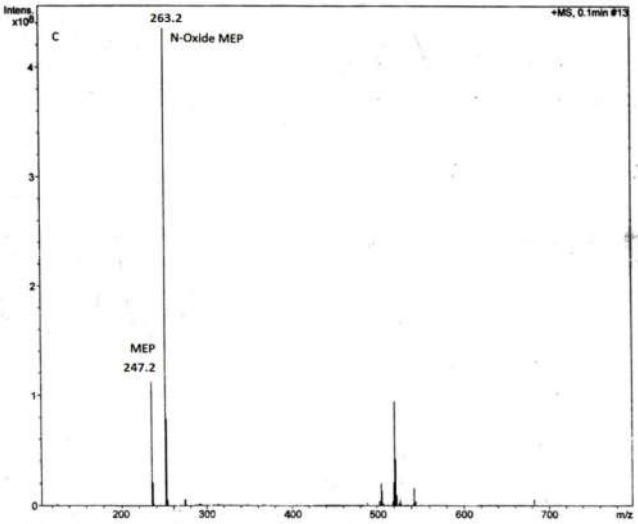

Figure 2C.

Figure 2. Comparison of LC/MS spectra chromatograph of (A) Oxidative degradation product on BUP, (B) Oxidative degradation product on ROP.(C)Oxidative degradation product on MEP

Test solution

Accurately weighted 25mg of test sample each BUP, ROP and MEP and transfer into a three different 50 mL volumetric flask, add about 10 mL of diluent and sonication to dissolve the content. Make the volume up to the mark with diluent and mix. Conc. of each BUP,ROP and MEP (500µg/mL, 500ppm).

2.4.3 Standard stock solution: Accurately weighted 25 mg each sample of BUP,ROP and MEP and transfer into a three different 100 mL volumetric flask, add about 50 mL of diluent into each of the flask and sonication to dissolve the content. Make the volume up to the mark with diluent and mix (each sample conc. of BUP,ROP and MEP (250µg/mL, 250ppm).

Method Validation

Validation of the developed method for the determination of BUP, ROP and Method MEP was performed according to the ICH guidelines "Validation of analytical procedures: text and Methodology Q2B(R),Q2B(R)" (International Conference on Harmonization, 1996; International Conference on Harmonization, 1996) with standards bulk drug thus system

suitability along with method selectivity, specificity , linearity, range, precision (repeatability) and intermediate precision, accuracy, limits of detection and quantification, BUP,ROP and MEP is short team and long term stability of the analysts in the prepared in the prepared solutions and robustness were demonstrated. The limit of quantification (LOQ) and limit of detection (LOD) were estimated using the following equations. LOQ=10s /s and LOD=3.3s /s

Where s is the standard deviation of intercept and s is the slope of the calibration curve

Forced Degradation Study

UV light, sunlight, thermal and humidity degradation

About 50mg of each sample BUP, ROP and MEP is taken in four different petri dishes. One petri dish is kept in UV light chamber for 200 watts-hours/square-meter, second petri dish in sunlight, third petri dish in oven at 105^0C for 72 hours for thermal and fourth petri dish in humidity at RT for degradation study.

Acid degradation

Accurately weighted 25mg of test samples of each BUP, ROP and MEP and transfer into a three different 50 mL volumetric flask, add about 5 mL of 5N hydrochloric acid solution and 10mL diluent and sonicate to dissolve the content. Make the volume up to the mark with diluent and mix. Conc. of each BUP, ROP and MEP (500µg/mL, 500 ppm). Heat it at 60^0C on water bath for 1 hr, cool it at RT and adjust the pH 7.0 with of 5N sodium hydroxide solution.

Alkali degradation

Accurately weighted 25mg of test sample each BUP,ROP and MEP and transfer into a three different 50 mL volumetric flask, add about 5 mL of 5N sodium hydroxide solution and 10mL diluent and sonicate to dissolve the content. Make the volume up to the mark with diluent and mix. conc. of each BUP,ROP and MEP (500µg/mL,500ppm). Heat it at 600C on water bath for 1 hr, cool it at RT and adjust the pH 7.0 with of 5N hydrochloric acid solution.

Oxidative degradation

Accurately weighted 25mg of test sample each BUP,ROP and MEP and transfer into a three different 50 mL volumetric flask, add about 5 mL of 10% hydrogen peroxide solution and 10mL diluent and sonicate to dissolve the content. Make the volume up to the mark with diluent and mix. conc. of each BUP,ROP and MEP (500µg/mL,500ppm).Heat it at 600C on water bath for 1 hr, cool it at RT .

RESULTS AND DISCUSSION

Optimization of chromatographic conditions and Forced degradation behavior

The development qualitative (% Purity) of LC-UV methods for determining drugs has received great attention in analytical research due to its use in quality control. The main objective of method development is to determine the BUP,ROP, MEP and degraded products of each BUP,ROP and MEP. The degradation products in chromatogram were present in the total- ion chromatogram, recorded by using the LC-UV method. Results shows that BUP,ROP, MEP did not degrade under acid, alkali ,thermal and sunlight stress conditions a mixture of each solution, we found very strange results that these products not degraded in alkali stress condition even if it contain amide linkage. In general amide group are highly sensitive towards alkali stress condition but here we observed that amide linkage in BUP, ROP, MEP is not broken, another important result we found is that it degraded under oxidative stress condition and one degradation product was formed in each BUP, ROP and MEP. Each BUP, ROP and MEP in oxidative degradation with 10% hydrogen peroxide solution. Every degradation product determination in LC-MS and to the m/z values and fragmentation pattern, The observed m/z values for ion peak $[M+H]^+$ and considerable fragments of the degraded products are 305.2,291.2,263.2. Based on these m/z value it is proposed that degraded products are the N-Oxide of BUP,ROP and MEP respectively. Results is tabulated and included in table 1. A typical chromatogram of the separation of the analysis under these conditions is presented in (Figure 2).

Method Validation

Method validation has been performed as per ICH guideline Q2A and Q2B includes several parameters like precision, linearity, accuracy , robustness, LOD,LOQ .These parameters all validation have been performed systematically both assay and purity on HPLC[17-18]. A typical chromatogram of the separation of the two analytes under these conditions is presented in (Figure 3A),(Figure 3B) . Results in memory tablets are included in table 2.

Linearity and range on LC-UV

All calibration curves for BUP presented coefficient of determination (R^2) 0.999, while for the ROP, MEP of determination (R^2) was greater than 0.995 as required. A lack-of-fit test was performed for all calibration curves and the calculated R^2-values of the representative curves, system suitability parameter was comply. The correlation coefficient would not be less than 0.98. Y- Intercept ≤25% referred to the calculated response of the x-value corresponding to the concentration of the specification limit. Representative linearity and range results in memory tablets are included in table 2 and linearity chart show in (Figure.4).

Stability on LC –UV

At the beginning of the method development it was observed that each of BUP, ROP and MEP stock solutions prepared in diluent seemed to be very stable and no additional peaks appeared in the chromatograms. Therefore based on this observation, a stability study on each BUP, ROP and MEP stock solution in diluent at RT and at 5^oC for initial,12hrs 24 hrs, 36hrs and 48hrs followed and found that they were stable and no degradation of each BUP, ROP and MEP were observed. Results for each BUP, ROP and MEP are included in table 3.

Robustness on LC-UV

The robustness of the method was evaluated by analyzing standards and test solutions at the nominal concentration of each BUP, ROP and MEP(100 µg/mL).

Table 2. Summary of system suitability test with linearity, t_R(retention time), coefficient of determination (R^2), Y-intercept, and LOQ data for proposed method validation (% Purity) of BUP, ROP, MEP

Substance	Range (µg/mL)	$t_{R,}$ (retention time)	$Rt_{R,}$ (relative time)	Coefficient of determination (R^2)	Y-intercept	LOQ	LOD	SLOPE
BUP	1 to 300	7.7	1	0.999	5469.× + 396.5	2.770	0.914	5469.14
ROP	1 to 300	6.2	0.80	0.999	1881.× - 50.84	1.990	0.657	1881.8
MEP	1 to 300	3.9	0.50	0.999	2335.× - 199.5	1.636	0.540	2335.15

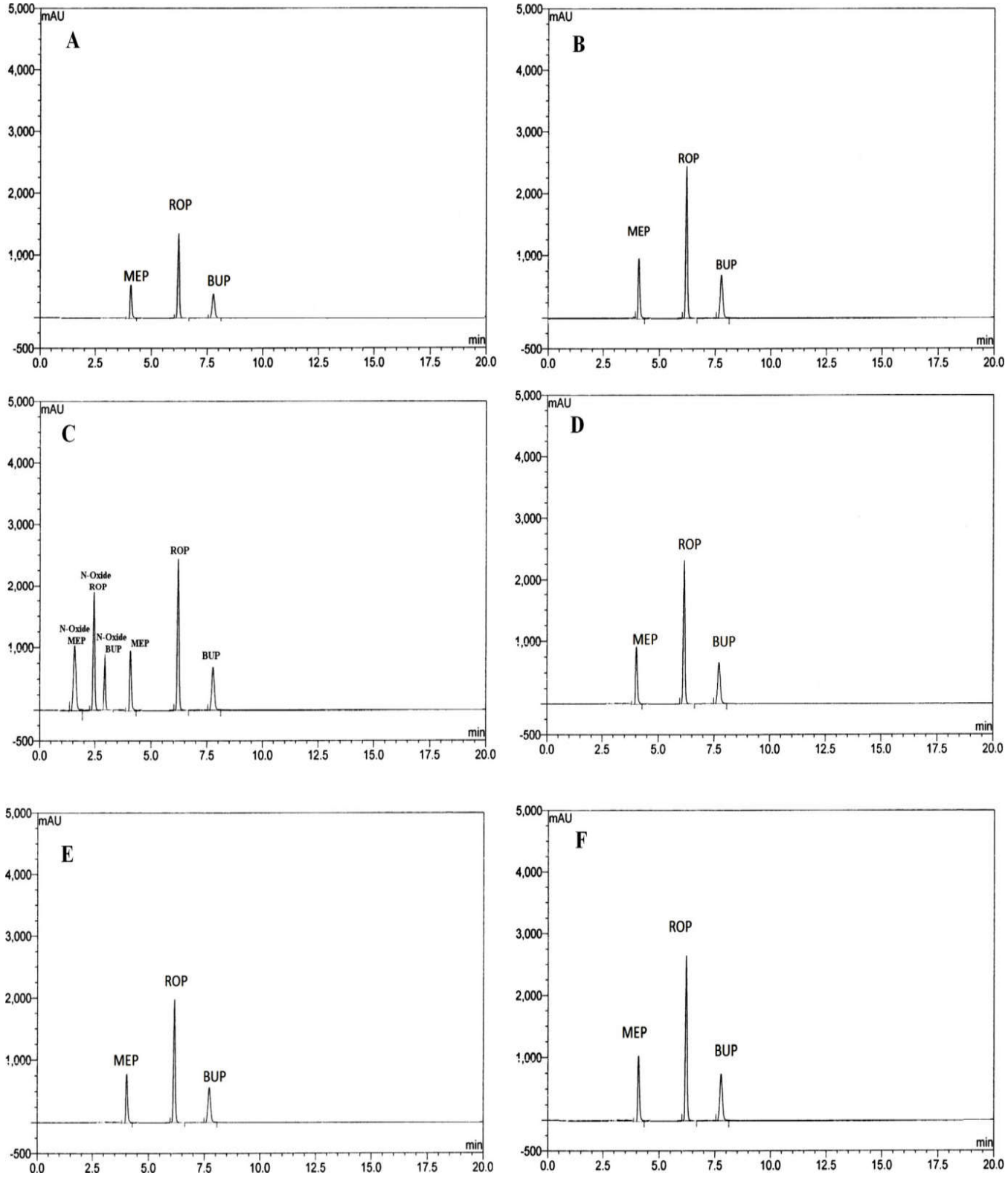

Figure 3. A chromatograph forced condition acid, alkali, oxidative, system suitability solution, test solution and thermal for HPLC method development and validation. Chromatograph of (A) Acidic degradation, (B)Alkali degradation, (C)Oxidative degradation, (D)System suitability solution, (E)Test solution, (F)Thermal degradation

Table 3. Summary of accuracy, recovery, robustness and stability test study data for proposed method validation (% Purity) of BUP, ROP, MEP

Parameter	Condition	Data	BUP	ROP	MEP
Flow rate	0.8 mL/min	% RSD	0.26	1.14	0.37
	1.0 mL/min	% RSD	0.28	0.49	0.27
pH in Mobile Phase	pH (7.4)	% RSD	0.91	0.26	0.28
	pH (7.6)	% RSD	0.18	0.50	0.39
UV	215 nm	% RSD	0.44	0.48	0.36
	225 nm	% RSD	0.76	0.23	0.37
Stability at ^{0}C	at RT				
	Initial		0.34	0.15	0.37
	12 hrs		0.40	0.29	0.40
	24 hrs		0.42	0.34	0.42
	36 hrs		0.54	0.37	0.47
	48 hrs	% RSD	1.17	0.42	0.53
	At 5^{0}C				
	Initial		0.08	0.72	0.20
	12 hrs		0.14	0.73	0.21
	24 hrs		0.24	0.80	0.22
	36 hrs		0.27	0.87	0.24
	48 hrs		0.29	0.88	0.28
Accuracy on level	Data				
50 %	% Recovery		100.57	100.34	100.71
	% RSD		0.20	1.04	1.65
100 %	% Recovery		100.56	100.08	101.35
	% RSD		0.90	0.26	0.49
150 %	% Recovery		100.16	100.33	100.35
	% RSD		0.30	0.45	0.14

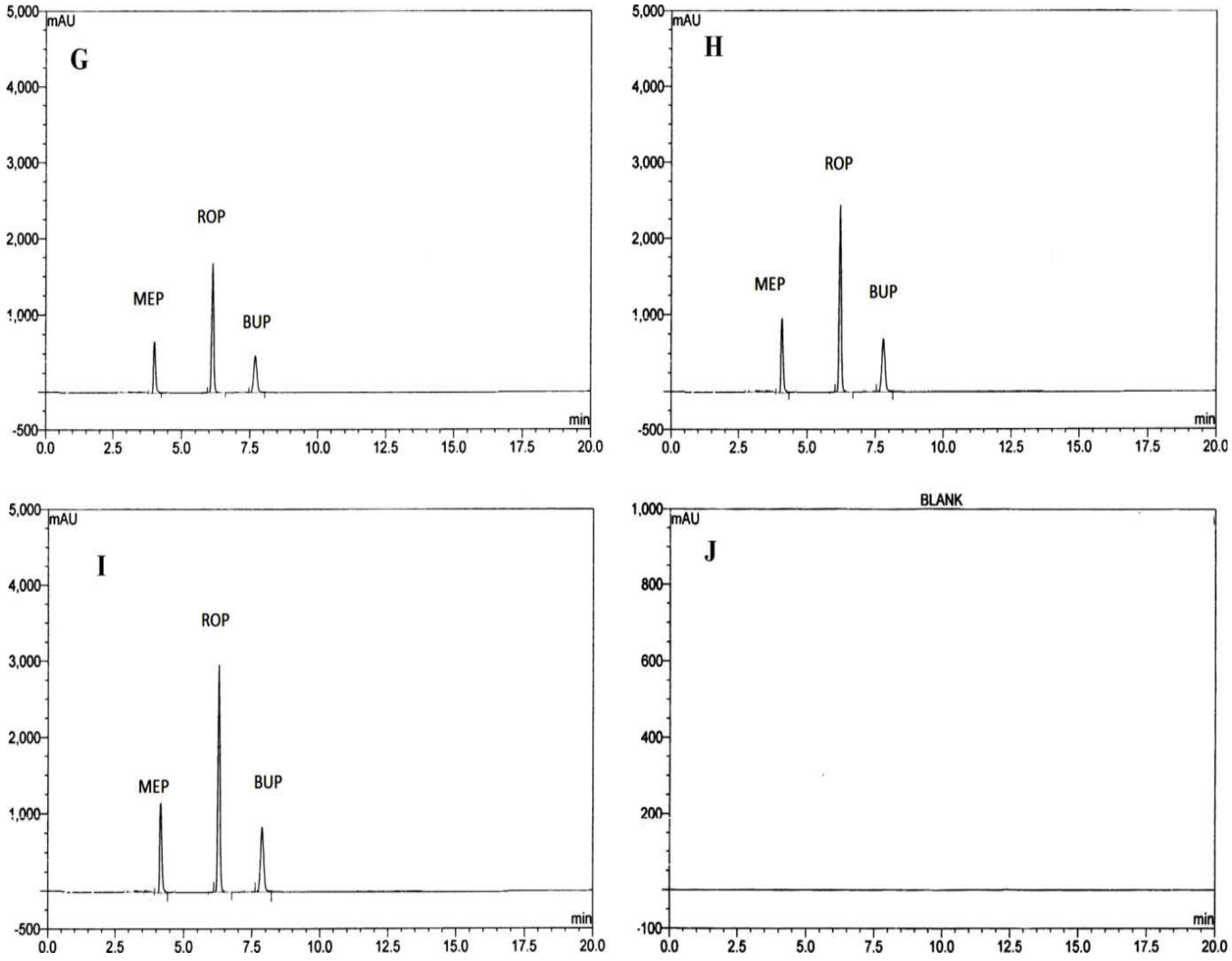

Figure 3. B Chromatograph forced condition UV degradation, sunlight degradation, humidity degradation, blank test (G)UV degradation,(H) sunlight degradation, (I) Humidity degradation, (J)Blank test

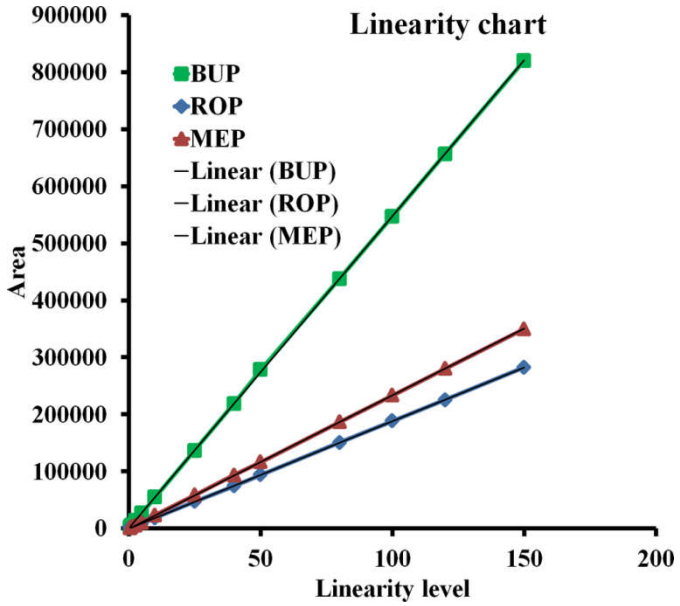

Figure 4. Plot of area verses linearity level (0.5% to 150%) and confidence level for BUP, ROP, MEP correlation coefficient would not be less than 0.995. Y- Intercept ≤25% referred to the calculated response of the x-value corresponding to the concentration (linearity level) of the specification limit

The parameters altered were the flow rate (±10%) 0.8,1.0 mL/min instead of 0.9mL/min, pH(±0.1unit)7.4, 7.6 instead of 7.5 and the different UV nm 215, 225, instead 220. Representative linearity and range results in each BUP, ROP and MEP drugs are included in the result were summarized in table 3.

Concluding Remarks

BUP, ROP and MEP was subjected to stress condition as per ICH guideline on LC-MS and LC-UV. It was found that these three local anesthetic drugs are stable in acidic, thermal, humidity, UV, sunlight and strange fully even if in alkali stress condition, mostly amide group is observed to be degrade in alkali stress condition but here we observed that amide linkage in BUP, ROP, MEP is stable in alkali stress condition. It is also concluded that these LA is unstable in oxidative condition and formed its respective N-Oxide impurities. The newly developed method is complies all the parameter including method validation as suggested in ICH guidelines Q2A and Q2B successfully. The method is applied successfully for stability testing as well as for commercially available BUP, ROP and MEP.

Abbreviations

BUP: Bupivacaine
ROP: Ropivacaine
MEP: Mepivacaine
LA: Local anesthetic
API:Active pharmaceutical ingredient;
ICH:International conference of Harmonization;
LOQ:limit of Quantification;
LOD: limit of detection;
RT:Room temperature;
t_R :Retention time;
Rt_R:Relative time;
R_S :Resolution;
R^2:Coefficient of determination;
ppm: Parts per million
RSD: Relative Standard Deviation
SD: Standard Deviation
mg: milli gram
mL: millilitre
nm: nano- meter
conc.: concentration
µg : micro gram
Hrs: hours

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