

# A Study of Method Development, Validation and Forced Degradation for Quantification of Buprenorphine Hydrochloride in a Microemulsion Formulation

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## ABSTRACT

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**Objective:** Development and validated of a simple, selective RP-HPLC method for the determination of buprenorphine hydrochloride in pharmaceutical microemulsion formulation. A forced degradation study of developed formulation was carried out in accordance with International Conference on Harmonization (ICH) guidelines Q1 (R2). **Method:** The chromatogram was obtained with 10 mmolL<sup>-1</sup> potassium phosphate buffer adjusted to pH 6.0 with triethanolamine and acetonitrile (17:83, v/v) as mobile phase, C18 HPLC column (250 × 4.6 mm i.d., 5 µm) kept at 30°C and UV detection at 284 nm. The compound was eluted isocratically at a flow rate of 1.0 mL min<sup>-1</sup>. **Results:** The average retention time for buprenorphine was 14.319 min. The method was validated according to the ICH guidelines. The validation characteristics included accuracy, precision, linearity, range, specificity, limit of Quantitation and robustness. The calibration curves were linear ( $R^2 > 0.999$ ) over the concentration range 1.0 – 500.0 µg mL<sup>-1</sup> for buprenorphine hydrochloride and recovery study for the compound was above 95 %. No spectral or chromatographic interferences from the microemulsion excipients were found. The drug was found to be labile under oxidative stress condition; whereas stable under all other stress conditions. **Conclusion:** This method is simple, rapid and suitable for routine quality control analysis.

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## INTRODUCTION

Buprenorphine hydrochloride (BU) is chemically known as (6R, 7R, 14S)-17cyclopropylmethyl- 7, 8-dihydro-7-[1S]-1-hydroxy-1, 2, 2 trimethylpropyl]-6-0-methyl-6, 14-ethano-17-normorphine hydrochloride (USP, 2007; Drugbank, 2016; Sweetman, 2009; Ash *et al.*, 1996). The molecular formula of BU is C<sub>29</sub>H<sub>41</sub>NO<sub>4</sub>, HCl and the molecular weight is 504.1 (Fig. 1). It is a potent semi-synthetic opiate analgesic with a potency of 20-40 times higher than that of morphine (Heel *et al.*, 1979). As an analgesic, it is used successfully by intramuscular, intravenous and sublingual routes to treat moderate to severe pain as well as chronic pain (Hoskin *et al.*, 1991). It is also indicated to treat opioid dependence by sublingual route (Dailymed, 2016).

Literature survey reveals various analytical methods available for the quantitative determination of BU, individually and in combination with other drugs.

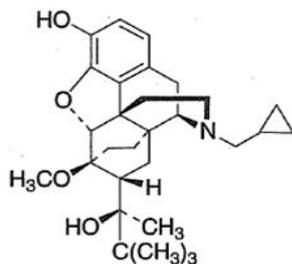
BU in biological samples analyzed mainly using chromatographic methods such as gas chromatography with electron-capture detector (Everhart *et al.*, 1997), HPLC with UV detector (Tebbett *et al.*, 1985; Hackett *et al.*, 1986); fluorescence detector (Liu *et al.*, 2005; Garrett *et al.*, 1985); electrochemical detector (Garcia-Fernandez *et al.*, 2001) and some hyphenated techniques like HPLC-MS (Polettini *et al.*, 1997; Moody *et al.*, 2002; Pirnay *et al.*, 2006; Rodriguez-Ross *et al.*, 2007). But, as these methods reported for analysis of biological samples, these involve sample preparation steps consisting of extraction and/or derivatization before analysis. Thus, these methods are not suitable for analyzing BU in bulk drug or formulations. Mostafavi *et al.* reported a simultaneous estimation method for BU along with naloxone and noroxymorphone for tablet formulation using HPLC

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with UV detector at 210 nm (Mostafavi *et al.*, 2009). But this method is suitable only for those formulations where no excipient absorbs at this wavelength range. To improve bioavailability of BU, researchers formulate it into novel drug delivery systems like microemulsion, which contain excipients like oils, surfactants, co-surfactants etc. Many of these components are known to absorb in the 200 – 250 nm region of UV spectrum (Baboota *et al.*, 2007; Wuelfing *et al.*, 2006; Johnson, 2013). In this case method reported by Mostafavi *et al.* (2009) and Tebbett *et al.* (1985) is not suitable. Thus, there is a need to develop analytical method for quantitative estimation of BU in microemulsion formulation.



**Fig.1:** Chemical structure of Buprenorphine (BU).

The aim of the present study was to develop a simple, efficient, selective and validated RP-HPLC procedure for estimation of buprenorphine in ME formulation. The method requires no extraction or derivatization steps reducing additional chromatographic interferences and complexity of the method. The method validated according to the International Conference on Harmonization (ICH) guidelines Q2 (R1) (ICH, 2005).

According to the current ICH “Stability Testing Guidelines” Q1 A (R2), forced degradation studies of BU were also carried out (ICH, 2003) to give additional information of stability of BU during formulation, analysis and storage (Reynolds *et al.*, 2002).

## MATERIALS AND METHODS

BU-ME developed in the Centre for Advanced Research and Innovation (CARIn), Zim Laboratories, used for analytical method development. All other reagents were of HPLC grade obtained from Merck Specialties Pvt. Ltd. Water purified via a Milli-Q® Reference System, Millipore SAS (Molsheim, France) used for all purposes.

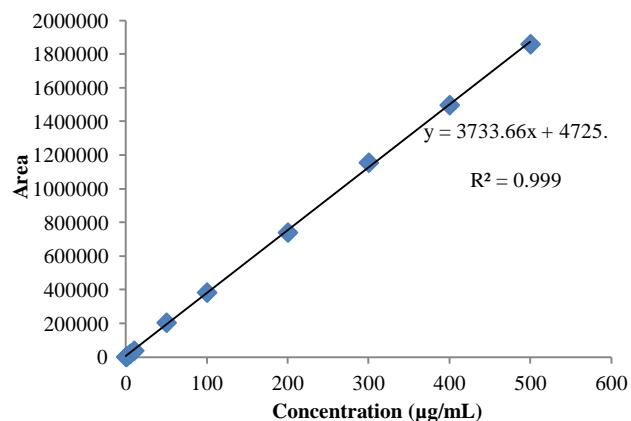
### Standard solution

Standard stock solution of BU was prepared in methanol to obtain a concentration of 500 µg mL<sup>-1</sup>.

### Working standard solutions

Working standard solutions at nine levels were prepared by appropriately diluting standard stock solution in the

concentration range of 1.0–500 µg mL<sup>-1</sup> for BU. Samples in triplicates were prepared for each concentration and peak areas were plotted against the corresponding concentrations to obtain the calibration graph as shown in Fig. 2.



**Fig. 2:** Calibration graph for Buprenorphine HCl

### Sample preparation

The accurately weighed quantity of ME containing equivalent to about 4.0 mg dose of BU was accurately weighed and transferred to a 100 mL volumetric flask. Approximately 15 mL of methanol was added to the flask and the contents were vortex-mixed for 10 min. The flask was adjusted to volume with the mobile phase and mixed well. The resulting solution was filtered using 0.45 µm Polytetrafluoroethylene (PTFE) filter into standard analytical glass vials and injected into the HPLC. Samples were prepared in triplicate and injected into the HPLC.

## METHOD DEVELOPMENT

### Instrumentation and chromatographic conditions

The HPLC system consisted of a LC-2010C HT LIQUID CHROMATOGRAPHY SHIMADZU (Kyoto, Japan) equipped with a quaternary pump, online degasser, column heater, autosampler and UV detector. Data collection and analyses were performed using SHIMADZU LC Solution software. Separation was achieved on C-18 column, PrincetonSPHER -100 C18 HPLC column (250 mm × 4.6 mm, 5 µm). The elution was isocratic with mobile phase consisting of acetonitrile and 10 mmol L<sup>-1</sup> potassium phosphate buffer adjusted to pH 6.0 with triethanolamine (83:17, v/v). The flow rate was 1.0 mL min<sup>-1</sup> that yielded a backpressure of about 1654 psi. The column temperature was maintained at 30°C, the detection was monitored at a wavelength of 284 nm and injection volume was 20 µL.

### Instrumentation and chromatographic conditions for forced degradation study

The forced degradation studies carried out using UFLC system consisted of a LC-30 AD Nexera Liquid Chromatography (Spinotech Pvt Ltd) equipped with a online degasser (DGU-20As

prominence degasser), column heater (CTO-20 AC prominence column oven), auto sampler (Sil-30AC Nexera auto sampler) and UV detector (SPD-20 A prominence UV/vis detector) and DAD detector (SPD-M 20A prominence diode array detector) was used to determine the peak purity. Analysis was performed at ambient temperature and detection was carried out in the range of 200 – 400 nm. Column specifications, mobile phase, flow rate and injection volume was same as mention under Instrumentation and chromatographic conditions.

#### Calibration graph for chromatographic measurement of BU

The area were measured for BU in the concentration range of 1.0, 5.0, 10.0, 50.0, 100.0, 200.0, 300.0, 400.0, 500.0  $\mu\text{g mL}^{-1}$  using above mentioned chromatographic conditions. Calibration graphs were plotted for BU at concentration range of 1.0-500  $\mu\text{g mL}^{-1}$  as shown in Fig. 2.

#### UV spectrum of Placebo ME

The accurately measured amount of placebo ME taken in 10.0 mL of volumetric flask and volume was made with methanol. This solution was then scanned from 200 to 400 nm with the UV spectrophotometer (Shimadzu 1800 UV–Vis double beam spectrophotometer with UV probe software) using methanol as a blank. The UV spectrum of placebo ME system containing Tween 20 was shown in the Fig.3.

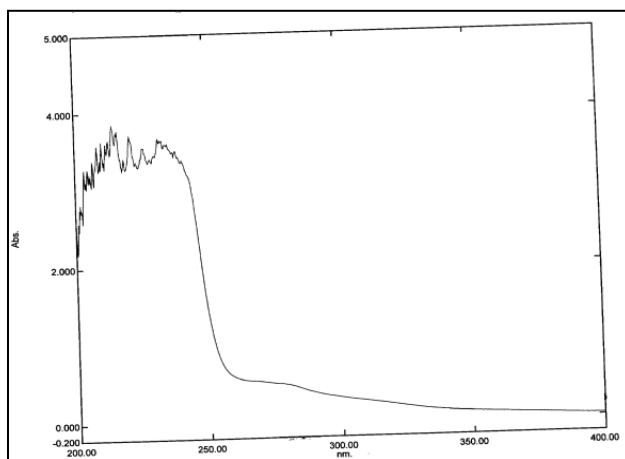


Fig. 3: UV spectrum of placebo ME system containing Tween 20.

#### UV spectrum of Buprenorphine HCl

Stock solution of BU was prepared by dissolving 20.0 mg of BU in 50 ml volumetric flask and volume was made with methanol. Further working standard solution was prepared by pipetting 5.0 ml of stock solution in 10 ml volumetric flask and volume was made with methanol to get concentration of 200.0  $\mu\text{g mL}^{-1}$  of BU. This solution was then scanned from 250 to 400 nm with the UV spectrophotometer (Shimadzu 1800 UV–Vis double beam spectrophotometer with UV probe software) using methanol as a blank. The UV spectrum of BU was shown in the Fig.4.

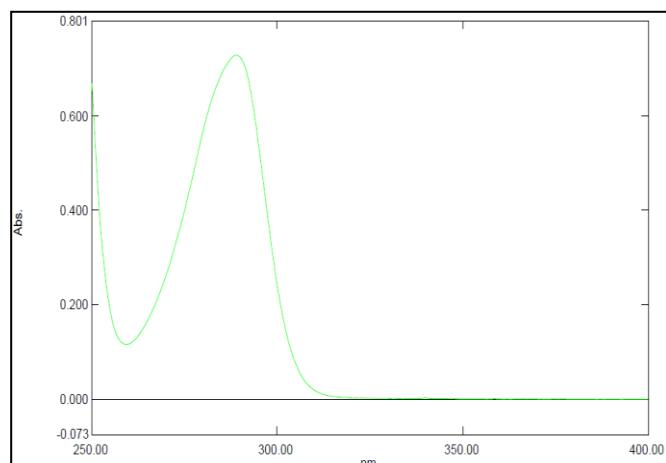


Fig. 4: UV spectrum of Buprenorphine HCl.

#### METHOD VALIDATION

The method was validated according to the ICH guidelines (ICH, 2005). The following validation characteristics were addressed: linearity, accuracy, precision, specificity, limits of detection and quantitation and robustness.

#### System suitability testing (SST)

System suitability standard solution containing 40  $\mu\text{g mL}^{-1}$  of BU was prepared by appropriately diluting and mixing the standard stock solution. System suitability was determined by injecting six injections of the system suitability standard before sample analysis.

#### Linearity and range

Standard calibration curve for BU was prepared with nine calibrations over a concentration range of 1.0 – 500.0  $\mu\text{g mL}^{-1}$  in triplicate. The data of peak area versus concentration were treated by linear least square regression analysis.

#### Accuracy

To study the reliability and suitability of the developed method, recovery experiments carried out. Placebo samples spiked with different amount of BU at 50, 100 and 150% in duplicate for each one ( $n = 6$ ) over the theoretical values. Measured values were compared with the theoretical concentration. Recovery for pharmaceutical formulations should be within the range  $100 \pm 5\%$ . The % R.S.D. of each measurement determined, which should be less than 2 %.

#### Precision

The precision of the developed method was assessed in terms of repeatability and intermediate precision by analyzing 40  $\mu\text{g mL}^{-1}$  BU sample solution in triplicate. The % R.S.D. values of the results corresponding to the peak area and retention time were expressed for intra-day precision and for 3 days for inter-day precision.

## Specificity

Placebo samples were analysed to demonstrate absence of interference with the elution of the BU. For determining selectivity of the method, placebo containing all the excipients used in the formulation of ME, was analyzed. All chromatograms were examined to determine if BU was co-eluted with any additional excipients peak.

## Limits of Detection and Quantization (LOD and LOQ)

LOD and LOQ were calculated based on the standard deviation of the response and the slope of the calibration curve. The standard deviation of y-intercepts of regression lines was used as the standard deviation of responses (ICH, 2005). These values were obtained using the following equations:

$$\text{LOD} = 3.3 \sigma / S \quad (\text{Eq. 1})$$

$$\text{LOQ} = 10.0 \sigma / S \quad (\text{Eq. 2})$$

Where,  $\sigma$  = the standard deviation of the response

$S$  = the slope of the calibration curve

## Robustness

The robustness of the method was evaluated by analyzing the system suitability standards and evaluating system suitability parameter data after varying, individually, the HPLC pump flow rate ( $\pm 10\%$ ) and column compartment temperature ( $\pm 14\%$ ).

## Forced degradation studies of buprenorphine HCl microemulsion

Forced degradation studies of BU ME carried out under stress conditions according to ICH regulatory guidance Q1A (R2) (ICH, 2003). The ME formulation and its placebo used for the forced degradation studies to show the stability of BU under stress conditions and specificity of proposed method.

## Hydrolytic condition: acid, base, water induced degradation

Appropriate quantity of BU ME containing 2.0 mg of BU was treated with 5.0 ml of 0.05 M HCl, 0.05 M NaOH and water separately. These samples were refluxed for 1 hr at 60°C in a heating bath (Equitron Round Bath, Medica Instrument Mfg co.). After cooling, the solutions were neutralized with 5.0 ml of 0.05 M NaOH and 0.05 M HCl for acid and base induced degradation studies respectively and the solutions were diluted with 10.0 ml of methanol and further volume was made up with mobile phase in a 50.0 mL volumetric flask. Accurately measured 4.0 mL of each solution was pipetted in 10.0 mL volumetric flask and volume was made up with mobile phase. These acid, base and water induced degradation studies were repeated for placebo ME giving similar treatment as given to BU ME. These samples were filtered using a 0.22 µm membrane syringe filter and injected into prominence UFC system with DAD detector. The total chromatographic run time was about double the retention of the drug peak.

## Oxidative condition: hydrogen peroxide induced degradation

Appropriate quantity of BU ME containing 2.0 mg of BU treated with 5.0 ml of 30 %  $H_2O_2$  and refluxed for 1 hr at 60°C in a heating bath (Equitron Round Bath, Medica Instrument Mfg co.). After cooling, same treatment was given to this solution as described under hydrolytic condition: acid, base, water induced degradation. The same experiment repeated for placebo ME.

## Thermal degradation study

Appropriate quantity BU ME containing 2.0 mg of BU and placebo ME weighed accurately and placed in glass vial and subjected to heat at 60°C in a hot air oven (Naano Lab India) for 5 hr, then diluted with methanol and mobile phase and analysed as described under hydrolytic condition: acid, base, water induced degradation.

## Photolytic degradation study

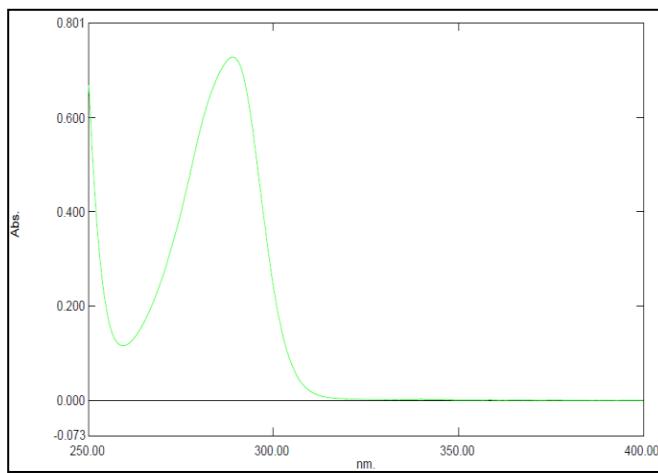
The photochemical stability of the drug studied by exposing same amount of BU ME and placebo ME to sunlight for 5 hr. Then the volume made upto 50.0 mL with methanol. 4.0 mL of this solution was pipetted in 10.0 mL of volumetric flask and volume made with mobile phase.

## RESULTS AND DISCUSSION

### Method development and optimization

BU being highly soluble in methanol i.e. 42.0 mg/mL, hence it is used as solvent for standard and sample preparation (Elephant Care International, 2016). The tween 20 present in the placebo ME system showed absorbance in the range of 200-250 nm (Fig. 3.), when scanned in the UV visible range of 200-400 nm. Hence the previously reported HPLC method for the determination of BU in tablet formulation using detection wavelength at 210.0 nm (Mostafavi *et al.*, 2009) was not suitable for estimation of BU in MEs. The UV spectrum of BU as shown in the Fig. 4, exhibits the  $\lambda_{\text{max}}$  of BU at 289.0 nm in methanol. But, the HPLC method was developed using 284.0 nm as detection wavelength for BU, since at this wavelength no interference of excipients was observed.

Typically, method development focused on identifying buffer type, strength and pH, organic solvent concentration and implementing small changes to optimize selectivity and enhance resolution. At the first stage, a C-18 column with water, methanol and 0.1 % orthophosphoric acid (65:25:10 v/v/v) at pH 3.2 was used as mobile phase. Though the column was previously washed and saturated with mobile phase, peak splitting of BU was observed. Subsequently, the splitting of the peak was overcome by increasing the pH of the mobile phase to 4.4, but the peak shape was not acceptable.



**Fig. 4:** UV spectrum of Buprenorphine HCl.

Then, in another trial, the pH of the mobile phase was kept at 4.4 and the ratio of methanol in the mobile phase increased to 30 % and then to 40 %, but the broadness of the BU peak further goes on increasing.

At the second stage, a C-18 column and potassium phosphate buffer, pH 3.0, was used with acetonitrile as the organic solvent. Though the column was base activated for improved peak shape of basic compound, the peak symmetry of the compound was poor. Subsequently, an acceptable peak shape was achieved by increasing the buffer pH to 6.0, using 83% of acetonitrile as organic solvent. For optimum resolution and peak symmetry, the mobile phase used consists of 10 mmol L<sup>-1</sup> potassium phosphate buffer adjusted to pH 6.0 with triethanolamine and acetonitrile (17:83, v/v). To improve repeatability of runs and reduce back pressure, which is important to extending the column life time, the column oven temperature was set at optimum 30 °C. In the optimized conditions, BU exhibited a sharp peak with better resolution. The optimal wavelength was established experimentally after measuring all spectra in mobile phase and testing the detector response using UV absorbance scanned over the range of 200 – 400 nm. Although the absorbance maxima recommended by BP for BU was 288 nm; it was shown that 284 nm is the optimal wavelength to maximize the sensitivity and has no interference with other components of the formulation.

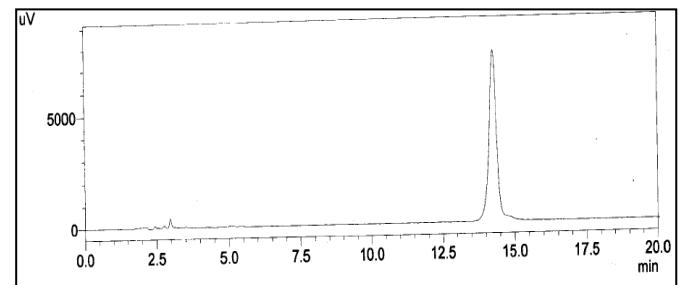
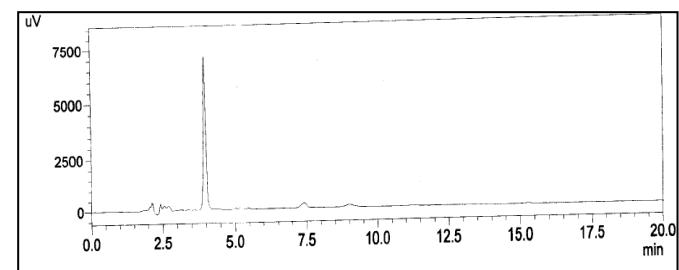
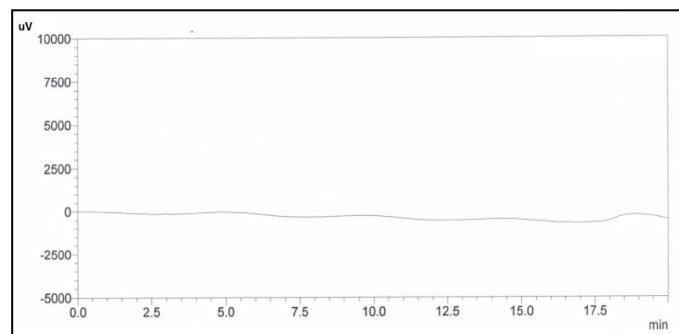
#### Method validation

Developed method must be validated before practical use. By following the ICH guidelines for analytical method validation, Q2 (R1), the SST was performed and the validation characteristics were addressed.

#### System suitability

The system suitability test ensures the validity of the analytical procedure as well as confirms the resolution between different peaks of interest. All critical parameters tested met the acceptance criteria on all days. Representative chromatograms obtained for the mobile phase, placebo and BU standard solution

of 40 µg mL<sup>-1</sup>, shown in the chromatogram, the analyte was eluted by forming symmetrical single peak and was well separated from the solvent front (Fig. 5).



**Fig. 5:** Representative chromatograms obtained for the mobile phase, placebo and BU standard corresponding to the 40 µg mL<sup>-1</sup>.

#### Linearity and range

The results, summarized in Table 1, shows that a good correlation exists between analyte peak area and concentration as denoted by R<sup>2</sup> value (0.999) and in the range of 1.0 µg mL<sup>-1</sup> to 500.0 µg mL<sup>-1</sup>. The Fig. 1 shows the average plot area versus concentration µg mL<sup>-1</sup> for the determination performed in triplicate.

**Table 1:** Linearity parameters for the estimation of BU.

Parameters (Units)	Buprenorphine hydrochloride
Linearity range (µg mL <sup>-1</sup> )	1.0 – 500
Slope	3733.66 ± 1.15
Intercept	4725.0 ± 5.29
Coefficient of determination (R <sup>2</sup> )	0.999 ± 0.0001

Values are reported as mean ± S.D. of three calibration curves generated on three consecutive days (n=3). Nine concentrations in the linearity range were evenly distributed.

## Accuracy

The reliability and suitability of the developed method studied using recovery experiments. For determining accuracy, placebo samples were spiked with different amount of BU at 50, 100 and 150 % in duplicate for each spiked amount over the theoretical values. The recovery was  $100 \pm 5\%$  for all samples with % R.S.D. less than 2 % (Table 2). This indicated that the developed method is accurate.

**Table 2:** Method validation results for BU.

Parameters (Units)	Buprenorphine hydrochloride
SST	
Theoretical plates	14856.925
Peak Area (A) (% R.S.D.)	0.561
Retention time ( $t_R$ ) (% R.S.D.)	0.218
Tailing Factor (% R.S.D.)	0.465
Repeatability <sup>a</sup> , Theoretical plates (% R.S.D.)	1.506
Repeatability <sup>a</sup> , (A) (% R.S.D.)	1.375
Repeatability <sup>a</sup> , ( $t_R$ ) (% R.S.D.)	1.046
Repeatability <sup>a</sup> , Tailing Factor (% R.S.D.)	1.257
Validation	
Precision <sup>b</sup> , Theoretical plates (% R.S.D.)	1.658
Precision <sup>b</sup> , (A) (% R.S.D.)	0.823
Precision <sup>b</sup> , ( $t_R$ ) (% R.S.D.)	0.604
Precision <sup>b</sup> , Tailing Factor (% R.S.D.)	0.754
Accuracy (50 % recovery studies)	99.75
Accuracy (50 % recovery studies) (% R.S.D.)	0.622
Accuracy (100 % recovery studies)	99.93
Accuracy (100 % recovery studies) (% R.S.D.)	0.436
Accuracy (150 % recovery studies)	95.09
Accuracy (150 % recovery studies) (% R.S.D.)	0.128
Specificity	No interference
Sensitivity	
LOD ( $\mu\text{g mL}^{-1}$ )	0.0046
LOQ ( $\mu\text{g mL}^{-1}$ )	0.0141

<sup>a</sup>Intra-day precision (repeatability) were tested with three replicate samples of 40  $\mu\text{g mL}^{-1}$  of BU under the guidance of ICH

<sup>b</sup>Inter-day precision were tested with three replicate samples of 40  $\mu\text{g mL}^{-1}$  of BU on three consecutive days.

## Precision

Intra and inter day precisions were established across the analytical range for BU. The intra- and inter-day precision was calculated using BU standard solution of 40  $\mu\text{g mL}^{-1}$  (Table 2). Repeatability (intra-day precision) of the analytical method was found to be reliable based on % R.S.D. (1.506, 1.375, 1.046 and 1.257) corresponding to the theoretical plates, peak areas, retention times and tailing factor. Similarly, repeatability (inter-day precision) was demonstrated on different days and evaluating the data for theoretical plates, peak areas, retention times and tailing factor that cover the assay method range. The % R.S.D. values were 1.658, 0.823, 0.604 and 0.754 for all evaluated data of inter-day precision and illustrated the good precision for the analytical method.

## Specificity

Injections of the placebo demonstrated absence of interference with the elution of the drug. These results proved that there was no interference from other materials in the ME

formulation; therefore, confirmed the specificity of the method (Fig. 5).

## Limits of Detection and Quantization (LOD and LOQ)

The limit of detection and limit of quantitation determine sensitivity of the method. The LOD and LOQ for BU were 0.0046  $\mu\text{g mL}^{-1}$  and 0.0141  $\mu\text{g mL}^{-1}$ , respectively. These values show that the proposed method has good sensitivity and results are presented in Table 2.

## Robustness

To make sure the insensitivity of the developed HPLC method to minor changes in the experimental conditions, it is important to show its robustness. None of the alterations caused a significant change in peak area and retention time for BU (Table 3). Although the changes in retention times and area were more significant, with increase or decrease in flow rate and temperature, but quantitation was still possible.

**Table 3:** Method validation data for robustness study of BU.

Parameter altered (Units)	Area	% of change Area	Retention time, $t_R$ (min)	% of change $t_R$ (min)
Increased flow rate (1.1 $\text{mL min}^{-1}$ )	140135	8.21	12.974	9.00
Decreased flow rate (0.9 $\text{mL min}^{-1}$ )	160199	4.92	15.609	9.47
Increased column temperature (35 °C)	144657	5.25	14.293	0.24
Decreased column temperature (25 °C)	138870	9.04	14.847	4.13

## Analysis of the developed microemulsion formulation

The validated method was used in the analysis of developed BU ME formulation with dose strength of 4.0 mg BU per unit. Representative chromatogram is shown in Fig. 6. The result for the drugs assay was in good agreement with the label claims. BU content was between 99.10 and 100.4%.

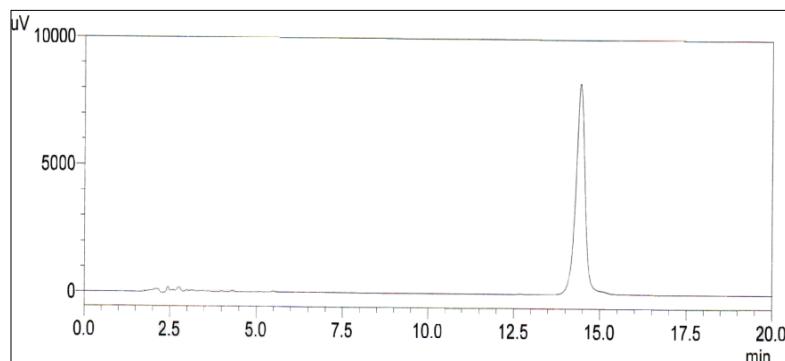
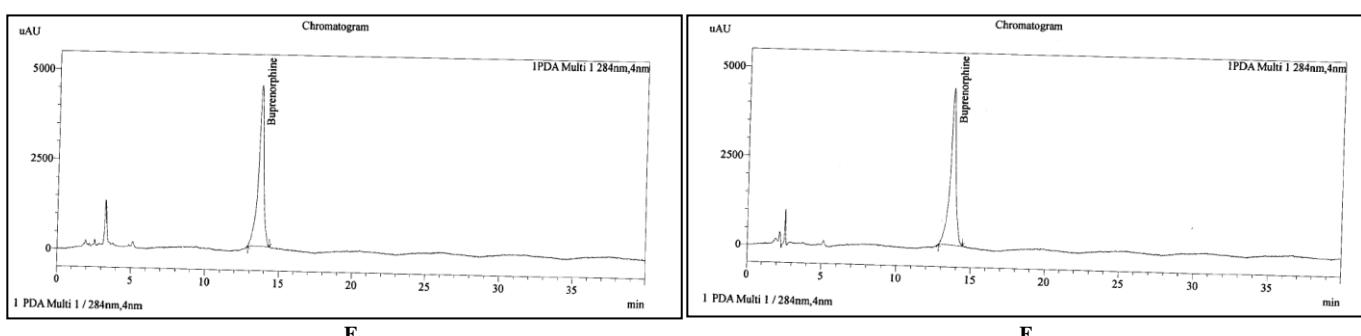
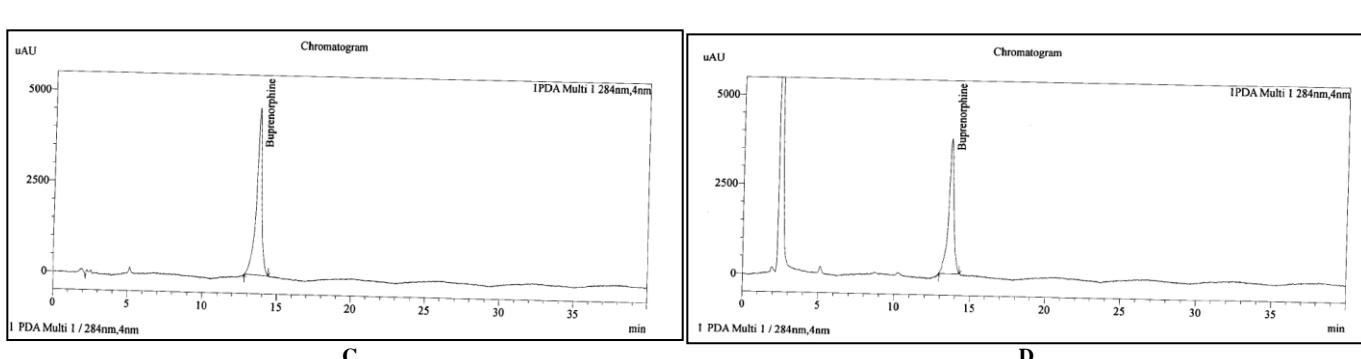
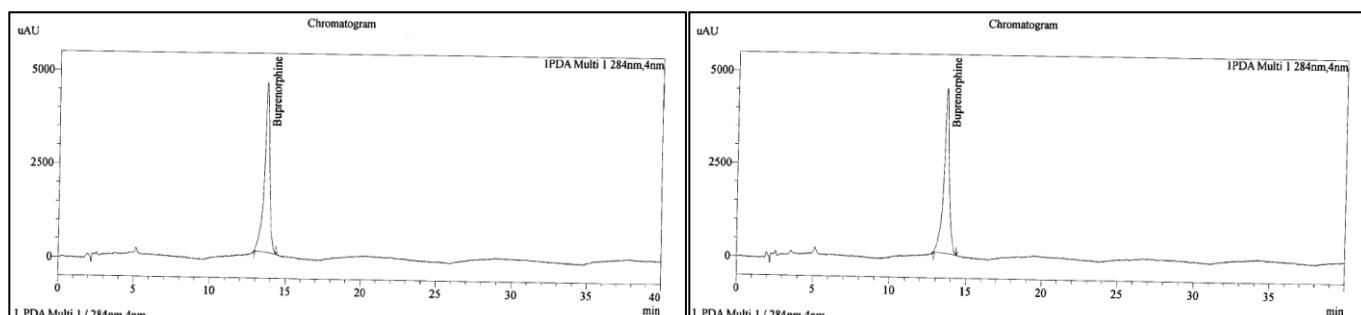
## Forced degradation study/specificity

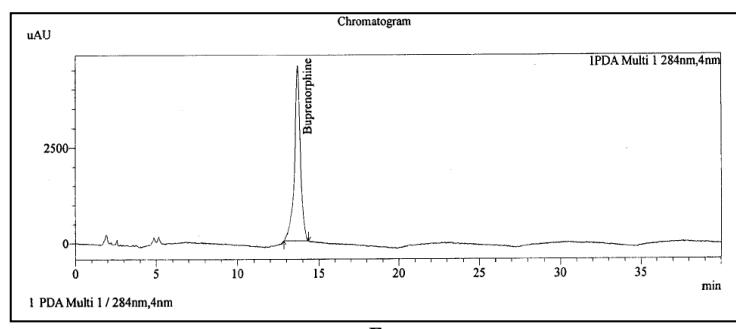
Forced degradation studies performed on BU ME and its placebo demonstrated selectivity and stability indicating capability of the proposed RP-HPLC method. Accordingly the degradation stress studies were conducted by exposing BU to acid, base, peroxide, water, light and heat.

Observed that around 2.42% and 3.59% drug degradation (Table 4) obtained on refluxing at 60°C in 0.05 M HCl and 0.05 M NaOH for 1 hr, respectively; but there was no elution of corresponding degradation products as compared to the standard unstressed solution of BU ME as well as its placebo (Fig. 7 (a, b) and Fig. 8 (a, b)). In neutral condition negligible amount of degradation observed i.e. 0.06 % on refluxing at 60°C for 1 hr (Fig. 7 (c) and Fig. 8 (c)). Peak purity index for BU peak in acidic, basic and neutral condition was within acceptance criteria i.e. not less than 0.990 as shown in Fig. 9 (a, b and c).

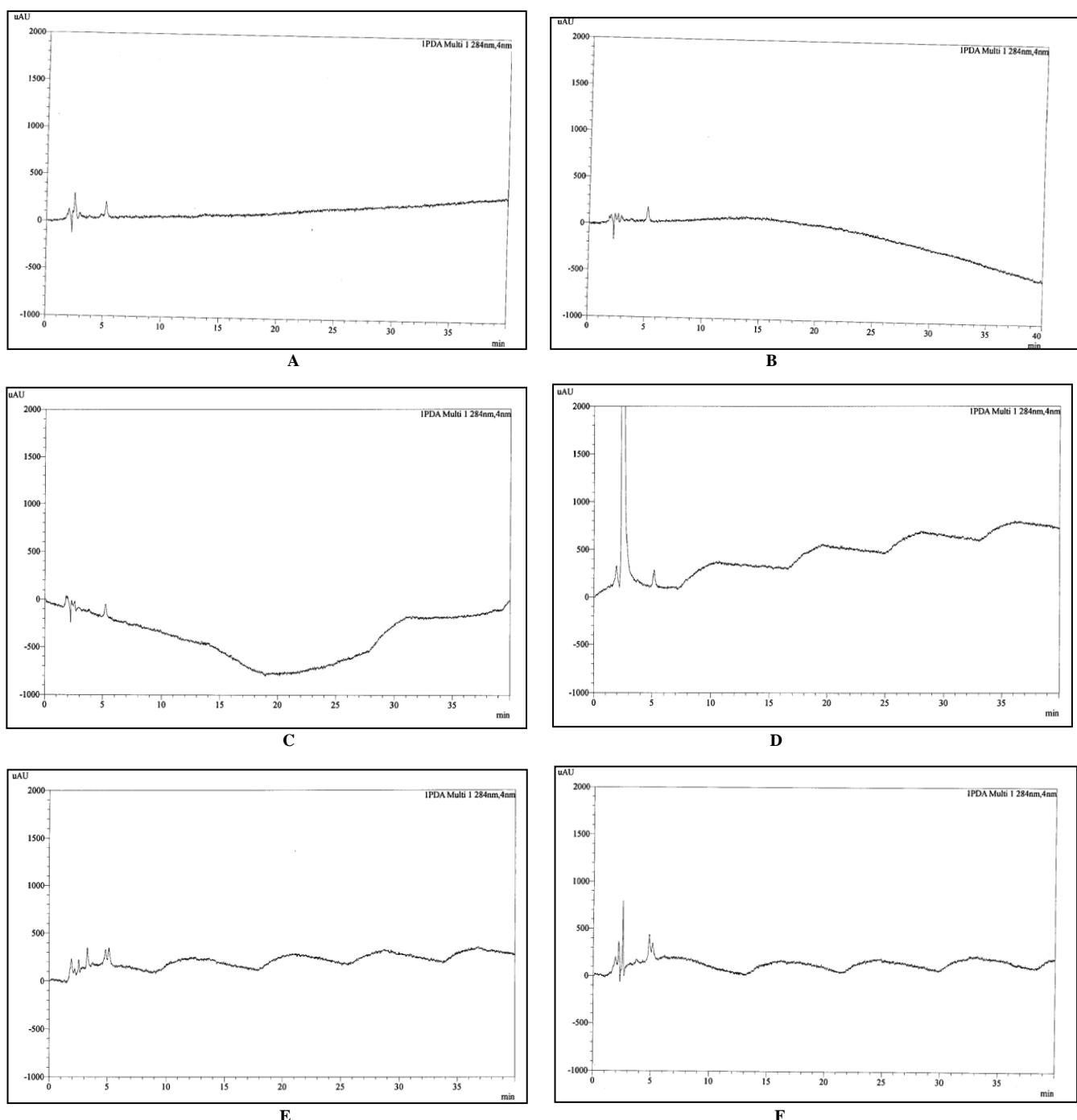
**Table 4:** Forced degradation study data for BU ME.

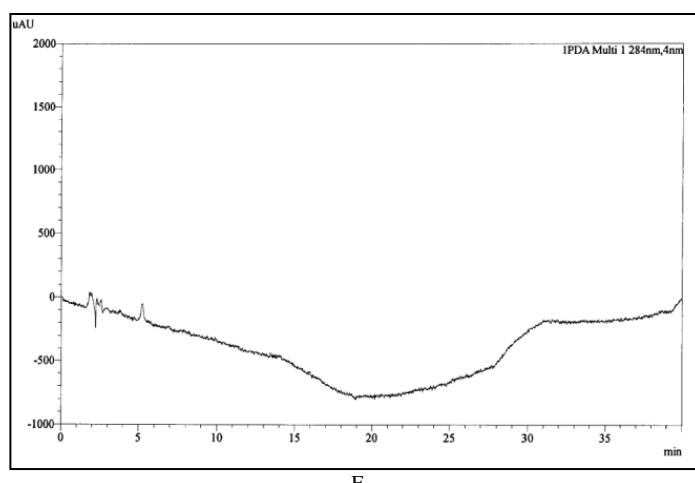
Stress condition	Assay degraded/ Unstressed sample (%)	Peak purity test for BU peak		
		Impurity	Peak purity index	Single point Threshold
Unstressed	99.29	Not Detected	1.00	0.999
Acidic (5.0 mL 0.05 M HCl) 60°C, 1 hr	96.87	Not Detected	1.00	0.999
Alkaline (5.0 mL 0.05 M NaOH) 60°C, 1 hr	95.71	Not Detected	1.00	0.999
Neutral (5.0 mL H <sub>2</sub> O) 60°C, 1 hr	99.23	Not Detected	0.99	0.999
Oxidation (5.0 mL 30 % H <sub>2</sub> O <sub>2</sub> ) 60°C, 1 hr	78.30	Not Detected	1.00	0.999
Thermal 60°C, 1 hr	97.95	Not Detected	1.00	0.999
Photolytic (daylight) 5 hr	99.27	Not Detected	1.00	0.999

**Fig. 6:** Representative chromatogram obtained for developed BU ME

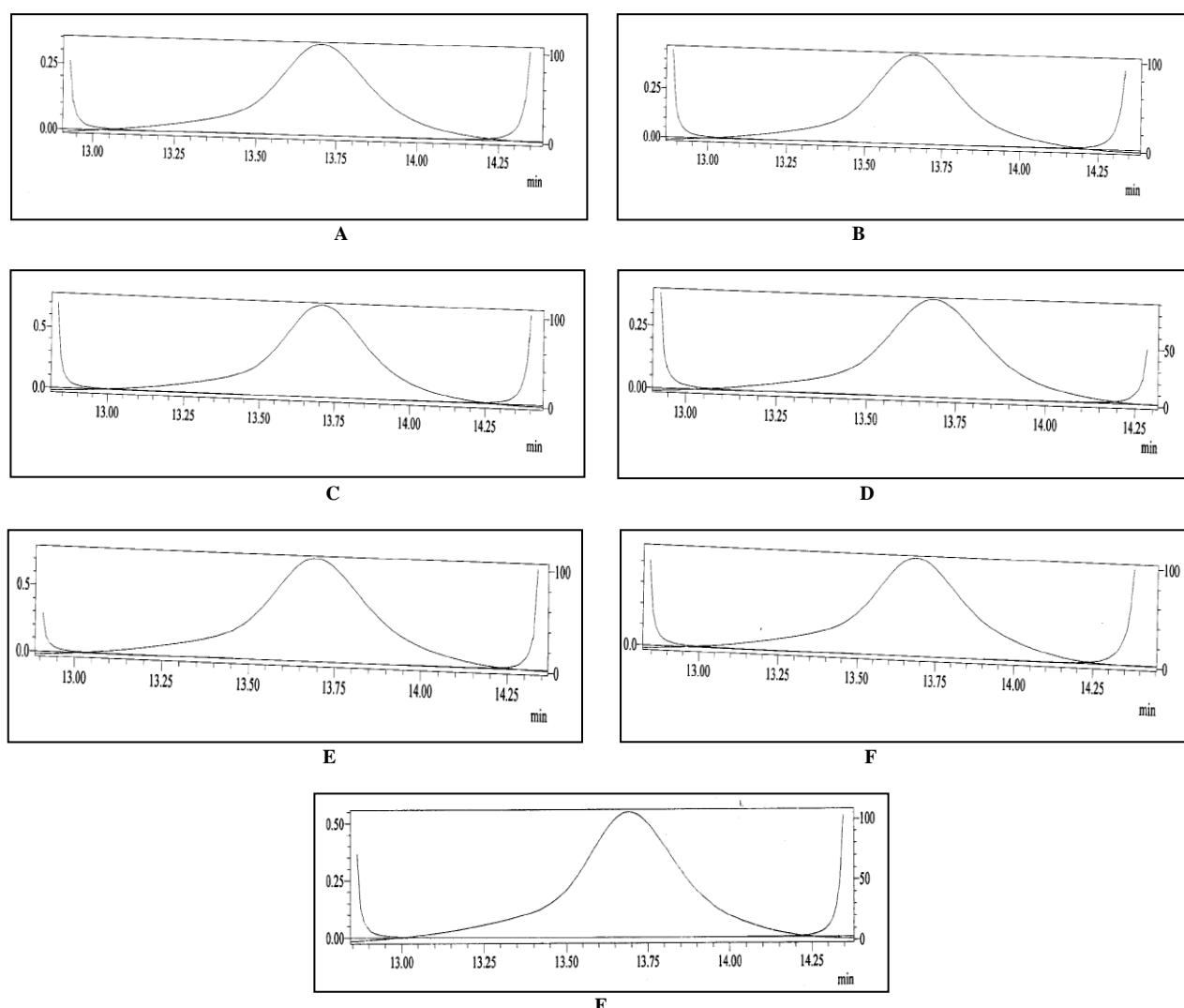


**Fig. 7: Fig. 7:** HPLC chromatogram of BU ME in (A) 0.05 HCl at 0°C (B) 0.05 M NaOH at 0 C; (C) neutral condition ( $\text{H}_2\text{O}$ ) at 0°C; (D) oxidative condition ( $\text{H}_2\text{O}_2$ ) at 0°C; (E) Thermal degradation condition 0 C; (F) Photolytic degradation condition (exposure to sunlight); (G) Unstressed condition.





**Fig. 8:** HPLC chromatogram of placebo ME in (A) 0.05 M HCl at 60 C; (B) 0.05 M NaOH at 60 C; (C) neutral condition ( $H_2O$ ) at 60 C; (D) oxidative condition ( $H_2O_2$ ) at 60 C; (E) Thermal degradation condition 60 C; (F) Photolytic degradation condition (exposure to sunlight); (G) Unstressed condition.



**Fig. 9:** Peak purity graph of buprenorphine HCl in (A) 0.05 M HCl at 60 C; (B) 0.05 M NaOH at 60 C; (C) neutral condition ( $H_2O$ ) at 60 C; (D) oxidative condition ( $H_2O_2$ ) at 60 C; (E) Thermal degradation condition at 60 C; (F) Photolytic degradation condition (exposure to sunlight); (G) Unstressed condition.

Observed that around 20.99 % drug degradation obtained on refluxing at 60°C in 30 % H<sub>2</sub>O<sub>2</sub> for 1 hr and there was no corresponding formation of degradation products as compared to its placebo when given the similar treatment as shown in Fig. 7 (d) and 8 (d). About 1.35 % and 0.02 % of degradation was observed in thermal and photolytic stress conditions as shown in Fig. 7 (e, f) and 8 (e, f), respectively. Peak purity index for BU peak in oxidative, thermal and photolytic condition was within acceptance criteria i.e. not less than 0.990, represented in Fig. 9 (d, e and f).

There was no interference of the sample diluents at the retention time of the BU peak. Moreover the peak purity test successfully passed for the BU peak in analysis of all the stressed samples and thus confirmed the spectral clearness of the principle peak. The purity and assay of BU was unaffected, which confirms that the developed method is stability-indicating method.

A simple, selective and precise stability indicating isocratic, reversed-phase HPLC method developed and found accurate, precise, linear and robust across the analytical range and robust. The method was specific for the determination of BU in a developed ME formulation. All the parameters met the criteria of the ICH guidelines for method validation. Forced degradation studies on BU ME and its placebo was conducted and the results revealed that the drug was liable under oxidative stress condition where as stable under acidic, basic and neutral hydrolysis, thermal and photolytic degradation conditions. The method could therefore be recommended for routine quality control analysis of raw material and various BU dosage formulations by assaying for potency and accuracy.

## PERMISSION

License granted to M/s Zim Laboratories Ltd. by food and Drug Administration M.S. for working with Narcotic Drugs and Psychotropic Substances.

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