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Synthesis and isolation of ion association complex of Bupivacaine HCl and Its Spectrophotometric Estimation in bulk and pharmaceutical Preparations

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ABSTRACT

A simple spectrophotometric method was developed by using the picric acid as an ion pair reagent with Bupivacaine HCl (BH) in bulk and injection dosage forms. The ion-Association complex formed between BH and PA was extracted with chloroform and identified its reaction sites by FTIR. The extracted yellow colored solution of complex showed maximum absorbance at 410 nm wave length. The bear and lambert law was obeyed at range of 10-100 ppm. The limit of detection, Limit of Quantification, slop and regression coefficient (r2) was respectively 5µg, 10-20µg, 0.455 and 0.9998. The developed and validated analytical method was successfully applied for the quantitation of pharmaceutical preparations. The recoveries and low standard deviations confirm the suitability of spiked analytical method.

Keywords: Ion-association complex, Bupivacaine HCL (BH), picric acid (PA), Validation, Stability

1. INTRODUCTION

Bupivacaine HCl is a local anesthetic drug, Chemical name is {(Rs)-1-butyl -N-(2,6 dimethyl) pipenidine-2-carboxamide}, (Fig-01). It is white crystalline powder that is freely soluble in water and 90% Ethanol, slightly soluble in Acetone and Chloroform [1]. It contains amino-Amide group, which is generally an anesthetic. The anesthetics which contain amino-amide group are more stable and have low side effects for allergic reaction [2]. The intracellular partisan of sodium channels and its influx into nerve cells are bonded by Bupivacaine. As compare to other local anesthetics, bupivacaine is cardio toxic

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however its adverse drug reactions are very rare if it is administered properly [3]. The side effects of bupivacaine are ringing in the ears, muscles twitching, sleepiness, change in vision, low blood pressure and irregular heart rate [4]. Extensive literature survey reveals that only few HPLC and spectrophotometric methods have been reported [5-7], (Fig 01).

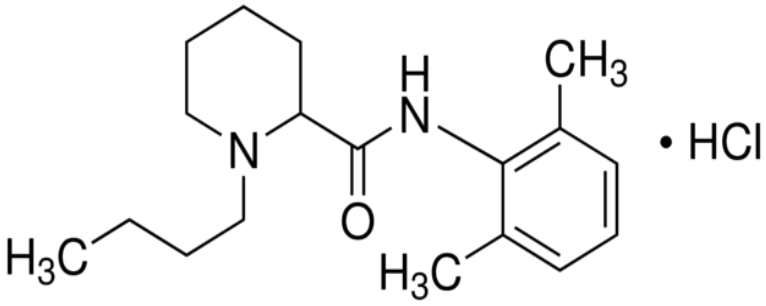

Fig 01;- Bupivacaine HCl

2. MATERIALS AND METHOD

Reagents Preparation

The bupivacaine active ingredient Samples were received from china (zing how pharma China). The picric acid and Lab Grade chloroform were purchased from fisher scientific. All purchased reagents were used without further purification.

Equipments

The spectrophotometer uv-vis-1600 (Shemadzu Japan Corporation) having a set of 1 cm quartz cell was used which is attached with EPISON LX 300 printer. The FTIR rang 650cm^{-1} – 4000cm^{-1} with minimum resolution limit 8cm^{-1} of Agilent Technologies were used throughout research work.

Procedure of ion-association complex synthesis

Weighed 0.229 gm picric acid and 0.329 gm of bupivacaine HCl, and transferred into separate beaker and dissolved in 10ml of chloroform and distilled water respectively. Transferred both solutions in separatory funnels and shacked reaction mixtures for about 5 to 10 mints, The organic layer was separated in another beaker, which was evaporated and dried.

Preparation of Solutions

Standard Stock Solution:

Accurately weighed 100mg bupivacaine HCl in 100ml volumetric flask, The distilled water was used to dissolve the sample, after sonication volume was made up to the mark with same solvent.

Preparation of Picric Acid Reagent:

0.5 g Sample of picric Acid was weighed accurately and transferred into 100ml volumetric Flask, chloroform was used as diluent, the final concentration of solution was 5mg/ml.

General procedures

The 0.2, 0.4, 0.8 and 1.0ml standard stock solution of Bupivacaine HCl was transferred in separatory funnels. 1 ml (5mg per ml) stock solution of picric acid and 1ml of phosphate buffer pH 7.4 were added in each solution and mixed well. The ion pair complex formed in the presence of buffer, between picric acid and Bupivacaine. All samples were extracted with chloroform. The organic layer was separated in 10 ml volumetric flask and made up the volume up to mark. Prepare blank without Bupivacaine HCl as per general procedure.

Pharmaceutical Preparation

The purposed testing procedure was applied on locally available pharmaceutical dosage forms, which are listed as following.

1. Bucaine® Ophth Pharma (Pvt). Ltd.
2. ABOCAN® ABBOTT Laboratories (Pvt.) Ltd.

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3. BUCAN® BOSH Pharmaceutical (Pvt.) Ltd.

3. RESULTS AND DISCUSSIONS

Selection of Maximum Wave Length

The λ max of ion-association complex was obtained by scanning between 350—500 nm of its 20mcg/ml solution which was prepared in chloroform. The yellow colored complex showed maximum absorbance at 410nm. The spectrogram obtained by scanning is referred in Fig;- 02.

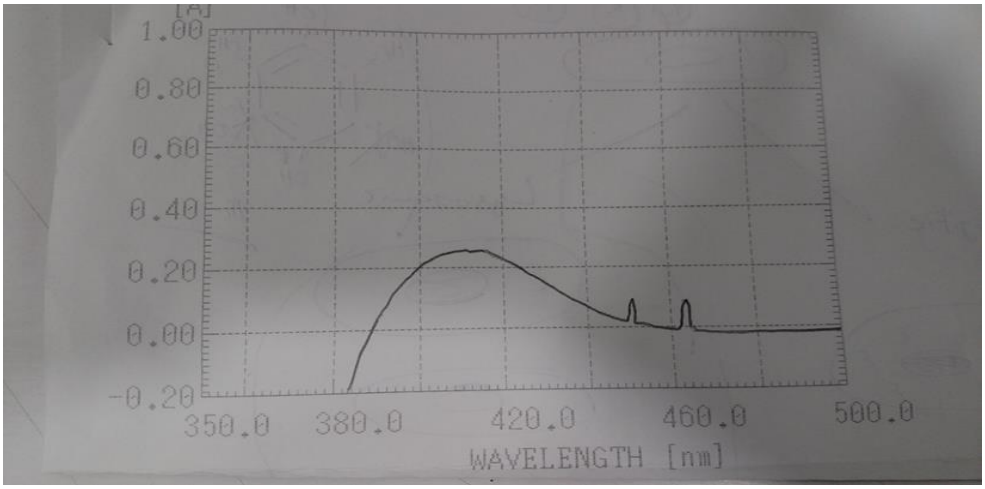
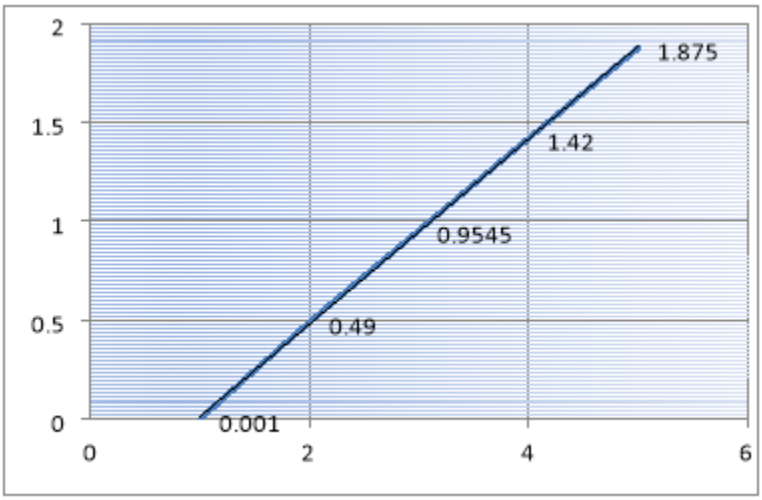


Fig 02;- Spectrogram



Fig; 03 Calibration curve of Bupivacaine

Table 01: Calibration Parameter

S.NO	*Parameters*	*Observations*
1	Slop	0.467
2	LOD	5 mcg
3	LOQ	10-20 mcg
4	r^2	0.9998
5	Intercept	0.455
6	Linear Equation Y=mx + c	Y= 0.467X + 0.455
7	λ Max	410 nm

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FTIR Spectra;-

For the purpose of identifying the reactions sites of Bupivacaine HCl and picric acid, the ion-association complex was synthesized and it was isolated by extraction. The FTIR spectra of isolated ion association complex, Bupivacaine HCL and picric acid are shown respectively in Fig 04a, b, and c. In the spectra of ion association complex the weak bands between 3300cm^{-1} and 2500cm^{-1} was recorded which can be attributed to the bond of ion association between BH and PA. These weak bands also confirm m (N + H_3) and migration of bonded protons of PA towards amino group of Bupivacaine HCl.

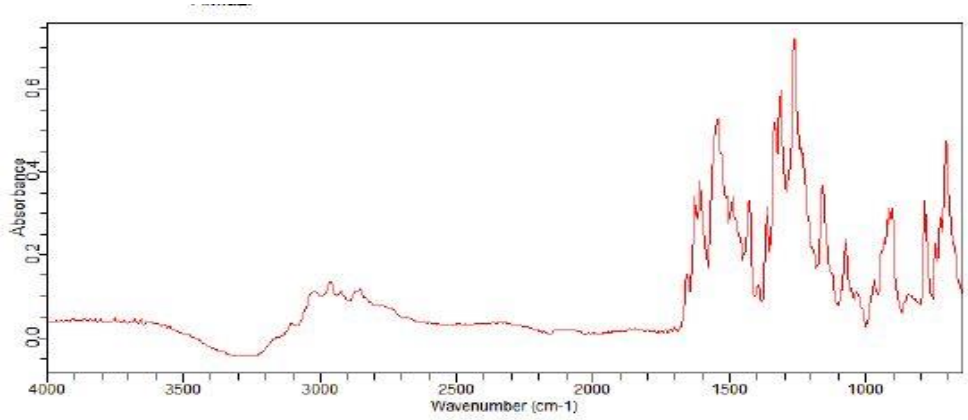

Fig-04a;- Ion Association Complex

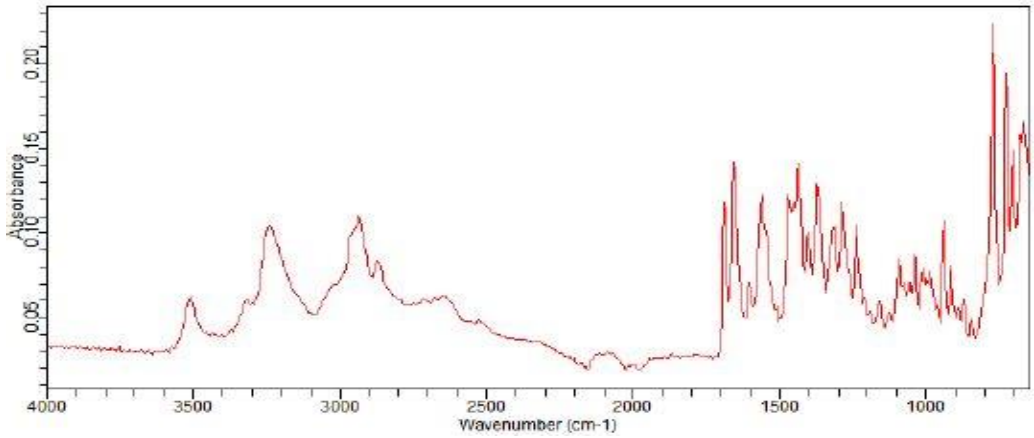

Fig-04b;- Bupivacaine HCl

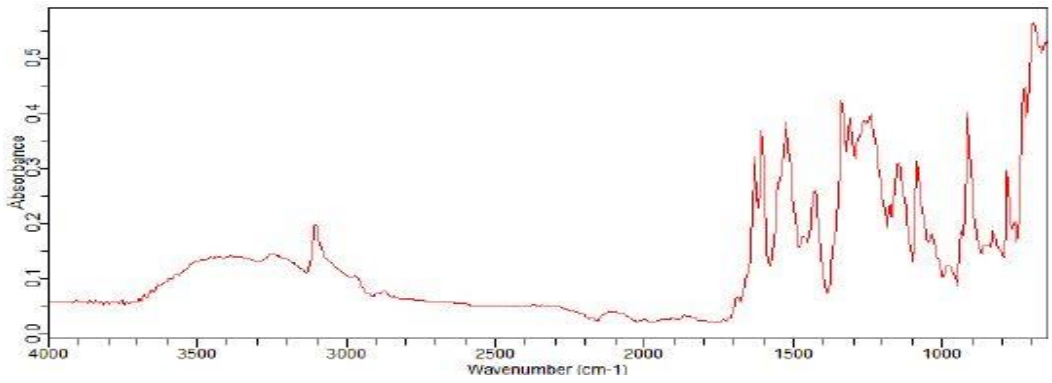

Fig-04c;- PICRICACID

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Determination of Mole Ratio between BH and PA;-

The mole ratio of ion-association complex of PA and BH was determined by using mole ratio method. The ratio 1:1 was determined as according to mole ratio plot which is shown in Fig-05.

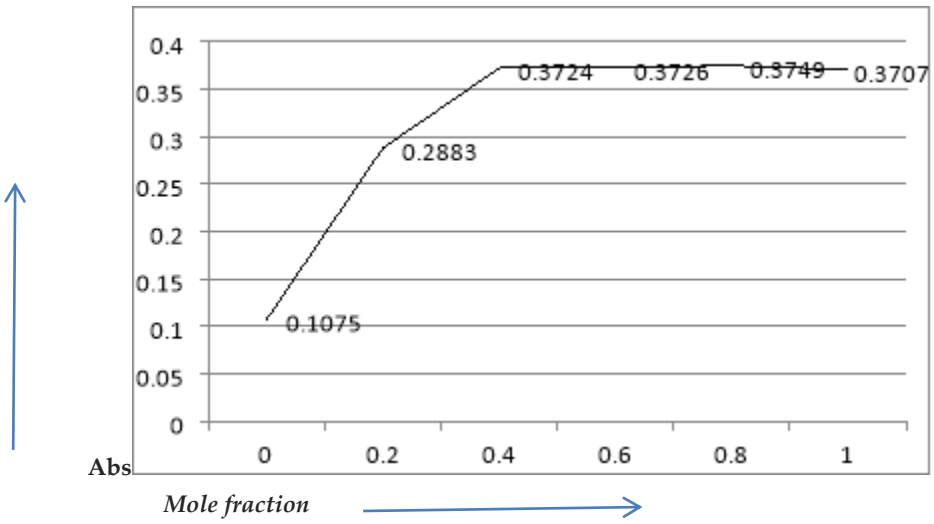

Fig 05: Mole Ratio Plot

Optimization of Effecting Parameters;-

The possible influencing factors are Buffer pH, amount of Buffer, time and temperature on purposed method.

1-Effect of Temperature;-

Three samples of same concentrations were prepared; the absorbances of Samples were taken at 4 °C, 15 °C, 20 °C, 25 °C and 35°C. The observed absorbance was plotted in Fig 06. In this Figure the higher absorbance observed at 4°c and 15°C was due to haziness of test solution developed due to cooling. The absorbance of solution could not be taken at higher temperature then 35°c because of the volatile nature of chloroform.

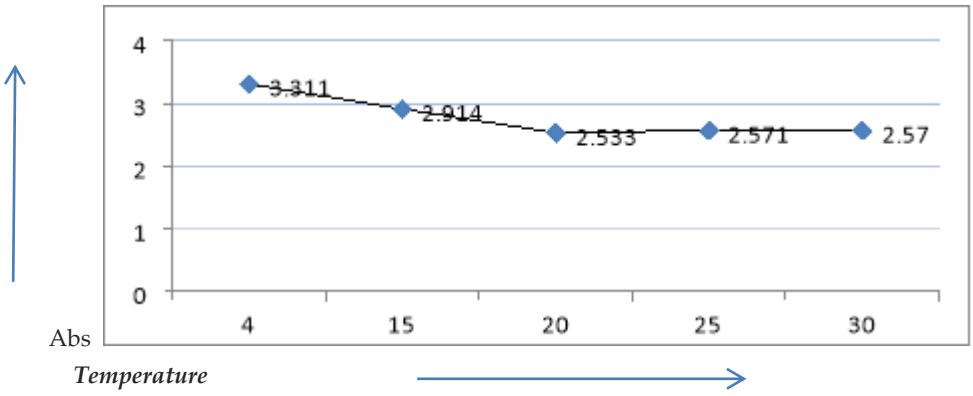

Fig 06; - Effect of temperature

2-Stability of complex with time;-

Three replicate samples (n=3) having same concentration (20mcg/ml). The stability of ion-pair complex with respective time intervals were studied by observing the absorbance of solution at 30, 60, 90 and 120 minutes. The results are presented in Fig07;- The observed results indicate that the complex is very stable, hence the purposed method is durable.

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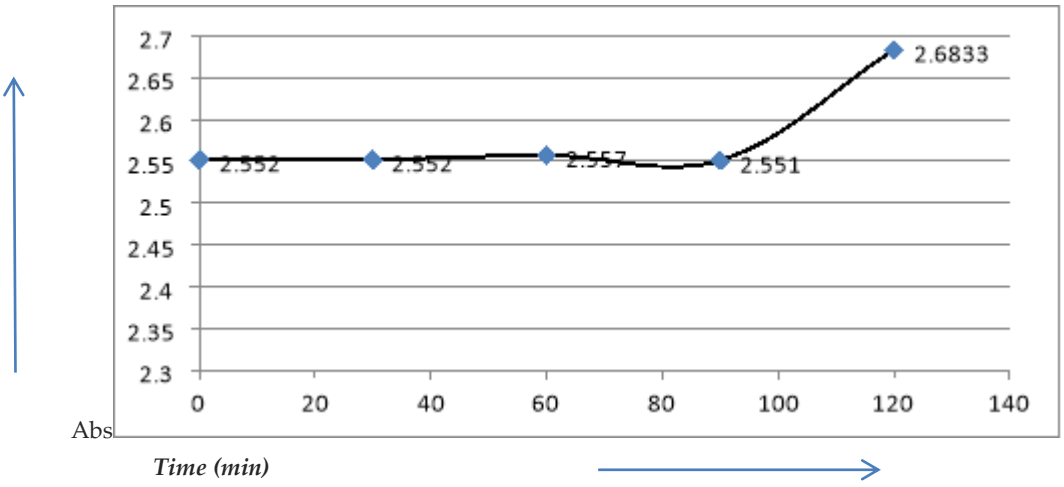

Fig 07;- Stability of Complex with Time

3-Effect of Buffer pH;-

There were four different samples, prepared by keeping the constant concentration of Bupivacaine HCl and PA. Buffer solution of pH 2, 4, 7, and 10 were added with different amounts in each set of samples. The extractions of samples were carried out by using the general procedure. The absorbance of each sample and their replicates were observed at 410 nm. The obtained results are tabulated in Fig 08.

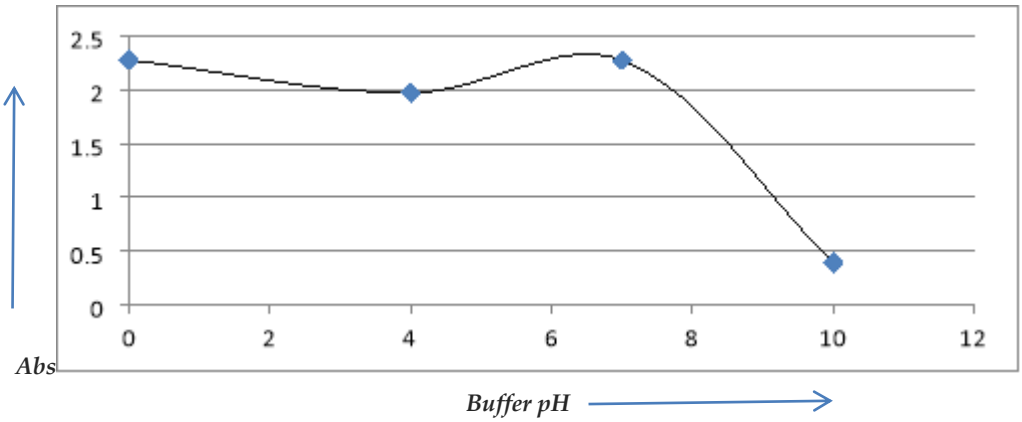

Fig 08;- Effect of pH

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4-Effect of Buffer Volume;-

The different volumes 0.5, 1.0, 1.5, 2.0 and 2.5 ml of buffer pH 7.4 were added in each reaction mixture. The recorded absorbances of complexes were plotted in Fig-09.

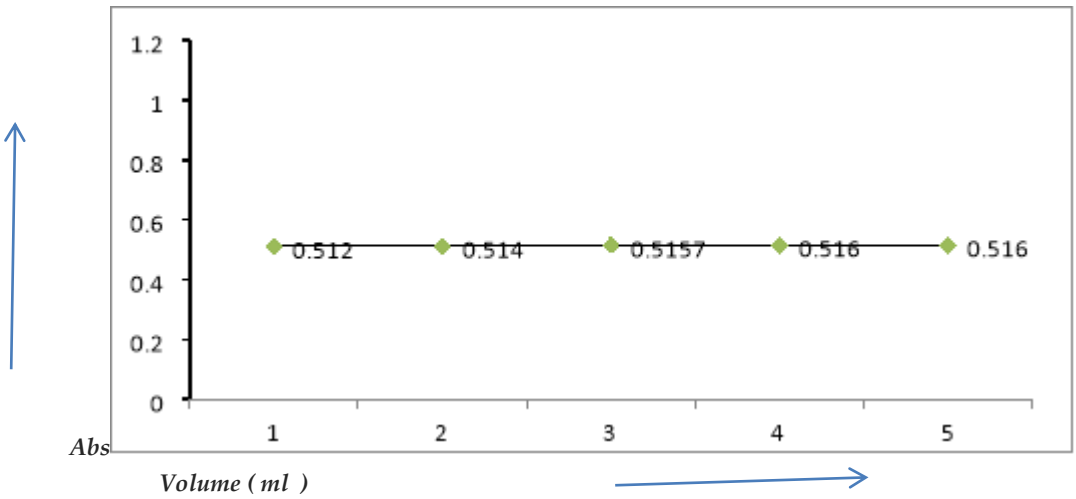

Fig 09;- Effect of Buffer Volume

5-Interference of Na^{+1}, Ca^{+2} and Mg^{+2};-

The 20,40,60,80 and 100 ppm concentration levels of sodium calcium, and magnesium were prepared. The 1.0 ml of each solution Na^{+1}, Ca^{+2} and Mg^{+2} was separately added in ion-association complex before extraction. The reaction mixture was shacked well and was separated by organic phase. The variation in absorbance were recorded against with pure BH and PA complex and plotted in Fig 10.

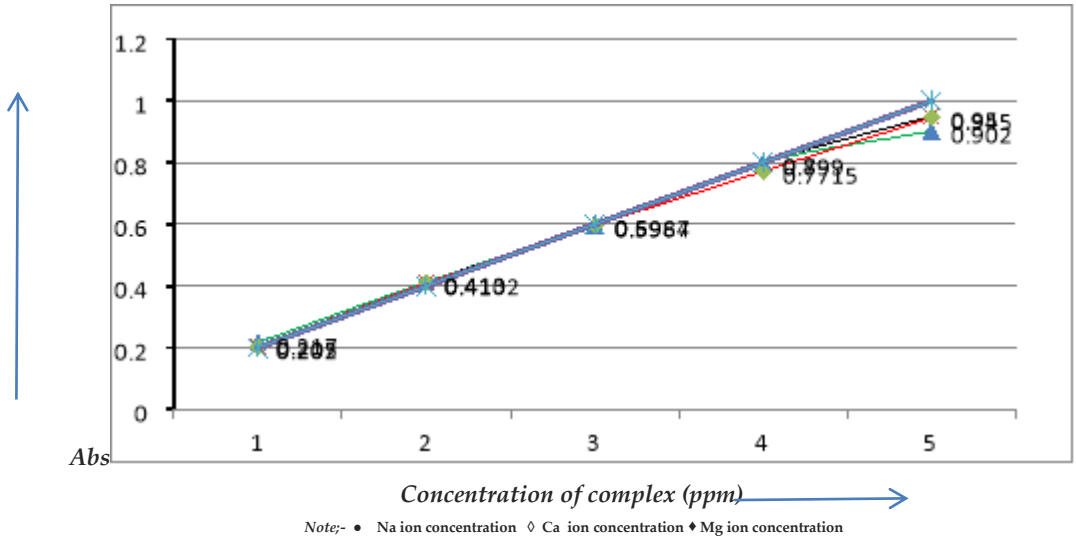

Fig10;- Interference of Na^{+1},Ca^{+2} and Mg^{+2} with respective of complex

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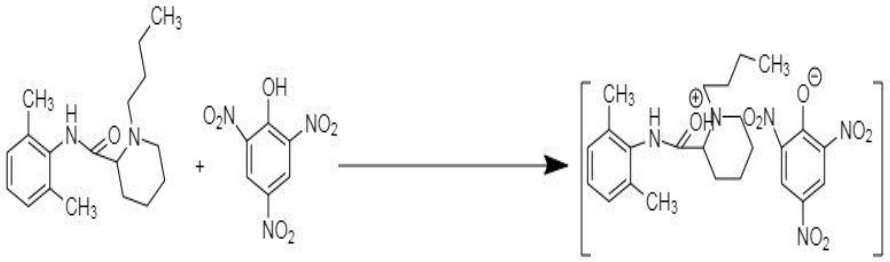
Reaction mechanism



Equation 1;- Ion association reaction between bupivacaine HCl and picric Acid

Analytical Method Validation;-

The purposed analytical method was validated by using the guide lines of ICH (9-10) and USP 40(2017)[8]. The key analytical parameters for method validation were accuracy, precision, Linearity and Ruggedness [9].

Accuracy

The accuracy of purposed method was evaluated in triplicate samples of five concentration levels, 20, 40, 60, 80 and 100 µg/ml [10]. The estimated percentage recovery of each solution was between 98-101.5%. The 0.99%RSD is the indication of good accuracy. The observed results with %RSD are presented in Table 02.

Table 02;- Accuracy

S.NO	Concentration of BH* Ug/ml	% RECOVERY	% RSD
1	20	99.2	0.99%
2	40	98.95	
3	60	100.5	
4	80	101.02	
5	100	99.96	

- BH* =Bupivacaine HCl

Linearity;-

The linearity of analytical method was determined on the concentration rang of 20-100µg from (n= 3) replicate samples [11]. Hence the obtained regression coefficient is 0.9998, slop 0.47 intercept 0.455 are the indication of good correlation between concentration and Absorbance, in purposed method.

LOD & LOQ

The LOD and LOQ of analytical method were determined by using the USP guidelines [12]. The Calculated values of LOD and LOQ are respectively 5µg and 10-20µg.These values are shown, high sensitivity of method.

Precision

The precision of analytical method is the repeatability and reproducibility of results. The precision of method was determined by intraday and Interday calculated test results of six different samples of having same concentration. The %RSD of all Interday and intraday precisions are not more than 2.0%. The results are presented in Table 03.

Table 03;- Precision

Precision	Sample NO	Concentrations of BH9 (µg)	% Results	% RSD
	1		100.1	

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Intraday Precision	2	20 µg	99.9	0.12%
	3		100.2	
	4		100.1	
	5		99.89	
Interday Precision	1	20µg	99.98	0.12%
	2		100.12	
	3		100.1	
	4		100.3	
	4		100.2	
	5		100.3	

Ruggedness

The ruggedness of analytical method was assessed by changing the temperature of reagent at 4c, 15c, 25c and 40c [13]. The results were evaluated as shown in Table -04;-.The %RSD which was less than 2.0%, fairly indicated, the high precession of proposed method.

Table-04;- Evaluation of Ruggedness

Temperature	% Recovery	% RSD
4 C^O	99.96	± 0.825
15 C^O	100.12	
25 C^O	99.48	
40 C^O	98.99	

Robustness

It is the capacity of analytical method, remains unaffected by varying small but deliberate changes in parameters. The results of recovery of Bupivacaine did not show any reasonable change in proposed method.

Application of method for % Recovery of Local Pharmaceutical formulations

Some pharmaceutical formulation available in Local pharmacy was purchased and tested by applying the analytical method and their obtained % Recoveries are tabulated in Table 05.

Table 05;- % Recovery

S.NO	Product Name	% Recovery	% RSD
1	BUCNE INJECTION	**99.9%**	**0.389%**
2	ABOCAN INJECTION	**100.1%**	
3	BUCAN INJECTION	**99.35%**	

4. CONCLUSION

A simple cheap, easy and precise spectrophotometric method has been developed for determination of Bupivacaine HCl in bulk and injection dosage form. The accuracy, precision, specificity and Linearity were optimized for the sack of validation of analytical method.

Funding:

This study has not received any external funding.

Ethical approval

Not applicable.

Conflict of Interest:

The authors declare that there are no conflicts of interests.

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Data and materials availability:

All data associated with this study are present in the paper.

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