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New Method For Spectrophotometric Determination of Lidocaine

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Abstract

This work include prepration the organic reagent 3-hydroxy-4-[(4-hydroxyphenyl)azo]-1-naphthalenesulfonic acid by coupling diazonium of 1-amino-2-naphthol-4-sulfonic acid with phenol .The reagent react with Lidocaine in acidic medium(PH = 4) to produce red colored complex with mole ratio 1:1 (L:M) and maximum absorbance at 510 nm . this method show obey to beers in the range of 1.44 – 69.31 ppm, with molar absorptivity of $4.1633 \times 10^3 \text{ L mole}^{-1} \text{ cm}^{-1}$ Sandell's sensitivity $0.0693 \mu\text{g.cm}^{-2}$ The effects of variables such as pH, time, concentration of color producing reagent, and stability of color were investigated to optimize the procedure. The results are validated statistically. The proposed method was applied to commercially available tablets, and the results were Pharmaceutical formulations.

Keywords : Lidocaine; determination, Spectrophotometry

Introduction

Lidocaine (2-(diethylamino)-N-(2,6-dimethylphenyl) acetamide) (LID). Lidocaine is an amide-type local anesthetic and is described to stabilize the membranes of nerve cells by inhibiting the ionic needed to start and deliver pulses flows and exerts its influence at higher heart rate by raising the threshold of electrical stimulation during ventricular diastole. In the usual therapeutic doses [1,2]

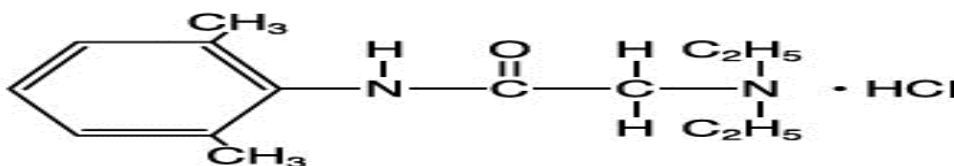


Fig. 1: Chemical structure of Lidocaine

Lidocaine has been determined by, GC [3,4] Spectrophotometric determination of Lidocaine in pharmaceuticals [5], with with bromocresol purple [6], bromocresol green [7] , sodium nitroprusside [8], Methylene Blue [9]. The objective of this study is to develop accurate, precise, sensitive, selective and reproducible spectrophotometric methods for the determination of Lidocaine in bulk and its pharmaceutical dosage form.

Experimental

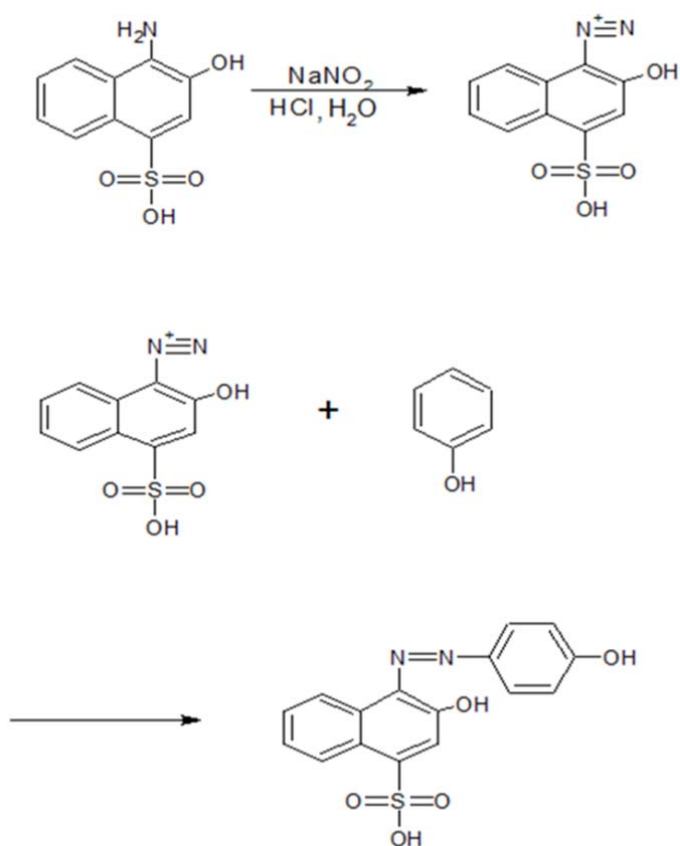
Reagents and apparatus

- LID (100.03% pure reference substance, produced by Lupin, India).
- stock solution (1 mg/mL): 100 mg LID was dissolved in 80%ml. water and 20% ethyl alcohol in a 100 mL volumetric flask.
- stock solution(1mg/mL): 100 mg HAN was dissolved in 80%ml water and 20% ethylalcohol in a 100 mL volumetric flask.
- HCl, NaOH, (Na₂CO₃), (NaNO₂), 1-amino-2-naphthol-4-sulfonic, Phenol, NaCl.
- Buffer Solution:
different buffer Solution used 0.2M, Acetate buffer , 0.2M Ammonium buffer,0.2M borate buffer and0.2M(PH=2.0-12.0)universal britton buffer solution.

- UV-Vis Spectrophotometer Model SP3000 OpTMA from Korea.
- IR,NMR, Elemental analysis.

Preparation of 3-hydroxy-4-[(4-hydroxyphenyl)azo]-1-naphthalenesulfonic acid (HAN)

This reagent is prepared according to the following scheme and depending on the reference [10,11]



Procedure

1. In test tube A, add approximately 4 mL of conc. HCl and place in an ice water bath.
2. In test tube B, place 4.78 g (0.02 mol) of 1-amino-2-naphthol-4-sulfonic acid, 0.92g of sodium carbonate (Na_2CO_3), and 35 mL of water and place in a hot water bath until a clear solution is obtained.

3. In test tube C, prepare a solution containing 1.42 g of sodium nitrite (NaNO_2) and 7 mL of water.
4. Remove test tube B from the hot water bath and pour the contents all at once from test tube C into test tube B.
5. Add the contents from test tube B to test tube A and place in an ice water bath until a significant amount of solid has precipitated.
6. In a 25 mL RBF, add 1.88 g (0.02 mol) of Phenol, 10 mL of 2.5 M NaOH and a magnetic stir bar and place in an ice-water bath. Turn on the stirrer and ensure the magnetic stir bar is working.
7. Add the contents of test tube A to the RBF while stirring and continue stirring and cooling the reaction for 10 minutes.
8. Remove the RBF from the ice water bath and heat the reaction using a thermowell until boiling commences (check with the instructor to ensure the set-up is correct).
9. Add 8 g of sodium chloride (NaCl) and continue heating until dissolved.
10. Stop stirring the reaction and cool to room temperature, then place in an ice water bath for 15 minutes.
11. Filter the solid using vacuum filtration with a Buchner funnel and wash with a saturated NaCl solution.
12. If no solid is precipitated, only keep the filtrate. If solid is precipitated, let the solid air dry and keep both the filtrate and the solid.

The synthesized compounds were identified and characterized by IR, $^1\text{H-NMR}$ and UV spectroscopy, Elemental analysis, R_f , M.P, C^0 . The physical properties are summarized in Table -1.

Materials and methods

We study the best volume and concentration of the LID, HAN, Buffer Solution, PH, solutions on the formation red complex was established.

LID solution forms with HAN solution Complex red coloured in presence universal britton buffer at PH=4.0 of which can be spectrometrically determined at 510nm.

LID-HAN method

To different aliquots of HAN solution corresponding to (0.5-4ml) were transferred into a series of 10 ml volumetric flasks. 0.05-2.4 ml of LID solution and Universal buffer Britton solution PH=4.0 was added to each flask diluted to volume with 1:4 H₂O:C₂H₅OH. The mixtures were cooled and the volume was completed to 10 mL with mixture solvent. Measured after 10 min of mixing against reagent blank.

Analysis of pharmaceutical formulations

20 tablets were accurately weighed were finely powdered and dissolved into sufficient volume of mixture solvent. The mixture was stirred well and filtered through Whatmann filter paper No. 42 and the filtrate was diluted with mixture solvent added universal Britton buffer pH=4.0. The mixtures were cooled and the volume was completed to 10 mL with mixture solvent and Absorbance was measured after 5min of mixing against reagent blank.

Results and discussion

The possible use of HAN for the detection and quantitative estimation depends on the formation complex .LID contains a primary aliphatic amino group which reacts with HAN reagent in mixture solvent medium. The primary amino group of LID reacted with HAN to form the colored complex, which absorbs a maximum at 510nm as shown in Fig.(1).

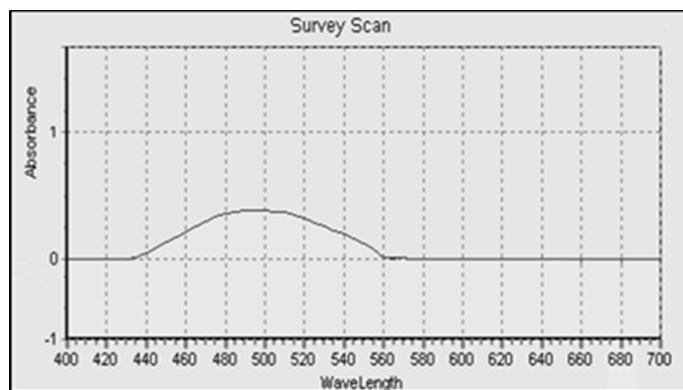


Fig (1) Absorption spectrum of LID- HAN formation

To optimize the reaction conditions, different parameter have been investigated such as, Buffer pH , Buffer Volume Fig.(2,3).

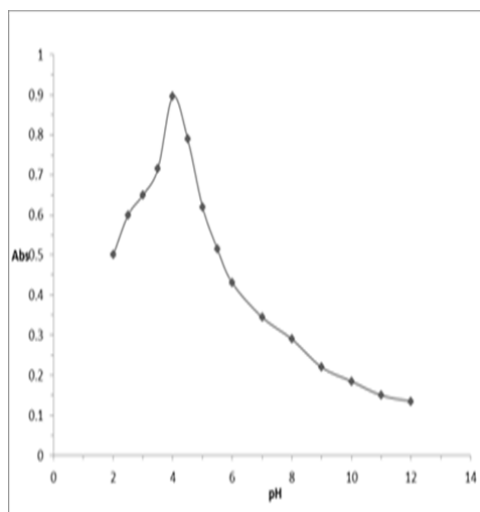


Fig. (2) Effect of Buffer pH on the color development.

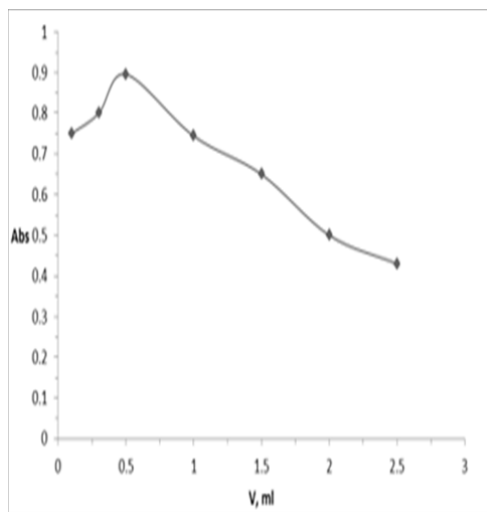


Fig. (3) Effect of the Buffer Volume

Effect of time

The effect of time on the formation and stability of the ion-associates was studied by measuring the absorbencies of the extracted ion-associates at increasing time intervals, the results show that the ion-associates were formed almost instantaneously in the cases at room temperature ($25 \pm 2^\circ\text{C}$). The color of the LID:HAN remained stable for 12 h. after these intervals, a slight decrease in color intensity occurred.

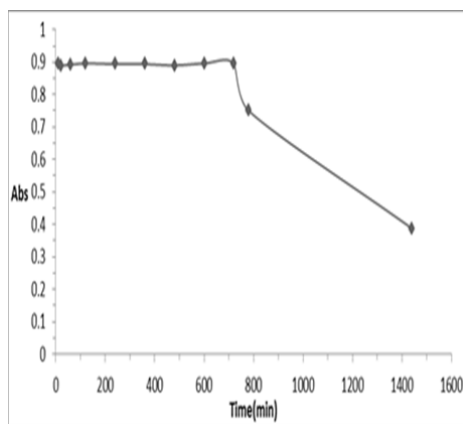


Fig.(4) Effect of time on color intensity

Effect of Reagent Volume

The effect of HAN concentration on the color development was investigated 2ml of HAN reagent produced maximum color intensity Fig .(5).

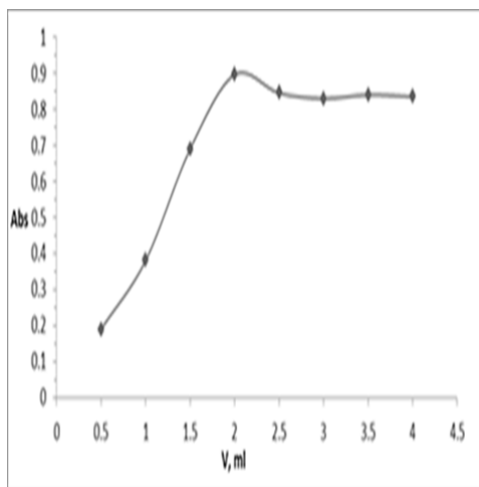
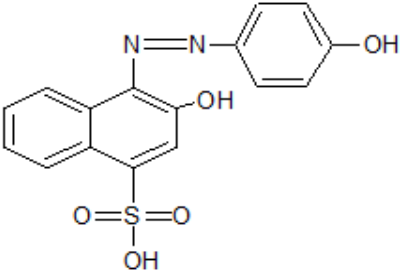


Fig. (5) Effect of Reagent Volume

Molar Ratios Determination of LID:HAN complexes

The molar ratio of the drug to dye of the colore complex was determined using the molar ratio [12] and continuous variation [13] methods. the ratio were found to be 1:1 for LID:HAN (Fig. 6), (Fig.7).The Beer's law limits, molar absorptivity, linear regression equation, correlation coefficient and detection limit determined for method is given in TABLE-2. A linear relationship was found between the absorbance at λ_{\max} and the concentration of the drug in the ranges 1.44–69.31 $\mu\text{g/ml}$.

Table(1): Physical properties of 3-hydroxy-4-[(4-hydroxyphenyl)azo]-1-naphthalenesulfonic acid

Structural formula																			
Yield(%)	90																		
M.P, C0	144-142																		
Rf	0.91																		
Mol. Formul)Mol.Wt(.)	C16H12N2O5S gr)344(<table border="1" style="width: 100%; border-collapse: collapse;"> <thead> <tr> <th>element</th> <th>% O</th> <th>% S</th> <th>% N</th> <th>% H</th> <th>% C</th> </tr> </thead> <tbody> <tr> <td>calcd</td> <td>23.25</td> <td>9.30</td> <td>8.13</td> <td>3.48</td> <td>55.81</td> </tr> <tr> <td>found</td> <td>23.89</td> <td>9.25</td> <td>8.11</td> <td>3.45</td> <td>55.30</td> </tr> </tbody> </table>	element	% O	% S	% N	% H	% C	calcd	23.25	9.30	8.13	3.48	55.81	found	23.89	9.25	8.11	3.45	55.30
element	% O	% S	% N	% H	% C														
calcd	23.25	9.30	8.13	3.48	55.81														
found	23.89	9.25	8.11	3.45	55.30														
Elemental analysis																			
IR data	1632 cm ⁻¹ (v N=N), 3402 cm ⁻¹ (v O-H), 1580cm ⁻¹ v (C=C), 3100 cm ⁻¹ (v C-H aromatic), 1105 cm ⁻¹ (v C-C);(v C-N), 1380 cm ⁻¹ (v SO ₂), 690cm ⁻¹ (v S-O)																		
¹ HNMR	δ 4.703(s, 2H, OH), δ 7.287- 7.898 (m, 8H, Ar-H), δ 6.954 (s, 1H, SO ₃ H), δ 8.484 (s.1H)																		
UV-Vis	λ _{max} (432nm, 229nm)																		

Table(2): Optical characteristics and statistical data for the regression equation of the proposed method

Parameter	Value
λ_{max}	510nm
Beer's law limit ($\mu\text{g}/\text{mL}$)	1.44 – 69.31
Molar absorptivity ($\text{L mole}^{-1} \text{ cm}^{-1}$)	4.1633×10^3
Sandell's sensitivity ($\mu\text{g}/\text{mL per } 0.001 \text{ A}$)	0.0693
Regression equation (Y^*)	
Slope (m)	0.0125
Intercept (c)	0.0101
Correlation coefficient	0.998
Relative Standard Deviation**	2.069
Limit of Detection ($\mu\text{g}/\text{mL}$)***	0.17
Limit of quantitation ($\mu\text{g}/\text{ml}$)	0.59

$$Y^* = mx + C$$

Where X is the concentration of analyte ($\mu\text{g}/\text{mL}$) and Y is absorbance unit.

Table(3): Study of the precision and of the accuracy of the method

Drug samples ($\mu\text{g}/\text{ml}$) Amount taken	Found ($\mu\text{g}/\text{ml}$)	Standard deviation SD	R.S.D %	Detection limit ($\mu\text{g}/\text{ml}$)	analytical Error $SD/(n)^{1/2}$	Relative Recovery (%) R
1.444	1.401	0.029	2.069	0.012	1.401 ± 0.033	97.022
11.552	11.450	0.102	0.890	0.045	11.450 ± 0.124	99.117
23.105	22.816	0.238	1.043	0.106	22.816 ± 0.2 94	98.749
28.882	28.731	0.401	1.395	0.179	28.731 ± 0.4 96	99.477
34.658	34.265	0.314	0.916	0.140	34.265 ± 0.3 88	98.866
40.434	40.677	0.485	1.192	0.216	40.677 ± 0.5 99	100.610
46.211	46.615	0.277	0.594	0.123	46.211 ± 0.3 41	100.874
57.764	57.359	0.478	0.833	0.213	57.359 ± 0.5 91	99.298
69.316	68.854	0.328	0.476	0.212	68.854 ± 0.5 88	99.333

Five independent analyses.

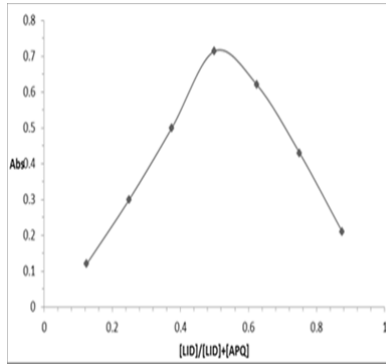
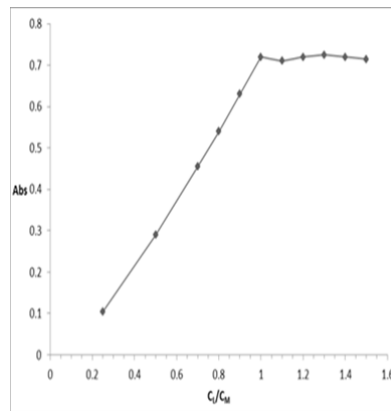


Fig .(6) The mole fractio

$(V_{LID})/(V_{LID} + V_{HAN}).$



Fig(7).The molar ratio[HAN] / [LID].

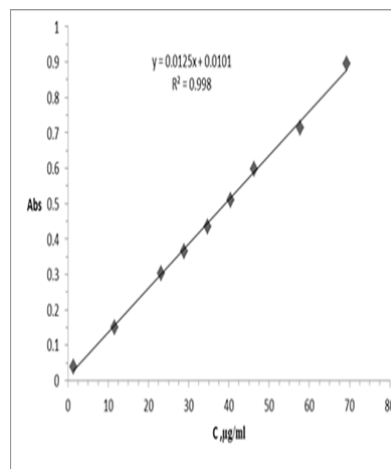


Fig. (8)Calibration rang for LID

Linearity and range

The graphs show negligible intercept and are described by the regression equation, $A = mC + b$ (where A is the absorbance of 1 cm layer, m is the slope, b is the intercept and C is the concentration of the measured solution in $\mu\text{g.ml}^{-1}$) obtained by the least-squares method [14]. The high molar absorptivity of the resulting colored complexes indicate the good sensitivity of the method (Fig. 8).

Conclusion

The results obtained are summarized in TABLE-3. The low values of relative standard deviation (RSD) indicate good precision and reproducibility of the method. The average percent recoveries obtained were 97.02 – 100.87%, indicating good accuracy of the methods

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