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EXTRACTION BEHAVIOUR OF COPPER LIGNOCAINE COMPLEX. Application for Determination of Lignocaine

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The extraction behaviour of coloured copper lignocaine complex was investigated. An organic solvent with medium dielectric constant (n-butanol) was chosen to extract the complex. A new procedure was suggested for the determination of lignocaine. It is based on the formation of coloured complex in alkaline medium (pH 10) followed by its extraction with n-butanol. The absorbance of organic layer is measured at 570 nm. Alternatively, determination of copper content of n-butanol extract via atomic absorption spectroscopy provides an indirect method for the determination of lignocaine. Both methods were applied to the analysis of pharmaceutical preparations with fair accuracy.

Key words: Solvent extraction of Cu-lignocaine complex, Spectrophotometry, Atomic absorption spectrophotometry (AAS).

Introduction

Lignocaine hydrochloride is an important local anaesthetic durg. Although its complexation with cupric ions attracted the attention of many authors [1-3], no studies on the extraction of its complex with Cu^{2+} were carried out. Extraction technique is applied in analytical chemistry to increase the selectivity of the method of analysis.

Among the general procedures available for determination of organic substances by using colorimetric and atomic absorption spectrometric (AAS) methods, those involving formation and extraction of metal chelates have been particularly popular in recent years owing to their facility for combining two different analytical techniques, extraction and spectrophotometry [4-6]. Many of the procedures are modifications of established spectrophotometric method involving metal chelate formation and solvent extraction, the metal being determined by atomic absorption spectrophotometry (AAS).

In continuation of our previous work for developing chemical methods in drug analysis [7-10], a new procedure is suggested for determination of lignocaine. It depends on the formation of copper lignocaine complex in slightly alkaline medium followed by its extraction into an organic solvent. The measurements were carried out by colorimetric and AAS methods.

Experimental

All chemicals used in the present investigation were pure BDH products. Lignocaine HCl was of sufficient purity and passed British Pharmacopea (B.P.) requirements. Copper sulfate solution was standardised by the recommended procedure [11]. A stock solution of lignocaine was prepared by dissolving a definite weight of lignocaine in the appropriate volume of water.

Apparatus. A Beckman DU-8 spectrophotometer and a Perkin Elmer atomic absorption spectrophotometric model 2380 were used. pH values were measured using a Hanna instrument type HI 8417.

General procedure. To the sample solution (5 ml) containing 5 mg of lignocaine HCl, 1 ml of $CuSO_4$(5%) and 1 ml of Na_2CO_3 solution (15%), were added and mixed. The volume was completed to 10 ml with distilled water and the solution was left for 5 min for complete complex formation. Added 10 ml of n-butanol equilibrated at the same alkalinity with Na_2CO_3. Then added 0.5g of anhydrous sodium sulfate to the mixture and shaked gently. The absorbance of organic layer was measured at wavelength 570 nm against a reagent blank and 1 cm silica cell was used for absorbance measurements at room temperature. The concentration of lignocaine is calculated from a calibration curve prepared in the same way in the range 0-2 mg/ml.

Atomic absorption spectroscopic procedure. Proceed as above as far as " Added 10 ml n-butanol.....". The n-butanol extract is then aspirated directly in the AAS under the following experimental conditions using a hollow cathode lamp of Copper = slit width 0.2 mm, wavelength 324.7 nm, lamp current 7 mA fuel gas acetylene 3 psi and air pressure 18 psi.

The concentration of lignocaine was calculated from the relevant calibration curve in the range 1-10 µg/ml lignocaine HCl.

Determination of lignocaine injection. To analyze lignocaine injection, 1 ml is diluted to 10 ml (for spectrophotometric determination) or 0.5 ml is diluted to 100 ml

(for AAS method).The recommended procedure was followed.

Results and Discussion

Lignocaine HCl reacts with cupric ions to give copper lignocaine complex. The formation of the complex is largely dependent on the pH of the medium and is favoured at pH 10. The violet coloured complex can be extracted with n-butanol. It must be taken into consideration that the determination of lignocaine HCl by this method is dependent on the extractibility of copper lignocaine complex, pH of the medium, the salting out effect and composition of the extracted species.

Figure 1 shows that the extracted complex reaches an absorption maximum at 570 nm in butanol layer. The study of salting out effect of anhydrous sodium sulfate on the extraction of copper lignocaine confirmed that the addition of 0.5 g of anhydrous Na_2SO_4 improve the extraction process. Salting out reagents are always used in solvent extractiion system in order to increase the sensitivity of the extracted layers and to accelerate the time of separation of the two layers. The presence of salting out reagents would also assist polymer formation in the organic phase which may lead to an increase in the tendency of extraction. Fig. 2 shows the effect of pH on the extraction of copper lignocaine complex. It is clear that absorbance of the organic layers increases with the pH of the medium till pH 10.

The effect of copper concentration on the extraction was also investigated. The results are shown in Fig. 3. The degree of extraction was more than 10^{-2}M, initially present in the aqueous phase when the concentration of lignocaine was kept constant at 10^{-2}M. For the determination of lignocaine an excess of copper over the stoichiometric ratio of copper to lignocaine complex (1:1) is used.

In the solvents of medium dielectric constants such as butanol (18%) association of complexes in the organic phase may take place. Such ion clusters correspond to new species of the complex or to polymer formation in the organic phase which may lead to an increase in the extent of extraction. The

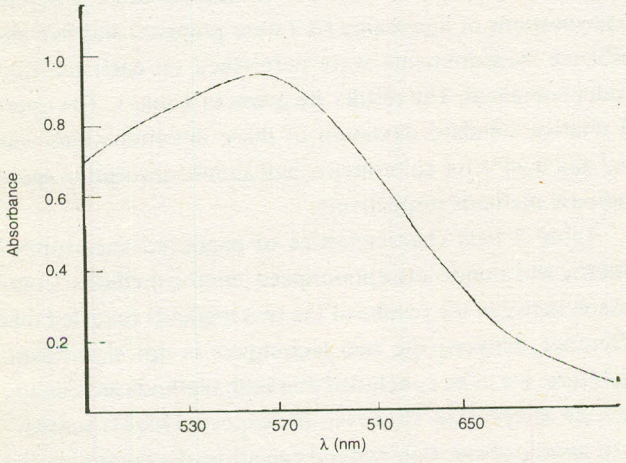

Fig.1. Absorption spectrum of the extracted Cu-lignocaine complex in n-butanol.

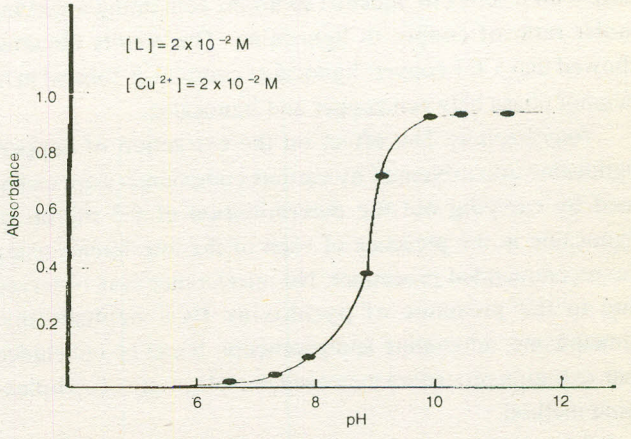

Fig. 2. Effect of pH on the extraction of Cu- lignocaine.

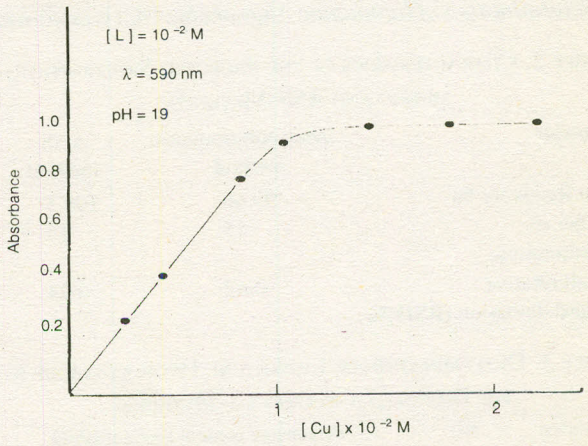

Fig.3. Effect of copper concentration on the extraction of Cu-lignocaine.

TABLE 1. DETERMINATION OF LIGNOCAINE HYDROCHLORIDE BY EXTRACTION PROCEDURE.

Solution No.	Spectrophotometric method			AAS-method		
	Taken (mg/ml)	found* (mg/ml)	%Recovery ± SD**	Taken (µg/ml)	found* (µg/ml)	%Recovery ±SD
1-5	0.5	0.5	100±0.54	2	2.03	101.5±0.32
6-10	1.0	0.99	99±0.7	5	5.01	100.2±0.46
11-15	1.5	1.5	100±0.762	10	9.98	99.8±0.28

*Average of five measurements. **Standard deviation.

advantages of extraction by n-butanol are that the solvent is less volatile, the complex is more soluble and the colour is stable for a week. The formation and extraction of the violet complex was investigated at temperatures ranging from 0 to 100°C and it was found that the absorbance values were not affected by temperature within that range.

Composition of extracted species. It has been found that the molar ratio of copper to lignocaine is 1:1 in alkaline medium (pH 10). Accordingly, continuous variation plots [12] were successfully made in order to confirm the composition of the extracted copper-lignocaine complex. The total concentration of copper and lignocaine was kept constant at 0.01 M and then the extraction was done as in the general procedure with a series of aqueous solutions containing a varying molar ratio of copper to lignocaine. The results obtained showed that a 1:1 copper: lignocaine complex is formed in n-butanol phase between copper and lignocaine.

Interference. The effect on the extraction of copper-lignocaine into n-butanol by various compounds were examined by carrying out the determination of 0.5 mg/ml of lignocaine in the presence of each of the interferents using the recommended procedure. No interference was observed due to the presence of pyridoxine HCl, naphazoline, dimethicone, adrenaline and panthenol. It can be concluded that extration procedure increased the selectivity of complexation method.

Spectrophotometric and atomic absorption spectrometric determination of lignocaine. Lignocaine HCl was allowed to react with Cu(II) ions in alkaline medium (carbonate medium) where the respective Cu(II) complex is formed and extracted with n-butanol. Then the analysis was completed by measuring the absorbance of organic layer at certain wavelength spectrophotometrically or measuring the copper content by AAS.

For colorimetric determination the absorbance of organic layer was measured at 570 nm. Beer's law was obeyed in the range 0-2mg. Calibration graph was linear and passed through the origin which was represented by the equation:

$$Y = 0.77X - 0.0$$

where Y = absorbance and X=lignocaine HCl concentration (mg/ml). Sandell's sensitivity for this method was 6.52 ppm cm^{-2}.

To determine this drug through copper content of the formed complex, atomic absorption spectroscopy was tried. The calibration graph was prepared by the procedure described above. Beer's law was obeyed in the range 1-10 μg ml^{-1} of lignocaine. The calibration graph was linear and passed through the origin which was represented by the equation:

$$Y = 0.71 X$$

Sandell's sensitivity was 0.03 ppm cm^{-2}

In order to determine the accuracy and precision of the two proposed methods, solutions containing three different concentrations of lignocaine HCl were prepared and five absorbance measurements were performed on each reaction product obtained. The results are given in Table 1. The overall relative standard deviation of these determinations was 0.62 and 0.34% for colorimetric and atomic absorption spectrometric methods respectively.

Table 2 lists characteristics of proposed spectrophotometric and atomic absorption spectrometric methods. Comparison between the results of the two methods revealed that difference between the two techniques is not significant. Therefore, it can be concluded that both methods are equally accurate and precise. However, the expected higher sensitivity of atomic absorption method can allow the determination of this compound in low concentrations range.

The proposed methods were applied to the determination of lignocaine HCl in pure form and in injection. As is evident from Table 3, the results of both methods are in good agreement with this official method [13]. Accordingly, both methods can be adopted in routine analysis.

TABLE 2. CHARACTERISTICS OF THE PROPOSED SPECTROPHOTOMETRIC AND ASS-METHODS.

Parameter	Spectrophotometric method	AAS method
Mean Recovery %	99.66	100.5
Number of determinations	15	15
Overall relative standard deviation (RSD)%	0.62	0.34

TABLE 3. DETERMINATION OF LIGNOCAINE HYDROCHLORIDE BY THE PROPOSED AND OFFICIAL METHODS.

Preparation	No	Percentage content found/method		
		Spectrophotometric*	AAS*	Official
Lignocaine pure	1	98.94±0.74	98.96±0.68	99.0
	2	99.84±0.66	99.95±0.55	99.3
	3	100.05±0.48	99.98±0.42	100.0
Xylocaine 2%	4	97.99±0.56	98.05±0.52	98.0
	5	98.75±0.78	98.94±0.47	99.0
	6	98.88±0.63	99.04±0.62	99.0
Xylocaine adrenaline	7	100.03±0.35	99.98±0.45	99.0
	8	99.38±0.82	100.00±0.77	100.0
	9	100.00±0.73	99.95±0.62	99.98

*Average of five measurements.

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