DEVELOPMENT OF VALIDATED STABILITY INDICATING HPTLC METHOD FOR LIGNOCAINE HYDROCHLORIDE AND ITS APPLICATION TO THE ASSAY OF FORMULATION AND DEGRADATION KINETICS

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ABSTRACT

HPTLC method was developed for lignocaine hydrochloride on silica gel 60 F$_{254}$ TLC plates using mobile phase comprising of toluene:methanol:ethylacetate (6:2:2%v/v/v) with two drops of ammonia and the detection was carried out at 270 nm using densitometer. The R$_f$ value of lignocaine hydrochloride was 0.68±0.02. The percentage recovery of the drug from injection formulation was carried out by standard addition method and was found to be close to 100 and %RSD less than 2% indicated good accuracy and precision of the method. The drug was subjected to acid, alkali and neutral hydrolysis, oxidation, thermal and photolytic degradation. The degradation study indicated lignocaine hydrochloride to be susceptible to acid hydrolysis and hydrogen peroxide. The degradation product was well resolved from the pure drug with significant differences in R$_f$ values. The developed validated method was applied to formulation and degradation kinetics of lignocaine hydrochloride. The rate of hydrolysis was found to increase with increase in the strength of hydrochloric acid. It was observed that the temperature had a major effect over acid hydrolysis of lignocaine hydrochloride compared with varying strength of hydrochloric acid. Further this study can be extended for the determination of shelf life of formulation containing lignocaine hydrochloride.

KEYWORDS: Lignocaine hydrochloride, HPTLC, stability indicating, degradation kinetics.
INTRODUCTION
Lignocaine Hydrochloride\cite{1}, [2 – diethylamino-aceto – 2’, 6’-xylidide hydrochloride] is a white crystalline powder, very soluble in water, freely soluble in chloroform and in ethanol (95%). Lignocaine hydrochloride is used as an effective local anaesthetic derived from esters of meta benzoic acid with pronounced antiarrhythmic properties. It has good activity of either preventing or relieving the pain by blocking the neuro transmission. Literature survey reveals simple non-aqueous titrimetric methods\cite{1,2}, NMR spectroscopy\cite{3} and HPLC methods\cite{4-8} reported for lignocaine Hydrochloride in single and multi dosage forms. The International Conference on Harmonization (ICH) guideline entitled “Stability testing of new drug substances and products” requires testing to be conducted to assess the inherent stability of the active substances\cite{9}. An ideal stability indicating method is one that quantifies the drug and resolves its degradation product. Unlike HPLC, HPTLC technique has several advantages\cite{10} which include possibility of analysis of several samples with small amount of mobile phase. This reduces analysis time and cost per analysis. The present work describes how forced degradation studies are used to develop stability-indicating method\cite{11} for lignocaine hydrochloride and its application to degradation kinetics.

MATERIALS AND METHODS
Materials
Lignocaine hydrochloride was received as a gift sample from Fourrts India Pvt Ltd. Chennai. Hydrochloric acid, hydrogen peroxide, toluene, ethyl acetate, methanol, sodium hydroxide, ammonia and all the above chemicals and solvents were supplied by S.D.Fine Chemicals Ltd., India, and Ranbaxy Chemical Ltd., New Delhi, India. Pre-coated silica gel 60F\textsubscript{254} on aluminium sheets were procured from Merck, Germany.

Instrumentation and chromatographic methods
Shimadzu Digital Electronics Balance, Jasco V-630 Spectrophotometer, CAMAG HPTLC System (with TLC Scanner, WinCATs software and Linomat 5 as application device), Hot air oven (Inlab Equipment Madras Ltd.), Sonicator (Leelasonic ultrasonic sonicator), Centrifuge (Remi).

The study was performed using a 20 x 10 cm aluminium sheet precoated with 0.2 mm layers of silicagel 60 F\textsubscript{254} plates which were prewashed with methanol followed by activation at 110ºC for 5 min. Samples were spotted as bands (6 mm wide and 6 mm apart) by means of CAMAG (Muttenz, Switzerland) Linomat 5 sample applicator equipped with a 100 µl
A twin trough glass chamber of 20 x 10 cm with toluene: methanol:ethylacetate (6:2:2%v/v/v) 2 drops of ammonia as the optimized mobile phase used to perform linear ascending development. Approximate time for development was 20 min and developed distance 8cm. Plates were dried and densitometric scanning was performed using CAMAG TLC scanner 3 at 270 nm, controlled by WinCATs software. Radiation source used was deuterium lamp. The slit dimension was 5.00× 0.45 mm, and the scanning speed was 20 mm/s.

**Methods**

**Forced degradation**\(^{[12]}\)

**Stress Studies**
The degradation study was performed in dark inorder to exclude the possible degradative effect of light on lignocaine hydrochloride.

**Acid Hydrolysis**
Ten milligram of lignocaine hydrochloride was weighed accurately and transferred into three different 10ml standard flasks. To this 5 ml of 1N, 0.5N, 0.1N HCl was added separately and made up to 10ml with methanol. This solution was refluxed for 6 hours at 80 °C. The samples were analyzed by HPTLC.

**Alkaline Hydrolysis**
Ten milligram of lignocaine hydrochloride was weighed accurately and transferred into three different 10 ml standard flasks. To this 5ml of 1N, 0.5N, 0.1N NaOH was added separately and made up to 10 ml with methanol. This solution was refluxed for 6 hours at 80ºC. The samples were analyzed by HPTLC.

**Neutral Hydrolysis**
Ten milligram of lignocaine hydrochloride was weighed accurately and transferred into 10 ml standard flasks. To this 5 ml of distilled water was added separately and made up to 10 ml with methanol. This solution was refluxed for 6 hours at 80ºC. The samples were analyzed by HPTLC.
Oxidative Degradation
Ten milligram of lignocaine hydrochloride was dissolved in methanol. To this 5 ml of 30% H₂O₂ was added and made up to 10 ml with methanol. This solution was refluxed for 6 hours at 80°C.

Thermal degradation
Ten milligram of lignocaine hydrochloride was weighed and transferred to a petridish. It was then placed in hot air oven at 50°C for 6 hours then dissolved and made up to 10 ml with methanol. The solution was analysed by HPTLC.

Photolytic degradation
The study was carried out by exposing the drug to sunlight for about 6 hours. The drug solution was prepared using methanol and was analyzed by HPTLC.

The various stressed samples were spotted on TLC plates in triplicates and analysed using the fixed chromatographic condition.

Validation[13]
The developed method was validated as per ICH recommendation in terms of linearity, precision, accuracy, LOD, LOQ, specificity and robustness. The linearity of response for lignocaine hydrochloride was assessed in the range of 0.5 – 3 µg/spot. The accuracy of the method is the similarity of results obtained by the developed method to the true values. Accuracy was expressed as % recovery. The intraday and interday precision was evaluated through replicate analysis of 2 different concentrations 6 times on the same day and 3 successive days. It was expressed in terms of %RSD.

Application to lignocaine hydrochloride injection
The proposed method was applied to assay of lignocaine hydrochloride in injection. The average % recoveries of different concentration were calculated. It was based on the average of 3 replicate determinations.

Degradation kinetics[14]
For studying the order of reaction
Ten milligram of lignocaine hydrochloride was dissolved in methanol in a 10 ml volumetric flask, and 5 ml of 1N hydrochloric acid was added and made up to the volume with methanol. This solution was then transferred into a clean round bottom flask and refluxed at 80°C for 6
hours. The degraded solution and standard solution of lignocaine hydrochloride were applied on HPTLC plates. Plates were placed in chromatographic tank previously saturated for 20 minutes with the mobile phase toluene: ethyl acetate: methanol (6:2:2%v/v/v) and 2drops ammonia and then air dried. Chromatograms were recorded. The concentration of lignocaine hydrochloride was calculated from the regression equation. Plotted % Log concentration of lignocaine hydrochloride remaining against time.

For studying the effect of concentration of hydrochloric acid on the reaction rate
Into a series of 10 ml volumetric flasks, 10 mg of lignocaine hydrochloride was dissolved in methanol and 5ml of 0.1, 0.5 and 1N hydrochloric acid was added and made up to the volume with methanol. These solutions were transferred into a clean round bottom flask and refluxed at 80°C for 6 hours. The degraded solution and standard solution of lignocaine hydrochloride were applied on HPTLC plate. The plate was placed in a chromatographic tank previously saturated for 20 minutes with the mobile phase toluene: ethyl acetate: methanol (6:2:2%v/v/v) and 2drops ammonia and then dried in the air. The chromatograms were recorded. The log of % of lignocaine hydrochloride remaining was plotted against time for different normalities of hydrochloric acid and the rate constant and half-life were calculated.

For studying the effect of temperature on the reaction rate
Ten milligram of lignocaine hydrochloride was taken in three different 10 ml volumetric flasks, dissolved in methanol and 5 ml of 0.1, 0.5 and 1N hydrochloric acid was added separately and the volume was made up to the mark with methanol. These solutions were transferred into clean dried round bottom flasks and then refluxed at 50°C, 60°C, 70°C and 80°C for 6 hours. The stressed sample solutions and standard solution of lignocaine hydrochloride were applied on HPTLC plate. The plate was placed in a chromatographic tank previously saturated for 20 minutes with the mobile phase toluene: ethyl acetate: methanol (6:2:2%v/v/v) and 2drops ammonia and then air dried. The chromatogram was recorded. The log of% of lignocaine hydrochloride remaining was plotted against time at different temperatures. The Arrhenius plot was also constructed for the effect of temperature on the rate of hydrolysis. The effect of temperature was studied by conducting the reaction at different temperatures using different concentrations of the acidic solution.

Degradation of Lignocaine hydrochloride in formulation
The fixed chromatographic condition was applied to estimate lignocaine hydrochloride from injection. The Lignocaine hydrochloride injection was subjected for acid hydrolysis, base
RESULTS AND DISCUSSION

Chromatographic conditions

A stability indicating HPTLC method for the analysis of lignocaine hydrochloride in bulk and formulation was developed. The solvent system that was fixed for HPTLC includes toluene: ethyl acetate: methanol (6:2:2%v/v/v) with 2 drops of ammonia which results in dense, compact spot with \( R_f \) value 0.68± 0.02. The study was carried out using silica gel 60 F\(_{254}\) aluminium sheet and lignocaine hydrochloride was detected at 270 nm (Fig. 1).

Forced degradation studies

In a preliminary study the degradation of lignocaine hydrochloride was studied in 0.1, 0.5 and 1 N HCl /NaOH and 10, 20 and 30% H\(_2\)O\(_2\). It was observed that lignocaine hydrochloride was more stable in sodium hydroxide. The degradation of lignocaine hydrochloride was investigated in acidic condition (Fig. 2) and the drug was also found to degraded in this condition at different temperature.
The chromatogram of lignocaine hydrochloride (bulk) which was obtained after treating with sodium hydroxide, light and thermal source showed no additional peaks. No significant change in the peak shape was observed. The sample degradation with 1N HCl, TFA and hydrogen peroxide showed one additional peak. For TFA additional peak at R_f 0.21± 0.02 and for 1N HCl additional peak was observed at R_f 0.26 ± 0.02. The peak of the degraded product was well resolved from drug peak. This proved the specificity of the method.

**Validation**

The calibration curve of lignocaine hydrochloride was found to be linear over the range of 0.5 – 3 µg/spot having the slope, intercept, and correlation coefficient values were found to be 1.021, 48.694 and 0.9999. The parameters like precision and accuracy were studied and the results are shown in table 1 & 2. The LOD and LOQ were found to be 2.61 ng/spot and 7.93 ng/spot respectively.

**Table 1: Precision studies**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Concentration(µl)</th>
<th>Precision</th>
<th>% RSD*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lignocaine hydrochloride</td>
<td>2.5</td>
<td>Interday precision</td>
<td>0.54</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Intraday precision</td>
<td>0.18</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Repeatability</td>
<td>0.41</td>
</tr>
<tr>
<td></td>
<td></td>
<td>i)Measurement</td>
<td>0.31</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ii)Sample application</td>
<td></td>
</tr>
</tbody>
</table>

* Mean of six determinations

**Table 2: Recovery studies**

<table>
<thead>
<tr>
<th>Level</th>
<th>% Recovery</th>
<th>% RSD*</th>
</tr>
</thead>
<tbody>
<tr>
<td>50%</td>
<td>100.6</td>
<td>0.83</td>
</tr>
<tr>
<td>100%</td>
<td>99.8</td>
<td>0.28</td>
</tr>
</tbody>
</table>

* Mean of six determinations

The proposed method was successfully applied to the injection formulation. The chromatogram was recorded as shown in fig. 3. Result is shown in table 3.
Figure 3: Chromatogram of formulation (1.5 µg/ml)

Table 3: Analysis of formulation

<table>
<thead>
<tr>
<th>Drug Brand</th>
<th>Amount (mg/ml)</th>
<th>% Label claim</th>
<th>% RSD*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Labelled</td>
<td>Estimated</td>
<td></td>
</tr>
<tr>
<td>LOX</td>
<td>21.3</td>
<td>21.28</td>
<td>99.9</td>
</tr>
</tbody>
</table>

*Mean of six observations

Kinetics of degradation

A regular decrease in lignocaine hydrochloride was observed with increasing time. A linear relationship was found by plotting the log concentration remaining versus time (Fig. 4).

Figure 4: First order plot of the hydrolysis of lignocaine hydrochloride with 1N HCl at 80°C
which indicates a pseudo first - order kinetics according to the equation: \( \log C_t = \log C_0 - \frac{kt}{2.303} \), where \( C_t \) is the percent remaining log concentration, \( C_0 \) is the initial percent of lignocaine hydrochloride (100%), \( k \) is the apparent first order rate constant with a–ve sign and “t” is the time. The values for rate constant and \( t_{1/2} \) are presented in table. The rate of degradation was decreased with reduction in temperature. No significant degradation was observed at lower temperature so kinetics of degradation could be monitored at higher temperature. The plot of \( \log k \) versus \( 1/T \) gave the Arrhenius plot which was found to be linear in the temperature range 50 – 80 °C. From the regression equation, the activation energy calculated was found to be 18.615 Kcal/mole. An extrapolation to Arrhenius plot is used to calculate the degradation rate constants at room temperature. The obtained data suggests that the lignocaine hydrochloride is susceptible to acidic degradation (Table 4).

**Table 4: Kinetic data for acid hydrolysis of lignocaine hydrochloride**

<table>
<thead>
<tr>
<th>Normality of HCl</th>
<th>Temperature (°C)</th>
<th>K (hrs(^{-1}))</th>
<th>( t_{1/2} ) (hrs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1N</td>
<td>50</td>
<td>0.0168</td>
<td>42.25</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>0.0205</td>
<td>33.80</td>
</tr>
<tr>
<td></td>
<td>70</td>
<td>0.0273</td>
<td>25.38</td>
</tr>
<tr>
<td></td>
<td>80</td>
<td>0.0404</td>
<td>17.15</td>
</tr>
<tr>
<td>0.5N</td>
<td>50</td>
<td>0.0190</td>
<td>36.37</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>0.0281</td>
<td>24.59</td>
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<tr>
<td></td>
<td>70</td>
<td>0.0359</td>
<td>19.26</td>
</tr>
<tr>
<td></td>
<td>80</td>
<td>0.0502</td>
<td>13.80</td>
</tr>
<tr>
<td>1N</td>
<td>50</td>
<td>0.0201</td>
<td>33.0</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>0.0310</td>
<td>21.82</td>
</tr>
<tr>
<td></td>
<td>70</td>
<td>0.0493</td>
<td>14.40</td>
</tr>
<tr>
<td></td>
<td>80</td>
<td>0.0629</td>
<td>11.01</td>
</tr>
</tbody>
</table>

**CONCLUSION**

HPTLC method was developed for lignocaine hydrochloride in bulk and injection formulation on silica gel 60 F\(_{254}\) TLC plates using mobile phase comprising of toluene:methanol:ethylacetate (6:2:2%v/v/v) and 2 drops ammonia at 270 nm. The \( R_f \) value of lignocaine hydrochloride was 0.68± 0.02. The percentage recovery of the drug from injection formulation was carried out by standard addition method and was found to be close to 100 and %RSD less than 2% indicated good accuracy and precision of the method. The drug was subjected to acid, alkali and neutral hydrolysis, oxidation, thermal and photolytic degradation. The degradation study indicated lignocaine hydrochloride to be susceptible to acid hydrolysis. The degradation product was well resolved from the pure drug with significant differences in \( R_f \) values. The \( R_f \) value of lignocaine hydrochloride was 0.68 ± 0.02.
and degradation product was 0.26 ± 0.02. The developed validated method was applied to formulation and degradation kinetics of lignocaine hydrochloride. The rate of hydrolysis was found to increase with increase in the strength of HCl. It was observed that the temperature had a major effect over acid hydrolysis of lignocaine hydrochloride compared with varying strength of HCl. Further this study can be extended for the determination of shelf life of formulation containing lignocaine hydrochloride.

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REFERENCES


