Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 4, Issue 2, 2012

Research Article

ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF LIDOCAINE IN OINTMENT FORMULATION BY U.V SPECTROPHOTOMETRIC METHOD

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Received: 14 Dec 2011, Revised and Accepted: 29 Jan 2012

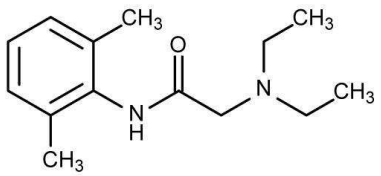
ABSTRACT

A simple and accurate UV method has been developed for the estimation of Lidocaine ointment formulation using SHIMADZU UV-Visible spectrophotometer, with 0.1NHcl as a solvent. The absorbance maxima were found to be 263nm. The percentage purity of ointment formulation was found to be 98.7%. This method was also validated by checking the accuracy, precision, Robustness and Ruggedness. The %RSD shows within specification limits. The linearity profile shows coefficient of variation 0.99.

Keywords: Lidocaine, UV Spectrophotometer, 0.1N Hcl.

INTRODUCTION

The first modern local anesthetic agent was lidocaine (trade name Xylocaine®). It was invented in the 1940s. Lidocaine is used topically to relieve itching, burning and pain from skin inflammations. Lidocaine, the first amino amide-type local anesthetic, was first synthesized under the name Xylocaine by Swedish chemist in 1943.



[2-(diethylamino)-*N*-(2,6-dimethylphenyl)acetamide]

Mechanism of Action[1]

Lidocaine stabilizes the neuronal membrane by inhibiting the ionic fluxes required for the initiation and conduction of impulses, thereby effecting local anesthetic action. Local anesthetics of the amide type are thought to act within the sodium channels of the nerve membrane.

Lidocaine ointment Formulation[1]

Lidocaine ointment 5% is a white to grayish-white ointment. The vehicle for the active ingredient is a water-miscible consisting of propylene glycol and polyethylene glycol.

Lidocaine ointment 5% is indicated for temporary relief of pain associated with minor burns and abrasions of skin, e.g. sunburns, herpes zoster, labialis, pruritus and insect bites and Anesthesia of mucous membranes, e.g. Various anal conditions such as hemorrhoids and fissures. Lidocaine is ineffective when applied to intact skin.

MATERIALS AND METHODS

Materials and Method

A SHIMADZU 1800 Double beam UV-VISIBLE spectrophotometer with 1.0 cm matching quartz cells, Lidocaine standard drug was procured as a gift sample from Aurobindo pharmaceuticals, Hyderabad, 0.1M Hcl, Standard volumetric flasks, Beakers, Pipettes and Measuring cylinder.

Analytical Specification[2]

Drug name: LIDOCAINE

Formulation: XYLOCAINE OINTMENT 5% USP

Table 1: Analytical specification of Lidocaine ointment formulation

S. no.	Test	Specification
1	Appearance	Colorless
2	Odour	Odorless
3	Crystal forms	Crystallizes from n-hexane as fine needles
4	Melting range	68-69^0 C
5	Solubility a. water b. Ethanol 95% c. Chloroform d. n-hexane	soluble in water and all organic solvents (g/ml, +25^0 C) 0.004 0.76 0.79 0.12
6	Average weight	1 gm
7	pH	6.0 to 7.0
8	Storage and stability	Store at 15-30^0 C. protect from freezing.

Analytical Method Development

Selection of Solvent

Selection of solvent was based on solubility and stability of drug in solvent system as well as extraction of drug from its formulation. Lidocaine pure form and its market formulation can be freely soluble in water and organic solvents. Hence Hcl of 0.1M concentration was selected as solvent for UV spectrometric determination.

Preparation of stock solution

A standard stock solution of Lidocaine was prepared by dissolving 10mg of drug in 10ml of 0.1M Hcl. Above stock solution was further diluted with same solvent to get the final concentrations of 100µg/ml and this was used as standard stock solution.

Determination of λ max

The standard stock solution of Lidocaine having the concentration 1000µg/ml was further diluted to 100µg/ml with 0.1M Hcl. The absorbance of resulting solution was scanned in the UV spectrometer ranging from 200-400nm. The plot shows the maximum absorbance at 263 ±1nm.

Linearity profile[3]

From the stock solution various dilutions were made to obtain solutions of 5, 10, 15, 20, 25 and 30 µg/ml. Absorbance values of

these solutions were measured at λmax 263 nm. The calibration curve was plotted between concentration of Lidocaine and respective measured absorbances. The stability of the drug in the solvent system and during actual analysis was also investigated.

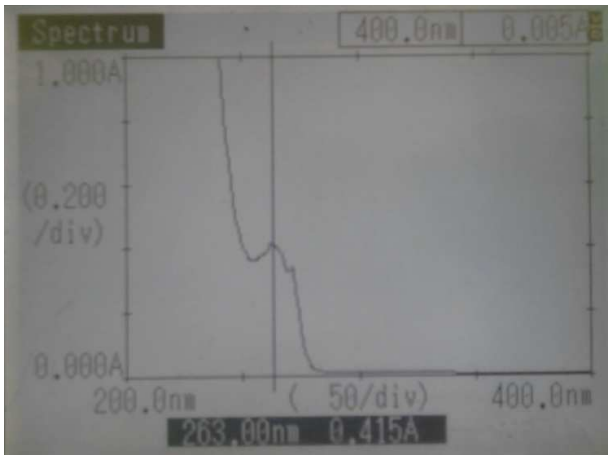


Fig. 1: UV spectrum of lidocaine in 0.1M Hcl at λ max 263.

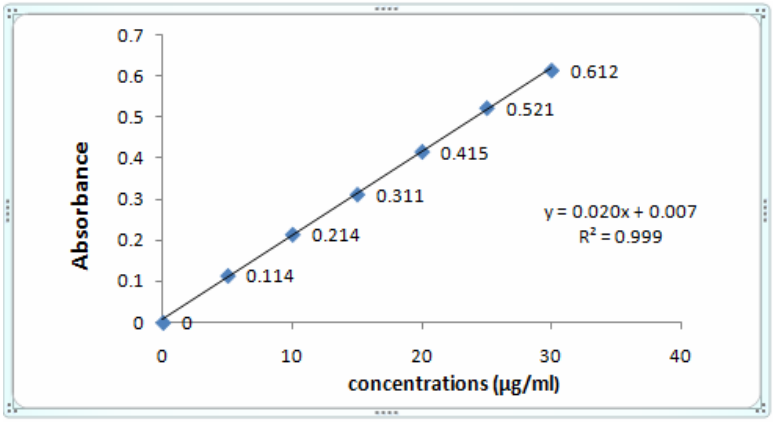


Fig. 2: Calibration curve of Lidocaine.

Table 2: Linearity profile

Statistical parameters	Results
Wavelength	**263 nm**
Slope	0.020364
Intercept	0.006964
Standard error	0.005456
Regression coefficient	Y=0.0204X+0.007
Correlation coefficient (r)	0.9995
Wavelength	263 nm

Assay of marketed formulation

Preparation of standard solution

Standard solution was prepared from the stock solution having the concentration 100µg/ml. from the above solution take 3ml of sample and diluted to 10ml with 0.1M Hcl. Measure the absorbance at 263nm.

Preparation of sample solution

Commercially available ointments of lidocaine XYLOCAINE OINTMENT 5% USP (ASTRA ZENECA) were selected for the estimation of total content by the proposed method. An amount equivalent to 0.01g of lidocaine was weighed accurately and transferred into 10ml volumetric flask and add 0.1M Hcl mix thoroughly and make up the volume. From this solution take 1ml and diluted to 10ml of the same solvent, from this take 3ml and diluted with same solvent to get the final concentration 30µg/ml. Measure the absorbance in UV spectrometer at 263nm.

Table 3: Absorbance of Standard and Test sample

Solutions	Absorbance at 263 nm
Standard sample	0.618
Test sample	0.610

$$\text{Formula} = \frac{\text{Sample absorbance} \times \text{standard dilution} \times \text{Average weight}}{\text{Standard absorbance} \times \text{sample dilution} \times \text{label claim}} \times 100$$

RESULTS AND DISCUSSIONS

The percentage purity of Lidocaine formulation was found to be 98.7 %. It complies as per specification.

Method Validation

Purpose: Validation is a process of establishing documented evidence, which provides a high degree of assurance that a specific activity will consistently produce a desired result or product meeting its predetermined specifications and quality characteristics[4].

Method validation procedure

Accuracy

Solutions were prepared in triplicate at levels 80%, 100% and 120% of test concentration using lidocaine working Standard as per the test method and taken absorbance of each solution in triplicate. The recovery results showed that the proposed method has an acceptable level of accuracy for lidocaine which is from 80% - 120% of test concentration is form 99 % - 101 %. Results were shown in table 4.

Table 4: Accuracy.

No. of preparations	Concentration (µg/ml)	% recovery	Mean	S.D	%RSD
A1:80%	24	98.72	99.38	0.49	0.49
A2:80%	24	99.69			
A3:80%	24	99.34			
B1:100%	30	101.82	100.01	1.10	1.09
B2:100%	30	99.21			
B3:100%	30	99.01			
C1:120%	36	100.8	100.73	0.84	0.83
C2:120%	36	101.54			
C3:120%	36	99.85			

Discussion

The differences between the assay obtained from average of 9 determinations (3conc/ 3replicates) are within the limits. The %RSD Acceptance criterion is 2%.

Precision

Precision of the method was demonstrated by Repeatability, intraday and interday variation studies. For repeatability study take nine samples of same concentration and observe the absorbance and calculate the %RSD. Results were shown in Table 5.

In intraday variation study nine different solutions of same concentration 30µg/ml were analyzed three times in a day i.e. from morning, afternoon and evening and the absorbance is noted. From the absorbance result mean, standard deviation and %RSD was calculated. The acceptable limit for intraday variation should be within 1% and results were shown in Table 6.

In the interday variation studies, solution of same concentration 30µg/ml were analyzed three times for the three consecutive days and the absorbance result mean, standard deviation and %RSD was calculated. The acceptable limit for interday variation should be within 2% and results were shown in Table 7.

Table 5: Repeatability

Concentrations (µg/ml)	Absorbance	Mean	S.D	%RSD
30	0.614	0.614	0.00216	0.35
30	0.616			
30	0.612			
30	0.612			
30	0.620			
30	0.616			
30	0.614			
30	0.614			
30	0.610			

Discussion

The assay results of 9 determinations having the same concentration are well and within limits. The %RSD was found to be 0.35 and Acceptance criterion is 2%.

Table 6: Intraday assay precision

Concentrations (µg/ml)	Time in Min.		
	10.00 Am	1.00 Pm	4.00 Pm
30	0.612	0.611	0.611
30	0.614	0.614	0.615
30	0.614	0.615	0.618
30	0.612	0.608	0.610
30	0.610	0.611	0.611
30	0.614	0.618	0.618
30	0.617	0.617	0.615
30	0.614	0.614	0.614
30	0.612	0.614	0.616
% RSD	**0.32**	**0.32**	**0.48**
Average %RSD	**0.37**		

Discussion

The intraday precision of 9 determinations having the same concentrations shows results within the limits. The Acceptance criterion is 2%.

Table 7: Interday assay Precision

Concentrations (µg/ml)	% R.S.D			Average
	Day 1	Day 2	Day3	% R.S.D
30	0.28	0.32	0.33	0.31

Discussion

The interday assay precision shows the %RSD results within the limits and the average %RSD is 0.31. The Acceptance criterion is 2%.

Linearity and Range[5]

Various aliquots were prepared from the secondary stock solution (100µg/ml) ranging from 5-30µg/ml. The samples were scanned in UV-Vis Spectrophotometer against 0.1M Hcl as blank. It was found that the selected drug shows linearity between the ranges of 5-30µg/ml.

The acceptable limit is, it should be linear in the specified range and the regression coefficient should not be less than 0.99.

Discussion

The relationship between the concentrations and the absorbances of lidocaine shows the linearity and the coefficient of variation not less than 0.99. (Table 8)

Limit of Detection[6]

The limit of detection (LOD) was determined by preparing solutions of different concentrations ranging from 0.1-0.5µg/ml. The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample, which can be detected but not necessarily quantification as an exact value.

Table 8: Linearity and Range.

Concentration (µg/ml)	Absorbance
5	0.114
10	0.214
15	0.311
20	0.412
25	0.521
30	0.612

Limit of Quantification

The limit (LOQ) was determined by preparing solutions of different concentrations of 30%, 50% and 80% of the 30 µg/ml of lidocaine working standard and labeled as LQC, MQC and HQC respectively. Measure 3 replicates of each of the concentration and calculate the mean, standard deviation and %RSD.

Robustness

Robustness of the method was determined by carrying out the analysis under different room temperature, wavelength conditions and varies in concentrations. The respective absorbances were noted and the result was indicated as %RSD and results were shown in Table 10.

Table 9: Robustness parameters

S.No.	Parameter	Limits		
		Minimum	Optimum	Maximum
1	Concentration (µg/ml)	25	30	35
2	Temperature (^{0}C)	22	25	28
3	Wavelength (nm)	261	263	265

Table 10: Robustness.

S.No.	Parameter	Absorbance		
1	Concentration (µg/ml)	At conc. 25	At conc. 30	At conc. 35
		0.514	0.612	0.721
2	Temperature (^{0}C)	At Temp. 22	At Temp. 25	At Temp. 28
		0.612	0.612	0.613
3	Wavelength (nm)	At 261	At 263	At 265
		0.605	0.610	0.606

Discussion

The assay was done at vary parameter conditions like Concentration, Temperature and wavelength. The result shows %RSD as 0.84 and Acceptance Criteria is 2%.

Ruggedness

Ruggedness of the method was determined by carrying out the analysis by different analyst and the respective absorbance of 30µg/ml was noted. The result was indicated as %RSD. It should be less than 2 of %RSD and results were shown in Table 11.

Table 11: Ruggedness.

Concentrations (µg/ml)	Analyst 1	Analyst 2
	Abs.	Abs.
30	0.614	0.611
30	0.613	0.615
30	0.616	0.611
30	0.608	0.618
30	0.611	0.608
30	0.613	0.611
Mean	0.612	0.612
SD	0.0027	0.0036
%RSD	0.44	0.68

Discussion

The assay results of two analysts with lidocaine same concentrations with 6 determinants shows within limits and Acceptance criteria is 2%.

CONCLUSION

The developed UV spectrophotometric method for the lidocaine is simple, sensitive and economical over the existing method with Ethanol as a solvent. This method was also validated by checking the Accuracy, Precision, Range and Linearity, Robustness and Ruggedness. The results were also reported as it shows High level of precision by low values of Standard deviation and Relative standard deviation. So this method can be applied successfully for the estimation of lidocaine in pharmaceutical formulation.

REFERENCES

1. Lidocaine ointment USP, Prescribing information, Astra Zeneca Companies, Submission control No. 106620.
2. Klaus Florey, Lidocaine Base and Hydrochloride, Analytical Profiles of Drug Substances, Chapter 8, page 207-243.
3. Deepak v. Bageshwar, avinash s. Pawar, vineeta v. Khanvilkar, vilasrao j. Kadam. Quantitative Estimation Of Mupirocin Calcium From Pharmaceutical Ointment Formulation By Uv Spectrophotometry, International Journal of Pharmacy and Pharmaceutical Sciences ISSN- 0975-1491 Vol 2, Issue 3, 2010.
4. ICH, Q2 (R1) validation of analytical procedures: text and methodology. International conference on harmonization: Nov.1996.
5. Suddhasattya Dey, K.Kalyani, B.Samyuktha, Sudhir Kumar Sahoo, Subhasis Mohapatra, P.N.Murthy, Dhiraj Kumar, Development And Validation of a Uv-vis Spectrophotometric Method For The Estimation And Degradation Monitoring Of Cefadroxil In Bulk And Pharmaceutical Dosage Forms, International Journal Of Chemistry Research Vol 1, Issue 1, 2010.
6. Vinay wamorkar, manjunath s.y., m. Mohan varma, Development And Validation Of Uv Spectroscopic Method For Determination Of Metoclopramide Hydrochloride In Bulk And Tablet Formulation, Int J Pharm Pharm Sci, Vol 3, Issue 3, 2011, 171-174.