World Journal of Pharmaceutical and Life Sciences
WJPLS
www.wjpls.org
SJIF Impact Factor: 4.223

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF BENZOXONIUM CHLORIDE AND LIDOCAINE HYDROCHLORIDE

Dr. Santosh V. Gandhi* and Ashwini D. Ighe

AISSMS College of Pharmacy, Kennedy Road, Near R. T. O., Pune 411001, Maharashtra, India.

***Corresponding Author: Dr. Santosh V. Gandhi**
AISSMS College of Pharmacy, Kennedy Road, Near R. T. O., Pune 411001, Maharashtra, India.

Article Received on 11/05/2017 **Article Revised on 01/06/2017** **Article Accepted on 22/06/2017**

ABSTRACT

The aim of the present study was to develop and validate reverse phase high performance liquid chromatography (RP-HPLC) method for simultaneous estimation of Benzoxonium chloride and Lidocaine Hydrochloride in bulk and combined dosage form. An isocratic, RP-HPLC method was developed on Nucleosil C18 (250 x 4.6 mm, 5 µm) column using 10 mM potassium dihydrogen phosphate buffer and acetonitritle (20:80 v/v) as mobile phase at flow rate of 1 ml/min at detection wavelength of 215 nm. The chromatographic conditions yield good separation between drugs with retention time (RT) of 5.28 ± 0.13 min and 9.76 ± 0.36 min for Lidocaine hydrochloride and Benzoxonium chloride, respectively. The method was validated with respect to linearity, precision, accuracy and robustness. The data of linear regression analysis indicated a good linear relationship over the range of 20-120 µg/ml for both drugs with a correlation coefficient (r^2) of 0.998 for Benzoxonium Chloride and 0.996 for Lidocaine hydrochloride. The developed method was found to be simple, sensitive, selective, accurate and repeatable for simultaneous analysis of Benzoxonium chloride and Lidocaine hydrochloride and can be adopted for routine analysis of these drugs in bulk and pharmaceutical dosage form.

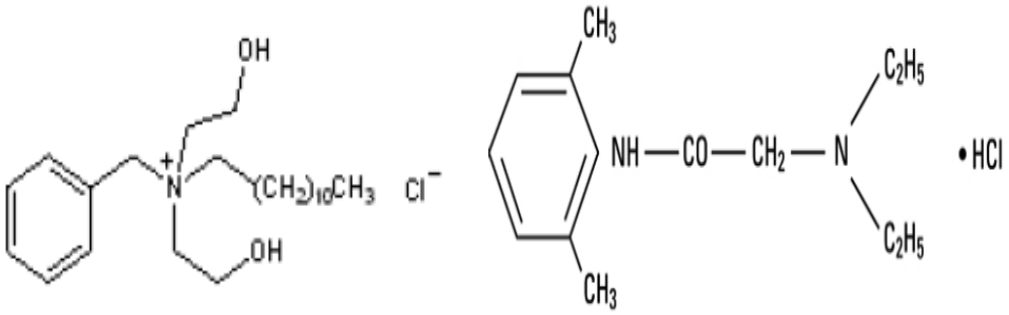
KEYWORDS: High performance liquid chromatography, Benzoxonium Chloride, Lidocaine Hydrochloride, Validation.

INTRODUCTION

Benzoxonium Chloride chemicaly is benzyl dodecyl bis(2hydroxyethyl)azaniumchloride and Lidocaine Hydrochloride chemically is Lignocaine Hydrochloride. Both the drugs are antiseptic and used in asthama treatment.[1-2]

Literature survey reveals few simple HPLC and stability indicating HPLC methods for estimation of lidocaine hydrochloride alone and in combinations with other drugs.[3-9]

To the best of our knowledge no RP-HPLC method has been reported for simultaneous estimation of Benzoxonium chloride and Lidocaine HCl combination. The present work describes a simple HPLC method for the simultaneous determination of Benzoxonium Chloride and Lidocaine Hydrochloride in bulk and pharmaceutical dosage form (LIDOCAM), according to the International conference on harmonization (ICH) guidelines.[10]



(A) **(B)**

Figure 1: Structure of (A) BenzoxoniumClorideand (B) Lidocaine Hydrochloride.

MATERIALS AND METHODS

Reagents and chemicals

Authentic sample of Benzoxonium Chloride and Lidocaine Hydrochloride was obtained from Ajanta Pharmaceuticals Ltd, Aurangabad. Acetonitritle (HPLC grade) was obtained from S. D. Fine Chem. Limited (Mumbai, India), HPLC grade water is collected at college using ELGA water purification system.

Chromatographic condition

HPLC system used was JASCO system equipped with Model PU 2080 Plus pump, Rheodyne sample injection port (20 µl), MD 2010 PDA detector and Borwin-PDA software (version 1.5). A chromatographic column Nucleosil C18 (250 x 4.6 mm, 5 µm) was used. Separation was carried out at a flow rate of 1 ml/min using 10 mM potassium dihydrogen phosphate buffer: acetonitritle (20:80 v/v) as mobile phase and detection at 215 nm. The representative Chromatogram is shown in Figure 2.

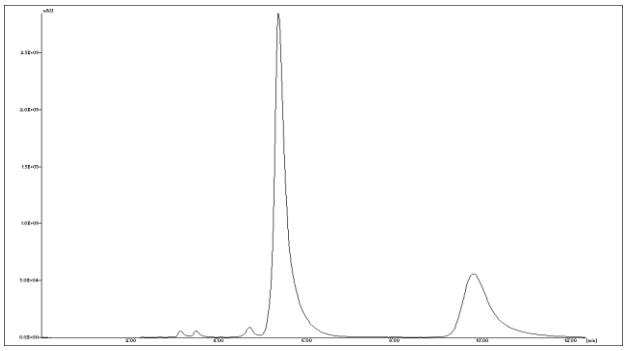


Figure 2: Chromatogram of mixture containing Lidocaine Hydrochloride (100µg/ml) and Benzoxonium Chloride (100 µg/ml).

Preparation of standard stock solution

Standard stock solution of each drug was prepared separately by dissolving 10 mg of the drug in 10 ml of methanol to get concentration of 1000 µg/ml. From the respective standard stock solution, then working standard solution was prepared containing 20-120 µg/ml of Benzoxonium chloride and Lidocaine hydrochloride separately in mobile phase.

Selection of detection wavelength

From the standard stock solution further dilutions were made using methanol and scanned over the range of 200-400 nm and the spectra was obtained. Both drugs showed considerable absorbance at 215 nm.

Preparation of sample solution (Formulation Analysis)

20 tablets were weighed and powdered. Powder equivalent to 10 mg of drug was transferred to 10 ml volumetric flask and volume was made up with methanol to get concentration (1000 µg/ml) and was sonicated for 10 min. Solution was filtered, 0.4 ml of filtrate was diluted to 10 ml with mobile phase. The resultant solution of concentration of 40 µg/ml was analyzed.

Validation of Analytical Method

Specificity

The specificity of the method was ascertained by peak purity profile studies. The peak purity values were found to be more than 995, indicating the no interference of any other peak of impurity or matrix.

Linearity

The linearity (relationship between peak area and concentration) was determined by analyzing six solutions over the concentration range of 20-120 µg/ml for Benzoxonium chloride and Lidocaine hydrochloride. Six replicates per concentrations were analyzed. The equation of calibration curve was found to be y = 17076x + 41558 for benzoxonium chloride with r^2 = 0.998 and y = 42191x + 162349 for Lidocaine Hydrochloride with r^2= 0.996. The peak area of drug was plotted against the corresponding concentrations to obtain the calibration curve as shown in Figure 3.

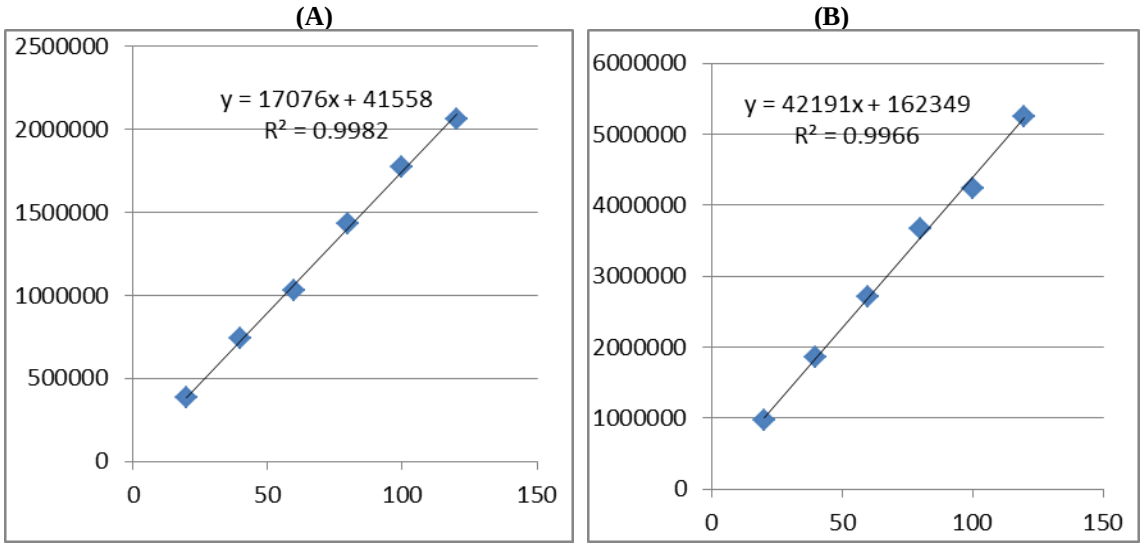


Figure 3: Calibration curve for (A) Benzoxonium chloride and (B) Lidocaine hydrochloride.

Precision

The precision of the method was demonstrated by Intra-day and Inter-day variation studies. For the intra-day studies, 3 replicates at 3 different concentrations (60,80,100µg/ml for Benzoxonium chloride and Lidocaine hydrochloride) were analyzed in a day and percentage relative standard deviation (%RSD) was calculated.

For the inter day variation studies, 3 different concentrations were analyzed on 3 consecutive days and % RSD was calculated. The results obtained for intraday and inter day variations were found to be within limits (less than 2% RSD). The results obtained are shown in Table 1 and 2.

Table 1: Intraday and Interday variation studies data for Benzoxonium Chloride.

Concentration (µg/ml)	Intra-day Precision		Inter-day Precision	
	Average area	% R.S.D	Average area	% R.S.D
60	1078098	1.8	1083345	1.31
80	1424351	0.69	1400572	0.58
100	1768324	0.98	1723648	0.65

Table 2: Intraday and Interday variation studies data for Lidocaine Hydrochloride.

Concentration (µg/ml)	Intra-day Precision		Inter-day Precision	
	Average area	% R.S.D	Average area	% R.S.D
60	2727212	1.68	2712212	0.44
80	3564257	0.79	3498257	0.70
100	4400261	0.67	4398261	0.61

Limit of detection (LOD) and limit of quantitation (LOQ)

From the linearity data the LOD and LOQ was calculated, using the formula LOD = 3.3 σ/S and LOQ = 10 σ/S where, σ = standard deviation of the y intercept of linearity equations and S = slope of the calibration curve of the analyte. LOD of Benzoxonium Chloride and Lidocaine Hydrochloridewas found to be 5.98 µg/ml and 4.48 µg/ml, respectively. LOQ of Benzoxonium Chloride and Lidocaine Hydrochloride was found to be 18.13 µg/ml and 13.57 µg/ml, respectively.

Assay

Assay was performed as per procedure mention under section sample preparation. Procedure was repeated for six times. The results obtained are shown in Table 3.

Table 3: Assay of marketed formulation.

Drug	Peak Area (Avg.)	Amount Recovered (µg/ml)	% Recovery	% RSD
Benzoxonium Chloride	721663.5	39.8281	99.57	0.58
Lidocaine Hydrochloride	1861292	39.9123	99.78	1.12

Accuracy

To check accuracy of the method, recovery studies were carried by spiking the standard drug to the sample solution, at three different levels around 50, 100 and 150 %. Basic concentration of sample solution chosen was 40 µg/ml of both drugs. % recovery was determined from linearity equation. The results obtained are shown in Table 4.

Table 4: Accuracy of Benzoxonium Chloride and Lidocaine Hydrochloride.

Level%	Sample		Standard		%Recovery, ±%RSD	
	Benzoxonium Chloride (µg/ml)	Lidocaine Hydrochloride (µg/ml)	Benzoxonium Chloride (µg/ml)	Lidocaine Hydrochloride (µg/ml)	Benzoxonium Chloride (µg/ml)	Lidocaine Hydrochloride (µg/ml)
50	40	40	20	20	100.12±1.83	100.51±1.68
100	40	40	40	40	100.71±0.69	99.94±0.79
150	40	40	60	60	100.17±0.98	99.99±0.67

Robustness

Robustness of the method was checked by carrying out the analysis under conditions during which mobile phase composition (± 2% Composition), detection wavelength (± 1 nm), flow rate (± 0.2 ml/min) were altered and the effect on the area were noted. Robustness of the method checked after deliberate alterations of the analytical parameters showed that areas of peaks of interest remained unaffected by small changes of the operational parameters indicating that the method is robust.

RESULTS AND DISCUSSION

The developed method was found to be simple, sensitive, selective, accurate and repeatable for simultaneous analysis of Benzoxonium Chloride and Lidocaine Hydrochloride in bulk and pharmaceutical dosage form without any interference from the excipients.

ACKNOWLEDGEMENT

Authors are thankful to the Principal and Management, AISSMS College of Pharmacy, Pune for providing required facilities for research work.

REFERENCES

1. https://www.drugbank.ca/drugs/DB06228 (Accessed on May 5, 2017).
2. https://en.wikipedia.org/wiki/Rivaroxaban (Accessed on May 5, 2017).
3. Belal TS, Haggag RS. J Chromatogr Sci., 2012; 50(5): 401-09.
4. Belal TS, Shaalan RA, Haggag RS. J AOAC Int., 2011; 94(2): 503-12.
5. Dubal KL, Ram VR., Kher GJ, Dave PN, Joshi HS., Khosla E, International Journal of Current Pharmaceutical Review and Research, 2016; 7(3): 141-50.
6. Eduardo RJ, Maria VLBB, Juliana MM, Rev. Bras. Cienc. Farm, 2002; 38(1): 107-11.
7. Sheikh S, Asghar S, Patni SA, International Journal of Scientific and Research Publications, 2012; 2(12): 10-8.
8. Prathyusha PCHGS, Shanmugasundaram P, Naidu PY, International Journal of Advances in Pharmaceutical Analysis, 2013; 3(1): 01-10.
9. Waraszkiewicz SM, Milao EA, DiRubio RJ, Pharm Sci., 1981; 70(11): 1215-8.
10. International Conference on Harmonization Guideline on Validation of Analytical Procedures Text and Methodology Q2 (R1), 2005.