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RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF LIGNOCAINE HYDROCHLORIDE AND CLOTRIMAZOLE HYDROCHLORIDE IN EAR DROPS

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ABSTRACT

A rapid and precise Reverse Phase High Performance Liquid Chromatographic method has been developed for the validated of Lignocaine and Clotrimazole, in its pure form as well as in ear drops. Chromatography was carried out on a Phenomenex C18 (4.6×250mm) 5µ column using a mixture of Methanol, Acetonitrile and potassium dihydrogen phosphate buffer (50:20:30) as the mobile phase at a flow rate of 1.2 ml/min, the detection was carried out at 220nm. The retention time of the Clotrimazole and Lignocaine was 2.266, 6.349min respectively. The method produce linear responses in the concentration range of 10-60µg/ml of Clotrimazole and 10-60µg/ml of Lignocaine. The method precision for the determination of assay was below 2.0% RSD. The method is useful in the quality control of pharmaceutical formulations.

KEYWORDS

Lignocaine HCl, Clotrimazole HCl, RP-HPLC and Validation.

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INTRODUCTION

Lignocaine is a synthetic aminoethylamide with local anesthetic and antiarrythmic properties.

MATERIAL AND METHODS

Chromatographic conditions

A prominence isocratic HPLC system (waters 515HPLC with auto sampler and UV Detector) column Phenomenex C18 (4.6 x 250mm, 5µm). A 20µL Rheodyne injection syringe was used for sample injection. HPLC grade Methanol, Acetonitrile and buffer were used for the preparing

the mobile phase. A freshly prepared Methanol, Acetonitrile and buffer (50:20:30% v/v) was used as the mobile phase. The solvents was filtered through a 0.45µ membrane filter and sonicated before use. The flow rate of the mobile phase was maintained at 1mL/min., column temperature was maintained at room temperature and the detection of the drug was carried out at 220nm.

Preparation of mobile phase

Mix a mixture of above HPLC grade Methanol500ml (50%), Acetonitrile 200ml and buffer 300ml of (30%) and degas in ultrasonic water bath for 10minutes. Filter through 0.45µ filter under vacuum filtration.

Diluent preparation

Mobile phase as diluent.

Standard solution preparation

Diluted 10 mg of Lignocaine and 10mg of Clotrimazole working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Sample solution preparation

10 mg equivalent weight of Lignocaine and Clotrimazole sample into a 10mL clean dry volumetric flask and add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent and filtered through 0.45micron injection filter by using a syringe. Further pipette 1ml of the stock solution into a 10ml volumetric flask and dilute up to the mark with Diluent.

METHOD VALIDATION[3-12]

Linearity

The linearity of the method was demonstrated over the concentration range of 10-60ppm of the Lignocaine target concentration. It includes 5-25ppm of Clotrimazole. Different concentrations of the pure drug were injected into the chromatographic system. Calibration curve of Lignocaine and Clotrimazole was constructed by plotting peak area versus applied concentration of Lignocaine and Clotrimazole. A typical chromatogram is shown in Figure No.1. The obtained results shown an excellent correlation between peak area and concentration of pure drug within the concentration range and it has shown in Figure No.2 and 3. The correlation coefficient for the average area at each level versus concentration of analyte was calculated and is presented in Table No.1 and 2 and their calibration parameters were shown in Table No.3 and 4.

Precision method

The precision of the method was demonstrated by inter-day and intra-day variation studies. In the intra-day studies, six repeated injections of standard solution was made and the response factor of drug peak and % RSD were calculated and present in Table No.5 and 6. The chromatogram was shown in Figure No.4. In the inter-day variation studies, six repeated injections of standard solution were made for six consecutive days and response factor of drug peak and % RSD were calculated shown in Table No.5 and 6. From the data obtained, the developed method was found to be precise.

Accuracy

A study of recovery of Lignocaine and Clotrimazole from spiked placebo was conducted at three different spike levels i.e.50%, 100% and 150% samples were prepared with Lignocaine and Clotrimazole raw material equivalent to about the target initial concentration of Lignocaine and Clotrimazole. Sample solutions were prepared in triplicate for each spike level and assayed as per proposed method. The % recovery was given in Table No.7 and 8. The mean recoveries of Clotrimazole spiked were found to be in the range of 98.90% - 100.20% and Lignocaine from spiked were found to be in the range of 98.42-99.52%.

System suitability

System suitability tests are an integral part of method development and are used to ensure adequate performance of the chromatographic system. Retention time (Rt), number of theoretical plates (N) and tailing factor (T) were evaluated for six replicate injections of the drug at a concentration of 30µg/ml. The results given in Table No.9 were within acceptable limits.

RESULTS AND DISCUSSION

In HPLC method, HPLC conditions were optimized to obtain, an adequate separation of eluted compounds. The objective of this study was to develop a rapid and sensitive RP-HPLC method for the analysis of Lignocaine and Clotrimazole in bulk dug and pharmaceutical dosage form by using the most commonly employed Phenomenex C-18 column with uv-detection.

The run time was set at 8min and the retention time for Clotrimazole and Lignocaine was 2.266, 6.349±0.2min respectively. Each sample was injected 6 times and the retention times were same. When the concentrations of Lignocaine and Clotrimazole and its respective peak areas were subjected to regression analysis by least squares method, a good linear relationship (r^2=0.999) was observed between the concentration of Lignocaine and Clotrimazole and the respective peak areas in the range 10-60µg /ml of Lignocaine and 5-25µg/ml of Clotrimazole. The regression equation was used to estimate the amount of Lignocaine and Clotrimazole, either in tablet formulations or in validation study (precision and accuracy). For the proposed RP-HPLC method, characteristic parameters were shown in Table No.2.

To analyse formulations, RP-HPLC method has been developed. Lignocaine and Clotrimazole tablets were analyzed as per the procedure described above. The low % RSD values (≤ 2) indicated that the method was precise and accurate. The mean recoveries found in the range of 98% – 100.20%. No interfering peaks were found in the chromatogram indicating that excipients used in the tablet formulation did not interfere with the estimation of the drug by the proposed RP-HPLC method.

Table No.1: Linearity results for Lignocaine Hcl

S.No	Conc. (µg / ml)	10	20	30	40	50
1	Avg. area	296060	592123	888176	1184235	1380300
2	Correlation	0.998				

Table No.2: Linearity results for Clotrimazole

S.No	Conc. (µg / ml)	10	20	30	40	50
1	Avg. area	693785	1385470	2081221	2675140	3368925
2	Correlation	0.999				

Table No.3: Characteristic parameters of Lignocaine for the proposed RP-HPLC method

S.No	Parameters	RP-HPLC
1	Calibration range (µg/ml)	10-60 of Lignocaine Hcl
2	Detection wavelength	220nm
3	Mobile phase (Methanol, Acetonitrile and buffer)	50:20:30
4	Retention time	2.266±0.02
5	Regression equation(Y*)	Y=22849x+94765
6	Slope (b)	22849
7	Intercept (a)	94765
8	Correlation coefficient (r^2)	0.998
9	Intraday precision (%RSD*) Interday precision (% RSD*)	0.72 0.60

Table No.4: Characteristic parameters of Clotrimazole for the proposed RP-HPLC method

S.No	Parameters	RP-HPLC
1	Calibration range (mcg/ml)	10-60 of Clotrimazole
2	Detection wavelength	220nm
3	Mobile phase (Methanol, Acetonitrile and buffer)	50:20:30
4	Retention time	6.349±0.02
5	Regression equation(Y*)	Y=66825x+38983
6	Slope (m)	66825
7	Intercept (c)	38983
8	Correlation coefficient (r^2)	0.999
9	Intraday precision (%RSD*) Interday precision (% RSD*)	0.28 1.30

Table No.5: Precision results for Lignocaine Hcl

Sl.No	Concentration (µg /ml)	Intraday precision (area)	Interday precision (area)
1	30	4795578	15814791
2	30	4781236	15698358
3	30	4816951	15958236
4	30	4702077	15759180
5	30	4839264	15705027
6	30	4756148	15762038
Mean		4789909	15782728
Std Dev		34597.82	239343.8
% RSD		0.72	0.60

Table No.6: Precision results for Clotrimazole

Sl.No	Concentration (µg /ml)	Intraday precision (area)	Interday precision (area)
1	30	4476639	707663
2	30	4467199	719232
3	30	4499734	699393
4	30	4463930	694851
5	30	4481893	712592
6	30	4480156	695616
Mean		4478259	704891.2
Std Dev		12714.15	9896.6
% RSD		0.28	1.30

Table No.7: Accuracy results for Lignocaine Hcl

Sample No	Spike level	Amount (ppm) found	Amount (ppm) added	% Recovery	Mean % Recovery
1	50%	29.52	10	98.40	98.40%
	50%	29.51	10	98.42	
	50%	29.53	10	98.38	
2	100%	39.49	20	98.70	98.72%
	100%	39.51	20	98.72	
	100%	39.48	20	99.9	
3	150%	49.76	30	99.52	99.52%
	150%	49.75	30	99.50	
	150%	49.77	30	99.54	

Table No.8: Accuracy results for Clotrimazole

Sample No	Spike level	Amount (ppm) found	Amount (ppm) added	% Recovery	Mean % Recovery
1	50%	20.04	10	100.20	100.20%
	50%	20.03	10	100.18	
	50%	20.02	10	100.22	
2	100%	29.98	20	99.93	99.93%
	100%	29.96	20	99.92	
	100%	29.94	20	99.91	
3	150%	39.56	30	98.90	98.90%
	150%	39.54	30	98.91	
	150%	39.58	30	98.92	

Table No.9: system suitability studies of Lignocaine and Clotrimazole by RP-HPLC method

S.No	Property	Lignocaine Values	Clotrimazole Values	Required limits
1	Retention time (R_t)	2.266±0.02	6.349±0.02	RSD ≤ 1%
2	Theoretical plates (N)	6230.13	9394.44	N > 2000
3	Tailing factor	1.10	1.12	T≤ 2

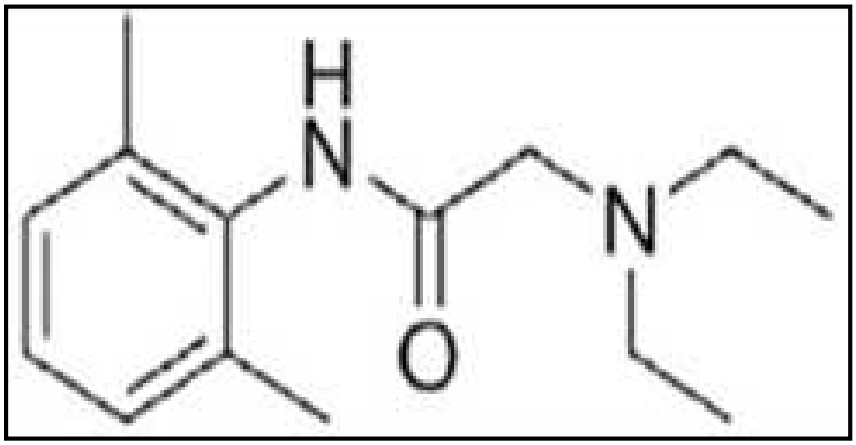

Figure No.1: Chemical structure of Lignocaine Hcl

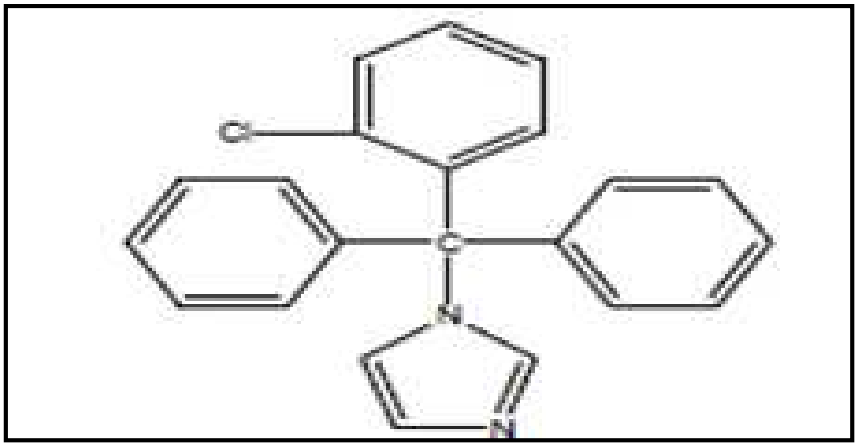

Figure No.2: Chemical structure of Clotrimazole

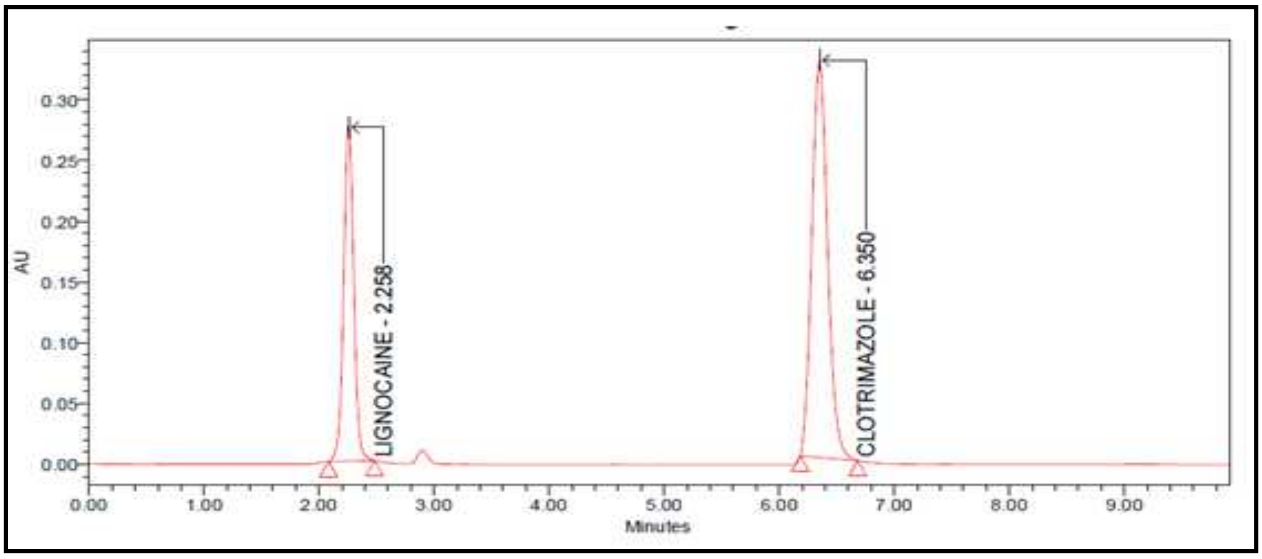

Figure No.1: Chromatogram of Lignocaine and Clotrimazole at 220nm

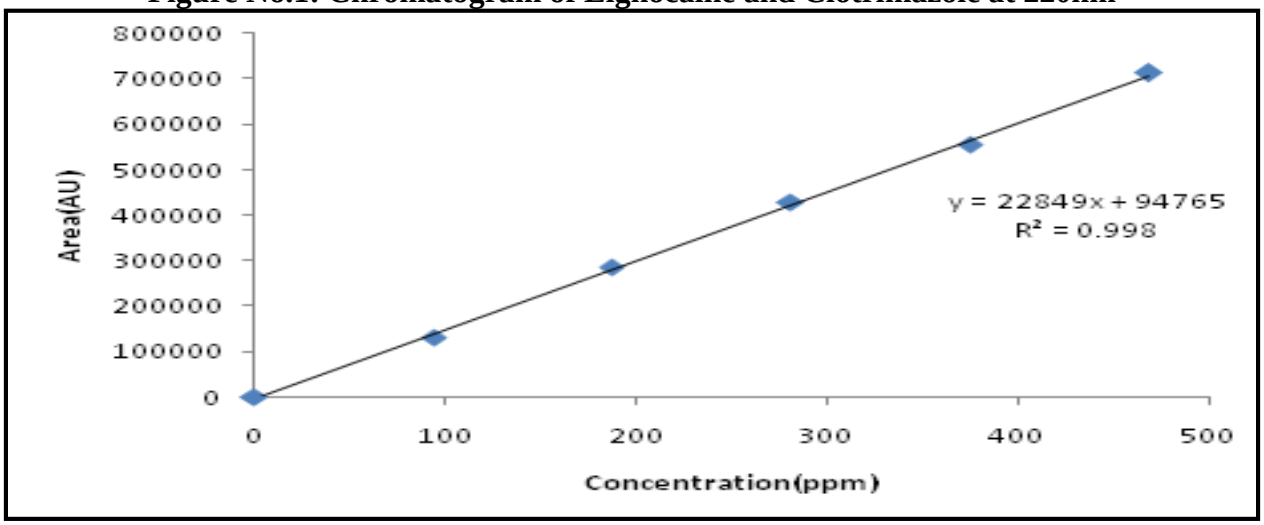

Figure No.2: Calibration curve of Lignocaine Hcl

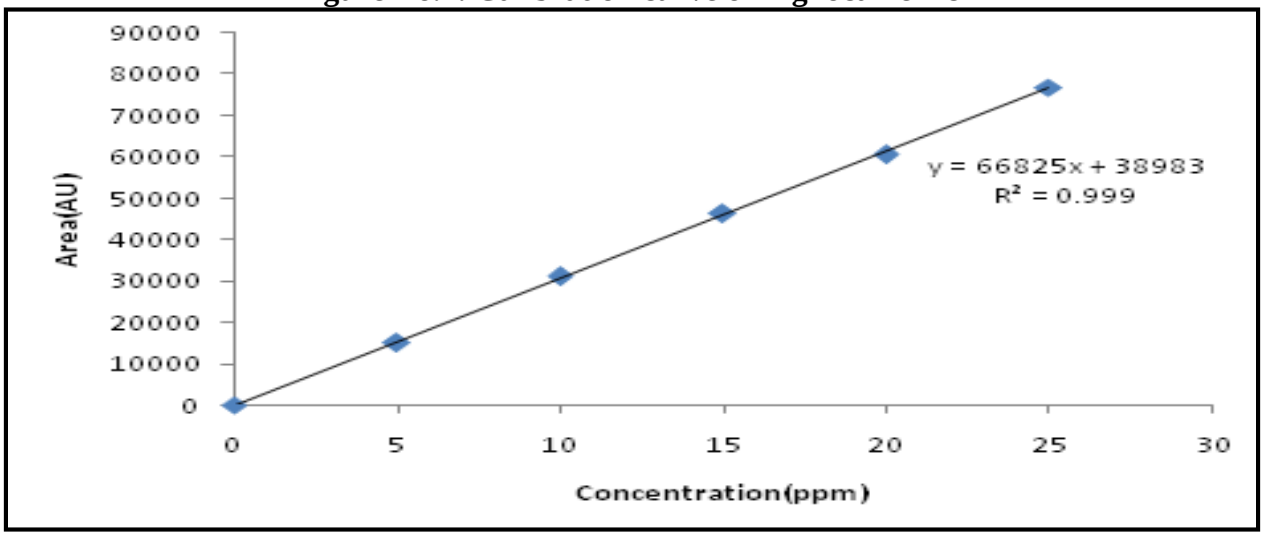

Figure No.3: Calibration curve of Clotrimazole

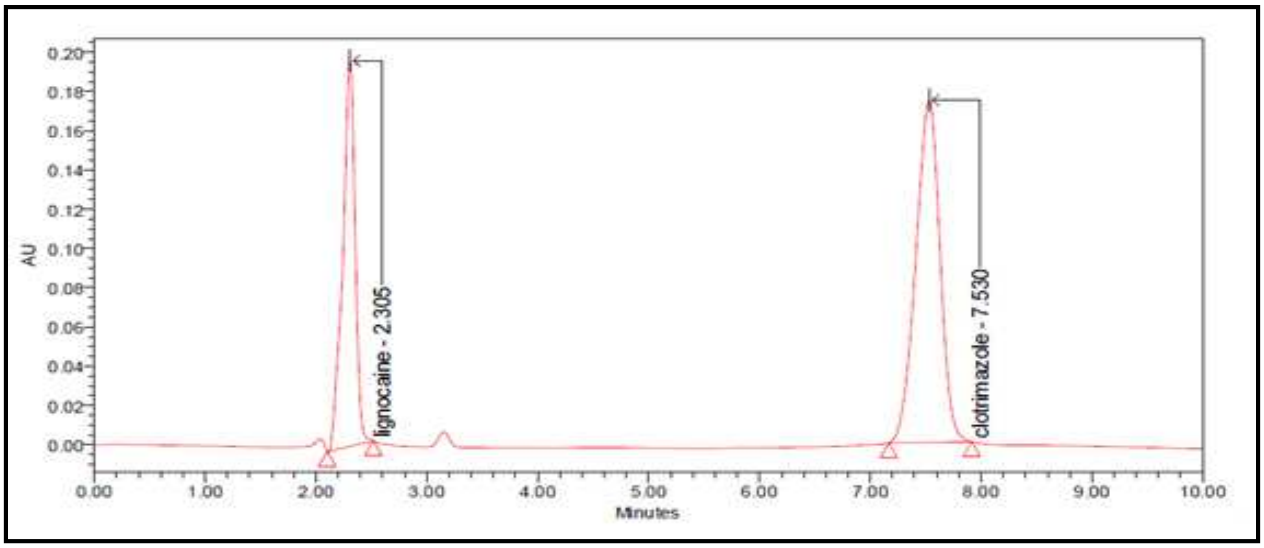

Figure No.4: Chromatogram of precision

CONCLUSION

The proposed RP-HPLC method was also validated for intra and inter-day variation. When the solution containing 30µg/ml of Lignocaine and 30 mcg/ml of Clotrimazole was repeatedly injected on the same day, the % RSD in the peak area for six replicate injections was found to be 0.28% for Lignocaine and 0.72% for Clotrimazole. Also the inter day variation (6 days and six injections) was found to be 1.29% for Lignocaine and 0.32% for Clotrimazole. The results are presented in Table No.3. The % RSD values were within 2 and the method was found to be precise. It can concluded the proposed HPLC method is rapid, sensitive, precise and accurate for the determination of Lignocaine and Clotrimazole and can be reliably adopted for routine quality control analysis of Lignocaine and Clotrimazole in Bulk and its pharmaceutical formulations.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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