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RP – HPLC Simultaneous Estimation of Diclofenac Diethylamine and Lidocaine in Pharmaceutical Gel Formulation

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ABSTRACT:-

A new specific, precise, accurate and robust RP-HPLC method has been developed for the simultaneous determination of Lidocaine and Diclofenac Diethylamine in a pharmaceutical gel formulation. The chromatographic separation was carried out at Jasco HPLC system consisting of Jasco PU 2075 pump having browin software.The stationary phase was PrincetonSPHER 100 C18 column (250mm X 4.6mm, 5µ).The mobile phase was Acetonitrile: Potassium dihydrogen phosphate (0.01M): Butane sulfonic acid sodium salt (45:55:0.1%), and adjust to pH 6.8± 0.05 with triethylamine. Detection was carried out at 261nm using Jasco UV 2075. The flow rate was 1.0ml/min and retention time was about 5.7min and 14.025min.for Diclofenac Diethylamine and Lidocaine respectively. The linearity was obtained in the concentration range of 20-300ug/ml and 50-500ug/ml for Lidocaine and Diclofenac Diethylamine respectively. Mean percentage recoveries were 99.84% for Lidocaine and 99.78% for Diclofenac Diethylamine. The LOD of Lidocaine and Diclofenac Diethylamine was found to be 20 µg/ml and 12.0 µg/ml whereas the LOQ was 35 µg/ml and 60 µg/ml respectively. The assay values of both the analytes was found to be well within the limits that is 101.5% and 98.25% for Lidocaine and diclofenac Diethylamine respectively. Percentage relative standard deviation of percent assay values for replicate sample preparation was 1.11% for Lidocaine and 0.95% for Diclofenac Diethylamine .The method was robust with respect to change in flow rate, and composition of mobile phase.

KEY WORDS: - Diclofenac Diethylamine, Lidocaine, HPLC-UV, Assay, Method Validation

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INTRODUCTION

Diclofenac Diethylamine[1] is diethylammonium 2-[(2, 6-dichloro anilino) phenyl] acetate. It is a white light beige crystalline powder. Sparingly soluble in water and acetone, freely soluble in ethanol (96%) and methanol; practically insoluble in *1M sodium hydroxide*, It melts about 154°C with decomposition. Diclofenac Diethylamine is official in *British Pharmacopoeia* which recommends potentiometric methods for its analysis.

Lidocaine[2] is 2-(Diethlamino)-N-(2, 6-dimethylphenyl) acetamide. It is a white or almost white crystalline powder. Practically insoluble in water, very soluble in ethanol (96%) and in methylene chloride. Lidocaine is also official in *British Pharmacopoeia* which recommends potentiometric non-aqueous method for its analysis.

Diclofenac Diethylamine[3] is a non-steroidal anti-inflammatory drug used for a variety of painful and inflammatory conditions. Lidocaine[3] is a local anesthetic of the amide type and has a fast onset and an intermediate duration of action. An extensive literature survey revealed GC, HPLC[4-16], and colorimetric [3], determination for Lidocaine, where as Diclofenac Diethylamine was determined by HPLC. A gradient HPLC[17] diode array detector stability indicating method described for the determination of Lidocaine hydrochloride and cetylpyridinium chloride in two combined oral dosage form. A method is also describes for simultaneous determination of Lidocaine and its principal metabolites by liquid chromatography on silica gel with aqueous eluent[18]. There is method for simultaneous determination of Aceclofenac[19] and Diclofenac in human plasma by narrow-bore HPLC using column switching. HPLC method for simultaneous[20] determination of Mephenesin and Diclofenac Diethylamine is also available. An HPLC[21-22], method utilized for the simultaneous determination of Diclofenac sodium and Lidocaine from injection dosage form. But there is no method which describes the simultaneous determination of Diclofenac Diethylamine and Lidocaine from gel dosage form meant for external application. The objective of this investigation was to develop[23] simple accurate and economical procedures for simultaneous estimation of Lidocaine and Diclofenac Diethylamine from a gel dosage form.

EXPERIMENTAL

Reagent and Chemical:

Acetonitrile HPLC grade and Potassium dihydrogen phosphate was procured from Merck and company.

Apparatus and Chromatographic Conditions:

Chromatographic separation was performed on a Jasco HPLC system consisting of Jasco PU 2075 pump, Jasco UV 2075 detector, Hemilton injection syringe with 20µl loop volume and window based Borwin software. An ODS PrincetonSPHER C18 RP-Column (250mm X 4.6mm, 5µm) was used for separation. The elution was carried out isocratically at flow rate of 1ml/min using Acetonitrile: 0.01 M Potassium dihydrogen phosphate: butane sulfonic acid sodium salt (45:55:0.1%) as mobile phase.

PREPARATION OF STANDARD SOLUTION:

Lidocaine

About 20mg of Lidocaine *RS* was accurately weighed and transferred to a 100ml, volumetric flask. Dissolved in 50ml of mobile phase and diluted to the volume, with mobile phase, to obtain Lidocaine concentration of 200mcg/ml.

Diclofenac Diethylamine:

A 100ug/ml of stock solution of Diclofenac Diethylamine *RS* was prepared in mobile phase.

Combine standard:

Weigh about 20mg of Lidocaine *RS* and 11.6mg of Diclofenac Diethylamine *RS* in 100ml of volumetric flask dissolved in 50ml of mobile phase, and diluted to volume with the mobile phase.

Sample Preparation:

Weigh the sample quantity equivalent to 20mg of Lidocaine and 11.6mg of Diclofenac Diethylamine and transfer to a 100ml volumetric flask. About 50ml of mobile phase was added and sonicated for 20min.with intermittent shaking. Then the solution was diluted up to the volume with mobile phase. The sample was filtered through PALL Ultipore N_{66} Nylon 6, 6 filter paper, and 20µl of clear filtrate was injected to estimate the percentage assay of Lidocaine and Diclofenac Diethylamine.

METHOD VALIDATION:

As per ICH guideline the method was validated and following parameters were evaluated, along with ruggedness[24-27].

Analysis of sample was carried out using the above method and the result are tabulated in table I.

Table I: Analysis of sample

Contents	Label claim %w/w	Found %w/w	Assay % of label amount
Diclofenac Diethylamine	1.16	1.14	98.28
Lidocaine	2.0	2.04	101.5

Sample – in house Production batch

CALIBRATIONS/LINEARITY:

Linearity of the method was established by analysis of combined standard solution. Several aliquots of standard Lidocaine and Diclofenac Diethylamine were taken in 10ml volumetric flasks and diluted up to the mark with mobile phase in such a way that the final concentration of Lidocaine and Diclofenac Diethylamine is 20-300µg/ml and 50-500µg/ml respectively(Figure:1). 20µl of each of solution was injected. Evaluations of two drugs were performed with UV detector at 261nm.Calibration curve was constructed by plotting peak area (Y-axis) against the amount of drug in mcg/ml (X axis) and the linear relationship was evaluated (Figure: 2).

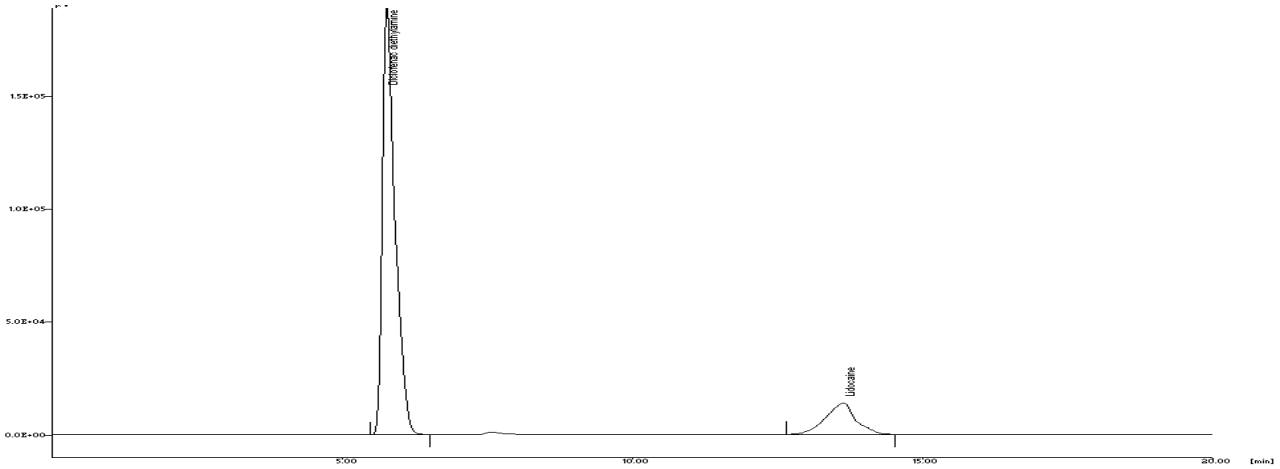

Figure: 1 Shows the HPLC graph of standard Diclofenac Diethylamine at the retention of 5.7 and Lidocaine at 14.03 simultaneously.

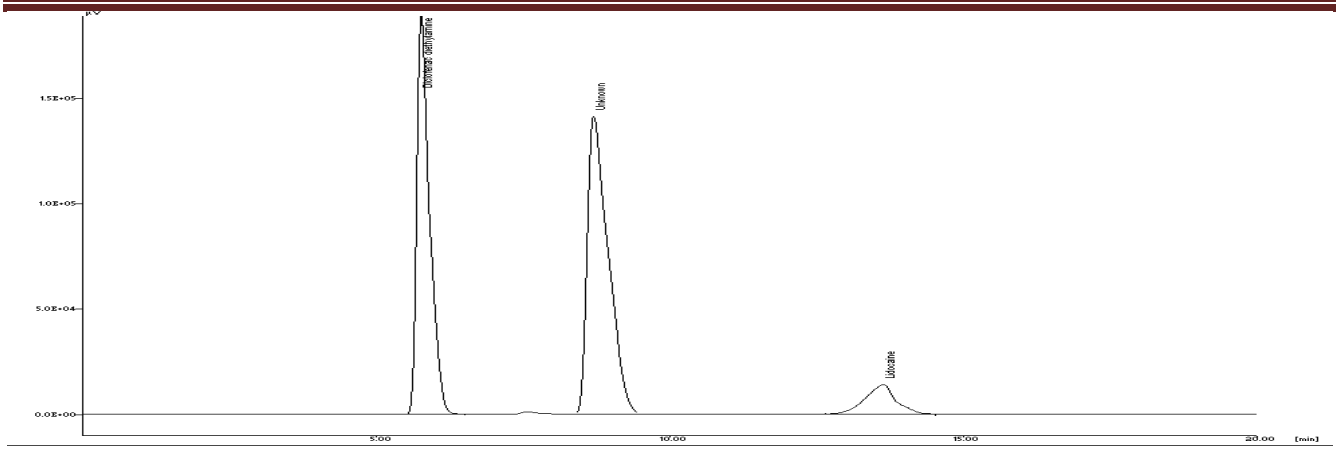

Figure: 2 Shows the HPLC graphs of sample consisting of Diclofenac Diethylamine at the retention of 5.7 and Lidocaine at 14.03simultaneously.

Limit of Detection (LOD) and Limit of Quantification (LOQ):

The limit of detection and limit of quantification of the developed method were determined by injecting progressively low concentration of the standard solutions using the developed RP-HPLC method. The LOD of Lidocaine and Diclofenac Diethylamine was found to be 20 µg/ml and 12.0 µg/ml respectively. The LOQ is the smaller concentration of the analyte response that can be quantified accurately the LOQ was 35 µg/ml and 60 µg/ml respectively.

RECOVERY STUDIES:

To study the accuracy and reproducibility of the proposed method recovery experiments were carried out. A fixed amount of pre-analyzed sample was taken and standard drug was added at 50%, 80% and 100% levels. Each level was repeated three times. The contents of Lidocaine and Diclofenac Diethylamine found by proposed method is shown in the Table II. The mean recoveries of Lidocaine and Diclofenac Diethylamine were 99.84% and 99.78% respectively which shows there is no interference from excipient.

PRECISION STUDIES:

Precision of method was studies by analysis of multiple sampling of homogeneous sample. The precision of analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogenous

Sample under the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility. Precision should be investigated using homogenous

authenticated sample. Precision Expressed as % RSD is given in Table-III which should be less than 2%.

Table II: Results of Accuracy Experiment

Amount of sample		Amount of drug added		Amount recovered		% Recovery	
Lidocaine (µg/ml)	Diclofenac Diethylamine (µg/ml)	Lidocaine µg/ml	Diclofenac Diethylamine (µg/ml)	Lidocaine (µg/ml)	Diclofenac Diethylam-ine (µg/ml)	Lidocaine (%)	Diclofenac Diethylam-ine (%)
200	100	50	50	249.59	149.56	99.83	99.70
200	100	50	50	250.02	149.86	100.00	99.90
200	100	50	50	249.68	148.96	99.87	99.30
200	100	80	80	279.88	180.15	99.95	100.08
200	100	80	80	280.11	179.89	100.03	99.93
200	100	80	80	278.99	179.86	99.63	99.92
200	100	100	100	299.69	199.12	99.89	99.56
200	100	100	100	299.00	199.76	99.66	99.88
200	100	100	100	299.10	199.63	99.70	99.81
Average percent recovery						99.84	99.78

Table III: Precision for Lidocaine and Diclofenac Diethylamine

Sample*	Diclofenac Diethylamine (%)	Lidocaine (%)
Sample 1	98.6	103.2
Sample 2	97.8	102.6
Sample 3	96.5	101.2
Sample 4	97.8	103.1
Sample 5	98.2	101.9
Sample 6	99.3	100.9
Average	98.0	102.1
%RSD	0.95%	1.11%

*sample – in house Production batch

SYSTEM SUITABILITY STUDIES:

System suitability testing is an integral part of many analytical procedures. The tests are based on the concept that the equipment, electronics, analytical operations and samples to be analyzed constitute an integral system that can be evaluated as such. In that the column efficiency, resolution and peak asymmetry were calculated for the standard solutions Table IV. The values obtained demonstrated the suitability of the system for the analysis of this drug combination.

Table IV: System Suitability Parameter

Parameter	Lidocaine	Diclofenac Diethylamine
Precision of the method (n = 6)	1.11 %	0.95%
Theoretical Plates	4224	4576
Resolution Factors	1.750	8.258
Asymmetric Factor	1.000	1.000
Retention time	14.025	5.767

ROBUSTNESS AND RUGGEDNESS OF THE METHOD:

Robustness of the method:

Robustness is a measure of its capacity to remain unaffected by small but deliberate variations in the chromatographic method parameters and provides an indication of its reliability. This was done by small deliberate changes in the chromatographic conditions at 3 different levels and retention time of Diclofenac Diethylamine and Lidocaine was noted. The factor selected were flow rate, pH and % Acetonitrile in the mobile phase. It was observed that there were no deliberate changes in the chromatogram, which demonstrated that the RP-HPLC method developed, are robust. Results describe in Table V.

Ruggedness of the method:

The USP guideline defines ruggedness as “the degree of reproducibility” of the test result obtained by the analysis of the same samples under a variety of normal test condition such as; different Laboratory, different analyst, different instrument etc. Here this was done by changing the instrument and analyst. Results, presented in the Table VI that indicates the selected factors are remained unaffected by small variations of this parameter.

Table V: Robustness of the method

Factor	Level	Retention time	
	Flow rate ml/min	Lidocaine	Diclofenac Diethylamine
1.1	-0.1	14.225	5.878
1.0	0	14.025	5.767
1.2	+0.1	13.996	5.676
pH of the mobile phase			
5.8	-1	14.009	5.673
6.8	0	14.025	5.767
7.8	+1	14.253	5.802
% Acetonitrile in the mobile phase			
43	-1	14.009	5.699
45	0	14.025	5.767
46	+1	14.110	5.823

Table VI: Ruggedness of the method

	Lidocaine Average of six values	**Diclofenac Diethylamine Average of six values**
Between Instrument I and II		
Instrument	% Content	% Content
I	97.2%	103.3%
II	98.9%	101.9%
% Error	1.7%	1.4%
Between Instrument I and II		
Analyst	% Content	% Content
I	97.2%	103.3%
II	98.8%	101.6%
%Error	1.6%	1.7%

CONCLUSION:

The proposed RP-HPLC method allows for accurate, precise and reliable estimation of Diclofenac Diethylamine and Lidocaine in combined semi solid dosage form. The developed method can be used for routine quantitative simultaneous estimation of Diclofenac Diethylamine and Lidocaine in Pharmaceutical Preparation.

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