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DEVELOPMENT AND VALIDATION OF A STABILITY-INDICATING RP-HPLC ASSAY METHOD AND STRESS DEGRADATION STUDIES ON DAPIPRAZOLE HYDROCHLORIDE IN OPHTHALMIC DOSAGE FORM

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ABSTRACT
A simple, rapid, accurate, precise, robust and reproducible reverse phase high performance liquid chromatographic method was developed for the determination of Dapiprazole in pure drug and pharmaceutical dosage form. The quantification was carried out using enable Inertsil ODS-3Vs column in a binary mode with mobile phase comprising of Acetonitrile: Methanol:Buffer (50:30:20) in the ratio of 50:30:20 %v/v at flow rate 1.0 mL/min, detection was carried out at 243 nm using UV-Visible detector with injection volume 20µl, the retention time was found to be 3.438min. The proposed method was validated as per ICH guidelines. The method produced linear response in the concentration range of 1-5µg/ml (R^2 0.999). The recovery studies were carried out and found to be within101%. %RSD was found to be 2%. LOD and LOQ of Dapiprazole for the method were found to be 3.64µg/ml and 11.04µg/ml respectively. The proposed method was statistically evaluated and can be applied for the routine analysis, quality control of raw materials, formulation of different strengths, dissolution studies and bioequivalence studies for the same formulation of Dapiprazole.

KEYWORDS: Dobutamine; RP–HPLC; Validation; Stability indicating: Ophthalmic Dosage form.

Dapiprazole: Chemically 1-[2-2[Hydroxy-3-propylamino)propoxy]phenylpropan-1-one hydrochloride is used class 1c anti arrythmic medication and acts as local anesthetic. Molecular Formula: $C_{21}H_{27}NO_3$.Hcl, Molecular weight: 377.909 g/mol, Solubility: Soluble in ethanol and in water (with warming*)*

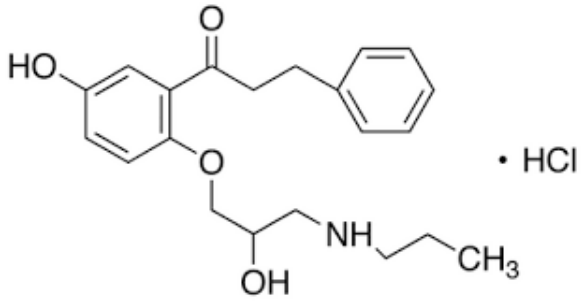

Fig. No.1. Chemical Structure of Dapiprazole *HCl.*

Dapiprazole works by slowing the influx of sodium ions into cardiac muscle cells, causing a decrease in excitability of the cells. Dapiprazole is more selective for cells with a high rate, but also blocks normal cells more than class1a or 1b. Dapiprazole differs from the prototypical class 1c antiarrhytmic in that it has additional activity as a beta-adrenergic blocker which can cause bradycardia and bronchospasm.

A RP-HPLC method was developed for the determination of Dapiprazole Hcland applied for in vitro permeability studies of self-emulsifying drug delivery system (SEDDS) formulations using Caco-2 cell line. Discovery® enable Inertsil ODS-3Vs column was used at 30 °C. Isocratic elution was performed with Acetonitrile: Methanol: Buffer (50:30:20). The flow rate was 1.0 ml/min and UV detection was at 243 nm. Dapiprazole eluted within 4 min.

MATERIALS AND METHODS
1.1 Instrumentation
HPLC instrument used was of Shimandzu Prominence Binary with Rheodyne injector and PDA Detector. Software used is LC-Solutions. UV-VIS spectrophotometer Lab India (UV-3000+)Double beam spectrophotometer with special bandwidth of 2mm and

10mm and matched quartz was be used for measuring absorbance forDapiprazolesolutions.

1.2 Chemicals
Dapiprazolereference standard was obtained from the tablets of brand *Pradil*(*Emcure)* of *150 mg*. Methanol,Acetonitrile was used as the solvent for the experiment.

1.3 Chromatographic conditions
Glass wares used in each procedure were soaked overnight in a mixture of chromic acid and sulphuric acid rinsed thoroughly with double distilled water and dried in hot air oven. Inertsil ODS-3Vs column was used at 30 °C. Isocratic elution was performed with Acetonitrile: Methanol:Buffer (50:30:20)mixture. The flow rate was 1.0mL/min and UV detection was at 243 nm. Dapiprazole eluted within 4 min.

1.4 Preparation of Standard stock solutions
Accurately weighed 10mg of Dapiprazole was transferred to 10ml volumetric flask. The volume was made upto mark with same solvent to 10ml (1000 µg/ml). Then 1ml of the above solution was diluted to 10ml with the solvent system (100µg/ml). The resultant standard solution (10µg/ml) was filtered through a 0.45 µm membrane filter and degassed under ultra-sonic bath prior to use. From the above standard solution several working standard solutions are made by serial dilution technique.

METHOD VALIDATION
The Proposed method was validated as per the ICH Q2 (R1) guidelines for linearity, range, accuracy, precision, ruggedness.

Robustness
Small deliberate changes in method like Flow rate,wavelength, and temperature are made but there were no recognized change in the result and are within range as per ICH Guide lines.

Precision
The precision is procedure expresses the closeness of agreement (degree of scatter) between the series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions.

Linearity
The ability (with in given range) to obtain test results, which are directly proportion alto the concentration (amount) of analyte in the sample.

Accuracy
To assess the accuracy of the proposed method, recovery studies were carried out three different levels i.e., 50%, 100% and 120%. To the pre- analyzed sample solution, a known amount standard drug solution was added at three different levels, absorbance was recorded. The % recovery was then calculated by using formula

% Recovery = A – B/ C

Where A = Total amount of drug estimated
B = Amount of drug found on preanalysed basis
C = Amount of Pure drug added

RESULTS AND DISCUSSION
Calibration of standards
The standard calibration curve was constructed for Dapiprazole HCl. Different volumes of stock solutions of each were accurately transferred in to 10mL volumetric flasks and diluted to mark to yield a concentration of 10µg/ml solutions. The calibration line was obtained by plotting the peak are against concentration of drug.

Robustness, limit of detection and limit of quantification, precision was calculated by percentage relative standard deviation. The accuracy was expressed in terms of percent recovery of the know amount of the standard drugs added to the known amount of the pharmaceutical dosage forms. Various validation parameters are per formed.

1. System Suitability
A Standard solution of Dapiprazole working standard was prepared as per procedure and was injected five times into the HPLC system. The system suitability parameters were evaluated from standard Chromatograms obtained by calculating the % RSD of retention time, tailing factor, theoretical plates and peak areas from five replicate injections are within range and Results were shown in table 1.

Table 1: System Suitability data.

	Peak Name	RT	Area	USP Plate Count	USP Tailing
1	Dapiprazole	3.282	35021	3385	1.225
2	Dapiprazole	3.329	68605	2997	1.123
3	Dapiprazole	3.328	97624	2813	1.051
4	Dapiprazole	3.329	128372	2817	1.022
5	Dapiprazole	3.364	159908	2383	0.980

2. Linearity
It is the ability of the method to elicit test result that is directly proportional to analytic concentration within a given range. It is generally reported as variance of slope or regression line. Appropriate volume of standard stock solution was transferred to volumetric flask of 10 ml capacity. The volumes were adjusted to the mark with mobile phase to give a solutions containing 2-10 ppm.

The slope, Y-intercept and correlation coefficient were calculated. The regression line relating standard concentrations of drug using regression analysis was calculated. The calibration curves were linear in the studied range and equations of the regression analysis were obtained.

Table 2: Linearity data of Dapiprazole.

Linearity level	Concentration (µg/ml)	Area			Mean
		Set-1	Set-2	Set-3	
I	2	35019	35039	35021	35021
II	4	68040	66572	66105	68605
III	6	97775	97695	97399	97624
IV	8	128390	128575	128172	128372
V	10	159932	15987	159895	159908
Y = 31723.2X+1997 R^2= 0.999751					

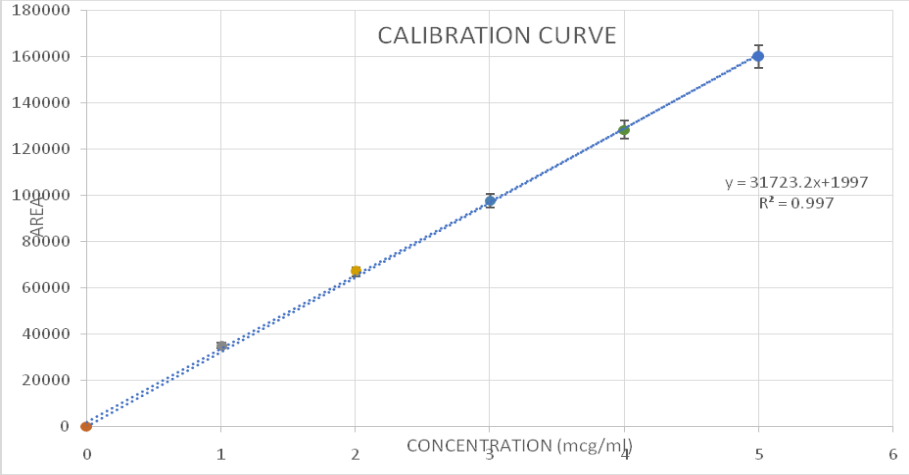

Fig. 02: Calibration curve of Dapiprazole.

3. Precision

Six working sample solutions of 20ppm are injected and the % Amount found was calculated and %RSD was found to be 0.9.

Table 3: Intraday precision studies of Dapiprazole HCl.

S. No	Amount (µg/ml)	Amount found (µg/ml)	Percentage%	% Mean	SD **	% RSD
1.	3	2.94	98.05	97.72	0.00089	0.000091
2.		2.83	94.33			
3.		2.95	98.33			
4.		2.92	97.33			
5.		3.00	100			
6.		2.95	98.33			

Table 4: Interday precision studies of Dapiprazole HCl.

S.No	Amount (µg/ml)	Amount found (µg/ml)	Percentage %	Mean	SD*	% RSD
1.	3	2.08	93.33	98.27	0.00008	0.0000814
2.		2.96	98.66			
3.		3.02	100.66			
4.		3.00	100			
5.		2.98	99.33			
6.		2.93	97.66			

Intermediate precision: Five working sample solutions of 20ppm are injected on the next day of the preparation of samples and the % Amount found was calculated and %RSD was found to be within the specified limits

Acceptance Criteria: The % RSD for all the five standard injections results should notbe more than2%

4. Accuracy

Accuracy: Three Concentrations of 50%, 100%, 120% are Injected in a triplicate manner and %Recovery was calculated as 100.8.

Table 5: Accuracy data of Dapiprazole.

S. No	% Spike Level	Amoun present (µg/ml)	Amount added (µg/ml)	Amount found (µg/ml)	Amount recovered (µg/ml)	% Recovery	Mean % Recovery	SD	% RSD
1.	50%	2.93	1.5	4.42	1.49	99.3	101.7	0.73	0.51
2.				4.46	1.53	102			
3.				4.49	1.56	104			
4.	100%		3	5.98	3.05	101.6	100.8	0.1	0.07
5.				5.95	3.02	100			
6.				5.94	3.01	100.3			
7.	150%		4.5	7.44	4.51	100.22	99.84	0.02	0.014
8.				7.41	4.48	99.55			
9.				7.42	4.49	4.49			

5. Robustness

Small Deliberate change in the method is made like Flow minus, flow plus, Mobile phase minus, Mobile phase plus, Temperature minus, Temperature Plus. %RSD of the above conditions was calculated.

6. LOD and LOQ

LOD and LOQ were calculated from the formula 3.3 x(σ/S) and 10 x (σ/S), respectively where, σ is standard deviation of intercept and S is the mean of slope. The LOD and LOQ can also be determined by S/N. The value for LODshouldbe3-5whilst for LOQ 10-15.

CONCLUSION

Based on the results obtained, it is found that the developed RP-HPLC technique is quite simple, accurate, precise, reproducible, sensitive and economical. They can become effective analytical tools for routine quality control of Dapiprazole in bulk and pharmaceutical dosage forms. The results obtained were well within the acceptance criteria.

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