Development Of Analytical Method For The Simultaneous Estimation Of Diclofenac Sodium And Pantoprazole In Pharmaceutical Formulation By RP-HPLC

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Abstract- A sensitive high-performance liquid chromatographic (HPLC) method were developed and validated for the estimation of Diclofenac sodium and Pantoprazole in bulk and pharmaceutical formulations. The chromatographic separation was achieved by RP- HPLC using a mixture of methanol: Phosphate buffer (10mM) in ratio 80:20 pH 3.5 as the mobile phase with isocratically system, a C_{18} column & the eluents was monitored using UV detector at 284 nm. The pH is adjested by ortho phosphoric acid. These methods were tested and validated for various parameters according to ICH guidelines. The proposed methods were successfully applied for the determination of Diclofenac sodium & Pantoprazole in pharmaceutical formulations. The results demonstrated that the procedure is accurate, precise and reproducible (relative standard deviation <2%), while being simple, cheap and less time consuming.

Index Terms- Diclofenac sodium; Pantoprazole, Marketed formulation, Spectrophotometer, HPLC.

I. INTRODUCTION

Diclofenac sodium is - 2-[(2, 6-Dichlorophenyl) amino] benzeneacetic acid monosodium salt is a N.S.A.I.D.(1) Pantoprazole is 5-(Difluoromethoxy)-2-(((3, 4-dimethoxy-2-pyridinyl)methyl) sulfinyl)-1H-benzimidazole is a proton pump inhibitor compound which has been developed for oral and parenteral use.(2)

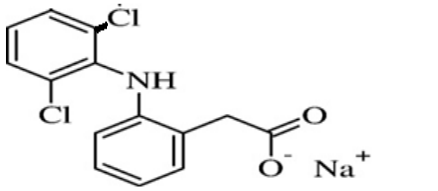
High-performance liquid chromatographic (HPLC) is the most frequently applied technique in the determination of drugs in biological fluids and dosage forms. We believe that the availability of this new method, with increased simplicity, sensitivity and selectivity, will be very useful for the determination of Diclofenac sodium and Pantoprazole in raw material and pharmaceutical preparations. This gradient HPLC method uses a simple mobile phase, UV detection and does not require complicated sample preparation. The aim of this study was to develop a simple, rapid and reproducible reversed-phase HPLC method.

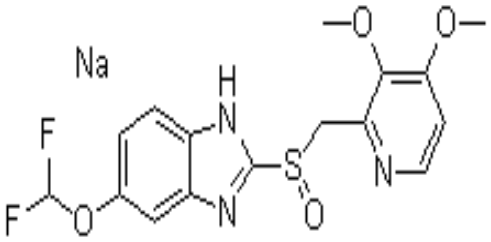
II. EXPERIMENTAL

2.1 Materials

DIC & PNT was supplied by Amoli organics Pvt, Ltd. and was used without further purification. Sodium hydroxide was purchased from Molychem (Mumbai). Hydrochloric acid and hydrogen peroxide was procured from LOBA Chemie Pvt. Ltd. (Mumbai). HPLC grade methanol was purchased from S. D. Fine-chem Ltd. (Mumbai) whereas HPLC grade water was purchased from Merck Ltd. All other chemicals were of analytical reagent grade.

2.2 Chemical structure :





Diclofenac sodium

Pantoprazole

2.3 Instrumentation

The HPLC system consisting of Thermo Separation Quaternary Gradient HPLC pump Spectra System P4000 with Variable UV-VIS detector of Spectra System UV1000, manual rheodyne injection system, the software was a Data ace software

version 6.1. The chromatographic separation was performed using Grace C_{18} (250mm × 4.6 mm i.d., 5mm particle size) Separation was achieved using a mobile phase consisting of buffer:methanol in the ratio (80:20 pH 3.5 adjusted with ortho phosphoric acid) at a flow rate of 1ml/min and UV detection at 284 nm. The column was maintained at ambient temperature with injection volume of 20 µl. The mobile phase was filtered through 0.45 µm Chrom Tech Nylon-66 filter and degassed in ultrasonic bath prior to use. A blank chromatogram was recorded before the studies. Quantization of result was performed using peak area counts.

2.4 Standard preparation

Stock solution of DIC & PNT was prepared. Accurately weighed quantity 5 mg of both was dissolved in methanol and volume was made up to 25 ml mark (200 µg/ml). The stock standard solution was diluted further with Methanol to get final concentration of about 10 µg/ml. then various trial are taken & mobile phase finalized where proper resolution of both the drug were seen. This was found that the sample preparation in mobile phase gives sharp resolution hence all samples were prepared in mobile phase. The stock solution was prepared in mobile phase of 100µg/ml.

III. Result & Discussion:

3.1 Preparation of calibration curve:-

The mobile phase was allowed to equilibrate with the stationary phase until steady baseline was obtained. The series of concentration from 2-20 µg/ml of both drug solutions were injected and peak area was recorded. The graph plotted as the concentration of the drug Vs peak area depicted in Fig. No.2 and 3.

3.2 Method Validation

3.2.1 Specificity (Selectivity)

Specificity was measured as ability of the proposed method to obtain well separated peak for DIC and PNT without any interference from component of matrix. The values obtained were very close to that in standard laboratory mixture in DIC no interference from the component of matrix. Mean retention time for –

DIC = 2.446

PNT = 6.678

3.2.2. Accuracy and precision

It was ascertained on the basis of recovery studies performed by standard addition method. The results of recovery studies and statistical data are recorded in Table No.1 Precision of an analytical method is expressed as S.D or R.S.D of series of measurements. It was ascertained by replicate estimation of the drugs by proposed method.

3.2.3 Ruggedness:

The studies of ruggedness were carried out under two different conditions-

a) Days
b) Analyst.

a) Interday (Different days):

Same procedure was performed as under marketed formulation analysis on different days. The % label claim was calculated. Data obtained for day 1, day 2, and day 3 is shown in Table No. 1

b) Different analyst:

The sample solution was prepared by two different analysts and same procedure was followed as described earlier. The % label claim was calculated as done in marketed formulation estimation.

3.3 Analysis of Pharmaceutical dosage form (eye drop):

The values of analysis of eye drop obtained by the proposed method were between 99.6% and 101.6% (Table 2), which showed that the estimation of dosage forms were accurate within the acceptance level of 95% to 105%. (Refer Table 2).

Tables:

Table 1: Summary of validation parameters for the proposed method

Validation Parameters	**DIC**	**PNT**
Linearity µg mL-1	0.5-5.0	0.2-2.0
Accuracy mean	0.065	0.055
Precision (% RSD)	0.047	0.100
Intraday (% RSD)	0.0360	0.115

Table 2: Results of analysis of formulation and recovery studies

Drug	Mean	S.D.	%RSD
DIC	99.89	99.89	99.89
PNT	99.88`	99.88`	99.88`

*Recovery amount was the average of six determinants

	% Label claim			
	ANALYST I		ANALYST II	
	DIC	PNT	DIC	PNT
%R.S.D	0.2033	0.4288	0.3465	0.2995

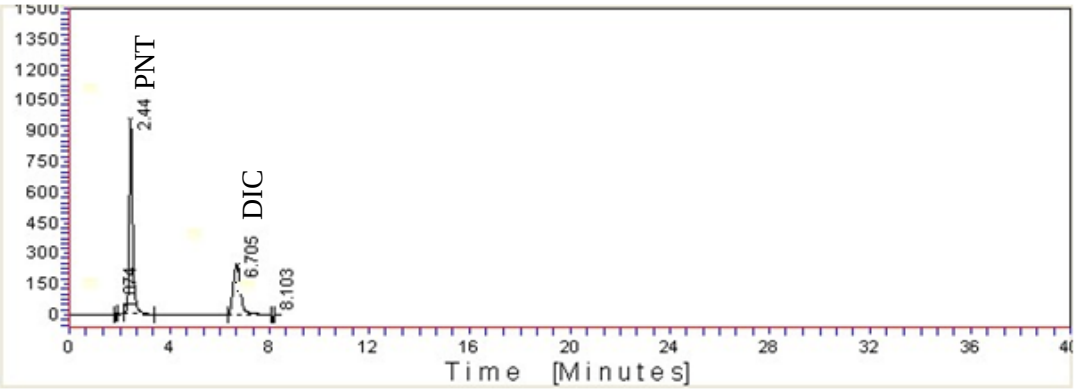


Fig. No.1: Table No. 3: Observations of Linearity and range study for DIC and PNT.

Sr.No.	%Label claim	Peak area	
		DIC	PNT
1	80	3500.941	1384.8
2	90	4015.44	1595.02
3	100	4567.858	1795.97
4	110	5042.207	1981.28
5	120	5544.819	2105.63

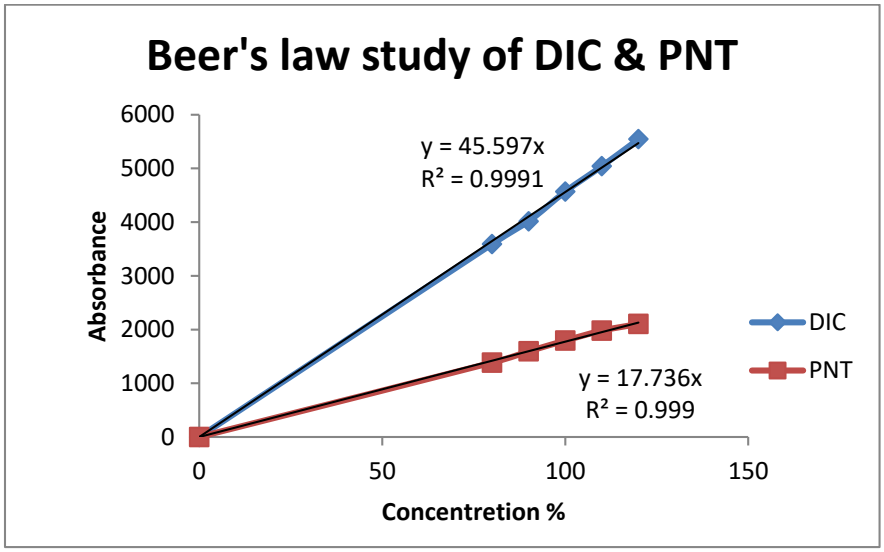
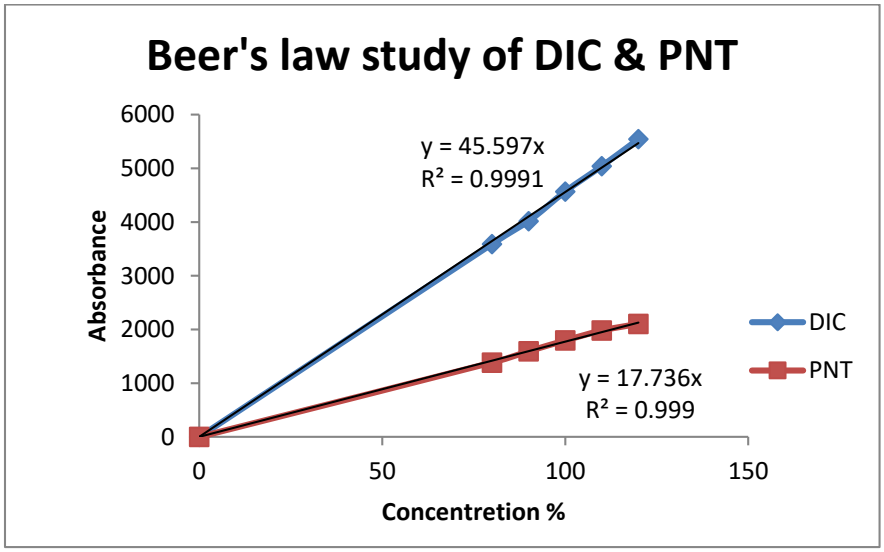


Fig. No:-24:-Plot of linearity and range study for DIC & PNT



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