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# DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF RABEPRAZOLE AND DICLOFENAC IN PURE AND TABLET DOSAGE FORM

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**ABSTRACT**

A simple, precise, accurate and rapid reverse phase high performance liquid chromatographic method was developed for the simultaneous determination of Rabeprazole and Diclofenac in pure and tablet dosage form. The method was carried out in isocratic using mobile phase, water and acetonitrile (50:50) and Phenomenex C-18 column having i.d of 240×4.6 mm and 5µm particle size was used. Flow rate was adjusted to 1.0 ml/min and effluents were monitored at 278 nm. The retention time obtained for Rabeprazole and Diclofenac was 2.6 and 3.7 min respectively. The calibration curves were linear in the concentration range of 5-30 µg/ml for Rabeprazole and Diclofenac 25-150µg/ml for. The developed method was validated in accordance to ICH guidelines.

**KEY WORDS**

Rabeprazole, Diclofenac and RP-HPLC.

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## INTRODUCTION

Rabeprazole (RAB) is chemically, 2-[[[4-(3-methoxypropoxy) -3- methyl -2- pyridinyl] -methyl] sulfinyl] -1H–benzimidazole sodium[1]. RAB a substituted benzimidazole, inhibits gastric acid secretion, used as an antiulcerative in treatment of duodenal ulcers, gastroesophageal reflux disease (GERD), Zollinger-Ellison syndrome etc. Diclofenac (DIC) is chemically, Sodium 2-[2-(2, 6-dichloroanilino) phenyl] acetate[1]. DIC derived from benzeneacetic acid, is a NSAID (nonsteroidal anti-inflammatory drug), used in the treatment of rheumatoid arthritis, osteoarthritis, and ankylosing

spondylitis and also for a variety of nonrheumatic inflammatory conditions. DIC is official in IP, BP and USP. The IP[2] describes titrimetry method, BP[3] and USP[4] describes potentiometric method for estimation of DIC. Literature survey reveals HPLC[5-6] methods for determination of DIC in pharmaceutical dosage forms. RAB is not official in IP, BP or USP. Literature survey reveals HPLC[7-8] methods for determination of RAB in pharmaceutical dosage forms and in human plasma. The combination of these two drugs is not official in any pharmacopeia; hence no official method available for estimation of RAB and DIC in their combined dosage forms.

## MATERIAL AND METHOD

### Chemicals and solvents

Dosage form used in this study was RACIDOL (by Macleods, INDIA) labeled to contain 20mg RAB and 100mg DIC. Pure samples of RAB and DIC were obtained from local pharmaceutical industry. Acetonitrile, water used is of HPLC grade.

### Instrumentation

The chromatographic separations were performed using HPLC-Waters (Model-2487) used a UV detector. The data was acquired through Empower-2-software. The column used was Phenomenex C18 (250×4.6mm i.d, 5µm particle size). Meltronics sonicator was used for enhancing dissolution of the compounds. All weighing was SIMADZU balance (model AUY-220).

### Chromatographic conditions

The chromatographic conditions have been optimized to achieve the best resolution and peak shape. Detection was carried out at 278 nm and flow rate of mobile phase was maintained at 1.0 ml/min. The column temperature was adjusted to ambient temperature. The injection volume was 20µl and total run time was 10 min. The peaks were identified by retention time; a typical chromatogram is shown in Figure No.1.

### Preparation of mobile phase

The water and acetonitrile was used as mobile phase in ratio 50:50 V/V.

### Preparation of standard stock solutions

Accurately Weigh10 mg of Rabeprazole (RAB) and 50mg of diclofenac (DIC) and transferred into 10ml of volumetric flask, and dissolved in HPLC Water that is equal to 1000 ug/ml of RAB and 5000 ug/ml of DIC. This solution further diluted to get take 5, 10, 15, 20, 25 and 30µg/ml of RAB and 25, 50, 75, 100, 125 and 150 µg/ml of DIC.

### Preparation of test solution

10 tablets of combined formulation of RAB and DIC were weighed, average weight was calculated and triturated in a mortar with pestle from that, powder equivalent to 10 mg of RAB and 50 mg of DIC was weighed and dissolved in HPLC water and test concentration was prepared by further dilution with same.

## METHOD VALIDATION

The developed method was validated as per the ICH (International Conference on Harmonization) guidelines with respect to System suitability, Precision, Linearity, Accuracy, Specificity, Robustness, Limit of detection and Limit of quantification.

### System suitability test

The system suitability was determined by making six replicate injections from freshly prepared standard solutions. The observed RSD values were well within usually accepted limits (≤2%). Theoretical plates, tailing factor, resolution between RAB and DIC were determined. The results were all within acceptable limits summarized in Table No.1.

### Linearity

Aliquots of 0.5, 1, 1.5, 2, 2.5, and 3 ml were taken from stock solution of concentration 100 µg/ml of RAB and 500 µg/ml of DIC and then diluted up to mark with HPLC water. Such that the final concentrations were in the range 5-30µg/ml for RAB and 25-150µg/ml for DIC. Volume of 20µl of each sample was injected in six times for each concentration level and calibration curve was constructed by plotting the peak area versus drug concentration. The calibration curves were shown in Figure No.2 & 3.

**Assay**

Accurately weighed tablet powder equivalent to 10mg of RAB and 50mg of DIC was transferred into 10ml volumetric flask and made up to the mark with diluent (HPLC water) to obtain solution of RAB (1000µg/ml) and DIC (5000µg/ml). From this solution 1ml was transferred to 10ml volumetric flask and made up to the mark with diluents water to obtain solution of RAB (100µg/ml) and DIC (500µg/ml), finally taken 1:5 ratio. The results were shown in Table No.2.

**Accuracy**

Accuracy of the method was done by recovery study. Sample solutions were prepared by spiking at about 50%, 100%, and 150% of specification limit to placebo and analyzed by the proposed HPLC method. Results are shown in Table No.3.

**Specificity**

The specificity of the method was performed by injecting blank solution (without any sample) and then a drug solution of 20 µl injected into the column, under optimized chromatographic conditions, to demonstrate the separation of both RAB and DIC from any of the impurities, if present. As there was no interference of impurities and also no change in the retention time, the method was found to be Specific.

**Limit of detection (LOD) and Limit of quantification (LOQ)**

The parameters LOD and LOQ were determined on the basis of response and slope of the regression equation. The linearity for RAB and DIC was performed from 5-30 µg/ml and 25-150 µg/ml respectively. The results for LOD and LOQ are in Table No.1.

**Precision**

Precision is the measure of closeness of the data values to each other for a number of measurements under the same analytical conditions. Standard solution of RAB (100µg/ml) and DIC (500µg/ml) were prepared as per test method and injected for 6 times. Results are shown in Table No.4. Three samples were prepared and analyzed as per the test method on same day and two different days and calculated the % RSD for assay of six preparations.

**Robustness**

Robustness studies were carried out by variations in flow rate and mobile phase compositions. It was observed that the small changes in these operational parameters did not lead to changes of retention time of the peak interest. The degree of reproducibility of the results proven that the method is robust. The results are in Table No.5.

## RESULTS AND DISCUSSION

The nature of sample, its molecular weight and solubility decides the proper selection of stationary phase. The drugs DIC and RAB were preferably analyzed by reverse phase chromatography and accordingly Phenominex $C_{18}$ column was selected. The elution of the compounds from column was influenced by polar mobile phase. The ratio of acetonitrile water was optimized to (50:50) to give well resolved and good symmetrical peaks with short run time. The retention time of DIC and RAB were found to be 2.6 and 3.7 min respectively. The calibration curve was linear over the concentration range of 5-30µg/ml (RAB) and 25-150µg/ml (DIC). The linearity of the method was statistically confirmed. RSD values for accuracy and precission studies obtained were less than 2% which revealed that developed method was accurate and precise. The analytical recovery at three different concentrations of RAB and DIC was determined and the recovery results were in the range of 10-20 µg/ml and 50-100 µg/ml. Therefore proposed validated method was successfully applied to determine RAB and DIC in tablet dosage form.

**Table No.1: Result of system suitability tests of RAB and DIC**

| S. No | Parameters | RAB | DIC |
|---|---|---|---|
| 1 | Linearity range | 5-30 µg/ml | 25-150 µg/ml |
| 2 | Correlation coefficient | 0.999 | 0.999 |
| 3 | Slope | 38129 | 703.6 |
| 4 | Intercept | -65099 | -36857 |
| 5 | Retention time(min) | 2.6 min | 3.7 min |
| 6 | USP plate count | 3850 | 7700 |
| 7 | Tailing factor | 1.3 | 1.2 |
| 8 | Limit of Detection (LOD) | 0.40 µg/ml | 3.29 µg/ml |
| 9 | Limit of quantification (LOQ) | 1.23µg/ml | 9.97µg/ml |

**Table No.2: Assay RAB and DIC in tablets by the developed method**

| S. No | Brandname | Content | Amount found mg/tablet | %Assay±S.D |
|---|---|---|---|---|
| 1 | Racidol | Rabeprazole 20mg | 100 | 100.1679±0.8943 |
| 2 | | Diclofenac 100mg | 500 | 100.28±0.6435 |

**Table No.3: Accuracy (% recovery) results of RAB and DIC**

| S.No | Sample | Spiked Amount (µg) | Recovered Amount (µg) | % Recovered | % Average Recovery |
|---|---|---|---|---|---|
| 1 | RAB | 10 | 5.02 | 100.7 | 100.06 |
| | | 15 | 10.01 | 100.27 | |
| | | 20 | 15.01 | 101.07 | |
| 2 | DIC | 50 | 49.76 | 99.54 | 99.90 |
| | | 75 | 100.03 | 100.03 | |
| | | 100 | 150.01 | 100.006 | |

**Table No.4: Precision Study**

| S. No | Repeatability (% RSD) (n=6) | Intermediate precision (% RSD) (n=6) | | | |
|---|---|---|---|---|---|
| | | Day 1 | | Day 2 | |
| | | Analyst 1 | Analyst 2 | Analyst 1 | Analyst 2 |
| 1 | RAB | 0.953 | 0.662 | 1.354 | 1.664 |
| 2 | DIC | 0.532 | 1.228 | 0.362 | 0.852 |

**Table No.5: Results for robustness test of RAB and DIC**

| S. No | Drug | Parameters count | Changes | RT(min) | USP Tailing | USP Plate |
|---|---|---|---|---|---|---|
| 1 | RAB | Flow rate (ml/min) | 0.9 | 2.485 | 1.2 | 3856 |
| | | | 1.1 | 2.752 | 1.3 | 3789 |
| 2 | DIC | Flow rate (ml/min) | 0.9 | 3.789 | 1.3 | 3850 |
| | | | 1.1 | 3.838 | 1.2 | 3758 |

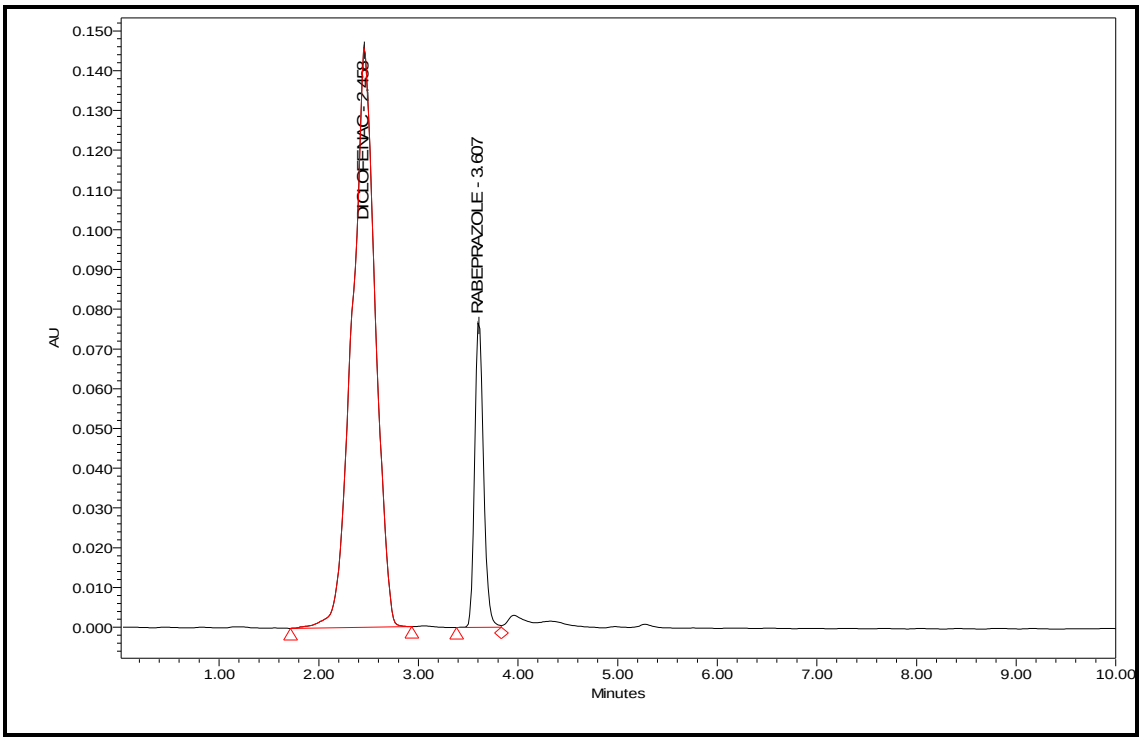


**Figure No.1: Typical Chromatogram of standard RAB and DIC**

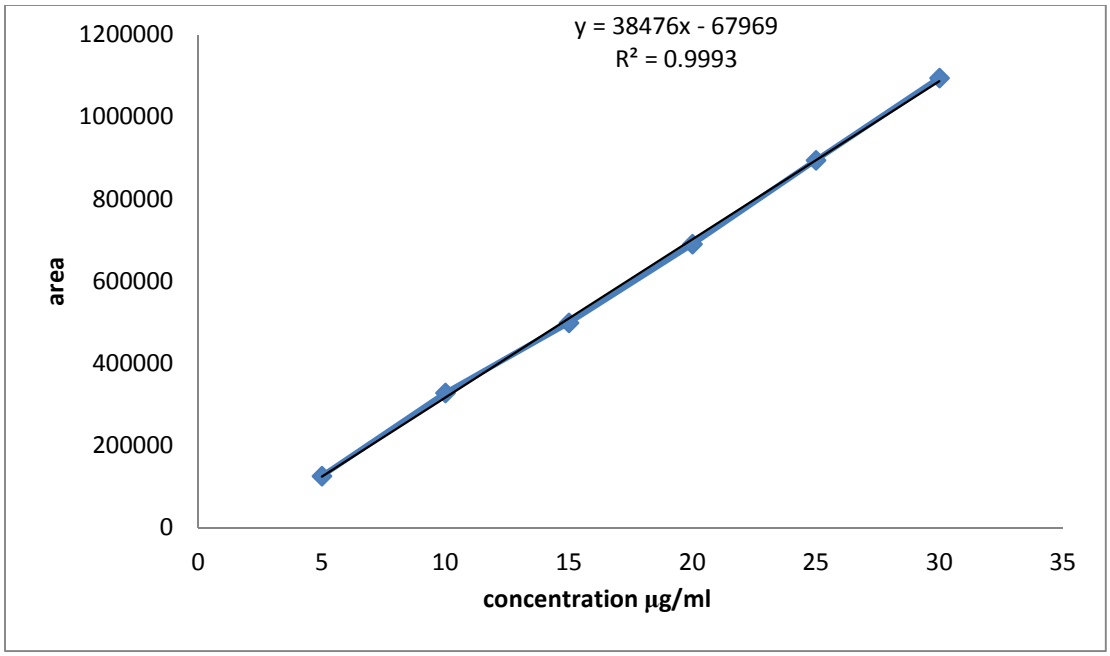


**Figure No.2: Linearity of RAB**

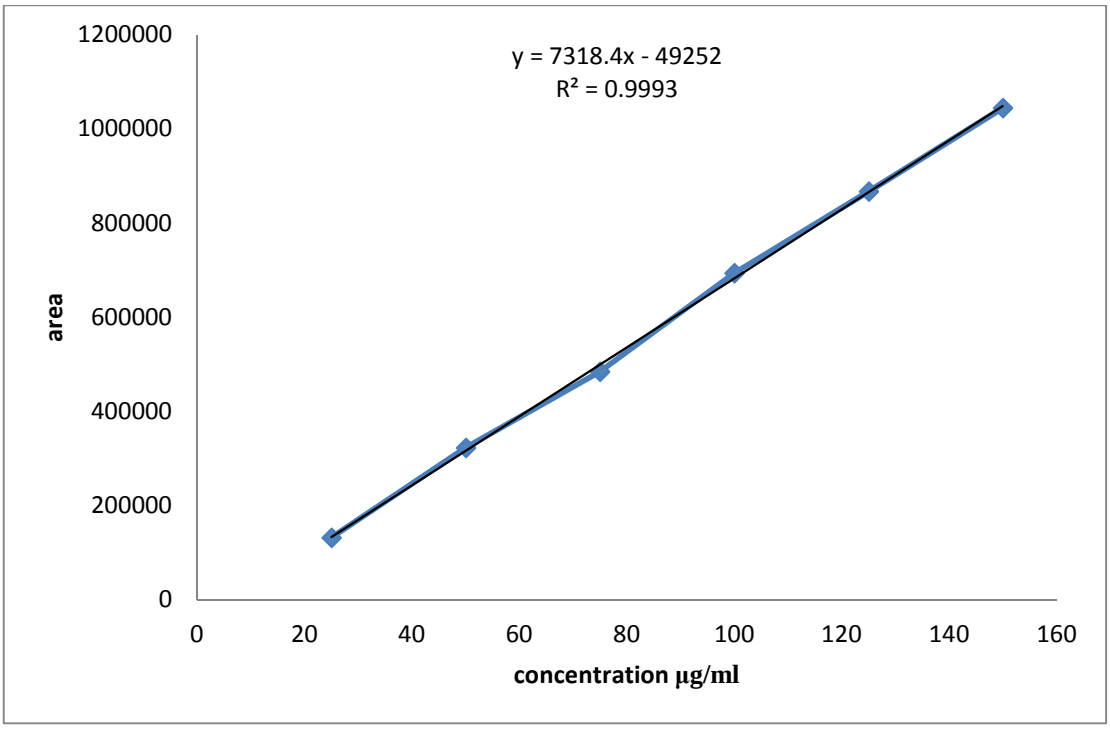


**Figure No.3: Linearity of DIC**

## CONCLUSION

The developed method is accurate, simple, rapid and selective for the simultaneous estimation of RAB and DIC in tablet dosage form. The excipients of the commercial sample analyzed did not interfere in the analysis, which proved the specificity of the method for these drugs. The sample preparation is simple, the analysis time is short and the elution is by isocratic method. Hence the proposed method can be conveniently adopted for the routine quality control analysis in the combined formulation.

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## CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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