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# A VALIDATED RP- HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF PARACETAMOL AND DICYCLOMINE HYDROCHLORIDE IN PHARMACEUTICAL FORMULATIONS

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**ABSTRACT:** A reversed-phase-liquid chromatographic (RP-HPLC) method was developed for the determination of Paracetamol (PCM) and Dicyclomine Hydrochloride (DICY) in their bulk drug and marketed formulations. A reversed-phase C-18 Phenomenex $C_{18}$ column (250 mm × 4.6 mm i.d., 5µm) with mobile phase consisting of Acetonitrile: Phosphate Buffer pH (5.5): triethylamine (75:25:0.02 v/v/v) was used with 1.0 ml/ min flow rate and detected at 218 nm. The retention times of Paracetamol and Dicyclomine Hydrochloride were found to be 2.65±0.3 min and 11.32±0.3 min, respectively. Developed methods were validated according to ICH guidelines. Linearity was observed at concentration range of 100-500 µg/ml for Dicyclomine Hydrochloride and 10-50 µg/ml for Paracetamol followed by Beer's law. The % recoveries of Paracetamol and Dicyclomine Hydrochloride were found to be between 101.05-101.20% and 100.55-100.95%. The percentage RSD for the method precision was found to be less than 2%. The proposed method is precise, accurate, selective and rapid for simultaneous determination of Dicyclomine Hydrochloride and Paracetamol.

**INTRODUCTION:** Dicyclomine Hydrochloride (DICY) chemically is 2-(diethyl amino) ethyl 1 cyclohexylcyclohexane-1-carboxylate (**Fig. 1**), is a muscarinic antagonist used as an anti spasmodic and in urinary incontinence[1]. This dual mode of action provides a specific anti cholinergic effect at Acetylcholine receptor and a direct effect upon smooth muscle, but with a rarely causes any side effect.

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Dicyclomine Hydrochloride is official in Pharmacopoeia. Paracetamol (PCM) chemically is 4-hydroxyacetanilide (**Fig. 2**), used as antipyretic and analgesic [2]. Paracetamol and Dicyclomine Hydrochloride are official in Indian Pharmacopoeia (IP), British Pharmacopoeia (BP) and United States Pharmacopoeia (USP) [2, 3, 4].

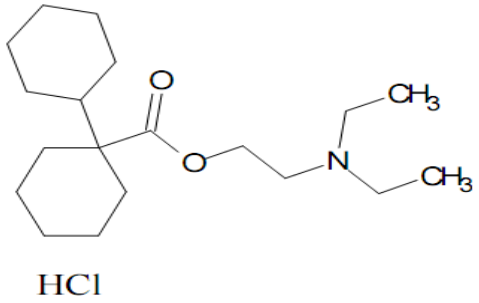


**FIG. 1: DICYCLOMINE HYDROCHLORIDE**

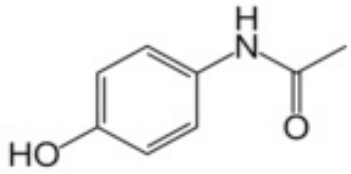


**FIG. 2: PARACETAMOL**

**Objective of Study**: Survey of literature revealed that numbers of method have been reported in literature for the individual analysis of Dicyclomine Hydrochloride and Paracetamol by UV spectrophotometric and RP-HPLC method. RP-HPLC methods are available in literature for simultaneous determination of Paracetamol with other drugs [5, 6, 7, 8, 9]. RP-HPLC and UV Spectrophotometric methods are available in literature for determination of Dicyclomine Hydrochloride with other drugs [10, 11, 12, 13]. However, to our knowledge, there is no reported RP-HPLC method available for simultaneous estimation of Dicyclomine Hydrochloride and Paracetamol.

The aim of the present work was to develop easy, economic, accurate, specific and precise RP-HPLC methods for simultaneous estimation of Paracetamol and Dicyclomine Hydrochloride in bulk drugs and combined pharmaceutical formulations and validation of newly developed analytical methods.

## MATERIALS AND METHODS:

**Apparatus and Software:** The RP-HPLC system consisted of Shimadzu LC-AT20, with isocratic pump with UV-VIS detector, Phenomenex $C_{18}$ column (250 mm × 4.6 mm i.d., 5µm), Rheodyne injector 7725I with 20 µl loop were used. Other equipments used were Digital pH meter (Global DPH 500), Balance (Contech CA 123), Sonicator (Frontline ultrasonication FS-2) and Milipore filter assembly.

**Reagents and Chemicals:**

**Solvent**: A gratuitous sample of pure Paracetamol was obtained from Yarrow Chem. (Mumbai), and Dicyclomine Hydrochloride from Palam Pharma (Ahemdabad), Potassium Dihydrogen Phosphate (Moly-Chem Limited, Mumbai, India), Double Distilled Water, and HPLC grade water, Acetonitrile and Triehylamine (Moly-Chem Limited, Mumbai, India).

**Year of Experiment:** 2013

**Site**- Department of Quality Assurance, N. R. Vekaria Institute of Pharmacy, C. L. College Campus, Bilkha Road, Junagadh-362001, Gujarat, India.

**Preparation of Mobile Phase and Stock Solution:** Mix Acetonitrile, Phosphate Buffer (pH 5.5) and Triethylamine in ratio of 75:25:0.02 (v/v/v) was used as diluents for the preparation of samples and used as mobile phase. Accurately weighed 10 mg Paracetamol and 100 mg of Dicyclomine Hydrochloride were transferred to 100 ml volumetric flask separately. It was dissolved with sufficient diluents and sonicated for 10 min then diluted up to mark with diluents to give concentration of 100 µg/ml of Paracetamol and 1000 µg/ml of Dicyclomine Hydrochloride.

**Preparation of sample solution of Mixture of PCM and DICY:** Accurately weighed 20 tablets and average weight was calculated, triturated them in glass mortar. Powder equivalent to 500 mg of PCM and 20 mg of DICY was weighed and transferred in to the 100 ml of volumetric flask, add 60 ml diluents and sonicate it for 30 minutes. Filter the solution through 0.2 µm membrane filter and diluted up to mark with diluents. It gives the solution of PCM 5000 µg/ml and DICY 200 µg/ml. From this solution suitable aliquots were pipette out in 10 ml volumetric flask and to this 4.98 mg standard DICY was added by standard addition method and volume was made up to the mark with diluents and 20 µl volume sample solution was injected into HPLC system under set chromatographic condition.

The same sample procedure was repeated for six times. The chromatographic conditions were found to yield good separation with satisfactory retention time of about 2.603 min for PCM and 11.327 min for DICY with sharp symmetrical peak (**Figure 3**) and analysis of marketed formulations shows in **Table 1**.

**TABLE 1: ASSAY OF TABLET FORMULATION**

| Parameter | Paracetamol | Dicyclomine Hydrochloride |
|---|---|---|
| **% Estimated** | 100.31±4.654 | 100.14±3.115 |
| **%RSD** | 0.643 | 0.487 |

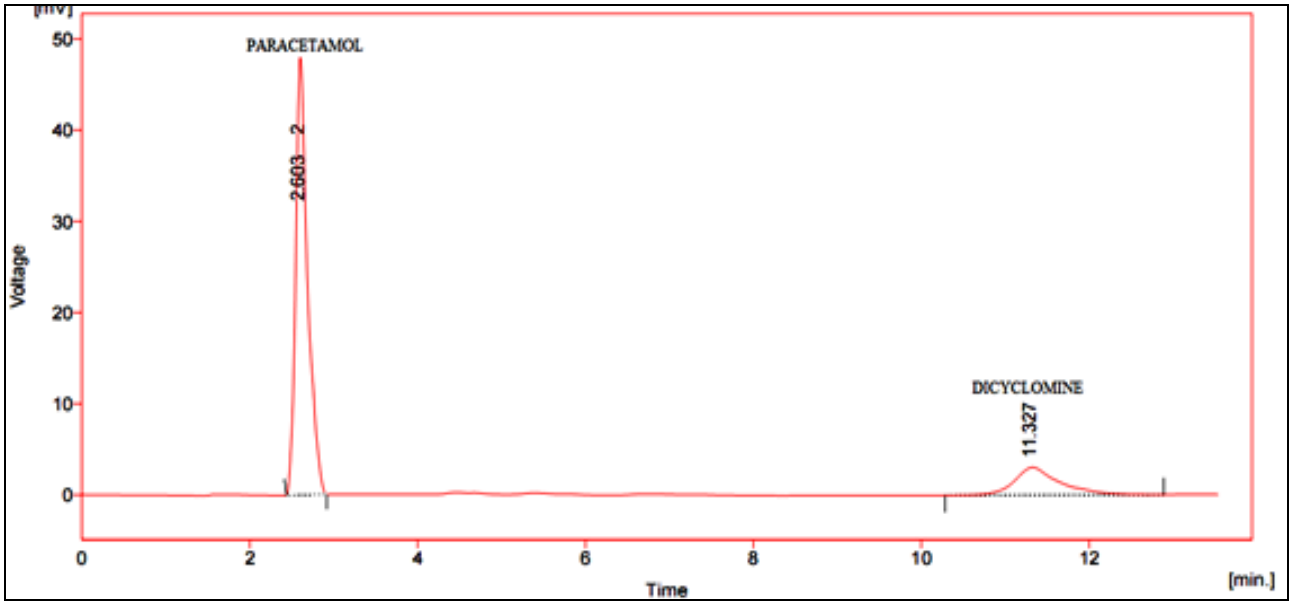


**FIG. 3: CHROMATOGRAM OF PCM AND DICY**

**Analytical method Development Validation**: Validation was done with respect to various parameters, as required under ICH guideline Q2 (R1).

**Linearity:** Several aliquots of standard solution of PCM and DICY were taken in different 10 ml volumetric flasks and diluted upto the mark with diluents such as final concentration of PCM and DICY were 10-50 µg/ml and 100-500 µg/ml respectively. These standards were tested six times replicates.

Calibration curves were constructed and the proposed method was evaluated by its correlation coefficient and intercept value, calculated in the corresponding statistical study (ANOVA) ($p < 0.05$).

**Precision:** The precision is a measure of the ability of the method to generate reproducible results. The precision of the assay was determined by repeatability (intraday) and intermediate precision (inter-day), system precision and method precision reported as %RSD. For this, 50µg/mL and 500 µg/ml of the solution were measured three times in a day, the same was repeated in next three days, same was repeated for bulk drug and marketed formulation.

**Accuracy:** Accuracy indicates the deviation between the mean value found and the true value. Accuracy was determined by means of recovery experiments, by the addition of active drugs to placebo formulations.

The accuracy was calculated from the test results as the percentage of the analyte recovered by the assay.

**Robustness:** To verify the robustness of the method, the analysis was done under variables wavelength, pH, mobile phase ratio and flow rate. Sample solution were injected and run under set chromatographic condition.

**System suitability parameter:** The system was evaluated by analyzing repeatability retention time, tailing factor and theoretical plats of the column.

**RESULTS AND DISCUSSION:** All of the analytical validation parameters for the proposed method were determined according to Conference on Harmonization (ICH) guidelines.

**Linearity:** The linearity of this method was determined at ranging from 10-50 µg/ml for PCM and 100-500 µg/ml for DICY. The regression equation were found to be Y = 1.916X-1.666 and Y = 53.25x-41.95 the correlation coefficient ($r^2$) 0.999 and 0.999 for DICY and PCM respectively shown in **figure 4 and 5**.

**Precision:** The precision (measurements of intraday, inter day, system precision and method precision) results showed good reproducibility with percent relative standard deviation (% RSD) was below 2.0% shown in **Table 2**. This indicated that method was highly precise.

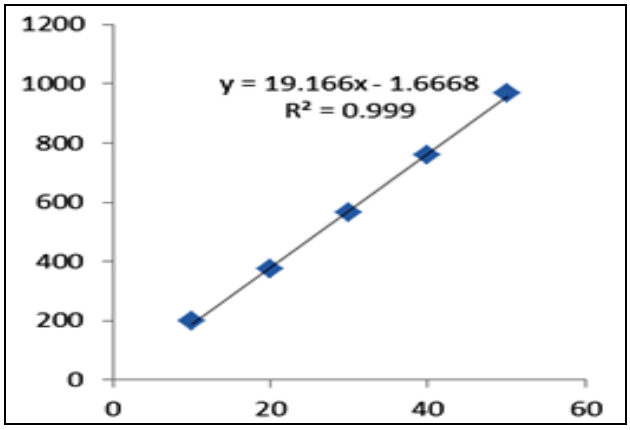


FIG. 4:CALIBRATION CURVE OF DICY

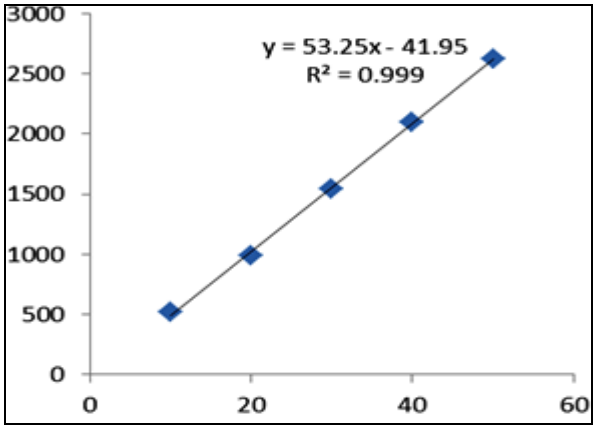


FIG. 5:CALIBRATION CURVE OF PCM

**TABLE 2: DATA OF PRECISION**

| PRECISION | PARACETAMOL* (%RSD±S.D.) | DICYCLOMINE HYDROCHLORIDE* (%RSD±S.D.) |
|---|---|---|
| Intraday | 0.535±5.37 | 0.830±4.8162 |
| Interday | 0.540±5.3631 | 0.963±3.638 |
| System precision | 0.85±22.63 | 0.39±3.821 |
| Method precision | 0.96±25.49 | 1.63±15.76 |

***Mean value of three determinations**

**Accuracy:** Recovery studies were performed to judge the accuracy of the method. The studies were carried out by adding a known quantity of pure drug to the pre-analyzed formulation and the proposed method was followed. From the amount of drug found, the percent recovery was calculated. Recovery study was carried out at three levels 80%, 100% and 120% for the formulation concentration of 20µg/ml for PCM and 200µg/ml for DICY shown in **Table 3**.

**TABLE 3: DATA OF ACCURACY**

| Level of Addition (%) | PARACETAMOL | | DICYCLOMINE HYDROCHLORIDE | |
|---|---|---|---|---|
| | Addition of pure drug | % recovery of pure drug | Addition of pure drug | % recovery of pure drug |
| 80 | 16 | 101.09 | 160 | 100.75 |
| 100 | 20 | 101.20 | 200 | 100.93 |
| 120 | 24 | 100.21 | 240 | 100.61 |

***Mean value of three determinations**

**System Suitability Test:** The parameters of system suitability study were presented in table 3. It was found that the average retention time ± standard deviation for PCM and DICY were found to be 2.605±0.03 min and 11.321±0.05 min for five replicate injections respectively. The asymmetry factor were found to be 1.01 and 0.611 for PCM and DICY respectively, which indicated asymmetric nature of the peak. The number of theoretical plates were found to be 5021 and 5922 for PCM and DICY respectively, which suggested an efficient performance of the column.The resolution was found to be 13.43 for both drug.These parameter shows in **Table 4**.

**TABLE 4: DATA OF SYSTEM SUITABLE PARAMETER**

| PARAMETERS | PARACETAMOL | DICYCLOMINE HYDROCHLORIDE |
|---|---|---|
| Retention time ± S.D. | 2.605±0.03 min | 11.321±0.05 min |
| Asymmetry factor | 1.01 | 0.611 |
| Theoretical plates | 5021 | 5922 |
| Resolution | 13.43 | |

***Mean value of three determinations**

**Robustness:** Robustness was performed by small but deliberate variation in the chromatographic conditions and was found to be unaffected by small variations like ±2% in volume of mobile phase composition, ±0.2 ml/min in flow rate of mobile phase and ±2% change in pH. ±2 in detection wavelength, it was observed that there were no marked changes in the criteria, which demonstrated that the proposed method was robust. These parameter shows in **Table 5**.

**TABLE 5: DATA OF ROBUSTNESS**

| Condition | %R.S.D. | | % Assay | Mean* | % Difference in % Assay | |
|---|---|---|---|---|---|---|
| | PCM | DICY | PCM | DICY | PCM | DICY |
| **(1) Change in the Mobile Phase Composition(± 2ml in organic Phase)** | | | | | | |
| Normal Condition (75:25:0.02 v/v/v) | 1.15 | 1.63 | 99.98 | 100.67 | - | - |
| Change in the organic phase (77:23:0.02 v/v/v) | 0.843 | 1.23 | 99.09 | 99.85 | 0.89 | 0.82 |
| Change in the organic phase (73:27:0.02 v/v/v) | 0.713 | 1.39 | 99.53 | 99.91 | 0.45 | 0.76 |
| **(2) Change in the Detection Wavelength(±2 nm)** | | | | | | |
| Normal Condition (218 nm) | 1.01 | 1.43 | 99.98 | 100.67 | - | - |
| Change in the Wavelength(220 nm) | 0.423 | 0.99 | 98.81 | 99.62 | 1.17 | 1.05 |
| Change in the Wavelength(216 nm) | 0.639 | 1.32 | 98.90 | 99.95 | 1.08 | 0.72 |
| **(3) Change in flow rate (±0.2 ml/min)** | | | | | | |
| Normal Condition1.0 ml/min | 0.231 | 0.64 | 99.98 | 100.67 | - | - |
| Change in the flow rate (1.2 ml/min) | 0.724 | 0.87 | 100.23 | 100.41 | 0.25 | 0.26 |
| Change in the flow rate (0.8ml/min) | 0.864 | 0.56 | 98.88 | 99.73 | 1.10 | 0.94 |
| **(4) Change in pH (±0.2)** | | | | | | |
| Normal Condition | 0.286 | 0.62 | 99.98 | 100.67 | - | - |
| Change in the pH(+0.2 ) | 0.722 | 0.73 | 99.77 | 99.12 | 0.21 | 1.55 |
| Change in the pH( -0.2) | 0.743 | 0.66 | 99.60 | 99.76 | 0.38 | 0.91 |

*****Mean value of three determinations

**CONCLUSION:** The RP-HPLC method has been developed for the simultaneous estimation of Paracetamol and Dicyclomine Hydrochloride in their combined marketed formulation and bulk drugs. The method gave good resolution for both the drugs with a short analysis time below 12 minutes which enables rapid quantification of many sample in routine and quality control analysis of tablets. The developed method was validated. It was found to be simple, precise, accurate and robust. The good % recovery in tablet suggests that the excipients present in the dosage forms have no interference in the determination. The proposed method can be used for routine analysis of Paracetamol and Dicyclomine Hydrochloride in combined dosage form. It can be also used in the quality control in bulk manufacturing.

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