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Simultaneous Estimation of Paracetamol and Dicyclomine Hydrochloride by Spectrophotometric Method

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ABSTRACT

A simple accurate, precise and reproducible spectrophotometric method has been developed and validated for the simultaneous estimation of Paracetamol and Dicyclomine hydrochloride in combined tablet dosage form by Q-Absorbance ratio method. Q-Absorbance ratio method involves two wavelengths the iso-absorptive point and λ_{max} of paracetmol i.e. 231.5 nm and 257 nm respectively in 6 : 4 ratio methanol: 0.1 N NaOH mixture. The linearity was detected in the range of 2-12 µg/ml (R^2 = 0.9997) for Paracetamol and 10-35 µg/ml (R^2 = 0.9989) for Dicyclomine Hydrochloride. The accuracy existed between 98-102% and the %RSD was less than 2%. The developed method was validated for linearity, accuracy and precision as per ICH guidelines.

Keywords: Paracetamol, Dicyclomine hydrochloride, Q-Absorbance ratio method.

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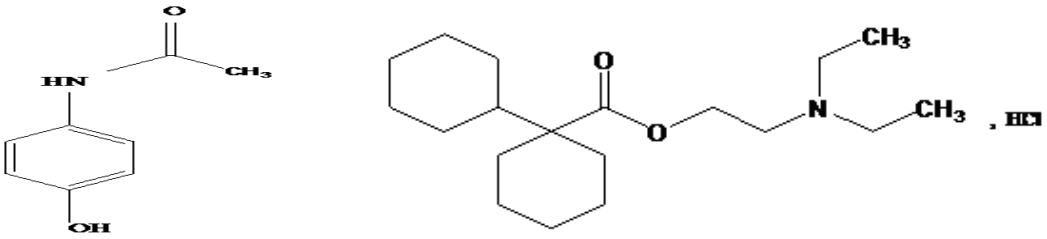
INTRODUCTION

Paracetamol is chemically N - (4 – hydroxyphenyl) acetamide and is used as analgesic and anti-pyretic agent. It acts by, increasing the pain threshold by inhibiting both isoforms of cyclooxygenase, COX-1, COX-2 and COX-3 enzymes involved in prostaglandin (PG) synthesis.[1]

Dicyclomine is chemically [bicyclohexyl]-1-carboxylic acid is an antispasmodic and anticholinergic agent. Its action is achieved via a dual mechanism: a specific anticholinergic effect at the acetylcholine-receptor sites, a direct effect upon smooth muscle.[2]

Both drugs are official in Indian pharmacopoeia 2010[3], United State Pharmacopoeia[4] and British Pharmacopoeia 2009.[5] Literature survey revealed that RP-HPLC,[6-8] Liquid Chromatography, UV- Spectrophotometric methods[9-11] were reported for the estimation of Paracetamol and RP-HPLC, [12] HPTLC [13] and spectrophotometric methods [14] were reported for the estimation of Dicyclomine Hydrochloride.

As per literature survey, no analytical method has been reported for simultaneous estimation of Paracetamol and Dicyclomine Hydrochloride in pharmaceutical dosage forms. In presented research work, we had developed a novel, simple, accurate, sensitive, reproducible, economical analytical method to estimate Paracetamol & Dicyclomine Hydrochloride in their combined dosage form in routine analysis. Chemical structure of drugs shown in figure.1



(A) (B)

Figure 1: Chemical Structure of (A) Paracetamol and (B) Dicyclomine Hydrochloride

MATERIALS AND METHOD

Reagents and Chemicals:

All the reagents used in this assay were analytical grade and the reagent solutions were prepared using pre analyzed distilled water. Tablets were purchased from local market.

Instruments:

Shimadzu UV/Vis-1700 double beam UV/Vis spectrophotometer and Lab India UV/Vis-3000$^+$ double beam spectrophotometer with a fixed slit width of 2 nm, 1 cm quartz cells was used. Class 'A' volumetric glassware were used.

PREPARATION OF STANDARD SOLUTION:

Preparation of stock solution of PCM:

Weighed accurately about 50mg of PCM & transferred into 50 ml volumetric flask, 30ml of methanol was added and sonicated for about 15 min then diluted up to the mark with 0.1N NaOH to get a stock solution having strength 1000 µg/ml.

Preparation of working Standard solution of PCM:

100 µg/ml solution of PCM was prepared by diluting 1ml stock solution to 10 ml with 0.1N NaOH and diluted further to get the concentration range of 2, 4, 6, 8, 10, 12 µg/ml of PCM.

Preparation of stock solution of DICY:

Weighed accurately about 10mg of DICY & transferred into 50 ml volumetric flask, 30ml of methanol was added and sonicated for about 15 min then diluted up to the mark with 0.1N NaOH to get a stock solution having strength 1000µg/ml.

Preparation of Working Standard Solution of DICY:

100 µg/ml solution of DICY was prepared by diluting 1ml stock solution to 10 ml with 0.1N NaOH. This solution was diluted further to get the concentration range of 10, 15, 20, 25, 30, 35 µg/ml of DICY.

Absorbance ratio method

The absorbance ratio method is a modification of the simultaneous equation method. It depends upon the principle that, for a substance which obeys beer's law at all wavelengths, the ratio of absorbance at any two wavelengths is constant value independent of concentration or path length. This ratio is called Q value. [16]

$$C_x = \frac{Q_M - Q_Y}{Q_X - Q_Y} \times \frac{A_1}{ax_1}$$

$$C_y = \frac{Q_M - Q_Y}{Q_X - Q_Y} \times \frac{A_2}{ay_2}$$

Where, $Q_0 = A_2/A_1$; $Q_X = a_{x2}/a_{x1}$; $Q_Y = a_{y2}/a_{y1}$, A_1 and A_2 are the absorbance of diluted samples at λ_1 and λ_2, a_{x1} and a_{x2} are the absorptivity of X. a_{y1} and a_{y2} are the absorptivity of Y.

Selection of wavelength

For Q-Absorbance method iso-absorptive point at 231.5 nm and the $\lambda_{max}$of PCM 257 nm was selected as working wavelength.

METHOD VALIDATION:

The method was validated with respect to linearity, precision, accuracy, robustness, LOD &

LOQ. [17]

Linearity:

Test solutions were prepared form standard stock solutions to get the final concentration of PCM and DICY in a range of 2-12 µg/ml and 10-35 µg/ml respectively and analyzed in five replicates. Absorbance vs. respective concentration for PCM and DICY were plotted as the calibration curve shown in figure. 2.

Precision:

The inter-day precision study was performed on three different days i.e. day 1, day 2 and day 3 at three different concentration levels, (n=9).The intra-day precision study was performed on the same day at 3 time intervals and at three different concentration levels (n=9). The %RSD of the obtained values was calculated.

Accuracy:

The accuracy of the method was evaluated as triplicate at three concentration levels (80, 100 and 120%), and the percentage recoveries were calculated.

Robustness:

The robustness of method was performed by change in wavelength. Three replicates were made for the concentration 8µg/ml of PCM and 25µg/ml of DICY and absorbance was record. The result is expressed in % RSD.

Limit of Detection and Limit of Quantitation:

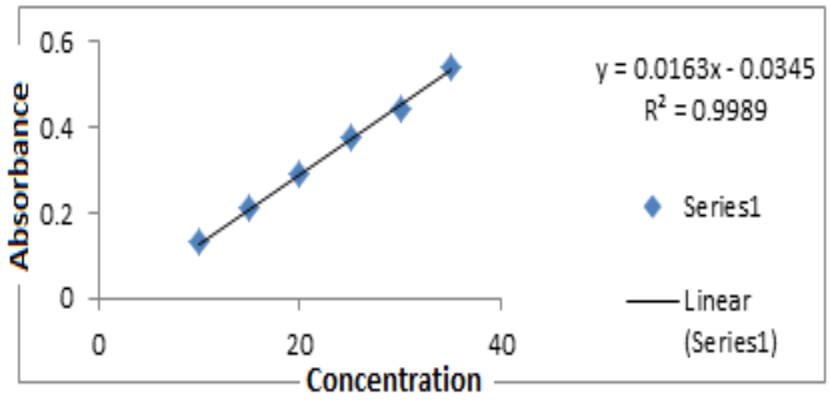
The LOQ and LOD were based on the standard deviation of the response and the slope of the constructed calibration curve (n=3), as described in International Conference on Harmonization guidelines Q2 (R1).

Application of Method on Marketed Formulation:

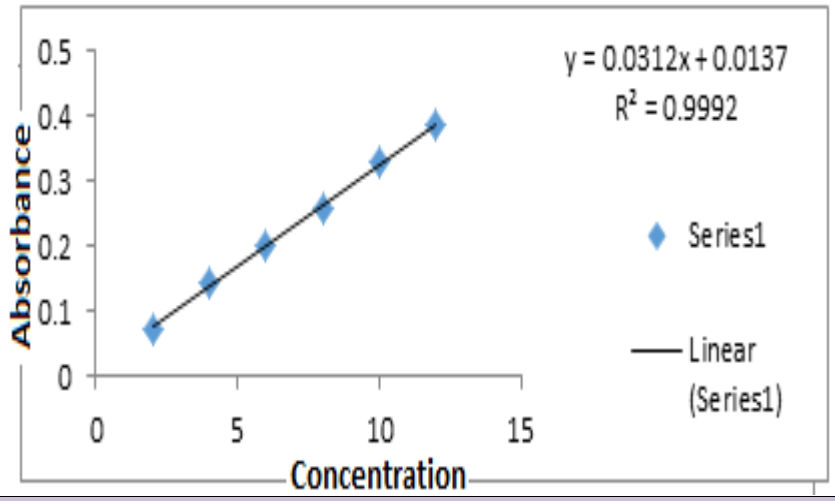
Accurately weighed twenty tablets of market formulation each of which containing 500 mg PCM, 20 mg DICY. A quantity of powder equivalent to 500 mg PCM and 20 mg DICY were accurately weighed, the difference in ratio of PCM and DICY is very high so to achieve the ratio in level, the mixture was spiked with 480 mg of pure drug of DICY and transferred to standard 100 ml volumetric flask and added 60 ml of methanol. The followed prepared solution was sonicated for 10 minutes by using ultra sonication and made up to mark with 0.1N NaOH. Aliquot portion of this solution was further diluted to achieve final concentration. The solution was filtered with a Whattman filter paper. First 5ml portion of filtrate was discarded and then absorbance was measured at respective their wavelengths.

RESULT AND DISCUSSION

The present work is precise and accurate for the estimation of PCM and DICY in combined dosage form. The calibration curve was linear over the concentration range 10-35µg/ml for DICY and 2-12µg/ml for PCM. The regression equation was found y = 0.0163x - 0.0345, r^2=0.9989 (DICY) andy = 0.0691x + 0.0353, r^2=0.9997 (PCM) at 231.5nm while y = 0.013x - 0.069, r^2 = 0.998 (DICY) and y = 0.069x + 0.035 r^2 = 0.9990 (PCM) at 257 nm (table 1). The %RSD in intra-day precision study was found 0.23-0.84% (PCM) and 0.85-1.88% (DICY) and % RSD for inter-day precision was 0.36-1.20% (PCM) and 1.09-1.50% (DICY) shown in table 2. The %recovery in accuracy study was found between 98-102% and the %RSD was less than 2% (table 3). The % RSD in robustness study was less than 2%, this indicates that the method is precise, accurate and robust. The LOD was found 0.098 µg/ml (PCM) and 0.83µg/ml (DICY) and the value of LOQ was found 0.299µg/ml and 2.53µg/ml for PCM and DICY respectively (table 4). This indicates that the method is accurate, precise and robust as per ICH guidelines. The method was successfully applied for marketed preparation and the result was shown in table 5.



(A)



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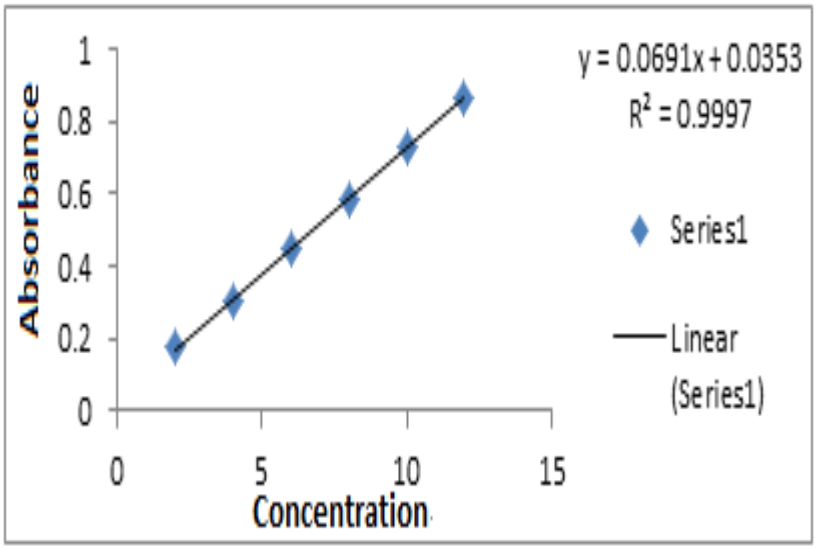
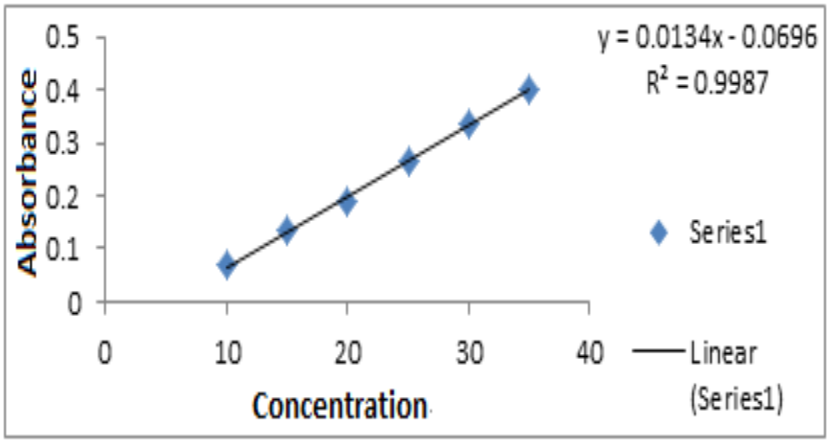


(C)



(D)

Figure 2: (A) Calibration curve of DicyclomineHCl at 231.5 nm (iso-absorptive point); (B) Calibration curve of Paracetamol at 231.5 nm (iso-absorptive point); (C) Calibration curve of Paracetamol at 257nm (λmax of paracetmol) (D) Calibration curve of DicyclomineHCl at 257nm (λmax of paracetmol)

Table 1: Linearity of assay Method

Parameters	DicyclomineHCl		Paracetamol	
	231.5nm	257nm	231.5nm	257nm
Linearity and Range	10-35 µg/ml	10-35 µg/ml	2-12 µg/ml	2-12 µg/ml
Regression Equation (y = mx+c)	y = 0.0163x - 0.0345,	y = 0.013x - 0.069	y = 0.0312x + 0.0137,	y = 0.069x + 0.035
Slope	0.0163	0.013	0.0312	0.069
Intercept	0.0345	0.069	0.0137	0.035
Correlation Coefficient (r^2)	0.9989	0.998	0.9997	0.9990

Table 2: Intra-day and inter-day precision studies of PCM & DICY.

S. No.	Concentration (µg/ml)		Intra-day precision*		% RSD*		Inter-day precision*		% RSD*	
	PCM	DICY	PCM	DICY	PCM	DICY	PCM	DICY	PCM	DICY
1	6	20	0.462	0.294	0.84	1.88	0.465	0.296	0.87	1.50
2	8	25	0.624	0.369	0.40	0.85	0.609	0.368	1.20	1.09
3	10	30	0.769	0.428	0.23	1.38	0.768	0.433	0.36	1.16

*Mean of nine replicates (n=9)

Table 3: Accuracy–Recovery study of PCM and DICY by standard-addition method.

S. No.	Amt. of Sample(µg/ml)		Spiked (µg/ml)	Concentration	Recovery* (%)		% RSD*	
	PCM	DICY	PCM	DICY	PCM	DICY	PCM	DICY
1	8	25	80%	80%	102.07	101.72	0.16	0.83
2	8	25	100%	100%	99.96	102.06		
3	8	25	120%	120%	100.91	99.54		

*Mean of three replicates (n=3)

Table 4: Validation Parameters for the method

Parameters	DicyclomineHCl	Paracetamol
Accuracy (% recovery)	102.06	99.96
Robustness	0.67	1.09
Specificity	0.27	0.11
LOD (µg/ml)	0.83	0.09
LOQ (µg/ml)	2.53	0.29

Table 5: Result of market formulation

Brand name	Label claim (mg/tablet)		Found (mg/tablet)		Percentage purity	
	PCM	DICY	PCM	DICY	PCM	DICY
SPASMAK	500	20	496	19.9	99.20	99.50

CONCLUSION:

The developed method was novel, simple, accurate, precise, reproducible and economical, which can be used to estimate PCM & DICY in their combined dosage form in routine analysis.

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