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DEVELOPMENT AND VALIDATION OF NEW ANALYTICALMETHODS FOR THE SIMULTANEOUS ESTIMATION OF PARACETAMOL AND DICYCLOMINE HYDROCHLORIDE IN BULK AND PHARMACEUTICAL DOSAGE FORMS BY USING RP-HPLC AND UV METHODS

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ABSTRACT

New simple, precise, accurate, economic and selective RP-HPLC and UV- Spectrophotometric method has been developed and validated for simultaneous estimation of Paracetamol (PCM) and Dicyclomine Hcl (DYCLO) in tablet dosage form.

1. RP-HPLC

Column : Agilent CN, 250mm x 4.6mm, 5µm.

Mobile phase : Buffer (1ml of Triethyl amine is dissolved into 1lt Water and

Adjust the pH-6.0 with Ortho Phosphoric acid: MEOH (60:40) v/v.

Flow rate : 1.5 mL/min

Detection : 220 nm

Retention time : Paracetamol is about 2.0 – 3.0 min.

Retention time : Dicyclomine is about 4.5 – 5.5 min.

Linearity : 0-487µg/ml and 0-150µg/ml.

2. UV-SPECTROPHOTOMETRY

Solvent : methanol and 0.1N NaoH

Wave length : 243nm of paracetamol and 345nm of Dicyclomine Hcl

Linearity : 2 -12µg/ml, 45-70 µg/ml

The development methods have been validated statistically as per ICH guidelines .The method showed good reproducibility and recovery with %RSD less than 2. So, the proposed

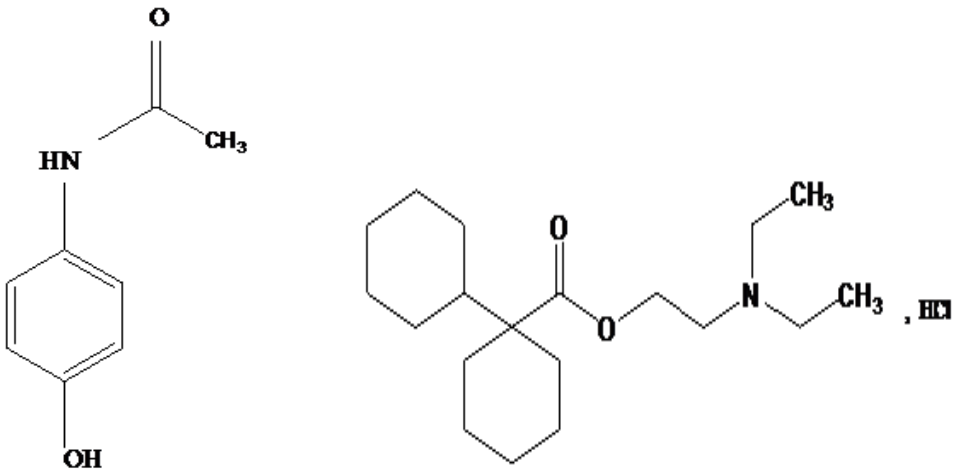
methods were found to be simple, specific, precise, accuracy, linear, and rugged. Hence it can be applied for routine analysis of Paracetamol and Dicyclomine Hcl in bulk drug and the pharmaceutical formulations.

KEYWORDS: Paracetamol, Dicyclomine hydrochloride, simultaneous estimation method.

INTRODUCTION

Paracetamol is chemically N - (4 – hydroxyphenyl) acetamide and is used as analgesic and antipyretic 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.

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.



(A) **(B)**

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

Both drugs are official in Indian pharmacopoeia 2010, United State Pharmacopoeia and British Pharmacopoeia 2009. Literature survey revealed that RP-HPLC, Liquid Chromatography, UV- Spectrophotometric methods were reported for the estimation of Paracetamol and RP-HPLC, 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

MATERIALS AND METHODS FOR RP-HPLC

The developed RP-HPLC method for the simultaneous estimation of Paracetamol and Dicyclomine hcl was carried out on: Agilent CN, 250mm x 4.6mm, 5µmColumn in Ambient mode using mobile phase composition of buffer (1ml of Triethyl amine is dissolved into 1lt Water and adjust the pH-6.0 with Ortho Phosphoric acid: MeoH (60:40 %v/v) with flow rate of 1.5 mL/min at 220 nm.

Meterials: Instruments used

Table no 1 Instruments used

Instrument	Specifications
HPLC	Waters,2695 separation module
Software	Empower ,Version 2.0
Detector	UV-Visible detector
Analytical balance	Sartorius
UV-Visible spectrophotometer	Shimadzu (UV-2450)
Sonicator	Biotechnics
pH meter	Cyberscan

Chemicals and reagents: Tri ethylamine (AR grade), Ortho Phosphoric Acid (HPLC grade), Acetonitrile (HPLC grade) ,HPLC grade Water (Milli Q or equivalent) was used in the buffer preparation.

Drug samples: Paracetamol and Dicyclomine Hcl pure drugs were procured from swasthik, analytical labs Pvt. Ltd., Vijayawada\

Formulation used: Commercial pharmaceutical preparations "**cyclopam"** Tablet which were claimed to contain 500mg of paracetamol and 20mg of Dicyclomine Hcl were obtained from local market.

METHOD DEVELOPMENT

Selection of the wavelength for simultaneous estimation : In setting up the conditions for development of the assay method, the choice of detection wavelength was based on the scanned absorption spectrum for Paracetamol and Dicyclomine Hcl. The UV-spectrum of Paracetamol and Dicyclomine Hcl was obtained separately by scanning the sample in the wavelength range 200-400nm against blank solution. From the overlay spectra of both the drugs, a wavelength was obtained at which both the drugs showed maximum absorbance. The wavelength selected was 220nm as shown in figure 2

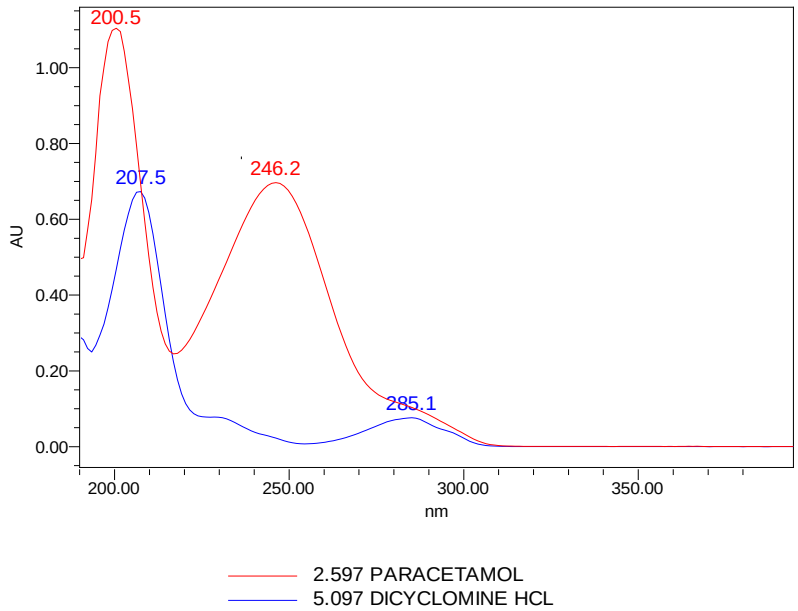


Figure No:2 UV spectrum of both Paracetamol and Dicyclomine hcl(220nm)

Selection of chromatographic method: The choice of chromatographic method is based on the nature of the sample (ionic or neutral molecule),its molecule weight and solubility. As drug are polar in nature, the reverse phase chromatographic technique was selected for the present work.

Optimized Method

Preparation of buffer : 1ml of Triethylamine is dissolved into 1lt Water and adjust the pH-6.0 with Ortho Phosphoric acid filtered and degassed.

Mobile phase: Prepared a mixture of Ortho phosphoric acid buffer : MEOH (60:40 v/v), filtered and degassed.

Preparation of Diluent: Mobile phase is used as a diluent.

Chromatographic condition

Used as suitable High Performance Liquid Chromatography equipped with UV-visible detector.

Column : Agilent CN, 250mm x 4.6mm, 5µm.

Wavelength : 220 nm

Injection Volume : 10µL

Column Temperature : **27˚ c**

Flow rate : 1.5 ml/min

Retention time of Paracetamol is about 2.0 – 3.0 min.

Retention time of Dicyclomine hcl is about 4.5 – 5.5 min.

Preparation of standard solution

Solution A

Paracetamol

Weighed accurately about 325mg of Paracetamol working standard into a 100 ml volumetric flask. Add 70 ml of diluent, sonicate to dissolve and dilute to volume diluent.

Solution A

Solution B

Dicyclomine hcl: Weighed accurately about 100 mg of Dicyclomine Hcl working standard into a 100 ml volumetric flask. Add 70 ml of diluent, sonicate to dissolve and dilute to volume diluents ml to 50 ml with Further dilute each 5mL of Solution-A , 1ml of Solution-B taken into 50ml with the diluent.

Preparation of Sample solution: Weigh 10 tablets and weigh powder then take into 5 tablets equivalent weight of sample into a 500 ml volumetric flask. Add 300 ml of diluent, sonicate to dissolve and dilute to volume diluent. Further dilute 5 ml to 50 ml with the diluent. Filter through 0.45µ Nylon syringe filter.

Procedure: Inject 10µL of Standard preparation five times and Sample preparation in the Chromatograph. Record the chromatograms and measure the peak responses for Paracetamol, Dicyclomine hcl. The System suitability parameters should be met. From the peak responses, calculate the content of Paracetamol, Dicyclomine hcl in the sample.

Evaluation of system suitability

1. Relative Standard Deviation of five replicate injections of Standard preparation for Paracetamol and Dicyclomine hcl peaks should not be more than 2.0%.
2. Theoretical plate count should not less than 2000.

Assay calculations

$$\frac{AT}{AS} = \frac{\text{Std wt (mg)}}{100} \times \frac{5}{50} \times \frac{1}{\text{wt taken}} \times \frac{500}{5} \times \frac{(P)\ \%\ \text{Potency of Std}}{50} \times \text{Avg wt}$$

Assay (%): Assay (mg/tab) x 100/ LC

Where

AT= Average area count of Paracetamol, Dicyclomine hcl peak in the chromatogram of sample solution.

AS= Average area count of Paracetamol, Dicyclomine hcl peak in the chromatogram of standard solution.

P=Percent potency of Paracetamol, Dicyclomine hcl working standard on as is basis.

LC= Label claim of Paracetamol, Dicyclomine hcl in mg

WT = Sample weight

Table no 2

Drug	Labeled claim(mg)	Amount present(mg)	% Assay
Paracetamol	325	325.25	100.3
Dicyclomine Hcl	20	20.25	100.9

METHOD VALIDATION

Analytical method validation is a process of performing several tests designed to verify that an analytical test method is suitable for its intended purpose and is capable of providing useful and valid analytical data. A validation study involves testing multiple attributes of a method to determine that it can provide useful and valid data when used routinely .There are several parameters that are considered in the method validation process as per International Conference of Harmonization(ICH)guidelines and as follows

1. System suitability
2. Linearity
3. Precision

4. Accuracy
5. Specificity
6. Roubustness
7. Ruggedness

SIMULTANEOUS ESTIMATION METHOD (VIERODT′S METHOD) OF PARACETAMOL AND DICYCLOMINE HCL FOR UV MATERIALS AND METHODS

Instruments used

A Single beam UV/Visible spectrophotometer (Thermo Scientific Aquamate plus) was used to measure absorbances of solutions. An electronic analytical balance (Shimadzu) and an ultrasonic bath sonicator (Cyber labs) were used in the study.

Reagents and chemicals

Analytical pure drugs of paracetamol and dicyclomine hydrochloride were obtained as gift samples from swasthic labs, vijayawada, india. The combined tablet formulation with a labelled claim of pacetamol mg and Dicyclomine hydrochloride mg respectively, were obtained from local drug store. Methanol of analytical grade purchased from.

Preparation of standard stock solution for paracetamol

Weighed accurately about 50mg of paracetamol and transferred into 50ml volumetric flask to this add 30ml of methanol and this is sonicated for about 15 min and dilute upto the mark with 0.1N NaoH finally to get a stock solution having strength 1000µg/ml.

Preparation of working standard solution of paracetamol

1000µg/ml solution of paracetamol was prepared by diluting 1ml stock solution to 10 ml with 0.1N NaoH and dilute further to get the concentration range **2,4,6,8,10,12 µg/ml** of paracetamol.

Preparation of stock solution of Dicyclomine Hydrochloride

Weighed accurately about 10mg of Dicyclomine Hcl and transferred into 50ml volumetric flask to this add 30ml of methanol was added and sonicated for about 15min .then diluted upto the mark with 0.1NNaoH finally to get stock solution having strength **1000 µg/ml**.

Preparation of working standard solution of Dicyclomine Hydrochloride

1000µg/ml solution of dicyclomine Hcl was prepared and this is diluting to 1ml stock solution to 10ml with 0.1N NaoH. This solution was diluted further to get concentration range of **10,15,20,25,30,35 µg/ml** of Dicyclomine Hcl.

Selection of wavelength

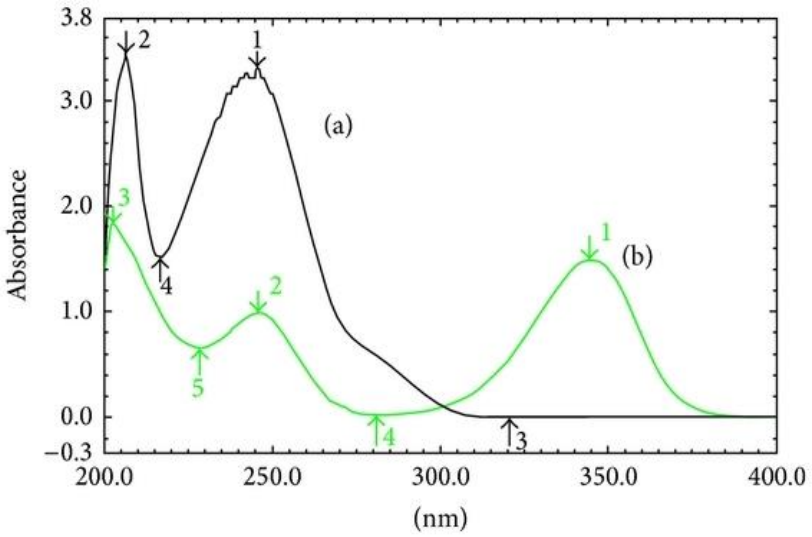
The standard stock solutions of paracetamol and Dicyclomine Hcl were scanned the range 200-400 nm in 1cm cell against methanol and NaoH as blank and the spectras were recorded. paracetamol showed λ_{max}243nm while Dicyclomine Hcl showed λ_{max}345nm.

Calibration curve

A series of dilutions were prepared from the standard stock solutions of paracetamol and Dicyclomine Hcl to obtain the concentration of 2-12µg/ml of paracetamol and 45-70µg/ml of Dicyclomine Hcl. Absorbances of the above solutions were measured at 243nm and 345nm and a calibration curve of absorbance against concentration was plotted.

Selection of suitable solvent

According to solubility profile and literature review, paracetamol was freely soluble in water, alcohol, acetone, methanol. Dicyclomine Hcl was soluble in water, alcohol, chloroform and methanol.



Over lay UV spectrum of both paracetamol and Dicyclomine Hcl (λ_{max} at 300nm)

In setting up the conditions for development of assay method, the choice of detection wavelength was based on the scanned absorption spectrum for paracetamol and Dicyclomine Hydrochloride was obtained separately by the sample over the wave length range 200-400

nm against blank. After through examination of the spectra, the wavelength 300nm(Iso-absorption point) was selected for further analysis, as shown in figure 3

Determination of absorptivity coefficients: The absorptivity coefficients were determined for both the drugs at the selected wavelengths by using the following formula

$A=A(^{1\%}$ 1cm) b c

The calculated values of $A(^{1\%}$ 1cm)are given below.

Table no 3 : Absorptivity values of Paracetamol and Dicyclomine Hcl

S.NO	Absorptivity values of paracetamol		Absorptivity values of Dicyclomine Hcl	
1	At 243nm ax_1	At 345nm ax_2	At 243nm ay_1	At 345nm ay_2
	8240	7420	4770	5690

The obtained absorptivity values were substituted in the following equations (1) and (2)

$A1 = ax_1C_x + ay_1C_y$

$A2 = ax_2C_x + ay_2C_y$

Where

A1 and A2 are absorbances of sample at 243nm and 345nm, Respectively.

ax_1 and ax_2 are absorptivities of paracetamol at 243nm and 345nm respectively.

ay_1 and ay_2 are absorptivitiesof Dicyclomine Hcl at 243nm and 345nm respectively.

C_x and C_y are concentrations of paracetamol and Dicyclomine Hcl.

Preparation of Sample solution: 20 tablets containing 325mg of paracetamol and 20 mg of Dicyclomine Hcl were weighed and average weight was calculated. The tablets were crushed and powdered in glass motar. For the analysis of drugs, a standard addition method was used. An accurately weighed Avg wt of tablets that is 602.235mg of powdered sample was transferred to 50ml volumetric flask, dissolved in sufficient quantity of methanol, sonicated and volume was adjusted up to mark with the same to obtain stock solution of 1000µg/ml of paracetamol and Dicyclomine Hcl. This solution was then filtered through whatmann filter paper.

Analysis of tablet dosage form: Aliquot portion of the above sample stock solution was diluted with methanol and the absorbance was measured at appropriate wavelengths and the

concentrations of the two drugs were estimated using below equations .The analysis procedure was repeated three times and the results are depicted in the table.

$$C_x = \frac{A_2\, ay_1 - A_1\, ay_2}{---------- \quad A_{x2}\, ay_1 - A_{x1}\, ay_2}$$

$$C_y = \frac{A_1\, ax_2 - A_2\, ax_1}{---------- \quad A_{x2}\, ay_1 - A_{x1}\, ay_2}$$

Table no 4: Assay results

Drug	Amount Found(mg)	Amount present (% Labeled claim)	% Assay
Paracetamol	496mg	500mg	99.20%
Dicyclomine Hcl	19.9mg	20mg	99.60%

METHOD VALIDATION

Analytical method validation is a process of performing several tests designed to verify that an analytical test method is suitable for its intended purpose and is capable of providing useful and valid analytical data. A validation study involves testing multiple attributes of a method to determine that it can provide useful and valid data when used routinely .There are several parameters that are considered in the method validation process as per International Conference of Harmonization(ICH)guidelines and as follows

1. System suitability
2. Linearity
3. Precision
4. Accuracy
5. Specificity
6. Roubustness
7. Ruggedness
8. LOD and LOQ

Forced degradation studies

The specificity of the method was demonstrated through forced degradation studies conducted on the sample using acid, alkaline, oxidative, reductive, thermal and photolytic degradations. The sample was exposed to these conditions and the main peak was studied for the peak purity, thus indicating that the method effectively separated the degradation procedures from the pure active ingredient regulatory guidance in ICH Q2A, Q2B,Q3B and

FDA 21 CFR section 211 requires the development and validation of stability –indicating potency assays.

Preparation and working standard solution

For solution A Three tabltes (3099mg) was taken into a 500ml Volumetric flask. For solution B further dilute 5ml of solution A to 50ml(To this add forced degradation solutions) then makeup to the volume with diluents.

1. Acid Degradation (5N HCl)

Procedure

Added 3ml of 5N HCl and heated at 70°C for 1 hours on a water bath. The flask was removed from the water bath and allowed to cool to room temperature. Added 3ml of 5N NaOH to neutralize the solution cooled to room temperature and diluted to volume with diluent and mixed.

2. Alkaline Degradation (5N NaOH)

Procedure

Added 3ml of 5N NaOH and heated at 70°C for 1 hours on a water bath. The flask was removed from the water bath and allowed to cool to room temperature. Added 3ml of 5N HCl to neutralize the solution cooled to room temperature and diluted to volume with diluent and mixed.

3.Peroxide Degradation (30% H_2O_2)

Procedure

Added 3ml of 30% H_2O_2 and heated at 70°C for 1 hours on a water bath. The flask was removed from the water bath, allowed to cool to room temperature and diluted to volume with diluent and mixed

4. Reduction Degradation (10% Sodium Bisulphate)

Procedure: Added 3ml of 10% Sodium Bisulphate and heated at 70°C for 1 hours on a water bath. The flask was removed from the water bath, allowed to cool to room temperature and diluted to volume with diluent and mixed.

5. Hydrolysis Degradation

Procedure: Heated at 70°C for 5 hours on a water bath. The flask was removed from the water bath, allowed to cool to room temperature and diluted to volume with diluent and mixed.

6. Thermal Degradation (105°C / 3 hrs)

Procedure: Sample was exposed at 80°C for at least 3 hrs and the exposed sample was analysed.

7.Humidity Degradation (25°C / 92% RH for 3 hrs)

Procedure: Sample was exposed at 25°C / 92% RH for at least 3 hrs and the exposed sample was analysed.

8. Photolytic Degradation

Procedure

Sample was exposed to 5 hours of sun light and the exposed

Acceptance criteria for forced degradation

Purity angle should be less than purity threshold. Ritonavir and its degraded substances should not have any flag in purity results table.

Observation: Purity angle is found to be less than threshold angle in all forced degradation studies without having signs of purity flags. The results were given in table No :5,6

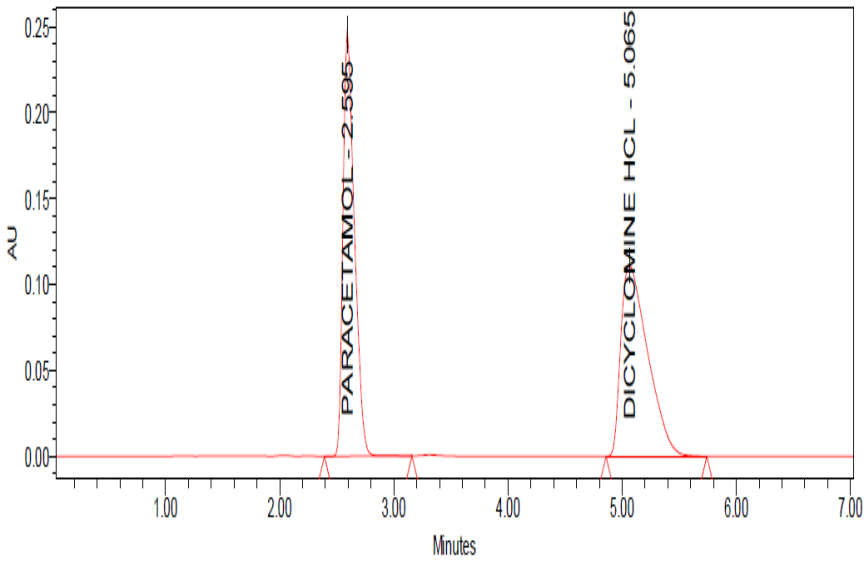


Fig No 4 : A representative Chromatogram of standard solution

Table No 5 : Results of Forced degradation of Paracetamol

S.NO	Stress Conditions	RT	Peak Area	Plate Count	USP Tailing	% Degraded	Purity Angle	Purity Threshold
1	Control	2.585	1744091	2560	1.374	-0.5	0.34	5.234
2	Acid	2.590	1051035	2739	1.406	26.1	0.163	5.184
3	Alkali	2.592	1055004	2763	1.381	26.3	0.153	5.163
4	Peroxide	2.591	1058328	2748	1.387	24.4	0.147	5.159
5	Reduction	2.588	1054944	2728	1.410	23.1	0.159	5.162
6	Thermal	2.589	1056611	2724	1.413	23	0.152	5.141
7	Photo	2.590	1049867	2741	1.405	23.6	0.165	5.159
8	Humidity	2.591	1057442	2749	1.386	23.1	0.146	5.154
9	Hydrolysis	2.592	1053805	2764	1.380	22.9	0.153	5.149
10	Heat	2.588	1054149	2729	1.409	23.2	0.157	5.150

Table No 6 : Results of Forced degradation of Dicyclomine Hcl

S.NO	Stress Conditions	RT	Peak Area	Plate Count	USP Tailing	% Degraded	Purity Angle	Purity Threshold
1	Control	5.050	1847729	2064	1.734	-0.6	0.274	5.732
2	Acid	5.078	1120401	2225	1.765	25.6	0.268	5.713
3	Alkali	5.080	1117872	2238	1.767	26.2	0.236	5.580
4	Peroxide	5.084	1122802	2213	1.784	24.2	0.222	5.561
5	Reduction	5.071	1119409	2214	1.804	22.9	0.212	5.533
6	Thermal	5.076	1123707	2221	1.791	22.6	0.217	5.567
7	Photo	5.078	1119126	2226	1.762	23	0.261	5.681
8	Humidity	5.084	1121823	2214	1.782	22.8	0.215	5.534
9	Hydrolysis	5.080	1117239	2239	1.766	22.7	0.236	5.563
10	Heat	5.071	1112449	2224	1.789	23.4	0.200	5.490

RESULTS AND DISCUSSION

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FORSIMULTANEOUS ESTIMATION OF PARACETAMOL AND DICYCLOMINE HYDROCHLORIDE

The development of an analytical method for the determination of drugs by HPLC has received considerable attention in recent years because of their importance in quality control of drugs and drug products. The objective of this study was to develop a simple, rapid, precise, accurate and sensitive HPLC method for the simultaneous estimation of paracetamol and Dicyclomine Hcl and its pharmaceutical dosage form.

The developed Rp-HPLC method for the Simultaneous estimation of paracetamol and Dicyclomine Hcl was carried out on cap cell pack C_{18} (250mm x 4.6mm, 5µm) column in isocratic mode using mobile phase composition of triethylamine : MEOH (60:40% v/v) with

flow rate of 1.5ml/min at 220nm.The average retention times for paracetamol and Dicyclomine Hcl was found to be 2.0-3.0min and 4.5-5.5 min respectively.

From the results shown in the assay value of paracetamol and Dicyclomine Hcl were found to be 100.3% and 100.9% respectively.

The linearity of paracetamol and dicyclomine Hcl was carried out at different concentrations ranging from 0-487 (ppm) and 0-450(ppm).Correlation coefficient was found to be 0.998,0.998,which indicates that the concentration had given good linearity.

The %RSD values of paracetamol and Dicyclomine Hcl for system precision and method precision was found to be 0.186,0.137 and 0.964,0.996 respectively shown . In these results are within the acceptance limit of less than 2%, indicates that the proposed method has good reproducibility. The results are good for both method precision and system precision.

From the results it was found that the mean percentage recovery values were found to be 99.6-99.9% and for paracetamol and 100.4-100.5% for Dicyclomine Hcl and the results are within the acceptance limit which indicates that the method was accurate.

The robustness of the developed method was evaluated by changing the flow rate, wavelength and mobile phase composition, All the parameters were within the limits at all variable conditions as like % RSD Values.This indicates that the method was robust.

Table 7 : Validation parameters of paracetamol and Dicyclomine Hcl by RP-HPLC

S.NO	PARAMETERS	PARACETAMOL	Dicyclomine Hcl
1	Linearity(ppm)	0-487	0-150
2	Correlation coefficient	0.998	0.998
3	Precision i)System precision ii)Method precision	 0.186 0.964	 0.137 0.996
4	Accuracy(%recovery)	99.9	100.5
5	Robustness (%RSD<2) Flow plus Flow minus Organic plus Organic minus Wavelength plus Wavelength minus	 0.832 0.730 0.658 0.556 0.200 0.159	 0.887 1.165 0.899 0.656 0.130 0.88
6	assay	99.2	99.6

DEVELOPMENT AND VALIDATION OF UV SPECTROPHOTOMETRIC METHOD FOR SIMULTANEOUS ESTIMATION OF PARACETAMOL AND DICYCLOMINE HYDROCHLORIDE IN BULK AND TABLET DOSAGE FORM

The simultaneous estimation of paracetamol and Dicyclomine Hcl in a commercially available tablet dosage form was developed by Uv- spectrophotometry.

After considering the solubility of both the drugs, 0.1N NaoH was selected as the common solvent. From the overlain spectra, 243nm, 345nm were selected as sampling wavelengths for paracetamol and Dicyclomine Hcl .this is a very simple method and requires knowledge molar absorptivities of the components of the components which should be determined very accurately. It only requires measurement of absorbances at 243nm and 345nm and few simple calculations, which can be done manually .The above said two wavelengths were selected to frame the simultaneous equation.

From the results in the amounts of paracetamol and Dicyclomine Hcl in the tablet dosage form were found to be 500mg for paracetamol and 20mg of Dicyclomine Hcl were found to be satisfactory.

The method validated as per ICH guidelines. Linearity was obtained in the concentration range 2-12µg/ml for paracetamol and 45-70µg/ml for DicyclomineHcl. The %RSD values of paracetamol and Dicyclomine Hcl for system precision and method precision was found to be 0.338,0.365 and 0.387,0.201 respectively and as these results are within the acceptance limit of <2% which indicates that the method was precise.

The mean percentage recovery values were found to be 100.15-100.15% for paracetamol and 99.49-100.62% for Dicyclomine Hcl and results are within the acceptance limit which indicates that the method was acuurate , LOD and LOQ for paracetamol and Dicyclomine hcl results are within the acceptance limit . Based on the results obtained the proposed method was accurate, precise, reproducible and can be employed for routine quality control of paracetamol and Dicyclomine Hcl in bulk and tablet dosage form.

Table no 8: Validation Parameters of Paracetamol and Dicyclomine Hcl by UV Spectrophotometry

S.NO	Parameters	Paracetamol	Dicyclomine Hcl
1	Linearity(µg/ml)	2-12	45-70
2	Correlation coefficient	0.998	0.999
3	Precision i)System precision ii)Method precision	 0.338 0.387	 0.365 0.201
4	Accuracy(% recovery)	100.15	100.62
5	LOD	O.4888 µg/ml	10.266 µg/ml
	LOQ	1.6133 µg/ml	33.87 µg/ml
6	Tablet Assay(%)	99.2	99.6

DEVELOPMENT AND VALIDATION OF UV METHOD FOR THE ESTIMATION OF DICYCLOMINE HYDROCHLORIDE IN BULK AND TABLET DOSAGE FORM

An effort has been made to identify a simple, precise, economic, rapid, specific and accurate method for the estimation of Dicyclomine Hcl and in formulation.

The solubility of Dicyclomine Hcl was determined as per Indian pharmacopoeia. Number of solvents tried to include distilled water, 0.1N NaoH, methanol, alcohol, chloroform. It is insoluble in ether. It is freely soluble in 0.1N NaoH, methanol, By considering the cost of methanol, solubility of the drug was tried with 0.1N NaoH. The solution was scanned in uv region in the wavelength range 200-400 nm against solvent as the blank. From the spectrum of Dicyclomine Hcl the wavelength maxima was found to be 345nm.

From the results in the amount of Dicyclomine Hcl in the tablet dosage form was found to be 19.9mg.

The method was validated as per ICH guide lines. Linearity was obtained in the concentration range of 45-70 µg/ml for Dicyclomine Hcl .The % RSD values of Dicyclomine Hcl for system and method precision was found to be 0.365,0.201 respectively and as these results are within the acceptance limit of <2 % which indicates that the method was precise.
The mean percentage recovery values were found to be 99.33% for Dicyclomine Hcl and the results were with in the acceptance limit which indicates that the method was accurate .Based on the results obtained the proposed method was accurate .precise, reproducible and can be employed for routine control of Dicyclomine Hcl in bulk and combined tablet dosage form.

Table no 9: Validation parameters of Dicyclomine Hcl by UV spectrophotometry

S.NO	Parameters	Dicyclomine Hcl
1	Linearity(µg/ml)	45-70
2	Correlation coefficient	0.999
3	Precision i)System precision ii)Method precision	 0.365 0.201
4	Accuracy(% recovery)	99.33
5	LOD LOQ	10.266 µg/ml 33.87 µg/ml
6	Tablet Assay(%)	99.6

CONCLUSION

A linear, Precise, accurate and analytical methods have been developed for the Paracetamol and Dicyclomine Hcl. The methods were very simple, Specific and reliable. All the results were within the limits. The developed methods were validated as per ICH guidelines.

Good agreement was seen in the assay results of pharmaceutical formulation by developed methods. Hence it can be concluded that the proposed methods were good approach for obtaining reliable results and found to be suitable for the routine analysis of Paracetamol and Dicyclomine Hcl in bulk and pharmaceutical dosage form.

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