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VALIDATED STABILITY INDICATING LIQUID CHROMATOGRAPHIC METHOD FOR SIMULTANEOUS ESTIMATION OF PARACETAMOL, TRAMADOL AND DICYCLOMINE IN TABLETS

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ABSTRACT: A new simple stability indicating reversed-phase liquid chromatographic method has been developed and validated for simultaneous estimation of Paracetamol, Tramadol and Dicyclomine in combined pharmaceutical dosage form. An Agilent Zorbax SB C18 (250mmx4.6mm, 5microns) column with mobile phase containing ammonium acetate: acetonitrile (700:300 v/v) was used. The flow rate was maintained at 1.0 mL/min, column temperature was 30°C and effluents were monitored by using a photodiode array detector at 233 nm. The retention times of Paracetamol, Tramadol and Dicyclomine were found to be 2.331, 3.182 and 7.650 min, respectively. Correlation co-efficient for Paracetamol, Tramadol and Dicyclomine were found to be 0.99, 0.99 and 0.99 respectively. The proposed method was validated with respect to linearity, accuracy, precision, specificity, and robustness. Recovery of Paracetamol, Tramadol and Dicyclomine in formulations was found to be in a range of 98-102%, 98-102% and 98-102% respectively. Paracetamol, Tramadol and Dicyclomine dosage form is also subjected to the stress conditions of oxidative, acid, base, hydrolytic, thermal and photolytic degradation. The degradation products were well resolved and peak purity test results confirmed that Paracetamol, Tramadol and Dicyclomine peaks were homogenous and pure in all stress samples, thus proving stability-indicating power of the method. Due to its simplicity, rapidness and high precision, this method can be applied for regular analysis.

INTRODUCTION: Paracetamol (INN) or acetaminophen (USAN) chemically named N-acetyl-p-aminophenol, is a widely used over-the-counter analgesic (pain reliever) and antipyretic (fever reducer). Paracetamol is classified as a mild analgesic. It is commonly used for the relief of headaches and other minor aches and pains and is a major ingredient in numerous cold and flu remedies.

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In combination with opioid analgesics, Paracetamol can also be used in the management of more severe pain such as post-surgical pain and providing palliative care in advanced cancer patients. Though Paracetamol is used to treat inflammatory pain, it is not generally classified as an NSAID because it exhibits only weak anti-inflammatory activity.

The onset of analgesia is approximately 11–29.5 minutes after oral administration of Paracetamol, and its half-life is 1–4 hours. Tramadol is a centrally acting opiod analgesic used to treat moderate to moderately severe pain. The chemical name for Tramadol hydrochloride is (±) cis-2-

[(dimethylamino) methyl]-1-(3methoxyphenyl) cyclohexanol hydrochloride. Structurally, Tramadol closely resembles a stripped down version of codeine. Both codeine and Tramadol share the 3-methyl ether group, and both compounds are metabolized along the same hepatic pathway and mechanism to the stronger opioid, phenol agonist analogs. For Codeine, this is Morphine, and for Tramadol, it is the O-desmethyltramadol. The drug has a wide range of applications, including treatment of rheumatoid arthritis, restless legs syndrome, motor neuron disease and fibromyalgia. Dicyclomine, also known as Dicycloverine is an anticholinergic that blocks muscarinic receptors.

It is chemically (bicyclohexyl)-1-carboxylic acid, 2-(diethylamino) ethyl ester, hydrochloride. Dicyclomine is used to treat intestinal hypermotility and the symptoms of irritable bowel syndrome (IBS) (also known as spastic colon). It relieves muscle spasms and cramping in the gastrointestinal tract by blocking the activity of acetylcholine on cholinergic (or muscarinic) receptors on the surface of muscle cells. It is a smooth muscle relaxant [1-2]. Structures of Paracetamol, Tramadol and Dicyclomine are depicted in **Figure 1**.

A literature survey revealed few liquid chromatography (LC) assay methods that have been reported for the determination of Paracetamol and Tramadol in bulk dug and pharmaceutical dosage forms, but there are no reported methods for simultaneous estimation of Paracetamol, Tramadol and Dicyclomine in combined pharmaceutical dosage forms [3-23].

The present International Conference on Harmonization (ICH) drug stability guidelines suggest that stress studies should be conducted on the drug product to establish its inherent stability characteristics, and the analytical method should able to separate all degradation impurities formed under stress studies to prove its stability-indicating power. In order to monitor possible changes to a product over time, the applied analytical chromatographic method must be stability-indicating. The best case for testing the suitability of a method is using real-time stability samples containing all relevant degradation products that might occur. But due to product development timelines, process characteristics, excipients, and other environmental factors, a forced degradation study (stress test) can serve as an alternative.

In a typical study, relevant stress conditions are light, heat, humidity, hydrolysis (acid / base influence) and oxidation or even a combination of described parameters. If it is necessary to form degradation products, the strength of stress conditions can vary due to the chemical structure of the drug substance, the kind of drug product, and product specific storage requirements. An individual program has to be set up in order to reach a target degradation of 5 to 20%.

A higher level of degradation will be out of the scope of product stability requirements and therefore unrealistic. The scope of the test is to generate degradation products in order to facilitate a method development for determination of the relevant products.

Therefore, samples will be stressed in a solid form and/or in solution. Typically, stress tests are carried out on one batch of material. For drug products the placebo should be stressed in a similar way in order to exclude those impurities that are not degradation products (e.g. impurities arising from excipients). The stability studies were determined by applying the physical stress (acid, base, peroxide, heat and light) to the product [24-33].

The aim of the present work is to focus on the development of an efficient stability indicating liquid chromatographic method for simultaneous estimation of Paracetamol, Tramadol and Dicyclomine in combined pharmaceutical dosage form such as Tablets in presence of its excipients and degradation products in a short chromatographic run.

The present work concerns the method development, method validation and forced degradation studies of Paracetamol, Tramadol and Dicyclomine in combined pharmaceutical dosage form. The developed Liquid Chromatographic method was validated with respect to specificity, limit of detection (LOD), limit of quantification

(LOQ), linearity, precision, accuracy and robustness. Forced degradation studies were performed on the placebo and drug products to show the stability-indicating nature of the method. These studies were performed in accordance with established ICH guidelines.

MATERIALS AND METHODS:

Instrumentation:

Samples were analyzed on Waters alliance 2695 HPLC system (Waters Corporation, Milford, MA) equipped with a with binary HPLC pump, Waters 2998 PDA detector and Waters Empower2 software. The separation was achieved on Agilent Zorbax SB C18 (250mmx4.6mm, 5microns) column.

Chemicals and Reagents:

Paracetamol, Tramadol and Dicyclomine standards were supplied by Dr. Reddy's Laboratories Ltd., Hyderabad. Acetonitrile of HPLC grade was purchased from E. Merck (India) Ltd., Mumbai. Ammonium acetate of AR grade was obtained from S.D. Fine Chemicals Ltd., Mumbai and milli Q water. Paracetamol, Tramadol and Dicyclomine tablets (Ultraspas: Aristo and Topspas: psychotropic's India) were procured from market.

HPLC Conditions:

The mobile phase consisting of Ammonium acetate: Acetonitrile (HPLC grade) were filtered through 0.45 µm membrane filter before use, degassed and were pumped from the solvent reservoir in the ratio of 700:300 v/v into the column at a flow rate of 1.0 ml/min. The column temperature was maintained at 30°C. The detection was monitored at 233 nm and the run time was 10 minutes. The volume of injection loop was 20 µl prior to injection of the drug solution.

Preparation of standard solution:

Take accurately 75 mg of Paracetamol, 650 mg of Tramadol and 40 mg of Dicyclomine HCl working standard and transfer it into 50 ml volumetric flask dissolve and diluted to volume with Mobile Phase. From the above solution take 5 ml into 25 ml volumetric flask make up the volume with Mobile Phase. (Concentration of Paracetamol : 0.3 mg/ml, Concentartion of Tramadol : 2.6 mg/ml, Concentration of Dicyclomine: 0.16 mg/ml).

Preparation of sample (drugs from marketed formulations) solution:

Take accurately sample powder equivalent to one and transfer it into 100 ml volumetric flask dissolve and diluted to volume with mobile phase. From the above solution take 5 ml into 25 ml volumetric flask make up the volume with Mobile Phase. (Concentration of Paracetamol: 0.3 mg/ml, Concentartion of Tramadol: 2.6 mg/ml, Concentration of Dicyclomine: 0.16 mg/ml).

Forced degradation studies:

Forced degradation studies were performed at a 1617.90 mg/mL concentration of Paracetamol, Tramadol and Dicyclomine in tablets to provide an indication of the stability-indicating property and specificity of the proposed method. A peak purity test was conducted for Paracetamol, Tramadol and Dicyclomine peaks by using a PDA detector on stress samples. All solutions used in forced degradation studies were prepared by dissolving the drug product in a small volume of stressing agents. After degradation, these solutions were diluted with mobile phase to yield a stated concentration approximately. Conditions employed for performing the stress studies are described below.

Acid degradation:

Tablet powder equivalent to 1617.90 mg was accurately weighed and dissolved in 5 ml of mobile phase, 5 ml of 5 N HCl was added and the mixture was kept at 70^0C for 5 min. The solution was brought to ambient temperature, neutralized by the addition of 5 ml of 5 N NaOH and diluted to 25 ml with mobile phase.

To prepare the blank, 5 mL of 5 N HCl and 5 mL of 5 N NaOH were diluted to 25 mL with mobile phase.

Base degradation:

Tablet powder equivalent to 1617.90 was accurately weighed and dissolved in 5 ml of mobile phase, 5 ml of 5N NaOH was added and the mixture was kept at 70^0C for 5 min. The solution was brought to ambient temperature, neutralized by the addition of 5 ml 5 N HCl and diluted to 25 mL with mobile phase. To prepare the blank, 5 mL of 5

N NaOH and 5 mL of 5 N HCl were diluted to 25 mL with mobile phase.

Oxidation degradation:

Tablet powder equivalent to 1617.90 mg was accurately weighed and dissolved in 5 mL of mobile phase, 5 mL of 3% hydrogen peroxide was added and the mixture was kept at 70^0C for 10 min. The solution was brought to ambient temperature and diluted to 25 mL with mobile phase.

To prepare the blank, 5 ml of 3% hydrogen peroxide was diluted to 25 mL with mobile phase.

Thermal degradation:

Tablet powder equivalent to 1617.90 was stored at 105^0C for 9 h, dissolved and diluted to 25 mL with mobile phase.

Photolytic degradation:

The susceptibility of the drug product to the light was studied; Tablet powder for photo stability testing was placed in a photo stability chamber and exposed to a white florescent lamp with an overall illumination of 1.2 million lux hours and near UV radiation with an overall illumination of 200 watt/m^2/h at 25^0C. Following removal from the photo stability chamber, the sample was prepared for analysis as previously described.

RESULTS:

Method Development:

The analytical procedure for the estimation of Paracetamol, Tramadol and Dicyclomine in marketed formulation was optimized with a view to develop a simple, precise and accurate assay method. Agilent Eclipse XDB (4.6*150 mm*3.5 mic), Agilent Zorbax SB C18 (4.6*250mm*5 mic) and Inertsil-ODS (4.6*250 mm*5 mic) were used to provide an efficient separation but appropriate chromatographic separation was achieved on Agilent Zorbax SB C18 (4.6*250mm*5 mic).

Various mobile phase systems were prepared and used to provide an appropriate chromatographic separation, but the proposed mobile phase containing Ammonium acetate: acetonitrile in the ratio of 700:300 (v/v) gave a better resolution. Using UV-visible PDA detector at 233 nm carried out the detection. Amongst the several flow rates tested, the flow rate of 1 ml/min was the best suited for both the drugs with respect to location and resolution of peaks. The retention time of Paracetamol, Tramadol and Dicyclomine was found to be 2.331, 3.182 and 7.650 min respectively. The chromatograms of standard and sample solution of Paracetamol, Tramadol and Dicyclomine were shown in **Figure 2**. The asymmetry factor of Paracetamol, Tramadol and Dicyclomine was 1.391, 1.410 and 1.246 found to be respectively, which indicates symmetrical nature of the peak.

The USP resolution of 5.884 was achieved between Paracetamol, Tramadol and 16.232 was achieved between Paracetamol and Dicyclomine. The USP plate count of Paracetamol, Tramadol and Dicyclomine was 7268, 5094 and 7277 found to be respectively, which indicates column efficiency for separation. System suitability parameters such as Peak asymmetry, Resolution and Number of theoretical plates are meeting ICH requirements. The percentage label claim of individual drugs found in formulations was calculated. The results of analysis shows that the amounts of drugs estimated were in good agreement with the label claim of the formulations [22-23].

Method Validation:

System Suitability Studies:

System suitability was determined before sample analysis from duplicate injections of the standard solutions of Paracetamol, Tramadol and Dicyclomine. The column efficiency, resolution and peak asymmetry were calculated for the standard solutions. The USP resolution of 5.884 was achieved between Paracetamol, Tramadol and 16.232 was achieved between Paracetamol and Dicyclomine. USP tailing (Peak Asymmetry) for Paracetamol, Tramadol and Dicyclomine were found to be 1.391, 1.410 and 1.246 respectively. Number of theoretical plates (USP plate count) for Paracetamol, Tramadol and Dicyclomine were found to be 7268, 5094 and 7277 respectively.

The values obtained demonstrated the suitability of the system for the analysis of this drug combinations, system suitability parameters may fall within ± 3 % standard deviation range during routine performance of the method [24-25].

Specificity: Specificity is the ability to assess unequivocally the analyte in presence of components which may be expected to be present. Typically these might include impurities, degradants, matrix, etc. Placebo interference was evaluated by analyzing the placebo prepared by the test method. No peak due to placebo was detected at the retention time of Paracetamol, Tramadol and Dicyclomine. The specificity of the developed method was also conducted in presence of its degradation products.

Precision: The precision of method was verified by repeatability and intermediate precision. Repeatability was checked by injecting six individual sample preparations of Paracetamol, Tramadol and Dicyclomine tablets. Percent relative standard deviation (RSD) of the area for each drug was calculated. The intermediate precision of the method was also evaluated using different analysts and different instruments and performing the analysis on different days. The results of precision study are provided in **Table 1**.

TABLE 1: PRECISION STUDIES OF PARACETAMOL, TRAMADOL AND DICYCLOMINE

S. No	Sample Wt	Area of Paracetamol	Area of Tramadol	Area of Dicyclomine	% Assay of Paracetamol	% Assay of Tramadol	% Assay of Dicyclomine
1	1617.90	4330461	4429029	12408435	99	100	99
2	1617.90	4337397	4422026	12435616	99	100	99
3	1617.90	4335747	4422920	12414268	99	100	99
4	1617.90	4337150	4421734	12441171	99	100	99
5	1617.90	4330018	4421768	12409800	99	100	99
6	1617.90	4335468	4428203	12442629	99	100	99
		Average			99	100	99
		STD			0.08	0.08	0.13
		%RSD			0.08	0.08	0.13

Accuracy: The accuracy of the method was determined by recovery experiments. The recovery studies were evaluated in triplicate using three concentration levels 50%, 100% and 150%. The percentage recovery data was obtained, added recoveries of standard drugs were found to be accurate **(Table 2, 3** and **4).**

TABLE 2: ACCURACY FOR PARACETAMOL

Spiked Level	Sample Weight	Sample Area	µg/ml added	µg/ml found	% Recovery	Mean
50%	808.95	2213785	148.654	151.85	102	
50%	808.95	2215303	148.654	151.96	102	102
50%	808.95	2219466	148.654	152.24	102	
100%	1617.90	4335104	297.307	297.36	100	
100%	1617.90	4331662	297.307	297.12	100	100
100%	1617.90	4339023	297.307	297.63	100	
150%	2426.90	6503147	445.970	446.07	100	
150%	2426.90	6506456	445.970	446.30	100	100
150%	2426.90	6506520	445.970	446.31	100	

TABLE 3: ACCURACY FOR TRAMADOL

Spiked level	Sample weight	Sample Area	µg/ml added	µg/ml found	% Recovery	Mean
50%	808.95	2165492	1296.132	1268.52	98	
50%	808.95	2161229	1296.132	1266.02	98	98
50%	808.95	2166071	1296.132	1268.86	98	
100%	1617.90	4425176	2592.264	2592.22	100	
100%	1617.90	4422496	2592.264	2590.65	100	100
100%	1617.90	4423686	2592.264	2591.35	100	
150%	2426.90	6637989	3888.476	3888.46	100	
150%	2426.90	6634891	3888.476	3886.65	100	100
150%	2426.90	6634705	3888.476	3886.54	100	

TABLE 4: ACCURACY FOR DICYCLOMINE

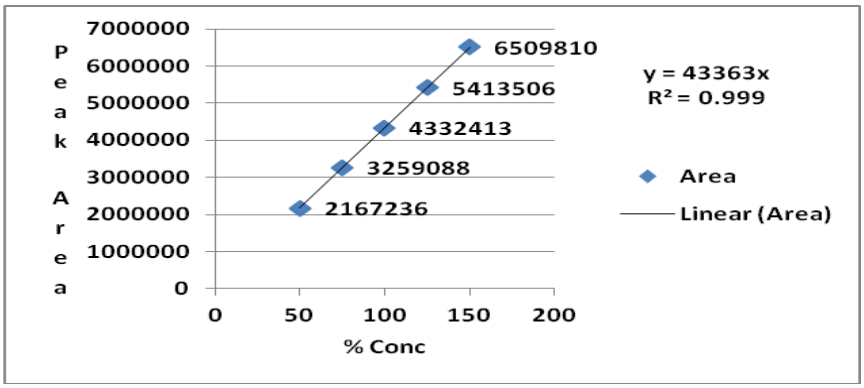
Spiked level	Sample weight	Sample Area	µg/ml added	µg/ml found	% Recovery	Mean
50%	808.95	6219497	79.282	79.29	100	
50%	808.95	6212658	79.282	79.21	100	98
50%	808.95	6213203	79.282	79.21	100	
100%	1617.90	12426515	158.564	158.43	100	
100%	1617.90	12422812	158.564	158.38	100	100
100%	1617.90	12473573	158.564	159.03	100	
150%	2426.90	18699275	237.851	238.40	100	
150%	2426.90	18606405	237.851	237.22	100	100
150%	2426.90	18688585	237.851	238.27	100	

Linearity and Range:

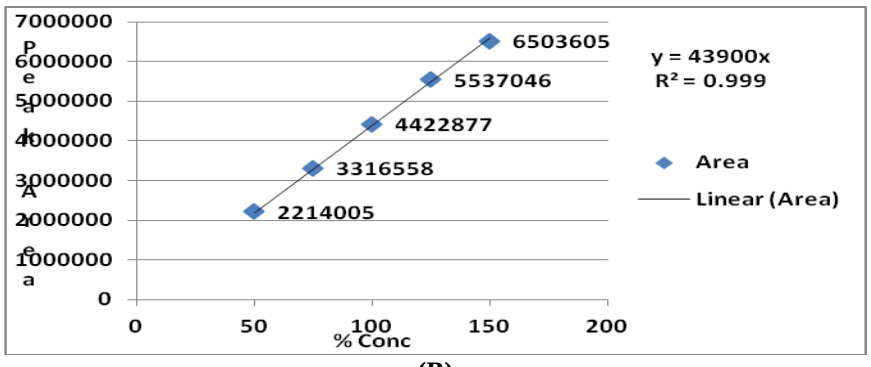
The linearity of the method was determined at five concentration levels (50%, 75%, 100%, 125% and 150%). Linearity test solutions were prepared by diluting the stock solutions to the required concentrations. The calibration curves were plotted between the responses of peak area versus concentration of analyte. The slope and intercept value for calibration curve was y = 43900 x (r2=0.99) for Paracetamol, y = 43363 x (r2=0.99) for Tramadol and y = 12428 x (r2=0.99) for Dicyclomine. The result (Table 5) shows that an excellent correlation exists between areas and concentration of drugs within the concentration range. Calibration curves are presented in **Figure 3**.

TABLE 5: LINEARITY OF PARACETAMOL, TRAMADOL AND DICYCLOMINE

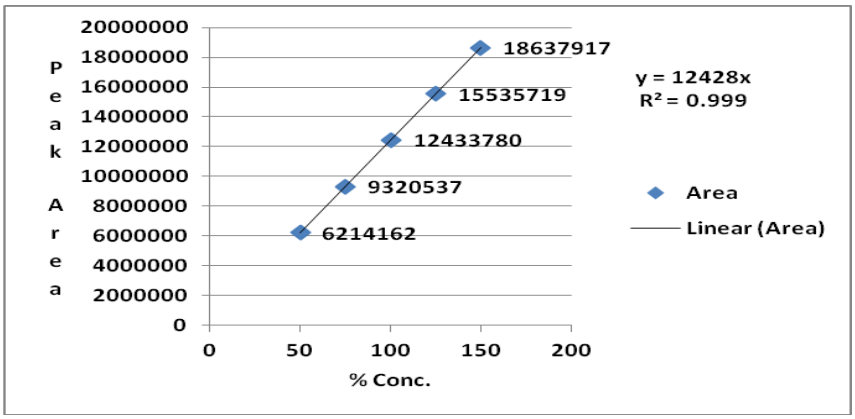
Paracetamol			Tramadol			Dicyclomine		
% Con.	Area	µg/ml	% Con.	Area	µg/ml	% Con.	Area	µg/ml
50	2167236	150	50	2214005	1300	50	6214162	80
75	3259088	225	75	3316558	1950	75	9320537	120
100	4332413	300	100	4422877	2600	100	12433780	160
125	5413506	375	125	5537046	3250	125	15535719	200
150	6509810	450	150	6503605	3900	150	18637917	240



(A)



(B)



(C)

FIGURE 3: LINEARITY CURVES (A) PARACETAMOL (B) TRAMADOL (C) DICYCLOMINE

Limit of detection & Limit of quantification (LOD & LOQ):

Limit of quantification and detection were predicted by plotting linearity curve for different nominal concentrations of Paracetamol, Tramadol and Dicyclomine **(Table 5)**. Relative standard deviation (σ) method was applied, the LOQ and LOD values were predicted using following formulas. Precision was established at these predicted levels.

(a) LOQ = 10 σ / S
(b) LOD = 3.3 σ / S

Where σ = residual standard deviation of response
S = slope of the calibration curve.

LOQ and LOD values for Paracetamol, Tramadol and Dicyclomine were found to be 2.922, 9.740; 2.846, 9.486 and 2.759, 9.195 respectively.

Robustness:

Robustness of the method was determined by making slight changes in the chromatographic conditions and system suitability parameters for Paracetamol, Tramadol and Dicyclomine standard and the resolution, USP Tailing and USP Plate count were recorded. The variables evaluated in the study were column temperature ($\pm 5^0$C), flow rate (±0.2 mL/min). It was observed that there were no marked changes in the chromatograms, which demonstrates that the method developed is rugged, and robust **(Table 6, 7 and 8)**.

TABLE 6: ROBUSTNESS-PARACETAMOL

Sample Name	Rt	Area	USP Tailing	USP Plate count	S/N
TEMP-1	2.329	4820461	1.418	7023	1213.20
TEMP-2	2.311	4867150	1.430	7153	1265.14
FLOW-1	2.344	4885747	1.414	7648	1190.58
FLOW-2	2.330	4827397	1.391	7577	1169.75

TABLE 7: ROBUSTNESS-TRAMADOL

Sample Name	Rt	Area	USP Tailing	USP Plate count	S/N
TEMP-1	3.182	4589029	5.494	4419	680.72
TEMP-2	3.160	4431734	5.686	4851	698.85
FLOW-1	3.198	4442920	5.764	5006	651.39
FLOW-2	3.176	4432026	5.715	4866	641.95

TABLE 8: ROBUSTNESS-DICYCLOMINE

Sample Name	Rt	Area	USP Tailing	USP Plate count	S/N
TEMP-1	7.656	12708435	1.249	6928	986.24
TEMP-2	7.597	12841171	1.277	7045	916.74
FLOW-1	7.668	12814268	1.231	7116	944.73
FLOW-2	7.642	12735616	1.291	6824	1017.73

Forced Degradation Studies:
Based on the results of the stress studies, the degradation behavior of Paracetamol, Tramadol and Dicyclomine is as follows.

Acid degradation:
Paracetamol, Tramadol and Dicyclomine were undergoing degradation in 5 N HCl at 70^0C for 10 min moderately. The impurities formed during this study are well separated from main drug peaks and mass balance is found to be in acceptable limit. Peak purity of drugs was also matches **(Table 9, Figure 4(A)**.

Base degradation:
Paracetamol, Tramadol and Dicyclomine were undergoing degradation in 5 N NaOH at 70^0C for 5 min moderately. The degradation peaks are well separated from main drug peaks and well resolved. Mass balance is found to be in acceptable limit. Peak purity of drugs was also matches **(Table 9, Figure 4(B)**.

Oxidation degradation:
Paracetamol, Tramadol and Dicyclomine were undergoing degradation in 3% hydrogen peroxide at 70^0C for 10 min. The degradation peaks were well resolved from main drug peaks. Mass balance is found to be in acceptable limit. Peak purity of drugs was also matches **(Table 9, Figure 4 (C)**.

Thermal degradation
Paracetamol, Tramadol and Dicyclomine were undergoing degradation upon thermal exposure moderately. Degradation peaks formed were well resolved from main drug peaks. Mass balance is found to be in acceptable limit. Peak purity of drugs was also matches **(Table 9, Figure 4 (D).**

Photolytic degradation
Upon subjecting the Paracetamol, Tramadol and Dicyclomine sample to both UV and visible light, only partial degradation of was observed. Testing of a placebo containing preservative leads to formation of number of different impurities with respect to an unstressed placebo. The amount of preservative decreased mainly by influence of oxidation, light and acid. Mass balance of preservative shows almost 100%. The active ingredients remain almost stable within tested period and mass balance matches **(Table 9, Figure 4(E)**.

TABLE 9: STRESS STUDIES (A) PARACETAMOL (B) TRAMADOL (C) DICYCLOMINE

Stress Condition	Sample weight	Paracetamol			Purity Angle	Purity Threshold
		Area	% Assay	% Deg.		
Acid	1617.9	3811061	87	12	0.748	0.954
Base	1617.9	3919190	90	9	0.724	0.925
Peroxide	1617.9	3910244	89	10	0.528	0.812
Heat	1617.9	3954892	90	9	0.547	0.812
Light	1617.9	3894973	89	10	0.578	0.745

(A)

Stress Condition	Sample weight	Tramadol			Purity Angle	Purity Threshold
		Area	% Assay	% Deg.		
Acid	1617.9	3474633	87	12	0.231	0.584
Base	1617.9	3516026	90	10	0.247	0.568
Peroxide	1617.9	3466318	89	10	0.214	0.487
Heat	1617.9	3520739	90	10	0.218	0.578
Light	1617.9	3458543	89	10	0.217	0.647

(B)

Stress Condition	Sample weight	Dicyclomine			Purity Angle	Purity Threshold
		Area	% Assay	% Deg.		
Acid	1617.9	9022435	72	27	0.526	0.748
Base	1617.9	9112190	73	26	0.512	0.841
Peroxide	1617.9	9997614	80	19	0.421	0.748
Heat	1617.9	10203882	81	18	0.514	0.645
Light	1617.9	11022173	88	11	0.621	0.714

(C)

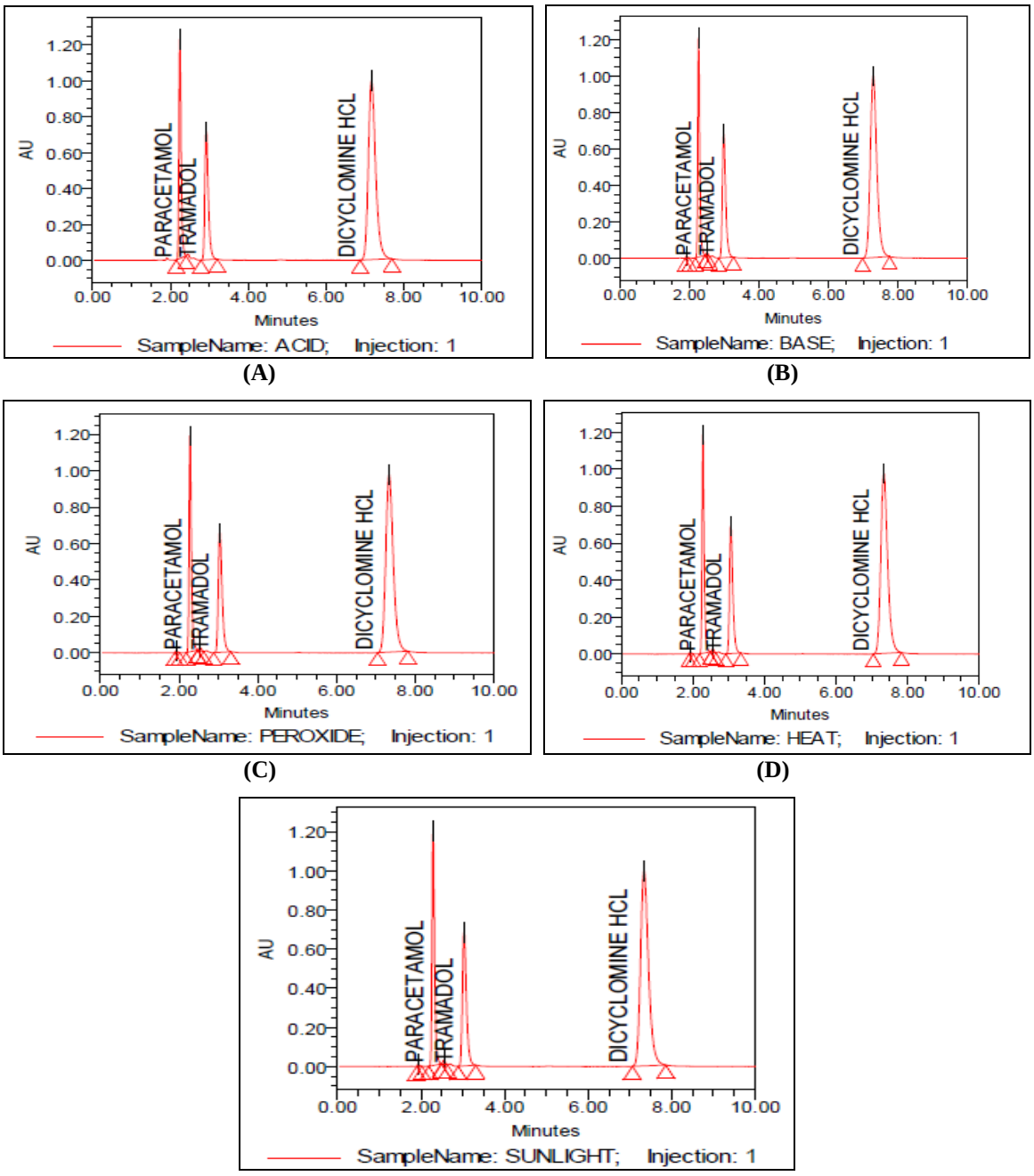


FIGURE 4: TYPICAL CHROMATOGRAMS (A) ACID DEGRADATION (B) ALKALI DEGRADATION (C) OXIDATIVE DEGRADATION (D) THERMAL DEGRADATION (E) PHOTOLYTIC DEGRADATION

DISCUSSION: System suitability parameters such as retention time, resolution, tailing and plate count were shown uniformity and %RSD was less than 1 so we can say system is suitable for analysis method specificity was concluded by **Figure 2**. Those figures are Paracetamol, Tramadol and Dicyclomine standard chromatogram and other one is formulation they were not observed in placebo and excipients peaks interference with standard and analytic peak so it proves method is selective. The result given in **Table 2** says that the method precision is passed for Paracetamol, Tramadol and Dicyclomine studies. The method accuracy was evaluated by recovery studies. Paracetamol, Tramadol and Dicyclomine recovery is meeting the ICH requirements and USP requirements 97%-103% and 98-102% respectively.

Percentage RSD is also very low so the method is accurate as shown in **Table 3, 4 and 5**. Linearity calibration curve was plotted for Paracetamol, Tramadol and Dicyclomine, the graph of

concentration versus peak areas to construct the linear regression equation and to calculate the value of correlation coefficient. The slope and intercept value for calibration curve was y = 43900 x (r^2=0.99) for Paracetamol, y = 43363 x (r^2=0.99) for Tramadol and y = 12428 x (r^2=0.99) for Dicyclomine. Method robustness results were also studied. Forced degradation studies of Paracetamol, Tramadol and Dicyclomine proves the method is also stability indicating and can be used for stability monitoring of the dosage forms to establish the re-test period.

CONCLUSION: The proposed HPLC method for the simultaneous estimation of Paracetamol, Tramadol and Dicyclomine in pharmaceutical dosage forms was found to be simple, sensitive, precise, accurate, linear, robust and rugged during validation. Further this method is stability indicating and can be used for routine analysis of production samples. Hence this method can easily and conveniently adopt for routine quality control of Paracetamol, Tramadol and Dicyclomine in pure and its pharmaceutical dosage forms.

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