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Research Article

Development and Validation of Stability Indicating RP-HPLC Method for Simultaneous Estimation of Trihexyphenidyl, Chlorpromazine and Trifluoperazine in Pharmaceutical Formulations

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ABSTRACT

A simple, rapid and accurate stability indicating High performance Liquid Chromatographic method was developed and validated for the simultaneous determination of Trihexyphenidyl (THP), Chlorpromazine (CLP) and Trifluoperazine (TFP) in pure and its Pharmaceutical formulations using Inertsil ODS C18 column (250mmx 4.6 mm, 5µ) and Methanol: Acetonitrile: Acetate Buffer 80:15:05 v/v as mobile phase at pH 6.1 and flow rate of 1 ml/min with isocratic elution. The eluted compounds were detected at a wavelength 239 nm. The retention times of THP, CLP and TFP are found to be 4.0, 5.9 and 7.7 min respectively with a correlation coefficient 0.999 each. The linearity range was found to be 0.4-4, 10-100 and 1-10 µg/ml for THP, CLP and TFP. The percentage recovery was found to be in range of 98.43 – 99.90%, 98.14-99.94 and 98.22-99.98% for THP, CLP and TFP respectively. Standard drugs were subjected to acid base, hydrolysis, Oxidation, photolytic and thermal degradation conditions. The degradation products of THP, CLP and TFP were well resolved from the pure drug with significant differences in their retention time values. This validated method was applied for the simultaneous estimation of THP, CLP and TFP in commercially available formulation sample.

Keywords: Trihexyphenidyl, Chlorpromazine, Trifluoperazine, HPLC method, ICH guidelines.

INTRODUCTION

Trihexyphenidyl is an antimuscarinic class drugs used as antiparkinsonian agent [1]. The drug is used as mono and combination therapy for treatment of symptomatic treatment of Parkinson's disease [2]. It is active in postencephalitic, arteriosclerotic, and idiopathic forms. Chlorpromazine is the first antipsychotic discovered in 1950. It is a prototypical phenothiazine antipsychotic drug available as a generic medication used for treatment of psychotic disorders such as schizophrenia [3]. Other uses include the treatment of bipolar disorder, attention deficit hyperactivity disorder, nausea and vomiting, anxiety before surgery, and hiccups that do not improve following other measures [4]. It is on the World Health Organization's List of Essential Medicines, the most effective and safe medicines needed in a health system [5]. Trifluoperazine is a typical antipsychotic primarily used to treat schizophrenia [6]. The drug belongs to phenothiazine chemical class used as an antipsychotic and an antiemetic. The drug blocks postsynaptic mesolimbic dopaminergic D1 and D2 receptors in the brain; depresses the release of hypothalamic and hypophyseal hormones and is believed to depress the reticular activating system thus affecting basal metabolism, body temperature, wakefulness, vasomotor tone, and emesis [7].

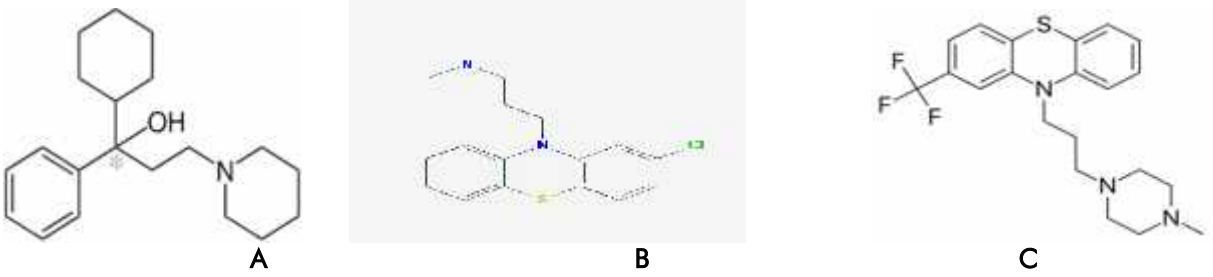

Figure 1: Chemical structures of A-Trihexyphenidyl HCl, B-Chlorpromazine, C-Trifluoperazine

Combination of Chlorpromazine, Trihexyphenidyl and Trifluoperazine used for Schizopherenia is available as tablet dosage form oral administration. The present work is aimed to analyze these drugs in combined dosage form by RP-HPLC method. Literature review reveals that various analytical methods have been reported with individual and combined dosage forms [8-24]. But no method has so far been reported in particular with selected combination of drugs in dosage form.

Experimental

Chemicals and Materials

Analytically pure THP, CLP and TFP were obtained as gift sample from reputed pharmaceutical companies. Methanol, acetonitrile, water (Merck, Mumbai, India) were of HPLC grade, while Acetate Buffer used for the preparation of mobile phase was of analytical grade (Merck Specialties Private Limited, Mumbai, India). The membrane filters 0.22 μm and syringe filters 0.45 μm for the analysis were supplied by Millpores (Millipores Ltd. Bangalore). Formulations of RELICALM SF containing 2mg of Trihexyphenidyl and 50mg of Chlorpromazine and 5mg Trifluoperazine tablets were procured from local market.

Equipment

To develop a High Pressure Liquid Chromatographic method for simultaneous estimation of THP, CLP and TFP isocratic PEAK HPLC instrument with Inertsil ODS C18 column (250mmx4.6mm, 5μ) and Electronic balance-DENVER (SI 234) was used. The instrument is equipped with a LC 20AT pump for solvent delivery and variable wavelength programmable LC 7000UV-detector. A 20μLRheodyne inject port was used for injecting the samples. Data was analyzed by using PEAK software.

Preparation of standard solution

Standard stock solution of THP, CLP and TFP pure drug (1mg/ml) was prepared by accurately weighing about 100 mg of each drug in 100 ml volumetric flask separately and dissolved with 25 ml of methanol and sonicated to dissolve them completely and made up to the mark with the same solvent. The contents were filtered through Nylon membrane sample filter paper. Required concentrations of THP, CLP and TFP were prepared separately using standard dilution method.

Procedure for pharmaceutical formulation

Sample solution was prepared by a composite of 20 THP, CLP and TFP combination tablets (RELICALM SF: 2mg of THP and 50 mg of CLP and 5 mg TFP) ,which were grinded to a fine, uniform size powder. An amount of drug equivalent to 100mg of CLP was accurately weighed and quantitatively transferred into 100 ml volumetric flasks. Approximately 30ml mobile phase was added and the solution was sonicated for 15 min. The flask was made up to volume with mobile phase and mixed well. Then the solution is filtered through 0.45 μm nylon 6, 6 membrane filter paper. Then an amount of the solution was diluted with mobile phase to a concentration of 50 μg/ml of CLP. Then based on the label claim of the both the drugs in the formulation, a concentration of 2 μg/ml of THP and 5 μg/ml of TFP solution were obtained.

Method development and Validation

Various Chromatographic conditions have been optimized in order for separation and identification of THP, CLP and TFP. The developed method was validated in terms of System Suitability, Specificity, Linearity and range, precision, accuracy, limit of detection, limit of quantification, solution stability and robustness as per USP and ICH guidelines [8-10].

Forced degradation studies

To perform the forced degradation study 50mg each drugs were subjected to acidic, alkaline, oxidizing, thermal and photolytic conditions. The drug was heated under reflux with 0.1 M HCl, 0.1N NaOH and 30% H_2O_2 (v/v) at 80^0C for 2 h separately in acidic, basic and peroxide degradation study. For thermal degradation, the powdered drug was exposed at 70^0C for 48 h. For photolytic degradation the powdered drug was exposed to sunlight for 48 h. After completion of the treatments the solutions were left to return to room temperature and diluted with solvent mixture to furnish standard solution concentration. The standard drug solutions after stress exposure were analyzed in the developed method and the % degradation was measured using unstressed standard results.

Formulation analysis

The sample solution prepared from marketed formulation brand RELICALM SF containing known concentration of THP, CLP and TFP was analyzed in the developed method. The % assay was calculated using standard calibration results of THP, CLP and TFP.

Results And Discussion

Method development

Several method development conditions were optimized for the simultaneous determination of THP, CLP and TFP. The optimized separation was achieved using mobile phase consisting of Methanol: Acetonitrile: Acetate buffer 80:15:05 v/v at pH 6.1 was found to be satisfactory. The drugs gave symmetric and sharp peaks with Inertsil ODS C18 column (250mm x 4.6mm, 5μm) at 4.0min, 5.9min and 7.7min for THP, CLP and TFP with good resolution, theoretical plates and acceptable tailing factor (figure 2), wavelength was set at 239nm, which provided better reproducibility with minimum interference.

Table.1: Chromatography conditions

Parameter	Results
MP	Methanol: Acetonitrile: Acetate Buffer 80:15:05 v/v
Wavelength	239nm
Stationary Phase	Inertsil ODS C18 column (250mm x 4.6mm, 5µm) column
pH of Mobile phase	6.1
Retention time THP CLP TFP	 4.0min 5.9min 7.7min
Flow Rate	1.0ml/min
Pump Mode	Isocratic
Pump Pressure	11.7±5MPa

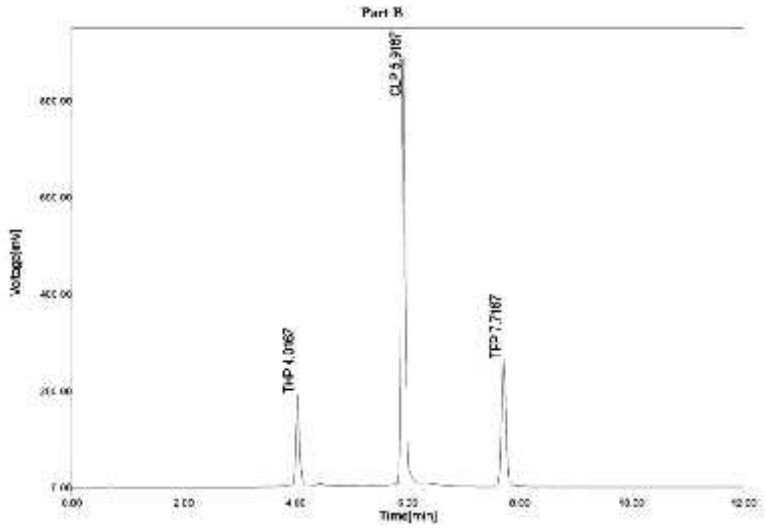

Figure.2: standard chromatogram of THP, CLP and TFP

System suitability

The system suitability was evaluated by calculating the %RSD values of peak area, retention time, asymmetry and theoretical plate's of six standard replicates of THP, CLP and TFP. The experimental results (Table 2) showed that the values were within the acceptable range for THP, CLP and TFP indicating that the system was suitable for the intended analysis

Table.2: System suitability test results

Parameter	THP	CLP	TFP
Api Concentration	2µg/ml	50µg/ml	5µg/ml
RT	4.0min	5.9min	7.7min
Resolution	….	7.53	11.65
Area	43658	263512	91352
Theoretical Plates	4265	5986	8138
Tailing Factor	0.71	1.42	1.25

Specificity

In specificity study, standard solutions of THP, CLP and TFP and formulation placebo were injected and in the results, peaks corresponding to THP, CLP and TFP were observed, which indicates that there was no interference from the excipients used also from the mobile phase. Thus specificity study ensures the selectivity of the developed analytical method which is able to separate and quantify THP, CLP and TFP in presence of different degradation products.

Linearity and range

The linearity of the developed method was determined at different concentrations ranging from 0.4-4µg/ml,10-100µg/ml and 1-10µg/ml THP, CLP

and TFP respectively. The regression analysis equation was y=21861x+653.4 and correlation coefficient (r^2) was 0.999 for THP y= 5219x+5698 and correlation coefficient (r^2) was 0.999 for CLP and y= 17488x+3970 and correlation coefficient (r^2) was 0.999 for TFP respectively, showing good linearity. The results confirmed the linearity of the standard curves over the range studied and the excellent reproducibility of the assay method. The results and linearity study were represented in Table3 and Figure 3.

Table 3: Linearity results

THP		CLP		TFP	
Concentration in µg/ml	Peak area	Concentration in µg/ml	Peak area	Concentration in µg/ml	Peak area
0.4	9982	10	65241	1	22354
0.8	19124	20	112547	2	41539
1.2	27135	30	162584	3	57248
1.6	36253	40	214851	4	75196
2	43658	50	263512	5	91352
2.4	52139	60	312054	6	109853
2.8	60982	70	372853	7	125478
3.2	69586	80	421582	8	142989
3.6	79521	90	482571	9	159826
4	89754	100	525355	10	179658

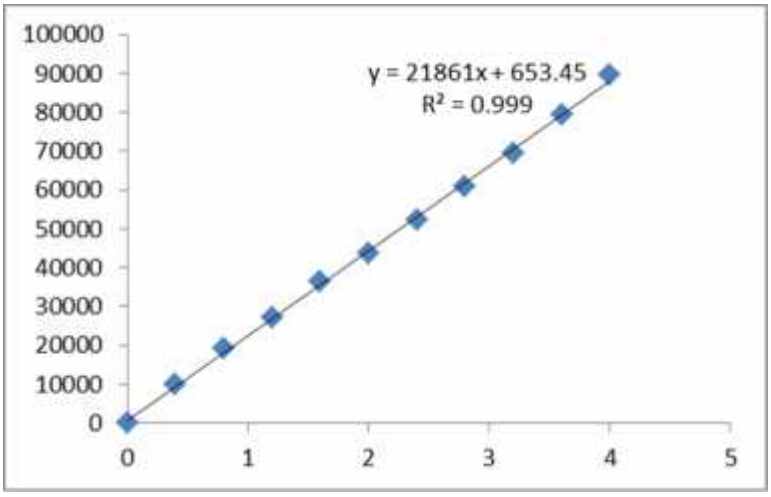

Fig.3.Calibration graph of linearity of THP

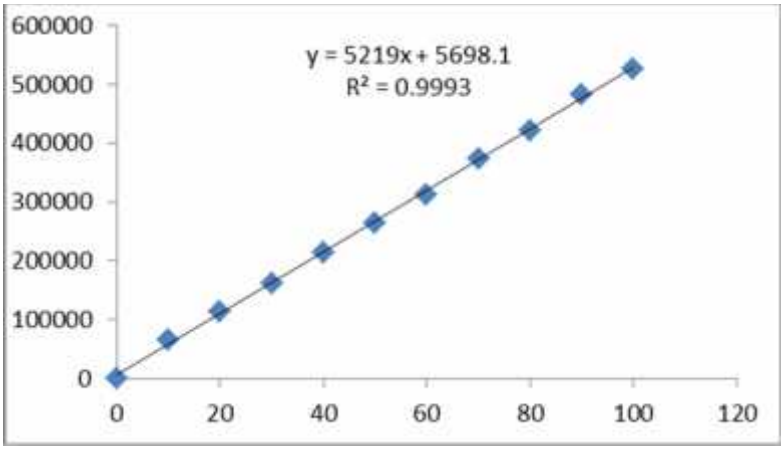

Fig.4.Calibration graph of linearity of CLP

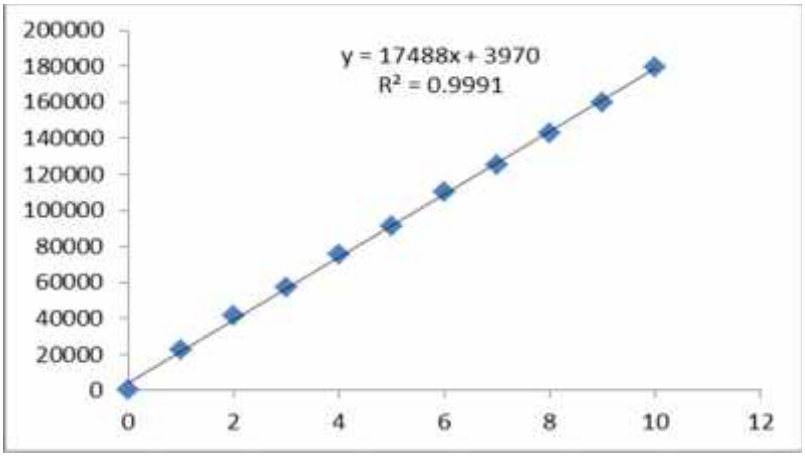

Fig.5.Calibration graph of linearity of TFP

Table 4: Summary of Validation Results

S. No	Parameter	Results
1	Linearity	THP : 0.4-4μg/ml CLP : 10-100μg/ml TFP : 1–10μg/ml
2	Intraday precision in % RSD	THP : 0.775 CLP : 0.738 TFP : 0.527
3	Interday precision in % RSD	THP : 0.786 CLP : 0.780 TFP : 0.536
4	Ruggedness in % RSD	THP : 0.951 CLP : 0.976 TFP : 0.667
5	Recovery in % assay	THP : 98.83-99.90 CLP : 98.14-99.94 TFP : 98.22-99.98
6	LOQ	THP : 0.033μg/ml CLP : 0.33μg/ml TFP : 0.004μg/ml
7	LOD	THP : 0.001μg/ml CLP : 0.01μg/ml TFP : 0.004μg/ml
8	Formulation assay (In %)	THP : 98.63 CLP : 98.53 TFP : 98.44

Method precision and intermediate precision

Precision studies were carried out by repeating the analysis of the sample six times and the results show that the mean assay value and %RSD to be 0.775, 0.738 and 0.527 for intraday precision and 0.786,0.780, 0.536 for interday precision for THP and CLP and TFP respectively.

Accuracy

Accuracy of the method was studied by applying the developed method to prepared synthetic mixtures of formulation excipients to which known amounts of THP, CLP and TFP were added. Mean recovery (Table 4) for THP was between 98.43-99.90%, 98.14-99.94 for CLP and 98.22-99.98% for TFP indicating that the developed method was accurate for the determination of THP, CLP and TFP in Pharmaceutical formulation.

LOD and LOQ

LOD and LOQ values were found to be 0.033μg/ml, 0.001μg/ml for THP, 0.33μg/ml, 0.01μg/ml for CLP and 0.013μg/ml, 0.004μg/ml for TFP respectively. This confirms that the method developed for the analysis of THP, CLP and TFP was found to be very sensitive.

Robustness

The change in mobile phase ratio, pH of mobile phase and detector wavelength was studied for the method developed for the analysis of THP, CLP and TFP. The % change in the peak area ratio of THP, CLP and TFP was studied in all the changed condition. The results confirm that they were not

affected by varying the conditions and were in accordance with the results for original conditions.

Solution stability

The solution stability of the standard and the test sample solution was checked by analyzing both the solutions at interval of 12 h till 24 h at room temperature. The results showed that both the retention time and area of THP, CLP and TFP were unchanged and no significant degradation was observed within the indicated period which was sufficient for performing analytical process.

Stress degradation study

The proposed validated Liquid Chromatographic method was successfully applied to study the stress degradation property of THP, CLP and TFP. The results of forced degradation studies were given in table 5 and figure D. In acidic, UV and Peroxide degradation study, the chromatogram shows three degradation products which confirms that three degradation products were observed along with THP, CLP and TFP. In Alkali, Sun Light and Thermal degradation study, the chromatogram shows two degradation products which confirms that two degradation products were observed along with THP, CLP and TFP. In all the stress degradation studies, clear separation of degradation compounds formed and standard THP, CLP and TFP was achieved. The % degradation of THP, CLP and TFP was found to be very less in all the stress conditions studied. Hence the proposed method could successfully separate the drugs THP, CLP, TFP and degradation products and the % degradation was found to be very less and hence the developed method was found to be stability indicating.

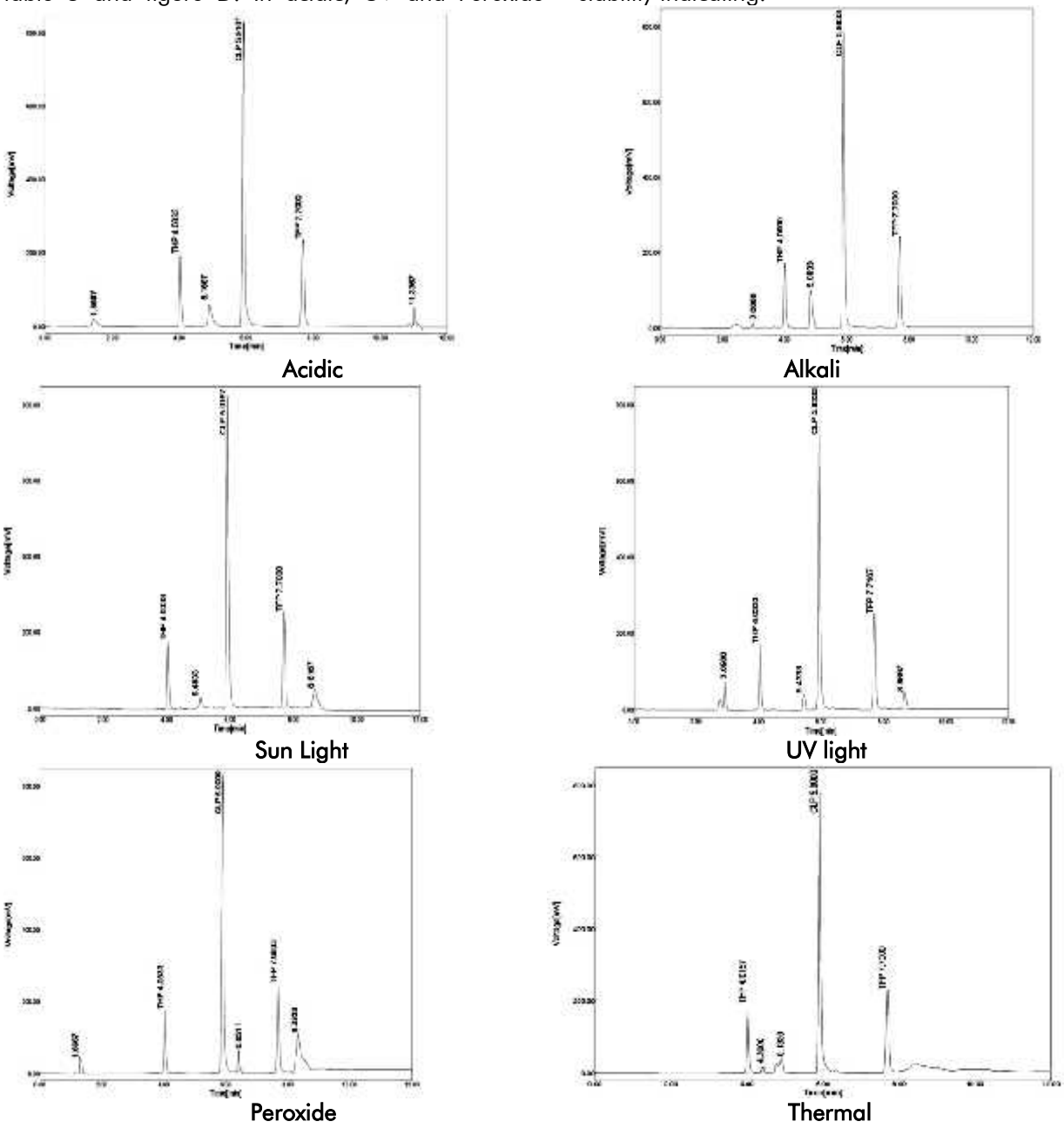

Figure.4: Forced degradation chromatograms of THP, CLP and TFP

Table .5: Forced Degradation results

Stress Condition	Trihexyphenidyl			Chlorpromazine			Trifluoperazine		
	Area	Assay	Degraded	Area	Assay	Degraded	Area	Assay	Degraded
Acidic	39506.8	90.4915	9.50845	250698	95.13703361	4.86297	88391.7	96.7595	3.24054
Base	40327.5	92.3714	7.62861	253917	96.3587237	3.64128	89743.9	98.2397	1.76033
Light	42513.1	97.3776	2.62243	254903	96.73290021	3.2671	89040.6	97.4698	2.53021
Peroxide	38589.7	88.3909	11.6091	251176	95.31869516	4.6813	88747.1	97.1485	2.8515
Thermal	42191.2	96.6402	3.35975	254313	96.50922918	3.49077	87013.7	95.251	4.74899
UV	40983.5	93.874	6.12603	237962	90.30400893	9.69599	88246.9	96.601	3.39905

Conclusion

A novel , simple, precise and stability indicating RP-HPLC method has been developed for the simultaneous estimation and stability studies of THP,CLP and TFP in combined formulation dosage forms. The isocratic method was found to be simple, specific, sensitive and robust on validation parameters. All results of method validation were found within the limit. The method separated drugs with high resolution and also from its depredated products. The method is stability indicating, can be continently used for the routine quality control analysis of THP, CLP and TFP in industries for batch studies.

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