International Journal of Botany Studies
www.botanyjournals.com
ISSN: 2455-541X
Received: 25-07-2021, Accepted: 08-08-2021, Published: 25-08-2021
Volume 6, Issue 4, 2021, Page No. 914-921

Development and validation of a forced degradation UPLC method for the simultaneous determination of Trifluoperazine HCl and Trihexyphenidyl HCL in bulk and pharmaceutical dosage form

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Abstract
An excellent method with simple, precise was developed for Trifluoperazine HCl and Trihexyphenidyl HCl by using Forced degradation UPLC method. The column used was C_{18} BEH _ 1.7 µm x 2.1 x 50 mm in ambient temperature. Flow rate was 0.8 ml/min, wavelength of 210 nm, mobile phase used was acetonitrile: water (60:40), Run time 4 min. The percentage purity and RT of Trifluoperazine HCl and Trihexyphenidyl HCl were found to be 99.86 & 99.90% and 1.4 &1.8 min respectively. The validation parameters was carried out, Linearity of Trihexyphenidyl HCl and Trifluoperazine HCl were found to be (5µg/ml to 40µg/ml) (2µg/ml to 16µg/ml) with the correlation coefficient of 0.998 and 0.995 respectively. Intermediate precision, Robustness, LOD LOQ was within the limit as per ICH guidelines. In accuracy the percentage recovery of Trihexyphenidyl HCl and Trifluoperazine HCl in bulk drugs samples was 99.45 to 99.97% and 99.45 to 99.86%respectively which indicates that the method was accurate. Forced Degradation was carried out in three conditions acidic, basic and peroxide condition, degradation takes at basic and peroxide. As per literature review there is no method developed for Trihexyphenidyl HCl and Trifluoperazine HCl in Forced degradation UPLC method.

Keywords: UPLC, trihexyphenidyl HCl, trifluoperazine HCl, ICH guide lines, forced degradation

Introduction
Trifluoperazine HCl is 10-[3-(4-methylpiperazin-1-yl) propyl]-2-(trifluoromethyl)-10H-phenothiazine.It is a Phenothiazine antipsychotics, Trifluoperazine blocks postsynaptic mesolimbic dopaminergic D1 and D2 receptors in the brain. Trihexyphenidyl HCl is 1-cyclohexyl-1-phenyl-3-(piperidin-1-yl) propan-1-ol. Muscarinic antagonists used for treatment of parkinsonian. Forced degradation Studies of drugs and materials play an integral role in production of pharmaceutical products. The results of degradation studies promotes development of Stability Indicating System (SIS), the design of formulations, the choice of storage and packaging conditions, the understanding of the chemistry of the drug molecule, and the problem solving of stability.Ultra Performance Liquid Chromatography (UPLC) system is an innovative product that brought revolution in high performance liquid chromatography by outperforming conventional high performance liquid chromatography (HPLC). UPLC decreases sample run times up to a factor of 10, uses up to 95 percent less solvent and significantly improves productivity in the laboratory.

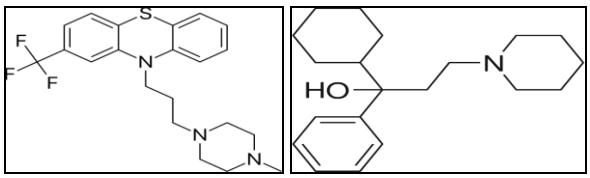

Fig 1: Structure of Trifluoperazine Structure of Trihexyphenidyl

Materials and Method
HPLC Water- Milli-Q grade, Acetonitrile- Fisher scientific, Methanol- Fisher scientific, Hydrochloric acid- Merck. These solvents were throughout method development and Validation. Trifluoperazine and Trihexyphenidyl are gift sample from Orchid Pharmaceuticals, Chennai.

Instrumentations
The UPLC method development and validation was done using Waters Acquity UPLC BEH Column. The dissolution apparatus Distek, UV-Visible spectrophotometer-Perkinelimer, Analytical balance-Sartorius.

ASSAY
Preparation of standard Stock solution:
Accurately weighed amount of Trihexyphenidyl HCl, 25 mg and Trifluoperazine HCl, 20 mg transferred into a clean, dry 50 ml volumetric flask with 20 ml of Acetonitrile: Water (40:60) mixture and sonicated for 5min. Further, the standard stock solution was made upto volume (50ml), using Acetonitrile: Water solution, to the final concentration of Trihexyphenidyl HCl and Trifluoperazine HCl of 100 µg/ml and 30 µg/ml, respectively. The prepared stock solutions were filtered through 0.45 µm membrane filter for further studies.

Preparation of sample solution
Ten tablets were accurately weighed and average weights of each tablet were calculated. Weighed tablets were then triturated in a mortar into a fine powder and accurately weighed amount of powder, equivalent to 25 mg and 20 mg of Trihexyphenidyl HCl and Trifluoperazine HCl, resp. was transferred into a clean, dry 50 ml volumetric flask with 50 ml of Acetonitrile: water mixture and sonicated for 5min. Further, the standard stock solution was made upto volume (100 ml), using Acetonitrile: water, to the final

concentration of Trihexyphenidyl HCl and Trifluoperazine HCl of 100 µg/ml and 30 µg/ml, respectively. The prepared stock solutions were filtered through 0.45 µm membrane filter for further studies.

Chromatographic condition
In the present study column used C_{18} BEH - 1.7 µm x 2.1 x 50 mm in ambient temperature. Flow rate was 0.8 ml/min, wavelength of 210 nm, mobile phase used was Acetonitrile water (40:60). Run time 4 min. The percentage purity of Trihexyphenidyl Hcl and Trifluoperazine HCl were found to be 99.90 and 99.86%.

Validation Parameter
- System Suitability
- Specificity
- Linearity and Range
- Precision
- Ruggedness
- Accuracy
- Robustness
- Limit of Detection and Limit of Quantitation
- Solution stability

System suitability
In this study, the system suitability was evaluated by injecting 5 replicate injections of the prepared Standard stock solution of Trihexyphenidyl HCl (100 µg/ml) and Trifluoperazine HCl (30 µg/ml). It was performed to determine the resolution, theoretical plates, tailing factor, repeatability of retention time etc. All parameters are within the range as per the ICH guidelines.

Specificity
Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. It was performed to identify the any impurity may present, it was done by using standard, sample, placebo dilutions of both Trihexyphenidyl HCl and Trifluoperazine HCl.

Calibration Curve (Linearity)
From the prepared Standard stock solution, 1 ml,2 ml,3 ml,4 ml,5 ml, 6 ml, 7 ml, 8 ml for Trihexyphenidyl HCl and 1 ml,2 ml,3 ml,4 ml,5 ml, 6 ml, 7 ml, 8 ml, for Trifluoperazine HCl, were pipetted out into 10 ml volumetric flask each and diluted with Acetonitrile: water mixture solution to obtain the final concentration of Trihexyphenidyl HCl and Trifluoperazine HCl, ranging from 8 µg/ml to 64 µg/ml of Trihexyphenidyl HCl and 20 µg/ml to 160 µg/ml of Trifluoperazine HCl respectively.

Precision
Intermediate precision was carried in both Trihexyphenidyl HCl, 25 mg and Trifluoperazine HCl, 20 mg transferred into a clean, dry 50 ml volumetric flask with 20 ml of Acetonitrile: Water (40:60) mixture and sonicated for 5min. Further, the standard stock solution was made upto volume (50ml), using Acetonitrile: Water solution, to the final concentration of Trihexyphenidyl HCl and Trifluoperazine HCl of 100 µg/ml and 30 µg/ml, respectively. With the same solution precision was carried out next day also. % RSD was with in the limit as per ICH guidelines.

Accuracy
Trihexyphenidyl HCl and Trifluoperazine HCl accuracy was study done in 50%, 100% and 150%.

Robustness
Robustness study was done by changing the wavelength, flow rate in both the drug. % RSD was with in the limit as per ICH guidelines. Tab

LOD and LOQ
Limit of detection was carried out in Trihexypheniyl HCl and Trifluoperazine, the value were found to be 4.18and 3.43respectively. Limit of quantification was carried out in Trihexypheniyl HCl and Trifluoperazine HCl, the value were found to be 11.97 and 10.43 respectively.

Forced degradation study
Acid Degradation Study (2N HCL)
To 5ml of the prepared standard stock solution of Trihexyphenidyl HCl (100 µg/ml) and Trifluoperazine HCl (30 µg/ml), 1 ml of 2N HCl was added and refluxed for 30 min at 75 °C. From the above solution10 µl was injected into the system and the chromatograms were recorded to detect the stability of the sample.

Base Degradation Study (2N NaOH)
To 5ml of the prepared standard stock solution of Trihexyphenidyl HCl (100 µg/ml) and Trifluoperazine HCl (30 µg/ml), 1 ml of 2N NaOH was added and refluxed for 30 min at 75 °C. From the above solution10 µl was injected into the system and the chromatograms were recorded to detect the stability of the sample.

Peroxide Degradation Study (2N H2O2)
Oxidative degradation study was performed using 5ml of the prepared standard stock solution of Trihexyphenidyl HCl (100 µg/ml) and Trifluoperazine HCl (30 µg/ml) mixed with 1 ml of H2O2 (20% w/v) was added and refluxed for 30 min at 75 °C. From the above solution10 µl was injected into the system and the chromatograms were recorded to detect the stability of the sample.

Photo degradation study
Photo degradation study was carried out by exposing the powdered sample, of Trihexyphenidyl HCl and Trifluoperazine HCl combination spreaded in a petri-plate, to 1.2 million lx and 200 Watts/h/m^2 per meter square of UV luminous light.

Thermal degradation study
Thermal degradation study was performed with powdered sample of Trihexyphenidyl HCl and Trifluoperazine HCl combination spreaded in a petri-plate, exposed to hot air at 100° C for 12 hours in oven.

Table 1: Assay of Trihexyphenidyl HCl and Trifluoperazine HCl

Commercial Formulation	Drug	Standard area	Sample area	Label Claim (mg)	Amount Present (mg)	% Purity
Trazine S	Trihexyphenidyl HCl	543911	547922	2 mg	1.95mg	99.90
	Trifluoperazine HCl	1482033	1483321	5 mg	4.97mg	99.86

The percentage purity of Trihexyphenidyl Hcl and Trifluoperazine HCl were found to be 99.90 and 99.86%.

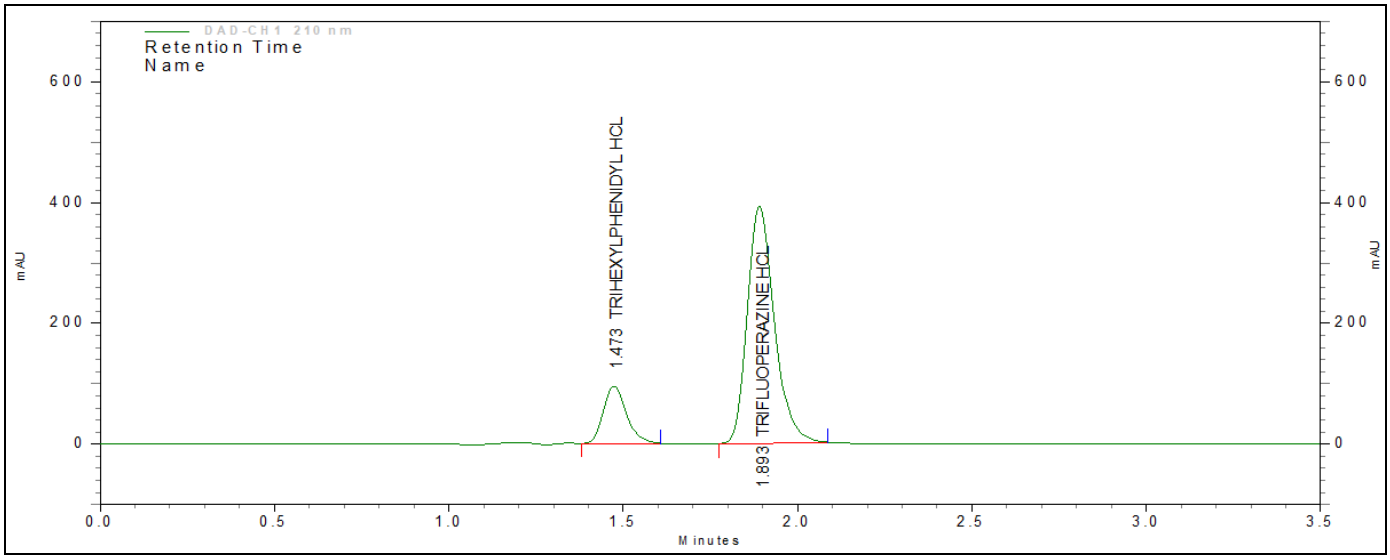

Fig 2: Chromtagram Trihexyphenidyl HCl and Trifluoperazine HCl

The Rt of Trihexyphenidyl HCl and Trifluoperazine HCl was found to be 1.473 and 1.893 respectively.

Table 2: Linearity of Trihexyphenidyl HCl and Trifluoperazine HCl

S. No	Trihexyphenidyl HCl		Trifluoperazine HCl	
	Conc (µg/ml)	Mean area	Conc (µg/ml)	Mean area
1	8	253791	20	1264423
2	16	616834	40	3068382
3	24	1016611	60	5088895
4	32	1386391	80	6807659
5	40	1802003	100	8879392
6	48	2190574	120	10772716
7	56	2574863	140	12440180
8	64	2946179	160	14276497

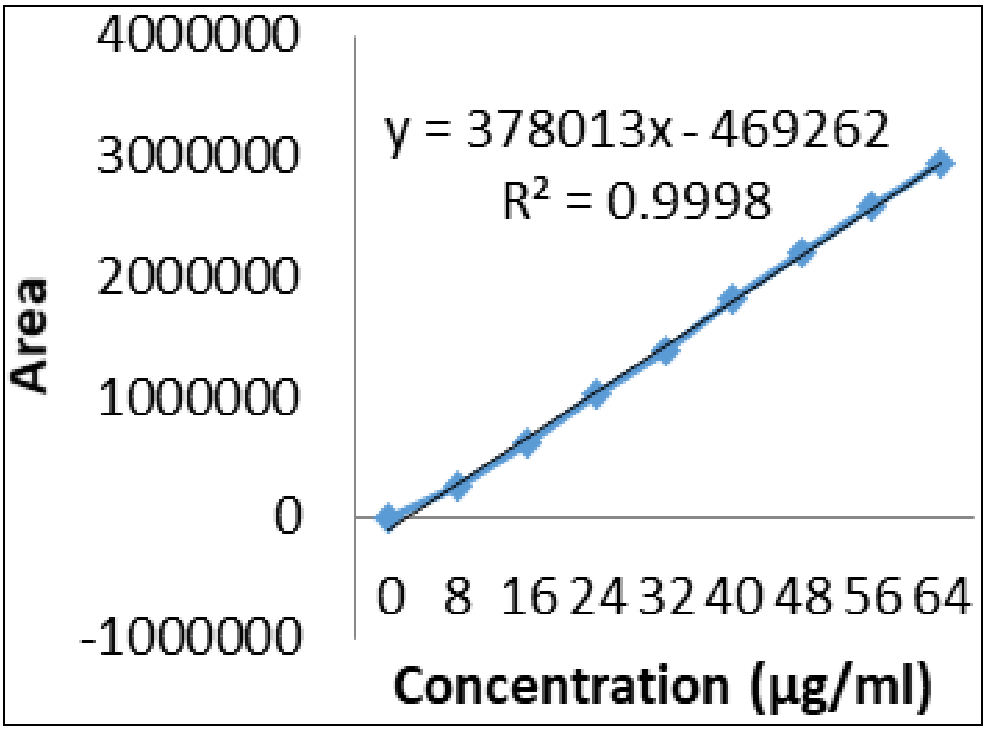

Fig 3: Linearity of Trihexyphenidyl HCl

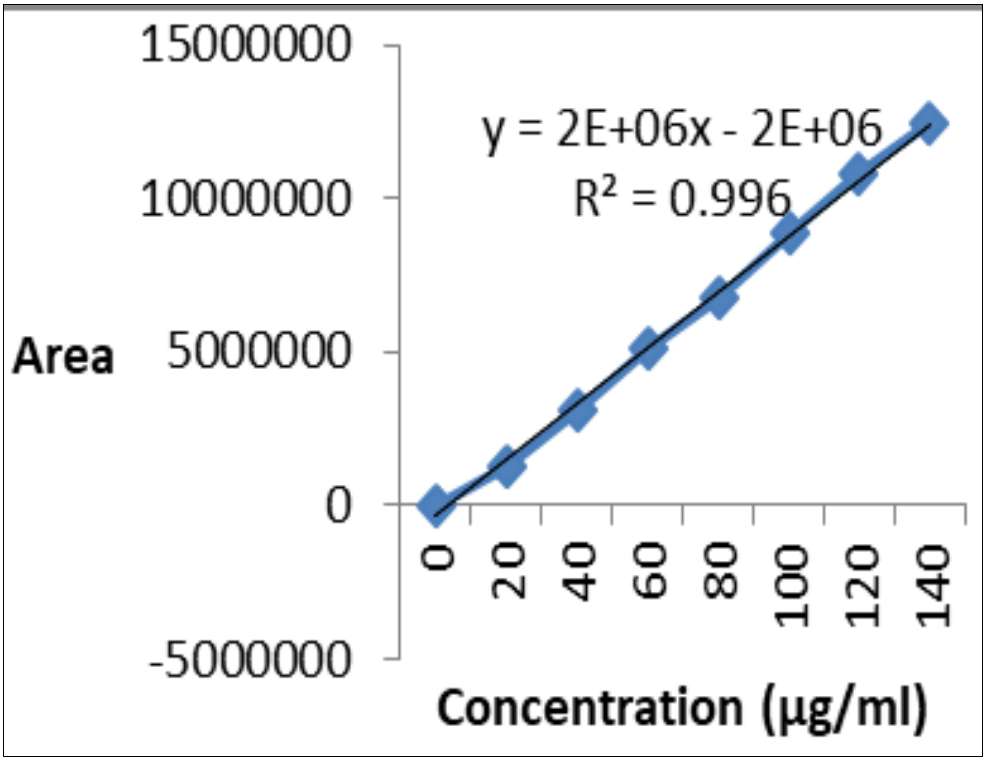

Fig 4: Linearity of Trifluoperazine HCl

Table 3: Intermediate precision of Trihexyphenidyl HCl and Trifluoperazine HCl

Analyst 1	Trihexyphenidyl HCl		Trifluoperazine HCl	
	Area	%Assay	Area	%Assay
1	8826017	99.86	1838525	99.70
2	8805130	99.78	1834384	99.65
3	8781363	99.84	1838113	100.2
4	8809344	99.87	1793737	99.89
5	8812989	99.86	1831749	99.74
6	8824499	99.96	1834915	100.4
Mean	8823467	99.83	1832112	99.99
%RSD	0.15		0.37	

Table 4: Intermediate precision of Trihexyphenidyl HCl and Trifluoperazine HCl

Analyst 2	Trihexyphenidyl HCl		Trifluoperazine HCl	
	Area	%Assay	Area	%Assay
1	8853552	99.60	1864161	98.90
2	8855016	100.00	1864915	99.10
3	8856980	98.10	1868265	100.10
4	8856580	99.10	1867265	99.60
5	8856415	98.04	1869565	100.50
6	8857382	99.01	1869460	100.10
Mean	8856759	99.70	1866375	99.60
%RSD	0.18		0.35	

Table 5: Accuracy of Trihexyphenidyl HCl and Trifluoperazine HCl

% Conc	Trihexyphenidyl HCl			Trifluoperazine HCl		
	Area		%Assay	Area		%Assay
	Standard	Sample		Standard	Sample	
50%	9038369	7304059	99.45	180718	1486275	99.74
100%	9038369	9052857	99.79	180718	1836113	99.88
150%	9038369	10777858	99.97	180718	2179193	99.86

Table 6: Robustness of Trihexyphenidyl HCl and Trifluoperazine HCl

S. no	Trihexyphenidyl HCl				Trifluoperazine HCl			
	Std area	Sample area	Assay	% RSD	Std area	Sample area	Assay	% RSD
Flow rate (0.6 ml)	10862221	10885863	99.91	0.16	2221621	2209843	99.58	0.23
Flow rate (1.0 ml)	76156267	7613126	99.60	0.31	1525376	1522975	99.92	0.67

Wavelength 208nm	10188601	10155910	99.71	0.28	201307	1994192	99.66	0.73
Wavelength 212nm	8834324	8854378	99.70	0.45	1845253	1843567	99.86	0.67

Table 7: Forced degradation of Trihexyphenidyl HCl and Trifluoperazine HCl

S. No	Condition	Trihexyphenidyl HCl			Trifluoperazine HCl		
		%Assay control	%Assay of degraded Sample	% Net degraded	%Assay control	%Assay of degraded Sample	% Net degraded
1.	Acid Degradation	99.8	96.5	3.3	99.9	97.5	2.4
2.	Base Degradation	99.8	114.2	14.4	99.9	117.5	17.6
3.	Oxidative Degradation	99.8	101.6	1.8	99.9	103.4	3.5
4.	Photolytic Degradation	99.8	99.4	0.4	99.9	104.6	4.7
5.	Thermal Degradation	99.8	101.9	2.1	99.9	104.6	4.7

Acceptance criteria: % Net degraded should be (NMT) not More than 20%

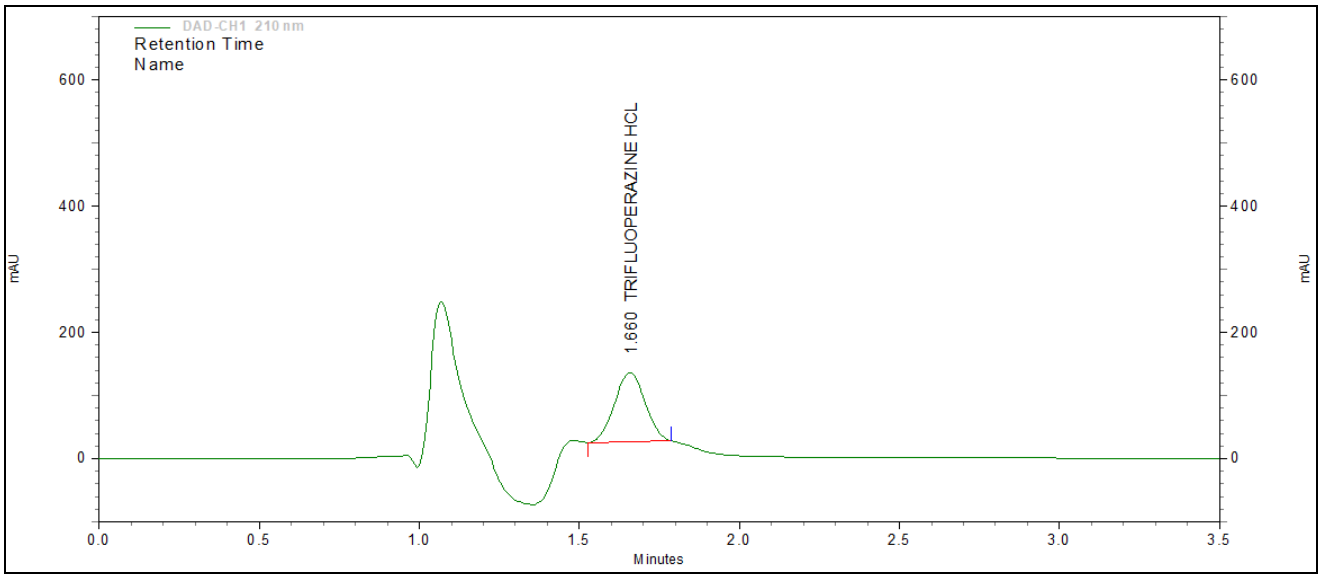

Fig 5: Acid Degradation chromatogram of Trihexyphenidyl HCl and Trifluoperazine HCl

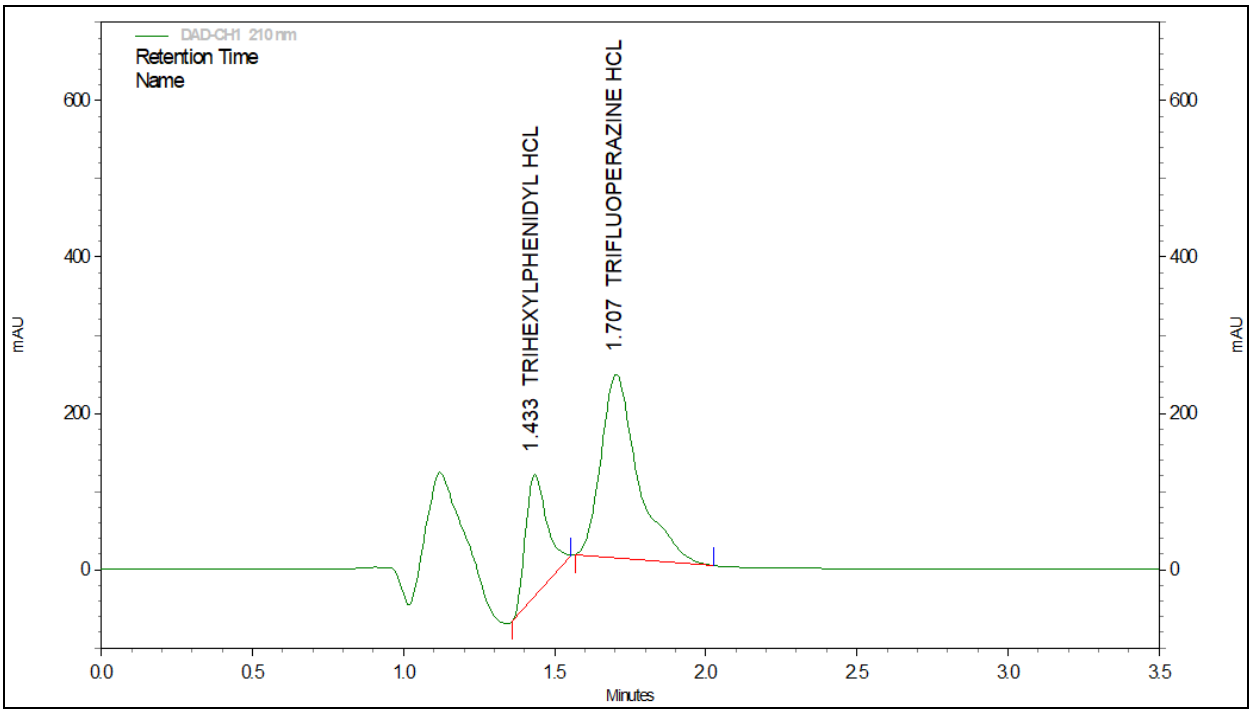

Fig 6: Base degradation Chromatogram of Trihexyphenidyl HCl and Trifluoperazine HCl

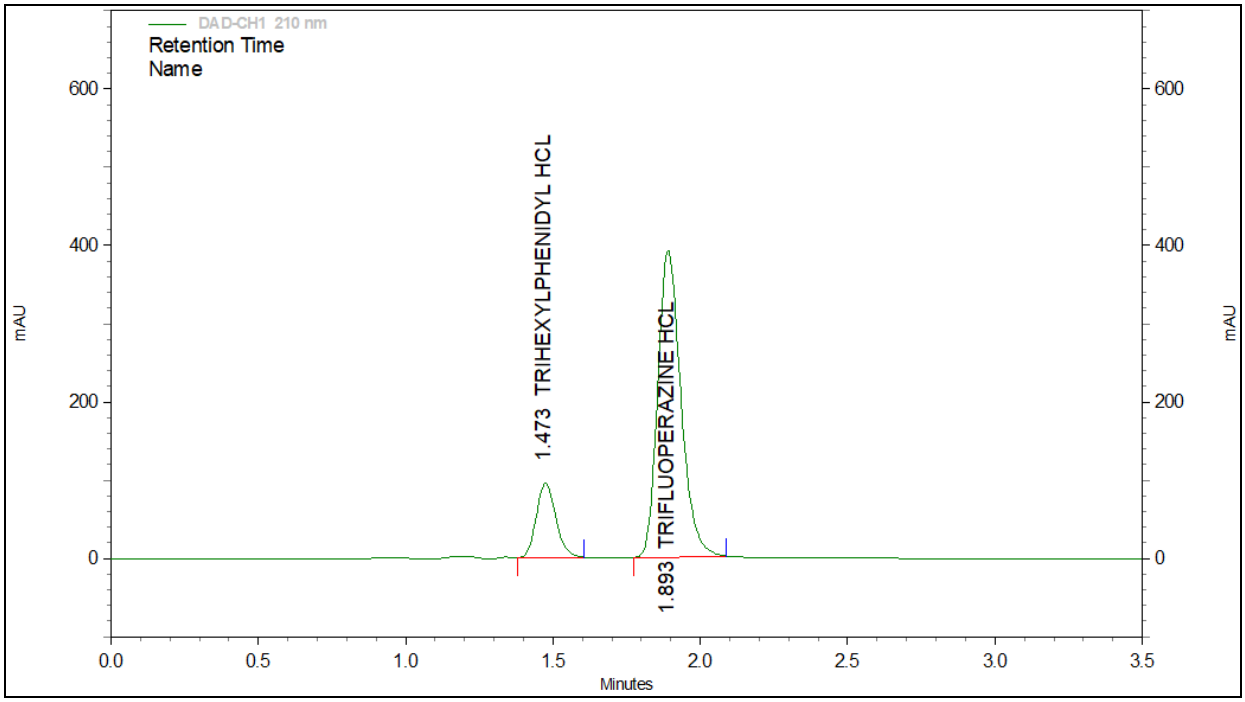

Fig 7: Oxidative Degradation chromatogram of Trihexyphenidyl HCl and Trifluoperazine HCl

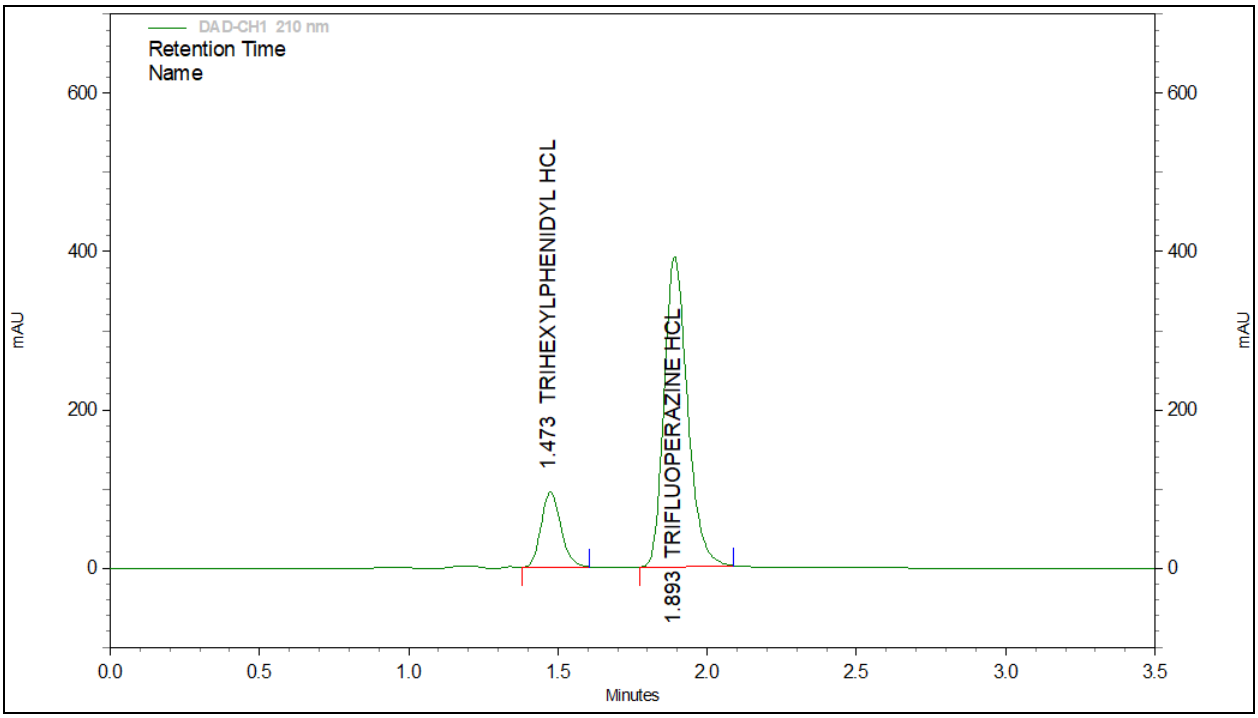

Fig 8: Photolytic Degradation chromatogram of Trihexyphenidyl HCl and Trifluoperazine HCl

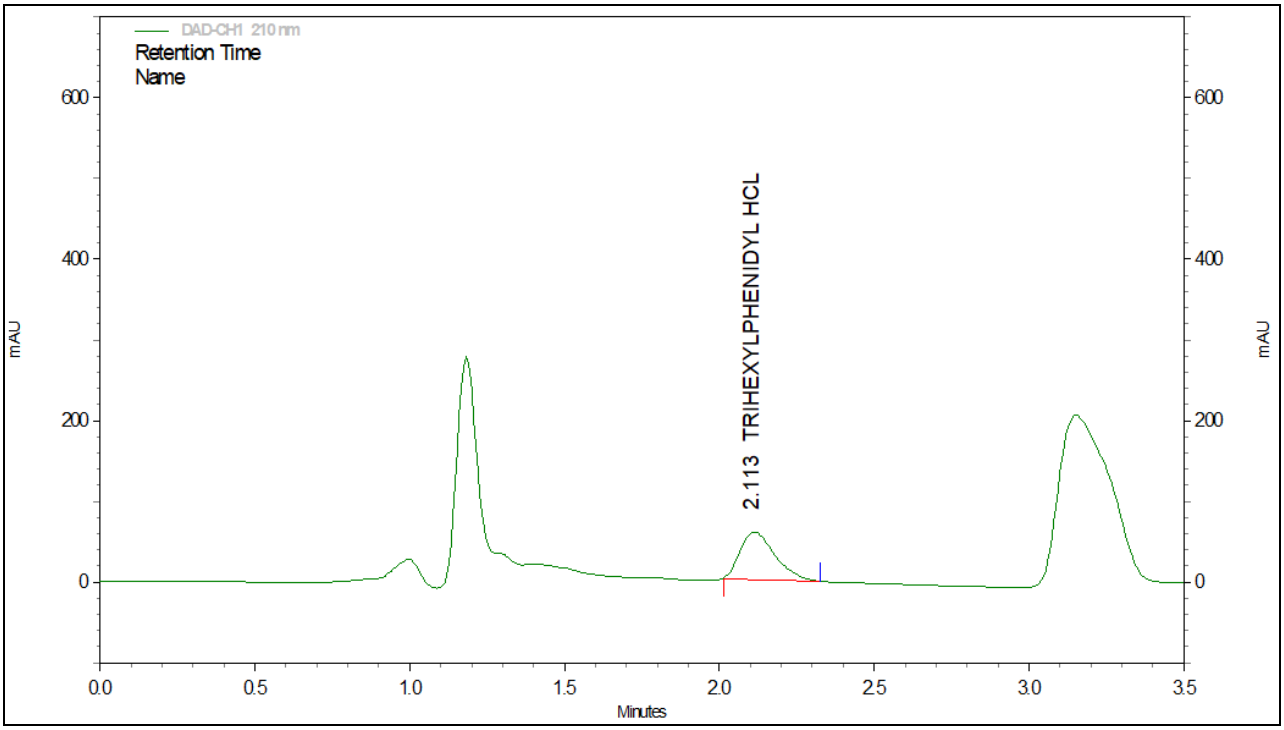

Fig 9: Thermal Degradation chromatogram of Trihexyphenidyl HCl and Trifluoperazine HCl

Conclusion

The present study was method development and validation of Trihexyphenidyl HCl and Trifluoperazine HCl by uplc method with forced degradation study. Since no method was developed in UPLC, RT of Trihexyphenidyl HCl and Trifluoperazine HCl was found to be 1.473 and 1.893 respectively. The Stress degradation of Trihexyphenidyl HCl and Trifluoperazine HCl was studied by exposing it to ICH defined degradation conditions. Degradation was studied under Acidic, Alkali, Oxidative, Photolytic and Thermal stress conditions. But there were no degradation taken place in any stressed conditions

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