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An Application Of Forced Degradation Studies Of Tipiracil With A Developed And Validated RP-HPLC Method As Per ICH Guidelines

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Abstract:

Tipiracil is a thymidine phosphorylase (TPase) inhibitor. Its function prevents the breakdown of the active component of trifluridine, thus increasing the bioavailability of trifluridine and boosting its systemic presence. The drug was subjected to forced degradation studies as per the conditions prescribed in ICH Q1 (R2) guideline. Tipiracil degraded in hydrolytic (acid and alkaline) and oxidative stress conditions. The drug was found to be stable in thermal and photolytic conditions. The novel degradation products were obtained and were well separated using an HPLC C18 stationary phase Cosmosil (250 × 4.6 mm, 5 μm) and mobile phase composed of Phosphate buffer (pH 3.0): Acetonitrile (30:70) pumped at 1.0 mL min−1 flow rate. The column temperature was set at 25°C and the detection at 257 nm using DAD detector. All the degradation products were isolated and characterised. A well resolved chromatographic method proposed in this study suggests that the proposed analytical method finds its application as a stability indicating assay method for the drug and can be used in routine analysis. The developed HPLC method will also suffice the suitability for impurity profiling and assay of Tipiracil in bulk drugs and pharmaceutical formulations.

Keywords: RP-HPLC, Tipiracil, ICH, Impurity, Forced Degradation Study.

INTRODUCTION:

The parent drug stability test guideline (Q1A) issued by International Conference on Harmonisation (ICH) requires that analytical test procedures for stability samples should be fully validated and the assays should be stability-indicating. Further, it is suggested that stress studies should be carried out to establish the inherent stability characteristics of the molecule such as the degradation pathways, leading to identification of degradation products and hence supporting the suitability of the proposed analytical procedures. [1-5,7]

The aims of the present study accordingly were to establish inherent stability of Tipiracil through stress studies under a variety of ICH recommended test conditions. Further, it is suggested that stress studies should be carried out to establish the inherent stability characteristics of the molecule such as the degradation pathways, leading to identification of degradation products and hence supporting the suitability of the proposed analytical procedures.

Tipiracil is a thymidine phosphorylase inhibitor. It is used in combination with Trifluridine, in a ratio of 1:0.5, to form TAS-102. The main function of Tipiracil in TAS-102 is to increase Trifluridine bioavailability by inhibiting its catabolism. TAS-102 is indicated for the treatment of metastatic colorectal cancer. Tipiracil prevents Trifluridine conversion into 5-trifluoromethyl-2, 4 (1H, 3H)-pyrimidinedione, which is an inactive major metabolite, by inhibiting the enzyme thymidine phosphorylase. Thus, Tipiracil is able to increase Trifluridine bioavailability. Tipiracil is a member of the class of pyrimidones that is uracil substituted by chloro and (2-iminopyrrolidin-1-yl)methyl groups at positions 5 and 6 respectively. Used (as the hydrochloride salt) in combination with Trifluridine, a nucleoside metabolic inhibitor, for treatment of advanced/relapsed colorectal cancer.

IUPAC nomenclature of Tipiracil is 5-Chloro-6-[(2-imino-1-pyrrolidinyl)methyl]-2,4(1*H*,3*H*)-pyrimidinedione.

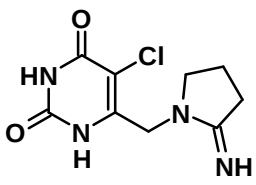


Fig-1: Chemical Structure of Tipiracil

MATERIALS AND METHODS:

Table-1: List of Instruments:

Sr. No.	Instruments	Make
1	HPLC	Waters e2695 separation module with PDA detector 2998
2	pH meter	Labindia
3	Analytical Weighing balance	Mettler Toledo
4	Digital ultra sonicator	Labman
5	UV – Vis Spectrophotometer	Schematzu

Table-2: List of Chemicals:

Sr. No.	Name of Chemicals	Manufacturer/Supplier
1	Tipiracil	Intas Pharmaceuticals Limited
2	Methanol (AR grade)	Merck
3	Hydrochloric Acid (AR grade)	Research Lab
4	Water (HPLC Grade)	Sd fine-Chem Ltd.
5	Acetonitrile (AR grade)	Merck
6	Sodium Hydroxide (AR grade)	Merck
7	Potassium Dihydrogen Phosphate (AR grade)	Thomas Baker

Preparation of mobile phase:
Accurately Weigh 6.8 g of Potassium dihydrogen ortho phosphate was transferred into 1000 ml volumetric flask. About 500 mL of double distilled water was added into the flask, dissolved the salt and finally water was added up to the mark. Then pH was adjusted to 3 by adding dilute sodium hydroxide solution. The mixture was sonicated for 10 minutes and then filtered through a 0.22 µm millipore filter. HPLC grade actonitrile was also filtered and degassed before use into the HPLC system.

RP-HPLC METHOD DEVELOPMENT: [6,8-18,19, 22-28]

Standard preparation:
10 mg tipiracil was weighed and transferred into 100 mL volumetric flask containing about 70 mL of mobile phase. The solution was sonicated for 15 min to dissolve the drug completely and the volume made up with mobile phase to get the concentration of tipiracil of 100 mg/mL solution. Further dilution was carried to get concentration of 20, 40, 60, 80, and 100 µg/mL of tipiracil.

Chromatographic conditions:
For quantitative analysis of tipiracil by RP-HPLC method, the mobile phase was comprised of Potassium dihydrogen ortho phosphate (pH 3) and acetonitrile in the ratio of 30:70 (v/v) at a flow rate of 1.0 mL/min. The injection volume was 10 µL for standard solutions. The run time was set for 07 min. Before analysis, every standard was filtered through 0.45 µm filter tips. The mobile phase was also filtered, sonicated and degassed before use. The column eluate was monitored with a PDA detector at 257 nm. All analyses were done at ambient temperature under isocratic condition.

Procedure:
Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

METHOD VALIDATION: [6,8-18, 22-28]

The methods were validated for different parameters like linearity, accuracy, precision, robustness, LOD, LOQ, etc.

Linearity:
The linearity of the developed method was performed with a concentration range of 20, 40, 60, 80 and 100 µg/mL by injecting repeated thrice times. The average peak areas were plotted against respective concentration. The linearity of the proposed method was evaluated by using calibration curves to calculate coefficient of correlation and intercept values.

Accuracy:
The accuracy of the method was evaluated by determination of recovery of tipiracil at three levels of concentrations at three times. The standard solutions were corresponding to 80%, 100% and 120% of nominal analytical concentrations.

Precision:
The precision of the method was demonstrated by intra-day and repeatability variation studies. In intra-day precision was established by analyzing three replicates over six concentrations (10 µg/mL) of tipiracil. Inter-day precision was carried out by six concentrations with three replicates for consecutive 3 days. The precision was expressed as %RSD amongst responses using the formula [%RSD = (standard deviation/mean) x 100 %].

Robustness:
Robustness of the proposed method was determined by small deliberate changes in flow rate (0.8, 1, 1.2 mL/min), change in organic composition of mobile phase ratio (±2%).

LOD and LOQ:
The limit of detection (LOD) and limit of quantification (LOQ) of the developed method were determined by injecting progressively low concentrations of the standard solutions for six times. LOD was calculated as 3.3*σ/S and LOQ was calculated as 10*σ/S.

System suitability:
A standard solution of tipiracil was prepared as per procedure and was injected six times into the HPLC system. The system suitability parameters were evaluated from standard chromatograms obtained by calculating the %RSD of retention times, tailing factor, theoretical plates and peak area from six replicate injections.

FORCED DEGRADATION STUDY OF TIPIRACIL: [5,7]

Forced degradation of the drug was carried out as per the ICH guideline. The forced degradation study of tipiracil was performed in acidic, alkaline and oxidant media, under UV radiation and thermal conditions.

Acidic degradation:
A 50 mL volumetric flask containing 50 mg of Tipiracil and 10 ml of 0.01M HCl was used to conduct the experiment. The mixture was neutralized with 10 ml of 0.01M NaOH after being held at room temperature for 30 minutes and the chromatogram was recorded.

Alkaline degradation:
In a 50 mL volumetric flask, 10 ml of 0.01M NaOH was added to 50 mg of Tipiracil. After being kept for half an hour, the mixture was neutralized with 10 mL of 0.01M HCl, and the chromatogram was taken.

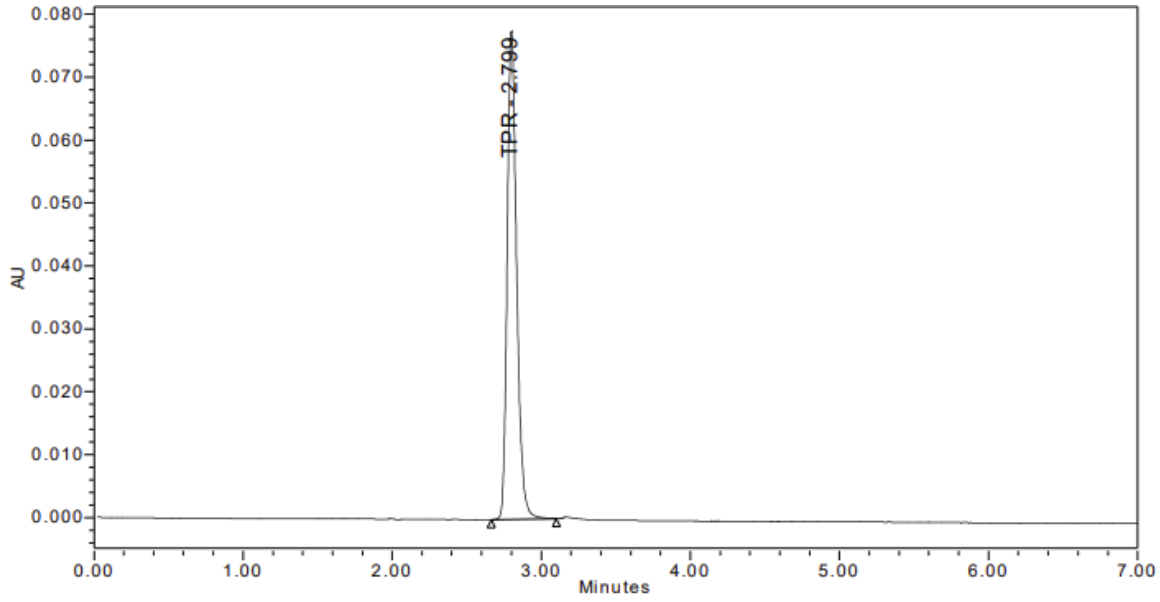
Oxidative degradation:
By adding 5 mL of 1% H_2O_2 to 50 mg of Tipiracil in a 50 mL volumetric flask, oxidative degradation was carried out. The chromatogram was then recorded while the mixture was maintained at ambient temperature for an hour.

Photolytic degradation:
Tipiracil was photolytically degraded by being exposed to 1.2M Lux hours while the chromatogram was recorded.

Thermal degradation:
Placed sufficient amount of tipiracil in petri dish and covered with aluminium foil and made holes on aluminium foil with pointed object. Kept it in hot air oven at 60º C for 72 hrs. After 72 hours sample was taken out and kept in desiccator to reach at room temperature subjected tipiracil prepared as per sample preparation method.

RESULT AND DISCUSSION:

Optimization of Mobile Phase:
The goal of method development was to produce sharp peaks for the active pharmaceutical ingredient with a resolution of more than two and a less asymmetric factor. Various mobile phases (water, methanol, acetonitrile, buffers of various pH ranges) were attempted in order to generate a distinct and well-defined drug peak. So the mobile phase ratio of Phosphate buffer (pH 3.0): Acetonitrile (30:70) which showed good chromatography and this ratio was finalized for the entire study.



	Peak Name	RT	Area	% Area	Height	USP Tailing	Symmetry Factor	K Prime	USP Plate Count
1	TPR	2.799	342343	100.00	77559	1.270582	1.270582	26.993115	9625.784782

Fig 2: Optimized chromatogram of tipiracil comprising of Phosphate buffer (pH 3.0): Acetonitrile (30:70) as a mobile phase

System suitability:
For the system suitability % RSD for the area of 6 replicates of standard solution was found to be NMT 2.0, theoretical plates were found to be NLT 2000 and Asymmetry was found to be NMT 2.0. Hence the system suitability passes all the criteria.

Table-3: System suitability of tipiracil

System Suitability							
Replicates	**Area**	**USP Tailing**	**USP Plates**	**RT**	**Mean**	**SD**	**%RSD**
Replicate 1	330190	1.27	9795	2.8	331599	2288.13	0.69
Replicate 2	332071	1.27	9751	2.8			
Replicate 3	328643	1.27	9790	2.8			
Replicate 4	331151	1.27	9780	2.8			
Replicate 5	335444	1.27	9758	2.8			
Replicate 6	332096	1.27	9749	2.8			

Linearity:
The calibration curves were obtained by plotting the peak area versus concentration over the range of 20 – 100 µg/mL with a correlation coefficient of 0.998.

Table-4: Linearity of tipiracil

Linearity				
% Level	**Area**	**RT**	**USP Plates**	**Mean**
20	342343	2.79	9625	931960
40	671229	2.8	9509	
60	951538	2.8	9554	
80	1205224	2.8	9629	
100	1489468	2.8	9583	

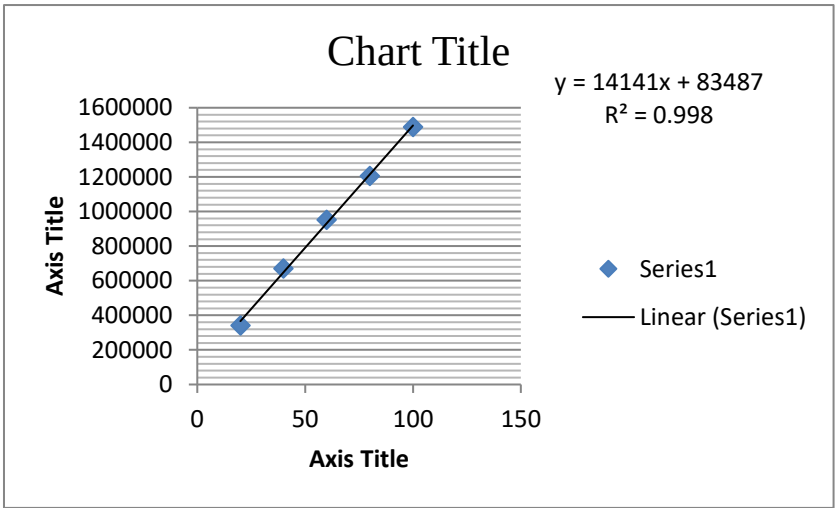


Fig-3: Calibration curve of tipiracil

Accuracy:
Accuracy at each levels 80 %, 100 % and 120 % was prepared in triplicate and recovered concentration verses the added concentration was determined and % recovery was found within the acceptable range of 98.0 % to 102.0 %.

Table-5: Accuracy study of tipiracil

Accuracy					
% Accuracy	**Area**	**Recovered Conc.**	**Added conc.**	**% Recovery**	**Mean Recovery**
80	553919	80.413	81.00	99.15	99.15
	550558	80.593	80.50	99.87	
	545774	80.224	81.50	98.43	
100	611463	100.042	100.5	99.54	99.49
	609665	100.59	100.5	100.09	
	613078	99.841	101.0	98.85	
120	674225	119.502	121.0	98.76	98.91
	682936	119.466	121.0	98.73	
	669903	119.596	120.5	99.25	

Precision:
Precision for six tests (About 100 µg/mL of tipiracil) was performed and % assay for individual as well as for mean assay value was found within the acceptable criteria 98.0 to 102.0% and % RSD for 6 samples was also NMT 2.0. Precision passes the criteria, no variation found by preparing six different samples. Results are good and reproducible.

Table-6: Precision of tipiracil

Intra Day Precision						
Sample	**Area**	**USP Plates**	**RT**	**Mean**	**SD**	**%RSD**
Sample 1	272831	9165	2.79	268302	3320.4	1.24
sample2	268000	9218	2.79			
sample 3	267502	9262	2.79			
sample 4	267685	9299	2.79			
sample 5	263052	9396	2.79			
sample 6	270739	9425	2.79			
Repeatability						
Sample	**Area**	**USP Plates**	**RT**	**Mean**	**SD**	**%RSD**
Sample 1	270067	9192	2.79	272555	2442.75	0.90
sample2	275138	9074	2.79			
sample 3	275138	9192	2.79			
sample 4	270573	9202	2.79			
sample 5	270457	9107	2.79			
sample 6	273959	9162	2.79			

Robustness:

Table-7: Robustness study of tipiracil

Change in Flow Rate								
Condition	**Parameters**	**Area**	**USP Plates**	**Change**	**RT**	**Mean**	**SD**	**%RSD**
Flow Rate	High	184939	8712	1.2 ml	2.33	184855	523.08	0.28
	High	185331	8895		2.33			
	High	184295	8559		2.33			
	Low	283160	10086	0.8 ml	3.49	283505	1972.80	0.70
	Low	281728	10007		3.49			
	Low	285628	10238		3.49			
Change in Composition								
Condition	**Parameters**	**Area**	**USP Plates**	**Change**	**RT**	**Mean**	**SD**	**%RSD**
Composition	High	225887	8753	ACN:Buffer (72:28)	2.768	224642	1483.75	0.66
	High	223000	9349		2.798			
	High	225038	9100		2.799			
	Low	225230	9832	ACN:Buffer (68:32)	2.814	224458	703.41	0.31
	Low	223853	9357		2.797			
	Low	224292	9354		2.796			

Chromatography was not compromised by changes in the wavelength, changes in the flow rate of mobile phase and changes in the column oven temperature and system suitability was found within acceptable range at each levels. So we can conclude that the method is robust.

Forced Degradation Study: [20,21]
Acid hydrolysis:

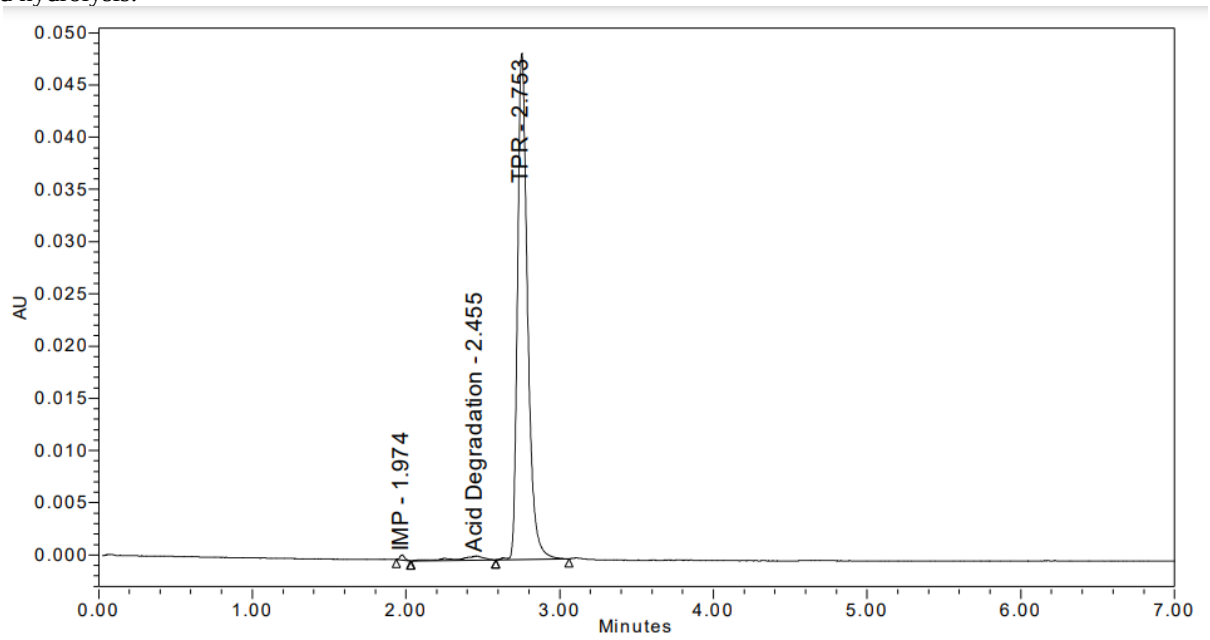


Fig-4: Chromatogram of Acid degradation for 0.01 N HCl for 30 minutes.

Chromatogram of acid hydrolysis (0.1 N HCl) performed at room temperature for 30 minutes showed 14.79 % of degradation with two degradation peaks at R.T. 2.455 for the first degradation peak and 1.974 for the second degradation peak.

Alkaline hydrolysis:

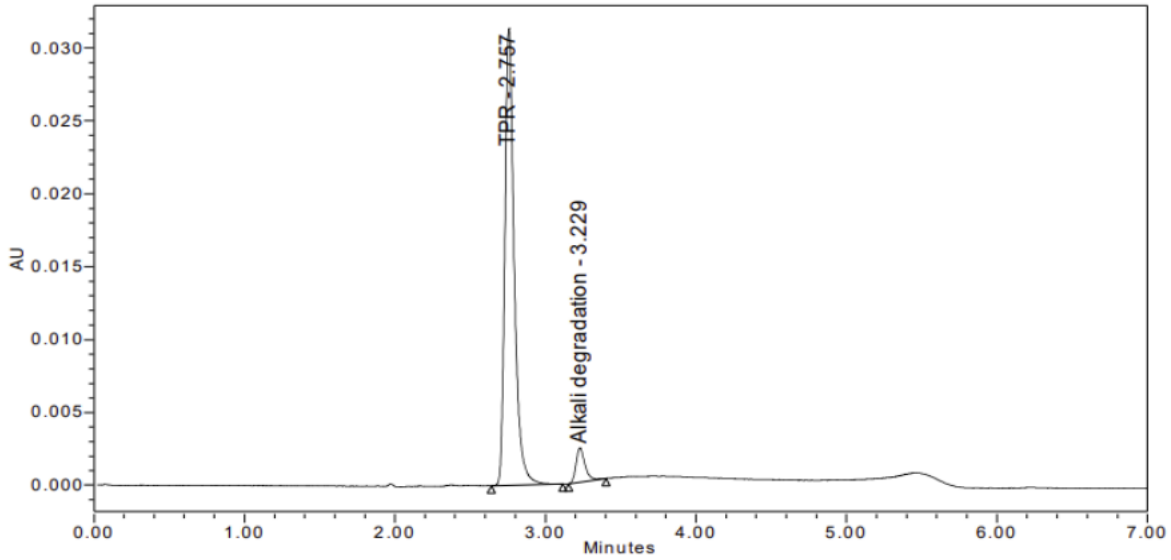


Fig-5: Chromatogram of base degradation of 0.01 N NaOH for 30 minutes.

Chromatogram of a base hydrolysis by using 0.01 N NaOH at room temperature for 30 minutes showed 9.16 % of degradation with degradation peak at R.T. 3.299 for the first degradation peak.

Oxidative degradation:

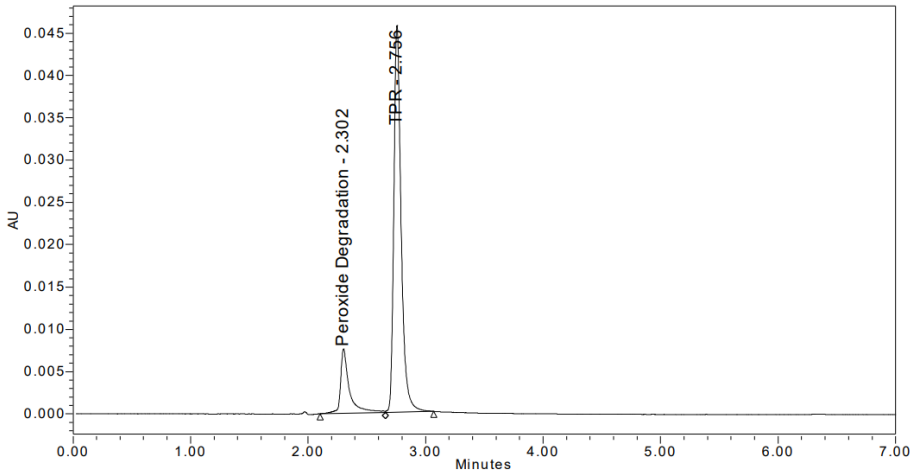


Fig 6: Chromatogram of peroxide degradation of 1% Hydrogen peroxide for 60 minutes.

Tipiracil showed 13.25 % degradation for peroxide stress study by using 1% H_2O_2 for 60 minutes at room temperature. For peroxide stress study degradation product was obtained with R.T. of 2.302.
The drug was found to be stable for thermal and photolytic degradation conditions.

SUMMARY OF STABILITY DATA:

Table-8: Summary of Forced Degradation Study of tipiracil

Sample Name	Treatment	Exposure condition	% Assay	% Degradation
API	Tipiracil	NA	100.00	NA
	Thermal	60°C for 72 Hours	100.00	NA
	Photolytic	1.2M Lux hours	100.00	NA
	Acid	0.01N HCl for 30 minutes at R.T.	85.21	14.79
	Base	0.01N NaOH for 30 minutes at R.T.	90.84	9.16
	Peroxide	1% H_2O_2 for 60 minutes at R.T.	86.75	13.25

Conclusion:

Forced degradation of tipiracil in various conditions like alkaline, acidic, oxidation, UV radiation and thermal degradation was observed in this investigation. The content of degradation of the drug was quantitatively analyzed by RP-HPLC. For this purpose a new RP-HPLC method was developed and validated that was also mentioned in this paper. It was found that degradation in acidic condition was greater than in alkaline and peroxide condition. In oxidation degradation of tipiracil was significant. The drug was not degraded under exposing UV radiation and in the thermal condition.

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Conflict of Interest: None.

References:

1. ICH Q1A (R2) Stability Testing of New Drug Substances and Products.
2. ICH Q2 (R1) Validation of Analytical Procedures.
3. ICH Q3A (R2) Impurities in New Drug Substances.
4. USP General Chapter <1225>: Validation of Compendial Procedures.
5. Branch, S.K., 2005. Guidelines from the international conference on harmonisation (ICH). Journal of pharmaceutical and biomedical analysis, *38*(5), pp.798-805.
6. Goday, S., Abdulrahaman, S.K. and Prameelarani, A., 2017. Development and validation of stability indicating RP-HPLC method for the simultaneous estimation of combination drugs trifluridine and tipiracil in bulk and pharmaceutical dosage forms. IJRANS, 5, pp.93-104.
7. ICH Q1A (R2) Stability Testing of New Drug Substances and Products.
8. Kaveri, S. and Harika, K.S.L., RP-HPLC method development and validation for the simultaneous determination of trifluridine and tirpiracil in bulk form and marketed pharmaceutical dosage form.
9. Aher P, Surana K, Ahire E, Patil D, Sonawane D, Mahajan S. Development and Validation of RP-HPLC Method for Quantitative Determination of 4-Amino Benzene Sulphonamide in Sulphonamide Hydrochloride. Trends in Sciences. 2023 Mar 15;20(6):5209.
10. Narasimhan, B., Abida, K. and Srinivas, K., 2008. Stability indicating RP-HPLC method development and validation for oseltamivir API. Chemical and Pharmaceutical Bulletin, 56(4), pp.413-417.
11. Çelebier, M., Reçber, T., Koçak, E. and Altinöz, S., 2013. RP-HPLC method development and validation for estimation of rivaroxaban in pharmaceutical dosage forms. Brazilian Journal of Pharmaceutical Sciences, 49, pp.359-366.
12. Patel, B.N., Suhagia, B.N. and Patel, C.N., 2012. RP-HPLC method development and validation for estimation of darunavir ethanolate in tablet dosage form. International Journal of Pharmacy and Pharmaceutical Sciences, 4(3), pp.270-273.
13. Ahire E, Thakkar S, Borade Y, Misra M. Nanocrystal based orally disintegrating tablets as a tool to improve dissolution rate of Vortioxetine. Bulletin of Faculty of Pharmacy, Cairo University. 2020 Dec 1;58(1&2):11-20.
14. Burin, V.M., Arcari, S.G., Bordignon-Luiz, A.M.T. and Costa, L.L.F., 2011. Determination of some phenolic compounds in red wine by RP-HPLC: method development and validation. *Journal of chromatographic science*, *49*(8), pp.647-651.
15. Kirthi, A., Shanmugam, R., Prathyusha, M.S. and Basha, D.J., 2014. A review on bioanalytical method development and validation by RP-HPLC. *Journal of global trends in pharmaceutical sciences*, *5*(4), pp.2265-2271.
16. Çelebier, M., Reçber, T., Koçak, E. and Altinöz, S., 2013. RP-HPLC method development and validation for estimation of rivaroxaban in pharmaceutical dosage forms. *Brazilian Journal of Pharmaceutical Sciences*, *49*, pp.359-366.
17. Debata, J., Kumar, S., Jha, S.K. and Khan, A., 2017. A New RP-HPLC method development and validation of dapagliflozin in bulk and tablet dosage form. *Int J Drug Dev Res*, *9*(2), pp.48-51.
18. Narasimhan, B., Abida, K. and Srinivas, K., 2008. Stability indicating RP-HPLC method development and validation for oseltamivir API. *Chemical and Pharmaceutical Bulletin*, *56*(4), pp.413-417.
19. Snyder, L., Kirkland, J., and Glajch, J. *Practical HPLC Method Development*. Wiley, 1997.
20. Kats, R. "Forced Degradation Studies: Regulatory Considerations and Implementation." *BioPharm International*, Jul. 01, 2005.

21. Reynolds D., et al. "Available Guidance and Best Practices for Conducting Forced Degradation Studies." *Pharmaceutical Technology*, Feb. 1, 2002.
22. Solanki, V.S., Bishnoi, R.S., Baghel, R. and Jain, D., 2018. RP-HPLC method development and validation for simultaneous estimation of Cilnidipine, Atenolol and Chlorthalidone. *Journal of Drug Delivery and Therapeutics*, *8*(6-s), pp.78-82.
23. Shah, Y., Iqbal, Z., Ahmad, L., Khan, A., Khan, M.I., Nazir, S. and Nasir, F., 2011. Simultaneous determination of rosuvastatin and atorvastatin in human serum using RP-HPLC/UV detection: Method development, validation and optimization of various experimental parameters. *Journal of Chromatography B*, *879*(9-10), pp.557-563.
24. Gupta, S., Verma, P., Mishra, A.P., Omar, N. and Mathur, R., 2021. A Review on Novel Analytical Method Development and Validation by RP-HPLC Method. *Indian Journal of Forensic Medicine & Toxicology*, *15*(4), pp.3479-3486.
25. Sri, K.V., Anusha, M. and Reddy, S.R., 2015. A rapid RP-HPLC method development and validation for the analysis of linagliptinin bulk and pharmaceutical dosage form. *Asian Journal of Pharmaceutical Analysis*, *5*(1), pp.16-20.
26. Peraman, R., Bhadraya, K., Reddy, Y.P., Reddy, C.S. and Lokesh, T., 2015. Analytical quality by design approach in RP-HPLC method development for the assay of etofenamate in dosage forms. *Indian journal of pharmaceutical sciences*, *77*(6), p.751.
27. Nirmala, K. and Raju, R.R., 2012. A novel method development for validation and detection of colchicine drug by RP-HPLC. *Rasayan J Chem*, *5*, pp.106-11.
28. Savadkouhi, M.B., Vahidi, H., Ayatollahi, A.M., Hooshfar, S. and Kobarfard, F., 2017. RP-HPLC method development and validation for determination of eptifibatide acetate in bulk drug substance and pharmaceutical dosage forms. *Iranian journal of pharmaceutical research: IJPR*, *16*(2), p.490.

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