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Stability Indicating Analytical method development & Validation of 2, 4-dihydroxy-5- Fluoro Pyrimidine in bulk drugs & its Injection formulation

S.Imampasha[1], Dr. Mohd. Ibrahim[2], Dr. V.Murali Balaram [1]

*[*1]Department of Pharmaceutical Analysis & Quality Assurance, Sultan-Ul-Uloom College of Pharmacy, Banjara Hills, Hyderabad-500034, TS, India*

[2]Department of Analytical Chemistry, Nizam Institute of Pharmacy Research Centre, Nalgonda T.S India

***Corresponding Author: S. Imampasha**

Email: impazam@gmail.com

ABSTRACT

Stability indicating HPLC method is developed for 2,4-dihydroxy-5-fluoropyrimidine bulk drug and its formulation, all validation parameters including specificity (interference, forced degradation), Precision (system, method, intermediate), Linearity, accuracy, range ,robustness studied, forced degradation (acid, base, peroxide, water, thermal ,humidity, photo stability effect studied for 2,4-dihydroxy-5-fluoropyrimidine,Retention time was found to be 3.4minutes at the wave length of 254nm.

INTRODUCTION

2,4-dihydroxy-5-fluoropyrimidine is an anti-metabolite used in the cancer chemotherapy, Estimation of the drug in bulk drug industry and formulation sites by liquid chromatography is essential to maintain quality maintenance &for regulatory affairs to detect the quantity of the drug at bulk level &pharmaceutical formulation [1].

Chromatographic parameters

Column : 250 x 4.6mm, 5 µm (YMC Pack ODS or equivalent to L1)
Flow rate : 1.0 mL/min
Wavelength : 254 nm
Injection Volume : 20 µL
Column Temperature : Ambient
Run time : 20 minutes

MATERIALS &METHODS

Details of Chemicals

Fluorouracil Standard (Batch IOG371.USP Grade), Fluorouracil 50mg/ml (Batch IFU-319, Ingénues, ACN (Merck)

Instruments (Columns, serial no.) :

HPLC

VLS-DR/HPLC/05
VLS-DR/HPLC/12
VLS-DR/HPLC/16

VLS-DR/HPLC/17

Analytical Balance

VLS-DR/BAL/01

pH Meter

VLS-DR/PHM/01

Preparation of Buffer

Accurately weigh about 6.8 gm of monobasic potassium phosphate and dissolve in 1 liter water. Adjust the pH of this solution to 5.7 with 5(M) Potassium hydroxide solution.

Preparation of Mobile Phase

Mix buffer and acetonitrile in the ratio of 95:5 %v/v.

Preparation of Standard solution

Accurately weigh and transfer about 10 mg of Fluorouracil standard into 100 mL volumetric flask, dissolve and dilute to the volume with diluent. Transfer 1 mL of this solution into a 10 mL volumetric flask, dissolve and dilute to volume with diluents.

Preparation of Sample

Take 1 mL of 50 mg /mL sample solution and transfer into a 50 mL volumetric flask, dissolve and dilute to volume with diluents. Transfer 1 mL of this solution into 100 mL volumetric flask, dissolve and dilute to volume with diluents [6].

Procedure:

Separately inject 20 µL of blank (diluents), standard solution in five replicates, check standard once and sample solution (each preparation). Record the chromatograms and measure the area response for fluorouracil peak [7].

System suitability

RSD for Fluorouracil peak area from five replicate injections of standard solution should be not more than 0.73, Tailing factor for Fluorouracil peak area from standard solution should be not more than 1.5, Ratio between Standard and Check standard solutions should be between 0.98 to 1.02.

Validation Results[2.3.4]

System Suitability

As per methodology, injected blank, standard solution for five times and checks standard solution into HPLC system [8].

Specify

Interference Study

As per methodology, injected blank, placebo solution once each and standard solution, sample solution and spiked sample solution and checked the peak interference of blank, placebo and impurities at the retention time of Fluorouracil and its Impurities. Prepared and injected each impurity at 1% level individually and checked the interference at each impurity retention time. If the degradation is not achieved at any of the condition, report the minimal values.

Precision

System Precision

As per methodology, injected blank and standard solution five times and check standard once into HPLC system.

Method Precision

Analyzed six test preparations of Fluorouracil injection 50 mg/mL as per the methodology and determined the % RSD of six sample preparations for Assay of Fluorouracil.

Linearity

Linearity for Fluorouracil was determined in the concentration range from 50 to 150 % levels of test concentration levels.

Accuracy

As per methodology, prepared and injected 50%, 100% and 150% level of target concentrations sample solutions of Fluorouracil and demonstrated the accuracy of the method. Calculated the system suitability parameters and % individual and % mean recovery at each level.

Range

From the results of Method Precision, Linearity and Accuracy it was concluded that the range of the Analytical method was established from 50 to 150% of target concentration.

Robustness (Effect of variation in PH)

System suitability preparation were analyzed as per the methodology at low PH (5.6) and high PH (5.8) variation in buffer.

Effect of Variation in Flow rate

System suitability preparations were analyzed as per the methodology at low column flow (0.9 mL/min) and high column flow (1.1 mL/min) variation in flow rate.

Effect of Variation in mobile phase composition:

System suitability preparations were analyzed as per the methodology at low mobile phase composition (93:7) and high mobile phase composition (97:3) variation in mobile phase composition.

Effect of Variation in Column Oven Temperature

System suitability preparations were analyzed as per the methodology at high column oven temperature (30°C) variation in column oven temperature.

Stability of analytical solution

Stability study of standard solution and sample preparation were performed at room temperature and 2-8 °C conditions.

Table 1: Complete Degradation Data [5]

S.No	Type of Stress	Assay (%w/w)	Degradation (%w/w)	Purity 1Angle	Purity 1 Threshold	Peak Purity (Pass/Fail)
1	**Acid**	99.7	-	0.663	1.089	Pass
2	**Base**	96.6	-	0.635	1.079	Pass
3	**Peroxide**	80.4	-	0.653	1.117	Pass
4	**Water**	96.8	-	0.446	1.106	Pass
5	**Thermal**	98.8	-	0.454	1.089	Pass
6	**Humidity**	100.9	-	0.462	1.083	Pass
7	**Photo stability**	95.9	-	0.452	1.087	Pass

Table 2: Method precision Results

Sample	Assay(%w/w)
01	101
02	101
03	101
04	101
05	101
06	102
Average	101
S.D	0.4082
%RSD	0.4

Table 3: Intermediate Precision

Parameter	Analyst-1	Analyst-2
Column ID Number	USNH040034	H-15-07
HPLC ID Number	VLS-DR/HPLC/14	HP2 (Agilent 1100)
Date of Analysis	2015.03.16	2015.04.09

Table 4: Intermediate precision Results

Sample	Assay(%w/w)
01	102
02	102
03	102
04	101
05	102
06	101
Average	102
S.D	0.516
%RSD	0.5

Table 5: Precision and Intermediate Precision Method

Preparation Sites	Analyst –I/Column-I/System-I (Transferring site)
1	101
2	101
3	101
4	101
5	101
6	102
Avg	101
SD	0.4082
%RSD	0.4
%RSD(12 Prep)	0.5

Table 6: Linearity Results of Fluorouracil

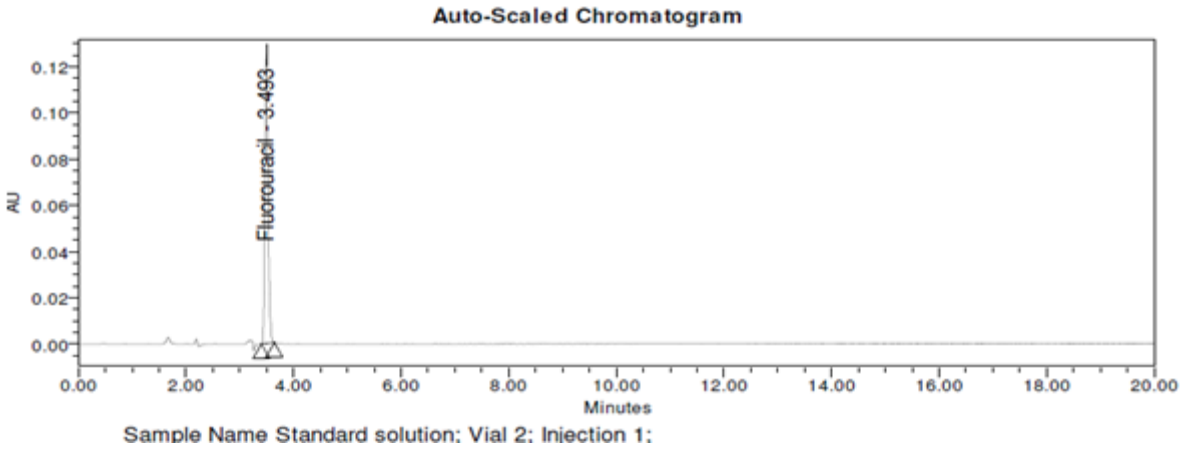
Level	Fluorouracil Concentration (ppm)	Fluorouracil Peak Area
50	5.501	268049
80	8.801	430817
100	11.002	539539
120	13.202	644928
150	16.503	805507
Correlation Coefficient	1.0000	

Table7: Accuracy of Fluorouracil (Assay)

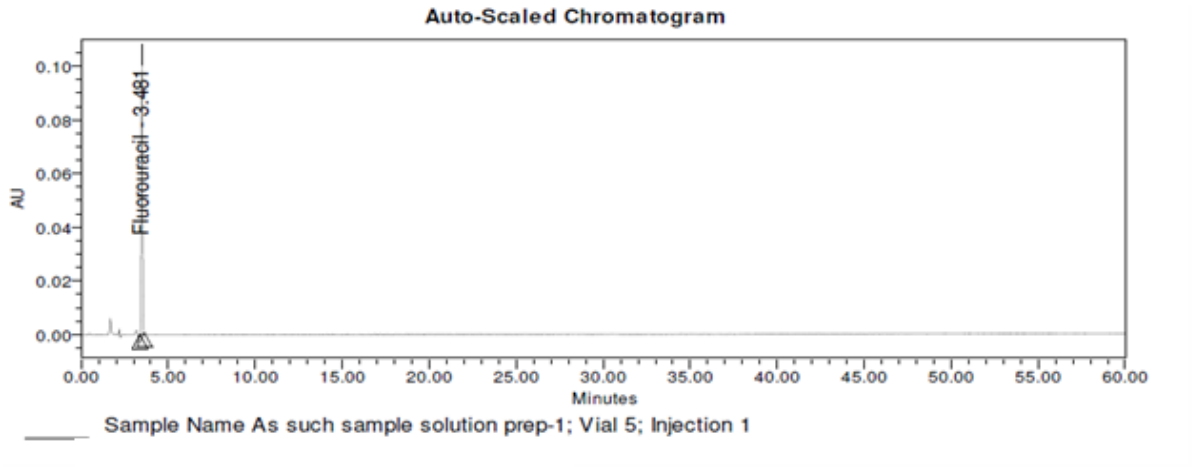
Sample No	Spike level	Added (ppm)	Found (ppm)	'%' Recovery	'%' Mean recovery	%RSD
1	50%	5.005	5.071	101	101	0.0
2	50%	5.005	5.048	101		
3	50%	5.005	5.051	101		
4	50%	5.005	5.060	101		
5	50%	5.005	5.070	101		
6	50%	5.005	5.048	101		
1	100%	10.010	9.893	99	99	0.0
2	100%	10.010	9.891	99		
3	100%	10.010	9.887	99		
1	150%	15.015	14.280	99	99	0.0
2	150%	15.015	14.890	99		
3	150%	15.015	14.883	99		
4	150%	15.015	14.897	99		
5	150%	15.015	14.903	99		
6	150%	15.015	14.839	99		

Table 8: Effect of Variation in Flow rate

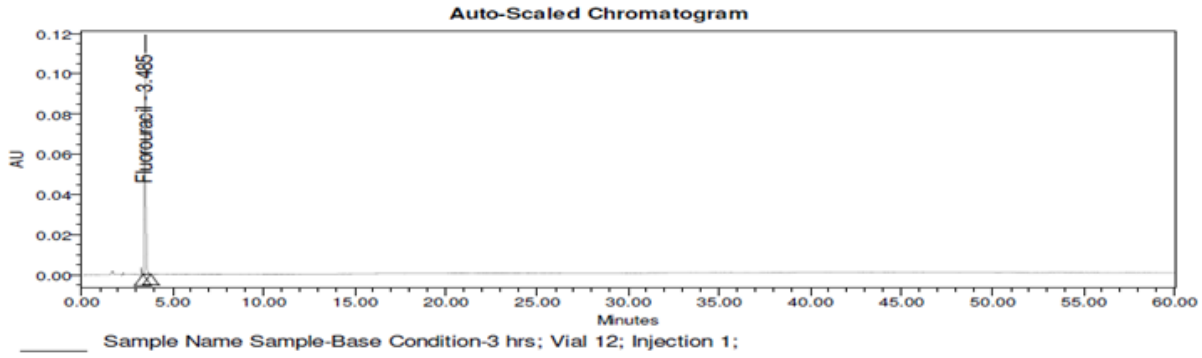
Parameter	Tailing Factor	% RSD	Ratio between Standard and Check standard
Low flow (0.9 mL/min)	1.3	0.14	1.00
High flow (1.1 mL/min)	1.3	0.09	1.00
Acceptance Criteria	**NMT 1.5**	**NMT 0.73**	**Between0.98 to 1.02**



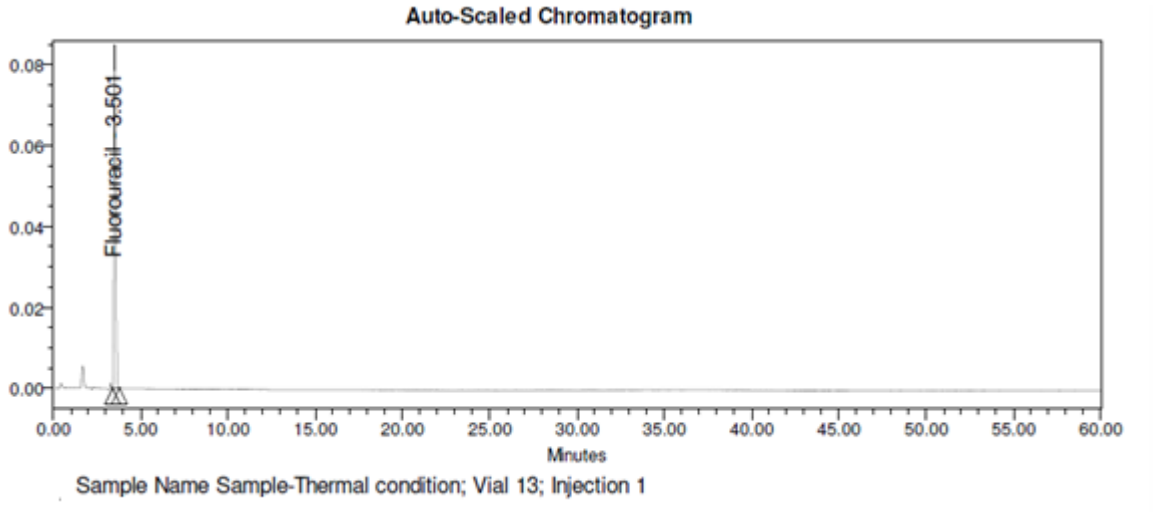
Spectra.1: Typical chromatogram of Assay Standard



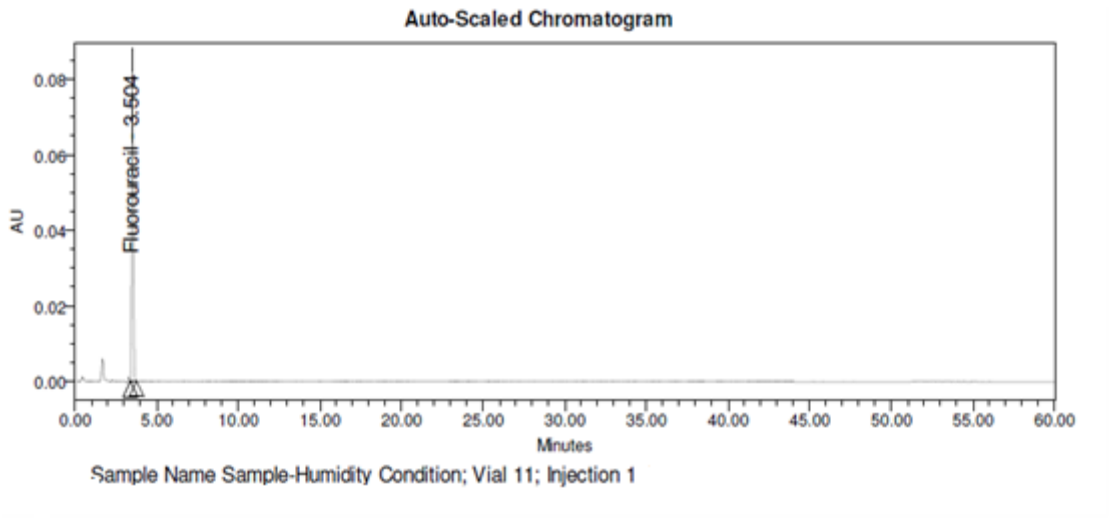
Spectra.2: Typical chromatogram of Sample



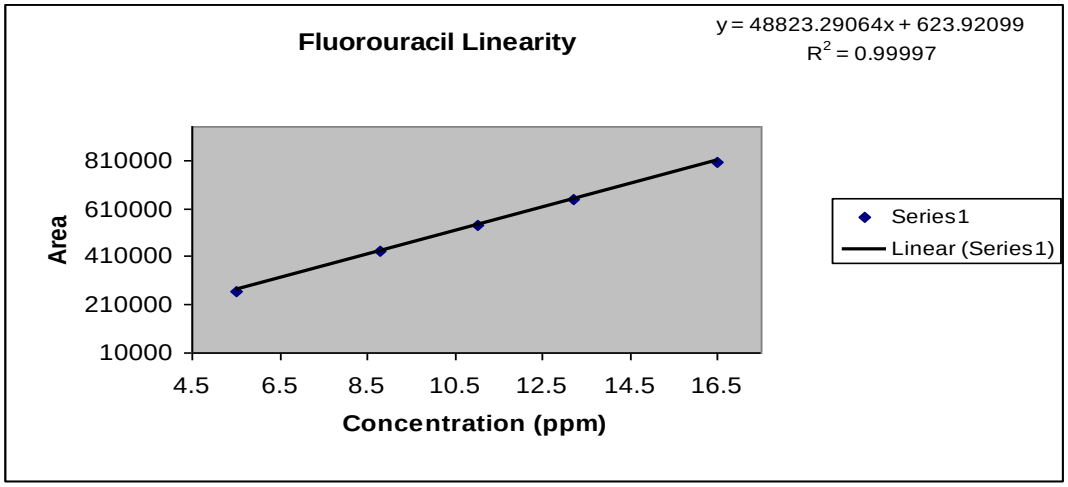
Spectra.3: Typical chromatogram of Base stress Sample



Spectra.4: Typical chromatogram of Thermal stress Sample



Spectra.5: Typical chromatogram of Humidity stress Sample



GRAPH 1: Fluorouracil Linearity graph

CONCLUSION

Analytical method was developed for estimation of 2,4-dihydroxy-5-fluoropyrimidine in bulk drug &its formulation ,validated for all the parameters . Hence, it was concluded that the analytical method is specific, precise, linear, accurate, rugged and robust. Hence, the present analytical method proved as stability indicating the results were within the acceptance criteria therefore can be used for regular analysis in Pharmaceutical bulk drug industry and at the formulation manufacturing industry for drug estimation ,There is no interference of excepients in the injection formulation such as diluents , solubulizers etc

Acknowledgement

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REFERENCES

[1]. Bakshi M, Singh S., Development of validated stability-indicating assay methods-critical review. J Pharma Biomed Anal. 28, 2002, 1011–40.

[2]. ICH, Stability testing of new drug substances and products. Geneva: International Conference on Harmonization, IFPMA; 2003.
[3]. ICH, Stability testing: Photo stability testing of new drug substances and products .Geneva: International Conference on Harmonization, IFPMA; 1996.
[4]. Gerber, F.; Krummen, M.; Potgeter, H.; Roth, A.; Siffrin, C.; Spoendlin, C. "Practical aspects of fast reversed-phase high-performance liquid chromatography using 3µm particle packed columns and monolithic columns" 2004.
[5]. Ettre, C.Milestones in Chromatography: The Birth of Partition, Chromatography". LCGC. **19**(5), 2001, 506–512. Retrieved 2016-02-2
[6]. Henry, Richard A. "The Early Days of HPLC at DuPont". Chromatography Online. Avanstar Communications Inc, 2009.
[7]. Giddings, J. Calvin Dynamics of Chromatography, Part I. Principles and Theory. Marcel, Dekker, Inc., New York. 1965, 281
[8]. V. R. Sinha,* R. V. Kumar, and J. R. Bhinge,A Stability-Indicating RP-HPLC Assay Method for 5-Fluorouracil,Indian journal of pharmaceutical Sciences 71(6), 2009, 630–637.