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Available online at: http://www.iajps.com ***Research Article***

DEVELOPMENT AND VALIDATION OF STABILITY INDICATING ASSAY METHOD FOR ESTIMATION OF TERIFLUNOMIDE IN TABLET DOSAGE FORM

Upadhyay Priya Shitalaprasad*, Dr Vinay C. Darji, Bhumi Patel, Jaymin Patel
Sharda School of Pharmacy, Pethapur, Gandhinagar, Gujarat-382421

Abstract:
A simple, rapid, economical, precise and accurate Stability indicating RP-HPLC method for Teriflunomide In its Pharmaceutical Dosage Form has been developed.
A reverse phase high performance liquid chromatographic method was developed for the Teriflunomide in its Pharmaceutical Dosage Form has been developed. The separation was achieved by Cosmosil (250mm x 4.6 mm) column and Buffer (pH 4.0): Methanol (40:60) as mobile phase, at a flow rate of 1 ml/min. Detection was carried out at 248 nm. Retention time of Teriflunomide was found to be 3.300 min. The method has been validated for linearity, accuracy and precision. Linearity observed for Teriflunomide 10-30 µg/ml. Developed method was found to be accurate, precise and rapid for simultaneous estimation of Teriflunomide in its Combined Dosage Form.
The drug was subjected to stress condition of hydrolysis, oxidation, photolysis and Thermal degradation, Considerable Degradation was found in Thermal degradation. The proposed method was successfully applied for the simultaneous estimation of both the drugs in commercial Combined dosage form.
Keywords: *Teriflunomide, Stability indicating RP-HPLC Method, Validation.*

Corresponding Author:
Upadhyay Priya Shitalaprasad.
B-2 Shyamdarsan society, shivaji chawk,
New naroda, Ahmedabad-382426
M-9574552593,Email ID- Pu477791@gmail.com

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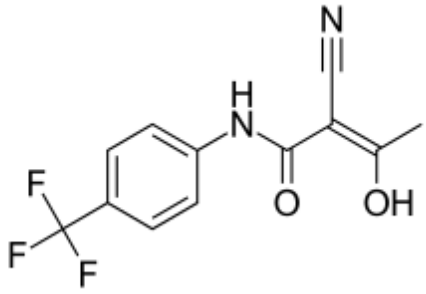
INTRODUCTION:

Teriflunomide is the primary active metabolite of leflunomide, a drug used in the treatment of rheumatoid arthritis.The mechanism of action of teriflunomide is not completely understood. It acts primarily as an inhibitor of dihydroorotate dehydrogenase, a mitochondrial enzyme involved in the novo synthesis of pyrimidines, thereby limiting the expansion of stimulated T cells and B cells and decreasing the migration of lymphocytes to the CNS.Furthermore, it is thought that teriflunomide has other immunological effects independent of pyrimidine synthesis inhibition, such as the inhibition of protein tyrosine kinases and of cyclooxygenase-2.Oral bioavailability is close to 100% and time to steady-state concentration is approximately three months. Teriflunomide is excreted by the liver.

Structure	
Chemical Formula	$C_{12}H_9F_3N_2O_2$
Mol. Weight	270.207 g/mol
IUPAC Name	(2Z)-2-cyano-3-hydroxy-N-[4-(trifluoromethyl)phenyl]but-2-enamide

- Two LC-MS methods and HPLC methods are reported for the analysis of Teriflunomide. But no Stability indicating HPLC method reported for this drug in Pharmaceutical dosage form. Therefore, it was thought worthwhile to develop stability indicating RP-HPLC Method for the Estimation of Teriflunomide.

Apparatus and equipments used in experiment:

Table-1 Chemical/ Reagent

Chemical/ Reagent	Grade	Manufacturer
Methanol	HPLC Grade	Spectrochem pvt Ltd.
Potassium Dihydrogen Phosphate	AR	Merck specialties pvt, Ltd., Mumbai
Water	HPLC Grade	Mili-Q Water
Acetic Acid	AR	Spectrochem pvt Ltd.

Table-2 Instrumentation for HPLC

Component	Brand / Model / Software
HPLC	Shimadzu LC20-AT
HPLC Column	Inertsil ODS- 3v (150*4.6mm)
Detector	UV detector
Ultrasonicator	Frontline machinery
Digital pH meter	Thermo lab
Analytical Balance	Mettler Toledo

Table -3 Instrumentation for UV spectrophotometer

Component	Brand / Model / Software
UV Visible spectrophotometer	Systronic 119

EXPERIMENTAL WORK

Table -4 RP-HPLC optimized chromatographic conditions

Parameters	Chromatographic Condition
Mode of elution	Isocratic
Mobile Phase	Phosphate Buffer (pH 4.0): Methanol (40:60)
Column	Inertsil ODS 3V (150× 4.6mn)
Flow rate	1.0 ml/min
Runtime	10 min
Injection volume	20 µL
Detection wavelength	248 nm

Preparation of Solutions

(A) Teriflunomide standard stock solution: (200µg/mL)

A 20 mg of Teriflunomide was weighed and transferred to a 100 mL volumetric flask. Volume was made up to the mark with Diluent.

(B) Preparation of Working standard solution of Teriflunomide (20µg/mL)

Take 1 mL from the Teriflunomide stock solution and transferred to 10 mL volumetric flask and volume made up to the mark with Diluent.

(C) Diluent: Take 6.8 gm of Potassium dihydrogen phosphate buffer in 1000ml volumetric flask.

Add 900ml of water,degas to sonicate for 10min,finaly make vol upto the mark. Adjust the pH of Buffer with the diluted o-phosphoric acid to make the pH of Buffer(4.5)

Results and Discussion:

(D) Buffer preparation: Take 6.8 gm of Potassium dihydrogen phosphate buffer in 1000ml volumetric flask.

Add 900ml of water,degas to sonicate for 10min,finaly make vol upto the mark.

Adjust the pH of Buffer with the diluted o-phosphoric acid to make the pH of Buffer(4.5)

Mobile phase:-

Potassium dihydrogen phosphate buffer 0.05M (pH 4.5): Methanol (40:60v/v)

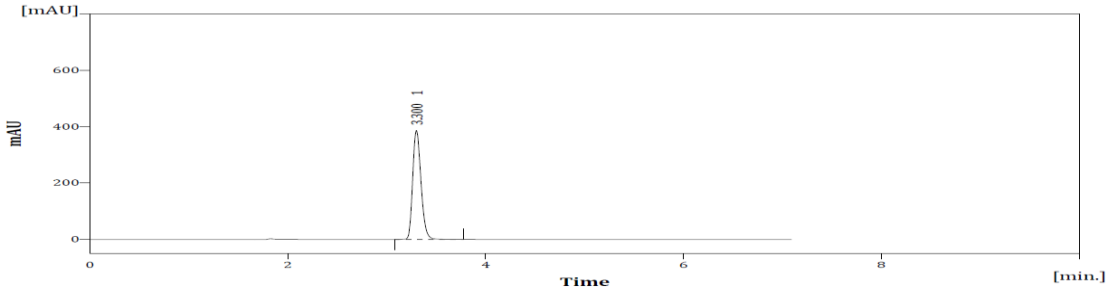


Fig.1 : Buffer 0.05M (pH 4.5): Methanol (40:60v/v)

Table-5 System suitability parameter

Parameters	Teriflunomide
Retention Time	3.300
Theoretical Plates	6926
Asymmetry	1.429

METHOD VALIDATION

Accuracy:

For Teriflunomide

10 µg/ml drug solution was taken in three different flask label A, B and C. Spiked 50% , 100%, 150% of standard solution in it and diluted up to 10ml. The area of each solution peak was measured at 248 nm. The amount of Teriflunomide was calculated at each level and % recoveries were computed

Table 6: Recovery data for Teriflunomide

SR. NO.	Conc. Level (%)	Sample amount (µg/ml)	Amount Added (µg/ml)	Amount recovered (µg/ml)	% Recovery	Average	% RSD
1	50 %	10	8	8.065	100.811	101.112	0.924
2		10	8	8.029	100.366		
3		10	8	8.173	102.160		
4	100 %	10	10	10.053	100.533	99.862	0.983
5		10	10	9.873	98.735		
6		10	10	10.032	100.317		
7	150 %	10	12	11.933	99.444	98.573	1.000
8		10	12	11.853	98.771		
9		10	12	11.700	97.504		

Precision

I. Repeatability

The data for repeatability of peak area measurement for Teriflunomide (20 µg/ml) The % RSD for Teriflunomide (20 µg/ml) was found to be 0.564

Table 7: Repeatability data for Teriflunomide

Teriflunomide (20 µg/ml)				
Sr. No.	Conc. (µg/ml)	Area	Mean ± S.D (n=6)	% R.S.D
1.	20	2269.981	2289.002±12.915	0.564
		2283.650		
		2295.139		
		2285.970		
		2308.867		
		2290.403		

II. Intraday precision

% RSD in Intraday precision for Teriflunomide was found in range of 0.252-502

Table 8: Intraday precision data for Teriflunomide

Sr. No.	Conc. (µg/ml)	Mean ± S.D (n=6)	% R.S.D
1	10	1129.620±5.673	0.502
2	20	2261.647±5.699	0.252
3	30	3380.538±12.250	0.362

III. Interday precision

% RSD in Interday precision for Teriflunomide was found in range of 0.475-1.010

Table 9: Interday data for Teriflunomide

Sr. No.	Conc. (µg/ml)	Mean ± S.D (n=6)	% R.S.D
1	10	1106.376±11.174	1.010
2	20	2282.849±12.613	0.553
3	30	3390.619±16.097	0.475

Linearity:

Correlation co-efficient for calibration curve Teriflunomidewas found to be 0.999

The regression line equation for Teriflunomide is as following:

For Teriflunomide **y = 113.51x - 10.985**

Table 10: Linearity data for Teriflunomide

Sr. No	Concentration (µg/ml)	Area
1	10	1136.791
2	15	1667.695
3	20	2276.669
4	25	2812.870
5	30	3401.921

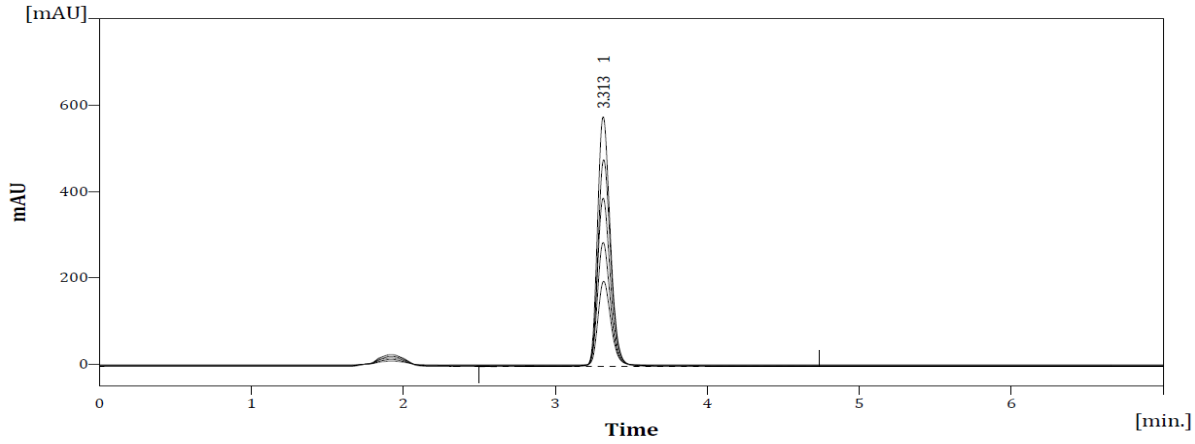


Fig. 2: Overlay chromatogram of different concentrations of Teriflunomide

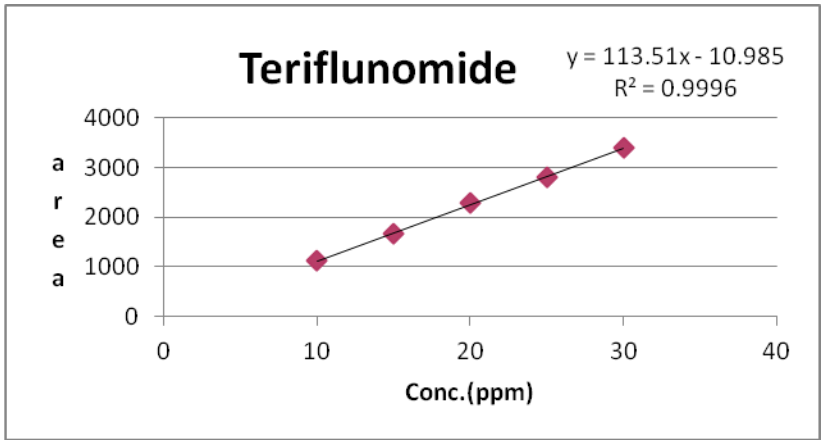


Fig. 3: Calibration Curve of Teriflunomide (10-30 µg/ml)

Specificity:

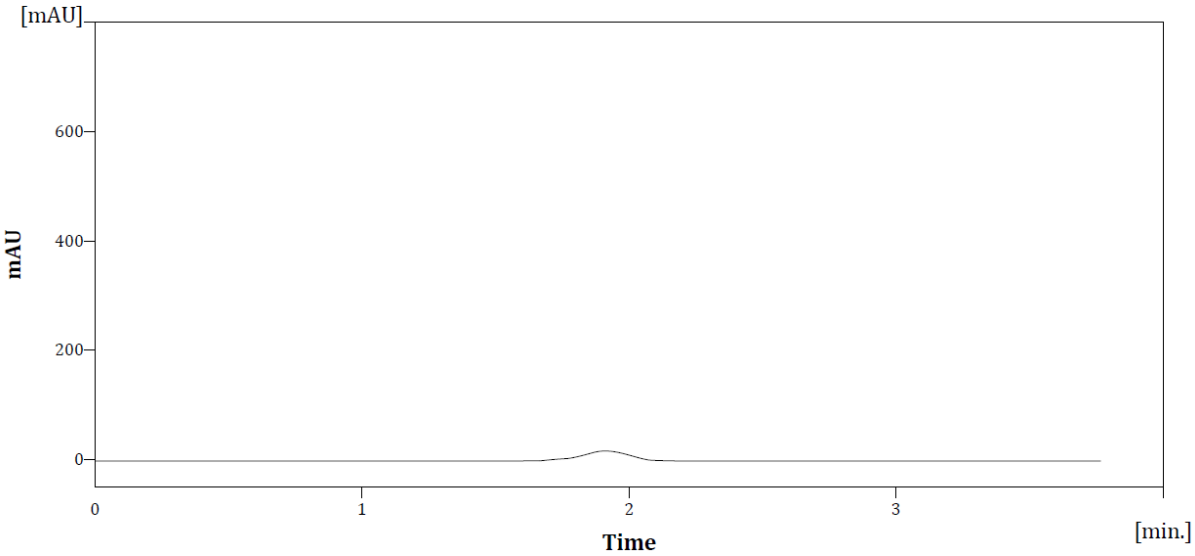


Fig. 4: Chromatogram of Blank

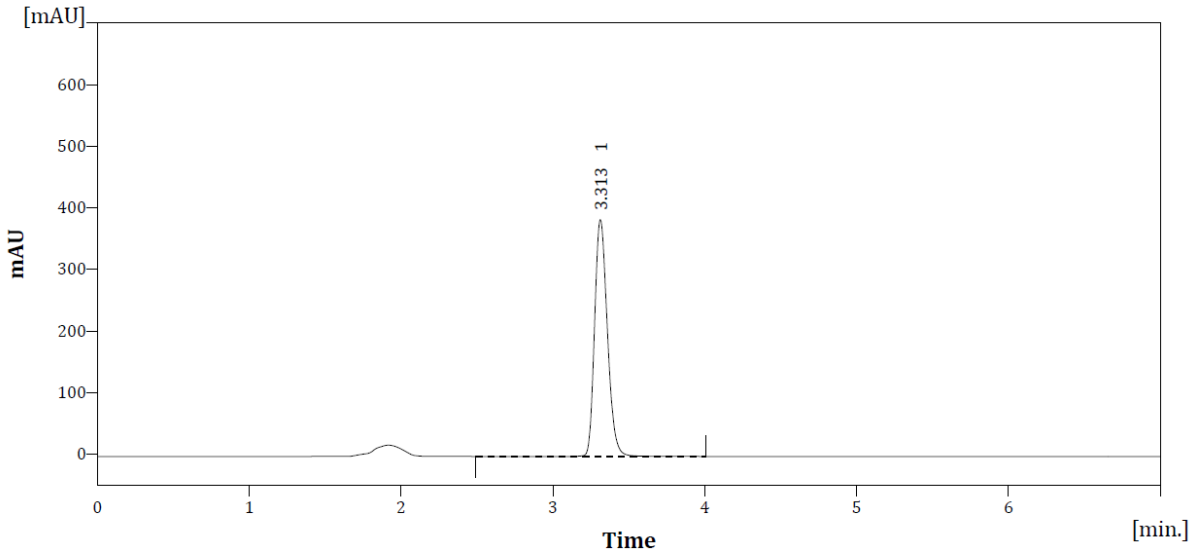


Fig. 5: Chromatogram of Standard

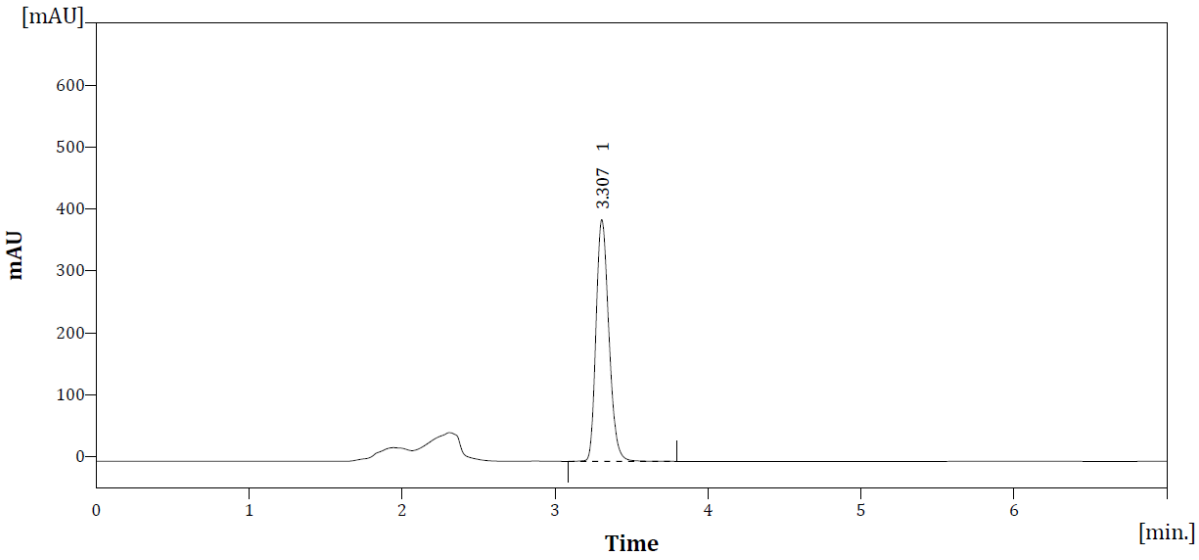


Fig. 6: Chromatogram of Sample

- Here The Chromatogram of Blank preparation does not interfere with the chromatogram of Standard and Sample preparation of Teriflunomide.
- So, the Developed Method is Specific for estimation of Teriflunomide
- **LOD and LOQ:**

Calibration curve was repeated for five times and the standard deviation (SD) of the intercepts was calculated. Then LOD and LOQ were calculated as follows:

LOD = 3.3 * SD/slope of calibration curve

LOQ = 10 * SD/slope of calibration curve

Where, SD = Standard deviation of intercepts

Table 12: LOD and LOQ data for Teriflunomide

LOD	LOQ
LOD = 3.3 x (SD / Slope) = 3.3 x (20.743/113.51) = 0.603 µg/ml	LOQ = 10 x (SD / Slope) = 10x (20.743/113.51) = 1.827 µg/ml

Robustness:

- Following parameters were changed one by one and their effect was observed on system suitability for standard preparation.

Table 13: Robustness data for Teriflunomide

SR NO.	Area at Flow rate (- 0.2 ml/min)	Area at Flow rate (+ 0.2 ml/min)	Area at Mobile phase(-2)	Area at Mobile phase(+2)	Area at pH (-0.2)	Area at pH (+0.2)
1	2276.739	2234.003	2299.125	2209.068	2245.274	2274.519
2	2299.579	2251.865	2322.160	2222.375	2227.228	2290.516
3	2283.494	2233.858	2336.091	2233.491	2238.356	2283.640
% R.S.D	0.513	0.462	0.805	0.550	0.407	0.352

Assay of Marketed formulation:

Take Tablet powder equivalent to 20mg Teriflunomide and Transfer to 100ml volumetric flask and add 60ml of mobile phase and shake the solution for 15minutes and make up the volume with mobile phase. (Stock solution 100ppm), take 1ml from this solution and transfer to 10ml volumetric flask and make the volume with mobile phase. (Working solution 10ppm)

Table 14: Assay of Marketed formulation

Sr. No.	Label claim (mg)	Result (mg)	% Assay	average % Assay	SD	%RSD
1	14	13.113	93.668	93.664	0.946	1.010
2	14	13.245	94.608			
3	14	12.980	92.716			

I. Acid degradation

Acid decomposition studies were performed by one ml of stock solution was transferred in to 10 ml of volumetric flask. Two ml of 0.1 N HCl solutions was added and mixed well and heated for 6 hrs at 70 ^{0}C. After time period the content was cooled to RT. Then the solution was neutralized with 2ml 0.1N NaOH. Then the volume was adjusted with diluent to get 20µg/ml for Teriflunomide.

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II Base degradation:- Base decomposition studies were performed by refluxing one ml of stock solution was transferred in to 10 ml of volumetric flask. Two ml of 0.1 N NaOH solutions was added and mixed well and put for 4 hrs at 70 ºC. After time period the content was cooled to RT. Then the solution was neutralized with 2ml 0.1N HCl Then the volume was adjusted with diluent to get 20µg/ml for Teriflunomide.

III Oxidative degradation:-Oxidative decomposition studies were performed by refluxing one ml of stock solution was transferred in to 10 ml of volumetric flask. Two ml of 3% H2O2 solutions was added and mixed well and put for 6 hrs at 70 ^{0}C. After time period the content was cooled to RT. Then the volume was adjusted with diluent to get 20µg/ml for Teriflunomide.

IV. Photolytic degradation:- One ml of stock solution was transferred in to 10 ml of volumetric flask. This Solution was put in UV Chamber for 12 hrs. Then the volume was adjusted with diluent to get 20µg/ml for Teriflunomide

V. Thermal degradation:- One ml of stock solution was transferred in to 10 ml of volumetric flask. This Solution was put in Oven 100 ^{0}C for 8 hrs. Then the volume was adjusted with diluent to get 20µg/ml for Teriflunomide

Table 15: standard for stability

Teriflunomide	
Area	2263.178

Table 6.13: % Degradation

Condition		**% Degradation**		**% Degradation**
	Area	**Standard**	**Area**	**Sample**
Acid	1975.946	12.69	2010.831	11.15
Base	1941.639	14.21	1915.379	15.37
Oxidation	1865.415	17.58	1913.755	15.44
Photo	2029.725	10.32	2029.449	10.33
Thermal	1892.472	16.38	1915.895	15.34

CONCLUSION:

- RP-HPLC method was developed for estimation of Teriflunomide. In RP-HPLC method, good resolution and separation of two drugs was achieved. Buffer (pH 4.0): Methano (40:60) was used as mobile phase. Retention time of Teriflunomide was found to be 3.300 min with a flow rate of 1 ml/min. The proposed method was accurate and precise. Therefore proposed method can be used for routine analysis of Teriflunomide in tablets.

Forced degradation study of Teriflunomide was performed by RP-HPLC method which includes Acid, Base, Oxidative, Photo and Thermal degradation. Results of degradation were found within limit.

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ABBREVIATIONS

ABBREVIATIONS USED	FULL FORM
SYMBOLS	
%	Percentage
Mcg or µg	Microgram
ml	Milli-liter
mm	Millimeter
µ	Micro
Fig.	Figure
gm	Gram
L	Liter
Min.	Minute
nm	Nanometer
Rs	Resolution
Others	
USP	United States Pharmacopeia
WHO	World Health Organization
CDSCO	Central Drug Standard Control Organization
ICH	International Conference on Harmonisation
FDA	Food and Drug Administration
DAAs	Direct Acting Anti-Viral
UV	Ultra Violet
HPLC	High Performance Liquid Chromatography
LC	Liquid Chromatography
IUPAC	International Union of Pure and Applied Chemistry
LOD	Limit of Detection
LOQ	Limit of Quantitation
SD	Standard Deviation