World Journal of Pharmaceutical Sciences
ISSN (Print): 2321-3310; ISSN (Online): 2321-3086
Available online at: https://wjpsonline.com/
Research Article

METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF UMBRALISIB USING RP-HPLC

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Received: 08-10-2025 / Revised Accepted: 11-10-2025 / Published: 14-10-2025

ABSTRACT
RP-HPLC methodology for quantifying Umbralisib in pharmaceutical formulations. The chromatogram was analysed using an Inertsil C18 column of 150 x 4.6 mm with a particle size of 5 µm. The mobile phase, including 0.1% TFA and acetonitrile in a 70:30 ratio, was injected through the column at a flow rate of 0.9 ml/min. The buffer utilised in this procedure was a 0.1% TFA (pH 4.8) solution. The temperature was sustained at 26°C. The selected optimised wavelength was 210.0 nm. The retention time of Umbralisib was determined to be 2.683 minutes. The %RSD of Umbralisib was determined to be 0.8, whereas the %RSD of the method precision for Umbralisib was discovered to be 0.4.The recovery for Umbralisib was determined to be 99.73%. The limits of detection (LOD) and quantification (LOQ) values derived from the regression equation of Umbralisib were 0.03 and 0.08, respectively. The regression equation for Umbralisib is y = 44120x + 4646.3. The retention periods and run times were reduced, indicating that the suggested approach is straightforward and cost-effective, suitable for frequent quality control testing in industries.

Key Words: Umbralisib, Method development, Validation, RP-HPLC.

INTRODUCTION[1-10]
Umbralisib is a novel, orally administered dual inhibitor of the PI3K-delta (phosphoinositide 3-kinase delta) and CK1-epsilon (casein kinase 1-epsilon) pathways, developed for the treatment of hematologic malignancies. Its therapeutic focus is primarily on B-cell malignancies, including chronic lymphocytic leukemia (CLL) and various forms of non-Hodgkin lymphoma (NHL), such as marginal zone lymphoma (MZL) and follicular lymphoma (FL). Umbralisib represents a targeted therapy that addresses critical signaling pathways involved in the survival, proliferation, and metastasis of malignant B cells.
The PI3K-delta pathway plays a key role in B-cell receptor signaling, which is critical for the proliferation and survival of B cells. Overactivation of this pathway is a hallmark of many B-cell malignancies. By inhibiting PI3K-delta, umbralisib disrupts this signaling, leading to reduced tumor cell growth and survival. Additionally, the inhibition of CK1-epsilon, a less commonly targeted pathway, contributes to the regulation of immune responses and may help in mitigating autoimmune and inflammatory side effects associated with other PI3K inhibitors.
Umbralisib received accelerated approval from the U.S. Food and Drug Administration (FDA) in February 2021 for the treatment of adult patients with relapsed or refractory MZL and FL who have received at least one prior anti-CD20-based therapy. Its approval is based on results from pivotal clinical trials, such as the UNITY-NHL study, which demonstrated significant efficacy in patients with relapsed or refractory MZL and FL, with manageable safety profiles.

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How to Cite this Article: Kaikasha Begum, METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF UMBRALISIB USING RP-HPLC, World J Pharm Sci 2025; 13(03): 266-273; https://doi.org/10.54037/WJPS.2022.100905

In comparison to other PI3K inhibitors, umbralisib's dual-inhibition mechanism aims to reduce the immune-mediated toxicities, such as colitis and pneumonitis, commonly associated with PI3K-delta inhibitors. Its oral formulation and relatively favorable side-effect profile make umbralisib an important treatment option for patients who have limited alternatives after failure of initial therapies.

Umbralisib selectively inhibits the PI3K-delta enzyme, blocking a key survival and growth signal in B cells. It also inhibits CK1-epsilon, which is involved in the regulation of inflammatory and immune responses. This dual mechanism of action not only promotes tumor cell death but may also reduce autoimmune side effects that are common with other PI3K inhibitors.

ANALYTICAL BACKGROUND[11]

Umbralisib acts against against marginal zone lymphoma by interrupting the PI3K pathway; this is an essential pathway for B-cell receptor signaling responsible for the progression of lymphoma. It is chemically known as 2-[(1S)-1-{4-amino-3-[3-fluoro-4-(propan-2-yloxy)phenyl]-1H-pyrazolo[3,4-d]pyrimidin-1-yl}ethyl]-6-fluoro-3-(3-fluorophenyl)-4H-chromen-4-one.

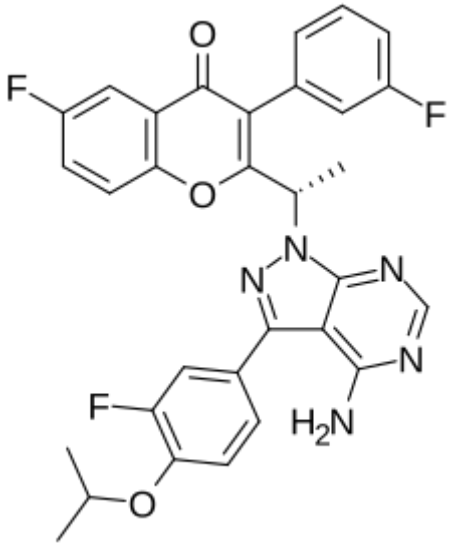

Figure 1 structure of Trilaciclib

High Performance Liquid Chromatography (HPLC) plays a crucial role in the validation of Umbralisib, In the review of literature, more economical methods were observed[12-13], hence a simple, cost-effective stability-indicating simultaneous estimation of Umbralisib by RP-HPLC in pharmaceutical dosage form must be developed and validated as per the guidelines of ICH (Q2 specification).

MATERIALS:

Umbralisib pure drug (API), Umbralisib formulation (Ukoniq), Distilled water,Acetonitrile, Phosphate buffer, Methanol, Potassium dihydrogen ortho phosphate buffer, Ortho-phosphoric acid. All the above chemicals and solvents are from Rankem.

INSTRUMENTATION

The development and method validation were conducted using a WATERS HPLC, specifically the model 2695 SYSTEM, equipped with a Photo diode array detector. The system also included an automated sample injector and the Empower 2 software.

Table 1: Chromatographic Conditions:

Mobile phase	0.1% TFA: Acetonitrile (70:30 v/v)
Flow rate	1.0 ml/min
Column	Inertsil C18 (4.6 x 150mm, 5µm)
wave length	210 nm
Column temperature	30°C
Injection volume	10µL
Run time	10.0 min
Buffer	0.1% TFA

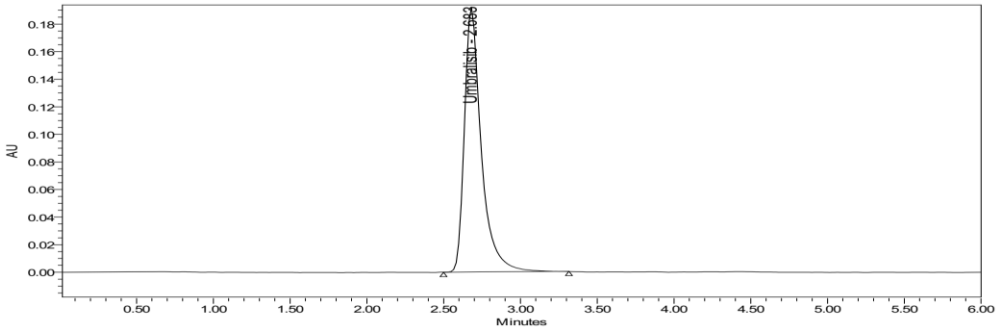

Figure 2: Optimized Chromatogram

Methods:

Preparation of Standard stock solutions: Accurately weighed 20mg of Umbralisib is transferred to 50ml volumetric flask. 3/4 th of diluents was added to the flask and sonicated for 10 minutes. Flask was made up with diluents and labeled as Standard stock solution. (400µg/ml of Umbralisib)

Preparation of Standard working solutions (100% solution): 1ml from stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (40µg/ml of Umbralisib).

Preparation of Sample stock solutions: 10 Tablets were weighed and the average weight of each Tablet was calculated, then the weight equivalent to 1 Tablet (200mg Tablet) was transferred into a 100ml volumetric flask, 50ml of diluents was added and sonicated for 25 min, further the volume was made up with diluent and filtered by HPLC filters (2000µg/ml of Umbralisib)

Preparation of Sample working solutions (100% solution): 0.2ml of filtered sample stock solution was transferred to 50ml volumetric flask and made up with diluent. (60µg/ml of Umbralisib)

Validation:

System suitability parameters:

The system suitability parameters were determined by preparing standard solution of Umbralisib (40 ppm) and the solution were injected six times and the parameters like peak tailing, resolution and USP plate count were determined.

The % RSD for the area of six standard injections results should not be more than 2%.

Specificity (Selectivity): Checking of the interference in the optimized method. We should not find interfering peaks in blank and placebo at retention times of these drugs in this method. So, this method was said to be specific. Representative chromatogram is shown in Figure 3 and experimental data is given in Table 2

Table: 2 System suitability parameters for Umbralisib

S no	Umbralisib		
Inj	**RT(min)**	**USP Plate Count**	**Tailing**
1	2.691	3305	1.57
2	2.696	3373	1.55
3	2.705	3545	1.53
4	2.711	3432	1.56
5	2.713	3485	1.56
6	2.714	3520	1.55

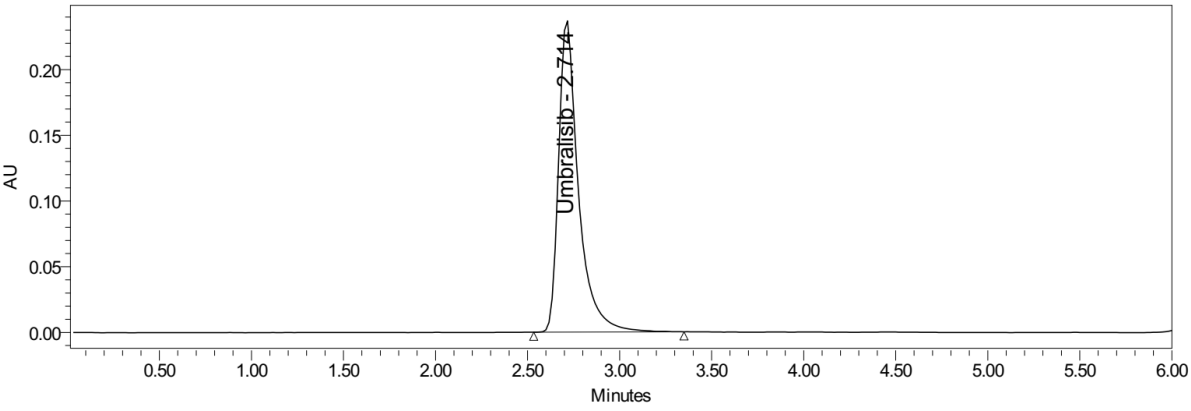

Figure 3: System Suitability Chromatogram of Umbralisib

Table 3: Specificity Data

Peak name	Rt	Area	USP plate count	Tailing
Umbralisib	2.683	1768051	3223.5	1.5

Specificity:

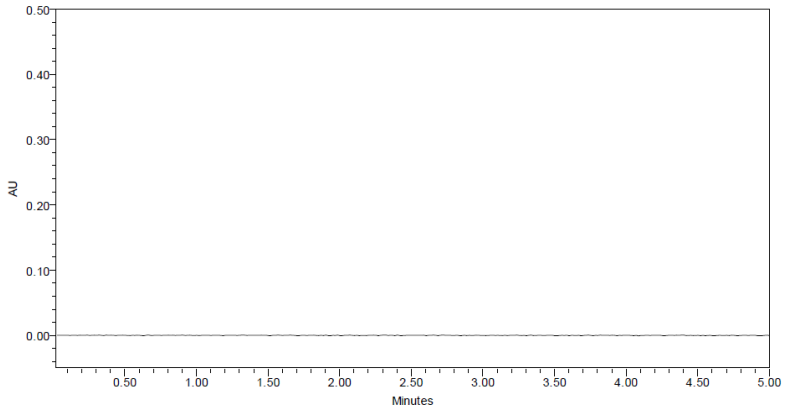

Figure 4 Chromatogram of blank.

The forced degradation conditions are mentioned in Table 4 and the results are mentioned in Table 5

Table 4: Forced degradation conditions for Umbralisib

Stress condition	Solvent	Temp(^{0}C)	Exposed time
Acid	2N HCL	60^{0}c	30 mins
Base	2N NAOH	60^{0}c	30 mins
Oxdation	20% H_2O_2	60^{0}c	30 mins
Thermal	Diluent	105^{0}c	6 hours
Photolytic	Diluent	-	-
Hydrolytic	Water	60^{0}c	

From the results, degradation peaks were observed when the samples were exposed to acid. According to the stress study, none of the degradant co-eluted with the active drug peaks formed.

Table 5: Degradation profile results

Degradation Condition	% Drug Un Degraded	% Drug Degraded
Acid	94.22	5.78
Base	98.48	1.52
Oxidation	96.62	3.38
Thermal	96.61	3.39
Photolytic	94.02	5.98
Hydrolytic	99.05	0.95

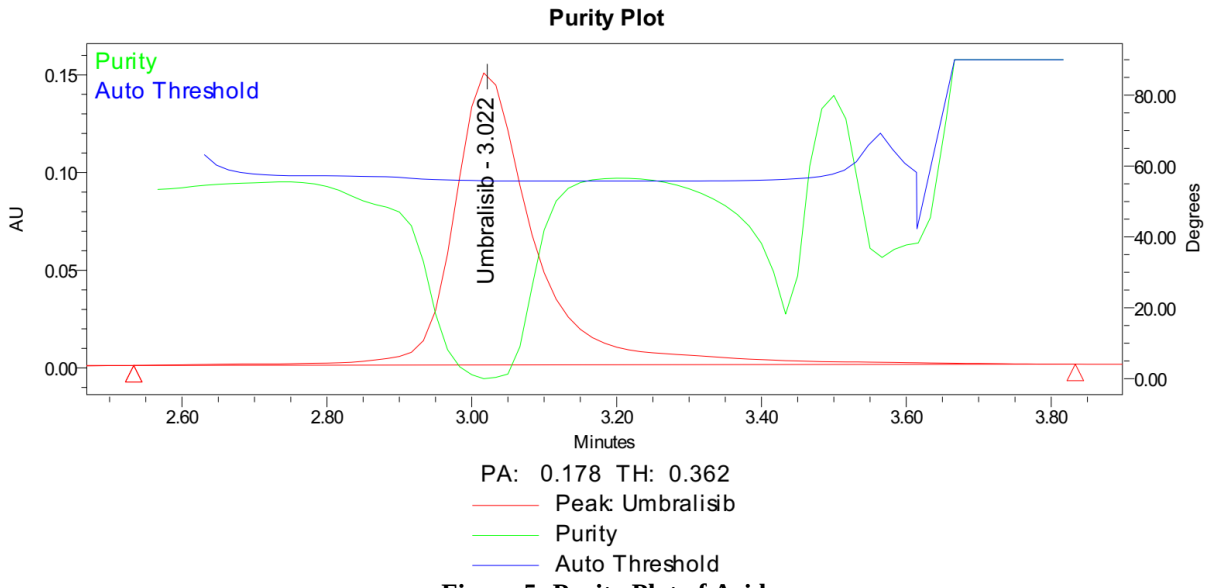

Figure 5: Purity Plot of Acid

Limit of detection (LOD) The detection limit is considered as very low level of concentration of an analyte in a sample that can be detected, but not necessarily quantitated.

Limit of quantitation (LOQ): The limit of quantitation is considered as the lowest concentration of an analyte in a sample that can be determined with acceptable precision and accuracy of the method.

The LOD values obtained for Umbralisib are listed in Table 6.

Table 6: Summary of limit of detection

Sample	Conc (µg/ml)
LOD	0.03
LOQ	0.08

Linearity: The linearity of the method was demonstrated for Umbralisib by analyzing the solutions ranging from 25% to 150% of the specification limit (Table 7). The correlation coefficient for Umbralisib was 0.999. This indicates good linearity

Linearity:

Calibration data is given in table 7 and regression data in table 8 and calibration curve in figure 6

Table 7: Calibration data of Umbralisib

Umbralisib	
Conc (µg/mL)	**Peak area**
0	0
10	448849
20	883051
30	1323558
40	1768322
50	2227807
60	2641418

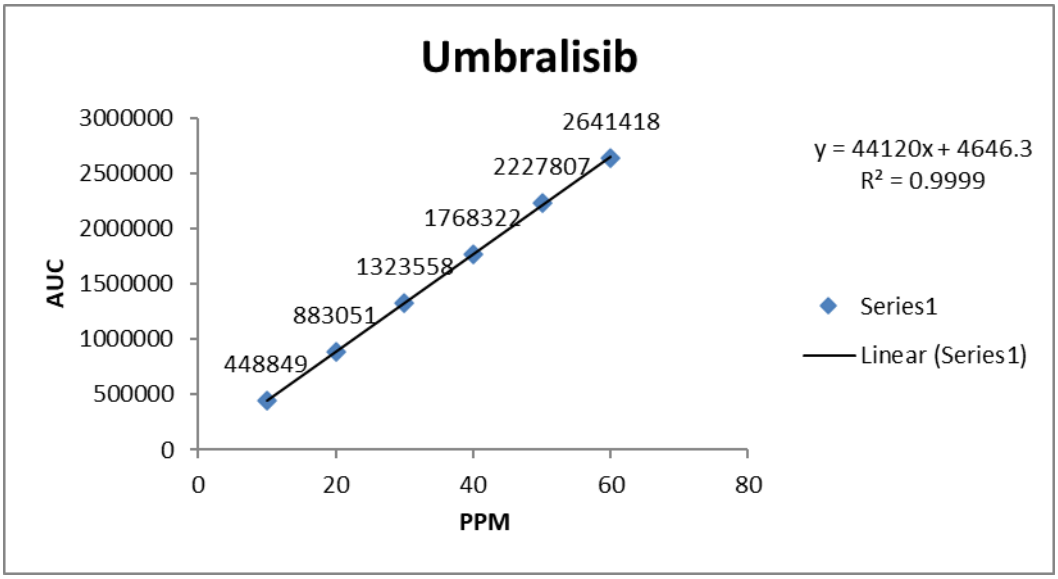

Figure 6: Calibration curve of Umbralisib

Table 8: regression data

Parameter	Umbralisib
Conc range (µg/mL)	1-60µg/ml
Regression Equation	y = 44120x + 4646.3
Co-relation	0.999

Accuracy: The accuracy of the method was determined by using solutions containing spiked samples of Umbralisib at 50%, 100% and 150% of the working strength. All the solutions were prepared in triplicate and analysed. The percentage recovery results obtained for each impurity was listed in Table 9

Table 9 Accuracy table of Umbralisib

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% recovery
50%	20	19.90	99.50
	20	19.90	99.52
	20	19.85	99.27
100%	40	40.21	100.51
	40	39.85	99.62
	40	39.62	99.04
150%	60	60.38	100.63
	60	59.92	99.87
	60	59.75	99.58
Mean % recovery			**99.73**

System Precision: The system precision was performed by analyzing six replicate injections of standard solution at 100% of the specified limit with respect to the working strength of Umbralisib. Results of peak area are summarized in Table 10

Table 10 System precision table of Umbralisib

S. No	Area of Umbralisib
1.	1761406
2.	1754027
3.	1766048
4.	1793826
5.	1774497
6.	1772996
Mean	**1770467**
S.D	**13712.6**
%RSD	**0.8**

Method Precision: The precision of the method was determined by analyzing a sample of Umbralisib). Data obtained is summarized in Table 11

Table 11 Repeatability table of Umbralisib

S. No	Area of Umbralisib
1.	1784836
2.	1777942
3.	1786787
4.	1768714
5.	1781352
6.	1784622
Mean	**1780709**
S.D	**6651.9**
%RSD	**0.4**

Intermediate precision: It is differently from the repeatability, the precision obtained within a single laboratory over a longer period (generally at least several months) and considers more changes than repeatability. Data obtained is summarized in Table 12

Table 12 Intermediate precision table of Umbralisib

S. No	Area of Umbralisib
1.	1727942
2.	1736787
3.	1738714
4.	1738714
5.	1761352
6.	1784622
Mean	**1748022**
S.D	**21071.1**
%RSD	**1.2**

Robustness: The chromatographic conditions were deliberately changed to evaluate the robustness of the existing method. To determine the robustness of method, system suitability solution is prepared as per methodology and injected into HPLC at different altered conditions to check the method's ability like flow rate (± 10%), column oven temperature (± 5°C) and Mobile phase (± 10%) from actual method conditions. No significant change is observed by changing flow, temperature, Mobile phase, and system suitability also complied as per methodology. The robustness results are summarized in Table 13.

Table 13 Robustness data for Umbralisib

Condition	%RSD of Umbralisib
Flow rate (-) 0.9ml/min	0.7
Flow rate (+) 1.1ml/min	0.4
Mobile phase (-) 65B:35A	0.5
Mobile phase (+) 75B:25A	1.0
Temperature (-) 27°C	1.2
Temperature (+) 33°C	0.7

Assay data: -

Ukoniq Tablet bearing the label claims Umbralisib 200 mg. Assay was performed with the above formulation. Average % Assay for Umbralisib obtained was 100.28% respectively. Assay data shown in table no 14.

Formula to calculate assay:

$$\% \text{ Assay} = \frac{AT}{AS} \times \frac{WS}{100} \times \frac{1}{10} \times \frac{10}{1} \times \frac{P}{5} \times \frac{FV}{100} \times \frac{}{L.C} \times 100$$

AT	Avergage peak area of sample in test solution
AS	Mean peak area of sample in standard solution
WS	Weight of sa,ple working standard taken in mg
P	Assay of sample working standard in % in dried basis
L.C	Label claim
FV	filled volume (1ml of a vail)

Table 14: Assay Data of Umbralisib

S.no	Standard Area	Sample area	% Assay
1	1761406	1784836	100.51
2	1754027	1777942	100.12
3	1766048	1786787	100.62
4	1793826	1768714	99.60
5	1774497	1781352	100.31
6	1772996	1784622	100.50
Avg	**1770467**	**1780709**	**100.28**
Stdev	**13712.6**	**6651.9**	**0.375**
%RSD	**0.8**	**0.4**	**0.4**

CONCLUSION

The Umbralisib HPLC investigation found that this approach can properly measure the drug's concentration and purity. This technology is ideal for pharmacokinetic studies and standard quality control since it may be used repeatedly with exact peak resolutions and steady retention times. HPLC analysis is used to determine Umbralisib's efficacy and safety in medicinal applications, as well as to verify its chemical makeup.

ACKNOWLEDGEMENT:

The authors are thankful to, Department of Quality Assurnce, Sultan-ul-uloom, Affiliated to JNTUH India and Spectrum Pharma Research Solutions, Hyderabad, Telangana, India for providing with the gift sample of Umbralisib Pure API.

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