# Method Development for Umbralisib by using RPHPLC

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*Abstract*- **A simple, rapid, precise, sensitive and reproducible reverse phase high performance liquid chromatography (RP-HPLC) method has been developed for the quantitative analysis of Umbralisib in pharmaceutical dosage form. Chromatographic separation of Umbralisib was achieved on Waters Alliance-e2695, by using Waters X-Terra RP-18 Column (150x4.6mm, 3.5µ) column and the mobile phase containing ACN and Ammonium formate pH-3.0/OPA in the ratio of 50:50% v/v. The flow rate was 1.0 ml/min; detection was carried out by absorption at 225nm using a photodiode array detector at ambient temperature. The number of theoretical plates and tailing factor for Umbralisib were NLT 2000 and should not more than 2 respectively. % Relative standard deviation of peak areas of all measurements always less than 2.0. The proposed method was validated according to ICH guidelines. The method was found to be simple, economical, suitable, precise, accurate & robust method for quantitative analysis of Umbralisib.**

*Key words*: **HPLC Umbralisib**

**INTRODUCTION:**

Umbralisib is used **to treat marginal zone lymphoma** (MZL; a slow growing cancer that begins in a type of white blood cells that normally fight infection) in adults whose cancer has returned or did not respond to a certain type of medication The PI3K pathway is a deregulated in malignancies, leading to the overexpression of p110 isoforms (p110α, p110β, p110δ, p110γ) that induces malignant transformation in cells. Umbralisib inhibits several protein kinases, including PI3Kδ and casein kinase CK1ε. PI3Kδ is expressed in both healthy cells and malignant B-cells. CK1ε is believed to be involved in the pathogenesis of malignant cells, including lymphomas. This results in reduced progression of relapsed or refractory lymphoma. In biochemical assays, umbralisib inhibited a mutated form of ABL1. In vitro, umbralisib inhibits malignant cell proliferation, CXCL12-mediated cell adhesion, and CCL19-mediated cell migration Umbralisib is rapidly absorbed in the GI tract. The Tmax of umbralisib is about 4 hours. After consumption of a high-fat, high calorie meal with umbralisib, the AUC increased by 61% and the Cmax increased by 115%.

**Method Development for Umbralisib** The wavelength of maximum absorption of the solution of the drug in mixture of Acetonitrile and Ammonium formate pH-3.0/OPA (50:50)were scanned using PDADetectorwithin the wavelength region of 200–400 nm against Acetonitrile and Ammonium formate pH-3.0/OPA (50:50)as blank. The absorption curve shows isobestic point at 225nm.Thus225 nm was selected as detector wavelength for the HPLC chromatographic method.

**Chromatographic conditions:**

During the selection of chromatographic conditions, numbers of trails were carried out and the best trail was selected for optimized method.

**Preparation of Ammonium formate buffer solution:**

6.30g of Ammonium formateis dissolved in 1litre ofHPLC grade water adjust pH-3.0 with OPA. Filter through 0.45µ nylon filter.

**Preparation of Mobile Phase:**Mobile phase was prepared by mixing Ammonium formate pH-3.0/OPA and ACN taken in the ratio 50:50. It was filtered through 0.45µ membrane filter to remove the impurities which may interfere in the final chromatogram.

**Chromatographic condition:**

Use Waters AllianceHPLC.

Column :Waters X-Terra RP-18(150mmx4.6, 3.5µm)
Mobile phase ratio :ACN and Ammonium formate pH-3.0/OPA (50:50)
Detection wavelength : 225 nm
Flow rate : 1ml/min
Injection volume :10µl
Run time : 5min

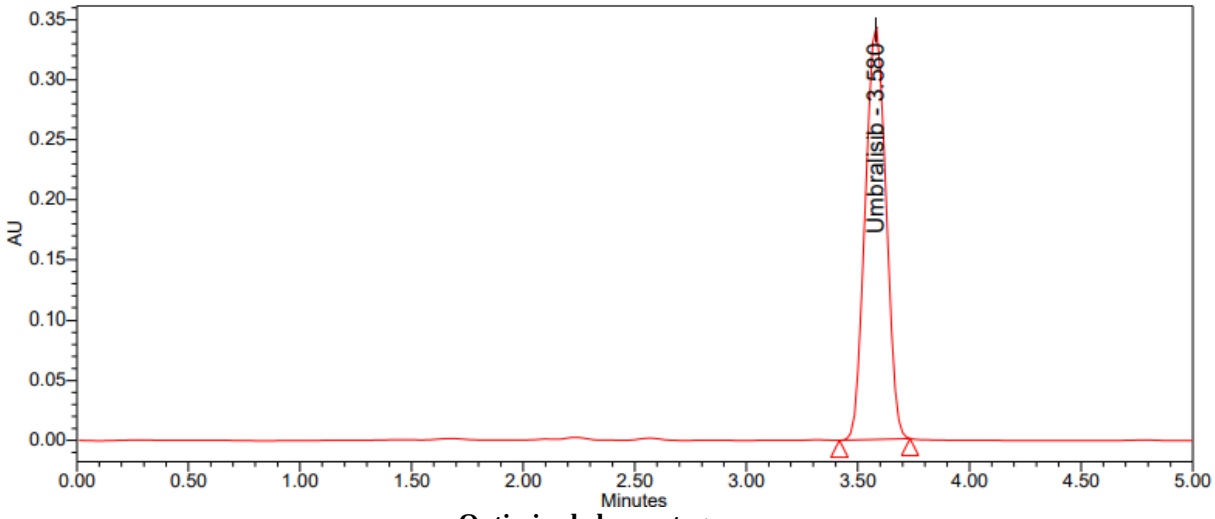
**Diluent:**Use mobile as diluent

**Preparation of standard solution**

Accurately weigh and transfer 50mg of Umbralisib working standard into a 100ml clean dry volumetric flask add Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.(Stock solution) Further pipette 5 ml of the above stock solutions into a 50 ml volumetric flask and dilute up to the mark with diluent. (50ppm ofUmbralisib)

**Sample Solution Preparation:**
Accurately weighed and transfer 99mg of Umbralisibsample into a 100mL clean dry volumetric flask add diluent and sonicate it up to 30 min to dissolve, and centrifuge for 30min to dissolve it completely and make volume up to the mark with the same solvent (Stock solution).Further pipette 5 ml of the above stock solutions into a50ml volumetric flask and dilute up to the mark with diluent (50ppm of Umbralisib).

**Procedure:**
Inject 10 µL of the standard, sample into the chromatographic system and measure the areas for Umbralisibpeak and calculate the %Assay by using the formulae.



**Optimized chromatogram**

**METHOD VALIDATION SUMMARY:**
**Specificity:**
Specificity of an analytical method is ability to measure specifically the analyte of interest without interference from blank and known impurities. For this purpose blank chromatogram, standard chromatogram and sample chromatogram were recorded. The chromatogram of blank shows no response at the retention times of drugs which confirms the response of drug was specific.
**LINEARITY:**
**Preparation of stock solution:**
Accurately weigh and transfer 50mg of Umbralisibworking standard into a 100 ml clean dry volumetric flask add diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.(Stock solution)
**Range:**
The Range of an analytical method is the interval between the upper and lower levels of analyte (including these levels) that have been demonstrated with precision, accuracy and linearity
**Preparation Accuracy Sample solutions:**
**For preparation of 50% solution (With respect to target Assay concentration):**
Accurately weigh and transfer 49.5mg of Umbralisibsample into a 100 ml clean dry volumetric flask add diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.(Stock solution)
Further pipette 5 ml of the above stock solutions into a 50ml volumetric flask and dilute up to the mark with diluent. (25ppm of Umbralisib)
**For preparation of 100% solution (With respect to target Assay concentration):**
Accurately weigh and transfer 99mg of Umbralisibsample into a 100 ml clean dry volumetric flask add diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.(Stock solution)
Further pipette 5 ml of the above stock solutions into a 50ml volumetric flask and dilute up to the mark with diluent. (50ppm of Umbralisib)
**For preparation of 150% solution (With respect to target Assay concentration):**
Accurately weigh and transfer 148.5mg of Umbralisibsample into a 100 ml clean dry volumetric flask add diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.(Stock solution)
Further pipette 5 ml of the above stock solutions into a 50ml volumetric flask and dilute up to the mark with diluent. (75ppm of Umbralisib)
**Precision**
Precision is the degree of repeatability of an analytical method under normal operation conditions. Precision is of 3 types
1. System precision
2. Method precision
3. Intermediate precision (a. Intra-day precision, b. Inter day precision)

System precision is checked by using standard chemical substance to ensure that the analytical system is working properly. In this peak area and % of drug of six determinations is measured and % RSD should be calculated.
In method precision, a homogenous sample of single batch should be analyzed 6 times. This indicates whether a method is giving constant results for a single batch. In this analyze the sample six times and calculate the % RSD.

**ROBUSTNESS:**

As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition, Temperature Variation was made to evaluate the impact on the method. A. The flow rate was varied at 0.9 ml/min to 1.1ml/min. Standard solution 50ppm of Umbralisibwas prepared and analysed using the varied flow rates along with method flow rate. On evaluation of the above results, it can be concluded that the variation in flow rate affected the method significantly. Hence it indicates that the method is robust even by change in the flow rate ±10%.
Standard solution of 50ppm of Umbralisibwas prepared and analysed using the varied in mobile phase ratio.

### 5.1.2 Limit of detection (LOD) and limit of quantification (LOQ):

The limit of detection (LOD) limit of quantification (LOQ) of the drug carry was calculated using the following equation as per international conference harmonization (ICH) guidelines. LOD for Umbralisibwas found to be 0.15µg/mL and LOQ for Umbralisibwas found to be 0.5µg/ml.

**DEGRADATION STUDIES:**

**Preparation of stock:**

Accurately weigh and transfer 99mg ofUmbralisibsample into a 100ml clean dry volumetric flask add Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

**Acid degradation:**

Pipette 5 ml of the aforementioned solution was added to a 50 ml vacuum flask, followed by 1 ml of 1N HCl. The vacuum flask was then maintained at 60°C for 1 hour before being neutralised with 1 N NaOH and diluted to 50ml with diluent. Filter the solution using 0.22 micron syringe filters and transfer to bottles.

**Alkali degradation:**

Pipette 5 ml of above solution into a 50ml volumetric flask and add 1ml of 1N NaOH was added. Then, the volumetric flask was kept at 60ºC for 1 hour and then neutralized with 1N HCl and make up to 50ml with diluent. Filter the solution with 0.22 microns syringe filters and place in vials.

**Thermal degradation**

Umbralisibsample was taken in petridish and kept in Hot air oven at $105^0$ C for 24 hours. Then the sample was taken and diluted with diluents and injected into HPLC and analysed.

**Peroxide degradation**

Pipette 5 ml above stock solution was added to a 50 ml vacuum flask, 1 ml of 3 percent w/v hydrogen peroxide was added to the flask and the volume was built up to the mark using diluent. The vacuum flask was then maintained at 60ºC for 1hour.After that, the vacuum flask was left at room temperature for 15 minutes. Filter the solution using 0.22micron syringe filters and transfer to bottles.

**Reduction degradation**

Pipette 5ml of above-stock solution was added to a 50ml vacuum flask, 1ml of 10% Sodium bisulphate was added to a flask and the volume was built up to the required volume with diluent. The vacuum flask was then maintained at 60ºC for 1 hour. After that, the vacuum flask was left at room temperature for 15 minutes. Filter the solution using 0.22 micron syringe filters and transfer to bottles.

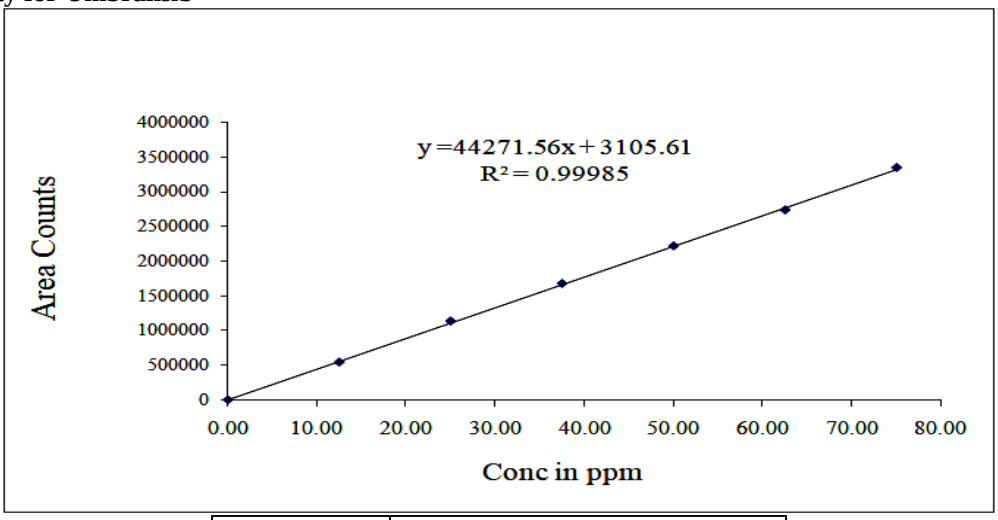
**Photolytic degradation**

Umbralisibsample was placed in sun light for 24 hours. Then the sample was taken and diluted with diluents and injected into HPLC and analysed.

**Hydrolysis degradation**

Pipette 5ml of above-stock solution was added to a 50ml vacuum flask, 1ml of HPLC grade water was added to a flask and the volume was built up to the required volume with diluent. The vacuum flask was then maintained at 60˚C for 1 hour. After that, the vacuum flask was left at room temperature for 15 minutes. Filter the solution using 0.22micron syringe filters and transfer to bottles.

**Results of linearity for Umbralisib**




| S.NO | Umbralisib | |
|---|---|---|
| | Conc.(µg/ml) | Peak area |
| 1 | 12.50 | 542492 |
| 2 | 25.00 | 1133316 |
| 3 | 37.50 | 1674077 |
| 4 | 50.00 | 2216525 |
| 5 | 62.50 | 2733019 |
| 6 | 75.00 | 3343594 |
| **Regression equation** | y=44271.56x+3105.61 | |
| **Slope** | 44271.56 | |
| **Intercept** | 3105.61 | |
| **$R^2$** | 0.99985 | |

**Assay of Umbralisib**

| Drug | Area | Average sample area | Std wt. (mg) | Sample wt. (mg) | Label amount (mg) | Std purity | Amount found (µg/ml) | assay |
|---|---|---|---|---|---|---|---|---|
| Umbralisib | 2231489 | 2234222 | 50 | 99 | 200 | 99.8 | 50.4 | 100.8 |
| | 2236955 | | | | | | | |

**Repeatability:**

| S. No. | Area for Umbralisib |
|---|---|
| 1 | 2245679 |
| 2 | 2202432 |
| 3 | 2223387 |
| 4 | 2227485 |
| 5 | 2212066 |

| | |
|---|---|
| 6 | 2241679 |
| **Average** | 2225455 |
| **Standard Deviation** | 16673.030 |
| **%RSD** | 0.75 |

**Precision**

| **Injection** | **Area** | |
|---|---|---|
| | **Day-1** | **Day-2** |
| 1 | 2234786 | 2241134 |
| 2 | 2212367 | 2225098 |
| 3 | 2231542 | 2233287 |
| 4 | 2228143 | 2219882 |
| 5 | 2256357 | 2240545 |
| 6 | 2242687 | 2248332 |
| **Average** | 2234314 | 2234713 |
| **Standard Deviation** | 14721.819 | 10725.287 |
| **%RSD** | 0.66 | 0.48 |

**Accuracy:**

| **%Concentration(at specification Level)** | **Average Area** | **AverageAmount Added (mg)** | **Amount Found (mg)** | **% Recovery** | **Mean %Recovery** |
|---|---|---|---|---|---|
| 50% | 1121441 | 25 | 25.3 | 101.2 | 101.2 |
| | 1123234 | 25 | 25.34 | 101.4 | |
| | 1119126 | 25 | 25.25 | 101.0 | |
| 100% | 2235921 | 50 | 50.44 | 100.9 | 100.9 |
| | 2256023 | 50 | 50.9 | 101.8 | |
| | 2217308 | 50 | 50.02 | 100.0 | |
| 150% | 3328308 | 75 | 75.09 | 100.1 | 100.2 |
| | 3321124 | 75 | 74.92 | 99.9 | |
| | 3343564 | 75 | 75.43 | 100.6 | |

**Robustness:**

| **Parameter** | **Umbralisib** | | | | |
|---|---|---|---|---|---|
| | **Condition** | **Retention time(min)** | **Peak area** | **Tailing** | **Plate count** |
| Flow rate Change (mL/min) | Less flow (0.9ml) | 3.955 | 2441736 | 1.09 | 13664 |
| | Actual (1ml) | 3.580 | 2217100 | 1.02 | 13592 |
| | More flow (1.1ml) | 3.277 | 2092709 | 0.95 | 13457 |
| Organic Phase change | Less Org (45:55) | 4.814 | 2542811 | 1.12 | 13710 |
| | Actual (50:50) | 3.574 | 2212025 | 1.03 | 13597 |
| | More Org (55:45) | 2.847 | 1871340 | 0.97 | 13396 |

**CONCLUSION:**

The developed HPLCmethod for the estimation of selected drug is simple, rapid, accurate, precise, robust and economical. The mobile phase and solvents are simple to prepare and economical, reliable, sensitive and less time consuming. The sample recoveries were in good agreement with their respective label claims and they suggested noninterference of formulation excipients in the estimation and can be used in laboratories for the routine analysis of selected drugs. Since the system validation parameters of HPLC method used for estimation of selected drug in pure and have shown satisfactory, accurate and reproducible results (without any interference of excipients) as well, it is deduced that the simple and short proposed methods be most useful for analysis purpose. The present work concluded that stability indicating assay method by RP-HPLC was simple, accurate, precise, and specific and has no interference with the placebo and degradation products. Hence these can be used for routine analysis of Umbralisib