ScienSage

Journal of Advanced Scientific Research

Available online through http://www.sciensage.info

ISSN
0976-9595
Research Article

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR THE SIMULTANEOUS DETERMINATION OF VENETOCLAX AND OBINUTUZUMAB IN BULK AND PHARMACEUTICAL DOSAGE FORM

S N Murthy Boddapati*[1], A Emmanuel Kola[2], Vamsi Krishna Suggisetti[1]

[1]*Department of Chemistry, Sir C R Reddy College, PG Courses, Eluru, Andhra Pradesh, India.*

[2]*Department of Chemistry, Sir C R Reddy Autonomous College, Eluru, Andhra Pradesh, India.*

**Corresponding author: snmurthyboddapati@gmail.com*

ABSTRACT

A simple, sensitive and rapid chromatographic method was developed and validated for quantification of Venetoclax and Obinutuzumab in bulk and pharmaceutical dosage form using Cliral cell ODH (4.6x150mm, 5µ) column. The mobile phase consists of isopropyl alcohol, n-hexane and tetra hydrofuran with 1ml of acetic acid in the ratio of 50:30:20v/v/v. The flow rate is maintained at 1.0 ml/min, detection was carried out by absorption at 221nm using photo diode array detector. The calibration curve was linear and regression coefficient (R^2) value was found to be 0.999 and concentration ranging from 3-45 µg/ml of Venetoclax and 0.75-11.25 µg/ml of Obinutuzumab respectively. The LOD and LOQ of the method were found to be 0.03 µg/ml, 0.0075µg/ml and 0.3µg/ml, 0.075µg/ml for Venetoclax and Obinutuzumab. The developed method was found to be simple, economical, suitable and validated according to ICH guidelines.

Keywords: Venetoclax, Obinutuzumab, HPLC, Development and Validation

1. INTRODUCTION

Venetoclax, sold under the trade name Venclexta and Venclyxto, is a medication used to treat chronic lymphocytic leukemia [1, 2]. Venetoclax is used for adults with chronic lymphocytic leukemia (CLL) or small lymphocytic lymphoma (SLL). Indication does not depend on mutation status. Venetoclax is also used as part of a combination therapy for acute myeloid leukemia (AML) [3-5]. For this purpose it is with azacitidine, decitabine or low-dose cytarabine for newly diagnosed adults over 75 or those with other health problems where intensive chemotherapy [6, 7] cannot be used. Common side effects of venetoclax include neutropenia (low white blood cell count) [8, 9], nausea [10, 11], anemia [12-14], diarrhea [15], upper respiratory tract infection [16], fatigue [17] and thrombocytopenia (low platelet count) [18]. Major side effects include tumor lysis syndrome [19] and severe neutropenia [20]. Additionally, this drug may cause fertility problems in males. Obinutuzumab is a humanized anti-CD20 monoclonal antibody, originated by GlycArt Biotechnology AG and developed by Roche as a cancer treatment. It can be used as a first-line treatment for chronic lymphocytic leukemia in combination with chemotherapy or with venetoclax, as a first -line treatment for follicular lymphoma [21, 22] in combination with chemotherapy, and as treatment for relapsed or refractory follicular lymphoma in combination with bendamustine [23] chemotherapy.

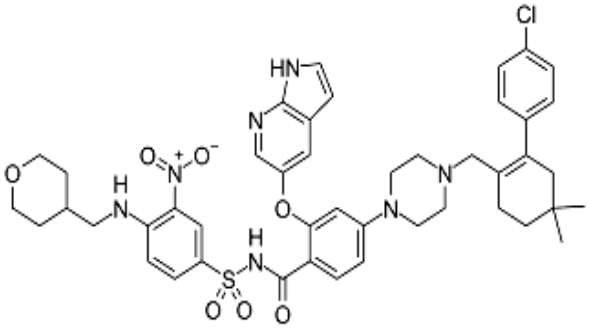


Fig. 1: Structure of Venetoclax

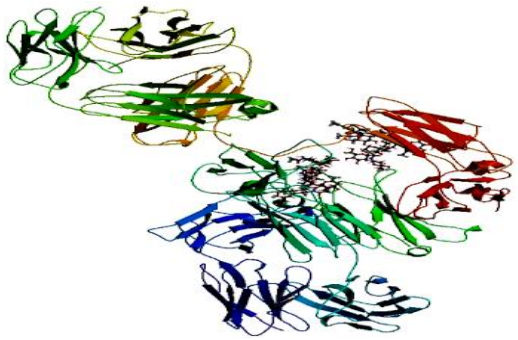


Fig. 2: Structure of Obinutuzumab

Obinutuzumab is used in combination with chlorambucil as a first-line treatment for chronic lymphocytic leukemia. Its progression-free survival is significantly better than rituximab in the same combination but its overall survival is not significantly better. It is also used in combination with bendamustine followed by obinutuzumab monotherapy for the treatment of patients with follicular lymphoma as a second line treatment to a regimen containing rituximab. It was not tested in pregnant women.

2. MATERIAL AND METHODS

Acetonitrile, Orthophosphoric acid and water (HPLC grade), were purchased from Merck Ltd. Worli, Mumbai, India. APIs of Venetoclax and Obinutuzumab as reference standards were procured from Spectrum Pharma research solutions pvt. Ltd, Hyderabad.

2.1. Instrumentation

Waters Alliance liquid chromatography (model 2695) monitored with empower 2 data handling system and fitted with a Chiral cell ODH (150x4.6mm, 5µ) and a detector of photo diode array (model 2998) was used for this study.

2.2. Preparation of Mobile phase

Isopropyl alcohol, n-hexane and tetra hydro furan were taken in 50:30:20 ratio, mixed thoroughly. To this added 1ml of acetic acid and sonicated for 5 min and filtered through 0.22 µm membrane filter and used as mobile phase. The HPLC analysis was performed on reversed-phase HPLC system with isocratic elution mode using a mobile phase of isopropyl alcohol, n-hexane and tetra hydro furan (50:30:20) on chiral cell ODH column (150x4.6mm, 5µ) with 1ml/min flow rate at 221nm using PDA detector.

Diluent: Mobile phase was used as diluent.

2.3. Preparation of standard solution (Venetoclax 30µg/ml and Obinutuzumab 7.5µg/ml)

Accurately weighed and transfered 30 mg of Venetoclax and 7.5 mg of Obinutuzumab working standard into 100ml volumetric flask and added 70 ml of diluent, sonicated to dissolve it for 30 min. and made upto the mark with diluent. This was used as stock solution.

Took 5 ml of the stock solution and transferred into 50 ml volumetric flask and made upto the mark with diluent.

2.4. Preparation of sample solution

Accurately weighed 51 mg of venetoclax tablet and 7.5 mg of obinutuzumab injection powder and transferred into 100 ml volumetric flask. Added 70 ml of diluent and sonicated to dissolve it for 15 min, made up to the mark with diluent.

Took 5 ml of the sample stock solution and transferred into 50 ml volumetric flask and made upto the mark with diluent.

2.5. Optimization of chromatographic conditions

Various combination of mobile phase was screened with respect to resolution, theoretical plate count, tailing and other system suitability parameters. Finally the separation was performed with freshly prepared mobile phase consists of isopropyl alcohol, n-hexane and tetra hydro furan in the ratio of 50:30:20 with a flow rate of 1.0 ml/min. 221nm wavelength, injection volume of 10 µl and ambient temperature was maintained during the entire process to obtain symmetric peak of venetoclax and obinutuzumab.

3. RESULTS AND DISCUSSION

To obtain the best chromatographic condition, different columns like C_{18}, C_8 and CN- propyl and mobile phases were tested. The best chromatographic separation occurred on chiral cell ODH column with a mobile phase consisting of isopropyl alcohol, n-hexane and tetra hydro furan in (50:30:20) at a flow rate of 1ml/min and PDA detection at 221nm. Finally the following conditions were found to be optimum after evaluating the column efficiency by parameters.

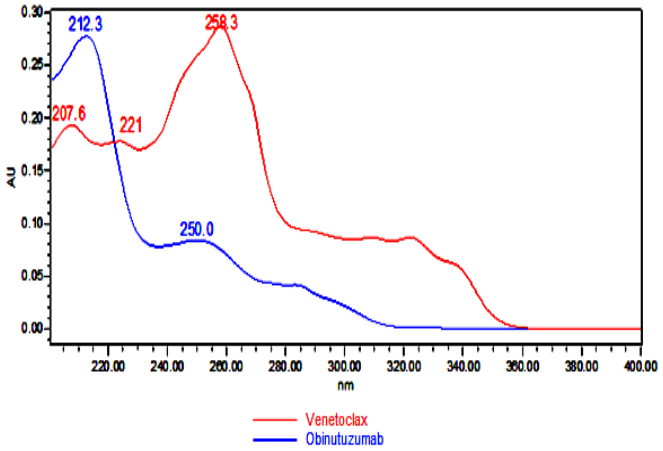


Fig.3: PDA Spectrum of Venetoclax and Obinutuzumab

Table 1: Optimized chromatographic conditions

Stationary Phase	Chiral cell column (150x4.6mm, 5µ)
Mobile Phase	Isopropyl alcohol: n-Hexane: Tetra hydro furan (50:30:20)
Injection volume	10 µl
Flow rate	1.0 ml/min
Column temperature	25°C
Wave length	221 nm
Run time	6 min.
Retention time of Venetoclax	2.457 min.
Retention time of Obinutuzumab	3.888 min.

Table 2: System suitability results

Parameter	Venetoclax	Obinutuzumab
Theoretical plate count	3111	6605
Tailing factor	1.03	1.01
Resolution	-	7.75
Retention time	2.451	3.883

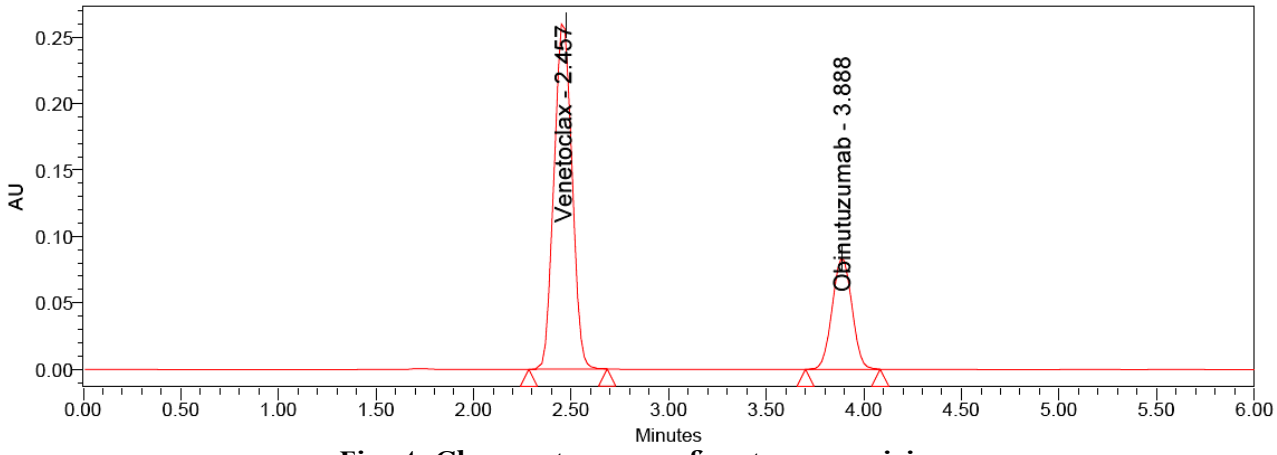


Fig. 4: Chromatogram of system precision

3.1. System suitability

The system suitability was performed by injecting standard solution containing 30 µg/ml of venetoclax and 7.5 µg/ml of obinutuzumab in six replicates. The result indicates that the system suitability parameter is within the limit. The results were as shown in table 2.

3.2. Linearity

The linearity of the method was established by determining the plotting a graph between concentration and corresponding peak area for venetoclax and obinutuzumab over a concentration range from 10-150 µg/ml and 5-75 µg/ml respectively. The correlation coefficient was found to be 0.999 for both drugs.

3.3. Limit of Detection (LOD) and Limit of Qunatification (LOQ)

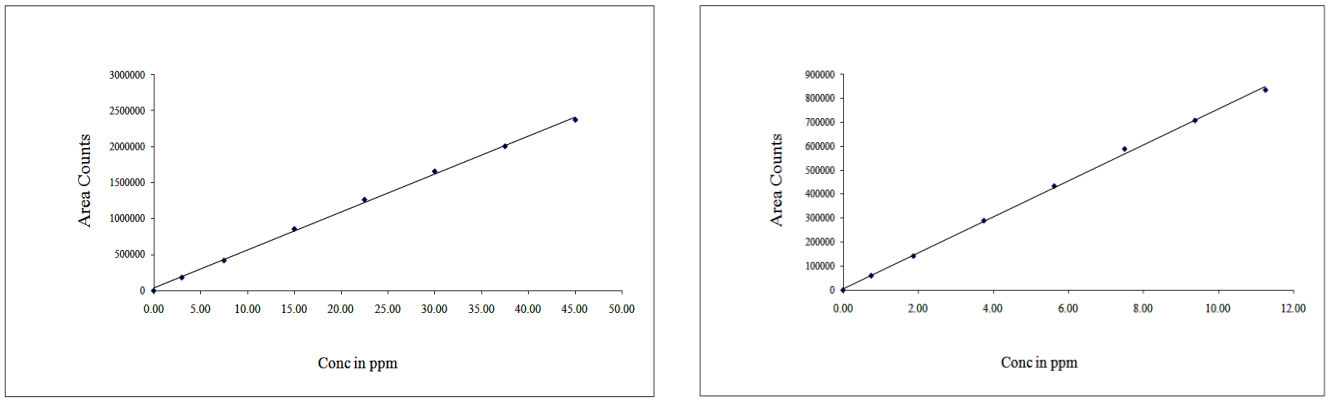
The LOD and LOQ were found to be 0.03µg/ml, 0.0075µg/ml and 0.3 µg/ml, 0.075µg/ml for Venetoclax and Obinutuzumab respectively. The results are given in Table 4.

3.4. Method Precision (or) Repeatability

The % RSD value for six replicate injections of known concentration of Venetoclax and Obinutuzumab carried out on the same day was found to be <2% which indicate that the method is repeatable.

Table 3: Results of linearity

S. No.	Venetoclax		Obinutuzumab	
	Concentration (µg/ml)	Area	Concentration (µg/ml)	Area
1	3	182238	0.75	59908
2	7.5	418812	1.88	141877
3	15	855675	3.75	289663
4	22.5	1263729	5.63	433475
5	30	1655329	7.5	588257
6	37.5	2003213	9.38	707096
7	45	2369046	11.25	834284



A **B**

Fig. 5: Calibration curve of (A) Venetoclax (B) Obinutuzumab

Table 4: Results of LOD and LOQ

Drug	LOD	LOQ
Venetoclax	0.03	0.3
Obinutuzumab	0.0075	0.075

Table 5: Results of method precision

S. No.	Area of Venetoclax	Area of Obinutuzumab
1	1665089	590947
2	1666873	589345
3	1670703	592887
4	1669657	591665
5	1670107	594267
6	1669209	591441
Mean	1668606	591759
Std Dev	2167.457	1682.784
%RSD	0.13	0.28

3.5. Accuracy

The concentrations of Venetoclax and Obinutuzumab were prepared in three levels of 50%, 100% and 150%. The percentage recovery obtained was found to be in the acceptable limit of 98%-102%. From the table it was found that the developed method is precise and accurate.

3.6. Robustness

Robustness of the chromatographic method was determined by varying flow rate and mobile phase composition. % RSD was found to be within the acceptable limit. Robustness results were tabulated in table 7.

Table 6: Accuracy results

Accuracy	Amount of Venetoclax	% Recovery	Amount of Obinutuzumab	% Recovery
50%	50	100.1	25	99.5
100%	100	99.9	50	99.3
150%	150	99.6	75	99.9

Table 7: Results of robustness

Parameter	% RSD of Venetoclax	% RSD of Obinutuzumab
Flow (1.2ml/min)	0.55	0.18
Flow (0.8ml/min)	0.95	0.38
Organic phase (55:45)	0.88	0.54
Organic phase (45:55)	0.28	0.39

4. CONCLUSION

Till today there is no HPLC method to estimate the combination of Venetoclax and Obinutuzumab. For the estimation of these two drugs simultaneously HPLC method was developed and validated according to ICH guidelines. All the validation parameters including system suitability, accuracy, method precision, LOD, LOQ and robustness are within the acceptable limits. The proposed method can be used for the routine analysis of Venetoclax and Obinutuzumab in the dosage form.

5. ACKNOWLEDGEMENT

The authors are thankful to the management committee of Sir C R R Educational Institutions for financial support and the authors are thankful to Icon Pharmaceutical Company, Vijayawada, A.P., for Instrumental support.

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