Int. J. Pharm. Investigation, 2025; 15(1):248-254.
https://www.jpionline.org

Original Article

QBD-Based Development of Experimental Design with Response Surface-Based Optimization of Siponimod by Chromatographic and Spectroscopic Method

Renjitha Jaya Raveendran[1,2,*], Prasanth Viswanadhan Vasantha[3], Chainesh Shah[1]

[1]Department of Pharmaceutical Sciences, Shri Jagdishprasad Jhabarmal Tibrewala University, Jhunjhunu, Rajasthan, INDIA.
[2]Department of Pharmaceutical Chemistry, Mount Zion College of Pharmaceutical Sciences and Research, Adoor, Kerala, INDIA.
[3]Department of Pharmaceutics, Mount Zion College of Pharmaceutical Sciences and Research, Adoor, Kerala, INDIA.

ABSTRACT

Background: A recent technique for characterizing Siponimod, a multiple sclerosis medication, was developed using Ultraviolet spectroscopic analysis, high-performance liquid chromatography, high-performance Thin-Layer Chromatography and spectrofluorimetric analysis. **Purpose:** To optimize the amount of Siponimod in Pharmaceutical formulation by parallel methods assisted with response surface design and to validate the method as per ICH guidelines. **Materials and Methods:** The UV absorbance and the fluorescence intensity were separately measured using the Cary 5000 double-beam spectrophotometer and the Cary Eclipse Spectrofluorometer. The Agilent C_{18} column was used for chromatographic separation and the mobile phase comprised of phosphate buffer and Acetonitrile at different concentrations. Using Digital Expert Stat-Ease -360 software, the response surface approach was used to optimize the methods' variables using the Box-Behnken design. HPTLC determination was carried out using a Camag Linomet densitometric scanner. **Results:** Wavelength maximums of Siponimod detected at 258 nm, the medication shows a chromatographic peak that is distinct and easy to interpret. There was a fluorescence excitation at 486 nm. The recovery percentage varied from 92% to 100% w/w with a relative standard deviation of less than 2. By ICH regulations, the procedures were confirmed and a transitional deterioration pattern was perceived while taking into account the medications' stability in a forced stress environment. **Conclusion:** It was discovered that the procedure was reliable and suitable for regular screening of Siponimod in bulk dose form as well as pharmaceutical formulation.

Keywords: Multiple Sclerosis, Siponimod, Analytical Method.

Correspondence:
Ms. Renjitha Jaya Raveendran
[1]Research Scholar, Department of Pharmaceutical Sciences, Shri Jagdishprasad Jhabarmal Tibrewala University, Jhunjhunu-333010, Rajasthan, INDIA.
[2]Associate Professor, Department of Pharmaceutical Chemistry, Mount Zion College of Pharmaceutical Sciences and Research, Adoor-691556, Kerala, INDIA.
Email: renjurenjithajr94@gmail.com

Received: 23-08-2024;
Revised: 27-08-2024;
Accepted: 04-10-2024.

INTRODUCTION

Multiple Sclerosis (MS) is a long-term autoimmune disease that affects the central nervous system and causes neurological symptoms including weakness in the muscles, abnormalities in vision and senses and deterioration in cognitive function. Although disease-modifying therapies can diminish the prospect of a relapse, they are not particularly efficacious in addressing the progressive loss of neurons that results in enduring debility. Utilizing Siponimod, a selective Sphingosine-1-Phosphate (S_1P) receptor modulator, is one feasible strategy to tackle this problem.[1,2] It has been demonstrated that Siponimod (SPD) rallies the course of multiple sclerosis, especially in those who have secondary progressive multiple sclerosis.[11,12] By confining lymphocytes in lymph nodes, SPD lessens the possibility that these cells will move to the central nervous system and fuel the inflammatory processes that advance the course of illness.[3,4] Several recent research suggests the effectiveness of SPD in treating multiple sclerosis. For instance, one study discovered that patients with multiple sclerosis may have better clinical and biomolecular results if SPD was used in conjunction with other therapies like the Bobath concept and reflex locomotion.[5-8]

The development and optimization of spectroscopic and chromatographic methods has customary a lot of attention recently due to its usefulness in a variety of applications, including metabolomics research. Due to the advancements in these instruments, researchers can look more closely at intricate systems, such as functional meals and herbal medicines. The study of phytomedicines has rapidly evolved as a result of substantial improvements in chromatographic methods.[9,10]

A methodical approach to the creation of analytical techniques known as "Quality by Design" places a focus on comprehending

DOI: 10.5530/ijpi.20250063

the essential qualities of a product and determining the crucial process variables that affect quality. Quality is enhanced and quality variations are decreased with the use of QbD. Design of Experiments, such as the Boxbehnken Design (BBD), is a crucial tool in the development of QbD-based analytical methods.[11,12]

There has been no research published on SPD optimization in drug dose formulations. The present investigations help build polynomial models to explore quadratic response surfaces and to develop an analytical approach with parallel exploration by combining Ultra Violet (UV) spectroscopic, High-performance liquid chromatography (HPLC), High-Performance Thin Layer Chromatography (HPTLC) and Spectrofluorimetric analysis (FS) for quantification of Siponimod in bulk and pharmaceutical formulations.[13,14]

MATERIALS AND METHODS

Instrumentation

Analytical Balance (Shimadzu, AP224W, New Delhi), Membrane Filter (Merck Millipore, SMWP04700, Haryana), HPLC (Agilent 1220 Infinity G4288C, New Delhi), Rheodyne injector (7725 D2-0, Scientific Laboratory Supplies, New Delhi), Double Beam Spectrophotometer (Cary 5000 Version 2.24, United States), Spectrofluorometer (Jasco FP-6200, Taiwan), HPTLC with Scanner (Camag 028.2000, German), Automatic Sample Applicator (Camag Linomat ATS 4, Mumbai), Precoated plate (Camag SG-60F_4, German), Digital Expert Stat-ease 360 Software.

Drugs and Reagents

SPD reference standard (Alkem Laboratories, India); HPLC-Grade Methanol and Water (Merck, India); Dihydrogen Orthophosphate (Arochem, Madhya Pradesh) and NaOH (Meruchem, Mumbai), Mayzent tablet (Novartis, Karnataka).

Preparation of Stock Solution

Pipette precisely enough stock solution (solution A) to achieve a concentration of 1 mg/mL using the reference standard, further dilution was made to get a 10 mcg/mL concentration of SPD standard solution.[14,15]

For preparation of the sample solution, Mayzent tablet (2 mg Siponimod) was weighed on average, precisely weighed an amount equaling 100 mg of powder on a glass stopper flask, diluted with 15 mL of methanol, sonicated for 10 min to extract the powder and filtered using Whatman No. 1 filter paper. An exact amount of the solution was pipetted and concoct a drug solution containing 100 µg/mL by dilution with methanol.[16,17]

Spectroscopic Method Development

The absorbance spectra of the SPD test and standard solutions were attained at 400-200 nm wavelength region using blank rectification with alcohol. After a 30 min initial stabilization time, a UV-visible spectrophotometer was used for the scanning process to determine the drug's lambda max.[17-19]

Spectrofluorimetric examination of SPD (10 µg/mL) was steered to ascertain the emission wavelength. Around 500 µL of this solution was combined with 10 mL of 0.1N NaOH and incubated for 10 min at 60°C. Measured 150 µL of the raised solution and diluted with 10 mL of distilled water, safranin solution was added as a fluorescent enhancing reagent. The spectrofluorometer was used to scan the prepared solution to determine the emission wavelengths. To determine the emission wavelength, scanning was done across a range of 220 nm to 800 nm then calculate the fluorescent intensity at attained emission wavelength.[11,12]

Chromatographic Method Development

Chemometric factorial design using the QbD technique led to the development of the High-Performance Liquid Chromatography (HPLC) technique. Selection of the analysis's factors was based on the Quality Target Product Profile. For the suggested strategy, the recovery % was chosen as a Critical Quality Attribute (CQA).[16,17] The chosen key parameters that had to be maintained to keep the QTPP within an acceptable response range were pH, flow rate and composition of the mobile phase. The HPLC technique's mobile phase, flow rate and pH were optimized and selected using Box Behnken-assisted surface design following the definition of the QTPP and CQAs.[15-20]

To optimize the chromatographic conditions, the Design Expert tools will be utilized. All of these parameters ensure that both the method's performance and the quality of the findings fit within the predefined analytical target profile.[20] Table 1 provides the coded range of the variables that were chosen.

To choose the Mobile Phase for High-Performance Thin Layer Chromatography (HPTLC) methods, a trial-and-error approach was used. The SPD standard stock solution (1000ng/spot) was individually spotted on an HPTLC plate using an automated sample applicator and it was then left dry at room temperature for 4-5 min.[20] The mobile phase was placed in a CAMAG glass chamber and given 20 min to saturate. Following the saturation phase, the spotted plate was left to continue developing in the saturated mobile phase. The Camag TLC Visualizer was used to observe spots related to band tailing, band form, band separation, etc., Several trials were carried out by varying the composition and ratio of the mobile phase. 0.1 µL of the supernatant layer (100 ng/spot) from the sample solution was applied to an HPTLC plate to evaluate the SPD content in the formulation of Mayzent tablets using the Chromatographic Method.[18-20]

By comparing the peak areas of the HPLC and HPTLC chromatograms with the conforming standard peaks, the concentration of each medication in the formulation was determined by observing and analyzing the chromatograms.[16]

Optimization of Veracity of the Developed Methods

The chromatograms were recorded, the interference with the standard was determined and the sample solution was examined by the technique to verify the specificity of the procedure. Blank and placebo samples were injected under the chromatographic conditions specified above.[20]

Testing the accuracy of the test procedure involved producing recovery samples of SPD at concentration levels of 20%, 100% and 150% of the stock sample, the percentage recovery of each sample was calculated for the quantity added.[9,10]

Under a range of conditions, including bench top at room temperature and in a refrigerator at 2 to 8ºC, the standard and sample solutions for solution stability were prepared. It was determined if standard and sample solutions were stable by comparing previously created standard and sample solutions with newly prepared solutions.[14] Calculating the unique, mean percentage of drug dissolved and the percentage of relative standard deviation for every set of samples was used to assess the method's validity.[12,13]

RESULTS

Spectroscopic Estimation

At the wavelength of UV maximum absorption (λ_{max}) of 258 nm, it was discovered that the SPD's chosen range was linear. Using an excitation wavelength of 258 nm and scanning throughout the range of 258 nm-620 nm, the fluorescence emission spectra were recorded in methanol and the intensity of the fluorescence was measured at 485 nm. In the concentration range of 2-20 µg/mL and 5-30 µg/mL separately, the fluorescence intensity and UV absorbance were linear. Figure 1 displays the ultraviolet and fluorescence spectra.

Chromatographic Assessment

Chemometric factorial design based on important quality parameters was used to confirm the HPLC mobile phase. The HPLC process was made more efficient by using response surface design at the low-high level. The various interactions and quadratic impacts of the organic modifiers, pH and flow rate were investigated by surface characterization using a quadratic equation, as shown in Figure 2.

The response surface plots demonstrated a functional link between the independent variables (organic phase modifier, pH and flow rate) and the dependent variable (percentage recovery). A little change in the coded factors causes a small impact on the answer, indicating the method's resilience.

For the persistence of optimizing the mobile phase, many statistical parameters were assessed, including the coefficient estimate, Variance Inflation Factors (VIF), *p*-value and f-value. The coefficient estimate, assuming that all other variables remain constant, shows the anticipated change in response for each unit change in factor value. Table 2 presents the findings of the statistical analysis.[17]

The model is deemed significant based on its F-value of 23.72. The probability that an F-value this great might be the result of noise is merely 0.39%. Model terms are considered significant when P-values are less than 0.0500. It was evident from these statistical characteristics that the petite variation was noted in line with the response's coded components. At pH 6.3, with an upsurge in flow velocity and a noticeable concentration of modifiers, the

Table 1: **Optimization Factors for Experimental Design in HPLC.**

Factors	Name	Units	Minimum	Maximum	Coded Low	Coded High	Mean	Standard Deviation
A	pH	-	6.50	7.50	-1 ↔ 6.50	+1 ↔ 7.50	7.00	0.3536
B	Organic Phase modifier	mL	10.00	30.00	-1 ↔ 10.00	+1 ↔ 30.00	20.00	7.07
C	Flow rate	mL/min	0.7500	1.25	-1 ↔ 0.75	+1 ↔ 1.25	1.0000	0.1768

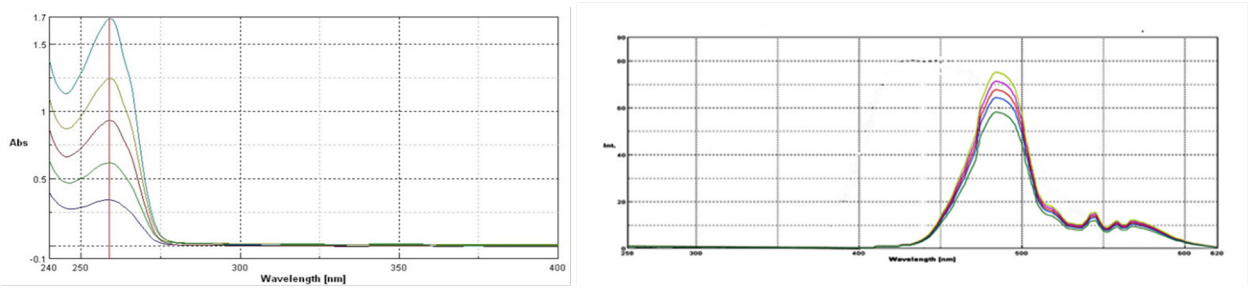

Figure 1: UV and Fluorescent Overlaid Spectrum of SPD.

chromatogram with the least amount of tailing factor and the most consistent response was achieved.

A study of several interactions led to the confirmation that the mobile phase was 0.05 M Phosphate Buffer: Acetonitrile in the ratio 75: 25 at 1.25 mL/min Flow rate at pH 6.3. The chromatogram at 10 mcg/mL was produced with a retention time of 6.623.

The chosen mobile phase for the HPTLC method was Ethyl acetate with formic acid, glacial acetic acid and water in the ratio 15:2:1.5:1.5 v/v/v/v. Camag TLC scanner IV was used to measure densitometric parameters at 248 nm. Comparing sections of HPLC and HPTLC chromatograms obtained for the sample drug solution collected in three different concentrations with that of the standard allowed for the quantitative measurement of the medication and it was discovered that the percentage recovery ranged from 92% to 100%w/w. Given in Figure 3 was the HPLC and HPTLC chromatogram.[18,19]

By comparing the chromatogram's peak area concerning a standard, the percentage recovery of the pharmaceutical formulation was determined. The outcomes of the parallel determination are displayed in Table 3.

Table 2: Outcomes of Statistical Parameters Based on Coded Factors.

Exceed Limits of Percentage Recovery				Coefficient of Coded Factors				
Run	Actual Value	Predicted Value	Residual	Factor	Coefficient Estimate	Standard Error	F-Value	*p* Value
1	0.1054	0.1049	0.0005	Intercept	0.1049	0.0002	23.72	0.0039
2	0.1048	0.1049	-0.0001	A-Ph	0.0011	0.0002	20.73	0.0104
3	0.1054	0.1054	0.0000	B-modifier	0.0007	0.0002	7.90	0.0483
4	0.1048	0.1048	0.0000	C-flow rate	0.0023	0.0002	90.82	0.0007
5	0.1054	0.1054	0.0000	AB	0.0001	0.0002	0.3622	0.5797
6	0.1048	0.1049	-0.0001	AC	-0.0011	0.0002	20.73	0.0104
7	0.1054	0.1054	0.0000	BC	0.0026	0.0002	114.79	0.0004
8	0.1054	0.1054	0.0000	A^2	-0.0004	0.0002	3.53	0.1336
9	0.1048	0.1048	0.0000	B^2	0.0008	0.0002	10.42	0.0320
10	0.1054	0.1049	0.0005	C^2	-0.0005	0.0002	4.25	0.1084
11	0.1048	0.1048	0.0000	ABC	ALIASED			
12	0.1043	0.1043	0.0000	A^2B	-0.0008	0.0003	5.82	0.0733
13	0.1048	0.1048	0.0000	A^2C	-0.0015	0.0003	19.10	0.0120
14	0.1010	0.1010	0.0000	AB^2	-0.0010	0.0003	7.81	0.0491

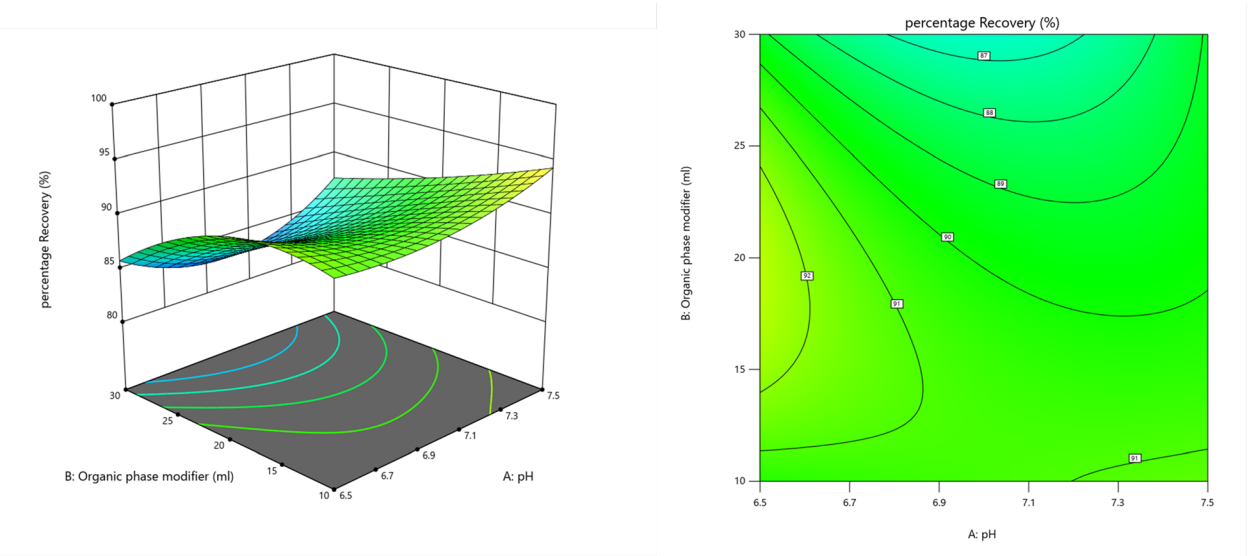

Figure 2: Response surface and corresponding Contour plot of SPD.

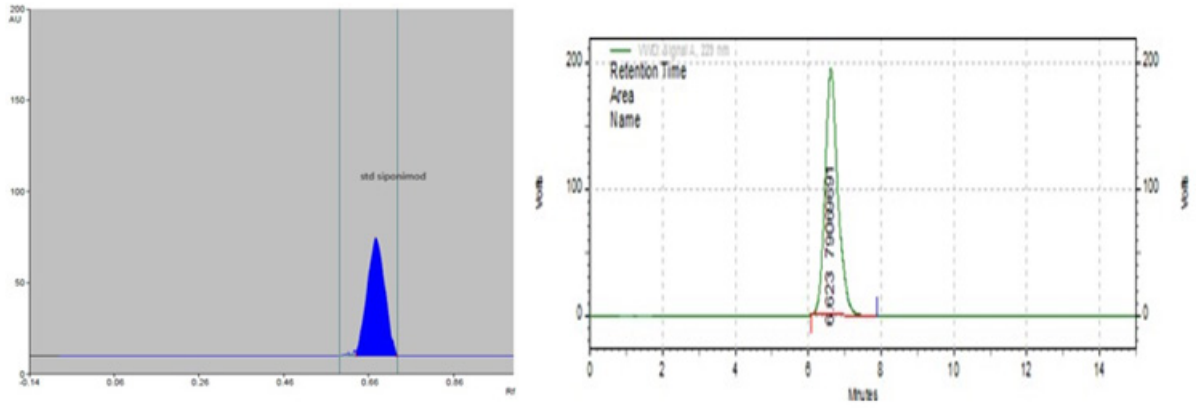

Figure 3: HPTLC and HPLC chromatogram of SPD.

Table 3: Assay results of Siponimod in Tablet formulation.

Fluorescence spectroscopy	Concentration (µg/mL)	Fluorescence Intensity		Amount in Tablet(mg)	Label Claim (%)
		Sample	Standard		
	2	162.98	160.66	1.9715	98.57
	4	322.13	320.96	1.9927	99.63
	8	634.25	626.89	1.9766	98.83
Ultra Violetspectroscopy	**Concentration (µg/mL)**	**Absorbance**		**Amt in Tablet(mg)**	**Label Claim (%)**
		Sample	**Standard**		
	5	0.3324	0.3526	1.8854	94.27
	15	0.9965	1.0625	1.8757	93.78
	30	1.8956	2.0253	1.8719	93.59
High Pressure Liquid Chromatography	**Concentration (µg/mL)**	**Peak Area**		**Amount in Tablet(mg)**	**Label Claim (%)**
		Sample	**Standard**		
	50	33654871	35642842	1.8884	94.20
	100	77849432	79006975	1.9706	98.53
	200	147658562	158245352	1.8661	93.30
High Performance Thin layer Chromatography	**Concentration (ng/spot)**	**Peak Area**		**Amount in Tablet(mg)**	**Label Claim(%)**
		Sample	**Standard**		
	100	4199.23	4534.27	1.8922	94.61
	200	8196.78	8462.36	1.9372	96.86
	400	15634.14	17462.76	1.8906	94.53

*Label claim of tablet was 2 mg.

Validation of the Developed Methods

When the Blank and placebo samples are compared to the standard for interference, the sample solution according to the approach displays no obtrusive peaks. Recovery at 20%, 100% and 150% recoveries are satisfactory. Calculating the individual, mean percent of drug dissolved and percentage of relative standard deviation for each set of samples validated the concordant results, which are achieved at repeated measurement at room temperature and reflect techniques accuracy. The validation outcomes were displayed in the Table 4.[14,16]

DISCUSSION

Four analytical techniques, including spectrophotometric, spectrofluorimetric, HPTLC and HPLC were used to assess the Siponimod tablet. The maximal absorbance and fluorescence emission of SPD are seen at 258 nm and 485 nm respectively, with

Table 4: Validation Results of Established Method.

Parameters	UV	Fluorimetry	HPTLC	HPLC
Linearity	5-30 µg/mL	2-20 µg/mL	100-500 ng	50-300 µg/mL
Intercept	0.0069	0.0467	0.0045	40635
Slope	0.0285	0.1027	0.0211	22100
Relative Standard Deviation (%)	0.8433	0.4021	0.7326	0.3509
Coefficient of Variation	0.0084	0.0040	0.0073	0.0035
Correlation Coefficient	0.9988	0.9893	0.9977	0.9941
Limit of Detection (µg/mL)	9.764	1.299	2.0738	0.5×10^{-4}
Limit of Quantitation (µg/mL)	10.6592	3.938	6.2843	0.6×10^{-4}
Drug Recovery (%)	98.77	97.25	98.49	98.90

linearities in UV absorption of 5-30 mcg/mL and fluorescence intensity of 2-20 mcg/mL.[9] RP-HPLC column with a non-polar mobile phase at pH 6.3, a flow rate of 1.25 mL/min and retention periods of 6.64 min are used to separate the aforementioned drugs. The varied interaction effects and quadratic impacts of the independent factors on percentage recovery (dependent variables) were investigated using statistical screening design utilizing Design Expert® and quadratic response.[20]

The method's robustness is demonstrated by the substantial response surface plots of certain quadratic model terms. The HPTLC method was created through a series of experiments employing the linear ascending development approach and a wide range of solvents in various ratios. Ethyl acetate, formic acid, glacial acetic acid and water (15:2:1.5:1.5 v/v/v/v), the optimized mobile phase, demonstrated an acceptable resolution at R_f 0.68.[17,18]

The linearity of the study supports the reliability of the approach with a regression equation that has a coefficient of 0.9999, stability for over 3 hr at room temperature and LOD and LOQ that are within the range. The low standard deviation of the recovery % served as further evidence of the method's dependability. At no point throughout the method's development was excipient interference seen.

The RP-HPLC technique was utilized to separate the drug in tablet dosage form for this study since the medication under examination was polar and could be separated utilizing reverse-phase chromatography. A C_{18} column served as the stationary phase, while the mobile phase consisted of 0.05 M phosphate buffer mixed with acetonitrile in a 75:25 ratio. To get the right resolution, several ratios were then applied. Pharmaceutical retention times are shortened when the proportion of organic modifiers such as KH_2PO_4 in the mobile phase is increased, as was shown during mobile phase optimization and selection.

A greater proportion of organic phase results in a sharper peak, whereas a greater proportion of aqueous phase creates a wider peak. The pH of the mobile phase was chosen such that it was at least two units away from the pKa of the medication. The wavelength of 254 nm, 400 bar pump pressure 15 min stop time were the chosen experimental conditions. The method yielded good resolution and an optimal retention time. The validity of the method was demonstrated and the suggested approach was found to be appropriate for both pharmaceutical formulation and routine SPD screening in bulk dose form correspondingly in the methods can use for biomedical screening of SPD.

CONCLUSION

Several methods were established for the quantitative measurement of Siponimod in pharmaceutical dose form, including UV, FS, HPLC and HPTLC. In addition to ethyl acetate, formic acid, glacial acetic acid and water at varying concentrations for HPTLC reveal distinct peaks for SPD, mobile phases for HPLC were diverse ratios of acetonitrile and phosphate buffer. Box Behnken validation indicates stability; it shows how changes in flow rate, pH and the presence of organic phase modifiers affect percentage recovery. A statistical analysis using variables revealed the method's significance. The new technique's uniqueness is indicated by the distinct peaks seen in the UV and fluorescence spectra.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

SPD: Siponimod; **UV:** Ultra Violet; **HPLC:** High-Performance Liquid Chromatography; **HPTLC:** High Performance Thin Layer Chromatography; **FS:** Fluorescence Spectroscopy; **LOD:** Limit of Detection; **LOQ:** Limit of Quantitation; **RSD:** Relative Standard Deviation; **RA:** Rheumatoid Arthritis; **BBD:** Box Behnken Design; **RSP:** Response Surface Plot; **CP:** Contour Plot.

REFERENCES

1. Bigaud M, Rudolph B, Briard E, Beerli C, Hofmann A, Hermes E, *et al.* Siponimod penetrates, distributes and acts in the central nervous system: preclinical insights. Mult Scler J Exp Transl Clin. 2021;7(4):20552-68.
2. Bobinger T, Manaenko A, Burkardt P, Beuscher V, Sprügel MI, Roeder SS, *et al.* Siponimod (BAF-312) attenuates perihemorrhagic edema and improves survival in

experimental intracerebral hemorrhage. Stroke. 2019;50(11):3246-54. doi: 10.1161/STROKEAHA.119.027134, PMID 31558140.

3. Chaoyang C, Xiu D, Ran W, Lingyun M, Simiao Z, Ruoming L, *et al.* Pharmacokinetic characteristics of siponimod in healthy volunteers and patients with multiple sclerosis: analyses of published clinical trials. Front Pharmacol. 2022;13(1):824232. doi: 10.3389/fphar.2022.824232, PMID 35620290.
4. Shakeri-Nejad K, Gardin A, Gray C, Neelakantham S, Dumitras S, Legangneux E. Safety, tolerability, pharmacodynamics and pharmacokinetics of intravenous siponimod: a randomized, open-label study in healthy subjects. Clin Ther. 2020;42(1):175-95. doi: 10.1016/j.clinthera.2019.11.014, PMID 31926605.
5. Glaenzel U, Jin Y, Nufer R, Li W, Schroer K, Adam-Stitah S, *et al.* Metabolism and disposition of siponimod, a novel selective S1P1/S1P5 agonist, in healthy volunteers and *in vitro* identification of human cytochrome P450 enzymes involved in its oxidative metabolism. Drug Metab Dispos. 2018;46(7):1001-13. doi: 10.1124/dmd.117.079574, PMID 29735753.
6. Bigaud M, Ramseier P, Tisserand S, Lang M, Urban B, Beerli C, *et al.* Central versus peripheral drug exposure ratio, a key differentiator for siponimod over fingolimod? Neurol Ther. 2023;12(4):1187-203. doi: 10.1007/s40120-023-00487-4, PMID 37195409.
7. Kappos L, Li DK, Stüve O, Hartung HP, Freedman MS, Hemmer B, *et al.* Safety and efficacy of siponimod (BAF312) in patients with relapsing-remitting multiple sclerosis: dose-blinded, randomized extension of the phase 2 BOLD study. JAMA Neurol. 2016;73(9):1089-98. doi: 10.1001/jamaneurol.2016.1451, PMID 27380540.
8. Díaz-Villamarín X, Piñar-Morales R, Barrero-Hernández FJ, Antúnez-Rodríguez A, Cabeza-Barrera J, Morón-Romero R. Pharmacogenetics of siponimod: A systematic review. Biomed Pharmacother. 2022;153(1):113536. doi: 10.1016/j.biopha.2022.113536, PMID 36076616.
9. Gentile A, Musella A, Bullitta S, Fresegna D, De Vito F, Fantozzi R, *et al.* Siponimod (BAF312) prevents synaptic neurodegeneration in experimental multiple sclerosis. J Neuroinflammation. 2016;13(1):207. doi: 10.1186/s12974-016-0686-4, PMID 27566665.
10. O'Sullivan C, Schubart A, Mir AK, Dev KK. The dual S1PR1/S1PR5 drug BAF312 (siponimod) attenuates demyelination in organotypic slice cultures. J Neuroinflammation. 2016;13(1):31. doi: 10.1186/s12974-016-0494-x, PMID 26856814.
11. Sabsabi S, Mikhael E, Jalkh G, Macaron G, Rensel M. Clinical evaluation of siponimod for the treatment of secondary progressive multiple sclerosis: pathophysiology, efficacy, safety, patient acceptability and adherence. Patient Preference Adherence. 2022;16:1307-19. doi: 10.2147/PPA.S221882, PMID 35637684.
12. Dobson R, Giovannoni G. Multiple sclerosis-a review. Eur J Neurol. 2019;26(1):27-40. doi: 10.1111/ene.13819, PMID 30300457.
13. Rudick RA, Cohen JA, Weinstock-Guttman B, Kinkel RP, Ransohoff RM. Management of multiple sclerosis. N Engl J Med. 1997;337(22):1604-11. doi: 10.1056/NEJM199711273372207, PMID 9371858.
14. Tömösi F, Kecskeméti G, Cseh EK, Szabó E, Rajda C, Kormány R, *et al.* A validated UHPLC-MS method for tryptophan metabolites: application in the diagnosis of multiple sclerosis. J Pharm Biomed Anal. 2020;185(1):113246. doi: 10.1016/j.jpba.2020.113246, PMID 32182446.
15. Alam P, Shakeel F, Taleuzzaman M, Foudah AI, Alqarni MH, Aljarba TM, *et al.* Box-Behnken Design (BBD) application for optimization of chromatographic conditions in RP-HPLC method development for the estimation of thymoquinone in nigella sativa seed powder. Processes. 2022;10(6):1082. doi: 10.3390/pr10061082.
16. Haque SM. Box-Behnken experimental design for optimizing the HPLC method to determine hydrochlorothiazide in pharmaceutical formulations and biological fluid. J Mol Liq. 2022;352:1187-89. doi: 10.1016/j.molliq.2022.118708.
17. Khorshidi N, Rahimi M, Salimikia I. Application of aeration-assisted homogeneous liquid-liquid microextraction procedure using Box-Behnken design for determination of curcumin by HPLC. J Sep Sci. 2020;43(13):2513-20. doi: 10.1002/jssc.202000001, PMID 32255267.
18. Alam P, Shakeel F, Iqbal M, Foudah AI, Alqarni MH, Aljarba TM, *et al.* Quantification of pomalidomide using conventional and eco-friendly stability-indicating HPTLC assays: A contrast of validation parameters. ACS Omega. 2023;8(33):30655-64. doi: 10.1021/acsomega.3c04382, PMID 37636909.
19. Wadie M, Abdel-Moety EM, Rezk MR, Marzouk HM. A novel smartphone HPTLC assaying platform versus traditional densitometric method for simultaneous quantification of alfuzosin and solifenacin in their dosage forms as well as monitoring content uniformity and drug residues on the manufacturing equipment. RSC Adv. 2023;13(17):11642-51. doi: 10.1039/d3ra01211e, PMID 37063718.
20. Alam P, Shakeel F, Alqarni MH, Foudah AI, Aljarba TM, Ghoneim MM, *et al.* Development and validation of a stability-indicating greener HPTLC method for the estimation of flufenamic acid. Separations. 2023;10(1):39. doi: 10.3390/separations10010039.

Cite this article: Renjitha JR, Prasanth VV, Shah CN. QBD-Based Development of Experimental Design with Response Surface-Based Optimization of Siponimod by Chromatographic and Spectroscopic Method. Int. J. Pharm. Investigation. 2025;15(1):248-54.