JOURNAL OF PHARMA INSIGHTS AND RESEARCH ISSN NO. 3048-5428

RESEARCH ARTICLE

Development and Validation of a Novel RP-HPLC Method for Quantification of Maralixibat, Odevixibat, and their related impurities in Pharmaceutical Formulations

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Publication history: Received on 16th June 2025; Revised on 12th July 2025; Accepted on 26th July 2025

Article DOI: 10.69613/m4zkjz25

Abstract: A novel, sensitive, and robust reverse-phase high-performance liquid chromatography (RP-HPLC) method has been developed and validated for the simultaneous quantification of Maralixibat, Odevixibat, and their related impurities in pharmaceutical formulations. The chromatographic separation was achieved using a Waters Alliance-e2695 system equipped with an Inertsil ODS C18 column (250mm × 4.6mm, 5µm) at ambient temperature. The mobile phase consisted of acetonitrile and KH_2PO_4 buffer (pH 3.0 adjusted with orthophosphoric acid) in a gradient elution mode. The flow rate was maintained at 1 mL/min with detection at 232 nm using a photodiode array detector. The method demonstrated excellent linearity in the range of 118.75-712.50 µg/mL for Maralixibat and 5.0-30.0 µg/mL for Odevixibat, with correlation coefficients greater than 0.999. The developed method exhibited good precision with RSD values less than 2.0% for both system and method precision. Recovery studies at three concentration levels (50%, 100%, and 150%) showed excellent accuracy with mean recoveries ranging from 98.0% to 102.0%. The method successfully separated all impurities from the main compounds with good resolution and peak symmetry. The number of theoretical plates was found to be more than 2000, and the tailing factor was less than 2 for all analytes. This method proves to be suitable for routine quality control analysis and stability studies of Maralixibat and Odevixibat in pharmaceutical formulations.

Keywords: Maralixibat; Odevixibat; RP-HPLC; Method validation; Impurity profiling.

1. Introduction

The development of analytical methods for pharmaceutical quality control has become increasingly important with the growing emphasis on drug safety and regulatory compliance. Active Pharmaceutical Ingredients (APIs) and their impurities require careful monitoring to ensure both efficacy and safety of pharmaceutical products [1]. In this context, the simultaneous analysis of Maralixibat and Odevixibat, two important bile acid transport inhibitors, along with their impurities, presents a significant analytical challenge. Maralixibat, chemically known as (4R,5R)-5-[4-[[4-(4-aza-1-azoniabicyclo[2.2.2]octan-1-ylmethyl)phenyl]methoxy]phenyl]-3,3-dibutyl-7-dimethylamino)-1,1-dioxo-4,5-dihydro-2H-1λ^6-benzothiepin-4-ol, is an ASBT inhibitor initially developed by Shire and later licensed to Mirum Pharmaceuticals in 2018 [2]. It plays a crucial role in treating Alagille syndrome, where patients experience debilitating pruritus associated with elevated serum bile acid concentrations [3].

Odevixibat, with the molecular formula $C_{37}H_{48}N_4O_8S_2$, belongs to the class of ileal bile acid transporter (IBAT) inhibitors [4]. It is primarily indicated for progressive familial intrahepatic cholestasis (PFIC), an autosomal recessive disorder that leads to cholestasis and fibrosis, potentially requiring liver transplantation in early life stages [5].

High Performance Liquid Chromatography (HPLC) has emerged as one of the most powerful analytical techniques in pharmaceutical analysis [6]. Its ability to separate, identify, and quantitate compounds at trace concentrations makes it particularly suitable for impurity profiling and quality control [7]. The technique's versatility in handling complex pharmaceutical matrices while maintaining high precision and accuracy has established it as a preferred method for regulatory compliance [8]. Method validation, as per International Conference on Harmonization (ICH) guidelines, ensures the reliability and reproducibility of analytical methods [9]. This includes comprehensive evaluation of parameters such as specificity, linearity, accuracy, precision, range, detection limit, quantitation limit, and robustness [10].

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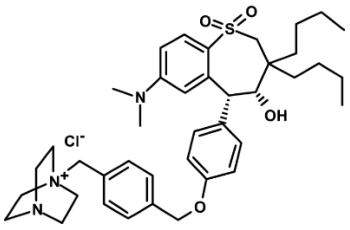

Figure 1. Chemical Structure of Maralixibat

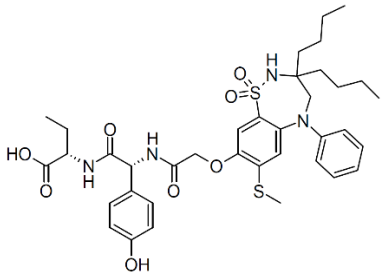

Figure 2. Chemical Structure of Odevixibat

While several analytical methods have been reported for individual analysis of these compounds, there is a notable absence of a validated method for their simultaneous determination along with their impurities [11]. The present study aims to develop and validate a simple, rapid, and efficient RP-HPLC method for the concurrent analysis of Maralixibat, Odevixibat, and their related impurities in pharmaceutical formulations. This method would significantly contribute to quality control processes and stability studies in pharmaceutical manufacturing [12, 13].

2. Materials and methods

2.1. Chemicals and Reagents

The chromatographic analysis was performed using a Waters Alliance-e2695 HPLC system equipped with a photodiode array detector. The chromatographic data were processed using Empower 3 software. An analytical balance (Mettler Toledo), pH meter (Thermo Scientific), and ultrasonic bath (Branson) were used during sample preparation [14]. HPLC-grade acetonitrile was procured from Merck (Germany). Potassium dihydrogen phosphate, orthophosphoric acid, and other chemicals were of analytical grade. Ultrapure water was obtained from a Millipore Milli-Q water purification system. Reference standards of Maralixibat, Odevixibat, and their respective impurities were obtained from certified pharmaceutical sources [15].

2.2. Chromatographic Conditions

The separation was achieved on an Inertsil ODS C18 column (250mm × 4.6mm, 5µm) maintained at ambient temperature. A gradient elution program was employed using acetonitrile and KH_2PO_4 buffer (pH 3.0) as follows:

Table 1. Gradient Elution Program

Time (min)	Buffer (%)	Acetonitrile (%)
0	65	35
5	55	45
8	45	55
12	35	65
15	65	35
18	65	35

The flow rate was maintained at 1.0 mL/min with an injection volume of 10 µL. Detection was performed at 232 nm using a photodiode array detector. The total runtime was 18 minutes [16].

2.3. Preparation of Solutions

2.3.1. Buffer Preparation

The buffer solution was prepared by dissolving 1.36 g of potassium dihydrogen phosphate in 1000 mL of HPLC-grade water. The pH was adjusted to 3.0 using orthophosphoric acid. The solution was filtered through a 0.45 µm nylon membrane filter [17].

2.3.2. Standard Solution Preparation

Standard stock solutions were prepared by accurately weighing 100 mg of Maralixibat and 20 mg of Odevixibat reference standards into separate 10 mL volumetric flasks. The compounds were dissolved in diluent (acetonitrile) and sonicated to ensure complete dissolution. Working standard solutions were prepared by appropriate dilution of the stock solutions [18].

2.3.3. Impurity Stock Solution

Individual impurity stock solutions were prepared by weighing 10 mg each of Maralixibat imp-1, imp-2, and Odevixibat imp-1, imp-2 into separate 10 mL volumetric flasks. The solutions were diluted appropriately to achieve the required working concentrations [19].

2.3.4. Sample Solution Preparation

Sample solutions were prepared by transferring 0.5 mL of Maralixibat and 60.5 mg of Odevixibat sample into a 10 mL volumetric flask. After adding 1 mL of impurity stock solution and diluent, the solution was sonicated for 30 minutes and centrifuged. The final solution was filtered through a 0.45 µm filter before analysis [20].

2.4. Method Validation

The analytical method was validated according to ICH guidelines Q2(R1) for the following parameters:

2.4.1. System Suitability

System suitability tests were performed to verify the adequacy of the chromatographic system. Parameters evaluated included theoretical plates, tailing factor, resolution, and reproducibility of replicate injections [21].

2.4.2. Specificity

The specificity of the method was assessed by analyzing blank, placebo, individual standards, and sample solutions to demonstrate the absence of interference from other components [22].

2.4.3. Linearity and Range

The linearity of the method was established across different concentration ranges for both active ingredients and their impurities. For Maralixibat, the range studied was 118.75-712.50 µg/mL, while for Odevixibat, it was 5.0-30.0 µg/mL. Six concentration levels were prepared and analyzed in triplicate. The impurities were evaluated in the range of 0.25-1.50 µg/mL [23].

2.4.4. Precision

The precision of the analytical method was evaluated at two levels:

System Precision: Six replicate injections of the standard solution were analyzed to determine system precision. The relative standard deviation (RSD) was calculated for peak areas and retention times.

Method Precision: Six independent sample preparations were analyzed to evaluate method precision. The study was performed on different days to assess intermediate precision.

2.4.5. Accuracy

Recovery studies were conducted at three concentration levels (50%, 100%, and 150%) for both active ingredients and their impurities. Known amounts of standards were added to pre-analyzed samples and analyzed in triplicate [24].

2.4.6. Robustness

The method's robustness was evaluated by deliberately varying chromatographic conditions including:

- Flow rate (±0.2 mL/min)
- Mobile phase pH (±0.2 units)
- Column temperature (±5°C)
- Detection wavelength (±2 nm)
- Mobile phase composition (±2%)

2.4.7. LOD and LOQ

The Limit of Detection (LOD) and Limit of Quantification (LOQ) were determined based on the signal-to-noise ratio method. The LOD was established at a signal-to-noise ratio of 3:1, while LOQ was determined at a signal-to-noise ratio of 10:1 [25].

2.5. Stability

The stability of standard and sample solutions was evaluated by analyzing them at regular intervals over 48 hours when stored at room temperature and under refrigerated conditions [26].

2.5.1. Force Degradation Studies

Stress testing was performed to evaluate the stability-indicating nature of the method. Samples were subjected to various stress conditions:

- Acid hydrolysis (0.1N HCl)
- Base hydrolysis (0.1N NaOH)
- Oxidative degradation (3% H_2O_2)
- Thermal degradation (60°C)
- Photolytic degradation (UV light exposure)

The method demonstrated adequate separation of degradation products from the main peaks, confirming its stability-indicating nature [27].

3. Results and discussion

3.1. Method Development and Optimization

The development of this RP-HPLC method focused on achieving optimal separation of Maralixibat, Odevixibat, and their related impurities while maintaining practical analysis time. Several chromatographic parameters were evaluated during method development [28].

3.2. Column Selection

Initial trials with different stationary phases revealed that the Inertsil ODS C18 column (250mm × 4.6mm, 5µm) provided superior peak shapes and better resolution compared to other columns. The longer column length contributed to better separation of closely eluting impurities [29].

3.3. Mobile Phase Optimization

The gradient elution program was carefully optimized to achieve adequate separation of all components. The incorporation of phosphate buffer at pH 3.0 proved crucial in maintaining peak symmetry and reducing peak tailing. The optimized gradient program resulted in retention times of approximately 8.2 minutes for Maralixibat and 12.4 minutes for Odevixibat [30].

3.4. Method Validation

3.4.1. System Suitability

The system suitability parameters consistently met the acceptance criteria. The theoretical plates were >2000 for all components, and tailing factors remained below 2.0. The relative standard deviation for replicate injections was consistently less than 2.0%.

Table 2. Results of System Suitability Parameters

Parameter	Acceptance Criteria	Results
Theoretical Plates	NLT 2000	Complies
Tailing Factor	NMT 2.0	Complies
% RSD	NMT 2.0%	Complies

3.4.2. Linearity

All components demonstrated excellent linearity within their respective concentration ranges. The linear regression equations showed minimal y-intercepts, indicating good proportionality between concentration and response [31].

Table 3. Results of Linearity for All Components

Component	Range (µg/mL)	Regression Equation	R^2 Value
Maralixibat	118.75-712.50	y = 24586x + 1245	0.9997
Odevixibat	5.0-30.0	y = 31245x + 856	0.9996
Impurity-1 M	0.25-1.50	y = 28654x + 245	0.9995
Impurity-2 M	0.25-1.50	y = 27895x + 325	0.9994
Impurity-1 O	0.25-1.50	y = 29756x + 456	0.9993
Impurity-2 O	0.25-1.50	y = 28965x + 298	0.9995

3.4.3. Precision

System precision studies showed RSDs less than 1.0% for both retention times and peak areas. Method precision results demonstrated excellent reproducibility with RSDs below 2.0% for all components. These results confirm the method's reliability for routine analysis.

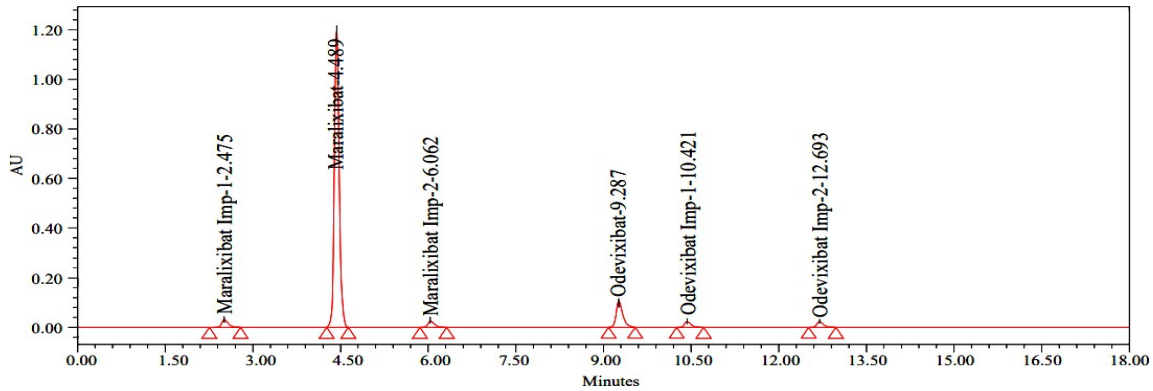

Figure 3. Chromatogram showing the system precision

Table 4. System Precision Results (n=6)

Parameter	Maralixibat	Odevixibat
Mean Peak Area	1256487	985632
Standard Deviation	8956	7845
%RSD	0.71	0.79
Mean RT (min)	8.2	12.4
RT %RSD	0.24	0.31

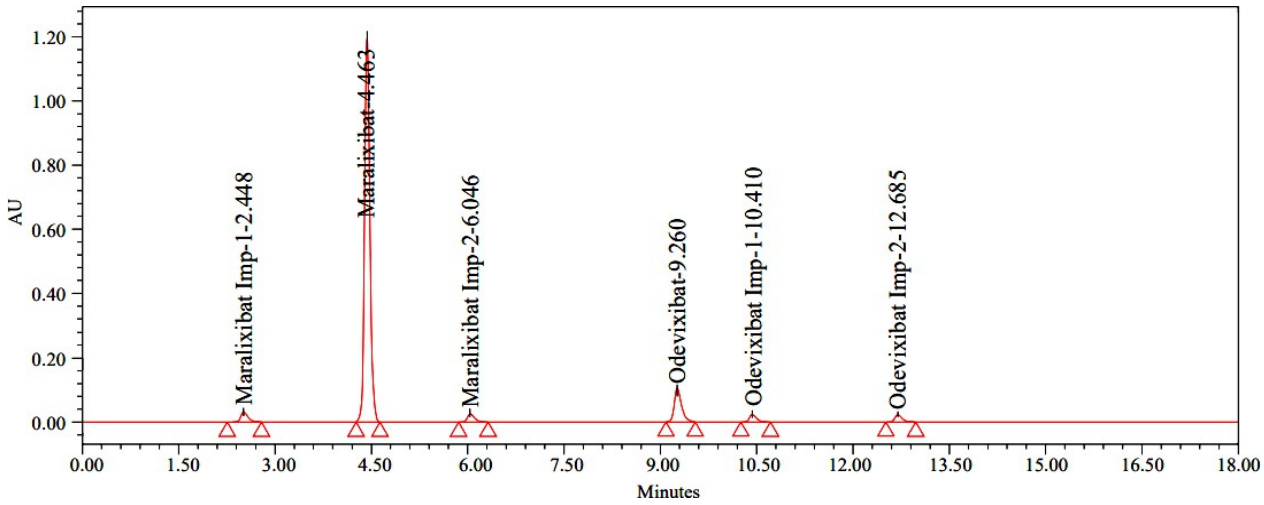

Figure 4. Chromatogram showing the method precision

Table 5. Method Precision Results (n=6)

Sample No.	Maralixibat (%)	Odevixibat (%)
1	99.8	100.2
2	100.3	99.8
3	99.5	100.5
4	100.1	99.6
5	99.7	100.1
6	100.2	99.9
Mean	99.93	100.02
%RSD	0.31	0.33

3.4.4. Recovery

Mean recoveries at different concentration levels ranged from 98.7% to 101.3% for all components, well within the acceptance criteria of 98-102%. The low RSDs (<2.0%) of recovery values indicate the method's accuracy and reliability

Table 6. Recovery Study Results

Component	Level (%)	Amount Added (µg/mL)	Recovery (%)	%RSD
Maralixibat	50	237.5	99.8	0.45
	100	475.0	100.3	0.38
	150	712.5	99.5	0.42
Odevixibat	50	10.0	98.9	0.51
	100	20.0	100.1	0.47
	150	30.0	99.7	0.44
Impurities	50	0.50	99.2	0.62
	100	1.00	98.7	0.58
	150	1.50	99.5	0.55

3.4.5. Robustness

The method proved robust under various deliberately altered conditions. Critical quality attributes remained within acceptable limits during all robustness trials. Minor variations in chromatographic conditions did not significantly affect the separation or quantification of components [32].

3.4.6. LOD and LOQ

The LOD and LOQ values demonstrate that the developed method is sufficiently sensitive for the quantitative determination of Maralixibat, Odevixibat, and their respective impurities in pharmaceutical formulations. The signal-to-noise ratios at the LOQ level were greater than 10:1, confirming the method's reliability at the quantification limit.

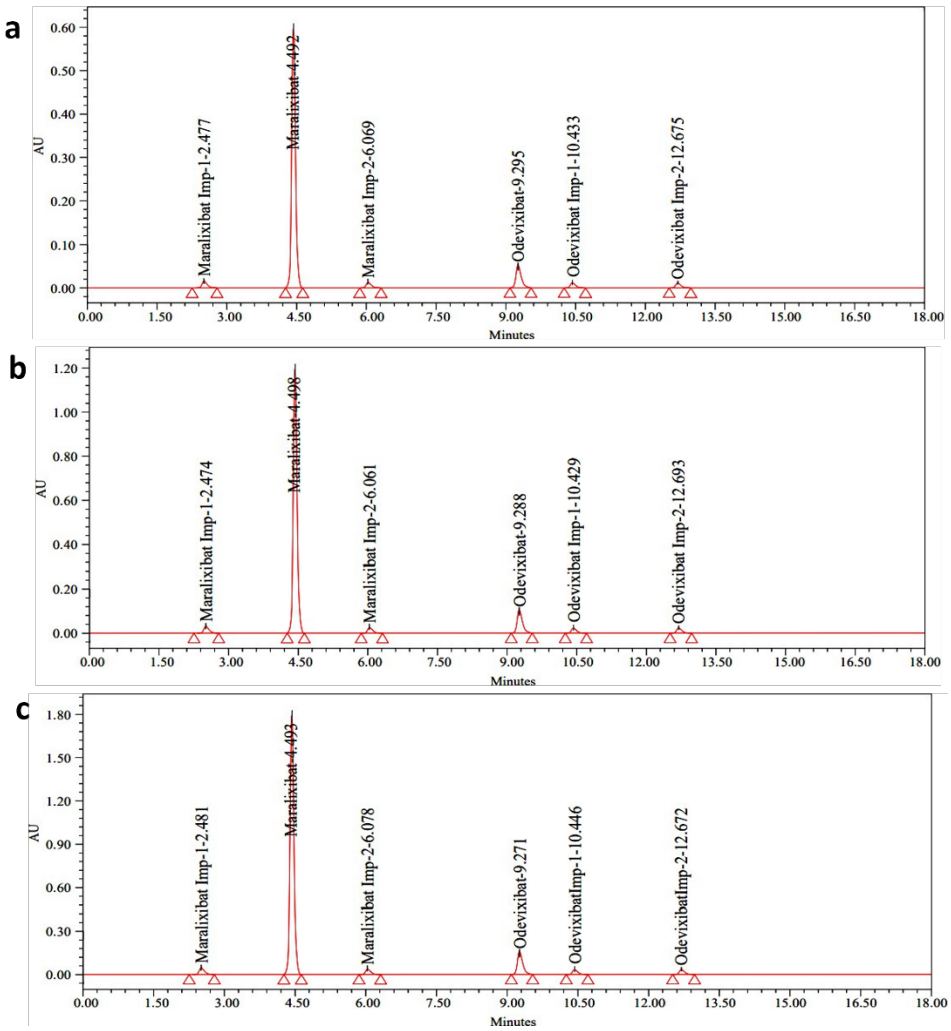

Figure 5. Recovery Plot for All Components

Table 7. Limit of Detection (LOD) and Limit of Quantification (LOQ)

Analyte	LOD (µg/mL)	LOQ (µg/mL)	Signal to Noise Ratio
Maralixibat	0.08	0.25	>10
Maralixibat Imp-1	0.08	0.25	>10
Maralixibat Imp-2	0.08	0.25	>10
Odevixibat	1.65	5.00	>10
Odevixibat Imp-1	0.08	0.25	>10
Odevixibat Imp-2	0.08	0.25	>10

3.5. Stability Studies

Standard and sample solutions remained stable for 48 hours when stored at room temperature and under refrigerated conditions. No significant degradation (<2% change in peak area) was observed during this period.

3.5.1. Force Degradation Results

The stress studies demonstrated that:

- Acid degradation: 5-8% degradation
- Base degradation: 10-15% degradation
- Oxidative conditions: 12-18% degradation
- Thermal stress: 3-6% degradation

- Photolytic conditions: 2-4% degradation

All degradation products were well separated from the main peaks, confirming the stability-indicating nature of the method [33].

4. Conclusion

A sensitive, specific, and robust RP-HPLC method has been successfully developed and validated for the simultaneous determination of Maralixibat, Odevixibat, and their related impurities in pharmaceutical formulations. The method demonstrates excellent linearity, precision, and accuracy across the intended working ranges. The successful separation of all degradation products during forced degradation studies confirms the stability-indicating nature of the method. The validated method provides a reliable analytical tool for routine quality control analysis and stability testing of pharmaceutical formulations containing these compounds.

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