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A Stability Indicating Method for Determination of Odevixibat using High Performance Liquid Chromatography Method

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Abstract

A sensitive, accurate and precise stability-indicating high performance liquid chromatography (HPLC) method was developed for determination of odevixibat in pure form and pharmaceutical formulation. In this study, comprehensive stress testing of odevixibat has been carried out according to ICH guidelines Q1A (R2). Odevixibate was subjected to forced degradation studies under hydrolytic (acid and base), oxidative, photolysis, and thermal stress conditions. The chromatographic separation was performed using a Phenomenex C_8 column, flow rate of 1.0 mL/min, and UV detection at 224 nm. The optimized HPLC system was achieved using mobile phase composition containing methanol:water (85:15, v/v). The proposed method showed well separation between the drug and its degradation products and had a good accuracy. The method was linear with 5-50 µg mL^{-1} for odevixibat. The method showed to be linear (r_2 > 0.999), precise (RSD < 0.350%), accurate (recovery of 99.95% for odevixibat), specific and robust. LOD and LOQ values were 1.09 µg mL^{-1} and 3.60 µg mL^{-1} for odevixibat. The method was validated according to ICH guidelines and applied for determination of the cited drugs in its pharmaceutical formulation.

Keywords: Odevixibat; HPLC; Stability indicating method; Pharmaceutical formulation.

1. Introduction

Odevixibat, (2S)-2-[[(2R)-2-[[2-[(3,3-dibutyl-7-methylsulfanyl-1,1-dioxo-5-phenyl-2,4-dihydro-1λ6,2,5-benzothiadiazepin-8-yl)oxy]acetyl]amino]-2-(4-hydroxyphenyl)acetyl]amino]butanoic acid, was approved for medical use in the United States and in the European Union for the treatment of pruritus in people three months of age and older with progressive familial intrahepatic cholestasis (PFIC).[1,2-4] Odevixibat is soluble in dimethyl sulfoxide and alcohol. It has a molecular formula of $C_{37}H_{48}N_4O_8S_2$, and molecular weight of 740.93 g/mol as shown in **Fig 1**. There are no published methods yet to assign this drug in pharmaceutical preparations.[1,2-9] The aim of the present work was to develop and validate a new HPLC method for determination of odevixibat in pure form and its pharmaceutical formulation. This study describes forced degradation of odevixibat as prescribed under international council for harmonization (ICH) guidelines to quantify the percentage of drug degradation.[10]The developed method was validated in accordance with ICH guidelines Q2 (R1).[11]

2. EXPERIMENTAL

2.1. Pure sample

Odevixibat 98% was kindly provided as a gift sample by Albireo Pharma, Inc, USA.

2.2. Pharmaceutical preparation

Bylvay® capsules was supplied as a gift sample by (US-based Biopharmaceutical Company Albireo Pharma), each Tablet contains 200 mcg of odevixibat.

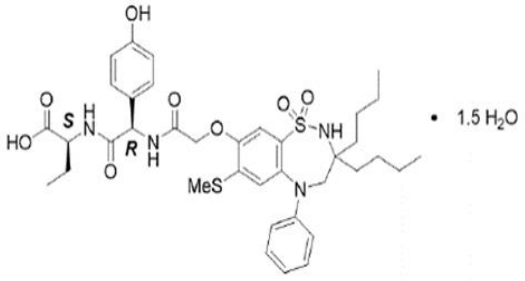

Figure 1: Structure formula of odevixibat

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2.3. Chemicals and solvents

Organic solvents methanol (HPLC gradient grade) was purchased from Sigma-Aldrich (Steinheim, Germany). Deionized water (WP 4100 reagent grade water purifier-SMEG) was used for standard and sample preparations.

Hydrogen peroxide (30%) (Sigma-Aldrich (Steinheim, Germany)), prepared as 3% H_2O_2 aqueous solution.

Hydrochloric acid (37%) (Sigma-Aldrich (Steinheim, Germany)), prepared as 0.1N HCL aqueous solutions.

Sodium hydroxide pellets (99.0%) (Sigma-Aldrich (Steinheim, Germany)), prepared as 0.1N NaOH aqueous solutions.

2.4. Instrumentation

The HPLC system used was Hitachi L-2000 series (Japan), equipped with a Model L-2130 pump, an L injection loop and a UV-Vis detector L2420. Theμon-line solvent vacuum degasser, an autosampler with mobile phase consisted of methanol: water (85:15, v/v). The system was operated isocratically at flow rate 1 min/mL and UV wavelength 224 nm.

2.5. Standard solutions

Standard stock solution (200 μg mL^{-1}) of odevixibate was prepared in methanol. Odevixibate working solutions in the desired concentration range was prepared by appropriate dilution of standard stock solution with mobile phase. The quality control (QC) samples were prepared from another prepared odevixibat stock solutions. The stock solutions were prepared once a month, kept at 2–8 ˚C in a refrigerator and brought to room temperature before use.

2.6. Procedure

2.6.1. Chromatographic condition

The separation was carried out in a Phenomenex C8 column Luna 10μ (150 x 4,6 mm). Methanol-water (85:15, v/v), flow rate 1.0 mL/min, and UV detector wavelength 224 nm have been chosen as the optimized HPLC condition for determination of odevixibat.

2.6.2. Construction of the calibration graph

Aliquots of 2 μL of analytes standard solutions at six different concentrations (5–50 μg mL^{-1}) of odevixibat were injected into HPLC system. The procedure was carried out in triplicate for each concentration. The analyte peak area obtained was plotted against the corresponding concentration of the analyte (expressed as μg mL^{-1}).

2.6.3. Application to pharmaceutical formulation

The contents of five Bylvay™ capsules (200mg of odevixibat) were weighed. Appropriate weight of powder equivalent to 20mg was accurately weighed, transferred to 100- mL volumetric flask and the volume was made up to 50 mL with methanol. The solution was shaken vigorously for 20 min then mixed for 30 min and filtrated. The volume was completed with methanol to produce a stock solution labeled to contain 200 μg mL^{-1} odevixibat.

2.4.4. PROCEDURE FOR STABILITY STUDIES

- *Acid degradation*

Accurately weights of 20mg of odevixibat were transferred to two 100-ml volumetric flask. To each flask, 5ml 0.1N HCL and 45ml methanol were added. One flask was kept at room temperature for 7 days while the other was kept at 50°c for 5 hrs on reflux chamber. The mixtures was neutralized with 5ml 0.1N NaOH and the volumes were made up to 100-ml with methanol. Finally 2.5 mL of each mixture was transferred to 10-mL volumetric flask and diluted to volume with the mobile phase and analyzed using the proposed procedure.

- *Base degradation*

Accurately weights of 20mg of odevixibat were transferred to two 100-ml volumetric flask. To each flask, 5ml 0.1N NaOH and 45ml methanol were added. One flask was kept at room temperature for 7 days while the other was kept at 50°c for 5 hrs on reflux chamber. The mixtures was neutralized with 5ml 0.1N HCL and the volumes were made up to 100-ml with methanol. Finally 2.5 mL of each mixture was transferred to 10-mL volumetric flask and diluted to volume with the mobile phase and analyzed using the proposed procedure.

- *Oxidative degradation*

Accurately weights of 20mg of odevixibat were transferred to two 100-ml volumetric flask. To each flask, 5ml 3%H_2O_2 and 45ml methanol were added. One flask was kept at room temperature for 7 days while the other was kept at 50°c for 10 hrs on reflux chamber. The volumes were made up to 100-ml with methanol. Finally 2.5 mL of each mixture was transferred to 10-mL volumetric flask and diluted to volume with the mobile phase and analyzed using the proposed procedure.

- *Photo degradation*

Small quantity of odevixibat powder was kept under UV chamber for 12 hrs; after that 20mg of odevixibat was weighed and transferred to 100- ml volumetric flask. The volume was made up to 100ml with diluent. Finally 2.5 mL of the degradation

product was transferred to 10-mL volumetric flask and diluted to volume with the mobile phase and analyzed using the proposed procedure.

- *Thermal degradation*

Small quantity of odevixibat powder was transferred to a Petri dish and kept at 50 °C in hot air oven for 12 hrs; after that 20mg of odevixibat was weighed and transferred to a 100-ml volumetric flask. The volume was made up to 100ml with diluent. Finally 2.5 mL of the degradation product was transferred to 10-mL volumetric flask and diluted to volume with the mobile phase and analyzed using the proposed procedure.

3. RESULTS AND DISSCUSION

3.1. Method development

Optimization of chromatographic conditions was achieved to develop and validate a selective and rapid assay method for the determination of odevixibat.

Optimization of experimental conditions

The chromatographic separation was optimized after taking intoaccount the resolution between the drugs and its degradation product. The separation was carried out in a Phenomenex C8 column Luna 10µ (150 x 4,6 mm). The separation was performed by a flow rate of 1.0 mL min^{-1} and an injection volume of 2 µL. Methanol: water (85:15, v/v) mobile phase was the best choice to separate the intact drugs from its degradation product. The Chromatogram of standard solution of odevixibat was shown in **Fig. 2.**

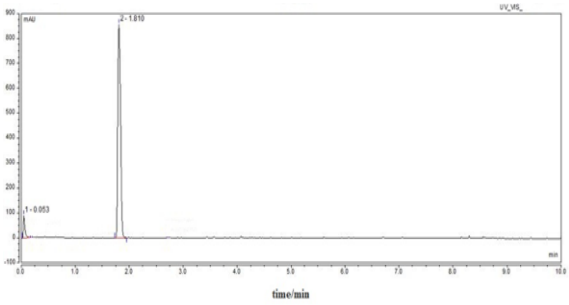

Figure 2: HPLC chromatogram of odevixibat standard (40µg mL^{-1}).

3.2. Method validation

3.2.1. Linearity and range

A linearity relationship was established by plotting the peak area values of the analyte versus the corresponding concentrations in µg mL^{-1}. The regression data results were summarized in **Table 1,** where the range of proposed method for the analysis of the studied drugs was showing. The high values of correlation coefficient indicate the linearity of the calibration graphs.

3.2.2. Limit of detection and limit of quantification. LOD and LOQ were calculated according to ICH by comparing measured signals from samples with known low concentration of analyte with those of blank samples and establishing the minimum concentration at which the analyte can be reliable detected. The analytical parameters of the proposed methods are summarized in **Table 1**.

3.2.3. Accuracy and precision.

Accuracy and precision were evaluated at three different concentration levels (5, 20, and 50) within the same day to obtain repeatability (intra-day precision) and over three different days to obtain intermediate precision (inter-day precision). The accuracy and precision were calculated and expressed in terms of percent recovery and standard deviation, respectively. All values were within the acceptance variability limits as shown in **Table 1**.

Table 1: Regression and validation data for estimation of odevixibat by the proposed method.

Parameter	Odevixibat
Linearity range (µg mL^{-1})	5-50
LOD (µg mL^{-1})	1.09
LOQ (µg mL^{-1})	3.6
Regression parameter*	Y= a+ b C
Correlation coefficient	0.9999
Slope (b)	542.07
Intercept (a)	69.171
Accuracy %	99.95
Precision (% RSD)	
Repeatability	0.294
Intermediate precision	0.332
Robustness (%RSD)	
Flow Rate	0.391
Temperature	0.444
Mobile phase composition	0.581

Y= a + bC, where Y is the peak area and C is the concentration in ng mL^{-1}.

3.2.4. Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components that may be expected to be present. The specificity was evaluated from the HPLC chromatogram. The HPLC chromatogram of the samples shows that the method has sufficient specificity to resolve all related substances and the odevixibat from each other. Furthermore, UV detector also showed excellent purity for odevixibat. Specificity of developed method was determined by chromatographic analysis of odevixibat by applying different stress conditions, thermal heating at 50 °C, 0.1N NaOH, 0.1N HCL, 3.0% H_2O_2, and photolytic degradation and the results were given in **Table 2**.

3.2.5. Robustness

Robustness is important to verify that the method performance is not affected by typical changes in normal experiments. Robustness was evaluated by changing the flow rate (1.0 ± 0.1ml min^{-}

[1]) and the composition of mobile phase. The effect of HPLC oven temperature was studied at 30 ± 1 ˚C. The measured response variances were the % RSDs. The % RSDs of peak area for odevixibat were given in **Table 1**. The degree of reproducibility of the results obtained as a result of small deliberate variations in the method parameters has proven that the method is robust.

Table 2: Summary of the forced degradation study.

Degradation Types	Condition	Time	% degradation Odevixibat
Thermal degradation	Dry heat (50 ºC, 10 hrs)	10 hrs	7.51
Basic hydrolysis	0.1 N NaOH (at room temp 25 ºC)	7 days	8.54
Basic hydrolysis	0.1 N NaOH (reflux at 50 ºC)	5hrs	10.99
Acidic hydrolysis	0.1 N HCl(at room temp 25 ºC)	7 days	7.89
Acidic hydrolysis	0.1 N HCl (reflux at 50 ºC)	5hrs	9.25
Oxidative hydrolysis	3% H_2O_2(reflux at 50 ºC)	10hrs	10.33
Photo degradation	Photolytic (UV, 5hrs)	12 hrs	8.12

Table 3: Recovery study of odevixibat by applying standard addition technique:

Drug	Pharmaceutical taken (µg mL^{-1})	Pharmaceutical Found (µg mL^{-1})	Pure added (µg mL^{-1})	Pure found (µg mL^{-1})	%Recovery
odevixibat	**20**	**19.92**	**16**	**15.98**	**99.88**
			20	**20.02**	**100.1**
			24	**23.97**	**99.88**
		Mean ± % RSD			**99.95 ± 0.251**

Table 4: Results obtained after determination of odevixibat in Bylvay capsule and comparison with the reported method.

Parameter	Odevixibat	
	Proposed method	**Reported method***
n[a]	5	5
%R	99.60	100.17
%RSD	0.251	0.281
SD	0.251	0.281
Variance	0.063	0.079
Student's t-test (2.306)[b]	0.796	------
F-value (6.388)[b]	1.322	------

[a] Experiments number. [b] Tabulated values of "t "and "F" at (P = 0.05). ***Reported method**

HPLC method for determination of odevixibat dosage form was achieved using C-18 column using DMSO mobile phase, and the flow rate was 1.5ml/min.[NODCAR]

3.3. Forced degradation:

Forced degradation of odevixibat was carried out to confirm that during stability study or throughout the shelf life, any degradation product if found will not interfere with the determination of odevixibat. In addition, the forced degradation study would help to identify the type of degradation pathway (whether oxidative, alkali hydrolysis, acid hydrolysis, photolytic etc.) for each one. Forced degradation was performed by degrading the sample with thermal heating at 50 ˚C, 0.1N NaOH, 0.1N HCL, 3.0% H_2O_2, and photolytic degradation, UV detector wavelength 224 nm have been chosen as the optimized HPLC condition for determination of odevixibat. in different water samples because it gave the best baseline of odevixibat peak (base to base), standard solution of odevixibat was shown in **Fig. 3**. From the forced degradation studies, the results of the forced degradation listed in table (2). It was found that the drug was labile to degradation under the used conditions as shown in **Fig. 3.**

3.4. Application to the finished product

Bylvay™ capsules can be determined using the proposed method. The obtained results showed absence of any interference from either excipients or additives. The proposed method showed good accuracy and precision for assay of odevixibat in Bylvay™ capsules and the values were listed in **Table 3 and 4**.

4. CONCLUSION

This method is sensitive, and selective, and can be successfully applied for determination of odevixibat in the pharmaceutical preparation.

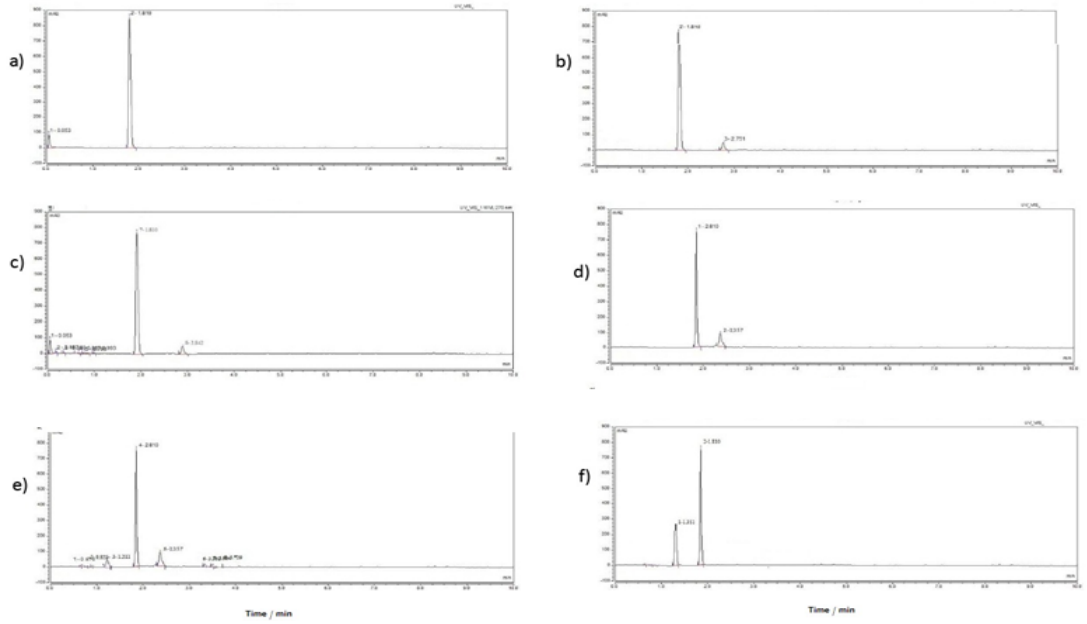

Figure 3: HPLC chromatograms of odevixibat under different stress conditions: (a) standard odevixibat, (b) acidic hydrolysis (0.1 N HCL at 25°c), (c) acidic hydrolysis (0.1 N HCL at 50°c), (d) basic hydrolysis (0.1 N NaOH at 25°c), (e) basic hydrolysis (0.1 N NaOH at 50°c), (f) oxidative hydrolysis (3.0% H_2O_2) Respectively.

5. DATA AVAILABILITY

The readers can access the data represented in this research article by contacting with the author on this E-mail:ahmedabdrabou31@yahoo.comasthe data presented is his own personal experiment and work.

6. CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this paper.

7. FUNDING STATEMENT

The authors declare that there is no Funding Statement regarding the publication of this paper.

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