

## Analytical method development and validation of Elobixibat by Reverse Phase High Pressure Liquid chromatography

Mr. Suyash Shashikant Ingle<sup>1\*</sup>, Misbah Sultana Abdul Kausar Badewale<sup>2</sup>, Sidharam Shankar Holikatti<sup>3</sup>, Ashlesha Nagendra Zade<sup>4</sup>, Suyash Sudhakar Mali<sup>5</sup>, Sakshi Shivanand Hiremath<sup>6</sup>, Priya Arvind Kondabattini<sup>7</sup>, Padmashri Pandurang Adam<sup>8</sup>

<sup>1</sup> Gandhi Natha Rangji College of Pharmacy, Solapur, Maharashtra, India, suyashingle07@gmail.com, ORCID: 0000-0002-8373-7336

<sup>2</sup> Gandhi Natha Rangji College of Pharmacy, Solapur, Maharashtra, India, misbahp1@yahoo.com, ORCID: 0009-0005-7687-6417

<sup>3</sup> Gandhi Natha Rangji College of Pharmacy, Solapur, Maharashtra, India, sidholikatti28@gmail.com, ORCID: 0000-0002-2941-1036

<sup>4</sup> Gandhi Natha Rangji College of Pharmacy, Solapur, Maharashtra, India, ashleshazade2000@gmail.com, ORCID: 0009-0000-0648-6919

<sup>5</sup> Gandhi Natha Rangji College of Pharmacy, Solapur, Maharashtra, India, suyashmali5599@gmail.com, ORCID: 0009-0008-9024-9497

<sup>6</sup> Gandhi Natha Rangji College of Pharmacy, Solapur, Maharashtra, India, sakshi.hiremath04@gmail.com, ORCID: 0009-0001-4120-0733

<sup>7</sup> Gandhi Natha Rangji College of Pharmacy, Solapur, Maharashtra, India, priyakondabattini03@gmail.com, ORCID: 0009-0004-1921-7661

<sup>8</sup> Gandhi Natha Rangji College of Pharmacy, Solapur, Maharashtra, India, adampadmashri@gmail.com, ORCID: 0009-0004-9247-0604

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### ABSTRACT

A precise and accurate analytical method for estimation of Elobixibat was developed and validated as per ICH guidelines. The mobile phase consisted of Methanol: 0.1% Formic acid in Water in the ratio 78:22 v/v by using Agilent Zorbax Bonus RP (250 x 4.6 mm, 5 $\mu$ ) as stationary phase. The wavelength was set at 282 nm with 10  $\mu$ l injection volume. The samples were allowed to run for 10 minutes. The Elobixibat peak eluted at 3.71 minutes. The % RSD for instrument precision was found to be 0.07% for Elobixibat. The % RSD of accuracy for Elobixibat at 80%, 100% and 120% was found to be 0.03%, 0.04% and 0.06%, respectively. The coefficient correlation was 0.999 for Elobixibat. The % RSD for inter and intra-day precision for Elobixibat was found to be 0.18% and 0.29%, respectively. The LOD and LOQ for Elobixibat was below 0.53  $\mu$ g/ml and 1.14  $\mu$ g/ml. From the validation data can it be concluded that method can be used for estimation of Elobixibat.

**Keywords:** Elobixibat, RP-HPLC, Validation, Method development, LOD and LOQ, Analytical estimation.

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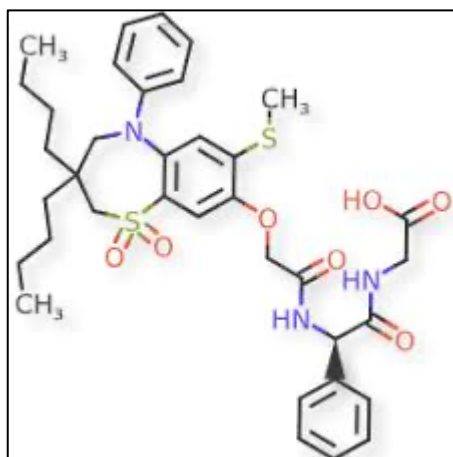
### INTRODUCTION

Constipation chronic and idiopathic constipation (CC) has a high prevalence across the world. A subgroup of CC patients exhibit decreased faecal (and by extension, intra-colonic) bile acids (BA). Elobixibat is a locally-acting ileal bile acid transporter (IBAT) inhibitor, which results in enhanced BA delivery to the colon and a new category of treatment of CC. BAs speed up the colonic transit and

augment colonic secretion. Thus, there is a possibility of IBAT inhibitors being used to treat CC patients.

Elobixibat is an IBAT (or apical sodium bile acid transporter) inhibitor, otherwise known as the SLC10A2 gene. ASBT/IBAT is a bile acid: sodium symporter that reabsorbs bile acids in the ileum, the first stage in their enterohepatic circulation. Elobixibat suppresses bile acid absorption, thus raising the level of bile acids in the small

intestine, thereby hastening intestinal clearance and loosening stool.



**Figure 1: Chemical structure for Elobixibat**

Elobixibat is an *in vitro* CYP3A4 inhibitor. The importance of this inhibition in drug-drug interactions is however limited, secondary to reduced bioavailability orally. It is highly selective for IBAT, with an affinity more than 400-fold higher than for human liver sodium-dependent BA transporter and more than 1000-fold higher than for neutral amino-acid transporter and Elobixibat was well tolerated and improved bowel movement frequency within two weeks of treatment in Indian patient population with chronic constipation. It is highly selective

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The table given below shows the Limits as per ICH guidelines.

**Table 1: Quality Target Profile for HPLC Method development**

Parameters	Limits
Theoretical Plates	Not less than 2000
Asymmetry	Not More than 2.0 (Fairly at 1.0)
Tailing Factor	Not More than 2.0 (Fairly at 1.0)
Run time	Not More than 20 minutes
Resolution	Not Less than 2.0

## 2. EXPERIMENTAL

### 2.1. Chemicals and Reagents:

A Sample of Elobixibat was taken from Aadhaar life sciences, Solapur, Maharashtra. HPLC-grade Formic acid (Merck) Mumbai, India and Methanol (Molychem, Mumbai) were purchased from a local supplier. HPLC grade RO water was provided via the internal Milli-Q system.

All of the weighing was carried out on scales that had been calibrated by NABL. The production of the samples was carried out with the use of the analytical balance and Type A glassware along with the 0.45  $\mu$ m Millipore syringe filters (Ultipor<sup>®</sup>N66<sup>®</sup>Nylon Membrane).

### 2.2. Instrumentation:

The instrument used for development and validation was an Agilent 1260 Infinity II equipped with a quaternary

pump and DAD detector. Software from Agilent called Open lab Ezchrom was used. Wet chemistry was conducted using the labman ultra-sonicator and the acet analytical balance.

### 2.3 Selection of Mobile phase:

Based on literature review the mobile phase of Methanol: 0.1% Formic acid in Water was chosen for the HPLC analysis of Elobixibat in order to gain better results, various ratio trials like 50:50, 45:55, 70:30, 78:22 v/v were performed with gradient type of elution technique.

### 2.4 Selection of Wavelength:

Standards were scanned from 190-400 nm on HPLC with DAD detector.

### 2.5 HPLC Method Development:

Multiple trials were done performed to achieve the system suitability criteria as per ICH guidelines as mentioned in table 1.

### 2.5.1 Preparation of Mobile Phase:

#### Mobile Phase: Methanol: 0.1% Formic acid in Water

In 1000 ml HPLC water, 0.1% formic acid was added and mixed well with methanol and filtered through 0.45-micron membrane filter and sonicated to degas for 10 minutes.

### 2.5.2 Preparation of Diluent:

Mixed separately measured 800 ml of methanol and 200 ml of 0.1% Formic Acid into a suitable container. Mixture was filtered through 0.45µm nylon membrane filter and briefly sonicated to degas.

### 2.5.3 Preparation of Standard Solution:

Prepare a Standard Stock Solution of Elobixibat by adding 3mg in 10 ml volumetric flask & add 5 ml diluent and Mix and sonicate for 5 minutes. Make up the volume to 10 ml with diluent. (Conc. = 300µg/ml).

## 2.6 Method validation:

### 2.6.1 Specificity:

Elobixibat was injected individually and in mixture at 100 µg/ml concentration each and peaks were identified by retention time. Blank injection ensured that the blank peak would not interfere with the major analyte peaks.

### 2.6.2 System Suitability:

The system was tested to for its suitability. The theoretical plate count, tailing factor, and resolution should meet ICH guidelines as mentioned in table 1.

### 2.6.3 Accuracy:

A technique's accuracy is determined by how closely its test results match the actual value. Three concentration levels were tested in recovery trials. Three replicate injections were performed at each level to calculate drug concentration, recovery percentage, and standard deviation.

### 2.6.4 Repeatability:

Analytical Instrument precision depends on test concordance. Multiple uniform samples were examined. After following the instructions, six injections were made from a single sample to test the system. Instrument precision was measured (how well the instrument replicates injections of equal concentration).

### 2.6.5 Linearity:

Methodological linearity is an analytical method's ability to give results proportional to analyte concentrations within a certain range. Five standard solutions were used to determine linearity. Plotting peak area against standard solution concentration on the calibration curve yielded the regression equation. This enabled equation development. For slope, intercept, and correlation coefficient, the least-squares approach was used.

### 2.6.6 LOD and LOQ:

Limits of detection (LOD) and quantification (LOQ) represent the method's capacity to detect and quantify the smallest analyte amounts. The following formulae determined the standard deviation and regression line slope needed to calculate LOD and LOQ.

$$LOD = \frac{3.3 \times Std. Error of Intercept}{Coefficients of X Variable 1}$$

$$LOQ = \frac{10 \times Std. Error of Intercept}{Coefficients of X Variable 1}$$

## 3. RESULTS AND DISCUSSION:

Using methanol and 0.1% formic acid in water as a mobile phase in HPLC is a common practice that offers several advantages. One of the primary benefits is the improvement in peak shape, as methanol helps in resulting improved peak shape and symmetry.

Additionally, the combination of methanol and 0.1% formic acid in water can provide high resolution and separation of closely related compounds, making it an ideal choice for analyzing complex mixtures. Furthermore, the use of methanol can also enhance the sensitivity of detection, especially for compounds that exhibit poor UV absorption, allowing for more accurate and reliable quantitation. Overall, the use of methanol and 0.1% formic acid in water as a mobile phase in HPLC is a valuable technique for achieving high-quality chromatographic separations and detections.

The trials were carried out by varying the ratio of methanol and 0.1% formic acid in water and the wavelength was finalised for analysis at 282 nm for appropriate intensity of Elobixibat peak based on the trials undertaken.

### 3.2 Final Chromatographic Conditions:

The Final chromatographic condition for estimation of Elobixibat was as follows (Table 2).

**Table 2: Final Chromatographic Conditions**

Parameters	Condition
HPLC Instrument	Agilent 1260 Infinity II
Column	Agilent Zorbax Bonus RP (250 mm x 4.60 mm, 5µm)

Wavelength	282 nm
Mobile Phase	Methanol: 0.1% Formic acid in Water (78:22)
Diluent	Methanol: 0.1% Formic acid in Water (80:20) v/v
Run time	10 minutes
Injection Volume	10 microliters
Flow Rate	1.0 ml/min
Column oven Temperature	30°C

**3.3 Method Validation:**

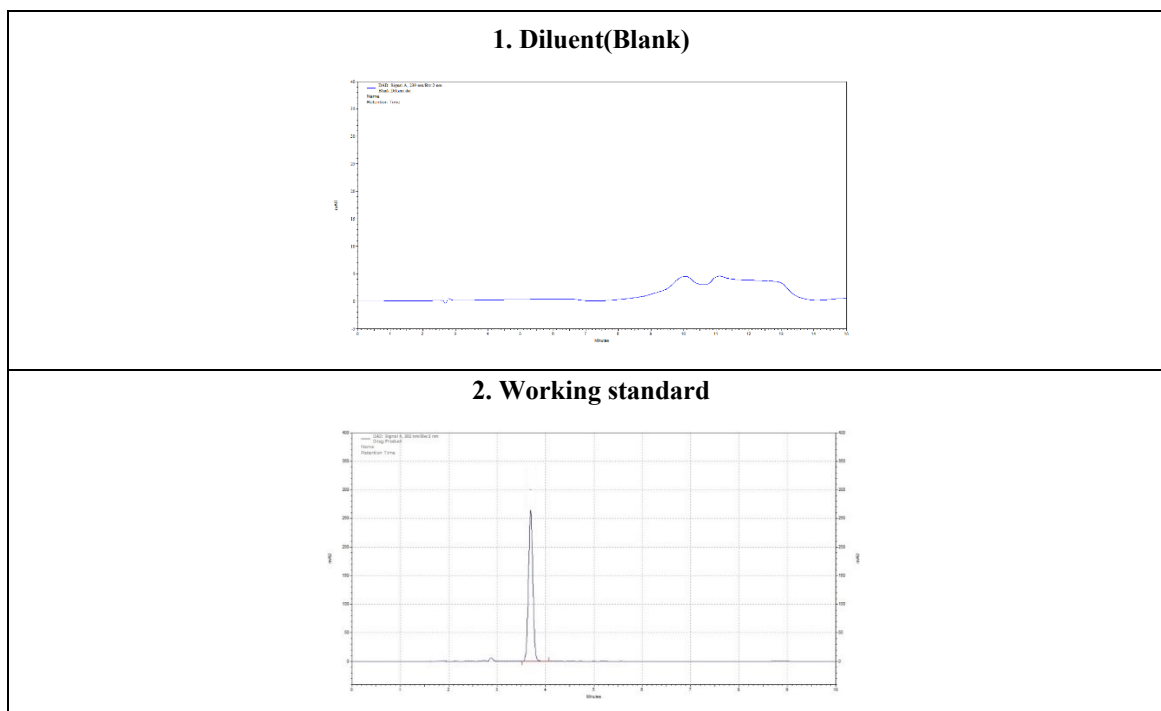
**3.3.1 Specificity**

Specificity was performed to check if there was any interaction between the peaks from blank or the API.

**Table 3: Specificity results of Elobixibat**

Sample ID	RT (min.)
Elobixibat	3.71

Based on specificity data, it was found that the Retention time of individual working standard was similar to that of mixture working standard and there is no interference of blank with main peak of Elobixibat.



**Figure 2: Chromatogram ID 1] Diluent, 2] Working Standard**

**3.3.2 Instrument Precision and System Suitability:**

The appropriateness of the HPLC instrument was assessed for the purpose of validation. According to the specified limits in table 1, the equipment was deemed appropriate for carrying out the validations. The precision test was conducted following the assessment of system

applicability. The data indicates that the % relative standard deviation for Instrument precision for Elobixibat are 0.07%. These % RSD demonstrates that the approach is highly precise and reliable when used by various analysts and for multiple sample preparations with the same concentration. (Table 4)

**Table 4: Results of Repeatability**

Sample ID	DFZ Peak Area
Replicate 1	3481717
Replicate 2	3483920
Replicate 3	3486356
Replicate 4	3480383
Replicate 5	3482396
Average	3482954
STDEV	2289.01
% RSD	<b>0.07</b>

**3.3.3 Accuracy:**

Accuracy for Elobixibat was performed in triplicates and it was observed that the method was accurate for the range 80%, 100% and 120%. The relative standard

deviation for 80%, 100% and 120% were 0.03%, 0.04% and 0.06% respectively. The accuracy determined the methods ability to analyses different concentration of drug in solution accurately.

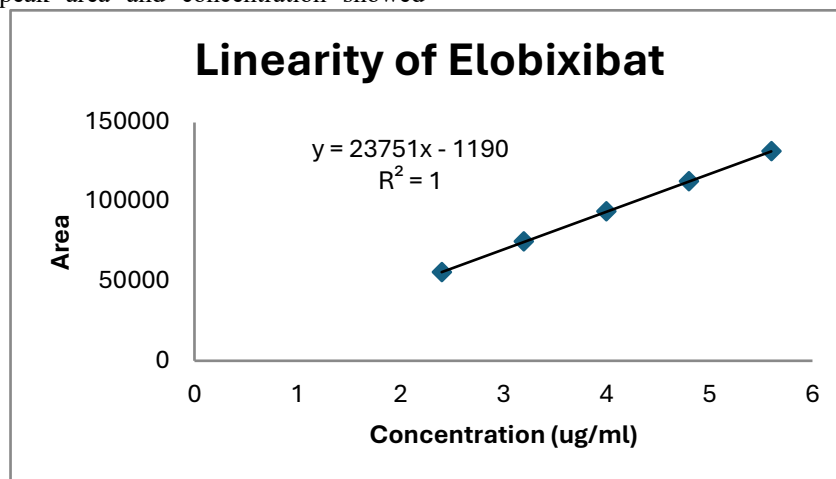
**Table 5: Results of Accuracy**

Sample ID	Reps	Spiked Conc. (ug/ml)	Area	Amt Recovered (ug/ml)	% Recovery	AVG	STDEV	RSD
80%	Rep 1	239.74	2798513	240.78	100.44	100.42	0.025403	0.03
	Rep 2	239.74	2797512	240.70	100.40			
100%	Rep 1	299.67	3481717	299.56	99.96	100.00	0.044725	0.04
	Rep 2	299.67	3483920	299.75	100.03			
120%	Rep 1	359.60	4167911	358.60	99.72	99.76	0.055813	0.06
	Rep 2	359.60	4171210	358.89	99.80			

**3.3.4 Linearity:**

Linearity was performed at different levels. The graph plotted between peak area and concentration showed

linearity with correlation coefficient as shown in table below. The linearity data in shown in graph in figure 3.



**Figure 3: Linearity of Elobixibat**

Linearity was performed for Elobixibat with concentration ranging from 80-120 µg/ml for Elobixibat and the Correlation Coefficient was found to be 1 for the API.

### 3.3.5 LOD and LOQ:

The Limit of Detection (LOD) and Limit of Quantitation (LOQ) were determined for Elobixibat. The results of analysis are shown in table 6.

**Table 6: Results of LOD and LOQ for Elobixibat**

Name	LOD (µg/ml)	LOQ (µg/ml)
Elobixibat	0.53	1.14

The LOD and LOQ were significantly low, implying the method to be very efficient in determining low concentration of drug. This value of LOD and LOQ can be used during cleaning validation in industry which can help companies know if the manufactured vessel or equipment is free from APIs stains.

#### 4. CONCLUSION

In this research article, a precise and accurate method was developed for estimation of Elobixibat bulk drugs by RP-HPLC technique. The developed method was validated for accuracy and precision as per ICH guidelines. The proposed method was found to be appropriate due to its simplicity, reliability, sensitivity, rapidness and selectivity for detection at very low concentrations of drug biomarkers. Based on validation data it is demonstrated and concluded that this method could be the highly sensitive and accurate method to be used in the routine analysis of Elobixibat for estimation with varied concentration in different formulations.

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