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**COMPARATIVE STUDY OF ULTRAVIOLET SPECTROSCOPY AND  
HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHIC  
METHODS FOR ELUXADOLINE**

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**ABSTRACT**

The aim of present research work is to develop and validate UV-spectrophotometric and High Performance Thin Layer Chromatography (HPTLC) methods for Eluxadoline. The estimation of Eluxadoline was performed by both UV and HPTLC method with detection wavelength of 238 nm. Chromatographic separation was carried on TLC aluminium plates pre-coated with silica gel G 60 F<sub>254</sub> with methanol: ethyl acetate: triethylamine (5:5:0.1 v/v) as mobile phase. The retardation factor (R<sub>F</sub>) was observed to be 0.45±0.02. The developed methods were successfully validated as per ICH Q2 (R1) guideline. Methods were found to be linear within the range of 2-12 µg/ml with correlation coefficient R<sup>2</sup> = 0.9963 (for UV method) and 200-1000 ng/band with correlation coefficient R<sup>2</sup> = 0.9982 (for HPTLC method). The methods were precise as %RSD < 2 % and accurate as % recovery were found to be in range of 98-102 %. LOQ of developed methods were found to be 2.27 µg/ml and 87.98 ng/band for UV and HPTLC method respectively. The results of assay and accuracy studies of both methods were evaluated by student t test. Statistical analysis shows that there is no significant difference (*p*>0.05) between UV and HPTLC methods regarding accuracy and assay results.

**Keyword: Eluxadoline, HPTLC, Student t test, UV method**

## INTRODUCTION:

IBS-D is diarrhea predominant - irritable bowel syndrome characterized by presence of loose or watery stools with at least 25 percent of bowel movements and hard or lumpy stools with less than 25 percent of bowel movements [1, 2]. Eluxadoline is a  $\mu$ - and  $\kappa$ -opioid receptor agonist and  $\delta$ -opioid receptor antagonist that acts locally in the enteric nervous system. It reduces symptoms of IBS-D such as belly pain and diarrhea by activating  $\mu$ -opioid receptor, which slows down the gastrointestinal motility, decrease visceral sensation and inhibit secretion. Antagonizing  $\delta$ -opioid receptor reduces some of the undesired effects of  $\mu$ -opioid receptor such as excessive slowing of gastrointestinal motility [3, 4]. Chemically it is 5-({[(2*S*)-2-amino-3-(4-carbamoyl-2,6-dimethylphenyl) propanoyl] [(1*S*)-1-(4-

phenyl-1 *H*-imidazol-2-yl) ethyl] amino} methyl)-2- methoxybenzoic acid (shown in **Figure 1**). Eluxadoline is soluble in methanol, sparingly soluble in 0.1N NaOH, 0.1 HCL, Practically insoluble in water (0.00268 mg/ml). After extensive literature survey it is reveals that two bioanalytical methods and RPHPLC-DAD, HPLC, degradation study with UPLC method [5-9] has been reported in literature but no UV-Spectrophotometry and HPTLC method has been reported yet, UV- Spectrophotometry and HPTLC methods are simple, economic, time-saving and labour-saving compared to sophisticated methods like UPLC, HPLC-MS. Hence, aim of the current research was to develop and validate UV-spectrophotometric and high-performance thin layer chromatography (HPTLC) method for Eluxadoline.

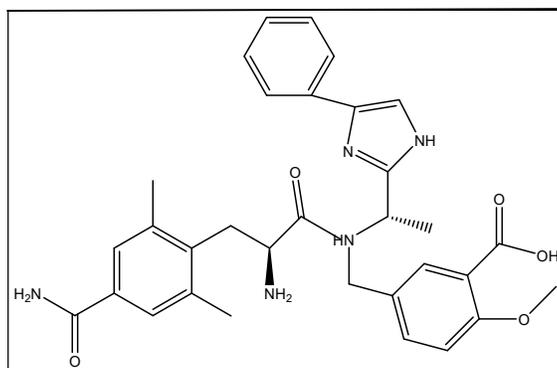


Figure 1: Structure of Eluxadoline

## MATERIALS AND METHODS:

### Chemicals and Reagents:

Eluxadoline working standard was received as gift sample from Zydus cadila healthcare

Ltd, Thane, India. Ethyl acetate AR grade, Methanol AR grade was purchased from Loba Chemie Pvt. Ltd. (India). TLC plates

Precoated with silica gel G 60 F<sub>254</sub> were purchased from Merck Pvt. Ltd.

**Instrument:**

The sample application was performed with help of CAMAG HPTLC system equipped with Linomat 5 sample applicator operated under a gentle stream of nitrogen, coupled with a Hamilton microliter syringe (100µl). CAMAG TLC SCANNER 3 was used for detection and densitometry scanning. Data acquisition was done by winCATS software (version 1.4.3). UV-spectral analysis was performed on UV-Visible spectrophotometer JASCO (Model- V730) with scanning speed of 400 nm/min, data interval of 1 nm, band width of 1 nm and detection wavelength of 238 nm.

**Preparation of Standard Stock Solution:**

For UV-spectrophotometric method and HPTLC method standard stock of Eluxadoline (1000 µg/ml) was prepared by accurately weighing 10 mg of Eluxadoline and transferred to 10 ml volumetric flask, then volume was made up to 10 ml with methanol. From this standard stock solution 1 ml was taken and diluted to 10 ml using methanol as solvent to get standard solution containing 100 µg/ml Eluxadoline.

**Selection of Analytical Wavelength:**

A solution containing Eluxadoline 10 µg/ml was prepared from the standard solution of Eluxadoline (100 µg/ml) using methanol as solvent and scanned over 200-400 nm in UV-Spectrophotometer.

**Optimization of Chromatographic****Conditions:**

To achieve optimized chromatographic condition, various trials were performed using different mobile phases in different solvent ratios, and after several trials, Methanol: Ethyl acetate: Triethylamine (5:5:0.1 v/v/v) was selected as the mobile phase, resulting in good peak shape with R<sub>F</sub> value 0.45±0.02. Chromatographic separation was carried on Merck's TLC aluminium plates pre-coated with silica gel G 60 F<sub>254</sub> (10 × 10 cm) using 40 µg/ml standard solution of Eluxadoline and development was carried out in twin trough TLC chamber. Chamber saturation was achieved by keeping developing chamber aside for 30 min at room temperature. The plate was developed and air dried at room temperature. Densitometric scanning was performed at wavelength 238 nm.

**Validation of analytical method:**

Validation of developed UV-spectrophotometric method and HPTLC was carried out as per as per the ICH Q2 (R1) guideline.

**Linearity:**

**For UV-Spectrophotometric method:** UV spectra of linearity solutions of 2-12 µg/ml were recorded in range of 200-400 nm and absorbance was noted at 238 nm. Calibration curve was obtained by plotting absorbance of drug solution against the corresponding concentrations.

**For HPTLC method:** A standard solution of Eluxadoline (40 µg/ml) was spotted on TLC plate with application volume 5, 10, 15, 20, 25 µl to achieve amount spotted in the range of 200-1000 ng/band. The plate was developed as per optimised procedure. The calibration curve was obtained by plotting peak areas vs. amount spotted.

**Precision:**

The precision of the developed methods was checked by intra-day precision and inter-day precision studies. For UV-Spectrophotometric method 6 replicates of Eluxadoline solution (4 µg/ml) and for HPTLC method 6 replicate of Eluxadoline solution (40 µg/ml) with application volume 10 µl (400 ng/band) of each solution were analyzed on same day after short interval of time and on consecutive days. %RSD was calculated.

**Accuracy:**

Accuracy of the developed methods was carried out by standard addition method. Synthetic mixture of product components (assay solution) was analyzed by adding known amount of the pure drug at 50, 100, 150 % level. This was evaluated utilizing the 3 replicates of 3 concentrations. (6 µg/ml, 8 µg/ml, 10 µg/ml for UV-spectrophotometric method and 600ng/band, 800ng/band, and 1000ng/band for HPTLC method) and % recovery was calculated.

**Assay:**

Assay procedure was carried on synthetic mixture of Eluxadoline with commonly used excipients. For HPTLC method, 6 replicates of sample solution (40 µg/ml) were prepared from 1000 µg/ml blend solution containing 10 mg Eluxadoline and 10µl (400 ng/band) application volume of each sample solution (40 µg/ml ) was applied on TLC plate. For UV-Spectrophotometric method 6 replicate of sample solution (4 µg/ml) were analyzed. % recovery was calculated.

**LOD and LOQ:**

The limit of Detection and limit of Quantitation of developed methods were calculated from calibration curves. The following equations were used to calculate LOD and LOQ.

$$\text{LOD} = 3.3 \times \sigma/S$$

$$\text{LOQ} = 10 \times \sigma/S$$

Where,  $\sigma$  = the standard deviation of y intercept. S = slope of the calibration curve.

**Robustness:**

Robustness was carried out by changing mobile phase composition, saturation time and time from application to development, time from development to scanning, change in scanning wavelength in HPTLC method and wavelength change in UV-Spectrophotometric method, to determine its effect on result. % RSD was calculated.

### Statistical Analysis

The developed UV-spectrophotometric and HPTLC methods are compared for assay and accuracy results. Student's t test (unpaired t test) was performed using Graphpad prism version 9.2.0.

### RESULTS:

Eluxadoline showed maximum absorbance at 238 nm. Hence it was selected as analytical wavelength **Figure 2** show UV spectrum of Eluxadoline (10 $\mu$ g/ml). Optimized chromatographic conditions for HPTLC method included mobile phase of Methanol: Ethyl acetate: Triethylamine (5:5:0.1 v/v) saturated for 30 min and detection was performed at 238 nm.  $R_F$

value of Eluxadoline was found to be  $0.45\pm 0.02$ . Densitogram is shown in **Figure 3**.

The developed methods was successfully validated as per ICH Q2 (R1) guideline and met to validation parameters. The results are shown in **Table 1** and calibration curve are shown in **Figure 4** and **Figure 5**.

### Statistical analysis:

The results of assay and accuracy studies of both methods were evaluated by student t test. Statistical analysis shows that there is no statistically significant difference ( $p>0.05$ ) between UV and HPTLC methods considering assay and accuracy results. Results are given in **Table 2**.

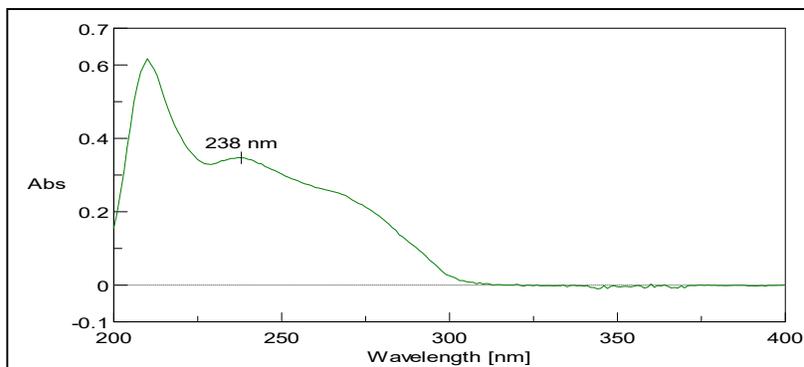


Figure 2: UV- Spectrum of Eluxadoline (10  $\mu$ g/ml)

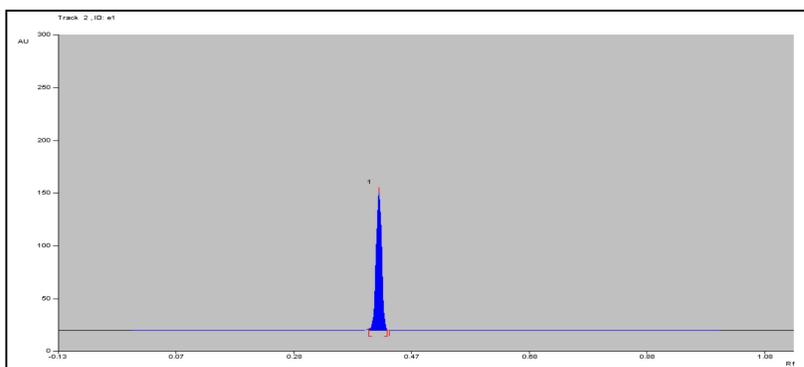


Figure 3: Densitogram of Eluxadoline (200ng/band,  $R_F = 0.45\pm 0.02$ )

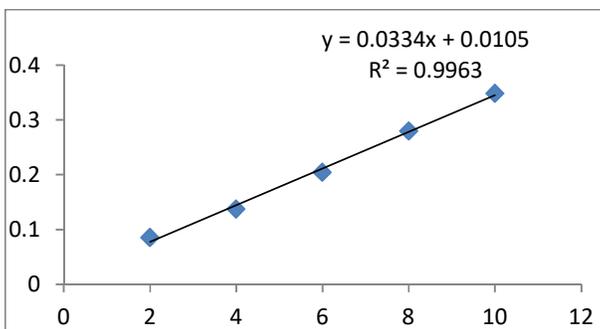


Figure 4: Calibration curve of Eluxadoline (2-12 µg/ml)

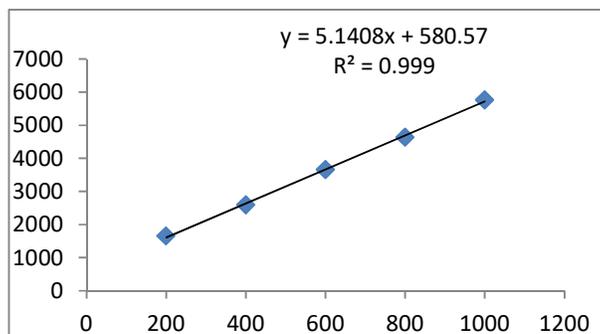


Figure 5: Calibration curve of Eluxadoline (200-1000 ng/band)

Table 1: Summary of Validation Parameters

Sr. No.	Validation parameters	UV method	HPTLC method
1	Linearity	y=0.0334x + 0.0105 R <sup>2</sup> =0.9963	y = 5.1408x + 580.57 R <sup>2</sup> = 0.9982
2	Range	2-12 µg/ml	200-1000 ng/band
3	Intra-day Precision (%RSD)	1.24	1.61
	Inter-day precision (%RSD)	1.38	1.78
4	% Assay	101.59	101.43
5	Accuracy (% Recovery)	50 % level	100.83
		100 % level	101.60
		150 % level	100.85
6	LOD	0.421 µg/ml	29.03 ng/band
7	LOQ	2.275 µg/ml	87.98 ng/band
8	Robustness (Change in detection wavelength 238 ± 1 nm)	Robust (%RSD < 2%)	Robust (%RSD < 2%)

Table 2: Statistical analysis of assay and accuracy results

Parameters	UV method		HPTLC method		Calculated t value	Table t value	P value by student's t test
	Average/mean	SD	Average/mean	SD			
Assay n=6)	101.59	0.88	101.41	0.71	0.40	2.23	0.69
Accuracy (n=3)	101.43	0.53	100.59	0.49	1.972	2.78	0.12

**DISCUSSIONS:**

Statistical analysis revealed that there is no statistically significant difference between the results of assay and accuracy studies obtained by the both methods. As the p

value was found to be >0.05 as well as calculated t value is smaller than critical value (table t value) at 95% confidence level indicating that there is no statistical

significant difference. This proves that both the developed methods are equivalent.

#### CONCLUSION:

The developed UV-Spectrophotometric and HPTLC methods are simple, economic and robust. These two methods can be alternatively used for analysis of Eluxadoline for quality assessment.

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#### Conflict of interest:

The authors have declared no conflict of interest.

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