Difference spectrophotometric method- hydrolytic degradation monitoring of eluxadoline

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ABSTRACT: Eluxadoline is antidiarrheal, intestinal anti-inflammatory / anti-infective agent for treatment of IBS-D (diarrhea predominant - irritable bowel syndrome). Literature survey revealed that Eluxadoline is prone to hydrolysis under acidic and basic condition. The aim of present research work was to develop and validate a simple Difference UV-spectrophotometric method for monitoring hydrolytic degradation of Eluxadoline under acidic condition. The Developed Difference Spectrophotometric method was based on the measurement of difference in absorbance of standard drug and acid degradation sample at 290nm. The developed method was successfully validated as per ICH Q2 (R1) guideline. The developed method was found to be linear in range of 10-60 µg/ml with correlation coefficient $R^2=0.9995$. Developed Difference spectrophotometric method is simple, linear and can be used as preliminary test in quality control laboratories to monitor hydrolytic degradation of Eluxadoline.

KEYWORD: Eluxadoline, Difference spectroscopy, Antidiarrheal, Hydrolysis, Anti-infective agent, IBS-D (diarrhea predominant - irritable bowel syndrome).

I. 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 µ- and κ-opioid receptor agonist and δ-opioid receptor1 antagonist that acts locally in the enteric nervous system. It reduces symptoms of IBS-D such as belly pain and diarrhea by activating u-opioid receptor, which slows down the gastrointestinal motility, decrease visceral sensation and inhibit secretion. Antagonizing δ-opioid receptor reduces some of the undesired effects of u-opioid receptor such as excessive slowing of gastrointestinal motility[3,4]. Chemically it is 5-({[(2S)-2-amino-3-(4-carbamoyl-2, 6-dimethylphenyl) propanoyl] [(1S)-1-(4-phenyl-1 H-imidazol-2-yl) ethyl] amino} methyl)-2- methoxybenzoic acid (shown in Fig.1). Eluxadoline is soluble in methanol, sparingly soluble in 0.1N Sodium Hydroxide (NaOH), 0.1NHydrochloric acid (HCl), Practically insoluble in water (0.00268 mg/ml). After extensive literature survey revealed that RPHPLC-DAD[5], two bioanalytical methods[6,7] and HPLC[8], degradation study with UPLC method[9]has been reported in literature.5-9Literature survey showed that Eluxadoline is prone to hydrolysis under acidic and basic condition. The aim of present research work was to develop and validate a simple Difference UV-spectrophotometric method for monitoring hydrolytic degradation of Eluxadoline under acidic condition. As UV- Spectrophotometry method is simple, economic, time-saving and labour-saving compared to sophisticated methods like UPLC, HPLC-MS. The Developed Difference Spectrophotometric method was based on the measurement of difference in absorbance of standard drug and acid degradation sample at 290nm.
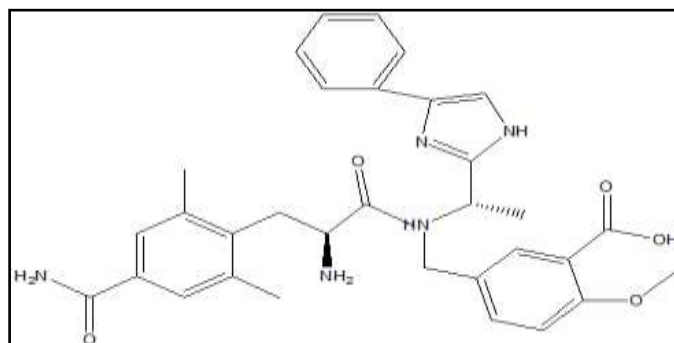
Fig. 1: Structure of Eluxadoline

II.   MATERIALS AND METHODS:

Chemicals and reagents:
Eluxadoline working standard was received as gift sample from Zydus cadila healthcare Ltd, Thane, India. Methanol AR grade, Hydrochloric acid (HCl) was purchased from LobaChemie Pvt. Ltd. (India).

Instrument:
UV-spectral analysis was performed on UV-Visible spectrophotometer JASCO (Model- V730).

Preparation of Standard Stock Solution:
A standard stock of Eluxadoline(1000 µg/ml) was prepared in 10 ml volumetric flask by accurately weighing 10 mg of Eluxadoline and dissolved in methanol, made up volume to 10 ml with methanol. Standard solution of Eluxadoline (500 µg/ml) was prepared from standard stock solution of Eluxadoline (1000 µg/ml) using distilled water as solvent.

Preparation of Standard Linearity Solution:
A standard linearity solution in range of 10-60 µg/ml was prepared from standard stock solution (500 µg/ml) using distilled water as solvent and zero order spectra was recorded and converted to first derivative spectra using software.

Preparation of Acid Hydrolysis Sample:
An equimolar acid hydrolysis sample (10-60 µg/ml) was prepared from standard stock solution (500 µg/ml) by adding 1 ml 0.5 N Hydrochloric acid (HCl) to each solution, made up volume to 10 ml with distilled water. These solutions were kept aside for 2 hrs at room temperature,after completion of specified time, zero order spectra were recorded and converted to first derivative spectra using software.

Degradation Study:
Eluxadoline showed degradation under acidic and basic condition. Initially the acid degradation sampleswere kept aside for 2 hrs and 24 hrs at room temperature.after completion of specified time, absorbance difference was calculated between the equimolar solution of standard and acid degradation sample at 290 nm. But it was observed that absorbance difference at 2 hrs and 24 hrs was nearly same, therefore degradation study was carried out only for 2 hrs.

Method Validation:
The developed difference spectroscopic method was validated as per ICH Q2 (R1), for linearity, precision, accuracy, LOD & LOQ, Robustness.

Linearity and Range of Standard and Acid Degradation Sample:
Linearity of standard and acid degradation sample were analysedwith 5 replicatesin range of 10-60 µg/ml and linear regression equation was plotted. The obtainedzero spectra were converted to first derivative using software and difference in absorbance of equimolar solution of standard and degradation sample were noted at 290nm. Results are given in Table 1.

Table 1: Linearity of Eluxadoline

Replicate	Concentration of Eluxadoline (µg/ml)					
	10	20	30	40	50	60
	UV absorbance					
1	0.00407	0.00711	0.0101	0.0143	0.0166	0.0197
2	0.00407	0.00721	0.0102	0.0142	0.0167	0.0196
3	0.00426	0.00738	0.0102	0.0129	0.0153	0.0184
4	0.00429	0.00694	0.0098	0.0132	0.0162	0.0179
5	0.00429	0.00714	0.0099	0.0121	0.0163	0.0188
6	0.00418	0.00724	0.0098	0.0122	0.0179	0.0183
AVG	0.00419	0.00715	0.0101	0.0132	0.0161	0.0187
SD	0.000104	0.000148	0.000188	0.000965	0.000603	0.000744

Precision:

Intra-day and Inter-day precision study was carried out by analyzing 6 replicates of equimolar solution of standard and acid degradation sample (20 µg/ml). Absorbance difference of equimolar solution of standard and acid degradation sample were noted at 290 nm and %RSD calculated.

Accuracy:

Accuracy of the method was determined by comparing results of degradation sample with result of analyte of known purity. Standard and Acid Degradation Sample solutions of 25, 35, 45µg/ml concentration were prepared in such way that it is covered by standard linearity solution(10-60 µg/ml). Concentration of these solutions was determined from linear regression line and % recovery was calculated. Results are given in Table 2.

Table 2: Recovery Studies

Nominal concentration (µg/ml)	Observed concentration (µg/ml)	% Recovery	Mean % Recovery
25	24.84	99.36	
35	34.75	99.28	99.95
45	45.55	101.22	

Limit of Detection (LOD) And Limit of Quantitation (LOQ):

The detection and quantitation limit were calculated from calibration curve. The following equations were used to calculate LOD and LOQ.

LOD = 3.3 × σ/S

LOQ = 10 × σ/S

Where, σ = the standard deviation of y intercept or standard deviation of responses at lowest concentration.   S = slope of the calibration curve.

Robustness:

Robustness is testing of the method by doing the small and deliberate changes to developed method. The optimized system is robust as %RSD is below 2 %.

III.   RESULTS AND DISCUSSION:

Zero order UV spectra of standard linearity and acid degradation sample (10-60 µg/ml) were recorded in range of 200-400nm. There was no spectral difference observed in zero order UV spectra of standard and acid degradation sample. Then these zero order spectra were converted to first derivative spectra. The first derivative spectra of equimolar solution of standard and acid degradation sample shows different spectral characteristics at 290nm, hence we decided to take absorbance difference i.e. absorbance of standard solution- absorbance of acid degradation sample solution. For this we overlay first derivative spectra of equimolar solution of standard and degradation sample shown in Fig.2 (A-F). At 290 nm wavelength overlay spectra of standard and

degradation sample show difference in absorbance in linear range and shown in Fig.3. Hence 290 nm selected as analytical wavelength for monitoring the hydrolytic degradation of Eluxadoline under acidic condition.
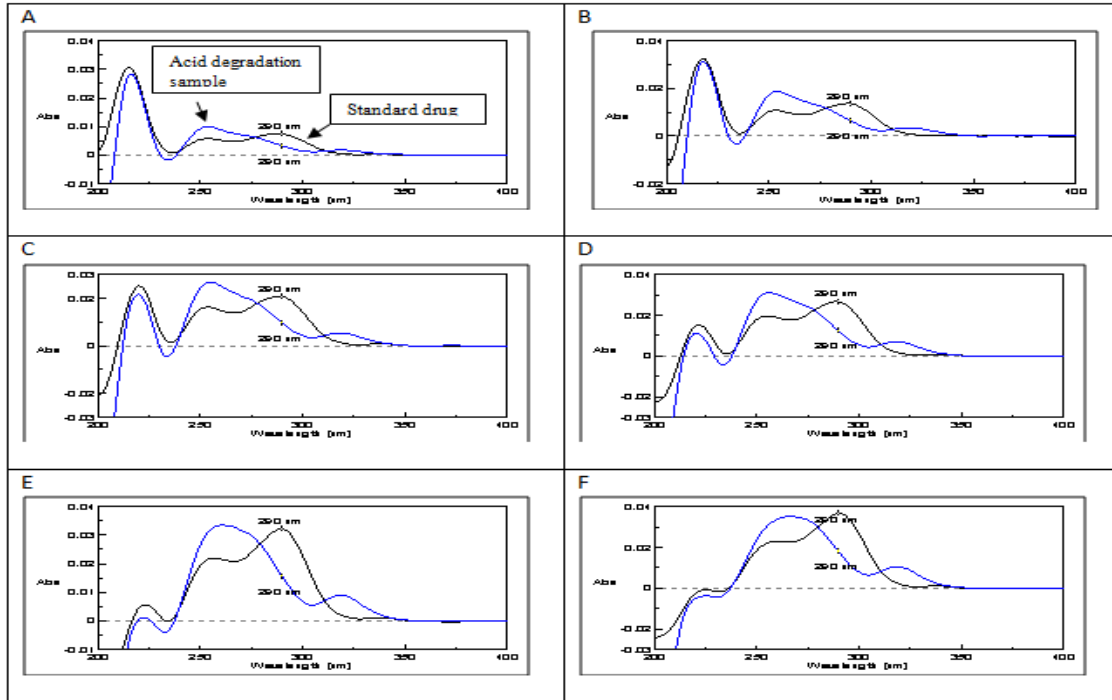

Fig. 2: First derivative overlay spectra of equimolar solution of standard and degradation sample (A-F: 10-60 μg/ml)
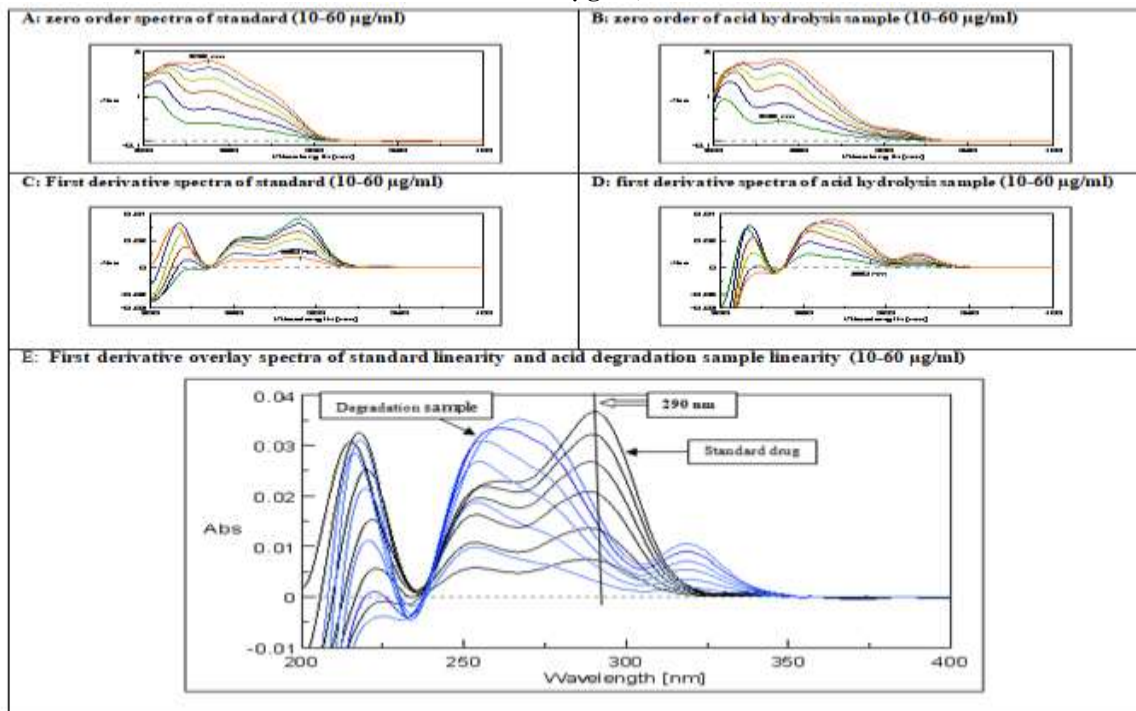

Fig.3: overlay spectra of standard and degradation sample

Developed method was successfully validated as per ICH Q2(R1) guideline, The developed method was found to be linear within the range of 10-60 µg/ml (r2 =0.9995), Precise as %RSD for Inter-day and Intra-day Precision were found to be < 2%, Accurate as % recovery value in accuracy study was found to be in range of 98-102% the And Sensitive as LOD and LOQ were found to be 1.14, 3.45 µg/ml and 3.54, 10.68 µg/ml respectively. Robust as %RSD <2%, Summary of validation parameter is summarized in Table 3.

Table 3: Summary of Validation Parameters

Sr.No.	VALIDATION PARAMETER		Result
1	Linearity		y= 0.0003x + 0.0013 R2= 0.9995
2	Range		10-60 µg/ml
3	Precision (%RSD)	Intra-day	1.35
		Inter-day	1.44
4	Accuracy (%Recovery)		99.95
5	LOD µg/ml	Using SD of responses at lowest concentration	1.14
		Using SD of y-intercept	3.45
6	LOQ µg/ml	Using SD of responses at lowest concentration	3.54
		Using SD of y-intercept	10.68
7	Robustness (wavelength change,290 ±1 nm)		Robust (%RSD < 2%)

IV. CONCLUSION:

The developed difference spectroscopic method is able to detect degradation of Eluxadoline under acidic condition. The developed difference UV-Spectrophotometric method for Eluxadoline met to validation criteria as per ICH Q2 (R1) guideline. Developed difference spectrophotometric method is simple, linear and can be used as preliminary test in quality control laboratories to monitor hydrolytic degradation of Eluxadoline.

V. ACKNOWLEDGEMENT:

CONFLICT OF INTEREST:
The authors have no conflicts of interest regarding this investigation**.**

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