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Estimation of Delafloxacin Using Derivative Spectrophotometry and Area Under Curve in Bulk Material and in Laboratory Mixture

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Abstract Simple, specific, rapid and accurate UV-spectrophotometric methods have been developed using a solvent acetonitrile (50 %) to determine delafloxacin in bulk material and in laboratory mixture. "Method A" is zero order derivative UV- spectrophotometry using absorbance, "Method B" is zero order derivative UV-spectrophotometry using Area Under Curve (AUC) technique, "Method C" is first order derivative UV-spectrophotometry using amplitude, "Method D" is First Order Derivative UV-spectrophotometry-AUC,"Method E" is Second Order Derivative UV-spectrophotometry using amplitude and "Method F" is second order derivative UV- spectrophotometry using (AUC) technique. The developed methods have shown excellent results in terms of linearity and range, accuracy, precision and Limit of Detection (LOD) and Limit of Quantification (LOQ). In all Methods, delafloxacin obeyed linearity in the concentration range of 2 - 12 µg/mL with ($r^2 > 0.999$). All these methods were applied for estimation of delafloxacin in laboratory mixture. All the above mentioned methods were validated considering linearity and range, accuracy, precision, ruggedness and sensitivity.

Keywords: Delafloxacin; UV-Spectrophotometry; Zero order Derivative; First order Derivative; Area under curve; Second Order Derivative

1. INTRODUCTION

Delafloxacin (DLF) is 1-(6-amino-3, 5-difluro-2-pyridyl)-8-chloro-6-fluoro-7-(3-hydroxyazetidin-1-yl)-4-oxoquinoline-3-carboxylic acid [1] (**Figure 1**). The ability of Delafloxacin, a fluoroquinolone, to form cleavable complexes with DNA and topoisomerase IV or DNA gyrase makes it dual-targeting ligand,

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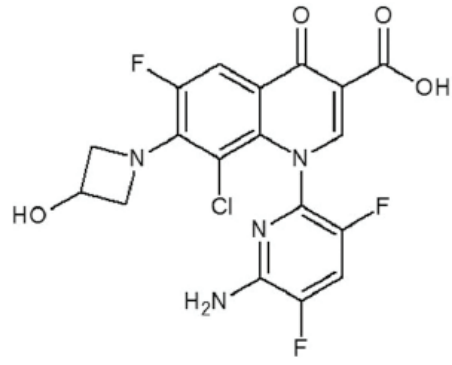


Figure 1: Chemical Structure of Delafloxacin.

which is able to inhibit these two enzymes in both Gram positive and negative bacteria [6]. It is primarily used to treat infections of skin and respiratory system [2]. In the literature, McEwen and co-workers have reported a LC-MS method for the analysis of delafloxacin and their metabolites in pooled urine, faeces and plasma [4].

The AUC method involves measurement of united value of area in the range of selected two wavelengths, 1 and 2. The wavelength range selection is a result of repeated observations until a linearity is achieved between AUC and concentration [5].

To our knowledge no method(s) were found in literature for determination of delafloxacin on bulk and formulation using derivative spectroscopic techniques. Therefore, our endeavor is to establish zero-, first- and second-order derivative UV-Spectrophotometry using amplitude and also AUC techniques. Further, methods were validated as per ICH guidelines [3].

2. EXPERIMENTAL WORK

2.1 Materials

Delafloxacin was received as free sample from Alkem Laboratories, Mumbai. All chemicals and reagents were purchased from Merck chemicals, Mumbai, India, of analytical grade.

2.2 Instrument

For present analysis, a double beam UV-VIS spectrophotometer (UV-2450, Shimadzu, Japan) was used, which was linked to computer installed with spectra manager software UV Probe 2.21 with 10 mm quartz cells. The spectra

were obtained with following set of instrumental parameters: wavelength range: 400 - 200 nm; scan speed: medium; sampling interval: 10 nm; band width: 1.0 nm; spectral slit width: 1 nm. An electronic balance (Model Shimadzu AUX 120) was used for weighing purpose.

2.3 Preparation of Stock Standard Solution (SSS)

The SSS was prepared by accurately weighing and dissolving 10 mg of delafloxacin in 100 ml of acetonitrile (50%) to achieve final concentration of 100 µg/mL.

2.4 Methods A (Zero Order Spectrophotometry) and Method B (Zero order Spectrophotometry–AUC)

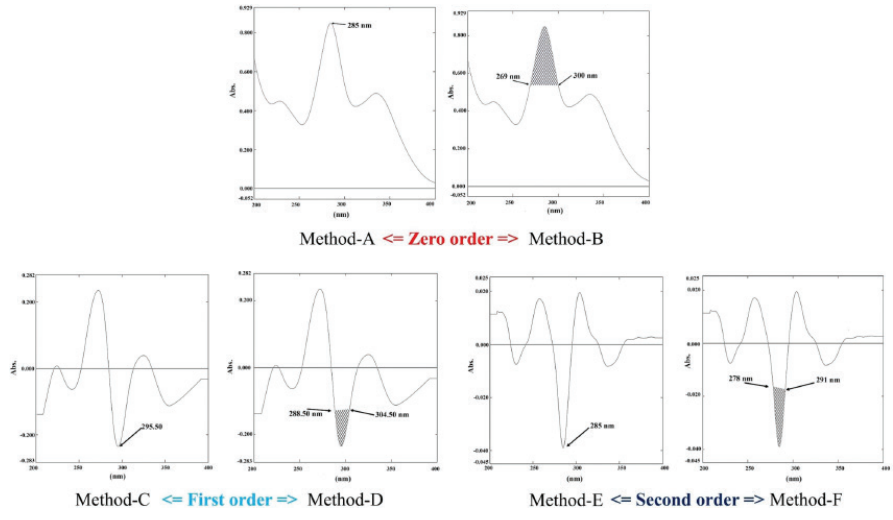
From the stock standard solution, an appropriate volumes 0.2 – 1.2 mL were transferred into a series of 10 mL volumetric flasks, followed by their volume make up to obtain final concentration ranging from 2 to 12 µg/mL. In Method A, absorbance was recorded at 285 nm while in Method B, area under curve was selected in the wavelength range of 269-300 nm. In method A, the linearity curve was plotted between concentration and absorbance while in method B, it was plotted between concentration and AUC.

2.5 Methods C (First order derivative –UV Spectrophotometry and D (First order Derivative –UV Spectrophotometry-AUC)

In Method C and D, spectra of above prepared solutions, in the range of 2 -12 µg/ mL, were derivatized into first order using software UV-Probe 2.21 with delta lambda 10 and scaling factor 10. In Method C, the amplitude was recorded at 295.50 nm while in Method D, AUC of the derivatized spectrum was studied at 288.50- 304.50 nm. The linearity curve was plotted between concentration and amplitude in Method C, while, on the other hand, in method D, it was plotted between concentration and AUC of first order derivative spectra between selected wavelengths.

2.6 Methods E (Second order Derivative UV Spectrophotometry) and F (Second order Derivative UV Spectrophotometry-AUC)

In method E and method F, spectra of above prepared delafloxacin solutions in the range of 2 - 12 µg/ mL were derivatized into second order using software UV-Probe 2.21 with delta lambda 10 and scaling factor 10. In “method E” the amplitudes were recorded at 285 nm while in “Method F” AUC was recorded in between two wavelengths 278-291 nm. The linearity curve was plotted between concentration and amplitude in Method E while, in method F, it was



plotted between concentration and AUC of second order spectra at selected wavelengths.

2.7 Preparation of Delafloxacin Laboratory Mixture

Laboratory mixture was prepared with 100 mg of delafloxacin and common excipients.

3. VALIDATION OF METHOD

All developed methods, i.e. A-F were validated as per ICG guidelines.

3.1 Linearity

In methods A-F, delafloxacin displayed linearity in concentration range of 2-12 µg/mL. The details of optical characteristics and linearity data is furnished in **Table 1.**

3.2 Recovery studies

The accuracy of methods used was confirmed by with percentage recovery studies. The known amounts of SSS were added into the pre-analyzed sample solutions (4 µg/mL), at levels of 80, 100 and 120%. The solutions were analyzed again using proposed methods, and the experiments were performed in triplicate at each level of all methods. The corresponding results are summarized in **Table 2.**

3.3 Precision

Method precision was ensured analyzing inter- and intra-day deviation among experiments. For this purpose, the solutions of 4, 6 and 8 µg/mL of delafloxacin were analyzed, and deviation was noted (**Table 2).**

3.4 Sensitivity

The sensitivity of delafloxacin measurement was determined in terms of LOD and LOQ denoted as limit of quantification and detection respectively.

Table 1: The Optical Properties.

Criterion	Method A	Method B	Method C	Method D	Method E	Method F
Beer-Lambert's range (µg/mL)	02-12	02-12	02-12	02-12	02-12	02-12
max (nm)/ Wavelength range (nm)	285	269-300	295.50	288.50-304.50	285	278-291
Slope	0.0835	0.5497	0.0226	0.1137	0.0038	0.0186
Intercept	0.0206	0.0927	0.0073	0.0011	0.0008	0.0011
Correlation coefficient	0.9992	0.9992	0.9993	0.9998	0.9991	0.9990

Table 2: Validation Parameters.

Parameters		A	B	C	D	E	F
Accuracy	80 %	100.07	100.59	98.95	98.99	97.86	98.90
	100%	99.85	99.76	99.74	98.99	97.58	99.10
	120%	98.35	100.70	98.02	98.24	98.84	98.71
Precision (% RSD)	**Intraday (n=3)**	1.01-1.20	0.91-1.32	0.42-1.05	0.52-1.11	0.81-0.83	0.95-1.18
	Interday	0.73-1.22	0.44-1.31	0.37-0.76	0.69-1.10	0.90-1.52	1.20-1.54
Repeatability (% RSD)		1.16	0.94	1.38	0.45	1.92	1.76
Ruggedness (% RSD)	**Analyst I**	0.29	0.75	1.36	0.41	1.51	1.69
	Analyst II	1.11	0.92	0.42	0.75	1.86	0.87
Limit of Detection		0.0472	0.0631	0.0581	0.0900	0.0750	0.0566
Limit of Quantification		0.1429	0.1913	0.1917	0.2729	0.2273	0.1714

The LOD and LOQ was calculated as per 3.3*N/B and 10*N/B equations respectively, wherein 'N' denoted standard deviation of absorbance, amplitude and peak areas of delafloxacin (n=3), taken as a measure of noise, and 'B' signifies the slope of corresponding calibration curve. Results are summarized in **Table 2.**

3.5 Repeatability

Repeatability analysis was performed by analyzing 6 µg/mL delafloxacin solutions for six times for all methods (**Table 2).**

3.6 Ruggedness

The ruggedness of developed methods was analyzed with 6 µg/mL solution of delafloxacin through evaluation of aliquots from a homogenous lots, performed by two different analysts under similar operational and environmental conditions for all methods. The results were in an acceptable range that is % RSD values <2 for all methods (**Table 2).**

3.7 Analysis of Laboratory Mixture

Laboratory mixture of Delafloxacin was prepared by using common excipients. The 100 mg of drug was accurately weighted and taken into a 100 mL volumetric flask containing Acetonitrile (50%), already sonicated for 15 min, followed by volume make up to the mark and filtration using Whatmann filter paper (number 41). From this solution; an appropriate volumes of 0.3 mL were diluted to 50 mL using same solution. The resulting solutions were scanned by using UV spectrophotometer in the range of 400-200 nm. The amounts of drug estimated using various proposed methods as determined from respective linearity equations and results are reported in **Table 3.**

4. RESULT AND DISCUSSION

In Acetonitrile (50%), delafloxacin displayed linearity for concentration range of 2-12 µg/mL. The maximum absorbance (λ max)/wavelength range and correlation coefficient for all methods given in **Table 1**. Laboratory mixture of delafloxacin were analyzed. The amounts of delafloxacin in mixture determined by all methods are given in **Table 3**.

In all methods, inter- and intra-day precision was studied (% RSD less than 2), and accuracy of all methods was determined calculating mean % recovery at 80, 100 and 120 % level. The results of accuracy study, repeatability, and ruggedness are represented in **Table 2.**

Table 3: Analysis of Laboratory Mixture.

Methods	Amount found (µg/mL)	% Amount found
A	5.9024	98.37 ± 1.39
B	5.9598	99.32 ± 1.55
C	5.8518	97.52 ± 1.07
D	6.0057	100.05 ± 1.15
E	6.0263	100.43 ± 1.51
F	5.8754	97.92 ± 1.61

CONCLUSION

A total of six methods were established to analyze delafloxacin based on different UV spectrophotometric derivative and AUC methods. The established methods were quite simple, and duly validated in terms of accuracy, sensitivity and precision. As such, these methods can benefit other researchers, involved in similar studies, to perform routine analysis of delafloxacin in bulk material as well as in in laboratory mixture.

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