ISSN: 2230-9926

Available online at http://www.journalijdr.com

IJDR

International Journal of Development Research
Vol. 5, Issue, 06, pp. 4702-4706, June, 2015

International Journal of
DEVELOPMENT RESEARCH

Full Length Research Article

DETERMINATION OF LEVOFLOXACIN IN PHARMACEUTICAL FORMULATIONTAVANIC BY VISIBLE SPECTROPHOTOMETRY OF ITS CHELATING COMPLEX WITH ALUMINUM ION (III)

***Ameen W. Qassim**

Department of Pharmaceutical Chemistry, Baghdad College of Pharmacy, Bab Al Muadha'am, Baghdad, Iraq

ARTICLE INFO

Article History:
Received 16th March, 2015
Received in revised form
19th April, 2015
Accepted 31st May, 2015
Published online 28th June, 2015

Key words:
Levofloxacin,
Spectrophotometry,
Chelating Complex.

ABSTRACT

A simple, sensitive and accurate spectrophotometric method has been developed for the analysis of levofloxacin (LEV) in pharmaceutical formulations, through formation of yellow-greenish chelating complex LEV-Al(III) of levofloxacin with aluminum (III) ion. The maximum absorbance (420)nm. Different variables affecting the reaction were carefully studied. The optimum concentration for metal ion (28)µg ml^{-1}, optimum temperature for the reaction (60) $C^0$and optimum time for the reaction (5) min Under optimized conditions, linearity was observed in the range of 5-45 µg mL^{-1} with detection limit (S/N) of 0.064 µg mL^{-1}, precision in range of 0.93-1.51 % , accuracy as the % E_{rel} of 1.16%, and recoveries ranged from 100.83 to 101.65 % with mean value of 101.16 ± 0.84. The proposed method was applied for the determination of LEV in the drug Tavanic by both direct calibration and standard additions procedures and found to be 4.95 and 4.89 mg per mL, respectively compared with the stated value of 5 mg per mL. All statistical calculations were implemented via the chem. software Minitab version 11.

INTRODUCTION

Levofloxacin (Fig. 1), (*S*)-(-)-9-fluro-2,3,-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7*H*-pyrido[1,2,3-*de*][1,4] benzoxazine-6-carboxylic acid (Wong *et al*., 1997). A third-generation fluoroquinolone, is the activelevo-isomer of ofloxacin and is twice as active as the parent drug. It's active against both Gram-positive and Gram-negative bacteria. Levofloxacin is administrated to patients with urinary, respiratory or cutaneous infections, in 500 mg/day doses. Levofloxacin is mainlyexcreted in urine (>85%), in unaltered form (Gonzalez *et al*., 2000). In Gram-negative bacteria ,the main target for fluoroquinolones is the complex between DNAand a topoisomerase II enzyme called DNA gyrase. It is requiredwhen the DNA double helix is being supercoiled after replication andtranscription. Thus, the main effects of fluoroquinolones are theinhibition of DNA supercoiling and the damage to DNA, whosesynthesis is required for bacterial growth (Patrick, 2009; Tripathi, 2008 and Jakob, 2003).Some papers have described the analysis of levofloxacin by Spectrophotometer (Ebraheem and Elbashir, 2012; Brashy *et al*., 2005; Sachan *et al*., 2012 and Shirkhedkar and Surana, 2009) and by other analytical methods (HPLC, LC/MS, HILIC-MS/MS) (Wong *et al*., 1997; Ji *et al*., 2006 and Watabe *et al*., 2010) capillary electrophoresis (CE) (Faria *et al*., 2006), spectrofluorimetry (Salem *et al*., 2007). The increasing utilization of this fluoroquinolone drug as anantibacterial agent demands the development of new and alternativemethods to successfully determine levofloxacin in raw material andpharmaceutical dosage forms. Therefore, an attempt to design amethod of estimation, which may be superior in some context to theexisting ones, was thought to be worth the effort. Thus, the aim of the study was to develop and validate analytical methods to quantify levofloxacin in bulk drug, and infusion, using UVspectrophotometer, for rapid determination of which should offersimplicity, reproducibility, sensitivity, and accuracy.

Corresponding author: Ameen W. Qassim
Department of Pharmaceutical Chemistry, Baghdad College of Pharmacy, Bab Al Muadha'am, Baghdad, Iraq

Experimental

MATERIALS AND METHODS

Levofloxacin and pharmaceutical formulation Tavanic (solution for intravenous infusion) were purchased from local market. Aluminium chloride waspurchased from local market. Ultraviolet/Visible spectrophotometer (Analytikjenaspecord 40 USA) with matched 1 cm quartz cell was used for all measurements. Infrared spectrum for the produced complex

was recorded on Shimadzu Fourier Transform Infrared model FT-IR8000.For pH measurement its used pH meter Hana (microprocessor pH meter pH210).

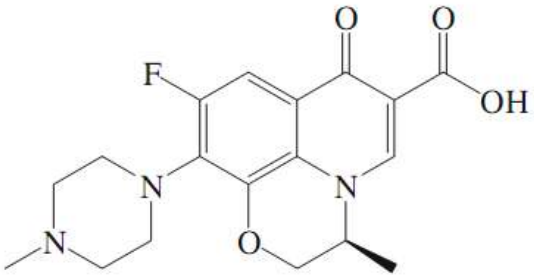

Fig.1. Levofloxacin

Standard solution and working solution preparation

Double distilled water was used as solvent. Stock standard solution of 500 µg $mL^{-1}$was prepared by dissolving 0.1g of levofloxacin in sufficient distilled water and diluted to 100 mL in volumetric flask. Working standard solution of different concentration of 5 µg mL^{-1}, 7 µg mL^{-1}, 10 µg mL^{-1}, 13 µg mL^{-1}, 16 µg mL^{-1}20 µg mL^{-1}, 25 µg mL^{-1}, 30 µg mL^{-1}, 35 µg mL^{-1}, 40 µg mL^{-1}, 45 µg ml^{-1}, was prepared by dilution of stock standard solution with distilled water. Stock standard solution was stable for several weeks at room temperature. A100 µg $mL^{-1}$of Aluminium chloride was prepared by dissolving 0.0490 g of $AlCl_3$ in distilled water and diluted to 100 ml into a volumetric flask.

General Procedure and Analytical Curves

Direct Calibration Method

Aliquots of (0.5-4.5 mL) of stock standard solution of levofloxacin (100 µg mL^{-1}) were transferred into eleven of 10 ml volumetric flask then 0.28 ml of 100 µg AL mL^{-1} was added to each flask. The solutions were put in a water bath at 60 C^0 for 5 min, and then diluted to 10 ml with distilled water. These solutions were corresponding to (5-45 µg LEV mL^{-1}). The absorbance were measured at 379 nm. The analytical curve was obtained by plotting absorbance against LEV concentration and the corresponding linear regression equation was used to convert absorbance into LEV concentration, for all analyzed Tavanic samples.

Sample Preparation of drug Tavanic (Infusion treatment)

Accurately measured 10 mLof infusion equivalent to 50 mg of levofloxacin was transferred into 100 mL volumetric flask and dilutes to mark with water then transferred 4 ml of this stock solution to 100 mL volumetric flask and diluted to mark with water (100µg mL^{-1}).

Standard Additions Method

Aliquots of 0.5 mL the above–prepared Tavanic sample solution were pipetted into thirteen of 10 ml calibrated flaks containing 0.000, 0.250, 0.500, 0.700, 1.000, 1.300, 1.600, 2.000, 2.500, 3.000, 3.500, 4.000, 4.500 mL of 100 µgLEV mL^{-1} then same steps were proceeded according to the procedure previously mentioned under direct calibration method.

RESULTS and DISCUSSION

Optimum Conditions

Effect of Al (III) concentration: It was found that the absorbance of LEV-Al (III) complex increases as the concentration ofAluminium (III) ions increases and then deviate towards the Al concentration axis Fig.2. Consequently, the optimum concentration of Al (III) of 28µg mLl^{-1} was selected to complete formation of chelating complex.

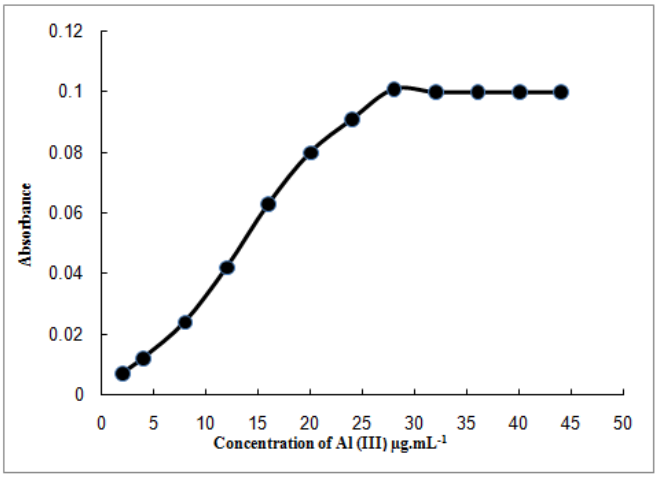

Fig.2. The effect of Al (III) ion concentration on the formation of LEV-Al(III) complex

Effect of temperature: it was found that the reaction rat increase by increase the temperature and reach maximum absorbance at 60 C^0 then the absorbance became decrease by increase temperature due to the dissociation of complex, consequently the optimum temperature was 60C^0 Fig.3.

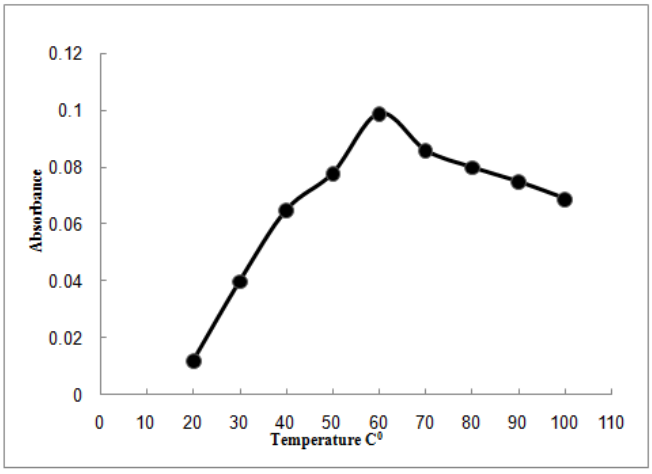

Fig.3. The effect of temperature on the formation of LEV-Al(III) complex

Effect of reaction time: The optimum reactiontime was determined by following the absorbance incrementat the λ max

of the formed complex Fig.4. It was found LEV-Al(III) completely developedafter heating in a water bath at 60 °C for 5 min.

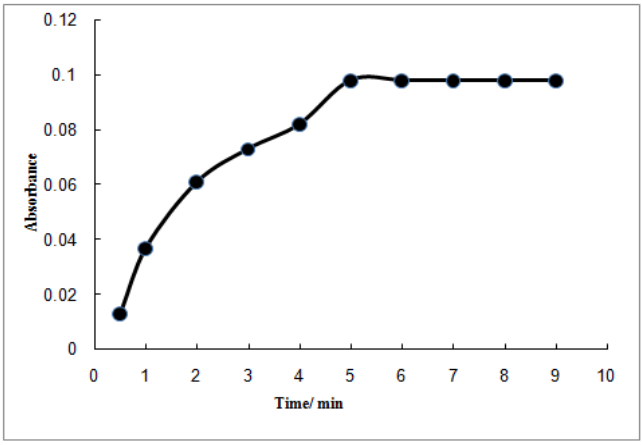

Fig.4. The effect of reaction time on the formation of LEV-Al(III) complex

Suggested Structure of the Complex

In the IRspectra of the complexes the absorption ofthe ν (C=O)carb has disappeared. Two very strong characteristic bands are present in the range 1601–1615 and1380-1395cm^{-1}assigned as ν (O–C–O) asymmetric andsymmetric stretching vibrations, respectively. The νvalues fall in the range 200–229 cm^{-1}indicating a mono-dentate coordination mode of the carboxylate group of the ligand (Efthimiadou *et al.*, 2007) The band observed at1623cm^{-1}is assigned to pure C=O stretching mode of ringcarbonyl group. The vibration $\nu(C=O)_p$ shifted from 1623 to1635cm^{-1}upon bonding which also suggests the binding oflevofloxacin to the metal ions through the ring carbonyloxygen atom (Lecomte *et al*., 1994). The IR spectra of all thecompounds exhibited a broad split band between 3600 and3100 cm^{-1}assigned to the O–H stretching vibrations ofwater molecules which also includes the N–H stretchingvibration of the piperazinylmoiety. The overall changes of theIR spectra suggest that the ligand is coordinated to metals viathe pyridone and carboxylate oxygen (Efthimiadou *et al*., 2006).

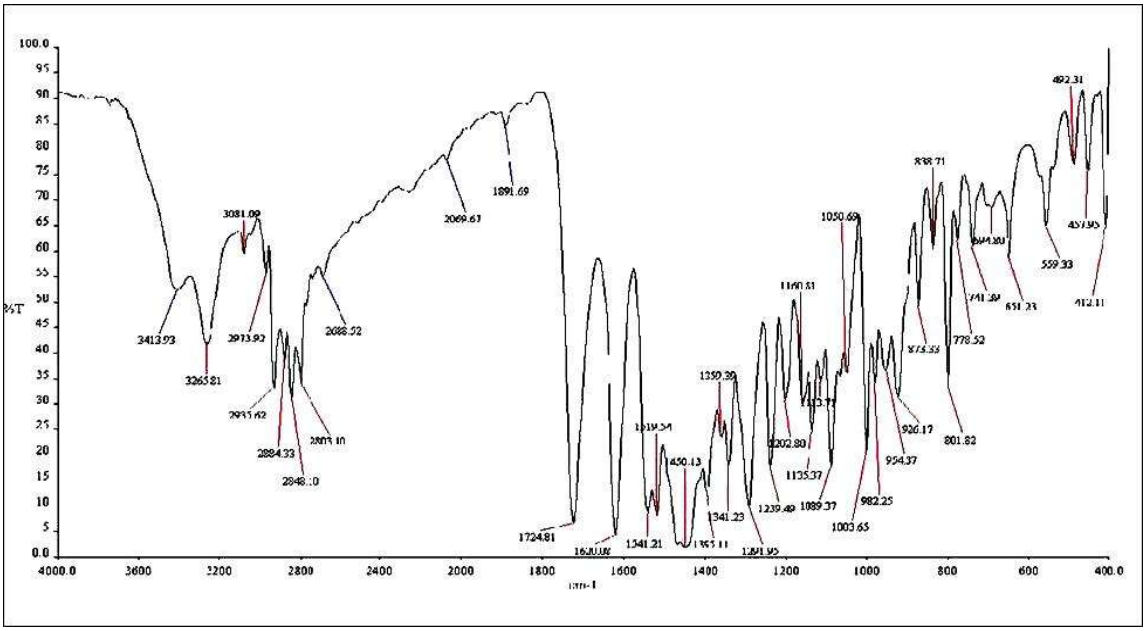

Fig.5. FTIR spectra of levofloxacin

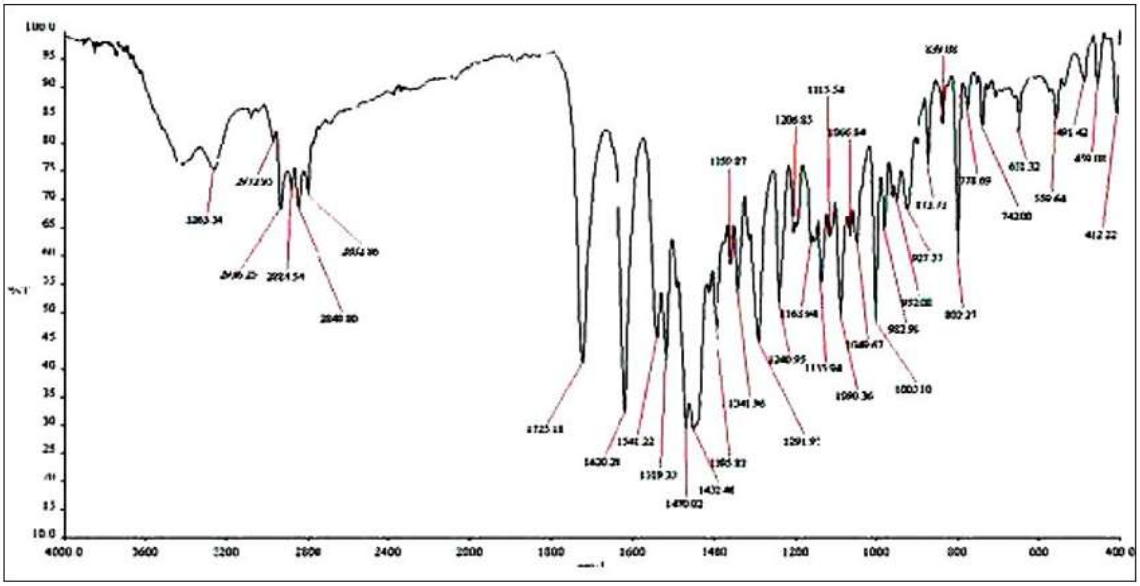

Fig.6. FTIR spectra of LEV-Al(III) complex

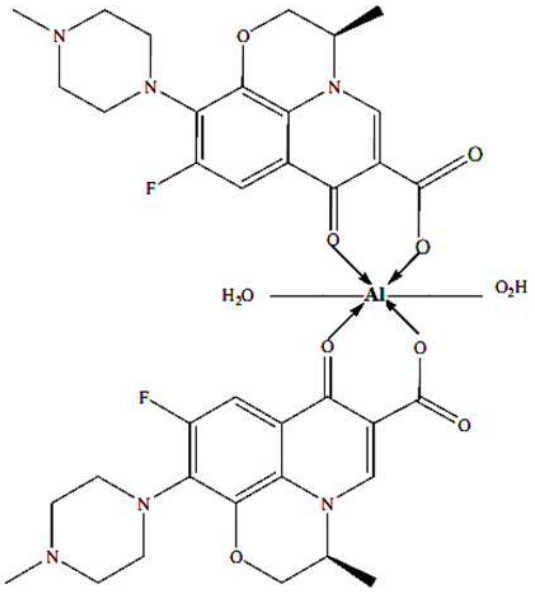

Fig.7. Predicted structure of LEV-Al(III) complex

Method Validation

Under the experimental conditions described above, the calibration graphs for the LEV-Al(III) constructed by plotting absorbance versus concentration in µg mL^{-1}Conformity with Beer's law was evident in the concentration ranges cited in Table1.

Table 1. Collective performance data for the analysis of levofloxacin by the proposed method

Analytical parameters	Values
λmax (nm)	420
Beer's law limits (µg.ml-1)	5 – 45
Correlation coefficient (r^2)	0.9993
Regression equation (y=bx+a)	y= 0.0022x + 0.0011
Slope (b)	0.0022
Intercept (a)	0.0011
LOD (µg.ml-1)	0.009
LOQ (µg.ml-1)	0.25
Molar absorptivity (Ɛ) (L mol-1 cm-1)	$111.15x10^3$
Sandell's sensitivity (S) (mg.cm-2)	$3.33x10^{-3}$

Determination of LEV in Tavanic

The proposed method was applied for the detection of LEV in Tavanic (solution for intravenous infusion) vials with stated value of 5 mg per unit by using direct calibration and standard additions procedures (Fig.8) under optimum conditions. The LEV was determined through measuring the absorbance of the complex results from the reaction LEV present in the pharmaceutical preparation with Aluminium (III) ion and found to be 4.93 and 4.89mg / unit with relative error of (-1.40%) and (-2.2%) respectively. It can also be observed from (Fig.8), that the ratio of the slopes of the direct calibration and standard additions is found to be the same, which indicates that the interferences resulting from drug constituents are insignificant using the proposed procedure. Thus, it is possible to use direct calibration procedure for the determination of LEV in drugs without need the standard additions method which requires more effort, more amount of sample and time-consuming. This is also support the specificity of the proposed method, indicating that the excipients did not interfere with the analysis of LEV.

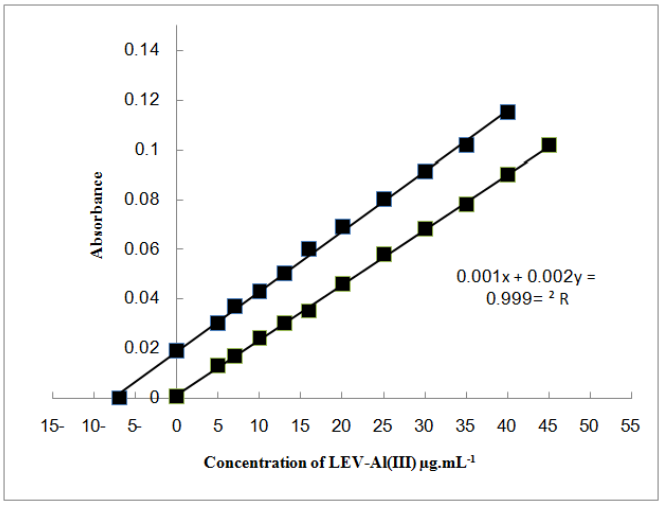

Fig.8. Determination of LEV in pharmaceuticals by using direct and standard additions Procedures

Table 2. The accuracy and precision of the proposed method for the determination of LEV in pharmaceutical preparation

Amount of Levofloxacin taken (µg.mL^{-1})	Amount of Levofloxacin found (µg.mL^{-1})	%Rec.	$\%E_{rel.}$	%RSD n = 5	Conf. Limit. for %Rec.±S.D	Mean $\%E_{rel.}$
10	10.10	101.00	1.00	1.51	101.16 ± 0.84	1.16
20	20..33	101.65	1..65	1.27		
40	40.63	100.83	0.83	0.93		

Regression equations, intercepts, slopes correlation coefficient, Limit of Detection (LOD), limit of quantification (LOQ) Sandell's sensitivity and The molar absorptivity for the complex for the calibration data were presented in Table 1. Theaccuracy in term of recovery percent and precision were achieved by spiking of 10, 20 and 40 µg ml^{-1} using the recommended procedure previously mentioned under section (2.2.4). The results were shown in Table 2. These data indicate that the visible spectrometric determination of LEV is not highly effected by the presence of other constituents in the drug sample.

Conclusions

The method that proposed in this work for the quantitation oflevofloxacin was simple, rapid, accurate and precise. The proposedmethod is also inexpensive due to use of distilled water for thedilution. Therefore, this method can be used for routine analysis oflevofloxacin in bulk and pharmaceutical formulations like tabletinfusion.

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