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ASSAY OF CIPROFLOXACIN BY ULTRAVIOLET-VISIBLE SPECTROSCOPY

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ABSTRACT

Ciprofloxacin belongs to a group of drugs referred to as fluoroquinolone class antibiotics, and is utilized to treat bacterial infections. This antibiotic was assayed by ultraviolet-visible spectroscopy by use of single-beam SpectroVis spectrometer in one-centimeter plastic cuvettes at 390 nm. Samples were analyzed from normal saline, aqueous mixture of dextrin/cellulose/starch/lactose, and aqueous solvent having 1% (v/v) ethanol, 1% (v/v) acetic acid (0.167 molar), 98% (v/v) distilled water. The standard curve ranged from 1.3919×10^{-3} molar to 1.4934×10^{-2} molar. The equation of line for standard curve is: $y = 52.388x + 0.1435$, with coefficient of determination R^2 of 0.9943, meaning 99.43% of the proportion of the variance in the dependent variable (absorbance) is predictable from the independent variable (concentration in molar). The Pearson r correlation coefficient is 0.9971. The limit of detection (LOD) is 1.0915×10^{-3} molar and the limit of quantitation (LOQ) is 3.6382×10^{-3} molar. The assay of this important broad-spectrum antibiotic that is active against both Gram-positive and Gram-negative bacteria is vital for manufacturing production assurance, patient compliance assurance, and prevention of adulteration of product.

KEYWORDS: ciprofloxacin, UV/Vis spectroscopy, antibiotic, Spectro-Vis.

INTRODUCTION

Ciprofloxacin is an important synthetic broad-spectrum antibiotic of the fluoroquinolone class, that is applied to treat respiratory infections, skin infections, infectious diarrhea, typhoid fever, as well as bone and joint infections.[1,2] It is a second-generation fluoroquinolone causing the death of the bacteria.[1,2] It is effective against both Gram-

positive and Gram-negative bacteria.[1] This antibiotic acts through the inhibition of DNA-gyrase, an enzyme that is critical to bacterial chromosome replication.[3,4] Ciprofloxacin, contains a piperazine group at position 7 of the 4-quinolone nucleus, which results in activity against Pseudomonas aeruginosa.[4]

Methods for the determination of ciprofloxacin have varied in the past. Ciprofloxacin is utilized is some ophthalmic solutions.[3] A microbiological assay applying the turbidimetric method for the determination of ciprofloxacin hydrochloride in ophthalmic solutions. This is a bioassay based on the inhibitory effect of CIPRO HCl upon a strain of Staphylococcus epidermidis ATCC 12228, which is used as the test microorganism.[3] Another methodology is a rapid method for the determination of ciprofloxacin hydrochloride in ophthalmic solution using ultraviolet light spectrophotometry.[4] The absorbance of ciprofloxacin was measured directly at 275 nm.[4]

Other methods include an ultraviolet spectrophotometric assay of samples of ground tablets prepared with the same solvent. The assay was determined by measuring the absorbance of stock solution against the solvent blank and comparing with the absorbance of ciprofloxacin at the wavelength of 278 nm by spectrophotometer.[5] Another is an assay of ciprofloxacin being accomplished by spectrofluorometric method, where the wavelengths of excitation and emission were 290 and 450 nm, respectively.[6] A high performance liquid chromatographic method for the quantitation of Ciprofloxacin in plasma using Acebutolol as the internal standard has been developed with a procedure involves protein precipitation with 7% perchloric acid. The drug and the internal standard were eluted from a 4-µm stainless steel Novapak C18 column.[7] A reversed-phase LC method for the determination of ciprofloxacin hydrochloride in ophthalmic solution form was achieved on a Symmetry Waters C18 column using UV detection at 275 nm.[8] A reversed-phase chromatography assay methodology used an RP-C18 column with an isocratic mobile phase of acetonitrile-2% acetic acid aqueous solution (16:84, v/v).[9] Another high-performance liquid chromatographic method for the determination of ciprofloxacin in serum utilized acetonitrile to precipitate serum protein. The drug were evaluated from a 10 microns U-Bondapack C-18 cartridge.[10]

The assay of this important broad-spectrum antibiotic is shown here by use of SpectroVis UV-Visible spectrophotometry to be highly accurate, sensitive, and reproducible. The assay of this drug is vital for manufacturing production assurance, patient compliance assurance, and prevention of adulteration of product.

MATERIALS AND METHODS

Reagents

All solvents were analytical grade and obtained from Sigma-Aldrich (St. Louis MO 63178 USA). The ciprofloxacin hydrochloride compound for use as standards and preparation of samples was obtained from Bayer Corporation, Pharmaceutical Division, 400 Morgan Lane, West Haven, CT, 06516 USA.

Instrumentation

SpectroVis Plus is a portable, single beam, visible to near-IR spectrophotometer and fluorometer. The SpectroVis Plus collects a full wavelength spectrum (absorbance, percent transmission, or intensity) as well as single wavelength measurements from wavelength Range: 380 nm–950 nm. The light source is incandescent (VIS), with wavelength accuracy: ± 2.0 nm. Generates full spectrum, Beer's law graph, and kinetics traces of visible samples, with or without USB interconnection. Cuvettes used were plastic and 1 centimeter width with absorbance readings obtained at 390 nm.

Samples were prepared and analyzed in various solvents. A stock solution of 1.6576×10^{-2} molar was prepared. Samples were analyzed by SpectroVis Plus from preparations with solvent of distilled water (98% v/v), 1% (v/v) ethanol, and 1% (v/v) acetic acid (0.167 molar). Samples were also analyzed having various amounts of dextrin, cellulose, starch, and lactose to represent excipients commonly used to administer this antibiotic.

Where necessary the filtering of mixtures having cellulose and/or starch excipients, was accomplished by Whatman 6900-2502 GD/X 25 Sterile Syringe Filter, 25mm, 0.2 Micron, PVDF Filtration Medium, with suitable plastic syringe.

Statistical analysis

Where indicated the numerical analysis for correlation, 95% ellipses, other parameters are determined by PAST version 2.06 (copyright Hammer and Harper 1999-2011). Microsoft EXCEL (copyright 2010 Microsoft Corporation, Microsoft Office Professional Plus 2010) and PAST v. 2.06 also performed summary statistical analysis. Evaluation of standard curve linearity, runs test, and other features was accomplished by GraphPad Instat version 3.06 (copyright © 1992-2003 by GraphPad software, Inc). Molecular properties of ciprofloxacin were determined utilizing Molinspiration cheminformatics http://www.molinspiration.com/ (Molinspiration Cheminformatics, Nova ulica, SK-900 26Slovensky Grob, Slovak Republic).

Outliers identified using Grubb's test (Graph Pad Quick Calcs, https: //www.raphpad. com/quickcalcs/). Evaluation of the standard curve by linear regression with parameters was accomplished by Method Validator (www.multiqc.com).

RESULTS AND DISCUSSION

Ciprofloxacin is an important broad-spectrum antibiotic of the fluoroquinolone class. It is effective for the treatment of infections originating from both Gram-positive and Gram-negative bacteria.[1,2] It's effectiveness includes those of bone and joint infections, intra-abdominal infections, certain type of infectious diarrhea, respiratory tract infections, skin infections, typhoid fever, and urinary tract infections, among others.[1,2] For the United States the number of prescriptions written for ciprofloxacin in 2016 is recorded as 7,192,190 prescriptions.[11] From 2006 to 2016, the number of ciprofloxacin prescriptions in the United States ranged from five million to as high as nine million.[11]

The molecular structure of ciprofloxacin is presented in Fig. 1. The structure contains a carboxylic acid group (-C(=O)OH), dihydro-quinoline, and piperazine rings. This antibiotic has formula of $C_{17}H_{18}FN_3O_3$. The structure has one hydroxyl group (-OH), two tertiary amine groups (-N<), and one secondary amine group (>NH). The fluorine atom places this drug into the fluoroquinolone class of antibiotics. IUPAC name of this important antibiotic is 1-cyclopropyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid. There are three rotatable bonds, 24 atoms, and a molecular volume of 285.5 Angstroms^3.

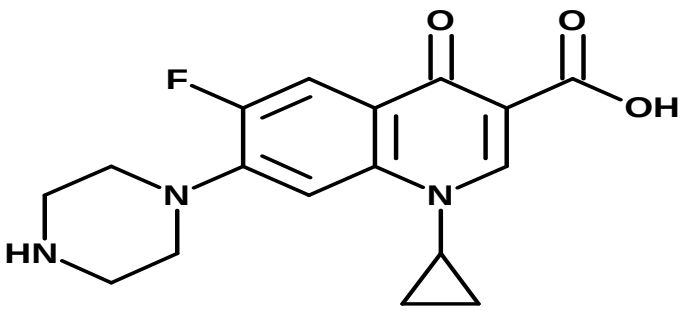

Fig. 1: Molecular structure of ciprofloxacin. IUPAC name is 1-cyclopropyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid with SMILES notation O=C(O)C2=CN(c1cc(c(F)cc1C2=O)N3CCNCC3)C4CC4. The molar mass is 331.35 grams per mole, Log P is -0.70, and polar surface area of 74.57 Angstroms^2.

Generally, the visible light region consists of a spectrum of wavelengths that range from approximately 900 nanometers (nm) to approximately 400 nm, with ultraviolet range for

spectroscopy at 200 nm to 400 nm. The SpectroVis instrument is a single-beam instrument, in that the cuvette containing only a solvent has to be measured first. The absorbance spectra for ciprofloxacin was obtained by SpectroVis spectrometer and shown in Fig. 2.

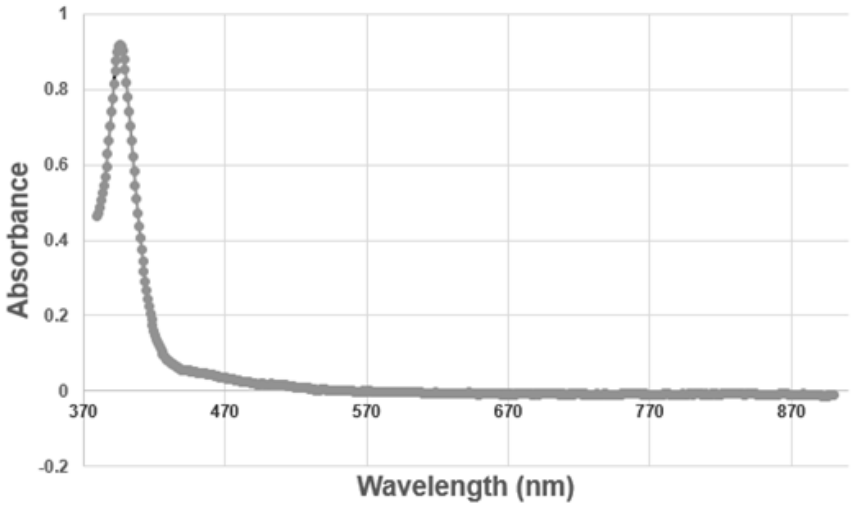

Fig. 2: The absorbance spectrum of ciprofloxacin from 380 nm to 900 nm. Ciprofloxacin at 1.6576 x 10^{-2} molar in solvent with 1% (v/v) ethanol, 98% (v/v) distilled water, and 0.167 molar acetic acid (1% v/v). Absorbance peak is located at 390 nm.

The absorbance spectrum for ciprofloxacin from 380 nm to 900 nm is easily acquired in the SpectroVis instrument (see Fig. 2). In this figure, the solvent is measured first as a "blank" calibration step (solvent: 1% (v/v) ethanol, 98% (v/v) distilled water, and acetic acid (1% v/v)). This is followed by measurement of the analyte solution at 1.6576 x 10^{-2} molar, which gave a strong absorbance peak at 390 nm. Determination of this wavelength peak for ciprofloxacin assay, was easily accomplished.

A standard curve was established for assay of this antibiotic, that was highly linear, and presented in Fig. 3. The range in concentration is from 1.3919 x 10^{-3} molar to 1.4934 x 10^{-2} molar. This line demonstrated a Pearson r correlation of 0.9971, indicating a very strong positive correlation. This indicates a coefficient of determination R^2 to be 0.9943, or 99.43% of the proportion of the variance for a dependent variable (absorbance) that is explained by an independent variable (concentration in molar).[12] The equation for this linear result is as follows: y = 52.388 L m^{-1} x + 0.1435. The slope is 52.388 L m^{-1}, with a 95% confidence interval of 50.359 L m^{-1} to 54.416 L m^{-1}. The y-axis intercept is 0.1435, with a 95% confidence interval of 0.1229 to 0.1641. This plot was found by the runs test to have 8 runs,

with *P*=.24, and showing no significant departure from linearity.[12] For this standard curve the standard deviation of residuals from the line, utilized for the determination of limit of detection (LOD) and limit of quantitation (LOQ), was found to be 0.01906.

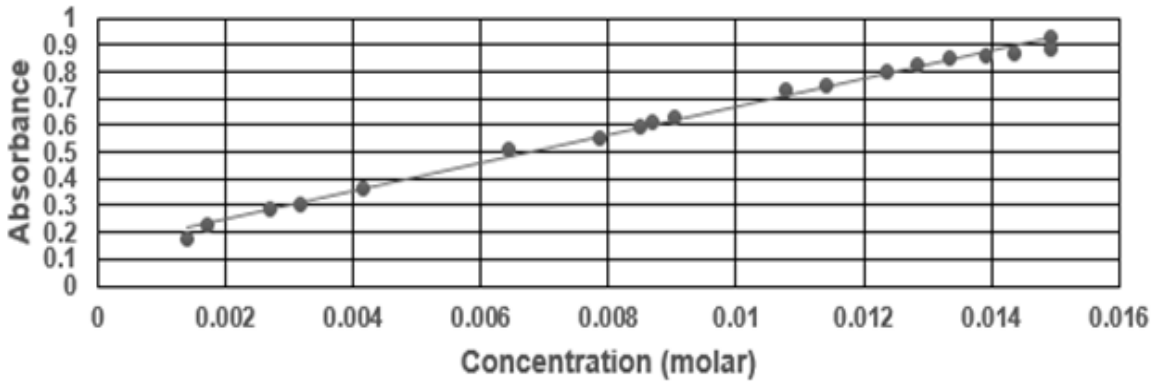

Fig. 3: Standard curve for ciprofloxacin assay. Equation of line is: y = 52.388 L m^{-1} x + 0.1435. The Pearson r correlation is 0.9971, with coefficient of determination R^2 is 0.9943. Range of concentration is 1.3919 x 10^{-3} molar to 1.4934 x 10^{-2} molar.

Data ellipses and confidence ellipses help to visualize relationships among variables in connection with linear models.[13] The data ellipses for the standard curve is presented in Fig. 4. Here the plotted points all fall within the 95% ellipses. All data that is applied for the standard curve falls within a 95% ellipses. This is the smallest ellipse that will cover 95 % of the data. This shows the consistency and linearity of the standard curve.

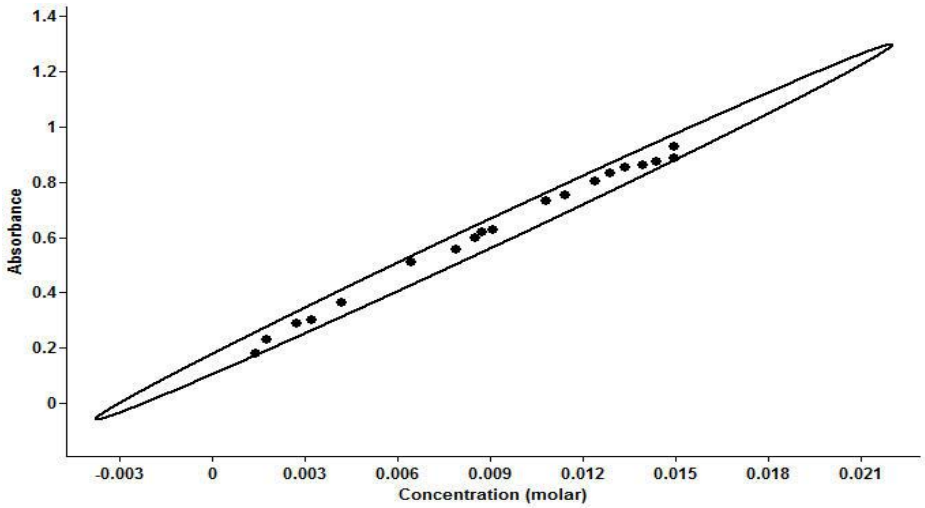

Fig. 4: 95% ellipses for standard curve for molar concentration versus absorbance. This plot shows that the data that is applied for the standard curve falls within a 95% ellipsis. This plot is the smallest ellipse that will cover 95 % of the data. All points of the standard curve are contained in the ellipses.

Analytical method development and validation procedures are vital for the discovery and development of drugs and pharmaceuticals.[14] Limit of detection (LOD) and limit of quantification (LOQ) are two important performance characteristics for analytic methodologies.[14] LOD and LOQ are characteristics describing the smallest concentration of an analyte that can be definitively measured through use of an analytical procedure.[14] The limit of detection (LOD) is 1.0915×10^{-3} molar and the limit of quantitation (LOQ) is 3.6382×10^{-3} molar.

For analytical assay the amount of analyte detected can be compared to the calculated determined amount of the analyte, to provide a observed percent recovery of analysis. It is a recovery near 100% (e.g. 80-110%) that gives confidence. The percent recovery (i.e. identification of ciprofloxacin present in a sample) for samples in solvent 98% distilled water, 1% ethanol (v/v), and 1% (v/v) (0.167 molar) acetic acid, is presented in Table 1.

Table 1: Percent Recovery From Assayed Samples.

RUN	Calculated Molar	Spectrometer SpectroVis Measured Molar	Percent Recovery
1	1.1603×10^{-2}	1.1424×10^{-2}	98.4
2	1.0498×10^{-2}	1.0871×10^{-2}	104
3	9.9456×10^{-3}	9.8019×10^{-3}	98.6
4	8.8405×10^{-3}	8.9429×10^{-3}	101
5	6.6304×10^{-3}	6.9959×10^{-3}	105
6	3.8252×10^{-3}	3.5791×10^{-3}	94.9
7	3.4897×10^{-3}	3.5982×10^{-3}	103
8	3.1360×10^{-3}	3.1018×10^{-3}	98.9
9	3.1360×10^{-3}	3.2614×10^{-3}	104
10	1.3531×10^{-2}	1.2264×10^{-2}	98.0
11	2.6661×10^{-3}	2.6056×10^{-3}	97.9
12	2.2658×10^{-3}	2.2658×10^{-3}	97.3
13	1.1471×10^{-2}	1.1673×10^{-2}	102
14	1.1315×10^{-2}	1.1615×10^{-2}	103
15	1.4861×10^{-2}	1.4517×10^{-2}	97.7
16	1.4366×10^{-2}	1.3925×10^{-2}	96.9
17	1.3902×10^{-2}	1.4269×10^{-2}	103
18	1.3902×10^{-2}	1.3562×10^{-2}	97.6
19	1.3468×10^{-2}	1.3295×10^{-2}	98.7
20	1.2432×10^{-2}	1.2322×10^{-2}	99.1
21	1.1718×10^{-2}	1.2092×10^{-2}	103
22	1.0774×10^{-2}	1.2140×10^{-2}	104
23	1.3813×10^{-2}	1.3371×10^{-2}	96.8
24	1.0774×10^{-2}	1.0623×10^{-2}	98.6
25	1.2708×10^{-2}	1.2551×10^{-2}	98.9
26	1.2156×10^{-2}	1.4207×10^{-2}	99.3

The average percent recovery is 100% having standard deviation of 2.8%, with range of 94.9% to 105%. The median of percent recovery in Table 1 is 98.9%. The calculated molar values are highly consistent with the molar values determined by SpectroVis spectrometer. By paired test the calculated and measured values of molar concentration have the same mean, by t-test (*P*=.59), and the medians are equal by Wilcoxon test (*P*=.34).[15,16] After ANOVA analysis, again, the calculated and measured values of percent recovery have the equal means (*P*=.95).[15,16] Therefore, this analysis for ciprofloxacin is efficient and reproducible.

Excipients are substances that are formulated alongside the actual active ingredient of a medication, and they are included for the purpose of long-term stabilization, bulking up solid formulations, or to confer a therapeutic enhancement on the active ingredient in the final dosage form.[17] Common excipients are dextrin, cellulose, starch, and lactose.[17] Presented in Table 2 are excellent percent recovery results for the assay of ciprofloxacin present at a concentration of 1.3261 x 10^{-2} molar, with the indicated excipient at the amount indicated in 2.500 milliliters of solvent solution (1% ethanol (v/v), 98% (v/v) distilled water, 1% (v/v) acetic acid) (see Table 2).

Results show statistically consistent percent recovery with no outlier by Grubb's test (*P*=.99, two-sided).[15,16] The average percent recovery is 103%, with standard deviation of 3.1%. The antibiotic ciprofloxacin was assayed accurately from excipient formulations having variation in content (i.e. cellulose varied 0.0026 grams to 0.0129 grams, starch varied from 0.0030 grams to 0.0178 grams). Excipients are an important part of drug formulations. The capability of this methodology to accurately assay ciprofloxacin from these formulations is a strong advantage.

Table 2: Percent recovery of ciprofloxacin (1.3261 x 10^{-2} molar) from tablet formulation.

Run	DEXTRIN (grams)	CELLULOSE (grams)	STARCH (grams)	Lactose (grams)	Recovered Molar Concentration	Percent Recovery
1	0.0101	0.0029	-	-	1.3659 x 10^{-2}	103
2	0.0089	-	0.0120		1.2929 x 10^{-2}	97.5
3	0.0177	-	-	0.0082	1.3261 x 10^{-2}	100
4	-	-	0.0161	-	1.4057 x 10^{-2}	106
5	-	-	-	0.0133	1.4189 x 10^{-2}	107
6	0.0104		0.0059	0.0066	1.3791 x 10^{-2}	104
7	0.0114	0.0030	-	-	1.3393 x 10^{-2}	101
8	-	0.0129	-	-	1.4057 x 10^{-2}	106
9	0.0213	-	-	-	1.3168 x 10^{-2}	99.3
10	0.016	-	-	-	1.3659 x 10^{-2}	103
11	-	0.0053	-	0.0147	1.4189 x 10^{-2}	107
12	-	0.0068	0.0178	-	1.3791 x 10^{-2}	104
13	-	0.0053	0.0074	-	1.3924 x 10^{-2}	105
14	0.0079	0.0026	0.0030	0.0050	1.4189 x 10^{-2}	107

The instrumental analysis of pharmaceutical products meant for human consumption is a vital function of analytical chemistry. The methodology presented here will be useful for assurance of isoniazid presence in aqueous, saline, or solid forms of pharmaceutical, and to recognize and avoid the problematic presence of adulterants. Analysis by HPLC will continue to permit confidence in the quality of pharmaceutical products available to medical facilities.

CONCLUSIONS

This study demonstrates the efficacy of visible spectroscopy for determination of ciprofloxacin, an important antibiotic for the treatment of broad range of bacterial infections. The study shows that ciprofloxacin can be assayed from aqueous mixtures as well as solid origin. Detection is consistent, accurate, and over a broad range of concentrations. Percent recovery of analyte is very high. The standard curve is linear and has extremely high positive correlation with absorbance of analyte at 390 nm. The limit of detection (LOD) is 1.0915 x 10^{-3} molar and the limit of quantitation (LOQ) is 3.6382 x 10^{-3} molar. This methodology will be useful for clinical monitoring for patient compliance and quality assurance for industrial manufacturing of this important drug.

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