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A Review: Different UV Spectrophotometric Methods for Determination of Quinolone Derivatives

Vishwa C Chauhan*, Divya D patel, Megha J Rana, Megha A Shah

Rofel shri G.M. Bilakhia College of Pharmacy, Namdha Campus, vapi-396195, Gujarat, India

ABSTRACT:

The quinolones are a family of synthetic broad-spectrum antibacterial drugs used in the treatment of urinary tract infections, respiratory tract infections, gastroentritis, and sexually transmitted diseases etc. Their antibacterial spectrum includes activity against gram negative organisms and also has been expanded to include activity against gram positive organisms. They act by inhibiting DNA synthesis by promoting cleavage of bacterial DNA in the DNA-enzyme complexes of DNA gyrase and type IV topoisomerase resulting in rapid bacterial death. The clinical and pharmaceutical analysis of this drug requires effective analytical procedures for quality control, pharmacodynamic and pharmacokinetic studies as well as stability study. An extensive survey of the literature published in various analytical and pharmaceutical chemistry related journals has been conducted and the instrumental analytical methods which were developed and used for determination of quinolones derivatives as single or combination with other drugs in bulk drugs and formulations have been reviewed. This review covers the time period from 2002 to 2014 during which six spectrophotometric analytical methods like simultaneous method, Q-absorbance method, dual wavelength method, derivative method, AUC method, difference spectrometry method were reported.

KEY WORDS: Quinolones, AUC (area under curve), difference spectrometry, Q-absorbance method, Simultaneous equation method, dual wavelength method, derivative method

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For Correspondence:
Vishwa C Chauhan
Student of Quality Assurance,
A-One college of Pharmacy, Anasan, Ahmedabad, Gujarat, India.
Email:jpsbronline@rediffmail.com

(www.jpsbr.org)

INTRODUCTION:

QUINOLONES [1]

With the recent introduction of agents such as gatifloxacin and moxifloxacin, the traditional gram-negative coverage of fluoroquinolone has been expanded to include specific gram-positive organisms. The new fluoroquinolone are rarely first-line agents and should be employed judiciously. The first Quinolones, nalidixic acid (Gram-negative), was introduced in 1962. Since then, structural modifications have resulted in second, third, and fourth-generation fluoroquinolones, which have improved coverage of gram-positive organisms.

Mechanism of Action: Quinolones rapidly inhibit DNA synthesis by promoting cleavage of bacterial DNA in the DNA-enzyme complexes of DNA gyrase and type IV topoisomerase, resulting in rapid bacterial death. As a general rule, gram-negative bacteria activity correlates with inhibition of DNA gyrase, and gram-positive bacteria activity corresponds with inhibition of DNA type IV topoisomerase.

Pharmacokinetics: Quinolones are well absorbed following oral administration, with moderate to excellent bioavailability. Elimination half-lives for the Quinolones vary from 1.5 to 16 hours. Therefore, most of these drugs are administered every 12 to 24 hours. The Quinolones are eliminated by renal and non renal routes. Quinolones are widely distributed throughout the body. Tissue penetration is higher than the concentration achieved in plasma, stool, bile, prostatic tissue, and lung tissue. Quinolones have a post antibiotic effect of about one to two hours.

Therapeutic Uses of Quinolones:

1. Genitourinary infections: Because of their extensive gram-negative coverage, Quinolones antibiotics were initially used to treat urinary tract infections.

2. Prostatitis: Quinolones are effective in the treatment of prostatitis because of their excellent penetration into prostatic tissue. Levofloxacin is an excellent first-line agent in the treatment of prostatitis.

3. Respiratory diseases: The U.S. Food and Drug Administration (FDA) have labelled gatifloxacin, moxifloxacin, sparfloxacin, and levofloxacin for use in the treatment of acute bacterial sinusitis.

4. Sexually transmitted diseases: Based on 1998 guidelines from the CDC, ceftriaxone is the agent of choice for treatment of uncomplicated Neisseria gonorrhoea urethritis and cervicitis. A single dose of ciprofloxacin or Ofloxacin should be considered as alternative treatment. Recently, gatifloxacin was reported to be as effective as Ofloxacin against N. gonorrhoea.

5. Gastroenteritis: Ciprofloxacin and Ofloxacin are the agents of choice for treatment of enteric typhoid fever.

Adverse Effects of Quinolones

Gastrointestinal: nausea, vomiting, diarrhoea, abdominal pain

CNS: headache, dizziness, drowsiness, confusion, insomnia, fatigue, malaise, depression, somnolence, seizures, vertigo, light-headedness, restlessness, tremor

Dermatologic: rash, photosensitivity reactions, pruritus

Other: QT prolongation, hepatotoxicity, abnormal or bitter taste, tendon rupture

METHODS FOR ANALYSIS OF QUINOLONES BY UV SPECTROSCOPY:

1. **Simultaneous equation method (Vierodt's method)**[2]:

Concentration of several components present in the same mixture can be determined by solving a set of simultaneous equation even if their spectra overlap. If Beer's law is followed, these equations are linear. If a sample contains two absorbing drugs (X and Y) each of which absorbs at the λmax of other drug, then this method may be application for determination of both the drugs.

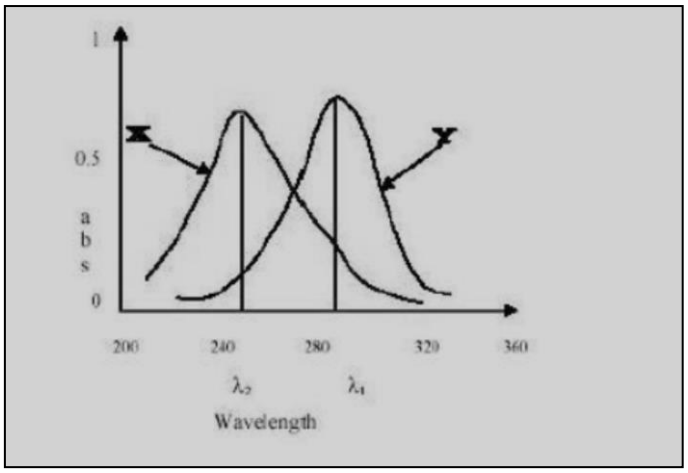

Figure 1: Selection of wavelength for Simultaneous equation method

The information required is:

1) The absorptivity of X at λ1 and λ2, ax1 and ax2 respectively.

2) The absorptivity of Y at λ1 and λ2, ay1 and ay2 respectively.

3) The absorbances of the diluted sample at λ1 and λ2, A1 and A2 respectively.

Let, CX and CY are the concentrations of X and Y respectively in the diluted sample. Two equations are constructed based upon the fact that at λ1 and λ2 the absorbance of the mixture is the sum of the individual absorbance of X and Y.

At λ1: A1 = ax1bCX + ay1bCY------ (1)

At λ2: A2 = ax2bCX + ay2bCY------- (2)

For measurement in 1 cm cell, b = 1

By rearranging equation (2) Cx = (A2*ay1– A1*ay2)/ (ax2*ay1–ax1*ay2)

Substituting CY in equation (1) and rearranging gives

CY = (A1*ax2– A2*ax1)/ (ax2* ay1– ax1 *ay2)

Criteria for obtaining maximum precision, based upon absorbance ratios, have been suggested that place limits on the relative concentrations of the components of the mixture.

The criteria are as follows;

(1) The ratios (A2/A1)/ (aX2/aX1) and (aY2/aY1)/ (A2/A1) should lie outside the range 0.1 – 2 for the precise determination of Y and X respectively. These criteria are satisfied only when the λmax of the two components are reasonably dissimilar.

Table for Simultaneous equation method:

Sr. no.	Drug	Description	Detection at	Ref. No.
1	Cefixime and Ofloxacin	**Solvent**: ethanol **Linearity**: 2- 10 mcg/ml (Ofloxacin) and 5-25 mcg/ml(Cefixime)	**Wavelength**: 297.4 nm (λmax of Ofloxacin) and 290.4 nm (λmax of Cefixime)	3
2	Cefixime and Ofloxacin	**Solvent**: methanol **Linearity**: 2-10 mcg/ml (Ofloxacin) and 4-20 mcg/ml (Cefixime)	**Wavelength:** 296.0 nm (λmax of Ofloxacin) and 234.0 nm ((λmax of Cefixime)	4
3	Cefixime and Ofloxacin	**Solvent**: a mixture of methanol and HCl (1:1). **Linearity**: 2.5-20 mcg/ml(Ofloxacin) and 5-40 mcg/ml(Cefixime)	**Wavelength:** 296.6 nm (λmax of Ofloxacin) and 285.8 nm (λmax of Cefixime)	5
4	Nitazoxanide and Ofloxacin	**Solvent**: ACN: phosphate buffer pH 2.5 AR grade **Linearity**: 1-30 mcg/ml (Ofloxacin) and 1-40 mcg/ml (Nitazoxanide)	**Wavelength**: 294.4 nm (λmax of Ofloxacin) and 345.6 nm (λmax of Nitazoxanide)	6
5	Ornidazole and Ofloxacin	**Solvent**: 0.5N acetic acid **Linearity**: 2- 10 mcg/ml (Ofloxacin) and 2-30 mcg/ml (Ornidazole)	**Wavelength**: 294.0 nm (λmax of Ofloxacin) and 317.0 nm (λmax of Ornidazole)	7
6	Nitazoxanide and Ofloxacin	**Solvent**: ethanol **Linearity**: 2- 10 mcg/ml (Ofloxacin) and 5-25 mcg/ml (Nitazoxanide)	**Wavelength**: 300 nm (Ofloxacin) and 344 nm (Nitazoxanide)	8
7	Ofloxacin and Ketorolac tromethamine	**Solvent**: acidic methanol **Linearity**: 1-11 mcg/ml (Ofloxacin) and 3-13 mcg/ml (Ketorolac tromethamine)	**Wavelength:** 300.0 nm (λmax of Ofloxacin) and 319.2 nm (λmax of Ketorolac tromethamine)	9
8	Ofloxacin and Ornidazole	**Solvent**: 0.1N HCl **Linearity**: 2-14 mcg/ml (Ofloxacin) and 5-35 mcg/ml Ornidazole	**Wavelength**: 293.0 nm (λmax of Ofloxacin) and 275.0 nm (λmax of Ornidazole)	10
9	Ofloxacin and Tinidazole	**Solvent**: 0.1N HCl **Linearity**: 5-30 mcg/ml (Ofloxacin) and 10-50 mcg/ml (Tinidazole)	**Wavelength** :227.0 nm (λmax of Ofloxacin) and 278.0 nm (λmax of Tinidazole)	11
10	Ciprofloxacin and tinidazole	**Solvent**: phosphate buffer(pH 6.8) **Linearity**: 10-35µg/ml(ciprofloxacin) and 10-80µg/ml(tinidazole) **Percentage recovery of both drugs**:98.1-99.7% **%RSD**: <2	**Wavelength:**271nm(λmax of ciprofloxacin) and 318nm(λmax of tinidazole)	12
11	Gatifloxacin and Ambroxol HCl	**Solvent**: methanol and 0.1M sodium hydroxide in the ratio of 8:2 **Linearity**: 2-10µg/ml (gatifloxacin) and 5-15µg/ml(ambroxol HCL) **Percentage recovery**: 99.45%-100.3% (gatifloxacin) and 99.73%-103.42% (ambroxol HCL) **%RSD**: less than 2%	**Wavelength:** 289nm(λmax of gatifloxacin) and 246nm (λmax of ambroxol HCL)	13
12	Gatifloxacin and ambroxol HCL	**Solvent**: methanol **Linearity**: 4-16µg/ml(gatifloxacin) and 10-50µg/ml(ambroxol HCL) **Percentage recovery**: 99.26% (gatifloxacin) and 99.32%(ambroxol HCL)	**Wavelength:**286nm(λmax of gatifloxacin) and 242nm(λmax of ambroxol HCL)	14

		%RSD: less than 2		
13	Levofloxacin	**Solvent**: methanol **Linearity**:3.0-8.0 µg/ml **%RDS**: below 1% **Average % recovery**: 101.42 ± 0.45%(tablets) and 100.34 ± 0.85%(injection) **LOD**: 0.08µg/ml **LOQ**:0.25µg/ml	Wavelength:298nm(λmax of levofloxacin)	15
14	Levofloxacin and ornidazole	**Solvent**:50%methanol **Linearity**:4-20µg/ml(levofloxacin) and 8-40µg/ml(ornidazole)	**Wavelength:**293.5nm(λmax of levofloxacin) and 318nm(λmax of ornidazole)	16
15	Moxifloxacin HCL and Bromfenac sodium(eye drops)	**Solvent**: Methanolic hydrochloride(0.1M) **Linearity**: 1-14 µg/ml(Moxifloxacin) and 1-14 µg/ml(Bromfenac sodium) **%percentage recovery**: 98%-102% of label claim for both drugs	**Wavelength:**294nm(λmax of moxifloxacin) and 265nm(λmax of Bromfenac sodium)	17
16	Norfloxacin and ornidazole	**Solvent**:0.1 N NaOH **Linearity**:4-20 µg/ml(Norfloxacin) and 5-25 µg/ml (ornidazole) **% label claim**: 98.15%-101.03%(for both drugs) **% recovery**:98.27%-101.07% (for both drugs)	**Wavelength:**273nm(λmax of norfloxacin) and 318.5nm(λmax of ornidazole)	18
17	Ofloxacin and Cefixime	**Solvent**:0.1 N NaOH **Linearity**: 10-50µg/ml.(for both drugs) **% recovery**:99.15 - 99.52%(Cefixime) and 99.43 - 99.85% (Ofloxacin) **%RSD**: 0.852 (for CEF) and 0.923 (for OFL).	**Wavelength:** 288 nm(λmax of Ofloxacin) and 237nm(λmax of Cefixime)	19
18	ciprofloxacin	**Solvent**: Water **Linearity**: 2.0–7.0 µg/ml **%RSD**: 1.55 to 2.47% (*n*=6)	**Wavelength:**275nm(λmax of ciprofloxacin)	20
19	Gatifloxacin	**Solvent**: distilled water **Linearity**: 4.0–14.0 mg/ml	**Wavelength**:287 nm(λmax of gatifloxacin)	21
20	Gemifloxacin mesylate	**Solvent**: methanol **Linearity**: 2.0 to 15.0 µg/ml	**Wavelength**: 272 nm and 343nm	22

(2) Two components do not interact chemically, thereby negating the initial assumption that the total absorbance is the sum of the individual absorbance.

The additivity of the absorbance should always be confirmed in the development of a new application of this technique. To reduce the random errors during measurements, sometimes instead of carrying out analysis of two components at two wavelengths, it is carried out at 3 or 4 wavelengths. The equations will no longer have a unique solution but the best solution can be found out by the least square criterion. The absorbance ratio and absorption factor method (Absorption correction method) are the modification of the simultaneous equation procedure.

1. **<u>Absorbance ratio method (Q-absorbance method)</u>**(2)**:**

Q-Absorbance method depends on the property that, for a substance which obeys Beer'slaw at all wavelength, the ratio of absorbances at any two wavelengths is a constant valueindependent of concentration or path length. For example, two different dilution of thesame substance give the same absorbance ratio A1/A2. In the USP, this ratio is referred toas Q value.

In the quantitative assay of two components in a mixture by the absorbance ratio method, absorbances are measured at two wavelengths. One being the max of one of thecomponent (2) and the other being a wavelength of equal absorptivities of the two components (As shown in **Figure 2**) i.e. an isoabsorptive point.

Two equations are constructed as described for the method of simultaneousequation. Their treatment is somewhat different, however, and uses the relationship ax1 =ay1 at 1. Assume b = 1 cm

A1 = ax1Cx + ax1Cy …….. (1)

A2 / A1 = (ax2Cx + ay2Cy) / (ax1Cx + ax1Cy)

Divide each term by Cx + C_Y and let F_X = Cx / (C_x + C_y) and F_Y = Cy / (Cx + Cy) i.e. Fx and F_Y are the fraction of X and Y respectively in the mixture:
A2 / A1 = (ax2 F_X + ay2Fy) / (ax1Fx + ax1Fy)
But F_Y = 1 – F_X
A2 / A1 = (ax2 F_X– F_X ay2 + ay2) / ax1
A2 / A1 = (ax2 F_X)/ ax1 - (F_X ay2)/ ay1 + (ay2) / ay1
Let QX = ax2 / ax1, QY = ay2/ ay1, and QM = A2 / A1
QM = F_X (QX - QY) + QY
F_X = (QM - QY) / (QX - QY) ……. (2)

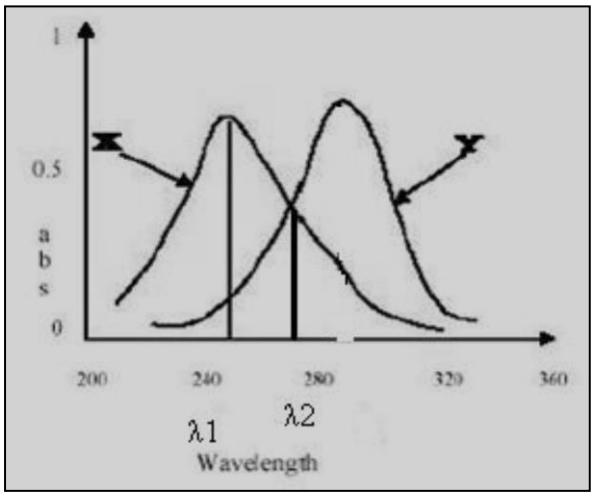

Figure2: **Selection of wavelength for Absorbance ratio method**.

Above equation gives fraction, rather than the concentration of X in the mixture in termsof absorbance ratios. As these are independent of concentrations, only approximate, rather than accurate, dilutions of X and Y and the sample mixture are required todetermine QX, QY, and QM respectively.

For absolute concentration of X and Y, eq. (1) is rearranged
A1 = ax1 (Cx +CY)
Cx +C_Y = A1 / ax1
From equation (2) Cx / (Cx +C_Y) = (QM - QY) / (QX - QY)
Cx / (A1 / ax1) = (QM - QY) / (QX - QY)
Cx = (QM - QY) A1 / (QX - QY) ax1
Equation 1 is also rearranged as:
CY= Cx – A1/ax1

Above equation gives the concentration of X in terms of absorbance ratios, the absorbance of mixture and the absorptivities of the compounds at the isoabsorptive wavelength.

Concentration of Y also finds out by put the value of concentration of X in equation 1.

Table for Q-absorbance method:

2. **Derivative spectrophotometry**(2)**:**

Derivative spectroscopy uses first or higher derivatives of absorbance with respect to wavelength for qualitative analysis and for quantification. For the purpose of spectral analysis in order to relate chemical structure to electronic transitions, and for analytical situations in which mixture contribute interfering absorption, a method of manipulating the spectral data is called derivative spectroscopy.

If a spectrum is expressed as absorbance, *A*, as a function of wavelength, λ, the derivative spectra are: **Zero order:** A= *f* (λ), **First order:** dA/dλ = *f* ′(λ), **Second order:** d^2 A/$d\lambda^2$ = *f* ″(λ) Derivative spectrophotometry involves the conversions of a normal spectrum to its first, second or higher derivative spectrum**(Figure3)**.In the context of derivative spectrophotometry, the normal absorption spectrum is referred to as the fundamental or zero order spectrum.

A first-order derivative is the rate of change of absorbance with respect to wavelength. A first order derivative starts and finishes at zero. It also passes through zero at the same wavelength as λmax of the absorbance band. Either side of this point is positive and negative bands with maximum and minimum at the same wavelengths as the inflection points in the absorbance band. This bipolar function is characteristic of all odd order derivatives. The most characteristic feature of a second-order derivative is a negative band with minimum at the same wavelength as the maximum order derivative shows positive band.

A strong negative or positive band with minimum or maximum at the same wavelength as λmax of the absorbance band is characteristic of the even-order derivatives. Note that the number of bands observed is equal to the derivative order plus one.

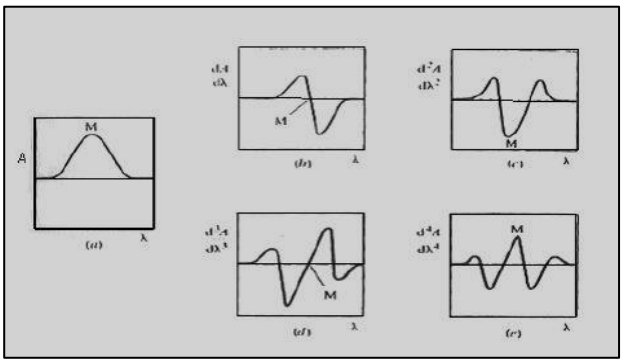

Figure 3: (a) Gaussian peak (b) First, (c) Second, (d) Third and (e) Fourth derivative Spectrum

- **Features**

A key feature of the derivative spectroscopy is that the derivative centroid peak width of a Gaussian peak decreases to 53 %, 41% and 34% of the original peak width in the 2nd, 4th and 6th order derivative respectively. These can increase the resolution of overlapping peaks.

A common, unwanted effect in the spectroscopy is baseline shift. This may arise either from instrument or sample handling effects. Because the first derivative of a constant absorbance offset is zero, using the first derivative spectra eliminates such baseline shifts and improves the accuracy of quantification.

Table for derivative method:

1. **Difference spectrometry**:(39-43)

Difference spectroscopy provides a sensitive method for detecting small changes in the environment of a chromophore or it can be used to demonstrate ionization of a chromophore leading to identification and quantitation of various components in a mixture. The selectivity and accuracy of Spectrophotometric analysis of samples containing absorbing interferent's may be markedly improved by the technique of difference spectrophotometry. The essential feature of a difference Spectrophotometric assay is that the measured value is the difference absorbance (ΔA) between two equimolar solutions of the analyte in different forms which exhibit different spectral characteristics.

The criteria for applying difference spectrophotometry to the assay of a substance in the presence of other absorbing substances are that:

A) Reproducible changes may be induced in the spectrum of the analyte by the addition of one or more reagents.

B) The absorbance of the interfering substances is not altered by the reagents.

The simplest and most commonly employed technique for altering the spectral properties of the analyte properties of the analyte is the adjustment of the pH by means of aqueous solutions of acid, alkali or buffers. The ultraviolet-visible absorption spectra of many substances containing ionisable functional groups e.g. phenols, aromatic carboxylic acids and amines, are dependent on the state of ionization of the functional groups and consequently on the pH of the solution.

If the individual absorbances, A_{alk} and A_{acid} are proportional to the concentration of the analyte and path length, the Δ A also obeys the Beer-Lambert law and a modified equation may be derived

Δ A = Δ ABC

Where, ΔA is the difference in absorptivity of the substance at the wavelength of measurement.

If one or more other absorbing substances is present in the sample which at the analytical absorbance Ax in the alkaline and acidic solutions, its interference in the Spectrophotometric measurement is eliminated

$\Delta A = (A_{alk} + Ax) - (A_{acid} + Ax)$

The selectivity of the ΔA procedure depends on the correct choice of the pH values to induce the spectral change of the analyte without altering the absorbance of the interfering components of the sample. The use of 0.1M sodium hydroxide and 0.1M hydrochloric acid to induce the ΔA of the analyte is convenient and satisfactory when the irrelevant absorption arises from pH-insensitive substances. Unwanted absorption from pH-sensitive components of the sample may also be eliminated if the pKa values of the analyte and interferent's differ by more than 4.

Table for difference spectroscopy method:

5. **Area under Curve Method**: (45)

The AUC (Area under Curve) method involves the calculation of integrated value of absorbance with respect to the wave-length between two selected wavelengths. Area calculation processing item calculates the area bound by the curve and the horizontal axis. The horizontal axis is selected by entering the wavelength range over which the area has to be calculated. The wavelength range is selected on the basis of repeated observations so as to get the linearity between area under curve and concentration.

Suitable dilutions of standard stock solution of the drug were prepared and scanned in the spectrum mode from the wavelength range 400-200 nm and the calibration curve was plotted.

Table for AUC spectroscopic method:

6. **Dual wavelength method: (46)**

The utility of dual wavelength data processing program is to calculate the unknown concentration of a component of interest present in a mixture containing both the components of interest and an unwanted interfering component by the mechanism of the absorbance difference between two points on the mixture spectra. This is directly proportional to the concentration of the component of interest, independent of the interfering components. The pre-requisite for dual wavelength method is the selection of two such wavelengths where the interfering component shows same absorbance whereas the component of interest shows significant difference in absorbance with concentration.

Table for Dual wavelength spectroscopic method:

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