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, Namda 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

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, 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)**

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](image)

The information required is:
1) The absorptivity of X at \( \lambda_1 \) and \( \lambda_2 \), \( a_{x1} \) and \( a_{x2} \) respectively.
2) The absorptivity of Y at \( \lambda_1 \) and \( \lambda_2 \), \( a_{y1} \) and \( a_{y2} \) respectively.
3) The absorbances of the diluted sample at \( \lambda_1 \) and \( \lambda_2 \), A\(_1\) and A\(_2\) respectively.

Let, \( C_X \) and \( C_Y \) are the concentrations of X and Y respectively in the diluted sample. Two equations are constructed based upon the fact that at \( \lambda_1 \) and \( \lambda_2 \) the absorbance of the mixture is the sum of the individual absorbance of X and Y.

At \( \lambda_1 \):
\[
A_1 = a_{x1}bC_X + a_{y1}bC_Y
\]

At \( \lambda_2 \):
\[
A_2 = a_{x2}bC_X + a_{y2}bC_Y
\]

For measurement in 1 cm cell, \( b = 1 \)

By rearranging equation (2) \( C_X = (A_2a_{y1} - A_1a_{y2}) / (a_{x2}a_{y1} - a_{x1}a_{y2}) \)

By rearranging equation (1) and rearranging gives
\[
C_Y = (A_1a_{x2} - A_2a_{x1}) / (a_{x2}a_{y1} - a_{x1}a_{y2})
\]

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 \(\lambda_{max}\) of the two components are reasonably dissimilar.

Table for Simultaneous equation method:

<table>
<thead>
<tr>
<th>Sr. no.</th>
<th>Drug Description</th>
<th>Detection at</th>
<th>Ref. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cefixime and Ofloxacin</td>
<td>Solvent: ethanol</td>
<td>Wavelength: 297.4 nm ((\lambda_{max}) of Ofloxacin) and 290.4 nm ((\lambda_{max}) of Cefixime)</td>
</tr>
<tr>
<td>2</td>
<td>Cefixime and Ofloxacin</td>
<td>Solvent: methanol</td>
<td>Wavelength: 296.0 nm ((\lambda_{max}) of Ofloxacin) and 234.0 nm ((\lambda_{max}) of Cefixime)</td>
</tr>
<tr>
<td>3</td>
<td>Cefixime and Ofloxacin</td>
<td>Solvent: a mixture of methanol and HCl (1:1).</td>
<td>Wavelength: 296.6 nm ((\lambda_{max}) of Ofloxacin) and 285.8 nm ((\lambda_{max}) of Cefixime)</td>
</tr>
<tr>
<td>4</td>
<td>Nitazoxanide and Ofloxacin</td>
<td>Solvent: ACN: phosphate buffer pH 2.5 AR grade</td>
<td>Wavelength: 294.4 nm ((\lambda_{max}) of Ofloxacin) and 345.6 nm ((\lambda_{max}) of Nitazoxanide)</td>
</tr>
<tr>
<td>5</td>
<td>Ornidazole and Ofloxacin</td>
<td>Solvent: 0.5N acetic acid</td>
<td>Wavelength: 294.0 nm ((\lambda_{max}) of Ofloxacin) and 317.0 nm ((\lambda_{max}) of Ornidazole)</td>
</tr>
<tr>
<td>6</td>
<td>Nitazoxanide and Ofloxacin</td>
<td>Solvent: ethanol</td>
<td>Wavelength: 300 nm (Ofloxacin) and 344 nm (Nitazoxanide)</td>
</tr>
<tr>
<td>7</td>
<td>Ofloxacin and Ketrolar tromethamine</td>
<td>Solvent: acidic methanol</td>
<td>Wavelength: 300.0 nm ((\lambda_{max}) of Ofloxacin) and 319.2 nm ((\lambda_{max}) of Ketrolar tromethamine)</td>
</tr>
<tr>
<td>8</td>
<td>Ofloxacin and Ornidazole</td>
<td>Solvent: 0.1N HCl</td>
<td>Wavelength: 293.0 nm ((\lambda_{max}) of Ofloxacin) and 275.0 nm ((\lambda_{max}) of Ornidazole)</td>
</tr>
<tr>
<td>9</td>
<td>Ofloxacin and Tinidazole</td>
<td>Solvent: 0.1N HCl</td>
<td>Wavelength: 227.0 nm ((\lambda_{max}) of Ofloxacin) and 278.0 nm ((\lambda_{max}) of Tinidazole)</td>
</tr>
<tr>
<td>10</td>
<td>Ciprofloxacin and tinidazole</td>
<td>Solvent: phosphate buffer (pH 6.8)</td>
<td>Wavelength: 271nm ((\lambda_{max}) of ciprofloxacin) and 318nm ((\lambda_{max}) of Tinidazole)</td>
</tr>
<tr>
<td>11</td>
<td>Gatifloxacin and Ambroxol HCl</td>
<td>Solvent: methanol and 0.1M sodium hydroxide in the ratio of 8:2</td>
<td>Wavelength: 289nm ((\lambda_{max}) of ciprofloxacin) and 246nm ((\lambda_{max}) of Ambroxol HCl)</td>
</tr>
<tr>
<td>12</td>
<td>Gatifloxacin and Ambroxol HCL</td>
<td>Solvent: methanol</td>
<td>Wavelength: 286nm ((\lambda_{max}) of ciprofloxacin) and 242nm ((\lambda_{max}) of Ambroxol HCL)</td>
</tr>
</tbody>
</table>
1. Absorbance ratio method (Q-absorbance method)\(^{(2)}\):

\[ Q_{\text{Absorbance}} \text{method} = \frac{A_1}{A_2} \]

Q-Absorbance method depends on the property that, for a substance which obeys Beer's law at all wavelength, the ratio of absorbances at any two wavelengths is a constant value independent of concentration or path length. For example, two different dilution of the same substance give the same absorbance ratio \(A_1/A_2\). In the USP, this ratio is referred to as Q value.

In the quantitative assay of two components in a mixture by the absorbance ratio method, absorbances are measured at two wavelengths. Two equations are constructed as described for the method of simultaneous equation. Their treatment is somewhat different, however, and uses the relationship

\[ ax_1 = ay_1 \]

Assume \( b = 1 \) cm

\[ A_1 = ax_1C_x + ax_1C_y \]

(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.

<table>
<thead>
<tr>
<th>No.</th>
<th>Drug Name</th>
<th>Solvent</th>
<th>Linearity</th>
<th>%RSD: less than 2</th>
<th>Wavelength: 298 nm ((\lambda_{\text{max}}) of levofloxacin)</th>
</tr>
</thead>
<tbody>
<tr>
<td>13</td>
<td>Levofloxacin</td>
<td>Solvent: methanol</td>
<td>Linearity: 3.0-8.0 (\mu g/ml)</td>
<td></td>
<td>Wavelength: 298 nm ((\lambda_{\text{max}}) of levofloxacin)</td>
</tr>
<tr>
<td>14</td>
<td>Levofloxacin and ornidazole</td>
<td>Solvent: 50% methanol</td>
<td>Linearity: 4-20 (\mu g/ml) (levofloxacin) and 8-40 (\mu g/ml) (ornidazole)</td>
<td></td>
<td>Wavelength: 293.5 nm ((\lambda_{\text{max}}) of levofloxacin) and 318 nm ((\lambda_{\text{max}}) of ornidazole)</td>
</tr>
<tr>
<td>15</td>
<td>Moxifloxacin HCL and Bromfenac sodium (eye drops)</td>
<td>Solvent: Methanolic hydrochloride (0.1 M)</td>
<td>Linearity: 1-14 (\mu g/ml) (Moxifloxacin) and 1-14 (\mu g/ml) (Bromfenac sodium)</td>
<td></td>
<td>Wavelength: 294 nm ((\lambda_{\text{max}}) of moxifloxacin) and 265 nm ((\lambda_{\text{max}}) of Bromfenac sodium)</td>
</tr>
<tr>
<td>16</td>
<td>Norfloxacin and ornidazole</td>
<td>Solvent: 0.1 N NaOH</td>
<td>Linearity: 4-20 (\mu g/ml) (Norfloxacin) and 5-25 (\mu g/ml) (ornidazole)</td>
<td></td>
<td>Wavelength: 273 nm ((\lambda_{\text{max}}) of norfloxacin) and 318.5 nm ((\lambda_{\text{max}}) of ornidazole)</td>
</tr>
<tr>
<td>17</td>
<td>Ofloxacin and Cefixime</td>
<td>Solvent: 0.1 N NaOH</td>
<td>Linearity: 10-50 (\mu g/ml) (for both drugs)</td>
<td></td>
<td>Wavelength: 288 nm ((\lambda_{\text{max}}) of Ofloxacin) and 237 nm ((\lambda_{\text{max}}) of Cefixime)</td>
</tr>
<tr>
<td>18</td>
<td>Ciprofloxacin</td>
<td>Solvent: Water</td>
<td>Linearity: 2.0-7.0 (\mu g/ml)</td>
<td>%RSD: 1.55 to 2.47%</td>
<td>Wavelength: 275 nm ((\lambda_{\text{max}}) of ciprofloxacin)</td>
</tr>
<tr>
<td>19</td>
<td>Gatifloxacin</td>
<td>Solvent: distilled water</td>
<td>Linearity: 4.0-14.0 (mg/ml)</td>
<td></td>
<td>Wavelength: 287 nm ((\lambda_{\text{max}}) of gatifloxacin)</td>
</tr>
<tr>
<td>20</td>
<td>Gemifloxacin mesylate</td>
<td>Solvent: methanol</td>
<td>Linearity: 2.0 to 15.0 (\mu g/ml)</td>
<td></td>
<td>Wavelength: 272 nm and 343 nm</td>
</tr>
</tbody>
</table>

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

Divide each term by Cx + Cy and let Fx = Cx / (Cx + Cy) and FY = Cy / (Cx + Cy) i.e. Fx and FY are the fraction of X and Y respectively in the mixture:

A2 / A1 = (ax2 Fx + ay2FY) / (ax1Fx + ax1FY)

But FY = 1 - Fx

A2 / A1 = (ax2 Fx - Fx + ay2 + ay2) / ax1

A2 / A1 = (ax2 Fx / ax1 - (Fx ay2) / ay1 + (ay2) / ay1

Let QX = ax2 / ax1, QY = ay2 / ay1, and QM = A2 / A1

QM = Fx (QX - QY) + QY

Fx = (QM - QY) / (QX - QY) ……….. (2)

Figure 2: Selection of wavelength for Absorbance ratio method.

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

For absolute concentration of X and Y, eq. (1) is rearranged

A1 = ax1 (Cx +Cy)

Cx +Cy = A1 / ax1

From equation (2) Cx / (Cx +Cy) = (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/dλ^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 spectrometry 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 interfering’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 ($\Delta A$) 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, $A_{\text{alk}}$ and $A_{\text{acid}}$ are proportional to the concentration of the analyte and path length, the $\Delta A$ also obeys the Beer-Lambert law and a modified equation may be derived:

$$\Delta A = \Delta ABC$$

Where, $\Delta A$ 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:

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

The selectivity of the $\Delta A$ 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 $\Delta A$ of the analyte is convenient and satisfactory when the irrelevant absorbance 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 interferer’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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