Chapter-V
Spectrophotometric determination of selected drugs by DDQ method
Charge transfer complex method is used for the estimation of drugs possessing secondary, tertiary amino groups or phenolic groups in pharmaceutical formulations. In this method the drug having an aromatic secondary or tertiary amino group or aromatic phenolic group or alkoxy group reacts with DDQ (2,3-dichloro 5,6-dicyano 1,4-benzoquinone) to form colored charge transfer complex. The resultant charge transfer complex is formed from donor-acceptor mechanism of Lewis acid-base reaction between the drug and DDQ chemical constituents. The transfer of an electron pair from donor to acceptor is readily possible during charge transfer phenomenon.

In charge transfer complex phenomenon the selected drugs acts as electron pair donors and DDQ acts as electron pair acceptor due to presence of strong electron withdrawing cyano group. The π electron pairs are transferred from drug as π-donor to DDQ as a π-acceptor. The solvents like methanol, acetonitrile, being polar solvents facilitates the complete transfer of charge from donor to acceptor with the formation of radical anion as the predominant chromogen.

Hence this reaction is chosen to develop a novel spectrophotometric method for the estimation of drugs having a phenol or aromatic amino
group. By using this method the following three drugs are determined.
1. Pragabalin 2. Ketorelac tromethamine 3 Tenofovir disoproxil fumarate
Section(i): Assay of Pragabalin

The method is based on the reaction of pragabalin with 2, 3-dichloro-5, 6-dicyano-1, 4-benzquinone (DDQ) to form a red colour charge-transfer complex. The red colour solution is used to determine the pragabalin spectrophotometrically.

(a) Spectrum of pragabalin treated with DDQ:

The wavelength of maximum absorbance of the pragabalin drug treated with DDQ solution is ascertained by the following procedure.

1.0 ml of pragabalin solution (100 µg/ml) is transferred into a standard flask. To this solution 3.0 ml of DDQ reagent is added to form red colour solution. The final volume is brought to 10 ml with methanol. The resultant solution is well mixed and allowed to stand for 5 min to complete the reaction. The absorbance of the red colour solution is measured in the wavelength range of 400 to 550 nm, against the reagent blank. The spectrum is given in fig.5.1.1.
Fig: 5.1.1: Spectrum of pragabalin
From fig 5.1.1, it is clear that the pragabalin drug treated with DDQ solution has maximum absorbance at 465 nm. Hence, all further studies are made at 465 nm.

The optimal conditions for the determination of pragabalin are arrived at by the following steps.

(b). Effect of concentration of DDQ solution on the absorbance of Charge transfer complex is studied by the following procedure.

In a series of standard flask 1.0 ml of pragabalin are taken and varying amounts of DDQ solution are added. The contents are made upto the mark with methanol. Reaction mixture was shaken gently for 5 min and allowed to stand for 5 min to complete the reaction. The absorbance of the resultant solutions is measured at 465 nm and the data are presented in table 5.1.1.
Table 5.1.1:

Effect of concentration of DDQ solution

<table>
<thead>
<tr>
<th>Volume of DDQ solution</th>
<th>Absorbance at 465 nm.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 ml</td>
<td>0.214</td>
</tr>
<tr>
<td>2 ml</td>
<td>0.308</td>
</tr>
<tr>
<td>3 ml</td>
<td>0.371</td>
</tr>
<tr>
<td>4 ml</td>
<td>0.281</td>
</tr>
<tr>
<td>5 ml</td>
<td>0.288</td>
</tr>
</tbody>
</table>

The data in table 5.1.1 indicate that 3.0 ml of DDQ is necessary for achieving maximum absorbance and hence maintained throughout the experimental studies.

(d) Assay Procedure:

To study the effect of drug concentration on the absorbance of the ion pair complex under optimal conditions now arrived is studied by the following method to know the suitability of the method for the assay of pragabalin.

Various aliquots of the standard pragabalin solution ranging from 0.5-2.5 ml are transferred into a series of standard flasks. To each flask, 3.0 ml
of DDQ solution is added. The final volume is brought to 10 ml with methanol. The reaction mixture in each flask is well shaken and allowed to stand for 5 min to complete the reaction. The absorbance of the red colour solution is measured at 465 nm, against the reagent blank prepared in similar manner omitting drug solution. Calibration graph is obtained by plotting absorbance values against the concentration of pragabalin solution. The calibration curve is found to be linear over a concentration range of 50 to 250 μg/ml of pragabalin. The amount of pragabalin present in the sample is read from the calibration graph. The results are presented in fig.5.1.2
Fig. 5.1.2: Calibration curve of pragabalin
(e) Assay of pragabalin in pharmaceutical formulations:

The method is then applied to the determination of the drug from the marketed tablet formulations. Tablets are weighed and contents are powdered and well mixed. The powder equivalent to 50 mg of pragabalin is dissolved in methanol, filtered, residue is washed with methanol and the volume is made upto 50 ml with methanol. Further dilution is made as described in the preparation of standard solution of pragabalin. Further analysis is carried out as per procedure described above and results are summarized in the Table.5.1.2. The amount of drug present in the sample is estimated from calibration graph.
Table 5.1.2:

Assay of pragabalin in tablets

<table>
<thead>
<tr>
<th>S.No</th>
<th>Sample (mg)</th>
<th><em>Amount Found (mg) ±S.D</em></th>
<th>Percentage of Label claim</th>
<th>C.V*</th>
<th>*t_cal</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>150</td>
<td>150.12±0.45</td>
<td>100.08</td>
<td>0.303</td>
<td>0.5899</td>
</tr>
<tr>
<td>2</td>
<td>150</td>
<td>150.02±0.37</td>
<td>100.01</td>
<td>0.2047</td>
<td>0.1208</td>
</tr>
<tr>
<td>3</td>
<td>150</td>
<td>149.98±0.23</td>
<td>99.98</td>
<td>0.1597</td>
<td>0.1874</td>
</tr>
</tbody>
</table>

*Average of five determination based on label claim*
(f) Results and discussion:

In this method the drug react with DDQ solution to form red coloured charge complex. The red coloured charge complex solution formed is measured at 465 nm against reagent blank. The amount of drug read from calibration curve. The calibration curve is linear over the range of 50-250 μg/ml of pragabalin. The values of Standard deviation, coefficient of variation values and $t_{cal}$ are shown in Table.5.1.2. The values of standard deviation and coefficient of variation values are low, indicates high accuracy and reproducibility of the method. The data of assay values of commercial formulations is subjected to statistical evaluation for student ‘t’ test to study the proposed method. The calculated ‘t’ values are less than ‘t’ theoretical values with 4 (n-1= 5-1) degrees of freedom at 5% level of significance indicate that there is no significant difference between proposed method and standard method.

The proposed method is found to be simple, precise, accurate and time saving, reproducible and can be conveniently adopted for routine analysis of estimation of pragabalin in bulk drugs samples and pharmaceutical formulations.
Section(ii): Assay of ketorelactromethamine

The method is based on the reaction of ketorelactromethamine with 2, 3-dichloro-5, 6-dicyano-1, 4-benzquinone (DDQ) to form an red colour charge-transfer complex. The red colour solution is used to determine the ketorelactromethamine spectrophotometrically.

(a) Spectrum of ketorelactromethamine treated with DDQ:

The wavelength of maximum absorbance of the ketorelactromethamine drug treated with DDQ solution is ascertained by the following procedure.

1.0 ml of ketorelactromethamine solution (100 μg/ml) is transferred into a standard flask. To this solution 3.0 ml of DDQ reagent is added to form red colour solution. The final volume is brought to 10 ml with chloroform. The resultant solution is well mixed and allowed to stand for 5 min to complete the reaction. The absorbance of the methanol colour solution is measured in the wavelength range of 350 to 550 nm, against the reagent blank. The spectrum is given in fig.5.2.1.
Fig: 5.2.1: Spectrum of ketorelac tromethamine
From fig 5.2.1, it is clear that the ketorelac tromethamine drug treated with DDQ solution has maximum absorbance at 460 nm. Hence, all further studies are made at 460 nm.

The optimal conditions for the determination of ketorelac tromethamine are arrived at by the following steps.

(b). **Effect of concentration of DDQ solution on the absorbance of Charge transfer complex is studied by the following procedure.**

In a series of standard flasks 1.0 ml of ketorelac tromethamine are taken and varying amounts of DDQ solution are added. The contents are made up to the mark with chloroform. Reaction mixture was shaken gently for 5 min and allowed to stand for 5 min to complete the reaction. The absorbance of the resultant solutions are measured at 460 nm and the data are presented in table.5.2.1.
Table.5.2.1:

Effect of concentration of DDQ solution

<table>
<thead>
<tr>
<th>Volume of DDQ solution</th>
<th>Absorbance at 460 nm.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 ml</td>
<td>0.388</td>
</tr>
<tr>
<td>2 ml</td>
<td>0.421</td>
</tr>
<tr>
<td>3 ml</td>
<td>0.634</td>
</tr>
<tr>
<td>4 ml</td>
<td>0.524</td>
</tr>
<tr>
<td>5 ml</td>
<td>0.581</td>
</tr>
</tbody>
</table>

The data in table.5.2.1 indicate that 3.0 ml of DDQ is necessary for achieving maximum absorbance and hence maintained throughout the experimental studies.

(d) Assay Procedure:

To study the effect of drug concentration on the absorbance of the ion pair complex under optimal conditions now arrived is studied by the following method to know the suitability of the method for the assay of ketorelactromethamine.

Various aliquots of the standard ketorelactromethamine solution ranging from 0.5-2.5 ml are transferred into a series of standard flasks. To
each flask, 3.0 ml of DDQ solution is added to produce red colour. The final volume is brought to 10 ml with chloroform. The reaction mixture in each flasks is well shaken and allowed to stand for 5 min to complete the reaction. The absorbance of the red colour solution is measured at 460 nm, against the reagent blank prepared in similar manner omitting drug solution. Calibration graph is obtained by plotting absorbance values against the concentration of ketorelac tromethamine solution. The calibration curve is found to be linear over a concentration range of 50 to 250 µg/ml of ketorelac tromethamine. The amount of ketorelac tromethamine present in the sample is read from the calibration graph. The results are presented in fig.5.2.2
Fig. 5.2.2: Calibration curve of ketorelac tromethamine
(e) Assay of ketorelac tromethamine in pharmaceutical formulations:

The method is then applied to the determination of the drug from the marketed tablet formulations. Tablets are weighed and contents are powdered and well mixed. The powder equivalent to 50 mg of ketorelac tromethamine is dissolved in methanol, filtered, residue is washed with distilled water and the volume is made upto 50 ml with chloroform. Further dilution is made as described in the preparation of standard solution of ketorelac tromethamine. Further analysis is carried out as per procedure described above and results are summarized in the Table.5.2.2. The amount of drug present in the sample is estimated from calibration graph.
Table 5.2.2:
Assay of ketorelac tromethamine in tablets

<table>
<thead>
<tr>
<th>S.No</th>
<th>Sample (mg)</th>
<th>Amount Found (mg)</th>
<th>Percentage of Label claim</th>
<th>S.D*</th>
<th>t_cal</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>10.06</td>
<td>100.6</td>
<td>0.3361</td>
<td>0.3984</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>10.04</td>
<td>100.4</td>
<td>0.4037</td>
<td>0.2216</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>9.94</td>
<td>99.4</td>
<td>0.2302</td>
<td>0.5830</td>
</tr>
</tbody>
</table>

*Average of five determinations based on label claim
(f) Results and discussion:

In this method the drug react with DDQ solution to form red coloured charge complex. The red coloured charge complex solution formed is measured at 460 nm against reagent blank. The amount of drug read from calibration curve. The calibration curve is linear over the range of 50-250 μg/ml of ketorelac tromethamine. The values of standard deviation and $t_{cal}$ are shown in Table.5.2.2. The values of standard deviation are low, indicates high accuracy and reproducibility of the method. The data of assay values of commercial formulations is subjected to statistical evaluation for student ‘t’ test to study the proposed method. The calculated ‘t’ values are less than ‘t’ theoretical values with 4 $(n-1= 5-1)$ degrees of freedom at 5% level of significance indicate that there is no significant difference between proposed method and standard method.

The proposed method is found to be simple, precise, accurate and time saving, reproducible and can be conveniently adopted for routine analysis of estimation of ketorelac tromethamine in bulk drugs samples and pharmaceutical formulations.
Section(iii): Assay of tenofovir disoproxil fumarate

The method is based on the reaction of tenofovir disoproxil fumarate with 2, 3-dichloro-5, 6-dicyano-1, 4-benzquinone (DDQ) to form red colour charge–transfer complex. The red colour solution is used to determine the tenofovir disoproxil fumarate spectrophotometrically.

(a) Spectrum of tenofovir disoproxil fumarate treated with DDQ:

The wavelength of maximum absorbance of the tenofovir disoproxil fumarate drug treated with DDQ solution is ascertained by the following procedure.

1.0 ml of tenofovir disoproxil fumarate solution (100 µg/ml) is transferred into a standard flask. To this solution 3.0 ml of DDQ reagent is added to form red colour solution. The final volume is brought to 10 ml with methanol. The resultant solution is well mixed and allowed to stand for 5 min to complete the reaction. The absorbance of the red colour solution is measured in the wavelength range of 400 to 550 nm, against the reagent blank. The spectrum is given in fig.5.3.1.
Fig: 5.3.1: Spectrum of tenofovir disoproxil fumarate
From fig 5.3.1, it is clear that the tenofovir disoproxil fumarate drug treated with DDQ solution has maximum absorbance at 465 nm. Hence, all further studies are made at 465 nm.

The optimal conditions for the determination of tenofovir disoproxil fumarate are arrived at by the following steps.

(b). Effect of concentration of DDQ solution on the absorbance of Charge transfer complex is studied by the following procedure.

In a series of standard flasks 1.0 ml of tenofovir disoproxil fumarate are taken and varying amounts of DDQ solution are added. The contents are made upto the mark with methanol. Reaction mixture was shaken gently for 5 min and allowed to stand for 5 min to complete the reaction. The absorbance of the resultant solutions is measured at 465 nm and the data are presented in table.5.3.1.
Table 5.3.1:

Effect of concentration of DDQ solution

<table>
<thead>
<tr>
<th>Volume of DDQ solution</th>
<th>Absorbance at 465 nm.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 ml</td>
<td>0.214</td>
</tr>
<tr>
<td>2 ml</td>
<td>0.308</td>
</tr>
<tr>
<td>3 ml</td>
<td>0.371</td>
</tr>
<tr>
<td>4 ml</td>
<td>0.281</td>
</tr>
<tr>
<td>5 ml</td>
<td>0.288</td>
</tr>
</tbody>
</table>

The data in table 5.3.1 indicate that 3.0 ml of DDQ is necessary for achieving maximum absorbance and hence maintained throughout the experimental studies.

(d) Assay Procedure:

To study the effect of drug concentration on the absorbance of the ion pair complex under optimal conditions now arrived is studied by the following method to know the suitability of the method for the assay of tenofovir disoproxil fumarate.

Various aliquots of the standard tenofovir disoproxil fumarate solution ranging from 0.4 to 2 ml are transferred into a series of standard flasks. To
each flask, 3.0 ml of DDQ solution is added. The final volume is brought to 10 ml with methanol. The reaction mixture in each flask is well shaken and allowed to stand for 5 min to complete the reaction. The absorbance of the red colour solution is measured at 465 nm, against the reagent blank prepared in similar manner omitting drug solution. Calibration graph is obtained by plotting absorbance values against the concentration of tenofovir disoproxil fumarate solution. The calibration curve is found to be linear over a concentration range of 40 to 200 µg/ml of tenofovir disoproxil fumarate. The amount of tenofovir disoproxil fumarate present in the sample is read from the calibration graph. The results are presented in fig.5.3.2
Fig. 5.3.2: Calibration curve of tenofovir disoproxil fumarate
(e) Assay of tenofovir disoproxil fumarate in pharmaceutical analysis:

. Pure sample equivalent to 50 mg of tenofovir disoproxil fumarate is dissolved in methanol, and the volume is made upto 50 ml with methanol. Further dilution is made as described in the preparation of standard solution of tenofovir disoproxil fumarate. Further analysis is carried out as per procedure described above and results are summarized in the Table.5.1.2. The amount of drug present in the sample is estimated from calibration graph.
Table. 5.3.2:
Assay of tenofovir disoproxil fumarate in tablets

<table>
<thead>
<tr>
<th>S.No</th>
<th>Pure sample taken (mg)</th>
<th>Sample Found(mg) S.D*</th>
<th>Percentage of Label claim</th>
<th>C.V*</th>
<th>t_cal</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>100</td>
<td>100.02±0.34</td>
<td>100.02</td>
<td>0.3491</td>
<td>0.1295</td>
</tr>
</tbody>
</table>

*Average of five determination based on label claim
(f) Results and discussion:

In this method the drug react with DDQ solution to form red coloured charge complex. The red coloured charge complex solution formed is measured at 465 nm against reagent blank. The amount of drug read from calibration curve. The calibration curve is linear over the range of 40-200 µg/ml of tenofovir disoproxil fumarate. The values of Standard deviation, coefficient of variation values and $t_{cal}$ are shown in Table.5.1.2. The values of standard deviation and coefficient of variation values are low, indicates high accuracy and reproducibility of the method. The data of assay values of commercial formulations is subjected to statistical evaluation for student ‘t’ test to study the proposed method. The calculated ‘t’ values are less than ‘t’ theoretical values with 4 (n-1= 5-1) degrees of freedom at 5% level of significance indicate that there is no significant difference between proposed method and standard method.

The proposed method is found to be simple, precise, accurate and time saving, reproducible and can be conveniently adopted for routine analysis of estimation of tenofovir disoproxil fumarate in bulk drugs samples and pharmaceutical formulations.