Spectrophotometric determination of diazepam via charge transfer complex formation reaction

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Received on:17-08-2014; Revised on: 21-09-2014; Accepted on:23-10-2014

ABSTRACT

Objective: A spectrophotometric method has been developed for the determination of diazepam (DZP) in bulk drug and in pharmaceutical formulations. Methods: This method is based on the formation of coloured charge transfer complexes of diazepam which act as electron donor with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) and 2,5-dichloro-3,6-dihydroxy-1,4-benzoquinone (p-CLA) reagents which act as π -acceptors in acetonitrile solvent. Results: DDQ and p-CLA were found to form charge-transfer complexes in a 1:1 stoichiometry [drug: reagent] with diazepam with a maximum absorption band at 550 and 480 nm, respectively. Optimization of temperature and time proved the supremacy of 20 and 30 °C and 20 and 5 minutes for DDQ and p-CLA reagents, respectively. Beer’s law was obeyed over the concentration ranges of 10-150 and 10-250 µg mL⁻¹ of DZP drug with high apparent molar absorptivities of 5.20x10² and 5.41 x10² and limits of detections are 6.83 and 9.60 µg mL⁻¹ using DDQ and p-CLA reagents, respectively. The results were compared with those given by the official method and showed that the developed methods are accurate, precise and reproducible. Conclusion: Thus the proposed methods are successfully applied to the determination of DZP in pharmaceutical formulations.

KEYWORDS: Diazepam; Charge-transfer complex; DDQ; p-CLA; Spectrophotometry.

1. INTRODUCTION

Diazepam (7-chloro-1,3-dihydro-1-methyl-5-phenyl-1,4-benzodiazepin-2(3H)-one¹ (Figure 1)) is a benzodiazepine drug, used in anxiety, insomnia, seizures including status epilepticus, muscle spasms, restless legs syndrome, alcohol withdrawal, benzodiazepine withdrawal and Ménière’s disease. It possesses anxiolytic, anticonvulsant, hypnotic, sedative, skeletal muscle relaxant, and amnestic properties². The pharmacological action of diazepam enhances the effect of the neurotransmitter GABA by binding to the benzodiazepine site on the GABAₐ receptor leading to central nervous system depression¹.

Diazepam has been determined in pharmaceutical preparations by spectrophotometric methods⁴-⁷, flow-injection chemiluminescence method⁸, chromatographic methods⁹-¹³, ultrasound-assisted emulsification microextraction method¹⁴, solid contact ion-selective electrodes (SC-ISE)¹⁵ and Sonogel-Carbon electrode (SngCE) modified with bentonite (BENT)¹⁶.

The aim of the present study was directed to develop simple, direct, sensitive and precise spectrophotometric methods for simultaneous determination of diazepam as electron donor via charge transfer complexation with π –acceptors p-CLA and DDQ in pure form and its dosage forms.

2. Experimental

2.1 Material

All chemicals and reagents used were of analytical reagent grade and some of them were used as such without any further purification. They included diazepam (DZP) provided by EL-Gomhoria Company, charge transfer complexing agents such as 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ) and p-chloranilic acid (p-CLA) were supplied from Arcos-USA. Absolute ethanol was supplied from Adwic,
while propan-2-ol and acetonitrile (AR) were supplied from Aldrich. Methanol, dimethyl formamide, tetrahydrofuran and 1,4-dioxane were supplied from El-Nasr Company, Egypt.

2.2. Preparation of Standard Solutions
1 mg mL⁻¹ Stock solutions of DZP drug was prepared by dissolving the accurate weighed amount in a definite volume of acetonitrile to get the required concentration. Dilute solutions were prepared by accurate dilution from the stock solution to get the desired concentrations. Valinil was manufactured by the Nile Company for Pharmaceutical and Chemical Industries, Cairo, Egypt (Each tablet contains 5 mg of diazepam) 0.1% (w/v) of DDQ and p-CLA reagents were prepared by dissolving 100 mg of DDQ and p-CLA in 100 mL acetonitrile. All solutions must be protected from light by keeping them in dark coloured quickfit bottles during the whole work.

2.3. Apparatus
The spectrophotometric measurements were carried out using the manual Unico 1200 spectrometer (United Products and Instruments, Inc.) in the wavelength range from 325-1000 nm and quartz cell of 1cm optical length was used. Small volumes were taken using automatic pipettes Socorex Swiss (50-200 μL).

2.4. Procedure
2.4.1. Selection of optimum wavelength
In calibrated 5 mL volumetric flask, 1 mL of DZP (1 mg mL⁻¹) was added to 1 ml of DDQ (1 mg mL⁻¹) or p-CLA (1 mg mL⁻¹) solution. The volume was completed to the mark with acetonitrile. The absorption spectra of the resulted CT complex products were scanned in the wavelength range from 400 to 640 nm from which the best wavelength for DZP drug was selected.

2.4.2. Optimization studies
2.4.2.1. Optimization of temperature and time
Aliquot of DZP stock solution (1 mL) was added to the 1 mL of DDQ (1 mg mL⁻¹) or p-CLA (1 mg mL⁻¹) solution and the volume complete to the mark with acetonitrile. The absorbance was measured at different time intervals in the range from 0–60 minutes then was measured at different temperatures in the range from 5 to 60 °C against the blank.

2.4.2.2. Optimization of diluting solvent
The effect of solvents on the absorptivity of the DZP-DDQ and DZP-p-CLA complexes was studied by using different organic solvents such as absolute ethanol, 1,4-dioxan, methanol, propan-2-ol, acetonitrile, tetrahydrofuran and dimethyl formamide.

2.4.2.3. Optimization of reagent concentration
Aliquot of DZP (1 mL) was added to different volumes of DDQ (1 mg mL⁻¹) or p-CLA (1 mg mL⁻¹), the volumes ranged from 0.25 to 5 mL and completed with the suitable solvents to 10 mL. The absorbance was measured at the selected wavelength.

2.4.3. Stoichiometric ratio determination
The stoichiometry of the CT complexes formed was examined by applying continuous variation and molar ratio methods. A series of solutions were prepared by mixing equimolar of DZP drug and reagent in varying portions, which keeps the total concentration constant at 4.40×10⁻³ and 4.79×10⁻³ mol L⁻¹ in case of DDQ and p-CLA reagents, respectively. The absorbance of the resultant charge transfer complexes was measured at 550 and 480 nm for DDQ and p-CLA reagents, respectively.

2.4.4. Validity of Beer’s law
Aliquot of 1 mg mL⁻¹ DDQ or p-CLA (3 mL) was added to different concentrations of DZP. The mixtures were completed up to 10 mL with acetonitrile. The absorbance of the coloured CT complex products were measured at the specific wavelength against reagent blank prepared similarly without drug.

2.4.5. Tablet analysis
For the analysis of DZP in the tablets, five tablets of pharmaceutical product (Valinil 5 mg) were weighed, and then dissolved in the minimum volume of acetonitrile, filtered, then transferred accurately to 25 mL measuring flask and completed to the mark with acetonitrile. To different concentrations of DZP was added 3 mL of 1 mg mL⁻¹ DDQ or p-CLA reagents. The volumes were made up to the mark with acetonitrile in 10 mL calibrated measuring flask. The absorbance of each was measured at its λmax against blank.

3. RESULTS AND DISCUSSION
3.1. Absorption spectra
The reaction of DDQ or p-CLA reagents as a π-acceptor with DZP drug as electron donor results in the formation of charge transfer complex which exhibits maximum at 550 and 480 nm, respectively (Figure 2). A solution of drug and DDQ in acetonitrile solvent yields an intense dark red colour which has a characteristic wavelength absorption band, frequently with two maxima at 550 (ε₁ = 5.20x10³ L mol⁻¹ cm⁻¹) and 590 nm (ε² = 4.83 x10² L mol⁻¹ cm⁻¹) in the electronic spectrum. The wavelength 550 nm was selected for further studies as it has the highest ε value.
3.2. Optimization of reagent concentration

3.2.1. Effect of time

Optimum reaction time was determined by monitoring the absorbance of the developed coloured complex at different time intervals (0-60 min) for both reagents. As shown in Figure (3) the absorbance reached its maximum after 20 and 5 minutes after addition of the DDQ and \( p \)-CLA reagents, respectively.

3.2.2. Effect of temperature

The effect of temperature on these CT complexes is shown in Figure (4). It is clear from the results that the absorbance attains maximum colour intensity at temperatures 20 and 30 °C for DDQ and \( p \)-CLA reagents, respectively. The colour of the CT complexes is remained constant for at least 24 hour.

3.2.3. Effect of solvent

Different solvents such as acetonitrile, methanol, ethanol, 1,4-dioxan, tetrahydrofuran, dimethyl formamide, and propan-2-ol were used to select elegant solvent for the analysis of the drug. Acetonitrile is found to be the most suitable solvent although ethanol and dimethyl formamide have high molar absorptivity than acetonitrile (Table (1)) but the stability and reproducibility of the absorbance values of the CT complexes are stable and reproducible in acetonitrile solvent.

Table (1). The molar absorptivity values of DZP-DDQ and DZP–\( p \)-CLA CT complexes in different solvents.

<table>
<thead>
<tr>
<th>Organic solvents</th>
<th>DZP-DDQ</th>
<th>( p )-CLA</th>
<th>( A ) ( \times 10^2 ) cm(^{-1} ) mol(^{-1} )</th>
<th>( \varepsilon ) ( \times 10^2 ) cm(^{-1} ) ( \times 10^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetonitrile</td>
<td>0.354</td>
<td>0.315</td>
<td>5.04 x 10^2</td>
<td>4.48 x 10^2</td>
</tr>
<tr>
<td>Methanol</td>
<td>0.021</td>
<td>0.249</td>
<td>0.30 x 10^2</td>
<td>3.54 x 10^2</td>
</tr>
<tr>
<td>Ethanol</td>
<td>0.956</td>
<td>0.533</td>
<td>13.61 x 10^2</td>
<td>7.59 x 10^2</td>
</tr>
<tr>
<td>Propan-2-ol</td>
<td>0.192</td>
<td>0.303</td>
<td>2.73 x 10^2</td>
<td>4.31 x 10^2</td>
</tr>
<tr>
<td>1,4-dioxan</td>
<td>0.103</td>
<td>0.198</td>
<td>1.47 x 10^2</td>
<td>2.82 x 10^2</td>
</tr>
<tr>
<td>Tetrahydrofuran</td>
<td>0.099</td>
<td>0.301</td>
<td>1.41 x 10^2</td>
<td>4.28 x 10^2</td>
</tr>
<tr>
<td>Dimethyl formamide</td>
<td>0.246</td>
<td>0.529</td>
<td>3.50 x 10^2</td>
<td>7.53 x 10^2</td>
</tr>
</tbody>
</table>

3.2.4. Effect of reagent concentration

The effect of the reagent concentration on the intensity of the colour at the selected wavelengths was ascertained by adding different amounts of the reagents DDQ and \( p \)-CLA to fixed concentration of 200 µg mL\(^{-1} \) of DZP drug. Figure (5) shows that 300 µg mL\(^{-1} \) of DDQ and \( p \)-CLA is the suitable concentration for quantitative determination of DZP drug. It also means that, maximum and reproducible colour
intensities are obtained and higher concentrations of reagents do not affect the colour intensity.

![Absorbance vs Concentration of reagents](image)

**Figure 5.** Effect of DDQ and p-CLA concentrations on the formation of DZP CT complexes.

3.3. Stoichiometric ratio determination

The composition of charge transfer complexes of studied DZP with DDQ and p-CLA were determined spectrophotometrically by applying continuous variation and molar ratio methods using equimolar solution which indicated that interaction of DZP drug with DDQ and p-CLA occurs on equimolar basis as shown in Scheme (1,2) (1:1 [DZP]: [DDQ] and [DZP]: [p-CLA]) (Figure (6,7)).

![Absorbance vs [DZP]/[DDQ] and [DZP]/[p-CLA]](image)

**Figure 7.** Stoichiometric ratio of the reaction of DZP with (a) DDQ and (b) p-CLA reagents using molar ratio method.

![Stoichiometric ratio of the reaction of DZP drug with DDQ and p-CLA reagents using the continuous variation method](image)

**Figure 6.** Stoichiometric ratio of the reaction of DZP drug with DDQ and p-CLA reagents using the continuous variation method.

**Scheme (1).** Structure of DZP-DDQ CT complex.
Scheme (2). Structure of DZP-p-CLA CT complex.

3.4. Validity of Beer’s law (Linearity, accuracy and precision)

According to the above described analytical conditions, linear calibration graph was obtained between absorbance versus concentration of DZP drug. Beer’s law is valid over the concentration range from 10-150 µg mL\(^{-1}\) of DZP drug while is valid over the concentration range from 10-250 µg mL\(^{-1}\) of DZP drug using p-CLA reagent. Table (2) shows the different analytical parameters obtained such as slope, intercept, correlation coefficient, Sandell sensitivity, molar absorptivity (\(\varepsilon\)), standard deviation, limit of quantification, limit of detection and relative standard deviation. The small value of Sandell sensitivity indicates the high sensitivity of the proposed method in the determination of the drug under investigation. Reproducibility of the method was measured for a series of four determinations at different concentration levels, data of percent relative standard deviation obtained for each reagent are reported in Table 2 which confirm the sensitivity of method.

Table (2). Analytical parameters for the determination of DZP drug using DDQ and p-CLA reagents.

<table>
<thead>
<tr>
<th>Reagents</th>
<th>DDQ</th>
<th>p-CLA</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\lambda_{\text{max}}) (nm)</td>
<td>550</td>
<td>480</td>
</tr>
<tr>
<td>time (min)</td>
<td>20</td>
<td>5</td>
</tr>
<tr>
<td>T (°C)</td>
<td>20</td>
<td>30</td>
</tr>
<tr>
<td>[Drug] (µg mL(^{-1}))</td>
<td>10-150</td>
<td>10-250</td>
</tr>
<tr>
<td>(\varepsilon) (L mol(^{-1}) cm(^{-1}))</td>
<td>5.20 \times 10^2</td>
<td>5.41 \times 10^2</td>
</tr>
<tr>
<td>S (µg cm(^{-1}))</td>
<td>0.548</td>
<td>0.526</td>
</tr>
<tr>
<td>% Recovery</td>
<td>98.79-99.57</td>
<td>100.7-101.5</td>
</tr>
<tr>
<td>(A = mC + z), m</td>
<td>0.0022</td>
<td>0.0008</td>
</tr>
<tr>
<td>z</td>
<td>0.145</td>
<td>0.0231</td>
</tr>
<tr>
<td>R</td>
<td>0.9983</td>
<td>0.9987</td>
</tr>
<tr>
<td>SD</td>
<td>0.013-0.018</td>
<td>0.006-0.01</td>
</tr>
<tr>
<td>RSD (%)</td>
<td>0.28-0.91</td>
<td>0.69-0.93</td>
</tr>
<tr>
<td>LOD (µg mL(^{-1}))</td>
<td>6.83</td>
<td>9.60</td>
</tr>
<tr>
<td>LOQ (µg mL(^{-1}))</td>
<td>22.78</td>
<td>32.00</td>
</tr>
</tbody>
</table>

*\(A = z + mC\); where C is the concentration in µg mL\(^{-1}\)

3.5. Between-day measurements

The validity and applicability of the proposed method and reproducibility of the results obtained was applied. Four replicate experiments at two concentrations of DZP drug are carried out. From Table (3), it is found that, the between-day relative standard deviations are less than 3%, which indicates that the proposed method is highly reproducible and DDQ and p-CLA reagents are successfully applied to determine DZP drug via the charge transfer reaction.

Table (3). Between–day precision of the determination of DZP drug using DDQ and p-CLA reagents.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Reagent</th>
<th>[DZP] (\mu)g mL(^{-1})</th>
<th>[DZP] (\mu)g mL(^{-1})</th>
<th>% Recovery</th>
<th>SD</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diazepam</td>
<td>DDQ</td>
<td>30.00</td>
<td>30.29</td>
<td>101.0</td>
<td>0.01</td>
<td>0.71</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100.00</td>
<td>99.73</td>
<td>99.73</td>
<td>0.03</td>
<td>0.95</td>
</tr>
<tr>
<td></td>
<td>p-CLA</td>
<td>30.00</td>
<td>29.30</td>
<td>97.67</td>
<td>0.01</td>
<td>2.61</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100.00</td>
<td>98.04</td>
<td>98.04</td>
<td>0.02</td>
<td>1.62</td>
</tr>
<tr>
<td>Valinil</td>
<td>DDQ</td>
<td>30.00</td>
<td>29.43</td>
<td>98.10</td>
<td>0.05</td>
<td>1.76</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100.00</td>
<td>101.7</td>
<td>101.7</td>
<td>0.02</td>
<td>0.83</td>
</tr>
<tr>
<td></td>
<td>p-CLA</td>
<td>30.00</td>
<td>30.63</td>
<td>102.1</td>
<td>0.06</td>
<td>1.95</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100.00</td>
<td>99.02</td>
<td>99.02</td>
<td>0.04</td>
<td>1.01</td>
</tr>
</tbody>
</table>

3.6. Analytical application

The applicability of the proposed method for the determination of DZP drug in commercial dosage form as tablets (5 mg/tablet) was examined by analyzing the marketed product (Valinil). The concentration of the drug in the dosage form is calculated from the appropriate calibration graph. There is no shift in the absorption maximum due to the presence of other constituents of the dosage form.

The determination of DZP drug in the dosage form is compared with those obtained by applying the official method [4] (Table 4). The results obtained are compared statistically by the % recovery with those obtained by official method on samples of the same batches. The values did not exceed the theoretical tabulated values indicating that there is no significant difference between accuracy of the proposed and the official method.
Table (4). Determination of DZP drug in pharmaceutical preparation using DDQ and p-CLA reagents and official method.

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Sample</th>
<th>Proposed [Drug] µg mL$^{-1}$</th>
<th>Official [Drug] µg mL$^{-1}$</th>
<th>% Recovery</th>
<th>SD$^*$</th>
<th>SD$^{**}$</th>
<th>F-test</th>
<th>t-test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Taken</td>
<td>Found</td>
<td>Taken</td>
<td>Proposed</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DDQ</td>
<td>Valinil</td>
<td>30.00 29.86</td>
<td>30.00 29.82</td>
<td>99.54</td>
<td>0.04</td>
<td>0.03</td>
<td>1.78</td>
<td>2.00</td>
</tr>
<tr>
<td>p-CLA</td>
<td>100.0</td>
<td>101.4</td>
<td>100.0 101.5</td>
<td>101.4</td>
<td>0.08</td>
<td>1.31</td>
<td>2.50</td>
<td></td>
</tr>
<tr>
<td></td>
<td>100.0</td>
<td>101.6</td>
<td></td>
<td>101.6</td>
<td>0.08</td>
<td>1.31</td>
<td>2.50</td>
<td></td>
</tr>
</tbody>
</table>

* Standard deviation values using proposed method (n = 4). ** Standard deviation values using official method (n = 4).
# Tabulated t value at 95% confidence limit = 2.77.
* Tabulated F value at 95% confidence limit = 6.388.

CONCLUSION

A charge-transfer complexation between diazepam with different reagents (DDQ and p-CLA) occurred with a 1:1 stoichiometry in each case, with maximum wavelength of absorption at 550 and 480 nm in case of using DDQ and p-CLA reagents, respectively. The proposed methods are beneficial over many of the reported methods due to its sensitivity, accuracy, wide application range, low relative standard deviation and high percentage of recovery and therefore can be used in rapid qualitative and quantitative determination of diazepam in both pure and dosage form.

REFERENCES


Source of support: Nil, Conflict of interest: None Declared