Charge Transfer Spectrophotometric Determination of Zolmitriptan in Pure and Dosage Forms

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Abstract

A facile, accurate, sensitive and validated spectrophotometric method for the determination of zolmitriptan (ZMT) in pure and dosage forms is described. The methods are based on the formation of charge transfer reactions between ZMT and chromogenic reagents 7,7,8,8-tetracyanoquinodimethane (TCNQ); p-chloranilic acid (P-CLA), quinalizarin (Quiz) and alizarin red S (ARS) producing charge transfer complexes which showed an absorption maximum at 840, 532, 554 and 534 nm for TCNQ, P-CLA, Quiz and ARS, respectively. The optimization of the reaction conditions such as the type of solvent, reagent concentration and reaction time were investigated. Beer’s law is obeyed in the concentration ranges 1.0-140 μg mL⁻¹. The molar absorptivity, Sandell sensitivity, detection and quantification limits are also calculated. The correlation coefficient was ≥0.9991 (n=6) with a relative standard deviation (R.S.D.) of ≤1.01. The proposed methods were successfully applied for simultaneous determination of zolmitriptan in tablets with good accuracy and precision and without interferences from common additives and the validity assesses by applying the standard addition technique, which compared with those obtained using the reported method.

Keywords: Charge transfer reaction; Zolmitriptan; Spectrophotometry; Dosage forms

Introduction

Zolmitriptan (ZMT), 4(S)-4-[3-(2-dimethyl aminoethyl)-1H-5-indolyl-methyl]-1, 3-oxazolan-2-one (Figure 1) belongs to a group of medicines known as Serotonin 5-HT1D Receptor Agonists. It works by stimulating serotonin receptors in the brain. Serotonin is a natural substance in the brain that, among other things, causes blood vessels in the brain to narrow. It is used to treat severe migraine headaches [1].

The assay of ZMT in pure and dosage forms, as far as we know, is not official in any pharmacopoeia, and therefore, requires much more investigation. The different analytical methods that have been reported for its determination include HPLC with mass spectrometry detection [2-7], with coulometric detection [8], electrospray ionization mass spectrometry [9], tandem mass spectrometry [10], fluorescence detection [11,12] in pharmaceutical preparations and biological fluids and spectrophotometric methods [13-18].

The molecular interactions between electron donors and acceptors are generally associated with the formation of intensely colored charge transfer complexes, which absorb radiation in the visible region [19]. A variety of electron donating compounds have been reported to yield charge-transfer complexes with various π-acceptors [20-25] and with alizarin derivatives [26-29].

The aim of the present study was directed to investigate simple, direct, sensitive and precise spectrophotometric methods for simultaneous determination of ZMT via complexation with π–acceptors; 7,7,8,8-tetracyanoquinodimethane (TCNQ) and p-chloranilic acid (p-CLA) or with alizarin derivatives; quinalizarin (Quiz) and alizarin red S (ARS) as chromogenic reagents in pure form and its dosage forms (tablets).

Materials and Methods

Apparatus

All the absorption spectral measurements were made using Kontron 930 (UV-Visible) spectrophotometer (Germany) with scanning speed 200 nm/min, and band width 1.0 nm equipped with 10 mm matched quartz cells. The PH values of different buffer solutions were checked using a Hanna pH-meter instrument (pH 211, Romania).

Materials and reagents

All chemicals and reagents were of analytical grade and water was always bi-distilled water.

Materials: Zolmitriptan (ZMT) and its formulation No-Migrain tablets (2 mg ZMT per tablet) were kindly supplied by Amoun Pharmaceutical Industries, Cairo, Egypt. Its purity was found to be 99.96 ± 0.64 (n=6) according to the HPLC procedures [1].

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Figure 1: The chemical structure of ZMT.
A stock solutions of ZMT (100 and 500 μg mL⁻¹) and (5.0×10⁻⁵ M) were prepared by dissolving the appropriate weight of ZMT in 5.0 mL methanol and the volume was diluted to the mark in a 100 mL calibrated flask with acetonitrile. Working standard solutions were prepared from suitable dilution of the standard stock solution. All solutions are stable for a period of 3.0 days when refrigerated (4°C).

Reagents: 7,8,8-tetracyanoquinodimethane (TCNQ), (Aldrich Chem. Co., Milwaukee, USA) and Chloranilic acid (p-CLA), (Fluka, Switzerland), were freshly prepared as (5.0×10⁻⁵ M) solutions in acetonitrile. Quinalizarin, 1,2,5,8-tetrahydroxanthraquinone (Quiz) and alizarin red S, 3,4-dihydroxy-9,10-dioxo-2-anthracene sulfonic acid (ARS) were Aldrich products and used without further purification. A stock solution (1.0×10⁻³ M) was prepared by dissolving the appropriate amount of the reagent in approximately 25 mL of methanol. After obtaining a solid-free solution, it was transferred to a 50 mL volumetric flask and completed to the mark with methanol. The solutions were stable for at least one week at 4°C.

General procedures

Using TCNQ and p-CLA reagents: Into 10 ml calibrated flasks were placed 0.2-2.8 mL of (500 μg mL⁻¹) ZMT using p-CLA method and 0.3-3.0 mL of (100 μg mL⁻¹) ZMT using TCNQ method, 1.0 mL of (5.0×10⁻⁵ M) p-CLA and TCNQ was added. The reaction mixture was heat in a water-bath at 60 ± 2°C for 5.0 and 15 min for p-CLA and TCNQ methods, respectively. Cool and then dilute to volume up to 10 mL with acetonitrile and the absorbance was measured at 532 and 840 nm for Quiz and ARS, respectively against a reagent blank. After obtaining a solid-free solution, it was transferred to a 50 mL volumetric flask and completed to the mark with methanol. The solutions were stable for at least one week at 4°C.

Using Quiz and ARS reagents: Aliquots of methanolic solutions containing (0.1-2.0 mL) of (100 μg mL⁻¹) ZMT are transferred to 10 mL volumetric flasks. To each flask 1.5 and 2.0 mL of (1.0×10⁻⁵ M) Quiz and ARS solution, respectively were added. Afterwards, the obtained mixture was shaken in order to promote the reaction and the volume was completed to the mark with methanol. The absorbance of this final solution was measured at 532 and 840 nm for Quiz and ARS, respectively against a reagent blank.

Procedures for pharmaceutical formulations (tablets): The contents of twenty tablets (No-Migrain, 2.5 mg ZMT per tablet) were crushed, finely powdered, weight out and the average weight of one tablet was determined for each drug. An accurate weight equivalent to 25 mg ZMT was transferred into a 100 mL calibrated flask dissolved in 20 mL methanol with shaking for 5.0 min and filtered through a sintered glass crucible (G4). The first 5.0 mL portion of the filtrate was rejected and the filtrate was diluted to 10 mL with acetonitrile using (p-CLA and TCNQ) or methanol using (Quiz and ARS) in a 100 mL measuring flask to give 250 μg mL⁻¹ stock solutions. Aliquot of the cited solutions was taken and analyzed as described under the above recommended procedures for construction of calibration curves. For the proposed methods, the content of a tablet was calculated using the corresponding regression equation of the appropriate calibration graph.

Stoichiometric relationship: Job’s method of continuous variation [30] was employed to establish the stoichiometry of the coloured products. A 1.0×10⁻³ M standard solution of ZMT and a 1.0×10⁻⁵ solution of reagents were used. A series of solutions was prepared in which the total volume of drug and reagent was constant (2.0 mL). The drugs and reagents were mixed in various proportions and diluted in a 10 mL calibrated flask with acetonitrile using (p-CLA and TCNQ) or methanol using (Quiz and ARS). Measure the absorbance at optimum wavelengths after treating each reagent at best time and temperature against a reagent blank following the above mentioned procedure.

Results and Discussion

In the present investigation, we investigate the development of simple, rapid, accurate, reproducible and adequately sensitive spectrophotometric methods for determination ZMT in bulk powder and pharmaceutical formulation based on the formation of charge-transfer complex between ZMT as electron-donor when they reacted with selected π-acceptors (TCNQ and p-CLA) in acetonitrile or with (Quiz and ARS) in methanolic medium. They produce a new band of absorption intensity at a suitable λmax which was characteristic for each complex (Table 1).

Absorption spectra

The reaction of ZMT with p-CLA results in the formation of a charge transfer complex of the π-π-type. This compound is considered to be an intermediate molecular association complex which dissociates in the corresponding radical anions in acetonitrile solvent. The p-CLA radical anion absorbs at 532 nm (Figure 2a). The experimental conditions should be carefully selected.

ZMT yields intense color with TCNQ in acetonitrile, absorbing maximally at wavelengths 840 nm (Figures 2b and 3) most probably due to the formation of charge-transfer complexes between the drug acting as n-donor (D) or Lewis base, and TCNQ, as π-acceptors (A) or Lewis acids:

![Figure 2a](image_url)

**Figure 2a:** Absorption spectra of reaction products of p-CLA with 100 μg mL⁻¹ ZMT.

![Figure 2b](image_url)

**Figure 2b:** Absorption spectra of reaction products of TCNQ with 25 μg mL⁻¹ ZMT.
The dissociation of DA complex is promoted by the high dielectric constant of the solvent, acetonitrile (37.5). Further support for the assignment was provided by the comparison of the absorption bands with those of the TCNQ and p-CLA.- radical anions produced by the iodide reduction method.

\[
\text{D} \quad \text{**} + \text{A} \rightarrow [\text{D} \quad \text{**} \cdot \text{A}^{-}] \rightarrow \text{D} \quad \text{**} + \text{A}^{-}
\]

At optimum conditions, the radical anion (absorbing species) was formed in the medium immediately after mixing of the reagents and showed maximum absorption at 554 and 534 nm using Quiz and ARS, respectively in methanol medium (Figures 4 and 5). Thus, these wavelengths were chosen for all further measurements in order to obtain highest sensitivity for the proposed methods. It is important to point out that the Quiz and ARS alone, in methanol medium, exhibits maximum absorption at 491 and 423 nm, respectively (Figures 2c and 2d). The high difference between maxima of the reagent and the product absorption bands ~ 63 or 106 nm for Quiz and ARS respectively allowed the measurement of the products with only a small contribution of the reagent that was added in excess in the medium.

**Optimization of the experimental conditions**

**Evaluation of the effect of the solvent nature:** Different solvents such as acetonitrile, acetone, chloroform, methanol, ethanol, 1,4-dioxane, dichloromethane, and 1,2-dichloroethane were examined (Table 2). Acetonitrile was found to be the best solvent for TCNQ and p-CLA reagents, because it has a high relative permittivity which ensures the maximum yield of TCNQ- and p-CLA-.- species, whereas, best sensitivity for Quiz and ARS was achieved with methanol, probably because of the capacity of this solvent to form stable hydrogen bonds with the radical anion.

**Effect of reagents concentration:** The optimum concentrations that give maximum color formation, 1.0mL of solution (5.0×10⁻³ M) TCNQ and p-CLA solution in acetonitrile was found to be sufficient for the production of maximum and reproducible color intensity. Higher concentrations of the reagent did not affect the color intensity (Figure 3). For Quiz and ARS, 1.5 and 2.0 mL of (1.0×10⁻³ M) Quiz and ARS reagents, respectively are a sufficient excess of the reagents to consume all drug present in the medium and were established for the method to attain satisfactory sensitivity besides.

**Effect of time and temperature:** The optimum reaction time was determined by following the color intensity at optimum wavelengths at ambient temperature (25 ± 2°C). Complete color development was attained after 90 and 50 min for TCNQ and p-CLA complexes, respectively. The complete color development was obtained by raising the temperature to 60 ± 2°C on a water-bath for 15 and 5.0 min using...
the interaction of ZMT and the reagents used takes place at two sites, which were the more sterically free terminal basic amino groups. In view of this result a reaction mechanism was proposed considering the transfer of free electron of the two nitrogen atom present in one molecule of drug to the charge-deficient center of reagent molecule from the total transfer of charge. On the basis of the literature data and our experimental results, tentative reaction mechanisms for ZMT-TCNQ and ZMT-Quiz complexes are proposed and given in schemes 1 and 2, respectively.

**Table 2:** Effect of solvent on the absorption intensity of reaction products of ZMT with TCNQ, p-CLA, Quiz and ARS.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>p-CLA</th>
<th>TCNQ</th>
<th>Quiz</th>
<th>ARS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetonitrile</td>
<td>0.51</td>
<td>0.42</td>
<td>0.38</td>
<td>0.35</td>
</tr>
<tr>
<td>Acetone</td>
<td>0.07</td>
<td>0.23</td>
<td>0.19</td>
<td>0.18</td>
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<tr>
<td>Chloroform</td>
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<td>0.24</td>
<td>0.23</td>
<td>0.27</td>
</tr>
<tr>
<td>Dichloromethane</td>
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<td>0.22</td>
<td>0.17</td>
<td>0.26</td>
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<tr>
<td>1,2-Dichloroethane</td>
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<td>0.17</td>
<td>0.14</td>
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<tr>
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<td>0.09</td>
<td>0.11</td>
<td>0.08</td>
</tr>
<tr>
<td>Methanol</td>
<td>0.29</td>
<td>0.28</td>
<td>0.77</td>
<td>0.68</td>
</tr>
</tbody>
</table>

**Figure 4:** Effect of time on the colour intensity at 60 ± 2°C for ZMT complexes with p-CLA and TCNQ.

**Figure 5:** Job's method of continuous variation graph for the reaction of ZMT complexes with reagents, respectively. Total molar concentration=1.0×10⁻⁴ M. Absorbance measurements were carried out at optimum wavelength (nm).

**Scheme 1:** Proposed mechanism for the reaction between ZMT and TCNQ charge transfer complex.

**Scheme 2:** Possible mechanism of radical anion formation from Quiz and ZMT reaction.
Validation of the proposed method

Linearity, detection, and quantitation limits: Following the proposed experimental conditions, the relationship between the absorbance and concentration was quite linear in the concentration ranges given in (Table 1). The regression equations were derived using the least-squares method [31]. The intercept (a), slope (b), correlation coefficient (r), molar absorptivities (ε), and sandell sensitivity values are summarized in (Table 1). The percentage recoveries of the pure drugs were evaluated by performing six replicate analyses on pure drug solution at four different concentration levels (within the working range). Percentage relative standard deviation (RSD %) as precision and percentage relative error (RE %) as accuracy of the proposed spectrophotometric methods were calculated. The relative standard deviation (RSD) values were less than 2% in all cases, indicating good repeatability of the suggested methods.

The detection limit (LOD) is defined as the minimum level at which the analyte can be reliably detected for the 3 drugs was calculated using the following equation, [32,33] and listed in Table 1.

LOD = 3s/k

where s is the standard deviation of replicate determination values under the same conditions as for the sample analysis in the absence of the analyte and k is the sensitivity, namely the slope of the calibration graph. In accordance with the formula, the detection limits were found to be 0.494, 1.97, 0.285 and 0.186 μg mL−1 using TCNQ, p-CLA, Quiz and ARS, respectively.

According to this equation, the limit of quantization is defined as the lowest concentration that can be measured with acceptable accuracy and precision [32,33]

LOQ = 10s/k

The limits of quantization LOQ, is defined as the lowest concentration of ZMT in No-Migain tablets (2.5 mg ZMT/tab) using the proposed methods.

Accuracy and precision: The accuracy and precision of the methods were evaluated by performing six replicate analyses on pure drug solution at four different concentration levels (within the working range). Percentage relative standard deviation (RSD %) as precision and percentage relative error (RE %) as accuracy of the proposed spectrophotometric methods were calculated. The relative standard deviation (RSD) values were less than 2% in all cases, indicating good repeatability of the suggested methods.
The proposed methods revealed no significant difference were found obtained by the reference method [16]. The results obtained by the reference methods were further ascertained by performing recovery studies. Pre-added tablet powder was spiked with pure ZMT at different levels and the total was determined by the proposed methods using standard addition technique. The percent recovery of pure ZMT added was in the range 98.50–100.50% with relative standard deviation of 0.319-0.716 (Table 4) indicating that the recoveries were good, and that the co-formulated substance and common excipients did not interfere in the determination.

Recovery studies: The accuracy and validity of the proposed methods were further ascertained by performing recovery studies. Pre-analyzed tablet powder was spiked with pure ZMT at different levels and the total was determined by the proposed methods using standard addition technique. The percent recovery of pure ZMT added was in the range 98.50–100.50% with relative standard deviation of 0.319-0.716 (Table 4) indicating that the recoveries were good, and that the co-formulated substance and common excipients did not interfere in the determination.

Interference studies

A study was performed in order to evaluate the effect of possible interfering species on the drug reaction with the reagents. The selectivity of the proposed spectrophotometric methods was investigated by observing any interference encountered from some common excipients of the pharmaceutical formulations such as starch, lactose, sucrose, glucose, gum acacia, and magnesium stearate. It was shown that these excipients did not interfere with the proposed methods. So, the proposed methods are able to determine the analyte in the presence of common excipients.

Application of the proposed methods to analysis of dosage forms

The proposed method was successfully applied to the determination of ZMT in its pharmaceutical formulation (Table 5). The results were compared statistically, by applying the t- and F-tests, with the results obtained by the reference method [16]. The results obtained by the proposed methods revealed no significant difference were found between the calculated and theoretical values of the proposed and reference methods at 95% confidence level. This indicated similar accuracy and precision in the analysis by the proposed and reported methods. It is evident from these results that the proposed methods are applicable to the analysis of ZMT in its dosage forms with comparable analytical performance.

Conclusions

The present study describes the successful evaluation of some π acceptors (TCNQ or p-CLA) and alizarin derivatives (Quiz or ARS) as an analytical reagent in the development of simple and rapid spectrophotometric methods for the accurate determination of ZMT in its dosage forms. The methods described herein have many advantages: it does not need expensive sophisticated apparatus, it is simple and rapid, and it has high sensitivity. The proposed methods used inexpensive reagents with excellent shelf life, and are available in any analytical laboratory.

References


