Section 7.0

DRUG PROFILE AND LITERATURE SURVEY

7.0.1 DRUG PROFILE

Zolmitriptan (ZMT) is chemically known as (4S)-4-[[3-[2-(dimethylamino)ethyl]-1H-indol-5-yl] methyl]-2-oxazolidinone. The molecular formula of ZMT is C_{16}H_{21}N_{3}O_{2} and its formula weight is 287.36 g mol^{-1}. Physically pure ZMT is a white crystalline powder [1]. It has the following chemical structure:

![Chemical structure of Zolmitriptan](image)

ZMT is soluble in acetic acid, acetonitrile, sulphuric acid and insoluble in water, chloroform, dichloromethane.

ZMT was first synthesized by a pharmaceutical company Laboratorios Vita [2]. ZMT is a selective agonist of serotonin (5-hydroxytryptamine; 5-HT) type 1B and 1D receptors. ZMT binds with high affinity to human 5-HT_{1B} and 5-HT_{1D} receptors leading to cranial blood vessel constriction. The therapeutic activity of ZMT for the treatment of migraine headache can most likely be attributed to the agonist effects at the 5HT_{1B/1D} receptors on intracranial blood vessels (including the arterio-venous anastomoses) and sensory nerves of the trigeminal system which result in cranial vessel constriction and inhibition of pro-inflammatory neuropeptide release [3].

ZMT is not official in any pharmacopoeia.
Chapter 7
Titrimetric and spectrophotometric assay of zolmitriptan

7.0.2 LITERATURE SURVEY OF ANALYTICAL METHODS FOR ZOLMITRIPTAN

7.0.2.1 Titrimetric methods

Literature survey revealed that no titrimetric methods have ever been reported for ZMT.

7.0.2.2 UV-Visible spectrophotometric methods

Only a few UV-spectrophotometric methods are found in the literature for ZMT when it is present in tablets. Acharjya et al [4] reported two UV-spectrophotometric methods for the determination of ZMT in bulk and pharmaceutical formulations. First method is the first derivative method in which the response of drug in 0.1 M H₂SO₄ measured at 298 nm and the second was based on calculation of area under curve (AUC) for drug in 0.1 M H₂SO₄ at 283 nm both in the linear range 0.5-100.0 µg mL⁻¹. In a UV-spectrophotometric method described by Murthy and Veditha [5], drug is dissolved in different solvents such as water, water: acetonitrile (1:1) and water: methanol (1:1) and the response measured at 283 nm in the case of water, 284 nm in the case of both acetonitrile:water, and MeOH: water within the Beer’s law range 10.0-50.0 µg mL⁻¹. One more UV- spectrophotometric method was developed by Sankar et al [6] in which drug in methanol showed absorption maximum at 226 nm and the method is applicable over the concentration range of 1.0-6.0 µg mL⁻¹.

There are only two visible spectrophotometric methods reported for the assay of ZMT in pharmaceuticals. The first method reported by Raza et al [7] is based on the charge-transfer reaction of ZMT with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone in acetonitrile medium to form a colored product peaking at 555 nm and Beer's law obeyed in the concentration range 10.0-250.0 µg mL⁻¹. The second report described by Zeynep and Ipek [8] consists two extractive methods which are based on the formation of yellow ion-pair complexes between ZMT and tropaeolin OO (TPOO) and bromothymol blue (BTB) in chloroform which are measured at 411.5 and 410 nm, respectively with corresponding linear range 2.0-20.0 and 1.5-17.0 µg mL⁻¹.
7.0.2.3 Other methods

High-performance liquid chromatography (HPLC) with UV-detection has been widely used for the quantitative determination of ZMT in pharmaceuticals [9-16]. Ultra-performance liquid chromatography (UPLC) [17] and liquid chromatography-mass spectrometry [18] for the assay of ZMT are also found in the literature.

Only one voltammetric [19] method has been previously reported for the quantification of ZMT in pharmaceuticals.

The literature survey presented in the foregoing paragraphs reveals that no titrimetric method has ever been reported for the assay of ZMT in dosage forms. This prompted the author to develop two simple, rapid and semi-micro scale titrimetric methods. Spectrophotometry is considered as the most convenient analytical technique in pharmaceutical analysis because of its inherent simplicity and availability in most quality control and clinical laboratories. But, only two visible spectrophotometric and three UV- spectrophotometric methods are reported for ZMT. The reported visible spectrophotometric methods suffer from one or other disadvantages and none of the reported UV- spectrophotometric methods is stability-indicating. Considering the importance of both the spectrophotometric techniques in pharmaceutical analysis, the author has applied these techniques for the assay of ZMT both in bulk drug and in dosage form and developed three visible spectrophotometric and two stability-indicating UV- spectrophotometric methods for ZMT. The details about the method development and validation are presented in Section 7.1 to 7.4 and a separate Section (7.5) has been devoted to compare the performance characteristics of the proposed methods with the existing methods.
Section 7.1

TITRIMETRIC ASSAY OF ZOLMITRIPTAN IN NON-AQUEOUS MEDIUM

7.1.1 INTRODUCTION

The theory and applications of titrations of nitrogenous bases in non-aqueous medium has been presented in Section 2.1.

In the literature no titrimetric procedures are available for the assay of ZMT. ZMT has weakly basic nitrogen functional group in its structure. The author succeeded to make use of nitrogenous basic nature of ZMT and proposed two simple, rapid, reliable and cost-effective non-aqueous titrimetric procedures. In these methods, the drug dissolved in glacial acetic acid, was titrated with acetous perchloric acid with visual and potentiometric end point detection, crystal violet being used as indicator for visual titration.

7.1.2 EXPERIMENTAL

7.1.2.1 Instrument

The instrument used is the same as that was described in Section 2.1.2.1.

7.1.2.2 Reagents and materials

All chemicals used were of analytical reagent grade. All solutions are made in glacial acetic acid (S. D. Fine Chem, Mumbai, India) unless mentioned otherwise. Perchloric Acid (0.005 M) was prepared as described in Section 2.1.2.2 and standardized using pure potassium hydrogen phthalate and crystal violet as indicator [20]. Crystal violet indicator (0.1 %) was prepared as described in Section 2.1.2.2. Standard ZMT solution (1.0 mg mL\(^{-1}\)): ZMT (pharmaceutical grade, 99.80 % pure) was procured from Jubilient life Sciences, Mysore, India, as a gift and was used as received. Stock standard solution containing 1.0 mg mL\(^{-1}\) drug was prepared by dissolving the required amount of ZMT in glacial acetic acid.

* This work has been communicated to International Journal of Pharmaceutical and Chemical Sciences.
One brand of tablet namely Zomig-2.5 (Intas Pharmaceuticals, Dehradun, India) was purchased from local commercial sources.

7.1.2.3 Assay procedures

Visual titration

An aliquot of the drug solution containing 1.0-10.0 mg of ZMT was measured accurately and transferred into a clean and dry 100 mL titration flask and the total volume was brought to 10 mL with glacial acetic acid. Two drops of crystal violet indicator were added and titrated with standard 0.005 M perchloric acid to a blue colour end point. An indicator blank titration was performed and corrections to the sample titration were applied. The amount of the drug in the measured aliquot was calculated from

\[ \text{Amount (mg)} = V M_w R/n \]

where \( V \) = volume of perchloric acid consumed (mL); \( M_w \) = relative molecular mass of the drug; \( R \) = molarity of the perchloric acid and \( n \) = number of moles of perchloric acid reacting with each mole of ZMT.

Potentiometric titration

An aliquot of the standard drug solution equivalent to 1.0-10.0 mg of ZMT was measured accurately and transferred into a clean and dry 100 mL beaker and the solution was diluted to 25 mL by adding glacial acetic acid. The combined glass-SCE (modified) system was dipped in the solution. The content was stirred magnetically and the titrant (0.005 M HClO₄) was added from a microburette. Near the equivalence point, titrant was added in 0.1 mL increments. After each addition of titrant, the solution was stirred magnetically for 30 s and the steady potential (e.m.f) was noted. The addition of titrant was continued until there was no significant change in potential on further addition of titrant observed. The equivalence point was determined by plotting the titration curves (volume of titrant versus e.m.f; first derivative curve or second derivative curve). The amount of the drug in the measured aliquot was calculated as described under visual titration.

Procedure for tablets

Fifty tablets each containing 2.5 mg of ZMT were weighed accurately and pulverized. An amount of powdered tablet equivalent to 100 mg of ZMT was transferred into a 100 mL calibrated flask and 60 mL of glacial acetic acid was added. The content
was shaken thoroughly for about 15-20 min, diluted to the mark with glacial acetic acid, mixed well and filtered using a Whatman No. 42 filter paper. The first 10 mL portion of the filtrate was discarded and a suitable aliquot was taken and assayed by following the general procedures described for visual and potentiometric end point detection.

**Procedure for selectivity study**

A placebo blank containing starch (10 mg), acacia (15 mg), hydroxyl cellulose (10 mg), sodium citrate (10 mg), talc (20 mg), magnesium stearate (15 mg) and sodium alginate (10 mg) was made and its solution was prepared as described under “Procedure for tablets” and then 50 mg of the above mixture extracted in acetic acid subjected to analysis by the proposed methods.

To 50 mg of the placebo blank described above, 100 mg of ZMT was added, homogenized and the solution of the synthetic mixture was prepared in a 100 mL calibration flask as described under “Procedure for tablets”. The filtrate was collected and analyzed by following the procedures of both visual and potentiometric titrations.

**7.1.3 RESULTS AND DISCUSSION**

**7.1.3.1 Chemistry**

The present methods are based on the neutralization reaction involving the basic property of ZMT and employ two techniques. The methods are based on the principle that substances, which are weakly basic in aqueous medium, when dissolved in non-aqueous solvents exhibit enhanced basicity thus allowing their easy determination. In the present titrimetric methods, the weakly basic property of ZMT was enhanced due to the non-levelling effect of glacial acetic acid and titrated with perchloric acid with visual and potentiometric end point detection. Crystal violet gave satisfactory end point for the amounts of analyte and concentrations of titrant employed. A steep rise in the potential was observed at the equivalence point with potentiometric end point detection ([Figure. 7.1.1](#)). With both methods of equivalence point detection, a reaction stoichiometry of 1:1 (drug:titrant) was obtained which served as the basis for calculation. Using 0.005 M perchloric acid, 1.0-10.0 mg of ZMT was conveniently determined. The relationship between the drug amount and the titration end point was examined. The linearity between two parameters is apparent from the correlation coefficients of 0.9972 and 0.9969.
obtained by the method of least squares for visual and potentiometric methods, respectively. From this it is implied that the reaction between ZMT and perchloric acid proceeds stoichiometrically in the ratio 1:1 in the range studied.

![Figure 7.1.1 Potentiometric titration curves for 6 mg ZMT Vs 0.005 M HClO₄. (a) Normal titration curve and (b) First-derivative curve.](image)

When a strong acid, such as perchloric acid, is dissolved in a weaker acid, such as acetic acid, the acetic acid is forced to act as a base and accept a proton from the perchloric acid forming an onium ion. The formed onium ion (CH₃COOH⁻) can very readily give up its proton to react with ZMT, so basic properties of the drug is enhanced and hence, titration between ZMT and perchloric acid can often be accurately carried out using acetic acid as solvent. The reactions occurring are as follows:

\[
\text{HClO}_4 + \text{CH}_3\text{COOH} \rightarrow \text{CH}_3\text{COOH}_2^+ + \text{ClO}_4^-
\]

\[
\text{CH}_3\text{COOH}_2^+ + \text{ZMT} \rightarrow \text{ZMT}^+ + \text{CH}_3\text{COOH}
\]

Overall, the reaction is:

\[
\text{HClO}_4 + \text{ZMT} \rightarrow \text{ZMT}^+ + \text{ClO}_4^-
\]
Chapter 7

Titrimetric and spectrophotometric assay of zolmitriptan

7.1.3.2 Method validation

The method validation was done according to the present ICH guidelines [21].

Accuracy and precision

The accuracy and precision of the methods was evaluated in terms of intermediate precision (intra-day and inter-day). Three different amounts of ZMT within the range of study in both methods were analyzed in seven replicates during the same day (intra-day precision) and five consecutive days (inter-day precision). The percentage relative standard deviation (RSD %) values were \( \leq 1.30\% \) (intra-day) and \( \leq 1.77\% \) (inter-day) indicating high precision of the methods. Also, the accuracy of the methods was evaluated as percentage relative error (RE %) and from the results shown in Table 7.1.1, it is clear that the accuracy is satisfactory (RE \( \leq 1.55\% \)).

<table>
<thead>
<tr>
<th>Method</th>
<th>ZMT taken, mg</th>
<th>ZMT found, mg</th>
<th>RE, %</th>
<th>RSD, %</th>
<th>ZMT found, mg</th>
<th>RE, %</th>
<th>RSD, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Visual titrimetry, (n=7)</td>
<td>4.0</td>
<td>4.01</td>
<td>0.29</td>
<td>0.86</td>
<td>4.02</td>
<td>0.61</td>
<td>1.31</td>
</tr>
<tr>
<td></td>
<td>6.0</td>
<td>6.03</td>
<td>0.50</td>
<td>0.57</td>
<td>6.06</td>
<td>0.92</td>
<td>0.74</td>
</tr>
<tr>
<td></td>
<td>8.0</td>
<td>8.04</td>
<td>0.45</td>
<td>0.43</td>
<td>8.06</td>
<td>0.76</td>
<td>0.65</td>
</tr>
<tr>
<td>Potentiometric titrimetry, (n=5)</td>
<td>4.0</td>
<td>4.05</td>
<td>1.24</td>
<td>1.30</td>
<td>4.06</td>
<td>1.55</td>
<td>1.77</td>
</tr>
<tr>
<td></td>
<td>6.0</td>
<td>6.07</td>
<td>1.13</td>
<td>0.87</td>
<td>6.08</td>
<td>1.34</td>
<td>1.18</td>
</tr>
<tr>
<td></td>
<td>8.0</td>
<td>8.05</td>
<td>0.61</td>
<td>0.70</td>
<td>8.09</td>
<td>1.08</td>
<td>1.02</td>
</tr>
</tbody>
</table>

RE: relative error, RSD: relative standard deviation.

Selectivity

The selectivity of the proposed methods was determined by placebo blank and synthetic mixture analyses. In the analysis of placebo blank solution, the titre value in both the cases was equal to the titre value of blank which revealed no interference. To assess the role of the inactive ingredients on the assay of ZMT, the general procedure was followed by taking the synthetic mixture extract at three different concentrations of ZMT (4, 6 and 8 \( \mu g \) mL\(^{-1} \) in both method A and method B). The percentage recovery values
obtained were in the range 98.78–103.4% with RSD < 2.68% with clear indication of non-interference by the inactive ingredients in the assay of ZMT.

**Ruggedness of the methods**

Method ruggedness was expressed as the RSD of the same procedure applied by four different analysts as well as using four different burettes. The inter-analysts RSD were ≤ 1.04% whereas the inter-burettes RSD for the same ZMT amounts ranged from 0.67 – 0.95% suggesting that the developed method was rugged. The results are shown in Table 7.1.2.

**Application to analysis of tablets containing ZMT**

The described titrimetric procedures were successfully applied to the determination of ZMT in its tablets (Zomig 2.5). The results obtained (Table 7.1.3) were statistically compared with the published reference method [11]. The reference method describes chromatographic separation of ZMT with UV-detection at 225 nm. The results obtained by the proposed methods agreed well with those of the reference method and with the label claim. The results were also compared statistically by a Student’s t-test for accuracy and by a variance F-test for precision with those of the reference method at 95% confidence level as summarized in Table 7.1.3. The results showed that the calculated t-and F-values did not exceed the tabulated values inferring that proposed methods are as accurate and precise as the reference method.

<table>
<thead>
<tr>
<th>Table 7.1.2 Results of method ruggedness study.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Method</strong></td>
</tr>
<tr>
<td>-------------</td>
</tr>
<tr>
<td>Visual end point detection</td>
</tr>
<tr>
<td>\</td>
</tr>
<tr>
<td>\</td>
</tr>
<tr>
<td>Potentiometric end point detection</td>
</tr>
<tr>
<td>\</td>
</tr>
<tr>
<td>\</td>
</tr>
</tbody>
</table>
Recovery studies

Accuracy and the reliability of the methods were further ascertained by performing recovery experiments. To a fixed amount of drug in formulation (pre-analyzed): pure drug at three different levels was added, and the total was found by the proposed methods. Each test was repeated three times. The results compiled in Table 7.1.4 show that recoveries were in the range from 98.00 to 102.0 \% indicating that commonly added excipients to tablets did not interfere in the determination.

Table 7.1.4 Results of recovery study via standard addition method.

<table>
<thead>
<tr>
<th>Tablet studied</th>
<th>Visual titrimetry</th>
<th>Potentiometric titrimetry</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ZMT in tablet extract, mg</td>
<td>Pure ZMT added, mg</td>
</tr>
<tr>
<td>Zomig 2.5</td>
<td>3.04</td>
<td>1.5</td>
</tr>
<tr>
<td>Zomig</td>
<td>3.04</td>
<td>3.0</td>
</tr>
<tr>
<td>Zomig 2.5</td>
<td>3.04</td>
<td>4.5</td>
</tr>
</tbody>
</table>

*Mean value of three determinations.
Section 7.2

EXTRACTIVE SPECTROPHOTOMETRIC METHODS FOR THE DETERMINATION OF ZOLMITRIPTAN IN BULK DRUG AND PHARMACEUTICAL FORMULATION USING BROMOCRESOL GREEN

7.2.1 INTRODUCTION

Extractive spectrophotometric analysis is mainly based on the measurement of absorbance of a complex formed between two oppositely charged molecules called ion-association or ion-pair complex. Ion-association is a chemical reaction whereby ions of opposite electrical charge come together in solution to form a distinct chemical entity. Ion-pairs are formed when a cation and anion, present in aqueous phase (aq), combine together [22-25] and these complexes are colored when extracted into an organic solvent (org).

\[ \text{A}^{n+}(aq) + \text{B}^{m-}(aq) \rightarrow \text{AB}^{(n-m)+}(org) \]

A hypothesis based on the possible role of solvated ion pair species in enhancing the extractive process, proposed by Higuchi [26], assumes that the free energy involved in the transference of the ionic components from the water phase to form simple ion pairs in the organic phase is predominant. Because of the accuracy and precision, and high selectivity and sensitivity, ion-pair extractive spectrophotometry has received considerable attention for the pharmaceutically important compounds and was widely used for the determination of many organic compounds which possess basic nitrogenous moiety [27-36].

From the literature survey presented in Section 7.0.2, it is noticeable that bromocresol green (BCG) was not used for the spectrophotometric determination of ZMT in pharmaceuticals and hence the author has used this dye in the present study. This section describes development and validation of two simple, sensitive and reproducible spectrophotometric methods for the determination of ZMT in pure form and in its tablets. The first method (method A) is based on the formation of a colored ion-pair complex (1:1 drug:dye) of ZMT with bromocresol green (BCG) at pH 4.20±0.01 and extraction of the complex into chloroform followed by the measurement of the yellow ion-pair complex at
435 nm. In the second (method B), the drug-dye ion-pair complex was dissolved in ethanolic potassium hydroxide and the absorbance of resulting base form of the dye was measured at 630 nm. These details are contained in this Section 7.2.

7.2.2 EXPERIMENTAL

7.2.2.1 Instrument

The instrument used is the same as that was described in Section 2.2.2.1. A digital pH meter Model Elico L1 120 was used for pH measurements.

7.2.2.2 Reagents and materials

All chemicals used were of analytical grade. Solvents used were of the spectroscopic grade. Distilled water was used throughout the investigation.

**Bromocresol green (BCG) (0.05 %):** Prepared by dissolving 50 mg of the dye (S.D.Fine Chem Ltd, Mumbai, India) in 10 mL of ethanol, and diluted to 100 mL with water.

**Walpole-buffer solution (pH 4.2):** Prepared by mixing 50 mL of 1 M sodium acetate and 35 mL of 1 M hydrochloric acid (Merck Specialities Pvt Ltd, Mumbai, India, Sp, gr. 1.18) and volume was made upto 250 mL, and pH was adjusted to 4.2 by using dilute NaOAc/HCl solution.

**Ethanolic KOH (1%):** One gram of the pure KOH (S.D.Fine Chem Ltd, Mumbai, India) was dissolved in and diluted with ethanol to 100 mL.

**Standard ZMT solution (40 µg mL⁻¹):**

The pharmaceutical grade ZMT and tablets used in this study are those mentioned in the Section 7.1.2.2.

A stock standard solution of ZMT (200 µg mL⁻¹) was first prepared by dissolving 20 mg ZMT in 20 mL acetic acid and diluting to 100 mL in calibrated flask with water. Stock solution was diluted with the water to get a working solution of 40 µg mL⁻¹.
7.2.2.3 Assay procedures

Method A

Into a series of 125 mL separating funnels, different aliquots 0.2, 0.5, 1.0,.....4.0 and 4.5 mL of standard drug solution (40 µg mL⁻¹) equivalent to 0.8 – 18.0 µg mL⁻¹ ZMT were accurately transferred and the total volume was brought to 10 mL by adding water to each funnel. To each funnel were added 5 mL of Walpole-buffer of pH 4.2 followed by 5 mL dye (0.05 %), and the content was mixed well. The funnels were shaken vigorously with 10 mL of chloroform (added from microburette) for 1 min and then allowed to stand for clear separation of the two phases. The separated organic layer was dried over anhydrous sodium sulphate and absorbance of the yellow ion-pair complex was measured at 435 nm against a reagent blank similarly prepared.

Method B

Varying aliquots 0.2,0.5,1.0,.....3.0 and 3.5 mL of ZMT-BCG ion-pair complex (2 µg mL⁻¹ in ZMT; prepared by following the procedure described in method A) equivalent to 0.08 – 1.4 µg mL⁻¹ with respect to ZMT were transferred into a series of 5 mL standard flasks and the total volume was brought to 3.5 mL by adding ethanol. To each flask, 1 mL of 1 % alcoholic KOH was added, the content mixed and kept aside for 5 min. Finally, the volume was made upto mark with ethanol and the absorbance measured at 630 nm against the reagent blank.

Procedure for tablets

The powder equivalent to 20 mg ZMT was weighed out and transferred into a 125 mL separating funnel, the extraction procedure was followed and a 200 µg mL⁻¹ ZMT solution was prepared as described under the general procedure for pure drug. A suitable aliquot was diluted to get a working concentration of 40 µg mL⁻¹ and used for the assay by following the general assay procedures described in method A and method B.

Procedure for selectivity study

A placebo blank was prepared as described under Section 7.1.2.3, and then 10 mg placebo blank extracted in acetic acid: water (1:4) was analyzed as done in “Procedure for tablets”.

A synthetic mixture was prepared by adding 20 mg of ZMT to about 20 mg of the placebo blank prepared above, homogenized and the solution was prepared as done under
“Procedure for tablets”. The filtrate was collected in a 100 mL flask. The synthetic mixture solution was diluted with water to get 40.0 µg mL⁻¹ ZMT solutions, and appropriate aliquot were subjected to analysis by method A. Two mL drug-dye ion-pair complex (0.8 µg mL⁻¹ in ZMT) was analysed by method B.

7.2.3 RESULTS AND DISCUSSION

7.2.3.1 Absorption spectra

Absorption spectra of the yellow colored ZMT-BCG ion-pair complex is shown in Figure 7.2.1 which has a maximum absorbance (λₘₐₓ) at 435 nm. This drug dye ion-pair complex was broken in ethanolic base to yield the base form of the blue dye which had maximum absorbance at 630 nm (Figure 7.2.1). In both the cases, the blanks had negligible absorbance.

Figure 7.2.1 Absorption spectra of the ion-pair complex of ZMT–BCG (10 µg mL⁻¹ ZMT) in method A and anionic form of the dye (0.8 µg mL⁻¹ ZMT-BCG ion-pair) in method B.

7.2.3.2 Chemistry

Chemically, the structure of ZMT features its basic nature and suggests the possibility of utilizing an anionic dye as chromogenic reagent. ZMT can be transferred from the aqueous phase into the organic phase as an ion-pair formed with the anionic form of the acid dyes. The extraction equilibria can be represented as follows:

\[ ZH^+_{(aq)} + D^-_{(aq)} \rightleftharpoons ZH^+D^-_{(org)} \]
where ZH\(^+\) and D\(^-\) represent the protonated ZMT and the anion form of the dye, respectively, and the subscript (aq) and (org) refer to the aqueous and organic phases, respectively. ZMT contains aliphatic tertiary amino group which is protonated in acid medium, while sulphonic acid group present in BCG is the only group undergoing dissociation in the pH range 2.5–5.0. The protonated ZMT forms ion-pair with the anionic form of BCG which is quantitatively extracted into chloroform. The drug-dye stoichiometric ratio as calculated by the Job’s continuous variations method \([37]\) is found to be 1:1. The possible reaction pathway is given in Scheme 7.2.1. In alcoholic alkaline medium, this ion-pair complex gets disturbed and it breaks to form a blue colored basic dye and the drug. The reaction pathway of this breaking is shown in Scheme 7.2.2.

**Scheme 7.2.1** The proposed reaction pathway for ion-pair complex formation.
Scheme 7.2.2 The proposed reaction pathway for the formation of anionic form of BCG.

7.2.3.3 Optimization of variables and method development

A number of preliminary experiments were performed to establish the optimum conditions necessary for rapid and quantitative formation of colored ion-pair complex and its extraction with a view to achieve the maximum stability and sensitivity. Optimum condition was fixed by varying one parameter at a time while keeping other parameters constant and observing its effect on the absorbance either at 435 nm in method A or at 630 nm in method B.

**Method A**

**Selection of the extracting solvent**

A number of organic solvents such as chloroform, dichloromethane, 1, 2-dichloroethane, carbon tetrachloride, hexane, toluene and benzene was examined for extraction of the ion-pair complex in order to provide an applicable extraction procedure. Chloroform (Table 7.2.1) was preferred for its efficient and quantitative extraction of ion-pair complex and the stability of the extracted ion-pair, its high sensitivity, and very low absorbance of the reagent blank and shortest time to reach the equilibrium between both phases.

**Table 7.2.1 Effect of the extracting solvent on absorbance of the formed ion-pair complex***

<table>
<thead>
<tr>
<th>Solvent</th>
<th>( A_{\text{blank}} )</th>
<th>( A_{\text{ion-pair}} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chloroform</td>
<td>0.020</td>
<td>0.539</td>
</tr>
<tr>
<td>1,2-dichloroethane</td>
<td>0.021</td>
<td>0.533</td>
</tr>
<tr>
<td>Dichloromethane</td>
<td>0.040</td>
<td>0.475</td>
</tr>
</tbody>
</table>

* Concentration of ZMT is 10 µg mL\(^{-1}\).
Effect of pH on the ion-pair complex formation

The effect of pH of the aqueous phase was studied by extracting the colored complex in the presence of either hydrochloric acid or acidic buffer (Walpole-buffer) of pH 2.7–4.6. It was noticed that the maximum absorbance of ion-pair complex and minimum absorbance of the reagent blank were observed with acidic buffer between pH ranges 3.8–4.6. The results are shown in Figure 7.2.2. The absorbance of complex and blank remain almost constant in this pH range. Thus, pH 4.2 was kept optimum for further studies. Effect of volume of buffer also studied and it was found that 5.0 mL buffer of pH 4.2±0.01 was optimum Figure 7.2.3.

![Figure 7.2.2](image-url)  
**Figure 7.2.2** Effect of pH of Walpole-buffer (method A)

![Figure 7.2.3](image-url)  
**Figure 7.2.3** Effect of volume of buffer (method A)
Effect of dye concentration

The effect of the dye concentration was studied by measuring the absorbance of solutions containing a fixed concentration of ZMT (10.0 µg mL\(^{-1}\)) and varied amounts of BCG. It is clear from Figure 7.2.4 that the maximum absorbance was found with 5.0 mL of 0.05 % BCG and beyond that absorbance was nearly constant. Thus, 5.0 mL of 0.05 % BCG in a total volume of 20 mL aqueous phase was used for ion-pair formation throughout the investigation.

Figure 7.2.4 Effect of BCG concentration on the color development (10 µg mL\(^{-1}\) ZMT).

Effects of contact and shaking time and sequence of addition

The effect of contact time between ZMT and BCG in the presence of buffer was studied in the time range 0-30 min before extraction and it was found that 5 min was sufficient to achieve maximum absorbance at 435 nm. Shaking times ranging from 0.5–3.0 min produced a constant absorbance, and hence a shaking time of 1 min was used throughout.

Effect of number of extractions

Under optimum conditions, the drug-dye complex in the aqueous phase was extracted with three 10 mL portions of chloroform and absorbance was measured each time. For the second extraction, the absorbance of the organic layer was negligibly small. Hence, a single extraction with 10 mL chloroform was selected for the extraction because of complete recovery of the complex.
Equilibration time and stability of the colored complexes

The organic and aqueous phases were clearly separated in less than 1 min. The drug-dye ion-pair complex was stable for more than 15 h at laboratory temperature (28±2°C).

Effect of order of addition of reactants

The sequence of order of addition of the reactants prior to extraction had small change in the absorbance values. So the order of addition of reactants should be in the described manner.

Composition of ion-pair complexes

The composition of the ion-pair complex formed between ZMT and BCG was established by applying Job’s method of continuous variations [37]. In this method, 1.054x10^-4 M solutions of ZMT and BCG were used and mixed in varying volume ratios in such a way that the total volume of each mixture was the same. The absorbance of each solution was measured at 435 nm and plotted against the mole fraction of the drug (Figure 7.2.5). The plot reached a maximum value at a mole fraction of 0.5 indicating that a 1:1 (ZMT:BCG) ion-pair complex is formed through the electrostatic attraction between protonated ZMT and BCG anion. The conditional stability constant (K_f) of the ion-association complex was calculated from the continuous variation data using the following equation [21]:

\[ K_f = \frac{A/A_m}{[1 - A/A_m]^{n+2} C_M (n)^n} \]

where A and A_m are the observed maximum absorbance and the absorbance value when all the drug present is associated, respectively. C_M is the mole concentration of drug at the maximum absorbance and n is the stoichiometry which BCG ion associates with drug. The log K_f value was found to be 6.73.
Chapter 7  Titrimetric and spectrophotometric assay of zolmitriptan

**Figure 7.2.5** Job’s Continuous-variations plot for ZMT-BCG complex

Method B

Studies on the effect of alkali concentration required to break the complex into its components revealed that 1 mL of 1 % alcoholic KOH (**Figure 7.2.6**) with a standing time of 5 min was sufficient to yield maximum absorbance at 630 nm. The stability of the resulted blue coloured dye was stable for more than 16 h at laboratory temperature (28±2°C).

**Figure 7.2.6** Effect of 1.0 % ethanolic KOH (0.8 µg mL⁻¹ ZMT-BCG ion-pair).

7.2.3.4 Method validation

The proposed methods were validated for linearity, sensitivity, selectivity, precision, accuracy, and recovery.
Linearity and sensitivity

A linear relation is found between absorbance and concentration of ZMT within the Beer’s law range given in Table 7.2.2. The calibration graphs (Figure 7.2.7) are described by the equation:

\[ Y = a + b \times X \]

(where \( Y \) = absorbance, \( a \) = intercept, \( b \) = slope and \( X \) = concentration in µg mL\(^{-1}\)) obtained by the method of least squares. Sensitivity parameters such as apparent molar absorptivity and Sandell sensitivity values and the limits of detection and quantification are calculated as per the current ICH guidelines [21] which are compiled in Table 7.2.2 that speaks of the excellent sensitivity of the proposed method.

![Figure 7.2.7 Calibration curves](image)

Accuracy and precision

In order to study the accuracy and precision of the proposed methods, three concentrations of pure ZMT within the linearity range were analyzed, each determination being repeated seven times (intra-day precision) on the same day and one time each for five days (inter-day precision). The percentage relative standard deviation (% RSD) was ≤ 1.64 % (intra-day) and ≤ 2.00 % (inter-day). In addition, the accuracy of the proposed method was measured by calculating the percentage relative error (% RE), which varied between 0.88 % and 1.77 %. The results of this study compiled in Table 7.2.3 indicate the high accuracy and precision of the proposed methods.
Table 7.2.2 Regression and sensitivity parameters.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Method A</th>
<th>Method B</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \lambda_{\text{max}} ), nm</td>
<td>435</td>
<td>630</td>
</tr>
<tr>
<td>Beer’s law limits (( \mu g \text{ mL}^{-1} ))</td>
<td>0.8-18.0</td>
<td>0.08-1.4</td>
</tr>
<tr>
<td>Molar absorptivity ((\varepsilon)) (L mol(^{-1}) cm(^{-1}))</td>
<td>1.50\times10(^4)</td>
<td>1.52\times10(^5)</td>
</tr>
<tr>
<td>Sandell sensitivity* (( \mu g \text{ cm}^{-2} ))</td>
<td>0.0191</td>
<td>0.0019</td>
</tr>
<tr>
<td>Limit of detection (( \mu g \text{ mL}^{-1} ))</td>
<td>0.19</td>
<td>0.02</td>
</tr>
<tr>
<td>Limit of quantification (( \mu g \text{ mL}^{-1} ))</td>
<td>0.57</td>
<td>0.07</td>
</tr>
<tr>
<td>Regression equation, ( Y^* )</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intercept, ((a))</td>
<td>-0.0037</td>
<td>-0.0123</td>
</tr>
<tr>
<td>Slope, ((b))</td>
<td>0.0537</td>
<td>0.5669</td>
</tr>
<tr>
<td>Correlation coefficient ((r))</td>
<td>0.9996</td>
<td>0.9998</td>
</tr>
<tr>
<td>Standard deviation of intercept ((S_a))</td>
<td>0.0071</td>
<td>0.0051</td>
</tr>
<tr>
<td>Standard deviation of slope ((S_b))</td>
<td>0.0006</td>
<td>0.0060</td>
</tr>
</tbody>
</table>

* Limit of determination as the weight in \( \mu g \) per mL of solution, which corresponds to an absorbance of \( A = 0.001 \) measured in a cuvette of cross-sectional area 1 cm\(^2\) and \( l = 1 \) cm. \( ^* Y = a + bX \) , where \( Y \) is the absorbance, \( a \) is the intercept, \( b \) is the slope and \( X \) is the concentration in \( \mu g \text{ mL}^{-1} \).

Table 7.2.3 Results of intra-day and inter-day accuracy and precision study.

<table>
<thead>
<tr>
<th>Method</th>
<th>ZMT taken (( \mu g \text{ mL}^{-1} ))</th>
<th>ZMT found(^a) (( \mu g \text{ mL}^{-1} ))</th>
<th>% RSD(^b)</th>
<th>% RE(^c)</th>
<th>ZMT found(^a) (( \mu g \text{ mL}^{-1} ))</th>
<th>% RSD(^b)</th>
<th>% RE(^c)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>4.00</td>
<td>3.96</td>
<td>1.61</td>
<td>1.03</td>
<td>3.95</td>
<td>1.85</td>
<td>1.16</td>
</tr>
<tr>
<td></td>
<td>8.00</td>
<td>8.08</td>
<td>0.45</td>
<td>0.94</td>
<td>8.08</td>
<td>0.67</td>
<td>1.04</td>
</tr>
<tr>
<td></td>
<td>12.0</td>
<td>12.21</td>
<td>0.71</td>
<td>1.77</td>
<td>12.18</td>
<td>0.74</td>
<td>1.47</td>
</tr>
<tr>
<td></td>
<td>0.40</td>
<td>0.41</td>
<td>1.64</td>
<td>1.30</td>
<td>0.41</td>
<td>2.00</td>
<td>1.33</td>
</tr>
<tr>
<td></td>
<td>0.80</td>
<td>0.79</td>
<td>0.90</td>
<td>1.03</td>
<td>0.80</td>
<td>0.98</td>
<td>1.07</td>
</tr>
<tr>
<td></td>
<td>1.20</td>
<td>1.19</td>
<td>0.92</td>
<td>0.88</td>
<td>1.20</td>
<td>1.09</td>
<td>0.91</td>
</tr>
</tbody>
</table>

\(^a\) Mean value of five determinations; \(^b\) Relative standard deviation (%); \(^c\) Relative error (%).

Robustness and ruggedness

The evaluation of the method robustness was done by slight alteration of volume of buffer for method A or volume of alcoholic KOH for method B and performing the analysis under the optimized experimental conditions. The effect of these changes on the absorbance reading of the colored systems in both methods was studied and found to be negligible confirming the robustness of the proposed methods. Method ruggedness was expressed as % RSD of the same procedure applied by three analysts and also by a single analyst performing analysis on three different cuvettes. The results presented in Table...
7.2.4 showed that no statistical differences between different analysts and instruments suggesting that the proposed methods were rugged.

**Table 7.2.4 Results of robustness and ruggedness study**

<table>
<thead>
<tr>
<th>Method</th>
<th>ZMT taken, µg mL⁻¹</th>
<th>Robustness</th>
<th>Ruggedness</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Volume of buffer / ethanolic KOH*</td>
<td>Inter-analysts (%) RSD, (n = 3)</td>
<td>Inter-instruments (%) RSD, (n = 3)</td>
</tr>
<tr>
<td>A</td>
<td>4.00</td>
<td>2.04</td>
<td>2.11</td>
</tr>
<tr>
<td></td>
<td>8.00</td>
<td>1.71</td>
<td>1.80</td>
</tr>
<tr>
<td></td>
<td>12.00</td>
<td>1.48</td>
<td>1.66</td>
</tr>
<tr>
<td></td>
<td>0.40</td>
<td>1.39</td>
<td>1.42</td>
</tr>
<tr>
<td>B</td>
<td>0.80</td>
<td>1.55</td>
<td>1.69</td>
</tr>
<tr>
<td></td>
<td>1.20</td>
<td>1.83</td>
<td>2.01</td>
</tr>
</tbody>
</table>

*In method A, the volumes of Walpole buffer were 4.8, 5.0 and 5.2 mL, and in method B the volumes of ethanolic KOH added were 0.9, 1.0 and 1.1 mL.

**Selectivity**

In the present methods, interference by the excipients often used in tablets as possible co-active substances was studied. Selectivity was evaluated by both placebo blank and synthetic mixture analyses. The placebo blank, consisting the composition as mentioned under “Procedure for selectivity study” was prepared and analyzed as described under the recommended procedures. The resulting absorbance readings for the methods were same as the reagent blank, inferring no interference from the placebo. The selectivity of the methods was further confirmed by carrying out recovery study from synthetic mixture. The percent recoveries of ZMT were 97.39±2.51 and 104.1±1.59, for method A and method B, respectively. This confirms the selectivity of the proposed methods in the presence of the commonly employed tablet excipients.

**Application to analysis of tablets containing ZMT**

The proposed methods were applied to the quantification of ZMT in commercial tablets. The results presented in Table 7.2.5 showed that the methods are successful to the determination of ZMT in pharmaceutical formulation without any detectable interference from the excipients present in the tablets. When the results were statistically compared with those of the reference method [11] by applying the Student’s *t*-test for
accuracy and \( F \)-test for precision, the calculated Student’s \( t \)-value and \( F \)-value at 95% confidence level did not exceed the tabulated values of 2.78 and 6.39, respectively. Hence, no significant difference exists between the proposed methods and the reference method with respect to accuracy and precision.

**Table 7.2.5** Results of assay of tablets and statistical comparison with the reference method.

<table>
<thead>
<tr>
<th>Tablet Brand name</th>
<th>Label claim mg/tablet</th>
<th>Found (Percent of label claim ±SD)*</th>
<th>Reference method</th>
<th>Proposed methods Method A</th>
<th>Method B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zomig-2.5</td>
<td>2.5</td>
<td>100.59± 0.90</td>
<td></td>
<td>101.4 ± 1.48 98.96± 1.06</td>
<td></td>
</tr>
</tbody>
</table>

*Mean value of five determinations.
Tabulated \( t \)-value at the 95% confidence level is 2.78.
Tabulated \( F \)-value at the 95% confidence level is 6.39.

**Recovery studies**

To further ascertain the accuracy of the proposed methods, a standard addition technique was followed. A fixed amount of drug from pre-analyzed tablet powder was taken and pure drug at three different levels (50, 100 and 150\% of that in tablet powder) was added. The total was found by the proposed methods. The determination at each level was repeated three times and the percent recovery of the added standard was calculated. Results of this study presented in **Table 7.2.6** reveal that the accuracy of methods was unaffected by the various excipients present in the formulations.

**Table 7.2.6** Results of recovery study via standard addition method.

<table>
<thead>
<tr>
<th>Tablets studied</th>
<th>Method A</th>
<th>Method B</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ZMT in tablets, ( \mu g ) mL(^{-1} )</strong></td>
<td><strong>Pure ZMT added, ( \mu g ) mL(^{-1} )</strong></td>
<td><strong>Total found, ( \mu g ) mL(^{-1} )</strong></td>
</tr>
<tr>
<td>Zomig-2.5</td>
<td>4.06</td>
<td>2.0</td>
</tr>
<tr>
<td></td>
<td>4.06</td>
<td>4.0</td>
</tr>
<tr>
<td></td>
<td>4.06</td>
<td>6.0</td>
</tr>
</tbody>
</table>

*Mean value of three determinations.
Section 7.3
SPECTROPHOTOMETRIC DETERMINATION OF ZOLMITRIPTAN IN BULK DRUG AND PHARMACEUTICAL USING VANILLIN AS A REAGENT*

7.3.1 INTRODUCTION

In 1864 Schiff Hugo Josef discovered the condensation products of aldehydes and amines; the products are now known as Schiff bases [38]. Schiff’s base is formed by a condensation reaction between an aromatic amine and an aldehyde or ketone, for example:

\[ \text{RNH}_2 + \text{R'CHO} \rightarrow \text{RN=CHR'} + \text{H}_2\text{O} \]

Similarly, secondary amines react with aldehydes or ketones to give carbinolamines which then dehydrate to give enamines [39].

\[ \text{RCH(OH)NH} \rightarrow \text{RCH\{\}}_{\text{N}} + \text{H}_2\text{O} \]

This reaction is of the interest to analytical chemist because of the formation of chromogenic product which is used for the spot test detection of aldehyde and ketone [40]. Based on this reaction many pharmaceutical compounds containing aromatic amine group have been determined [41-45].

The literature survey presented in Section 7.0.2 showed that enamine formation reaction has not been applied for the spectrophotometric assay of ZMT. In this Section 7.3, the author describes a method for the spectrophotometric determination of ZMT using an aldehyde containing compound, vanillin as a chromogenic agent. The method is based on the formation of a red colored enamine between vanillin with aromatic secondary amine present in ZMT in sulphuric acid medium. The proposed method has been demonstrated to be superior to the reported methods with respect to simplicity, speed and cost-effectiveness.

* This work has been published in ISRN Analytical Chemistry, Volume 2013, Article ID 790382, 7 pages, doi.org/10.1155/2013/790382
7.3.2 EXPERIMENTAL

7.3.2.1 Instrument

The instrument used is the same that was described in Section 2.1.2.1.

7.3.2.2 Reagents and materials

All chemicals were of analytical reagent grade and distilled water was used to prepare solutions.

Vanillin (4 %): The solution was prepared by dissolving 4 g of vanillin (Loba Chemie, Mumbai, India) in 100 mL methanol (Merck Ltd., Mumbai, India).

Sulphuric acid: Concentrated H₂SO₄ (Merck, Mumbai, India; sp. gr. 1.84) used as such.

Standard ZMT solution:

The pharmaceutical grade ZMT and tablets used in this study are those mentioned in the Section 7.1.2.2.

A stock standard solution equivalent to 200 µg mL⁻¹ ZMT was prepared by dissolving 50 mg of pure drug in methanol and diluting to 250 mL in calibrated flask with the same solvent.

7.3.2.3 Assay procedure

Different aliquots (0.25, 0.5, 1.0, ...., 4.5 mL) of 200 µg mL⁻¹ ZMT solution were accurately measured and transferred into a series of 10 mL volumetric flasks and the total volume was brought to 4.5 mL with methanol. To each flask was added 1 mL of 4 % vanillin followed by 1 mL of concentrated H₂SO₄ and kept aside for 10 minutes, finally the volume was brought up to mark with methanol. The absorbance was measured at 580 nm vs reagent blank. A calibration graph was prepared by plotting the measured absorbance vs concentration. The concentration of the unknown was read from the calibration graph or computed from the regression equation derived using the Beer’s law data.

Procedure for tablets

Twenty tablets were weighed and pulverized into a fine powder. An amount of tablet powder equivalent to 20 mg of ZMT was weighed into a 100 mL calibrated flask, 40 mL of methanol added and the mixture shaken for 20 min; then the volume was made up to the mark with same solvent, mixed well and filtered using Whatman No. 42 filter
paper. The filtrate equivalent to 200 µg mL\(^{-1}\) was subjected to analysis using procedure described above.

**Procedure for selectivity study**

A placebo blank was prepared as described under Section 7.1.2.3 in, and then 20 mg placebo blank extracted with 10 mL methanol was analyzed as done in “Procedure for tablets”.

A synthetic mixture was prepared by adding 20 mg of ZMT to about 20 mg of the placebo blank prepared above, homogenized and the solution was prepared as done under “Procedure for tablets”. The filtrate was collected in a 100 mL flask. The synthetic mixture solution was subjected to analysis by using the above procedure.

**7.3.3 RESULTS AND DISCUSSION**

**7.3.3.1 Chemistry**

Enamines are formed by a condensation reaction between a secondary amine and an aldehyde or ketone in the presence of an acid catalyst [46,47]. The formation of enamine forms the basis for the spectrophotometric determination of compounds of pharmaceutical significance. Vanillin, an aromatic aldehyde, has been applied to the quantification of drugs with primary or secondary amine in acidic medium using spectrophotometry [48,49]. The proposed method is based on the formation of chromogenic enamine between the secondary amino group of ZMT and aldehyde group of vanillin. The most probable condensation step for the formation of enamine between ZMT and vanillin is presented in **Scheme 7.3.1**.
Scheme 7.3.1 *The proposed reaction pathway for enamine formation.*

7.3.3.2 Absorption spectra

The absorption spectra of the chromogen formed between ZMT and vanillin, was recorded between 400-760 nm against respective reagent blank and the same is shown in Figure 7.3.1. The red colored enamine exhibits $\lambda_{\text{max}}$ at 580 nm. The reagent blank showed negligible absorbance at 580 nm. The measurements were thus made at this wavelength.

*Figure 7.3.1* Absorption spectra of: (a) the colored product (40 $\mu$g mL$^{-1}$ ZMT); (b) blank.
7.3.3.3 Optimization of experimental variables

Various experimental variables were optimized to achieve maximum sensitivity.

**Effect of vanillin**

Vanillin is insoluble in water and H$_2$SO$_4$. In methanol, both vanillin and ZMT were found to dissolve and the red colored reaction product was also obtained in this medium. Hence, methanol was used to prepare vanillin and ZMT solutions. The effect of vanillin on the sensitivity of the reaction was studied by using 4% vanillin and it was observed that when 0.5-2.0 mL was used, the absorbance readings were nearly constant; below and above this range there was a decrease in absorbance (Figure 7.3.2). Hence, considering minimum blank absorption and maximum chromogen absorption, 1 mL of 4% vanillin was used as optimum in a total volume of 10 mL.

![Figure 7.3.2 Effect of volume of 4% vanillin (40 µg mL$^{-1}$ ZMT)](image)

**Effect of acid**

The reaction was very slow in dilute acidic medium, thus concentrated sulphuric acid was used. The intensity of the red colored product was found to remain constant when 0.5-2.0 mL concentrated sulphuric acid was added and even no change in intensity of blank was observed (Figure 7.3.3). Hence, 1 mL concentrated acid in a total volume of 10 mL was fixed as the optimum.
Reaction time

The resulting red colored enamine was developed completely in 10 min (*Figure 7.3.4*) and remained stable for another 20 min, thereafter.

7.3.3.4 Method validation

Linearity and sensitivity

A linear relation was found to exist between absorbance and the concentration of ZMT in the range (5.0 - 90.0) µg mL⁻¹ (*Figure 7.3.5*). The calibration graph is described by the equation:

\[ Y = a + bX \]

(where \( Y \) = absorbance, \( a \) = intercept, \( b \) = slope and \( X \) = concentration in µg mL⁻¹) obtained by the method of least squares. Correlation coefficient, intercept and slope for
the calibration data are summarized in Table 7.3.1. Sensitivity parameters such as apparent molar absorptivity and Sandell sensitivity values, the limits of detection and quantification calculated as per the current ICH guidelines [21] are compiled in Table 7.3.1 and are indicative of the excellent sensitivity of the method. The limits of detection (LOD) and quantification (LOQ) were calculated according to the same guidelines.

Table 7.3.1 Regression and sensitivity parameters.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Proposed Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\lambda_{\text{max}}$, nm</td>
<td>580</td>
</tr>
<tr>
<td>Beer’s law limits ($\mu g \text{ mL}^{-1}$)</td>
<td>5.0-90.0</td>
</tr>
<tr>
<td>Molar absorptivity ($\varepsilon$) (L mol$^{-1}$ cm$^{-1}$)</td>
<td>$3.3 \times 10^3$</td>
</tr>
<tr>
<td>Sandell sensitivity* ($\mu g \text{ cm}^{-2}$)</td>
<td>0.0872</td>
</tr>
<tr>
<td>Limit of detection ($\mu g \text{ mL}^{-1}$)</td>
<td>1.26</td>
</tr>
<tr>
<td>Limit of quantification ($\mu g \text{ mL}^{-1}$)</td>
<td>3.81</td>
</tr>
<tr>
<td>Regression equation, $Y**$</td>
<td></td>
</tr>
<tr>
<td>Intercept, (a)</td>
<td>-0.0101</td>
</tr>
<tr>
<td>Slope, (b)</td>
<td>0.0117</td>
</tr>
<tr>
<td>Correlation coefficient (r)</td>
<td>0.9994</td>
</tr>
<tr>
<td>Standard deviation of intercept ($S_a$)</td>
<td>0.0098</td>
</tr>
<tr>
<td>Standard deviation of slope ($S_b$)</td>
<td>0.0002</td>
</tr>
</tbody>
</table>

*Limit of determination as the weight in $\mu g$ per mL of solution, which corresponds to an absorbance of $A = 0.001$ measured in a cuvette of cross-sectional area 1 cm$^2$ and $l = 1$ cm. **$y = a + bX$, where $Y$ is the absorbance, $a$ is the intercept, $b$ is the slope and $X$ is the concentration in $\mu g \text{ mL}^{-1}$. 

Figure 7.3.5 Calibration curve.
Accuracy and precision

Accuracy was evaluated as percentage relative error between the measured concentrations and taken concentrations for ZMT (Bias %). The results obtained are compiled in Table 7.3.2 and show that the accuracy is good. The precision of the method was calculated in terms of intermediate precision (intra-day and inter-day). Three different concentration of ZMT were analyzed in replicates during the same day (intra-day precision) and five consecutive days (inter-day precision). The % RSD values of intra-day and inter-day studies showed that the precision was good (Table 7.3.2).

Table 7.3.2 Results of intra-day and inter-day accuracy and precision study.

<table>
<thead>
<tr>
<th>ZMT taken (µg mL(^{-1}))</th>
<th>Intra-day (n = 7)</th>
<th>Inter-day (n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ZMT found(^a) (µg mL(^{-1}))</td>
<td>% RSD(^b)</td>
</tr>
<tr>
<td>---------------------------</td>
<td>------------------</td>
<td>----------------</td>
</tr>
<tr>
<td>20.0</td>
<td>19.70</td>
<td>1.45</td>
</tr>
<tr>
<td>40.0</td>
<td>40.71</td>
<td>0.83</td>
</tr>
<tr>
<td>60.0</td>
<td>60.61</td>
<td>1.22</td>
</tr>
</tbody>
</table>

\(^{a}\) Mean value of five determinations; \(^{b}\) Relative standard deviation (%); \(^{c}\) Relative error (%).

Robustness and ruggedness

Method robustness was tested by making small incremental changes in concentrated sulphuric acid concentration and reaction time. To check the ruggedness, analysis was performed by four different analysts and on three different instruments by the same analyst. The robustness and the ruggedness were checked at three different drug levels. The intermediate precision, expressed as % RSD, which is a measure of robustness and ruggedness was within the acceptable limits as shown in the Table 7.3.3.

Table 7.3.3 Results of robustness and ruggedness study.

<table>
<thead>
<tr>
<th>ZMT taken, µg mL(^{-1})</th>
<th>Robustness</th>
<th>Ruggedness</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Volume of acid(^a) (n=3)</td>
<td>Reaction time(^b) (n=3)</td>
</tr>
<tr>
<td>---------------------------</td>
<td>-------------</td>
<td>-------------</td>
</tr>
<tr>
<td>20.0</td>
<td>1.15</td>
<td>1.78</td>
</tr>
<tr>
<td>40.0</td>
<td>1.43</td>
<td>2.19</td>
</tr>
<tr>
<td>60.0</td>
<td>2.01</td>
<td>2.85</td>
</tr>
</tbody>
</table>

\(^{a}\) The volumes of conc. HCl solution were 0.9, 1.0 and 1.1 mL; \(^{b}\) reaction times were 9,10 and 11 min.
Selectivity

The selectivity of the proposed method for the analysis of ZMT was evaluated by placebo blank and synthetic mixture analyses. The recommended procedure was applied to the analysis of placebo blank and the resulting absorbance readings were same as that of the reagent blank, confirming no interference from the placebo.

The analysis of synthetic mixture solution yielded percent recoveries which ranged between 98.38 and 104.1 with standard deviation of 1.06 – 2.40. The results of this study show that there is no interference from the commonly added excipients in pharmaceutical formulations and confirmed the selectivity of the method.

Application to analysis of tablets containing ZMT

In order to evaluate the analytical applicability of the proposed method to the quantification of ZMT in commercial tablets, the results obtained by the proposed method were compared to those of the reference method [11] by applying Student’s $t$-test for accuracy and $F$-test for precision. The results (Table 7.3.4) show that the Student’s $t$- and $F$-values at 95% confidence level are less than the theoretical values, indicating that there is a good agreement between the results obtained by the proposed method and the reference method with respect to accuracy and precision.

<table>
<thead>
<tr>
<th>Tablet Brand name</th>
<th>Label claim mg/tablet</th>
<th>Found (Percent of label claim ±SD)$^a$</th>
<th>Reference method</th>
<th>Proposed method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zomig-2.5</td>
<td>2.5</td>
<td>100.59± 0.90</td>
<td></td>
<td>99.22 ± 1.50</td>
</tr>
</tbody>
</table>

$^a$Mean value of five determinations.
Tabulated $t$-value at the 95% confidence level is 2.78.
Tabulated $F$-value at the 95% confidence level is 6.39.

Recovery studies

The accuracy and validity of the proposed method were further ascertained by performing recovery studies. Pre-analyzed tablet powder was spiked with pure ZMT at three levels (50, 100 and 150% of that found in tablet powder) and the total was
determined by the proposed method. The percent recovery of pure ZMT added was in the range of 97.58-104.8% with standard deviation of 1.12 – 2.45 (Table 7.3.5) indicating that the recovery was good, and that the co-formulated substance did not interfere in the determination.

Table 7.3.5 Results of recovery study via standard addition method.

<table>
<thead>
<tr>
<th>Tablets studied</th>
<th>ZMT in tablets, µg mL⁻¹</th>
<th>Pure ZMT added, µg mL⁻¹</th>
<th>Total found, µg mL⁻¹</th>
<th>Pure ZMT recovered*, Percent±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zomig-2.5</td>
<td>19.84</td>
<td>10.0</td>
<td>31.27</td>
<td>104.8 ±1.78</td>
</tr>
<tr>
<td></td>
<td>19.84</td>
<td>20.0</td>
<td>40.13</td>
<td>101.4±1.12</td>
</tr>
<tr>
<td></td>
<td>19.84</td>
<td>30.0</td>
<td>48.63</td>
<td>97.58±2.45</td>
</tr>
</tbody>
</table>

*Mean value of three determinations
Section 7.4

DEVELOPMENT AND VALIDATION OF STABILITY-INDICATING UV-SPECTROPHOTOMETRIC METHODS FOR THE DETERMINATION OF ZOLMITRIPTAN IN PHARMACEUTICALS*

7.4.1 INTRODUCTION

The utility of UV-spectrophotometry for the assay of pharmaceutical compounds was discussed under the Section 2.5.

From the literature survey presented in Section 7.0.2 it is evident that only three methods [4-6] have been reported for the assay of ZMT and none of them is stability indicating. Thus, author made an attempt to develop two simple, rapid and stability indicating method for the quantification ZMT. The methods are based on the measurement of absorbance of ZMT solution either in 0.1 M H₂SO₄ at 222 nm in method A, or in acetonitrile at 224 nm in method B. The details of the methods are presented in this Section 7.4.

7.4.2 EXPERIMENTAL

7.4.2.1 Instrument

The instrument used is the same that was described in Section 2.5.2.1.

7.4.2.2 Reagents and materials

All chemicals and reagents used were of analytical or pharmaceutical grade and distilled water was used throughout the experiment

Spectroscopic grade acetonitrile (Merck Pvt. Ltd., Mumbai, India) used as such.

**Sulphuric acid (0.1 M)**: Aqueous solution of 1.0 M H₂SO₄ was prepared by diluting the concentrated H₂SO₄ (Merck, Mumbai, India; Sp. gr. 1.84).

**Hydrochloric acid (5.0 M) sodium hydroxide solution (5.0 M) and hydrogen peroxide (5 %)** was prepared as described in Section 2.5.2.2.

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* This work has been communicated to J App Spec.,
Standard ZMT solution:

The pharmaceutical grade ZMT and tablets used in this study are those mentioned in the Section 7.1.2.2.

Stock standard solutions equivalent to 20 µg mL\(^{-1}\) and 10 µg mL\(^{-1}\) ZMT were prepared separately in 0.1 M H\(_2\)SO\(_4\) and acetonitrile, and used for the assay in method A and method B, respectively.

7.4.2.3 Assay procedures

Method A (using 0.1 M H\(_2\)SO\(_4\))

Aliquots of standard ZMT solution (0.2-5.0 mL of 20 µg mL\(^{-1}\)) were accurately transferred into a series of 10 mL calibrated flasks and the volume was made up to the mark with 0.1 M H\(_2\)SO\(_4\). The absorbance of each solution was measured at 222 nm against the same solvent.

Method B (using acetonitrile)

Different volumes (0.2-5.0 mL) of standard 10 µg mL\(^{-1}\) ZMT solution were accurately transferred into a series of 10 mL calibrated flasks and the volume was made up to the mark with acetonitrile. The absorbance of each solution was measured at 224 nm against acetonitrile.

Procedure for tablets

An amount of the powder equivalent to 10 mg of ZMT was accurately weighed and transferred to a 100 mL calibrated flask, 60 mL of the respective solvent (0.1 M H\(_2\)SO\(_4\) in method A and acetonitrile in method B) was added and the content was shaken thoroughly for about 20 min. The volume in each flask was diluted to the mark with the same solvent, mixed well and filtered using Whatman No. 42 filter paper. First 10 mL portion of the filtrate was rejected and a suitable aliquot of the filtrate (100 µg mL\(^{-1}\) ZMT) was diluted appropriately to get a working concentration of 20 and 10 µg mL\(^{-1}\) ZMT and then subjected to analysis by applying the assay procedures described above.

Procedures for selectivity study

A placebo blank was prepared as described under Section 7.1.2.3 in, and then 10 mg placebo blank extracted in 0.1 M H\(_2\)SO\(_4\) or acetonitrile was analyzed as done in “Procedure for tablets”.
A synthetic mixture was prepared by adding 10 mg of pure ZMT to 10 mg of the above mentioned placebo blank and the mixture was homogenized. Following the procedure described under procedure for tablets, the synthetic mixture solution (100 µg mL\(^{-1}\) ZMT) was prepared, and then diluted with the respective solvent to get working concentrations of 20 and 10 µg mL\(^{-1}\) in ZMT before subjecting to analysis following the procedures described above.

**Procedure for stress degradation by hydrolysis under acidic, alkaline and neutral conditions**

A 2.0 mL of 20 µg mL\(^{-1}\) standard ZMT solution in 0.1 M H\(_2\)SO\(_4\) (method A) or 4.0 mL of 10 µg mL\(^{-1}\) standard solution of ZMT in acetonitrile (method B) was taken separately in three 25 mL calibrated flasks, 5.0 mL each of 5.0 M HCl (acid hydrolysis), 5.0 M NaOH (alkaline hydrolysis) and water (neutral hydrolysis) were added separately to each flask. The flasks were kept on a water bath for 2.0 h at 80 °C, and then cooled to room temperature. The first and second flasks were neutralized with 5.0 mL of 5.0 M NaOH (for acid hydrolysis) and 5.0 mL of 5.0 M HCl (for alkaline hydrolysis) followed by making all the flasks to the mark with the respective solvent (0.1 M H\(_2\)SO\(_4\) in method A or acetonitrile in method B). The absorption spectrum of solution in each flask was recorded from 400-200 nm versus the corresponding blank.

**Procedure for oxidative degradation**

To 2.0 mL of standard ZMT solution (20 µg mL\(^{-1}\)) in 0.1 M H\(_2\)SO\(_4\) (method A) or to 4.0 mL of 10 µg mL\(^{-1}\) standard ZMT solution in acetonitrile (method B) taken in a 25 mL calibrated flask, 5 mL of 5 % hydrogen peroxide was added. The flasks were kept on a water bath at 80 °C for 2.0 h. The flasks were cooled to room temperature, made up to the mark with the respective solvent and the absorption spectrum was run from 400-200 nm against the corresponding blank.

**Procedure for dry heat and photo-degradation**

The powdered sample (0.1 g) of ZMT was taken on a Petri dish and kept in the oven at 105 °C for 24 h, the sample cooled to room temperature and used to prepare 20 µg mL\(^{-1}\) ZMT in 0.1 M H\(_2\)SO\(_4\) (method A) and 10 µg mL\(^{-1}\) ZMT in acetonitrile (method B). To study the photostability powdered sample (0.1 g) of ZMT was taken on a Petri dish, exposed to UV radiation in a UV chamber of 1200 flux-hr for 48 h and then used to
prepare 20 µg mL⁻¹ ZMT in 0.1 M H₂SO₄ (method A) and 10 µg mL⁻¹ ZMT in acetonitrile (method B). The absorption spectrum of each solution was run from 400-200 nm against the corresponding solvent.

7.4.3 RESULTS AND DISCUSSION

7.4.3.1 Absorption spectra

ZMT solution in 0.1 M H₂SO₄ (method A) and acetonitrile (method B) exhibited absorption peaks at 222 and 224 nm for method A and methods B, respectively (Figure 7.4.1) and the absorbance at these wavelengths was found to be linearly dependent upon the concentration of drug. In both the cases, the corresponding blank solutions showed negligible absorbance. Therefore these wavelengths were used as analytical wavelength throughout the investigation.

![Figure 7.4.1 Absorption spectra of ZMT in a) 0.1 M H₂SO₄ (method A) and b) in acetonitrile (method B).](image)

7.4.3.2 Forced degradation study

The absorption spectra of the ZMT solutions in 0.1 M H₂SO₄ and acetonitrile treated with acid, base and water hydrolysis, hydrogen peroxide, dry heat and UV radiation were run in the range of (200-400 nm). The degradation was evaluated based on the comparison of the UV spectra of “ZMT after subjected to various degradation conditions” with those of the “standard ZMT solution”.
The resulting UV spectra of stress ZMT solutions (4 µg mL\(^{-1}\) in 0.1 M H\(_2\)SO\(_4\) and acetonitrile) subjected to acid hydrolysis showed the same spectra (Figure 7.4.2) of the standard solution which indicated that ZMT does not undergo degradation under this condition.

![Figure 7.4.2 Acid degradation. a) (method A), b) (method B)](image1)

Under alkaline conditions ZMT solutions in 0.1 M H\(_2\)SO\(_4\) and in acetonitrile undergoes degradation in both methods (Figure 7.4.3). Also, the absorption spectra of ZMT solutions in 0.1 M H\(_2\)SO\(_4\) and in acetonitrile treated with hydrogen peroxide showed that ZMT undergoes significant degradation in both methods (Figure 7.4.4).

![Figure 7.4.3 Base degradation. a) (method A), b) (method B)](image2)
The UV spectra of stress ZMT samples subjected to dry heat treatment and UV-degradation were similar to that of the standard ZMT sample in both methods and it showed that ZMT did not undergo degradation under these conditions (Figure 7.4.5). The results of degradation study are summed up in Table 7.4.1.
**Table 7.4.1** Forced degradation summary

<table>
<thead>
<tr>
<th>Degradation condition</th>
<th>% Assay* (method A)</th>
<th>% Assay* (method B)</th>
<th>Observation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control sample</td>
<td>99.8</td>
<td>99.8</td>
<td>Not applicable</td>
</tr>
<tr>
<td>Acid hydrolysis (5M HCl, 80°C, 2 hours)</td>
<td>99.8</td>
<td>98.7</td>
<td>No degradation observed</td>
</tr>
<tr>
<td>Base hydrolysis (5M NaOH, 80°C, 2 hours)</td>
<td>-</td>
<td>-</td>
<td>Extensively degraded</td>
</tr>
<tr>
<td>Oxidation (5% H₂O₂, 80°C, 2 hours)</td>
<td>-</td>
<td>-</td>
<td>Extensively degraded</td>
</tr>
<tr>
<td>Thermal (105°C, 3 hours)</td>
<td>98.9</td>
<td>98.4</td>
<td>No degradation observed</td>
</tr>
<tr>
<td>Photolytic (1.2 million lux hours)</td>
<td>100.0</td>
<td>98.7</td>
<td>No degradation observed</td>
</tr>
</tbody>
</table>

* Percentage against standard ZMT

### 7.4.3.3 Method validation

#### Linearity and sensitivity

Under optimum conditions a linear relation was obtained between absorbance and concentration of ZMT in the range of 0.4 - 10.0 and 0.2 - 5.0 µg mL⁻¹ for method A and method B, respectively (Figure 7.4.6). The calibration graph is described by the equation:

\[ Y = a + b X \]

(Where \( Y \) = absorbance, \( a \) = intercept, \( b \) = slope and \( X \) = concentration in µg mL⁻¹) obtained by the method of least squares. Correlation coefficient, intercept and slope for the calibration data are summarized in Table 7.4.2. Sensitivity parameters such as apparent molar absorptivity and Sandell sensitivity values, the limits of detection and quantification calculated as per the current ICH guidelines [21] are compiled in Table 7.4.2 speak of the excellent sensitivity of the proposed methods.
Figure 7.4.6 Calibration curves.

Table 7.4.2 Regression and sensitivity parameters.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Method A</th>
<th>Method B</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\lambda_{\text{max}}$, nm</td>
<td>222</td>
<td>224</td>
</tr>
<tr>
<td>Beer’s law limits (µg mL$^{-1}$)</td>
<td>0.4-10.0</td>
<td>0.2-5.0</td>
</tr>
<tr>
<td>Molar absorptivity ($\varepsilon$) (L mol$^{-1}$ cm$^{-1}$)</td>
<td>4.00×10$^4$</td>
<td>4.74×10$^4$</td>
</tr>
<tr>
<td>Sandell sensitivity* (µg cm$^{-2}$)</td>
<td>0.0072</td>
<td>0.0061</td>
</tr>
<tr>
<td>Limit of detection (µg mL$^{-1}$)</td>
<td>0.06</td>
<td>0.06</td>
</tr>
<tr>
<td>Limit of quantification (µg mL$^{-1}$)</td>
<td>0.19</td>
<td>0.19</td>
</tr>
<tr>
<td>Regression equation, $Y**$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intercept, ($a$)</td>
<td>0.0034</td>
<td>-0.0021</td>
</tr>
<tr>
<td>Slope, ($b$)</td>
<td>0.1371</td>
<td>0.1702</td>
</tr>
<tr>
<td>Correlation coefficient ($r$)</td>
<td>0.9997</td>
<td>0.9999</td>
</tr>
<tr>
<td>Standard deviation of intercept ($S_a$)</td>
<td>0.0237</td>
<td>0.0289</td>
</tr>
<tr>
<td>Standard deviation of slope ($S_b$)</td>
<td>0.0042</td>
<td>0.0076</td>
</tr>
</tbody>
</table>

*Limit of determination as the weight in µg per mL of solution, which corresponds to an absorbance of $A = 0.001$ measured in a cuvette of cross-sectional area 1 cm$^2$ and $l = 1$ cm. **$y = a + bx$**, where $Y$ is the absorbance, $a$ is the intercept, $b$ is the slope and $X$ is the concentration in µg mL$^{-1}$.

Accuracy and precision

In order to evaluate the accuracy and precision of the proposed methods, solutions containing three different concentrations of the ZMT were prepared and analyzed in seven replicates. The analytical results obtained from this investigation are summarized in Table 7.4.3. The low values of the relative standard deviation (% R.S.D) and percentage relative error (% R.E) indicate the high precision and the good accuracy of the proposed methods.
The assay procedure was repeated seven times, and percentage relative standard deviation (% R.S.D) values were obtained within the same day to evaluate repeatability (intra-day precision) and over five different days to evaluate intermediate precision (inter-day precision).

**Table 7.4.3 Results of intra-day and inter-day accuracy and precision study.**

<table>
<thead>
<tr>
<th>Method</th>
<th>ZMT taken (µg mL(^{-1}))</th>
<th>ZMT found(^a) (µg mL(^{-1}))</th>
<th>% RSD(^b)</th>
<th>% RE(^c)</th>
<th>ZMT found(^a) (µg mL(^{-1}))</th>
<th>% RSD(^b)</th>
<th>% RE(^c)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Method A</td>
<td>2.00</td>
<td>2.03</td>
<td>0.93</td>
<td>1.48</td>
<td>2.03</td>
<td>1.09</td>
<td>1.61</td>
</tr>
<tr>
<td></td>
<td>4.00</td>
<td>3.95</td>
<td>1.25</td>
<td>1.20</td>
<td>3.95</td>
<td>1.31</td>
<td>1.31</td>
</tr>
<tr>
<td></td>
<td>6.00</td>
<td>5.96</td>
<td>0.83</td>
<td>0.67</td>
<td>6.08</td>
<td>1.12</td>
<td>1.25</td>
</tr>
<tr>
<td></td>
<td>1.00</td>
<td>1.01</td>
<td>1.08</td>
<td>0.92</td>
<td>1.01</td>
<td>1.33</td>
<td>1.25</td>
</tr>
<tr>
<td>Method B</td>
<td>3.00</td>
<td>3.04</td>
<td>1.04</td>
<td>1.19</td>
<td>3.04</td>
<td>1.38</td>
<td>1.36</td>
</tr>
<tr>
<td></td>
<td>5.00</td>
<td>4.96</td>
<td>1.35</td>
<td>0.84</td>
<td>4.95</td>
<td>1.62</td>
<td>0.97</td>
</tr>
</tbody>
</table>

\(^a\) Mean value of five determinations; \(^b\) Relative standard deviation (%); \(^c\) Relative error (%).

**Selectivity**

The proposed methods were tested for selectivity by placebo blank and synthetic mixture analyses. The placebo blank solution was subjected to analysis according to the recommended procedures. The resulting absorbance readings for both the methods were same as the reagent blank, inferring no interference from placebo.

A separate experiment was performed with the synthetic mixture. The analysis of synthetic mixture solution at three concentration levels yielded percent recoveries which ranged from 97.58 to 102.7 with standard deviation of 0.96 – 2.33 in both the cases.

**Robustness and ruggedness**

The robustness of the methods was evaluated by measuring the absorbance at three different wavelengths whereas the method ruggedness was performed by four different analysts and also using three different cuvettes by a single analyst. Intermediate precision values (% RSD) were in the range 0.95 – 1.81% indicating acceptable ruggedness. These results are presented in **Table 7.4.4.**
Chapter 7  Titrimetric and spectrophotometric assay of zolmitriptan

Table 7.4.4 Results of robustness and ruggedness study.

<table>
<thead>
<tr>
<th>Method</th>
<th>ZMT taken, µg mL⁻¹</th>
<th>Method robustness</th>
<th>Method ruggedness</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wavelengths, nm²</td>
<td>Inter-analysts RSD, % (n = 3)</td>
<td>Inter-cuvettes RSD, % (n = 3)</td>
</tr>
<tr>
<td>A</td>
<td>2.00</td>
<td>1.17</td>
<td>1.81</td>
</tr>
<tr>
<td></td>
<td>4.00</td>
<td>1.24</td>
<td>1.06</td>
</tr>
<tr>
<td></td>
<td>6.00</td>
<td>1.09</td>
<td>1.33</td>
</tr>
<tr>
<td>B</td>
<td>1.00</td>
<td>0.95</td>
<td>1.52</td>
</tr>
<tr>
<td></td>
<td>3.00</td>
<td>1.53</td>
<td>0.98</td>
</tr>
<tr>
<td></td>
<td>5.00</td>
<td>0.83</td>
<td>1.10</td>
</tr>
</tbody>
</table>

²Wavelengths used were 221, 222 and 223 in method A and 223, 224 and 225 in method B.

Application to analysis of tablets containing ZMT

In order to evaluate the analytical applicability of the proposed methods to the quantification of ZMT in commercial tablets, the results obtained by the proposed methods were compared to those of the reference method [15] by applying Student’s t-test for accuracy and F-test for precision. The reference method describes a HPLC method using symmetry C₁₈ (250 x 4.6 mm, 5 µm in particle size) with mobile phase of 0.01 % triethylamine: acetonitrile: 0.02 M NH₄H₂PO₄; 28.2:25:46.8 (V/V/V) and the UV detection at 225 nm. The results (Table 7.4.5) show that the calculated Student’s t- and F-values at 95 % confidence level are less than the tabulated values, which confirmed that there is a good agreement between the results obtained by the proposed methods and the reference method with respect to accuracy and precision.

Table 7.4.5 Results of assay of tablets and statistical comparison with the reference method.

<table>
<thead>
<tr>
<th>Tablet Brand name</th>
<th>Label claim mg/tablet</th>
<th>Found (Percent of label claim ±SD)²</th>
<th>Reference method</th>
<th>Proposed methods</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Method A</td>
</tr>
<tr>
<td>Zomig-2.5</td>
<td>2.5</td>
<td>100.59 ± 0.90</td>
<td>99.37 ± 1.34</td>
<td>101.6 ± 1.46</td>
</tr>
</tbody>
</table>

²Mean value of five determinations.
Tabulated t-value at the 95% confidence level is 2.78.
Tabulated F-value at the 95% confidence level is 6.39.
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 three concentration levels (50, 100 and 150 % of that in tablet powder) and the total was found by the proposed methods. In both cases, the added ZMT recovery percentage values ranged of 98.20 – 102.0 % with standard deviation of 0.57 – 2.46 (Table 7.4.6) indicating that the recovery was good, and that the co formulated substance did not interfere in the determination.

Table 7.4.6 Results of recovery study via standard addition method.

<table>
<thead>
<tr>
<th>Tablets studied</th>
<th>Method A</th>
<th>Method B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ZMT in tablets, ( \mu g \ mL^{-1} )</td>
<td>Pure ZMT added, ( \mu g \ mL^{-1} )</td>
</tr>
<tr>
<td>Zomig- 2.5</td>
<td>1.99</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>1.99</td>
<td>2.00</td>
</tr>
<tr>
<td></td>
<td>1.99</td>
<td>3.00</td>
</tr>
</tbody>
</table>

*Mean value of three determinations.
SECTION 7.5
SUMMARY AND CONCLUSIONS – Assessment of methods

To the best of author’s knowledge no titrimetric methods have ever been reported for the assay of ZMT. The author has developed two titrimetric methods based on the visual and potentiometric titration of ZMT using 0.005 M perchloric acid as titrant. Crystal violet and modified glass-saturated calomel electrode system were used to locate the end point in visual and potentiometric titration, respectively. The proposed titrimetric methods are rapid, simple, precise and accurate; and to top all, inexpensive and can be used over a semimicro scale (1.0-10.0 mg) thus offering an additional cost advantage. It is worth noting that the author has developed the first ever visible titrimetric and potentiometric methods for ZMT.

The spectrophotometric methods are easier to perform and use an instrument which is inexpensive compared to those used in the previously reported techniques for zolmitriptan. All the methods are rapid and don’t involve any stringent experimental conditions which would affect the reliability of the results. The spectrophotometric method based on the breaking of ZMT-BCG ion-pair in KOH medium is the most sensitivity method has ever been reported for ZMT which surpasses the sensitivity of most of the previously reported HPLC methods. Molar absorptivity of the method is $1.52 \times 10^5$ L mol$^{-1}$ cm$^{-1}$. The method developed using vanillin as a reagent is the simplest method among the other developed visible spectrophotometric methods.

Two UV-spectrophotometric methods were developed and found to be superior compare to the reported methods [4-6] in terms of simplicity and sensitivity and application. The developed methods were subjected to forced studies under different stress conditions. For the first time, author developed a stability-indicating UV-spectrophotometric method, which can be considered as the simplest method to evaluate the stability of the drug under various stress conditions.

All the proposed methods were validated according to the ICH guidelines [21]. Method selectivity was evaluated by placebo blank, synthetic mixture and tablet analyses. Robustness and ruggedness of the methods were evaluated. Inter-day and intra-day accuracy and precision, statistical comparison with the literature method using Student’s
t-test and $F$-test, and recovery in the presence of tablet matrix were done. The results are presented in respective sections.

In conclusion, the author has developed and validated two titrimetric, three visible spectrophotometric and two UV- spectrophotometric methods for the assay of ZMT. A comparison of performance characteristics of the proposed spectrophotometric methods with those of the existing methods is presented in Table 7.5.1.
Table 7.5.1 Comparison of performance characteristics of the proposed spectrophotometric methods with the existing methods.

I. Spectrophotometric methods

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Reagent/s used</th>
<th>Methodology</th>
<th>( \lambda_{\text{max}} ) (nm)</th>
<th>Linear range, ( \mu \text{g mL}^{-1} ) and ( \varepsilon, \text{L mol}^{-1} \text{cm}^{-1} )</th>
<th>Remarks</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2,3-Dichloro-5,6-dicyano-1,4-benzoquinone</td>
<td>Charge-transfer complex measured</td>
<td>555</td>
<td>10.0-250.0 (1.70×10^3)</td>
<td>Less sensitive</td>
<td>7</td>
</tr>
</tbody>
</table>
| 2      | a) Tropaeolin OO  
|        | b) Bromothymol blue                  | Dichloromethane extractable ion-pair complexes measured | 411.5  
|        |                                      |                                      | 410                              | 2.0-20.0 (1.42×10^3)  
|        |                                      |                                      |                                  | 1.5-17.0 (1.60×10^3)  | -      | 8    |
| 3      | a) Bromo cresol green (BCG)  
|        | b) ZMT-BCG ion pair broken with ethanolic KOH | Chloroform extractable ion-pair complex measured | 415                              | 0.8-18.0 (1.50×10^4)                                              | Simple, highly sensitive and wide linear range, economical, no standardization | Present methods |
|        |                                      | breaking of the yellow ZMT-BCG ion-pair complex and measurement of blue colored anionic form of the dye in alkaline medium | 630                              | 0.08-1.4 (1.52×10^5)                                              |         |      |
| 4      | Vanillin in acid medium              | Enamine formed with drug measured    | 580                              | 5.0-90.0 (3.30×10^3)                                               | Sensitive, no heating or extraction step, inexpensive instrumental setup | Present method |
| 5      | UV  
|        | a) 0.1 M H\(_2\)SO\(_4\)  
|        | b) Acetonitrile                       | Absorbance measured in UV-spectrophotometer | 222                              | 0.4-10.0 (4.0×10^4)                                               | Simple, sensitive, easy to perform, stability-indicating. | Present methods |
|        |                                      |                                      | 224                              | 0.2-5.0 (4.74×10^4)                                               |         |      |
REFERENCES
5. T.E.G.K. Murthy, K. Veditha, Indian Pharmacist (New Delhi, India), 2011, 9, 57.


