Research Article

Titrimetric Assay of Zolmitriptan in Non-Aqueous Medium

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ABSTRACT

Based on the nitrogenous base or tertiary amine moiety in zolmitriptan (ZMT), an anti-migraine drug, two highly accurate and selective titrimetric methods are proposed for the determination of ZMT in bulk drug and pharmaceuticals. In these methods, the drug dissolved in glacial acetic acid, was titrated with acetous perchloric acid and end point determined either visually by using crystal violet indicator (method A) or potentiometrically by using combined glass-SCE electrode (method B). Both the methods are applicable over the concentration range of 1.0-10.0 mg of ZMT. Calculations are based on 1:1 molar ratio i.e., ZMT: $HClO_4$, owing to the presence of one tertiary nitrogen atom. The procedures were applied to determine ZMT in its tablets and the results were found to be in good agreement with those obtained by the reference method. Associated pharmaceutical materials did not interfere. The precision results, expressed by inter-day and intra-day relative standard deviation values, were satisfactory (≤ 1.77%). Accuracy was satisfactory as well. The accuracy and reliability of the methods were further ascertained by recovery studies via standard addition technique with percent recoveries in the range 98.00 - 102.0 % with standard deviation of less than 0.15%.

Keywords: Zolmitriptan; Non-aqueous titrimetry; Pharmaceuticals; Crystal violet; Potentiometry.

INTRODUCTION

Zolmitriptan (**Figure 1**) is a selective agonist of serotonin (5-hydroxytryptamine; 5-HT) type 1B and 1D receptors and chemically known as (4S)-4-[[3-[2-(dimethylamino) ethyl]-1H-indol-5-yl] methyl]-2-oxazolidinone. 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[1].

ZMT is not included in any pharmacopeia. Literature survey reveals that few analytical methods have been published for analysis of ZMT in human plasma and include high-performance liquid chromatography (HPLC) with coulometric[2], mass spectrometric detection[3-5] and liquid chromatography-mass spectrometry[1,6,7].

High-performance liquid chromatography (HPLC) with UV-detection has been widely used for the quantitative determination of ZMT in pharmaceuticals[8-15]. Ultra- performance liquid chromatography (UPLC)[16], liquid chromatography-mass spectrometry[17], voltammetry[18], UV-spectrophotometric methods[19-21] and visible spectrophotometric methods[22,23] are the other techniques applied for the assay 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 to develop two simple, rapid and semi-micro scale titrimetric methods.

EXPERIMENTAL

Apparatus

An Elico 120 digital pH meter provided with a combined glass-SCE electrode system was used for potentiometric titration. The KCl of the salt bridge was replaced with saturated solution of KCl in glacial acetic acid.

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): The stock solution of (~0.1 M) perchloric acid (S. D. Fine Chem, Mumbai, India) was diluted appropriately with glacial acetic acid to get a working solution of 0.005 M perchloric acid and standardized with pure potassium hydrogen phthalate and crystal violet as indicator[63].

Crystal violet indicator (0.1 %): Prepared by dissolving 50 mg of dye (S. D. Fine Chem, Mumbai, India) in 50 mL of glacial acetic acid.
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.
One brand of tablet namely Zomig-2.5 (Intas Pharmaceuticals, Dehradun, India) was purchased from local commercial sources.

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

$$\textbf{Amount (mg)} = VM_wR/n$$

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 $HClO_4$) was added from a micro burette. 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.

RESULTS AND DISCUSSION
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 2**). 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.

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 ($CH_3COOH_2^+$) 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 shown in **Scheme 1**.

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 ≤ 1.30% (intra-day) and ≤ 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 1**, it is clear that the accuracy is satisfactory (RE ≤ 1.55%).

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 µg 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 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 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 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.

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 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.

CONCLUSION

The methods provide two simple procedures for the determination of ZMT in bulk drug and in its dosage form. 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. Most of the reported methods require highly expertise personnel to handle the instrument and time consuming whereas the proposed methods are easy to operate without

consuming longer analysis time. Hence, the methods should find easy acceptance in industrial quality control laboratories for routine analysis.

Table 1: Results of intra-day and inter-day accuracy and precision study

Method	ZMT taken, mg	Intra-day accuracy and precision			Inter-day accuracy and precision		
		ZMT found, mg	RE, %	RSD, %	ZMT found, mg	RE, %	RSD, %
Visual titrimetry, (n=7)	4.0	4.01	0.29	0.86	4.02	0.61	1.31
	6.0	6.03	0.50	0.57	6.06	0.92	0.74
	8.0	8.04	0.45	0.43	8.06	0.76	0.65
Potentiometric titrimetry (n=5)	4.0	4.05	1.24	1.30	4.06	1.55	1.77
	6.0	6.07	1.13	0.87	6.08	1.34	1.18
	8.0	8.05	0.61	0.70	8.09	1.08	1.02
RE: relative error, RSD: relative standard deviation.							

Table 2: Results of method ruggedness study

Method	ZMT taken, mg	Ruggedness	
		Inter-analysts (% RSD): (n=4)	Inter-burettes (% RSD): (n=4)
Visual end point detection	4.0	1.04	0.67
	6.0	0.86	0.95
	8.0	0.79	0.83
Potentiometric end point detection	4.0	0.52	0.78
	6.0	0.49	0.90
	8.0	0.71	0.88

Table 3: Results of assay of tablets and statistical comparison with the reference method

Brand name	Label claim, mg/tablet	Found* (Percent of label claim ± SD)		
		Reference method	Proposed methods	
			Visual titrimetry	Potentiometric titrimetry
Zomig- 2.5	2.5	100.59±0.90	101.3±1.49 *t*=0.91 *F*=2.74	99.28±1.28 *t*=1.87 *F*=2.02
*Average of five determinations. Tabulated t value at the 95% confidence level is 2.77. Tabulated F value at the 95% confidence level is 6.39.				

Table 4: Results of recovery study via standard addition method

Tablet studied	Visual titrimetry				Potentiometric titrimetry			
	ZMT in tablet extract, mg	Pure ZMT added, mg	Total ZMT found, mg	Pure ZMT recovered* %	ZMT in tablet extract, mg	Pure ZMT added, mg	Total ZMT found, mg	Pure ZMT recovered* %
Zomig 2.5	3.04	1.5	4.56	100.7±0.15	2.98	1.5	4.47	99.53±0.04
	3.04	3.0	6.08	101.3±0.06	2.98	3.0	6.05	102.3±0.05
	3.04	4.5	7.45	98.00±0.09	2.98	4.5	7.45	99.33±0.05
*Mean value of three determinations.								

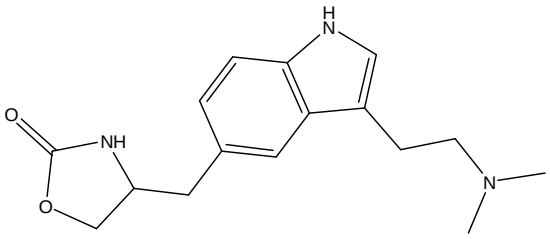
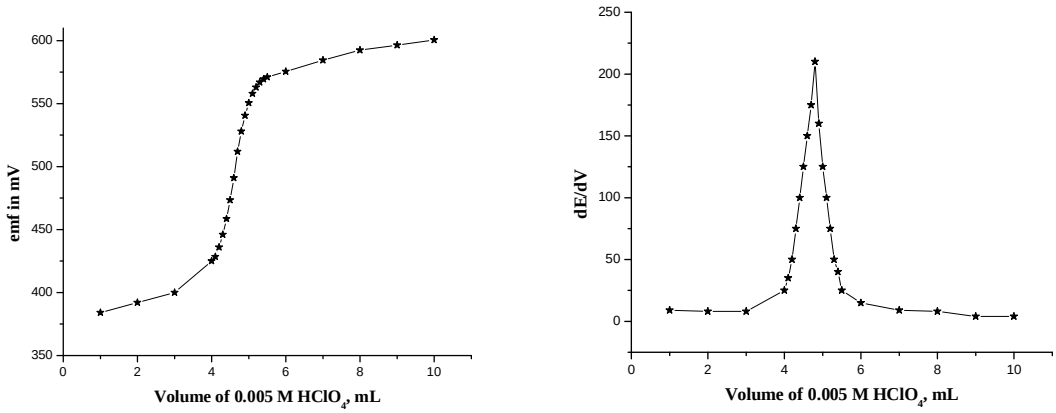
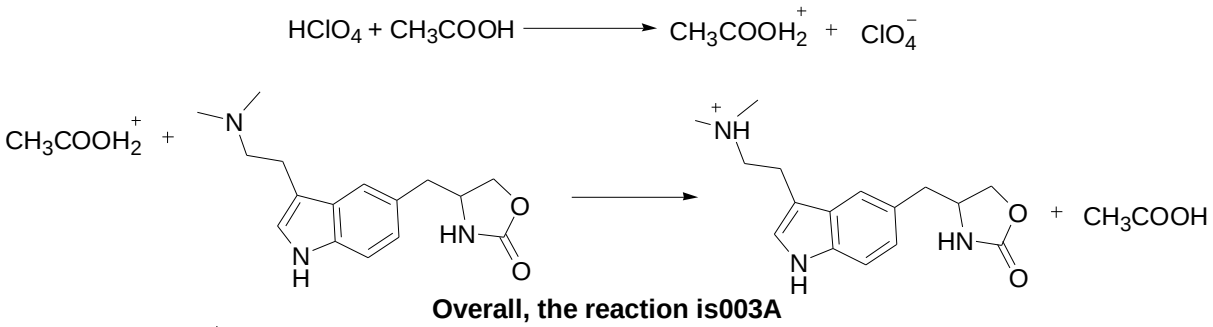


Fig. 1: Structure of Zolmitriptan

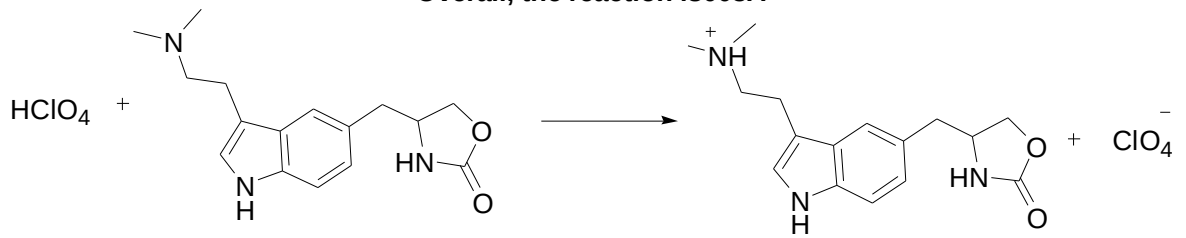


(a) **(b)**

Fig. 2: Potentiometric titration curves for 6 mg ZMT Vs 0.005 M $HClO_4$.
(a) Normal titration curve and (b) First-derivative curve



Overall, the reaction is003A



Scheme. 1: Probable reaction pathway

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