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Application of Folin-Ciocalteu reagent for the spectrophotometric determination of zolmitriptan in pharmaceutical preparations

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Abstract : A fast and selective spectrophotometric method for the determination of zolmitriptan in pharmaceutical bulk and dosage forms is described. The method is based on the formation of blue colored chromogen due to reduction of tungstate in Folin-Ciocalteu reagent by zolmitriptan in alkaline medium. The colored species has an absorption maximum at 750 nm and the Beer's law obeys over the concentration range of 3-50 μg mL^{-1}. The limit of detection and quantification value is 3.43 and 11.33 μg mL^{-1} respectively. Application of the proposed method to bulk powder and commercial pharmaceutical tablets is also presented. No significant difference was obtained between the results of the proposed method and the official USP method. The procedure described in this paper is simple, rapid and extraction free.

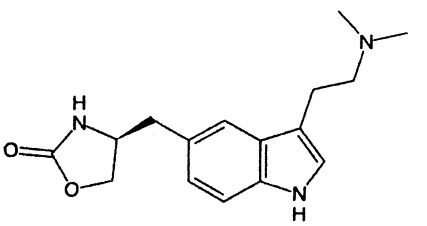
Keywords : Zolmitriptan, Folin-Ciocalteu reagent, pharmaceutical formulations, spectrophotometry.

Introduction

Zolmitriptan (IUPAC name (4*S*)-4-({3-[2-(dimethylamino)ethyl]-1*H*-indol-5-yl}methyl)-1,3-oxazolidin-2-one) is a selective agonist of serotonin (5-hydroxytryptamine; 5-HT) type 1B and 1D receptors. It is structurally and pharmacologically related to other selective 5-HT1B/1D receptor agonists. It is a drug for treating migraine headaches. Migraine headaches are believed to result from dilation of the blood vessels in the brain. Zolmitriptan causes constriction of the blood vessels and thereby relieves the pain of a migraine headache[1]. Zolmitriptan was approved by the FDA in November of 1997.

In our knowledge the assay of zolmitriptan in pure and dosage forms is not still official in any pharmacopoeia. But very recently a USP pending monograph draft-1 for public comment is published on internet.

The different analytical methods that have been reported for its determination include HPLC with mass spectrometry detection[2-7], with coulometric detection[8], electrospray ionization mass spectrometry[9], tandem mass spectrometry[10], fluorescence detection[11,12] in pharmaceutical preparations and biological fluids. Very few spectrophotometric methods are available in the literature for the quantitative determination of zolmitriptan in pharmaceuticals formulations[13-18].



Scheme 1. Chemical structure of zolmitriptan.

Aydogmus *et al.*[13] presented spectrophotometric method involving color development with tropaeolin and bromothymol blue in citrate-phosphate buffer of pH 4.0 and 6.0, respectively, but this involve stringent pH conditions. Rao *et al.*[14] and Sankar *et al.*[15] described the UV method in ethanol and methanol solvent respectively. Rani *et al.*[16] presented visible method by using the chromogens like MBTH at 570 nm and Brucine at 520 nm that involve extraction and specific buffer conditions to develop color. Our previous work[17] was the novel spectrophotometric method for the determination of zolmitriptan using DDQ reagent. However very recently Gouda *et al.*[18] used 7,7,8,8-tetracyanoquinodimethane (TCNQ) and *p*-chloranilic acid (*p*-CLA) as charge transfer reaction for spectrophotometric determination of zolmitriptan in pharmaceutical formulation.

Reported methods are not fair to middling, showing linearity over dynamic range, pH dependent, using tedious and time consuming extraction step that consume large amount of organic solvent. So there is a need to develop simple, sensitive and extraction free spectrophotometric method for the determination of zolmitriptan in pharmaceutical formulations. In the present study we have used Folin-Ciocalteu reagent as a color producing reagent for determination of zolmitriptan in pharmaceuticals and fortunately we have obtained the excellent results in term of accuracy and sensitivity. The careful survey of the literature shows that quantitative determination of zolmitriptan using Folin-Ciocalteu reagent has never been exploited for spectrophotometric determination.

Aim of the present study is to develop a sensitive and cost effective spectrophotometric method that rely on the use of cheap chemicals and simple technique but provides accuracy compared to costly and sophisticated technique like HPLC.

Experimental

Equipment :

All absorption spectra were made using (U 1100 Hitachi, Japan) spectrophotometer equipped with 1 cm matched quartz cells.

Chemicals and reagents :

Chemicals used were of analytical grade. Distilled water was used throughout the investigation. Folin-Ciocalteu reagent (Fluka, Germany), sodium hydroxide (Merck, Germany) were of analytical reagent grade and used without further purification. A pure zolmitriptan (pharmaceutical grade) sample was kindly provided by BioFine Pharmaceuticals Pvt. Ltd., Multan, Pakistan. Three brands of tablets, namely, zominat (BioFine Pharmaceuticals), ziptan (Medisure Pharma, Karachi, Pakistan) and onset (Pharmedic Pvt. Ltd., Lahore, Pakistan) were obtained from commercial sources.

Drug and reagent solutions :

A stock solution of zolmitriptan (1 mg/ml) : Prepared by dissolving 100 mg of pure drug in 40 ml water then volume was made up to mark with distilled water in a 100 ml volumetric flask. Working concentration of zolmitriptan was prepared by dilution of the above stock solution with water.

FC reagent solution (2.0 N) : was used as such.

NaOH solution (1.0 N) : Prepared by dissolving 4.0 g of sodium hydroxide in 100 mL of distilled water.

Determination of absorption spectra :

Zolmitriptan solution equivalent to 10 μg mL^{-1} was mixed with 2 mL of sodium hydroxide solution and 2 mL of F-C reagent in a 10-mL volumetric flask. After 10 min, the volume was made up to the mark with water and the content was mixed thoroughly. A blank solution was prepared in the same way in the absence of zolmitriptan. This solution was scanned in the range of 400–800 nm against the regent blank. Maximum absorption was observed at 750 nm and was fixed as analytical wavelength.

General analytical procedure :

Different aliquots of the working standard of zolmitriptan ranging from 3–50 μg mL^{-1}, were transferred into a series of 10-mL volumetric flasks and the total volume was brought to 10 mL with water. To each flask, 2.0 mL of sodium hydroxide solution and 2.0 mL of F-C reagent solution were successively added by means of a micropipette. The flasks were stoppered, contents were mixed and kept at room temperature for 10 min. The volume was made up to the mark with water and the absorbance of each solution was measured at 750 nm against a reagent blank.

Assay procedure for tablets :

An amount of finely ground tablet powder equivalent to 100 mg of zolmitriptan was accurately weighed into a 100-mL volumetric flask, the flask was shaken after addition of a 50 mL of water for about 2.0 min and finally the volume was made up to the mark with water. The content was kept aside for 5 min, and filtered using Whatman No. 42 filter paper. The first 10-mL portion of the filtrate was discarded and a suitable aliquot was used for the assay as described under "General analytical procedure".

Results and discussion

Many scientists have used Folin-Ciocalteu reagent as a chromogenic reagent for the quantitative determination of pharmaceuticals[19,20]. The structural features of zolmitriptan allowed the use of Folin-Ciocalteu reagent for its assay. The proposed method is based on the formation of a blue colored chromogen when zolmitriptan

reacted with the Folin-Ciocalteu reagent in the presence of sodium hydroxide. Colour formation may be explained as follows based on the analogy reported by Peterson[21]. Mixed acids in the F-C reagent involve the following chemical species :

$3H_2O{\cdot}P_2O_5{\cdot}13WO_3{\cdot}5MoO_3{\cdot}10H_2O$ and

$3H_2O{\cdot}P_2O_5{\cdot}14WO_3{\cdot}4MoO_3{\cdot}10H_2O$

The exact chemical nature of the FC reagent is not known, but it is believed to contain heteropolyphosphotunstates-molybdates. Sequences of reversible one or two-electron reduction reactions lead to blue species, possibly $(PMoW_{11}O_{40})^{4-}$. In essence, it is believed that the molybdenum is easier to be reduced in the complex and electron-transfer reaction occurs between reductants and Mo^{VI} [22].

$$Mo^{IV} + e \longrightarrow Mo^{V}$$

Zolmitriptan probably causes a reduction of molybdate in the Folin-Ciocalteu reagent, thereby producing one or more reduced species that have a characteristic intense blue color that show absorption maximum at 750 nm as shown in Fig. 1.

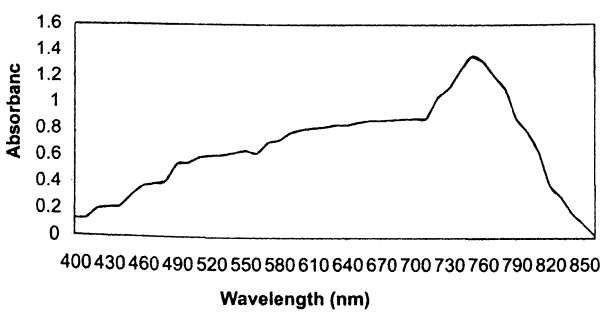


Fig. 1. Absorption spectrum of zolmitriptan and Folin-Ciocalteu complex.

Optimization of the reaction conditions :

Optimum conditions were fixed by varying one parameter at a time while keeping other parameters constant and observing their effect on the absorbance at 750 nm.

Effect of color producing regent on the formation of color complex was studied. Different volume of 2 *N* Folin-Ciocalteu reagent was used without further dilution. It was observed that 2 ml volume of Folin-Ciocalteu reagent is sufficient for the maximum color development as shown in Fig. 2. To find a suitable medium for the reaction different aqueous bases were investigated. Best results were obtained with 1 *N* sodium hydroxide. It was found that maximum and constant absorbance was obtained in the concentration range of 0.5 to 5 ml of 1 *N* sodium hydroxide thus 2.0 mL of 1 *N* sodium hydroxide was fixed as optimal as shown in Fig. 3.

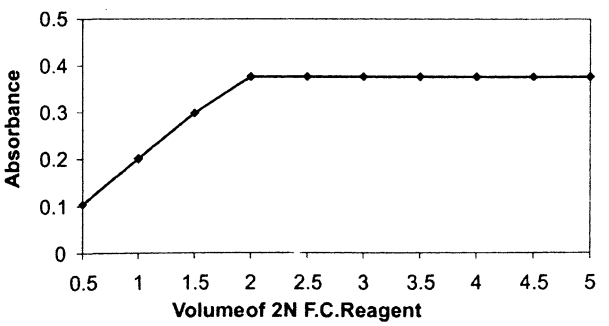


Fig. 2. Effect of reagent volume on color development.

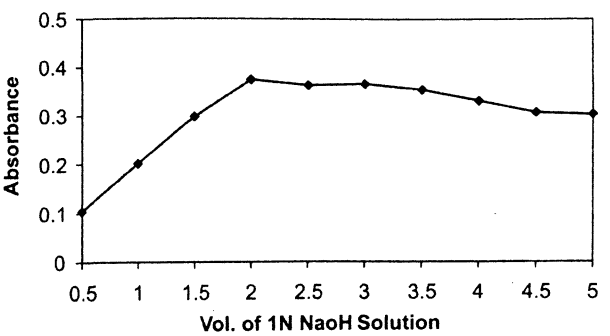


Fig. 3. Effect of base volume on color development.

The reaction time was studied by measuring the absorbance of the blue chromogen after mixing the reactants over a period from 1 to 30 min. Maximum color developed in 15 min and was stable for at least 90 min as shown in Fig. 4. Measurements were therefore made only after 10 min throughout the investigation.

Highest sensitivity was obtained when the order of reactants addition was maintained as described in the general analytical procedure and the same was followed throughout the investigation.

The analytical parameters and the optical characteristics for the spectrophotometric determinations of zolmitriptan by the proposed method are given in Table 1.

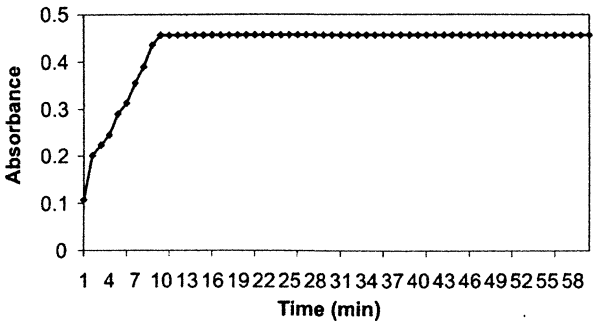


Fig. 4. Time required for color formation and stability of color.

Table 1. Analytical data for the complex of zolmitriptan with Folin-Ciocalteu reagent

Parameters	Values
λ_{max} (nm)	750
Beer's law measuring range (μg/ml)	3-50
Molar absorptivity (L mol^{-1} cm^{-1})	9.97×10^3
Sandell's sensitivity (μg/ml)	2.88×10^{-2}
Limit of Detection (μg/ml)	3.43
Limit of Quantification (μg/ml)	11.33
Slope	0.037
Intercept	-0.019
Correlation coefficient	0.9999

Interference effect :

More than 99.90% recovery of zolmitriptan was obtained in the presence of various excipients and additives used in tablet formulations such as microcrystalline cellulose, lactose, magnesium stearate and sodium starch glycolate. Under the experimental conditions applied, to a known amount (10 μg/ml) of drug excipients in different concentrations were added and analyzed. Results of the recovery analysis are presented in Table 2. Excipients up to the concentrations shown in the Table 2 have no interfere with the assay. In addition recoveries in most cases were 100% and the lower values of the RSD indicate the good precision of the proposed method.

Table 2. Percent recovery of zolmitriptan in presence of excipients/additives

Excipient/additive	Amount taken (μg/ml)	Recovery (% ± S.D.)
Microcrystalline cellulose	600	101.71 ± 0.34
Lactose	460	100.23 ± 0.36
Magnesium stearate	350	100.24 ± 0.72
Sodium starch glycolate	300	100.89 ± 0.57

Analytical applications :

Pharmaceutical formulations (zominat, ziptan and onset tablets) containing zolmitriptan 2.5 mg/tab were analyzed by the proposed method and the accuracy was tested by the standard additions method in which variable amounts of pure drug were added to the previously analyzed portion of pharmaceutical formulations. The results shown in Table 3 confirm that the excipients and additives used in tablet formulations do not interfere with the assay of zolmitriptan by the proposed method. The proposed method is highly sensitive; therefore it may be easily used for the routine analysis of zolmitriptan in pure form and in its pharmaceutical preparations. The proposed method was applied to determine zolmitriptan in pharmaceutical preparations, the official U.S.P. method[23] being used for comparative assay. The results are presented in Table 4. The performance of the method was estimated by Student-*t* values and *F*-ratio tests. At a 95% confidence level, the calculated *t*- and *F*-values did not exceed the theoretical values, indicating that the proposed and the official methods are equally accurate.

Validation of the proposed method :

The proposed spectrophotometric method was validated with respect to linearity range, accuracy, precision, limit of detection (LOD) and limit of quantitation (LOQ).

Linearity and range :

The prepared aliquots of drug solution (3-50 μg mL^{-1}) were scanned for absorbance maximum value at 750 nm. The absorbance range was found to be 0.104-1.87. These solutions obeyed Beer's law in over the concentration range of 3-50 μg mL^{-1} with regression of 0.9999 as shown in Fig. 5.

Accuracy and precision :

Accuracy and precision were investigated by analyzing zolmitriptan tablets (i.e. 2.5 mg tablet) in three independent replicates on the same day (Intra-day accuracy and precision) and on three consecutive days (Inter-day accuracy and precision).

To validate prediction ability of suggested method, different concentrations of zolmitriptan samples were prepared and analyzed. The results were satisfactory. Using standard addition technique, the method was further validated. The standard addition technique was carried out

Table 3. Determination of zolmitriptan in pharmaceutical dosage forms applying the standard addition technique

Proposed method	Taken amount (μg/ml)	Added amount (μg/ml)	Proposed method recovery[a] (%) Zominat tablets	Ziptan tablets	Onset tablets
	10	-	100.16	99.89	100.08
		5	100.14	100.08	99.63
		10	99.96	99.58	99.25
		15	100.09	100.05	99.86
Mean ± S.D.			100.11 ± 0.13	99.9 ± 0.22	99.70 ± 0.35

[a]Average of five determinations.

Table 4. Determination of zolmitriptan in pharmaceutical formulations

Sample	Recovery[a] (± S.D.%) Official method (USP)	Proposed method
Zominat tablets[b]	101.25 ± 0.56	99.17 ± 0.85
t		0.33
F		1.12
Ziptan tablets[b]	99.24 ± 0.85	100.98 ± 0.36
t		0.65
F		1.22
Onset tablets[b]	100.78 ± 0.23	99.96 ± 0.71
t		0.71
F		1.15

[a]Mean ± standard deviation of five determinations.

[b]All tablets contain zolmitriptan 2.5 mg per tablet.

The student *t*-test and *F*-test were calculated using MS Ecxel 2007.

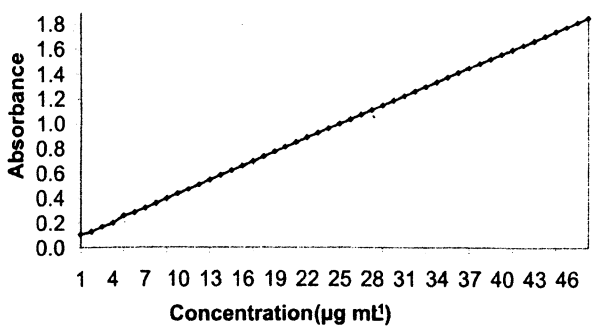


Fig. 5. Beer's law verification range.

by adding excipients with the addition of zolmitriptan at 25% (5 mg), 50% (10 mg) and 75% (15 mg), respectively in sample solution. The percent recoveries of the three concentrations were found to be close to 100%, indicative of high accuracy.

Limit of detection and limit of quantification :

LOD (k = 3.3) and LOQ (k = 10) of the method were established according to ICH definitions. LOD and LOQ of method are reported in Table 1. In this study, LOD and LOQ were based on the standard deviation of the response and the slope of the corresponding curve using the following equations :

$$LOD = 3.3\ S/M;\ LOQ = 10\ S/M$$

where S is the standard deviation of the absorbance of the sample and M is the slope of the calibrations curve.

Conclusion

Based on the results obtained, it can be concluded that the newly proposed spectrophotometric method for the determination of zolmitriptan is rapid, accurate, extraction free and economical. The simplicity, sensitivity and selectivity make the method a suitable alternative to the HPLC methods. Other characteristics such as short performance time, ease of handling and non-usage of organic solvents, also suggest this procedure as a routine laboratory method. Therefore, the proposed method can be adopted for the assay of zolmitriptan in quality control laboratories where modern instruments are not available.

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