Monatsh Chem 139, 1005–1010 (2008)
DOI 10.1007/s00706-008-0894-4
Printed in The Netherlands

Monatshefte für Chemie
Chemical Monthly

Kinetic and maximum-absorbance spectrophotometric methods for the determination of olanzapine

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Received 16 December 2007; Accepted 11 January 2008; Published online 18 February 2008

Abstract Two simple and sensitive spectrophotometric methods were developed for the determination of olanzapine (*OLZ*) based on its oxidation with potassium iodate in a sulfuric acid medium to produce an intense violet-colored species exhibiting a maximum absorbance at 537 nm. The initial rate of formation and the maximum absorbance of the violet-colored oxidized product were monitored, in the first and second methods. The various experimental parameters affecting the rate of development and stability of the oxidized product were carefully studied and optimized. *Beer*'s law was obeyed up to 4.0 and 7.0 $\mu g\,cm^{-3}$ *OLZ* with correlation coefficients of 0.998 and 0.996 ($n = 6$) and detection limits of 0.1 and 0.15 $\mu g\,cm^{-3}$, for the initial rate and maximum absorbance methods. The proposed methods were conveniently applied to the determination of *OLZ* in its dosage forms and in spiked serum samples.

Keywords Kinetic determination; Oxidation; Olanzapine; Dosage forms; Human serum.

Introduction

Olanzapine, 2-methyl-4-(4-methyl-1-piperazinyl)-10*H*-thieno[2,3-*b*][1,5]-benzodiazepine (Scheme 1) is an atypical antipsychotic drug that since its introduction in 1996, has been approved in over 84 countries for treating schizophrenia and related disorders [1].

Published methods for the determination of *OLZ* in pharmaceutical preparations and biological fluids include high-performance liquid chromatography (HPLC) with UV [4–7], electrochemical [8–10] or mass spectrometric detection [11–15], gas chromatography [16, 17], and capillary zone electrophoresis [18, 19]. However, only one spectrophotometric method based on derivatization has been developed for the determination of *OLZ* by its reactions with excess of Ce(IV) or $K_3[Fe(CN)_6]$ and the subsequent determination of the unreacted oxidizing agent [20].

On the other hand, kinetic methods of analysis are becoming increasingly popular because of their simplicity, speed, and precision compared to equilibrium methods [21]. When coupled with spectrophotometric monitoring, kinetic methods offer enhanced selectivity arising from the fact that measurements are carried out at the beginning of the reaction where the perturbation of other agents present in the system is less likely to occur. Moreover, the initial rate is derived from measuring the absorbance change with time instead of measuring a fixed absorbance value and therefore eliminating the possibility of any interference from any absorbing species in the complex sample matrix.

The aim of the present work is to develop simple, sensitive, and low-cost kinetic methods for the determination of *OLZ* in pharmaceutical formulations and spiked human serum samples. The methods,

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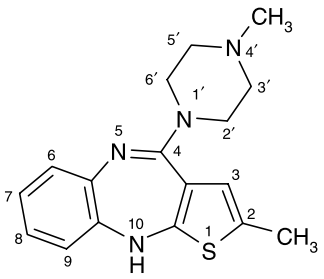

Scheme 1

reported here for the first time, are based on the reaction of the drug with iodate in a sulfuric acid medium to produce an intense violet-colored oxidized product whose initial rate of formation and maximum absorbance were monitored spectrophotometrically.

Results and discussion

Preliminary studies

Acidic aqueous solutions of *OLZ* have no absorption maxima in the visible range of the spectrum (Fig. 1, curve 1). However, in preliminary experiments, these solutions gave a transient violet-colored oxidized product in the presence of oxidizing agents, such as $K_2S_2O_8$, $Ce(SO_4)_2$, $KBrO_3$, KIO_4, KIO_3, HNO_3, and $(NH_4)VO_3$. The stability and rate of development of that product were greatly affected by the type and concentration of the acid and the oxidizing agents used. In the presence of the stronger oxidizing agents, $K_2S_2O_8$, $Ce(SO_4)_2$, $KBrO_3$, and KIO_4, the oxidized product was unstable and decolorized very rapidly. However, in the presence of $(NH_4)VO_3$, the color of the oxidized product was very weak and developed only after warming. Therefore, the effects of KIO_3 and HNO_3 concentrations were thoroughly investigated, and were found to give the same oxidized product that exhibited three absorption maxima in the visible range of the spectrum, at 537, 431, and 341 nm (Fig. 1, curve 2). The band at 341 nm is not well resolved though it has the highest intensity; moreover, it shrinks to a small shoulder and finally disappears completely upon using lower concentrations of *OLZ*. Therefore, the band at 537 nm, that is well resolved and did not disappear upon using lower concentrations of *OLZ*, was used for further studies. The absorbance of the violet-colored oxidized product increased with time reaching a maximum (equilibrium) value that remained constant for a while and then began to decrease resulting eventually in the formation of a colorless product that is believed to be a sulfoxide derivative [1, 2]. The maximum absorbance and the initial rate of color development were found to be directly proportional to *OLZ* concentration; therefore, the maximum absorbance and the initial rate methods were adopted for *OLZ* quantification.

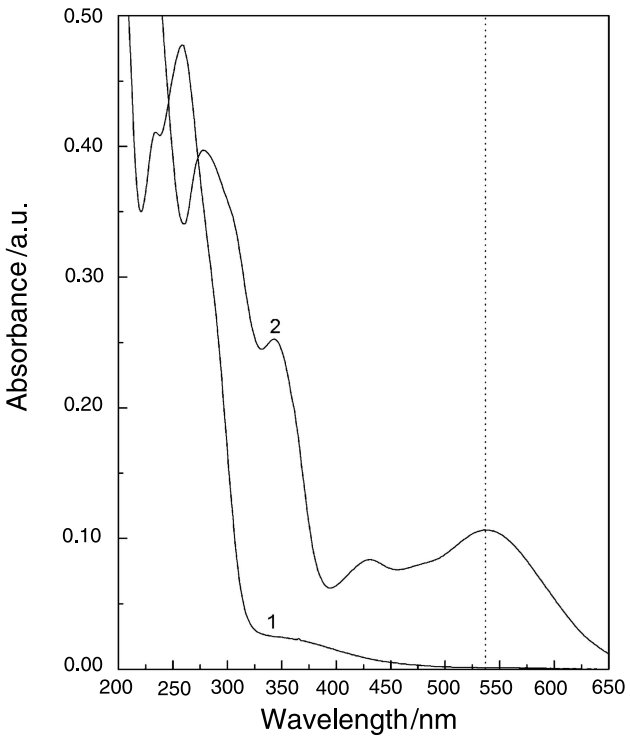

Fig. 1 Absorption spectrum of 7.0 μg cm^{-3} olanzapine in the presence of 3.0 *M* H_2SO_4 (*curve 1*) and its oxidized product with 0.4 m*M* iodate at 25°C (*curve 2*), against water as a reference

The properties of the colored oxidized product that was believed to be a 10-$N^{+\bullet}$ radical cation derivative of *OLZ* [1, 2], as well as the various experimental parameters affecting its development and stability were carefully studied and optimized. Such factors were changed individually while the others were kept constant. The studied factors included the type and concentration of the acid medium and the oxidizing agent, the reaction temperature, and the presence of excipients.

Effect of acid type and concentration

Nitric and perchloric acids were tested in preliminary experiments as acid media and oxidizing agents as well. In comparison with perchloric acid, nitric

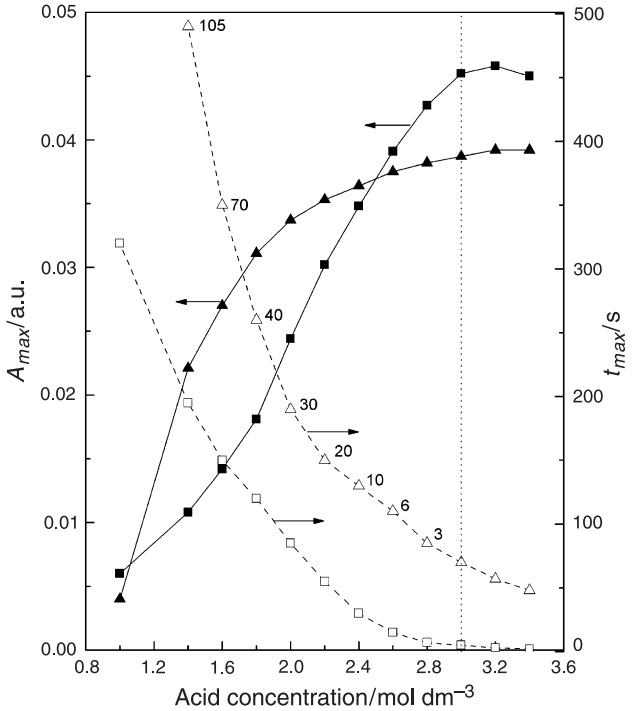

Fig. 2 Effects of H_2SO_4 acid (■, □) and HNO_3 acid (▲, △) concentrations on A_{max} (■, ▲) and t_{max} (□, △). Induction periods, in seconds, recorded with various concentrations of HNO_3 were given close to t_{max} data points. Conditions were: 3.0 μg cm^{-3} olanzapine and 0.4 m*M* iodate at 25°C. Iodate was not added in case of HNO_3 that acted as an acid and an oxidizing agent as well

acid gave much higher maximum absorbance values. The effects of various concentrations of nitric acid were studied at 25°C. The maximum absorbance values (A_{max}) increased with nitric acid concentrations up to 3.0 mol dm^{-3} and remained almost constant up to 3.4 mol dm^{-3} HNO_3 (Fig. 2). However, the times required to reach such maximum absorbances (denoted as t_{max}) were very high especially at low HNO_3 concentrations; moreover, such reactions were characterized by long induction periods that decreased with increasing the nitric acid concentration (the induction period is defined as the time interval between the addition of the last reactant and the appearance of the reaction product, and throughout which the reaction does not seem to proceed [21]). To reduce such t_{max} and induction periods, sulfuric acid was tested based on its ability to stabilize similar short-lived radical cations [22, 23].

Therefore, the effects of various concentrations of sulfuric acid were studied in the presence of 0.40 mmol dm^{-3} iodate as an oxidizing agent (Fig. 2). The oxidation reaction in the presence of sulfuric acid was much faster than that in the presence of nitric acid so that t_{max} values greatly reduced and the induction periods completely disappeared. The A_{max} values increased with sulfuric acid concentration up to 3.0 mol dm^{-3} and then remained almost constant up to 3.4 mol dm^{-3}. At higher H_2SO_4 concentrations the oxidized product became unstable so that the developing violet color reached its maximum absorbance almost instantaneously and after that it faded very rapidly forming a colorless species. Therefore, a sulfuric acid concentration of 3.0 mol dm^{-3} was adopted in the recommended procedure based on maximum absorbance measurements. On the other hand, the rate of color development increased exponentially with sulfuric acid concentration over the entire range but the linearity of the *A*-*t* graphs became poor at concentrations $\geq$ 3.2 mol dm^{-3}; therefore, a sulfuric acid concentration of 3.0 mol dm^{-3} was adopted in the recommended procedure based on rate measurements.

Effect of iodate concentration

The effect of iodate concentration was studied over the range of 0.005–0.60 mmol dm^{-3} IO_3^-.

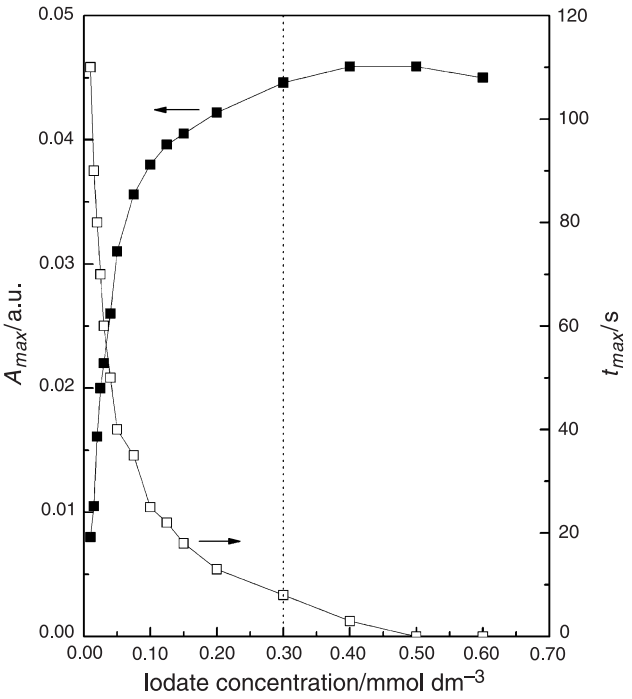

Fig. 3 Effects of iodate concentrations on A_{max} (■) and t_{max} (□). Except for the abscissa variable conditions were as given in Fig. 2

The maximum absorbance of the oxidized product (A_{max}) increased with iodate concentrations up to 0.30 mmol dm^{-3} and then remained almost constant up to 0.60 mmol dm^{-3} IO_3^-. Meanwhile, the time required to reach such maximum absorbance (t_{max}) rapidly decreased with iodate concentration reaching 3 s only at 0.40 mmol dm^{-3} IO_3^- (Fig. 3). Therefore, an iodate concentration of 0.40 mmol dm^{-3} was adopted in the recommended procedure. On the other hand, the rate of color development increased gradually with iodate concentrations in the range of 0.005–0.07 mmol dm^{-3} iodate; however, at iodate concentrations $\geq$0.05 mmol dm^{-3} the linearity of the *A-t* graphs became poor; therefore, an iodate concentration of 0.03 mmol dm^{-3} was adopted in the recommended procedure for the initial rate measurements.

Effect of reaction temperature

The oxidation of *OLZ* with iodate was studied at different temperatures (20–40°C). The initial rates of formation and maximum absorbance values of the oxidized product decreased slightly with increasing temperature (Fig. 4). This may be attributed to the increased instability of the formed radical cation with increasing temperature. Therefore, working at a room temperature of 25°C was adopted in the recommended procedure.

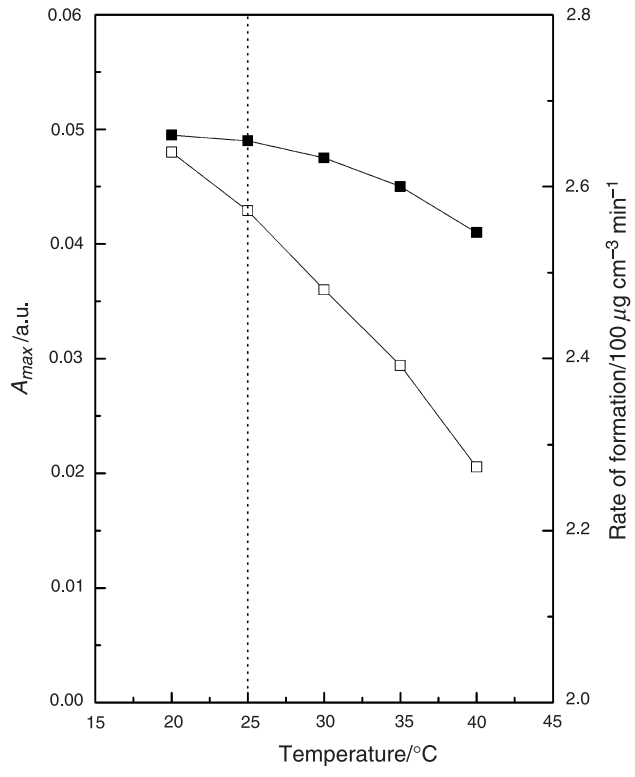

Fig. 4 Effects of temperature on A_{max} and initial rate of formation of the oxidized product of olanzapine. Except for the abscissa variable conditions were as given in Fig. 2

Effects of excipients

A systematic study of the effects of excipients was performed by adding a known amount of the excipient to a 10 cm^3 of 10 μg cm^{-3} *OLZ*, filtering off the insoluble excipient when necessary, washing the residue, diluting the combined filtrates and washings in a 25 cm^3 volumetric flask, and analyzing an aliquot of 1500 mm^3 following the recommended procedure. The results revealed that no interference was observed from 200 fold excess of excipients, such as lactose, glucose, mannitol, starch, gelatin, aspartame, povidone, crosspovidone, hydroxypropylcellulose, sodium citrate, magnesium chloride, magnesium stearate, talc, and titanium dioxide that are commonly found in pharmaceutical formulations of *OLZ*. Moreover, 20 fold excess of indigo carmine and ferric oxide did not interfere (the 2.5–10 mg tablets are imprinted with a blue ink containing indigo carmine-aluminum complex; the 15 mg tablets are coated with a blue-colored very thin film containing indigo carmine and the 20 mg tablets are coated with a red-colored thin film containing ferric oxide).

Calibration, limit of detection, and precision

Calibration graphs (100tan α *vs.* conc. and A_{max} *vs.* conc.) obtained, following the recommended procedures were found to be linear up to 4.0 and 7.0 μg cm^{-3} *OLZ*, with detection limits (based on the $3S_b$-criterion, three times the standard deviation of the blank divided by the slope of the respective calibration graph [22]) of 0.10 and 0.15 μg cm^{-3} *OLZ*, for the initial rate and maximum absorbance methods. Linear regression analysis of the data at 25°C gave the following equations:

$$100\tan\alpha = [0.01 \pm 0.027] + [0.85 \pm 0.029][C] \quad (r = 0.998)$$

$$A_{max} = [0.001 \pm 8.3 \times 10^{-4}] + [0.0165 \pm 7.4 \times 10^{-4}][C] \quad (r = 0.996)$$

where $[C]$ is the *OLZ* concentration in μg cm^{-3}.

Table 1 Determination of olanzapine in tablets and spiked human serum samples[a]

Sample	HPLC method [14]	Initial rate method			Maximum absorbance method		
	Found % ± S.D.	Found % ± S.D.	t[b]	$F_{6,6}$[b]	Found % ± S.D.	t[b]	$F_{6,6}$[b]
Zyprexa 5 mg/tablet[c]	98.7 ± 1.3	99.2 ± 1.2	0.63	1.2	97.5 ± 1.6	1.30	1.5
Zyprexa 10 mg/tablet[c]	101.2 ± 1.7	100.4 ± 1.5	0.79	1.3	99.7 ± 2.0	1.28	1.4
Serum 1[d]	99.1 ± 1.6	97.6 ± 1.8	1.39	1.3	97.1 ± 1.9	1.80	1.4
Serum 2[d]	98.9 ± 1.5	97.2 ± 1.7	1.68	1.3	100.5 ± 2.1	1.39	2.0

[a] Data are averages of six replicate determinations ($n = 6$). Reaction conditions were those given in the recommended procedure
[b] The tabulated student's t-test ($t_{(n=6)}$) values and the variance ratio ($F_{6,6}$) values at the 95% confidence level are 2.45 and 4.28
[c] Product of Eli Lilly and Company, Basingstoke, Hampshire, UK
[d] Serum samples from healthy human targets were spiked to contain 3 $\mu g\,cm^{-3}$ olanzapine and directly analyzed

The small values of standard deviations of the slopes and intercepts indicate the high precision of the proposed methods. Moreover, the precision of the method was further assessed according to the IUPAC recommendations [24] by analyzing 0.3, 1.0, 2.0, and 4.0 $\mu g\,cm^{-3}$ *OLZ* in aqueous solutions following both methods and gave recoveries of $\geq$99.3%, where the within-day *RSD*s were $\leq$1.3% and the between-day *RSD*s were $\leq$1.7% ($n = 6$) and the student's t-test values were $\leq$2.0, showing that the t-test could not detect any systematic error and revealed the high accuracy and precision of the proposed method (the tabulated t-value for the 95% confidence level and $n = 6$ is 2.45 [24]).

Applications

The proposed methods were directly applied to the quantification of *OLZ* in commercial tablets of Zyprexa and in spiked human serum samples from healthy targets (Table 1). The data were compared with those obtained following a published HPLC method [14]. Statistical analysis of the results did not detect any significant difference between the performances of the proposed methods and the reference HPLC method regarding accuracy and precision as revealed by the student's t-test and the variance ratio F-test [24].

Conclusion

It can be concluded that the proposed methods have the advantages of high sensitivity (small values of the *LOD*), simplicity, no need for solvent extraction or separation steps before the analysis, and the absence of any interference from tablet excipients. In addition, the proposed methods are accurate and precise as indicated by the good recoveries of the drug, the low *RSD* values (both within-day and between-day), and the good agreement with a typical reference HPLC method. The above findings substantiate the usefulness of the proposed methods for the assay and quality control of *OLZ* both in the pure and dosage forms.

Experimental

Apparatus

Maximum absorbance and kinetic measurements were made on a UV-VIS 1601 Shimadzu double beam spectrophotometer (Kyoto, Japan) equipped with a thermostated cell holder with 10 mm matched cells. The cell compartment of the spectrophotometer was thermostatically controlled at 25 ± 0.1°C by circulating water from a PolyScience (IL, USA) water bath. Eppendorf vary pipettes (Westbury, NY, USA), 10–100 and 100–1000 mm^3 were used to deliver accurate volumes.

Reagents

All reagents were of analytical grade and were used as received. Fresh distilled, deionized water was used throughout. Olanzapine was supplied by Eli Lilly and Co. Ltd. (Basingstoke, England) and was used as received. Tablets containing the drug were obtained from the local market. Potassium iodate, sulfuric acid, and nitric acid were purchased from Merck, Darmstadt, Germany. A stock standard solution of 250 $\mu g\,cm^{-3}$ *OLZ* was prepared in 0.01 $mol\,dm^{-3}$ H_2SO_4 and was further diluted when required. Aqueous working solutions of 6.0 $mol\,dm^{-3}$ HNO_3, 9.0 $mol\,dm^{-3}$ H_2SO_4, 12.0 $mmol\,dm^{-3}$ KIO_3, and 0.9 $mmol\,dm^{-3}$ KIO_3 were also prepared.

Treatment of tablets

The contents of ten tablets under investigation were weighed, ground into fine powder, and mixed well. An accurately weighed portion of the powder equivalent to one tablet of *OLZ* was transferred to a 50 cm^3 beaker containing about 25 cm^3 water and 1.0 cm^3 of the working sulfuric acid solution, placed in the ultrasonic bath for 10 min, and filtered into

a 100-cm^3 volumetric flask. The residue and filter paper were thoroughly washed with several few cm^3 of water. The washings and extracts were combined in the same measuring flask, completed to the mark with water, and further diluted if required.

Recommended procedure for the maximum absorbance method

Transfer aliquot volumes, containing $\leq 21\,\mu g$ of the unknown *OLZ* solution or the working *OLZ* standard solution, into a clean, dry, and thermostated spectrophotometric cell. Add $1000\,mm^3$ of the working sulfuric acid solution, dilute with water to $2900\,mm^3$, and add $100\,mm^3$ of the $12.0\,mmol\,dm^{-3}$ KIO_3 working solution. Shake well, return the cell to its holder, and press the start button of the spectrophotometer to record the maximum absorbance that develops within a few seconds, at 537 nm against water as a reference. Determine the content of the tablet either from the similarly constructed calibration graph or its linear regression equation.

Recommended procedure for the kinetic method

Transfer aliquot volumes, containing $\leq 12\,\mu g$ of the unknown *OLZ* solution or the working *OLZ* standard solution, into a clean, dry, and thermostated spectrophotometric cell. Add $1000\,mm^3$ of the working sulfuric acid solution, dilute with water to $2900\,mm^3$, and add $100\,mm^3$ of the $0.9\,mmol\,dm^{-3}$ KIO_3 working solution. Shake well, return the cell to its holder, and start recording the absorbance change with time at 537 nm, against water as a reference. Calculate the rate of development of the colored oxidized product ($100\tan\alpha$) in min^{-1} from the initial linear part of the *A-t* graph within 30 s of pressing the start button. Determine the content of the tablet either from the similarly constructed calibration graph or its linear regression equation.

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