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DEVELOPMENT OF THE KINETIC-SPECTROPHOTOMETRIC METHOD FOR QUANTITATIVE DETERMINATION OF ZOPICLONE IN TABLETS BY THE PERHYDROLYSIS REACTION

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Key words: Zopiclone; perhydrolysis; kinetic spectrophotometric determination; 3,3',5,5'-tetramethylbenzidine

A simple and express method for the quantitative determination of zopiclone in model solutions of the substance and in "Zopiclone" tablets, 7.5 mg, by the kinetic-spectrophotometric method according to 3,3',5,5'-tetramethylbenzidine oxidation has been developed. It is based on the system of two coupled reaction: 4-methyl-1-piperazineperoxycarboxylic acid generated in zopiclone perhydrolysis reacts with the excess of hydrogen peroxide in the weak alkaline medium with formation of coloured 3,3',5,5'-tetramethyldiphenylquinone diimine (λ_{max} = 420 nm, pH = 8.4). The reaction is performed spectrophotometrically by measuring the rate of change of the absorbance at 420 nm. The method was used for constructing the calibration graph. The initial rate of the reaction was obtained from the linear site of the slope of the initial tangent to the absorbance-time curve. In the pH range of 8.2-8.5 the rate of the coloured product formation becomes maximum. The calibration graph for zopiclone has a linear dependence in the range of 6-36 mg/l with the limit of detection (LOD) and quantitation (LOQ) of 1.81 and 6.04 mg/l, respectively. For five determinations of 18, 24 and 30 mg/l of zopiclone RSD is 1.81, 1.46 and 1.69%, respectively. The analytical performance of the method was validated statistically with respect to LOD, LOQ, accuracy, precision and linearity for zopiclone estimation in a pure substance and the results were satisfactory. "Zopiclone" tablets compared to the reference method contain 99.83±1.19% of $C_{17}H_{17}ClN_6O_3$ (RSD = 0.96% , δ = -0.17%). The assay of zopiclone in the presence of its hydrolysis products without preliminary separation is an important advantage of the method.

Zopiclone (6-(5-chloro-2-pyridyl)-6,7-dihydro-7-oxo-5H-pyrrolo-[3,4-b]pyrazin-5-yl-4-methylpiperazine-1-carboxylate) is an ester with similar sedative, anxiolytic, muscle relaxant, amnestic, and anticonvulsant properties to those of benzodiazepines. It is used as a hypnotic in the short-term treatment of insomnia [10]. Zopiclone is the subject of the British Pharmacopoeial monographs. The B.Ph. describes a non-aqueous titrimetric method for its determination in its bulk and liquid chromatography for tablets [4].

Some analytical procedures have been described for zopiclone determination in bulk, pharmaceutical formulations and/or biological fluids, which include liquid chromatography coupled with different highly sensitive detectors [5-8, 12], gas chromatography [9], capillary electrophoresis [11].

A simple kinetic method of the zopiclone determination by its perhydrolysis product with *p*-phenetidine as a chromogenic substrate was previously proposed [1]. The assay of zopiclone in the presence of its hydrolysis products and sensitivity is a distinctive advantage of this method.

The aim of this work was to develop a new kinetic method of the quantitative determination of zopiclone using the indicator reaction of 3,3',5,5'-tetramethylbenzidine (TMB) oxidation by hydrogen peroxide in the weak alkaline medium.

Materials and Methods

97% 3,3',5,5'-tetramethylbenzidine dihydrochloride hydrate (Aldrich, Germany), 96% ethyl alcohol (Dubovyazivskiy distillery, Ukraine), 30% solution of hydrogen peroxide prepared from 50% hydrogen peroxide of medical quality ("Inter-Synthesis" LLC, Borislav, Ukraine), double-distilled water (DDW). "Zopiclone" tablets, 7.5 mg, No. 10 ("Lubnypharm" JSC, Ukraine), batch No. 10313 (quality certificate No. 316) were used for the analysis.

To create and maintain the pH required 0.2 M phosphate buffer with pH 8.4 prepared according to Green was used. For this purpose 12 g of NaH_2PO_4 was dissolved in 450 ml of DDW and 50.6 ml of 1.9 mol/l NaOH was added, pH was monitored potentiometrically.

Preparation of TMB working solution with the molar concentration of 1×10^{-2} mol/l. Dissolve 0.313 g of the accurately weighed portion of TMB in a 100 ml volumetric flask in 50 ml of 96% ethanol. Dilute to the volume with DDW at 20°C and shake thoroughly.

Preparation of zopiclone working standard solution (WSS), 0.3 mg/ml ($c = 7.7\times10^{-4}$ mol/l). Dissolve 0.0300 g of the zopiclone substance in 96% ethanol in a 100 ml volumetric flask, dilute the solution to the volume with the same solvent and mix thoroughly.

All the chemicals and reagents were of analytical grade and the solutions were freshly prepared.

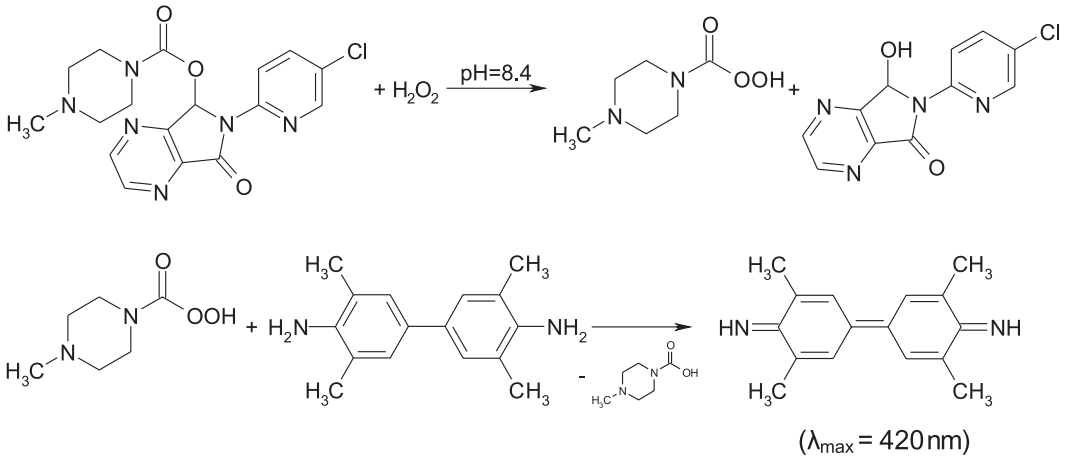

Fig. 1. Chemistry of zopiclone perhydrolysis and coupled TMB peroxyacid oxidation.

All spectrophotometric measurements were made on a SF-46 spectrophotometer (LOMO, USSR) with 1 cm matched quartz cells. The pH of the solutions was monitored by a glass electrode of ESL 43-07 type (the reference electrode – a silver/silver chloride electrode of EVL-1M3.1 type) on a laboratory I-130 ionometer ("Analytpribor" Research and Production Association).

The method of obtaining the data for the calibration curve. Transfer consistently 10 ml of 0.2 mol/l buffer solution (pH = 8.4) into a 25 ml volumetric flask adding from 1 to 5 ml of 0.3 mg/ml WSS, 6.0 ml of 1×10^{-2} mol/l TMB solution, 2 ml of 5,6 mol/l H_2O_2, dilute to the volume with DDW at 20°C and shake thoroughly for 30 sec. Measure the optical density of the solution obtained at 420 nm vs. blank solution (without the substance to be examined). Control the time from the moment of mixing the solution with the stop-watch.

The results obtained were processed according to the recommendations of the International Union of Pure and Applied Chemistry (IUPAC) [3] and the State Pharmacopeia of Ukraine (SPhU) [2] using mathematical statistics methods. Accuracy verification was performed by "input-output" analysis of the model solution. The content of the active substance in "Zopiclone" tablets, 7.5 mg, was determined by the method of standard.

Results and Discussion

The reaction involved in the present study is based on the perhydrolysis reaction of zopiclone with the excess of hydrogen peroxide in the weak alkaline medium forming 4-methyl-1-piperazinepercarboxylic acid (PA). Then PA formed reacts with TMB to give coloured 3,3',5,5'-tetrametyldiphenoquinone diimine derivative, which exhibits absorption maxima at 420 nm, pH = 8.4 (Fig. 1).

The present study was devoted to the involvement of this colour reaction in the determination of zopiclone.

It has been shown that hydrolytic cleavage products that are present do not interfere the zopiclone determination. The maximal rate of the coloured product formation was observed in the pH range of 8.2-8.5. The initial rate of the reaction was obtained from the linear site (3-10 min) of the slope of the initial tangent to the absorbance–time curve (Fig. 2).

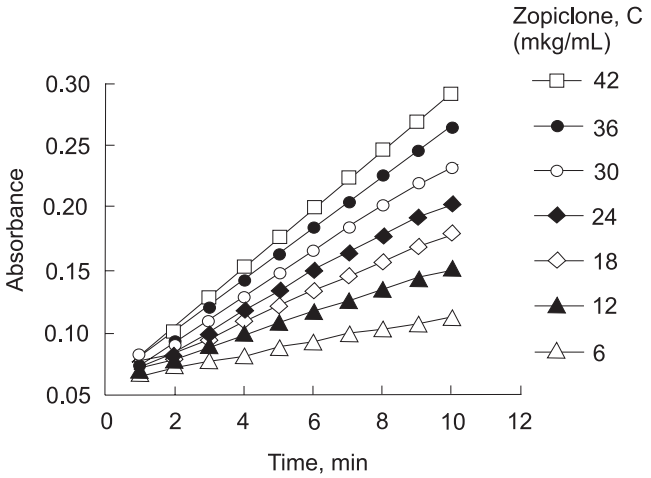

Fig. 2. Absorbance-time curves of 3,3',5,5'-tetrametyldiphenoquinone diimine accumulation for the reaction of different concentrations of zopiclone with TMB (2.4×10^{-3} mol/l) and H_2O_2 (0.448 mol/l) at pH=8.4.

As can be seen from Fig. 2, the initial rate increases with the increase of zopiclone concentration. Since the concentration of TMB and hydrogen peroxide is much higher than the analyte concentration, the course of the indicator reaction became of the pseudo first order with respect to zopiclone, therefore, its perhydrolysis is the limiting stage of the process. The calibration curve obtained by plotting the conditional initial rate of the reaction versus the final concentration of zopiclone under the optimum conditions showed a linear relationship in the range of 6-36 mkg/ml (Fig. 3).

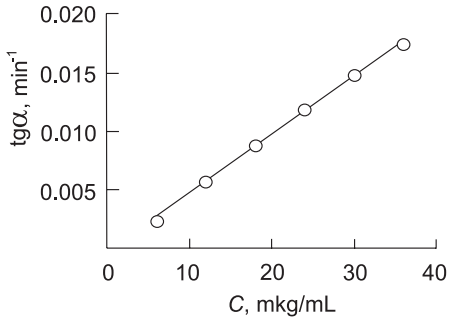

Fig. 3. The calibration curve of the zopiclone determination. *c* (TMB) = 2.4×10^{-3} mol/l; *c* (H_2O_2) = 0.448 mol/l; pH = 8.4.

Table 1

The data of regression analysis

Parameters	Data
Beer's Law Limit (mg/ml)	0.006-0.036
Regression equation*	tgα = 0.5058×*C* – 0.0004
S_b	0.013224
S_a	0.000309
Correlation coefficient (r)	0.9986
LOD (mkg/ml)	1.81
LOQ (mkg/ml)	6.04

* tgα = b × *C* + *a* where *C* is the concentration of zopiclone, mg/ml, tgα is the conditional initial reaction rate, min^{-1}.

The method of least square was used to estimate the regression characteristics of the calibration curve obtained (Tab. 1).

The results of the analysis of zopiclone in model solutions and in tablets are shown in Tab. 2 and Tab. 3, respectively.

The linearity of the method proposed was estimated in the normalized coordinates (r=0.9998). The values *a* and |b-1| do not exceed the confidence intervals of their uncertainty (the requirement of statistical insignificance), $a \le t(95\%, n\text{-}2) \times s_a$ (-3.20 ≤ 8.01) and $|b\text{-}1| \le t(95\%, n\text{-}2) \times s_b$ (0.00023 ≤ 0.08222).

The assay for the active substance in tablets. Shake carefully the accurately weighed portion of the tablet powder equivalent to 7.5 mg of zopiclone with 10 ml of 96% ethyl alcohol, filter to a 25 ml volumetric flask, dilute to the volume with the same solvent, wash the precipitate and mix thoroughly.

Transfer consistently 10 ml of 0.2 mol/l buffer solution (pH = 8.4) into a 25 ml volumetric flask adding 2 ml of the test zopiclone solution and then continue as when plotting the calibration curve. Similarly perform the experiment with WSS.

The zopiclone content in one tablet, *X* (g), was calculated by the formula:

$$X = \frac{C_{st} \cdot tg\alpha \cdot 25 \cdot 25 \cdot \overline{m}}{tg\alpha_{st} \cdot 2 \cdot 1000 \cdot m_w},$$

where C_{st} – is the concentration of zopiclone in WSS, mg/ml; tgα – is the tangent of the angle slope in the experiment with the test solution of zopiclone, min^{-1}; $tg\alpha_{st}$ – is the tangent of the angle slope in the experiment with WSS, min^{-1}; 25 – is the volume of the volumetric flask, ml; m_w – is the amount of zopiclone in a tablet, g; $\overline{m}$ – is the avarage tablet weight, g.

Table 2

Metrological characteristics of the results of the kinetic determination of zopiclone in model solutions (*n* = 5, *P* = 0.95)

Metrological characteristics	Amount taken, mkg/ml		
	0.018	0.024	0.030
$\bar{x}$	0.0183	0.0243	0.0303
s	3.31×10^{-4}	3.54×10^{-4}	5.12×10^{-4}
$s_{\bar{x}}$	1.48×10^{-4}	1.58×10^{-4}	2.29×10^{-4}
$\Delta\bar{x}$	4.11×10^{-4}	4.39×10^{-4}	6.36×10^{-4}
RSD, %	1.81	1.46	1.69
ε, %	2.24	1.81	2.10
δ, %	1.79	1.24	1.04

Table 3

Metrological characteristics of the results of the kinetic determination of zopiclone in tablets (*n* = 5, *P* = 0.95)

The zopiclone content in one tablet, g	Recovery		Metrological characteristics
	g	%	
0.00759* $^{+7.5\%}_{-7.5\%}$	0.007513 0.007513 0.007386 0.007576 0.007449	100.17 100.17 98.485 101.01 99.33	$\bar{x}$ =0.007487 (99.83%) s = 7.20×10^{-5} s= 3.22×10^{-5} $\Delta\bar{x}$ = 8.94×10^{-5} RSD = 0.96% ε = 1.19%; δ = -0.17%

Note: *determined by the current QC method.

CONCLUSIONS

1. A simple and express kinetic spectrophotometric method for determination of zopiclone has been proposed.

2. The assay of zopiclone in the presence of its hydrolysis products without preliminary separation is an important advantage of the method described.

3. The analytical method for quantitative determination of zopiclone in the substance has been validated statistically according to the criteria of LOD, LOQ, accuracy, precision and linearity. The results obtained are satisfactory; LOQ is 6.04 mg/l.

4. Zopiclone tablets compared to the reference method contain 99.83±1.19% of $C_{17}H_{17}ClN_6O_3$ (RSD = 0.96%, δ = -0.17%).

REFERENCES

1. *Блажеєвський М.Є. // Збірник доповідей та повідомлень науково-практичної конференції «Стан і розвиток сухопутних військ на сучасному етапі. Проблеми розвитку та озброєння військової техніки». – Х.: ХІТВ НТУ «ХПІ», 2006. – С. 94-96.*
2. *Державна фармакопея України / Державне підприємство «Науково-експертний фармакопейний центр». – 1-е вид. – Доп. 2. – Х.: Державне підприємство «Науково-експертний фармакопейний центр», 2008. – 620 с.*
3. *Экспериандова Л.П., Беликов К.Н., Химченко С.В., Бланк Т.А. // ЖАХ. – 2010. – Т. 65, №3. – С. 229-234.*
4. *British Pharmacopoeia. – London: The Stationery Office on behalf of the Medicines and Healthcare products Regulatory Agency, 2009. – 10952 p.*

5. *Gebauer M., Alderman C. // Biomed. Chromatography. – 2002. – Vol. 16, №4. – P. 241-246.*
6. *Mistri H., Jangid A., Pudage A., Shrivastav P. // J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. – 2008. – Vol. 864, №1. – P. 137-148.*
7. *Nirogi R., Kandikere V., Mudigonda K. // Biomed. Chromatogr. – 2006. – Vol. 20, №8. – P. 794-799.*
8. *Rossi S., Anzillotti L., Castrignanò E., Frison G., Zancanaro F., Chiarotti M. // Drug Testing and Analysis. – 2014. – Vol. 6, №3. – P. 226-233.*
9. *Shu C., Zhang L., Dong Y., Wang S., Niu X., Liu J. // Chinese J. of Forensic Medicine. – 2013. – Vol. 28, №1. – P. 46-48.*
10. *Sweetman S (Ed), Martindale: The complete drug reference. London: Pharmac. Press. Electronic version, (Edition 2007)*
11. *Tonon M., Bonato P. // Electrophoresis. – 2012. – Vol. 33, №11. – P. 1606-1612.*
12. *Tonon M., Jabor V., Bonato P. // Anal. Bioanal. Chem. – 2011. – Vol. 400, №10. – P. 3517-3525.*

РОЗРОБКА КІНЕТИКО-СПЕКТРОФОТОМЕТРИЧНОЇ МЕТОДИКИ КІЛЬКІСНОГО ВИЗНАЧЕННЯ ЗОПІКЛОНУ В ТАБЛЕТКАХ ЗА РЕАКЦІЄЮ ПЕРГІДРОЛІЗУ

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Ключові слова: *зопіклон; пергідроліз; спектрофотометрія; кількісне визначення; 3,3',5,5'-тетраметилбензидин*

Розроблена проста за апаратурним оформленням та експресна методика кількісного визначення зопіклону у модельних розчинах субстанції і таблетках «Зопіклон» по 7,5 мг кінетико-спектрофотометричним методом за індикаторною реакцією окиснення 3,3',5,5'-тетраметилбензидину. В її основу покладено систему двох спряжених реакцій: генерована в реакції пергідролізу (з надлишком гідрогену пероксиду) зопіклону 4-метил-1-піперазинпероксикарбонова кислота реагує з індикаторною речовиною з утворенням забарвленого 3,3',5,5'-тетраметилдифенохінондііміну, λ_{max}=420 нм при pH=8,4, за світловбиранням якого і здійснюють визначення. Тангенс нахилу лінійної ділянки (3-10 хв) залежності зростання поглинання у часі було взято за умовну початкову швидкість реакції. Максимальна швидкість утворення 3,3',5,5'-тетраметилдифенохінондііміну спостерігалась в інтервалі pH 8,2-8,5. Градуювальний графік лінійний в межах 6-36 мкг/мл. Межа виявлення та кількісного визначення становить 1,81 та 6,04 мкг/мл відповідно. Для п'ятиразових визначень 18, 24 і 30 мкг/мл зопіклону RSD становить 1,81, 1,46 і 1,69% відповідно. Здійснена валідація аналітичної методики за критеріями МВ, МКВ, правильності, збіжності і лінійності при визначенні зопіклону у субстанції. Вміст діючої речовини у таблетках «Зопіклон» по 7,5 мг у порівнянні зі знайденним його значенням за чинною МКЯ становить 99,83±1,19% (RSD = 0,96%, δ = -0,17%). Важливою перевагою, яка вигідно відрізняє новоопрацьовану методику, є можливість здійснення визначення вмісту схильного до гідролітичного розкладення препарату в присутності продуктів його гідролізу без попереднього розділення.

РАЗРАБОТКА КИНЕТИКО-СПЕКТРОФОТОМЕТРИЧЕСКОЙ МЕТОДИКИ КОЛИЧЕСТВЕННОГО ОПРЕДЕЛЕНИЯ ЗОПИКЛОНА В ТАБЛЕТКАХ ПО РЕАКЦИИ ПЕРГИДРОЛИЗА

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Ключевые слова: *зопиклон; пергидролиз; спектрофотометрия; количественное определение; 3,3',5,5'-тетраметилбензидин*

Разработана простая и экспрессная методика количественного определения зопиклона в модельных растворах субстанции и таблетках «Зопиклон» 7,5 мг кинетико-спектрофотометрическим методом по индикаторной реакции окисления 3,3',5,5'-тетраметилбензидина. В основу ее положена система двух сопряженных реакций: генерированная в реакции пергидролиза зопиклона 4-метил-1-пиперазинпероксикарбоновая кислота реагирует с индикаторным веществом с образованием окрашенного 3,3',5,5'-тетраметилдифенохинондиимина, λ_{max} = 420 нм при pH = 8,4, по светопоглощению которого и осуществляют определение. Тангенс наклона линейного участка (3-10 мин) зависимости роста поглощения во времени был принят как условная начальная скорость реакции. Максимальная скорость образования 3,3',5,5'-тетраметилдифенохинондиимина наблюдалась в интервале pH 8,2-8,5. Градуировочный график линейный в пределах 6-36 мкг/мл. Предел обнаружения и количественного определения составляет 1,81 и 6,04 мкг/мл соответственно. Для пятикратных определений 18, 24 и 30 мкг/мл зопиклона RSD составляет 1,81, 1,46 и 1,69% соответственно. Осуществлена валидация аналитической методики по критериям предела обнаружения, предела количественного определения, правильности, сходимости и линейности при определении зопиклона в субстанции. Содержание действующего вещества в таблетках «Зопиклон» 7,5 мг в сравнении с найденным его значеним по действующей МКК составляет 99,83±1,19% (RSD = 0,96%, δ = -0,17%). Важным преимуществом, которое выгодно отличает разработанную методику, является возможность определения содержания склонного к гидролитическому разложению препарата в присутствии продуктов его гидролиза без предварительного разделения.