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Original Scientific Article

Novel Kinetic Spectrophotometric Method for Determination of Tiopronin [*N*-(2-Mercaptopropionyl)-Glycine]

Lea Kukoc-Modun* and Njegomir Radić

Faculty of Chemistry and Technology, University of Split, Teslina 10/V, 21000 Split, Croatia

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Abstract. Novel simple kinetic spectrophotometric method for the determination of tiopronin [*N*-(2-mercaptopropionyl)-glycine, MPG] in pharmaceutical formulation has been developed and validated. The proposed method is based on the coupled redox-complexation reaction whose first step is reduction of Fe^{III} by MPG, while second one includes the complexation of Fe^{II}, resulted from preceding redox reaction, with 2,4,6-trypyridyl-*s*-triazine (TPTZ). The stable $Fe(TPTZ)_2^{2+}$ complex exhibits an absorption maximum at $\lambda = 593$ nm.

The initial rate and fixed time (at 3 min) methods were utilized for constructing the calibration graphs. The graphs were linear in concentration ranges from 1.0×10^{-6} to 1.0×10^{-4} mol L^{-1} for both methods with limits of detection 1.3×10^{-7} mol L^{-1} and 7.5×10^{-8} mol L^{-1} for the initial rate and fixed time method, respectively. The proposed methods were successfully applied for the determination of MPG in its commercial pharmaceutical formulation.

Keywords: tiopronin, *N*-(2-mercaptopropionyl)-glycine, kinetic spectrophotometry, initial rate method, fixed time method, pharmaceutical analysis

INTRODUCTION

N-(2-mercaptopropionyl)-glycine (MPG), also named tiopronin, is a synthetic aminothiol antioxidant. Studies have shown that MPG can function as a chelating, cardioprotecting and radioprotecting agent.[1,2] It is also used in treatment of cystinuria,[3,4] rheumatoid arthritis, liver and skin disorders,[5] and as an antidote to heavy metal poisoning.[6] Along with its desired effects, MPG may cause some side effects such as muscle pain, yellow skin or eyes, sore throat or fever, change in taste and smell, etc. Moreover, this drug could induce a dose-related nephrotic syndrome.[7] Therefore, the sensitive determination of MPG in biological samples and pharmaceutical preparation is highly desirable.

Various methods have been proposed for the determination of MPG, such as chemiluminescence,[8] chromatographic,[9–13] classical (equilibrium) spectrophotometric methods[14–16] and also spectrophotometric method with catalytic approach.[17]

Spectrophotometric technique is the most widely used in pharmaceutical analysis. The widespread of spectrophotometric methods is attributed to their inherent simplicity, economic advantages, and wide availability in most quality control laboratories. Kinetic spectrophotometric methods are becoming of a great interest for the pharmaceutical analysis.[18,19] The application of these methods offers some specific advantages over classical spectrophotometry, such as improved selectivity due to the measurement of the evolution of the absorbance with the reaction time. The authors have recently published a kinetic potentiometric method for the determination of MPG in pharmaceutical formulations.[20]

Surprisingly, to authors' knowledge, there are no published kinetic spectrophotometric methods for the determination of MPG. Also, none of the cited methods for determination of MPG or other thiols has used Fe^{III} and 2,4,6-trypyridyl-*s*-triazine (TPTZ) as reagent solution. In this report a novel, simple and sensitive kinetic spectrophotometric method with TPTZ as the chromogenic reagent for the determination of MPG is described and validated. The initial rate and fixed time methods, after their optimization and validation, are adopted for the determination of MPG in its pharmaceutical formulation.

EXPERIMENTAL

Reagents

All chemicals were of analytical-reagent grade and solutions were prepared in deionised water (Milli Q,

* Author to whom correspondence should be addressed. (E-mail: lkmodun@gmail.com)

Millipore, Saint Quentin, Yvelines, France).

The stock solution of MPG, 1.0×10^{-2} mol L^{-1}, was prepared by dissolving 163.2 mg of MPG (Sigma-Aldrich, St. Louis, USA) in deionised water up to 100.0 mL volume and stored in the dark bottle at 4 °C. Under these conditions the solution is stable for at least 30 days. Working solutions of lower concentrations were prepared daily by appropriate dilution of the stock standard solution with deionised water.

Stock solution of Fe^{III}, 1.0×10^{-2} mol L^{-1}, was prepared by dissolving 270.3 mg of $FeCl_3 \times 6\ H_2O$ (Kemika, Zagreb, Croatia) in a portion of deionised water. Concentrated hydrochloric acid (0.5 mL) was added before making up to a volume of 100.0 mL.

Stock solution of TPTZ (Merck, Darmstadt, Germany), 1.0×10^{-2} mol L^{-1}, was prepared by dissolving 312.3 mg in 2.0 mL HCl [c(HCl) = 6.0 mol L^{-1}], and diluted to 100.0 mL with deionised water. Stock solution of TPTZ was stored in the dark bottle at 4 °C.

Acetate buffer, pH = 3.6, was prepared by mixing 934.8 mL 0.5 mol L^{-1} acetic acid with 65.1 mL 0.5 mol L^{-1} sodium acetate. This buffer was used throughout the study. The pH values of another acetate buffer in optimization stage were adjusted by addition of solutions: 0.5 mol L^{-1} acetic acid or 0.5 mol L^{-1} sodium acetate until the target pH value was reached. For pH range 1.0–2.0 the solutions of 0.1 mol L^{-1} HCl (pH = 1.0) and 0.01 mol L^{-1} HCl (pH = 2.0) were used.

Sample solutions

Ten tablets of MPG containing drug, Captimer (MIT Gesundheit GmbH, Germany), were dissolved in 300.0 mL of deionised water, filtrated through filter paper (Blue ribbon, S&S, Germany) and diluted to 0.5 L. This solution should be analysed within 24 hours. Additional dilutions (10/100) were necessary to obtain a final concentration of sample solution that was added to reaction vessel.

Instrumentation and apparatus

The kinetic manifold for spectrophotometric determination of MPG is shown in Figure 1. The set-up consisted of a Shimadzu UV-1601 (Shimadzu, Kyoto, Japan) UV-Vis spectrophotometer equipped with Hellma (Jamaica, NY, USA) flow cell of 160 μL internal volume and 10 mm optical path. The instrument was set at 593 nm for all absorbance measurements and the output signals were recorded by coupling the spectrophotometer to a computer equipped with Hyper UV-Vis software provided by Shimadzu. The reaction solution (25 mL) was delivered from the double walled thermostated reaction vessel to flow cell with an Ismatec IPC eight-channel peristaltic pump (Ismatec, Zurich, Switzerland) at constant flow of 6 mL min^{-1} (in order to obtain 3 circulation cycles per min of liquid in all tube path; total tubing inner volume was 2 mL). In addition, the inner tubing

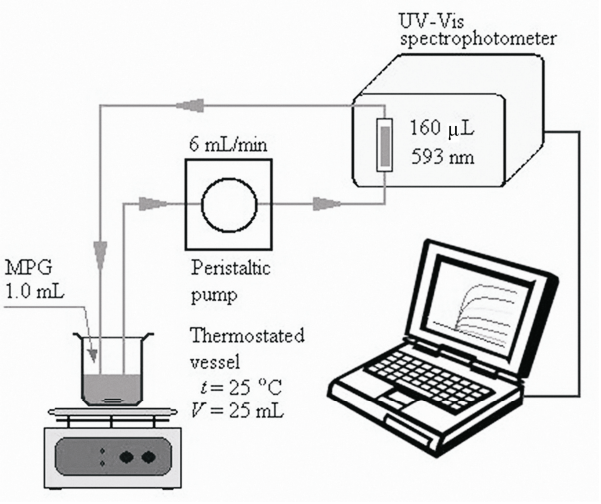

Figure 1. Kinetic manifold for spectrophotometric determination of MPG.

volume between the vessel and the entrance of the spectrophotometric cell was 1 mL. Measurements were performed under constant mixing of reaction solution.

A thermostated constant temperature water pump (MGW Lauda, Germany), accurate to ± 0.5 °C was used.

Measurements of pH were carried out with a Mettler Toledo SevenMulti potentiometer (Mettler Toledo, Schwerzenbach, Switzerland) equipped with combined glass electrode Mettler Toledo InLab®413.

Kinetic spectrophotometric method

For determination of MPG, a mixture of reagents was prepared as follows. In reaction vessel with 20.0 mL of acetic buffer (pH 3.6); 1.25 mL of Fe^{III} [c (Fe^{3+}) = 1.0×10^{-2} mol L^{-1}], 1.25 mL of TPTZ [c (TPTZ) = 1.0×10^{-2} mol L^{-1}] and 1.50 mL of deionised water were added. In this reagent solution, 1 minute after beginning of measurement, a 1.00 mL of analyte or sample was added to start the reaction. The final volume of reaction solution in the thermostated vessel (25 °C) was 25.0 mL. At the flow rate of 6 mL min^{-1} and λ = 593 nm the absorbance of the produced Fe^{II}-TPTZ complex was continuously recorded as the function of time. Frequency of data recording was 10 min^{-1}.

Data acquisition and processing

The kinetic data that has been recorded were transformed to the GraphPad Prism version 4.03 for Windows (GraphPad Software, San Diego, California, USA) for curve fitting, regression analysis and statistical calculations. The initial rate (K) of the reaction at different concentrations was obtained from the slope of the tangent to the absorbance-time curve. The calibration curve with excellent linearity was constructed by plotting the logarithm of the initial rate (log K) of reaction versus logarithm of the concentration (log c) of MPG. Alterna-

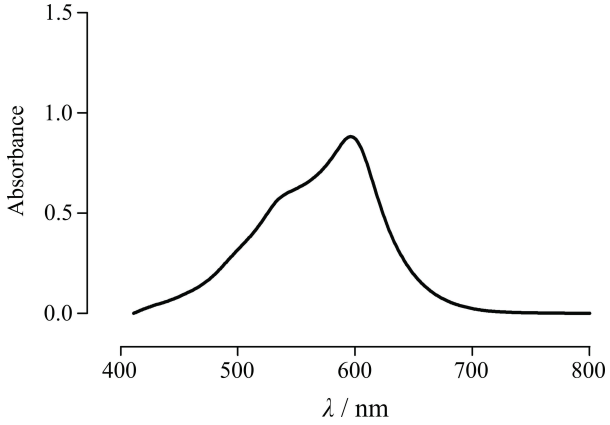

Figure 2. Typical absorption spectra of $Fe(TPTZ)_2^{2+}$ complex. Experimental conditions: c(MPG) = 4.0×10^{-5} mol L^{-1}, $c(Fe^{3+}) = 5.0 \times 10^{-4}$ mol L^{-1}, c(TPTZ) = 5.0×10^{-4} mol L^{-1}, pH = 3.6; t = 25 °C. The cell path length b is 10 mm.

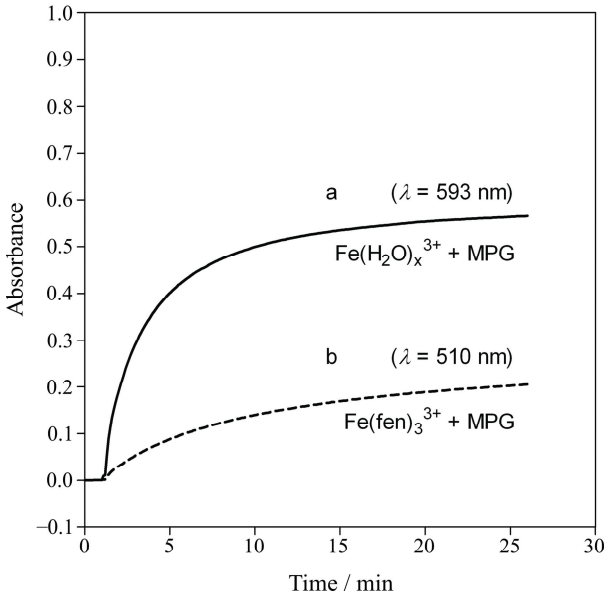

Figure 3. Absorbance as a function of time. Experimental conditions: a. $c(Fe^{3+}) = 3.0 \times 10^{-4}$ mol L^{-1}, c(TPTZ) = 9.0×10^{-5} mol L^{-1}, c(MPG) = 3.0×10^{-5} mol L^{-1}, pH = 3.6; b. $c(Fe^{3+}) = 3.0 \times 10^{-4}$ mol L^{-1}, c(phen) = 1.2×10^{-4} mol L^{-1}, c(MPG) = 3.0×10^{-5} mol L^{-1}, pH = 3.6.

tively, the calibration curve was constructed by plotting the absorbance measured at a fixed time of 3 min versus c(MPG).

RESULTS AND DISCUSSION

The proposed method is based on the coupled redox-complexation reaction. In the first (redox) step of the reaction, MPG (RSH compound) reduces Fe^{III} to Fe^{II} (Eq. (1)). In the second step of the reaction, the reduced Fe^{II} is rapidly converted to the highly stable, deep-blue coloured $Fe(TPTZ)_2^{2+}$ complex (Eq. (2)) with λ_{max} at 593 nm. The absorption spectrum for the reaction product is given in Figure 2.

$$2\,Fe^{3+} + 2\,RSH \rightleftarrows 2\,Fe^{2+} + RSSR + 2\,H^{+} \quad (1)$$

$$2\,Fe^{2+} + 4\,TPTZ \rightleftarrows 2\,Fe(TPTZ)_2^{2+} \quad (2)$$

TPTZ forms very stable complex with Fe^{II} but does not form a coloured complex with Fe^{III}. This notion was previously reported[21] and also confirmed in our laboratory.

In many published procedures Fe^{II} produced in the redox-reaction is determined through a second step using 1,10-phenantroline (phen) as an chromogenic reagent.[22]

Using a kinetic procedure the coupled redox-complexation reaction between MPG, Fe^{III} and TPTZ or phen was examined. The results, absorbance versus time, obtained in this experiment are shown in Figure 3. As can be seen, the rate of redox-reaction was increased and analytical signal was enhanced when Fe^{III} was in aqueous complex instead complex with phen. This could indicate that the use of TPTZ as a chromogenic reagent is more suitable for spectrophotometric determination of MPG in kinetic experiment.

Optimization of reaction conditions

The effect of pH was investigated over the range 1.0–4.0 using 0.1 mol L^{-1} HCl (pH = 1.0), 0.01 mol L^{-1} HCl (pH = 2.0) and acetate buffer for pH range 3.2–4.0. The absorbance increased simultaneously with increasing pH up to 3.6. However, precipitation of iron hydroxide occurred at pH above 3.8. Therefore, buffered reaction medium of pH 3.6 has been chosen as a compromise of keeping Fe^{III} in the solution by preventing the formation and precipitation of iron hydroxide and achieving quantitative $Fe(TPTZ)_2^{2+}$ complex formation since the complex is stable in the pH range 3.4–5.8.[23]

The influence of Fe^{III} concentration on determination of 4×10^{-5} mol L^{-1} MPG was studied in the range from 2×10^{-5} mol L^{-1} to 4×10^{-4} mol L^{-1}, allowing molar ratio Fe^{III} / MPG from 0.5 to 10 under experimental conditions: c(TPTZ) = 2.0×10^{-4} mol L^{-1}, pH 3.6, t = 25 °C. The results show that, by increasing the Fe^{III} concentration, the reaction can be forced to completion, as indicated by the constant value of absorbance when $[Fe^{3+}]$/[MPG] ratio was higher than five.

The influence of TPTZ concentration on determination of 4×10^{-5} mol L^{-1} MPG was studied in the range from 2×10^{-5} mol L^{-1} to 4×10^{-4} mol L^{-1}, allowing molar ratio TPTZ / MPG from 0.5 to 10 under experimental conditions: $c(Fe^{3+}) = 2.0 \times 10^{-4}$ mol L^{-1}, pH 3.6, t = 25 °C. The results show that, by increasing the TPTZ

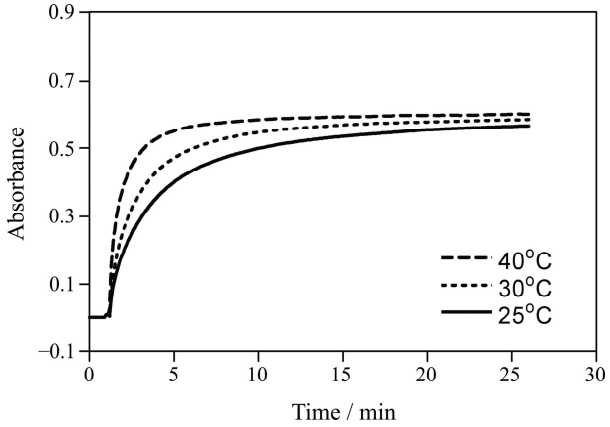

Figure 4. Absorbance as the function of time for the coupled redox-complexation reaction, measured at different temperatures (25, 30 and 40 °C). Experimental conditions: c(MPG) = 3.0×10^{-5} mol L^{-1}, c(Fe^{3+}) = 2.0×10^{-4} mol L^{-1}, c(TPTZ) = 2.0×10^{-4} mol L^{-1}, pH = 3.6, analyte added 1 min after beginning of the measurement.

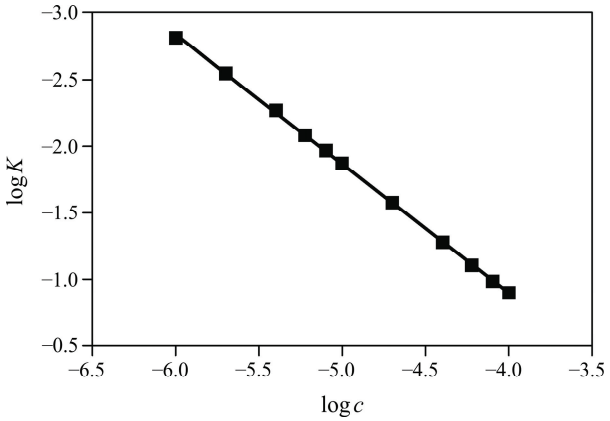

Figure 5. Linear plot for log c vs. log K for the kinetic reaction of MPG with Fe^{III} (5.0×10^{-4} mol L^{-1}) and TPTZ (5.0×10^{-4} mol L^{-1}). c is [MPG]: (1.0×10^{-6} to 1.0×10^{-4} mol L^{-1}); K is the reaction rate (s^{-1}).

concentration, the reaction can be forced to completion, as indicated by the constant value of absorbance when [TPTZ]/[MPG] ratio was higher than five.

The effect of reaction temperature on signal intensity was examined by varying the temperature from 25 to 40 °C using the thermostated water pump. The results showed that the coupled redox-complexation reaction is temperature-dependent, as the rate of the reaction increases with the elevation of the temperature (Figure 4). Therefore, to improve reproducibility of the method, it is necessary to keep the temperature of the reaction vessel constant (thermostated). In spite of heating the reaction mixture to 40 °C, the slight positive effect on the reaction was observed. For reasons of practical analytical measurements, the room temperature (25 °C) was chosen in the experiment.

All observed and optimized reaction conditions are summarised in Table 1.

Kinetics of the reaction

Under the above described optimum conditions, the absorbance-time curves for the reaction at varying MPG concentrations (1.0×10^{-6} to 1.0×10^{-4} mol L^{-1}) with the fixed concentration of Fe^{III} (5.0×10^{-4} mol L^{-1}) and TPTZ (5.0×10^{-4} mol L^{-1}) were generated.

Table 1. Optimization of reaction conditions for MPG determination

Variable	Studied range	Optimum conditions
Wavelength / nm	400–800	593
pH of buffer solution	1.0–4.0	3.6
Molar ratio Fe^{III} / MPG	0.5–10.0	5.0
Molar ratio TPTZ / MPG	0.5–10.0	5.0
Reaction temperature/°C	25–40	25

The initial reaction rates (K) were determined from the slopes of these curves. The logarithms of the reaction rates (log K) were plotted as a function of logarithms of MPG concentrations (log c) (Figure 5.). The regression analysis for the values was performed by fitting the data to the following equation:

$$\log K = \log k' + n \log c \tag{3}$$

where K is reaction rate, k' is the rate constant, c is the molar concentration of MPG, and n (slope of the regression line) is the order of the reaction. A straight line with slope values of 0.9686 (≈ 1) was obtained confirming the first order reaction. However under the optimized reaction conditions, the concentrations of Fe^{III} and TPTZ were much higher than concentrations of MPG in the reaction solution. Therefore, the reaction was regarded as a pseudo-first order reaction.

Quantitation methods

Initial rate method

The initial rates of the MPG reactions would follow a pseudo-first order, and were found to obey the following equation:

$$K = \Delta A / \Delta t = k' + c^n \tag{4}$$

where K is reaction rate, A is absorbance, t is the measuring time, k' is the pseudo-first order rate constant, c is the molar concentration of MPG, and n is the order of the reaction. The logarithmic form of the above equation is written as follow:

$$\log K = \log \Delta A / \Delta t = \log k' + n \log c \tag{5}$$

Table 2. Analytical parameters for the proposed fixed time spectrophotometric method for determination of MPG

Parameters	Fixed time method								
	1 min	2 min	3 min	4 min	5 min	10 min	15 min	20 min	25 min
Linear range $\times 10^4$ (mol L^{-1})	0.010−1.0	0.010−1.0	0.010−1.0	0.010−1.0	0.010−1.0	0.010−1.0	0.010−1.0	0.010−1.0	0.010−1.0
Intercept	0.0007	0.0029	0.0043	0.0060	0.0056	0.0094	0.0111	0.0123	0.0121
Slope	15350	19333	20559	20946	21240	21318	21310	21318	21388
Determination coefficient r^2	0.9995	0.9996	0.9997	0.9996	0.9997	0.9997	0.9997	0.9996	0.9998
LOD (mol L^{-1})	1.0×10^{-7}	8.0×10^{-8}	7.5×10^{-8}	7.3×10^{-8}	7.3×10^{-8}	7.2×10^{-8}	7.2×10^{-8}	7.2×10^{-8}	7.2×10^{-8}
LOQ (mol L^{-1})	3.3×10^{-7}	2.7×10^{-7}	2.5×10^{-7}	2.5×10^{-7}	2.4×10^{-7}	2.4×10^{-7}	2.4×10^{-7}	2.4×10^{-7}	2.4×10^{-7}
Molar absorptivity (L mol^{-1} cm^{-1})	14660	18936	20421	21077	21493	22208	22539	22813	23017
Sandell's sensitivity (ng cm^{-2})	11.13	8.62	7.99	7.74	7.59	7.35	7.24	7.15	7.09

Regression analysis using the method of least squares was performed to evaluate slope, intercept and determination coefficient.

The graph was linear for MPG concentrations ranging from 1.0×10^{-6} to 1.0×10^{-4} mol L^{-1} with intercept log $k' = 2.978$ and determination coefficient $r^2 = 0.9997$. The value of n (slope) 0.9686 (≈ 1) in the regression equation confirmed the first order reaction with respect to the MPG concentration. The limit of detection (LOD) and limit of quantification (LOQ) were calculated and found to be 1.3×10^{-7} and 1.0×10^{-6} mol L^{-1}, respectively. This low value confirmed the good sensitivity of the method and consequently its capability to determine low amounts of MPG.

Fixed time method

In this method, the absorbance of the reaction solution containing varying amounts of MPG was measured at a pre-selected fixed time. Calibration plots of absorbance versus the concentration of MPG were established at fixed periods of time for the reaction. The regression equations, coefficients of correlation, detection limits and other analytical parameters are given in the Table 2.

Although the concentration range obtained at all fixed time intervals was the same (from 1.0×10^{-6} to $\times 10^{-4}$ mol L^{-1}), the time interval of 3 min was recommended for practical reasons: less time required for analysis. Also, at fixed time of 3 min, lower LOD as well as a better correlation coefficient was obtained in comparison to fixed time of 1 or 2 min. Obtained results indicate that linear concentration range of the method might be even extended to concentration of MPG lower than 1.0×10^{-6} mol L^{-1}. Also for practical laboratory measurements the calibration graph absorbance measured at a fixed time of 3 min versus c(MPG) can be used.

Interferences Studies

The effect of some possible interfering cations and anions on the determination of 4.0×10^{-5} mol L^{-1} MPG was investigated for the maximum molar ratio of foreign ions. The influence of excipients that can commonly accompany MPG in pharmaceutical formulations was also studied. The tolerable concentration of K^+; NO_3^-; Na^+; SO_4^{2-} was 4.0×10^{-2} mol L^{-1} (molar ratio 1000:1) and the tolerable concentration of glucose, fructose, sucrose, boric acid, acetic acid was 2.0×10^{-3} mol L^{-1} (molar ratio 500:1). The tolerance is defined as the foreign-ion / excipient concentration causing an error smaller then ±5 % for determination of the analyte of interest. It should be emphasized that this contamination/analyte concentration ratio which has been studied is much higher than those normally found in the commercial pharmaceutical products.

Application

In order to evaluate the potential of the proposed methods for analysis of real sample, both methods were applied to pharmaceutical sample for the determination of MPG. The accuracy of the methods was checked by carrying out recovery studies, when known amounts of the MPG standard were added to the sample before determination by the recommended methods. As shown in the Table 3, there were no significant differences between the values obtained by the reported method[14] and those obtained by the two proposed methods ($P > 0.1$, Student t-test). The recoveries were approximately 100 % for both methods, indicating that the proposed kinetic methods are reliable for the determination of MPG in pharmaceutical preparations (Table 3).

These results are the proof of accuracy of the proposed methods and absence of interferences from common excipients. It is worth to mention that the proposed

Table 3. Determination of MPG in pharmaceutical preparation and recovery experiments

Method	Added γ / μg mL^{-1}	Found [a] γ / μg mL^{-1}	Recovery / %	Found by the reported method[14] γ / μg mL^{-1}
Initial rate	-	99.9 ± 0.7 [b]	-	99.0 ± 1.0
	50.0	150.0 ± 0.6	99.9	
	100.0	200.2 ± 0.8	100.2	
	150.0	50 ± 1	100.2	
	200.0	301 ± 2	100.3	
Fixed time	-	100.3 ± 0.6 [b]	-	99.0 ± 1.0
	50.0	150.1 ± 0.6	100.2	
	100.0	200.1 ± 0.8	100.1	
	150.0	250 ± 1	99.9	
	200.0	300 ± 2	100.2	

[a] Average of three determinations ± SD.
[b] Labeled content was 100 μg mL^{-1}.

kinetic spectrophotometric method was performed in the visible region away from the UV-absorption region of the UV-absorbing interfering excipient materials that might be dissolved from the MPG-containing pharmaceutical formulations.

CONCLUSION

Simple, rapid and sensitive kinetic spectrophotometric method for the determination of MPG has been successfully developed and validated. This method was based on the coupled redox-complexation reaction. The optimized values of factors affecting the signal forming reaction were: pH = 3.6; [Fe^{3+}]/[MPG] = 5; [TPTZ] / [MPG] = 5; t / °C = 25. The initial rate and fixed time (at 3 min) methods were utilized in this experiment. Both methods can be easily applied to the determination of MPG in pure form or in tablets. The proposed methods have several advantages over the previously reported classical spectrophotometric methods: wide linearity range of the calibration curve, sensitivity, selectivity and speed (3 min for the proposed method in comparison to 20 min for reported method).[14] In addition, proposed methods are sensitive enough to enable determination of near nanomole amounts of the MPG. Furthermore, the proposed methods do not require expensive instruments and/or critical analytical reagents. These advantages encourage the application of the proposed methods in routine analysis of MPG in quality control laboratories.

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REFERENCES

1. A. Garner, Z. Jamal, and T. F. Slater, *Int. J. Radiat. Biol. Relat. Stud. Phys. Chem. Med.* **50** (1986) 323−335.
2. S. I. Ayene, R. K. Kale, and P. N. Srivastava, *Int. J. Radiat. Biol. Relat. Stud. Phys. Chem. Med.* **53** (1988) 629−639.
3. F. Barbey, D. Joly, P. Rieu, A. Mejean, M. Daudon, and P. Jungers, *J. Urol.* **163** (2000) 1419−1423.
4. G. K. Chow and S. B. Streem, *J. Urol.* **156** (1996) 1576−1578.
5. D. Jarrar, P. Wang, W. G. Cioffi, K. I. Bland, and I. H. Chaudry, *J. Trauma* **49** (2000) 879−885.
6. J. L. Domingo, *Reprod. Toxicol.* **9** (1995) 105−113.
7. S. Lecoules, C. Duvic, M. Herody, and G. Nedelec, *Presse Med.* **28** (1999) 273−275.
8. J. Lu, C. Lau, S. Yagisawa, K. Ohta, and M. Kai, *J. Pharm. Biomed. Anal.* **33** (2003) 1033−1038.
9. K. Matsuura, K. Murai, Y. Fukano, and H. Takashina, *J. Pharm. Biomed. Anal.* **22** (2000) 101−109.
10. K. Matsuura and H. Takashina, *J. Chromatogr.* **616** (1993) 229−234.
11. S. Penugonda, W. Wu, S. Mare, and N. Ercal, *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.* **807** (2004) 251−256.
12. V. Springolo, W. Bertani, and G. Coppi, *J. Chromatogr.* **232** (1982) 456−460.
13. Y. Zhao, W. R. Baeyens, X. Zhang, K. Nakashima, A. C. Calokerinos, and G. Van der Weken, *Biomed. Chromatogr.* **11** (1997) 115−116.
14. M. A. Raggi, V. Cavrini, and A. M. Di Pietra, *J. Pharm. Sci.* **71** (1982) 1384−1386.
15. M. A. Raggi, M. R. Cesaroni, and A. M. Di Pietra, *Farmaco* **38** (1983) 312−316.
16. M. A. Raggi, L. Nobile, V. Cavrini, and A. M. Di Pietra, *Boll. Chim. Farm.* **125** (1986) 295−297.
17. Q. Li and L. Gao, *Anal. Sci.* **25** (2009) 89−93.
18. M. A. Chamjangali, V. Keley, and G. Bagherian, *Anal. Sci.* **22** (2006) 333−336.
19. I. A. Darwish, M. A. Sultan, and H. A. Al-Arfaj, *Talanta* **78** (2009) 1383−1388.
20. L. Kukoc Modun and N. Radic, *Croat. Chem. Acta* **79** (2006) 533−539.
21. G. S. R. Krishnamurti and P. M. Huang, *Talanta* **37** (1990) 745−148.
22. P. D. Tzanavaras and D. G. Themelis, *Anal. Chim. Acta* **588** (2007) 1−9.
23. P. F. Collins, H. Diehl, and G. F. Smith, *Anal. Chem.* **31** (1959) 1862−1867.

SAŽETAK

Nova kinetička spektrofotometrijska metoda za određivanje tiopronina [*N*-(2-merkaptopropionil)-glicina]

Lea Kukoč-Modun i Njegomir Radić

Kemijsko-tehnološki fakultet, Sveučilište u Splitu Teslina 10/V, 21000 Split, Hrvatska

Razvijena je i validirana nova i jednostavna kinetička spektrofotometrijska metoda određivanja tiopronina [*N*-(2-merkaptopropionil)-glicin, MPG] u čistom obliku ili u farmaceutskom pripravku. Predložena metoda temelji se na ukupnoj reakciji čiji je prvi korak redoks-reakcija u kojoj se odvija redukcija Fe^{III} djelovanjem MPG, dok drugi dio reakcije predstavlja kompleksiranje Fe^{II}, koji je nastao u redoks-reakciji, s 2,4,6-tripiridil-*s*-triazinom (TPTZ). Stabilan kompleks $Fe(TPTZ)_2^{2+}$ pokazuje apsorpcijski maksimum pri $\lambda = 593$ nm. Metoda početne brzine i metoda odabranog vremena korištene su za izradu kalibracijskih grafova. Grafovi su linearni u području koncentracija od $1{,}0 \times 10^{-6}$ do $1{,}0 \times 10^{-4}$ mol L^{-1} za obje metode, s granicama detekcije od $1{,}3 \times 10^{-7}$ mol L^{-1} za metodu početne brzine i $7{,}5 \times 10^{-8}$ mol L^{-1} za metodu odabranog vremena. Predložena kinetička spektrofotometrijska metoda uspješno je primijenjena kod određivanja MPG u komercijalnom farmaceutskom pripravku.