A novel and simple method for the determination of penicillamine (PEN), tiopronin (mercaptopropionyl glycine, MPG) and glutathione (GSH) in pharmaceutical formulations by kinetic spectrophotometry has been developed and validated. It is based on the redox reaction where the thiol compound (RSH) reduces Cu\textsuperscript{II}-neocuproine complex to Cu\textsuperscript{I}-neocuproine complex. The non-steady state signal of the formed Cu\textsuperscript{I}-neocuproine complex is measured at 458 nm. The initial rate and fixed time (at 1 min) methods were validated. The calibration graph was linear in the concentration range from $8.0 \times 10^{-7}$ to $8.0 \times 10^{-5}$ mol L\textsuperscript{-1} for the initial rate method and from $6.0 \times 10^{-7}$ to $6.0 \times 10^{-5}$ mol L\textsuperscript{-1} for the fixed time method, with the detection limits of $2.4 \times 10^{-7}$ and $1.4 \times 10^{-7}$ mol L\textsuperscript{-1}, resp. Levels of PEN, MPG and GSH in pharmaceutical formulations were successfully assayed by both methods. The advantages of the presented methods include sensitivity, short analysis time, ease of application and low cost.

**Keywords:** penicillamine, tiopronin, glutathione, kinetic spectrophotometry, pharmaceutical formulations

Penicillamine (PEN) is a synthetic amino acid that contains an additional SH group and is capable of forming non-toxic, water-soluble chelates with heavy metals, which are then excreted in the urine. PEN was the first chelator used for Wilson’s disease and it can be also used for lead, mercury and arsenic poisoning (1, 2). Tiopronin (mercaptopropionyl glycine, MPG) is a synthetic aminothiol with reducing and complexation properties used primarily for the treatment of cystinuria, as it increases cystine solubility (3, 4). Glutathione (γ-L-glutamyl-L-cysteinyl-glycine, GSH) is the most important hydrophilic intracellular antioxidant that protects cells against reactive oxygen (ROS) and nitrogen (RNS) species. Supplementation with GSH showed antiaging and hepatoprotective effects in humans (5–7).

Official methods (from the British Pharmacopoeia) for determination of these thiol compounds (RSH) are acid-base titration in a non-aqueous medium for PEN and redox titration for MPG and GSH (8). Other reported methods for determination of these RSH compounds in pure form and pharmaceutical formulations include spectrophotometry.
Nevertheless, these methods are insufficiently sensitive and selective, or overly expensive due to expensive instrumentation. In fact, the spectrophotometric technique is the one routinely used in the pharmaceutical analysis for quality control, due to its simplicity, availability, and low price. Kinetic methods allow sensitive and selective determination of many analytes within a few minutes, usually with no sample pretreatment. The application of kinetic spectrophotometric methods offers an advantage over classical spectrophotometry, such as improved selectivity, and shorter time per analysis.

The literature of kinetic spectrophotometric methods for the determination of the PEN, MPG and GSH in pharmaceutical formulations is relatively limited. At the best of our knowledge, there are only four published kinetic spectrophotometric methods for determination of PEN (36–39), and none for MPG or GSH; besides, the methods for determination of PEN (40) and MPG (41) were developed by our team.

Here we describe a new method for the determination of PEN, MPG and GSH by kinetic spectrophotometry.

EXPERIMENTAL

Reagents and chemicals

All reagents and chemicals used in the present study were of analytical grade and were used without further purification. Milli-Q (Millipore) double deionized water was used as an appropriate diluent.

Stock solutions of thiol compounds RSH \( (c(RSH) = 1.0 \times 10^{-2} \text{ mol L}^{-1}) \) were prepared by dissolving the appropriate amount of thiol compound: 0.1492 g of PEN (Fluka Chemika, Switzerland), 0.1632 g of MPG (Sigma-Aldrich, USA), 0.3073 g of GSH (Sigma-Aldrich) in Britton-Robinson buffer solution (pH = 2) and diluted to a nominal volume of 100.0 mL in the volumetric flask. The stock solutions were stored at 4 °C in a dark bottle and were stable for at least 30 days. Working standards of lower concentrations were prepared daily by diluting the stock solutions with Britton-Robinson buffer solution (pH = 3).

The oxidizing solution of copper(II)-neocuproine reagent was prepared by dissolving 25.0 mg of copper(II) sulfate pentahydrate (Kemika, Croatia) and 50.0 mg of neocuproine hydrate (Nc, Sigma-Aldrich) (1.0 mmol L\(^{-1}\) Cu(II) + 2.4 mmol L\(^{-1}\) Nc) in 100.0 mL Britton-Robinson buffer solution (pH = 3). Neocuproine (2,9-dimethyl-1,10-phenantroline) is slightly soluble in water, thus its solubility is improved when complex copper(II)-neocuproine, Cu(Nc)_2\(^{2+}\), is formed. Copper(II)-neocuproine reagent was stable for at least 30 days when stored at 4 °C.

Three commercially available pharmaceutical preparations were analyzed in this work: Metalcaptase tablets, 300 mg of PEN (HEYL Chemisch-Pharmazeutische Fabrik GmbH & Co. KG, Germany), Captimer tablets, 100 mg of MPG (MIT Gesundheit GmbH, Germany) and L-glutathione capsules, 50 mg of GSH (Solaray, USA). Ten PEN-containing tablets, or ten MPG-containing tablets, were weighed and pulverized. A powder quantity equivalent to 300 mg of PEN, or 100 mg of MPG, was dissolved in 300 mL of water, filtered through filter paper, and the filtrate collected in a 500-mL volumetric flask and diluted.
with water. The content of ten GSH-containing capsules was weighed and mixed. A powder quantity equivalent to 50 mg of GSH was dissolved in 500 mL of water. These solutions are not stable and should be analysed within 24 hours.

Iodine, sodium thiosulfate and perchlorate acid solutions were prepared and standardized according to the literature (8).

**Apparatus and procedure**

Spectrophotometric kinetic measurements were carried out by the instrumentation consisting of the peristaltic pump (IPC Ismatec, Switzerland) with the appropriate PTFE tubing of 0.8 mm i.d. that allows a continuous flow of reagent solution, magnetic stirrer that provides equal mixing of the total volume of reagent solution and a double beam Shimadzu UV-1601 UV/Vis spectrophotometer (Shimadzu, Japan) as a detector, equipped with a 160-µL inner volume quartz flow cell. Six mL of Britton Robinson buffer (pH = 3.0), 2.5 mL of copper(II) neocuproine complex and 15.5 mL of deionized water were added in a thermostated double-wall vessel placed on the magnetic stirrer. Constant temperature was maintained by a thermostated water pump (Julabo, Germany) with an external flow. The reaction was started by adding 1.0 mL of analyte 1 minute after the beginning of the experiment yielding the final volume of the reaction mixture of 25.0 mL. The absorbance of the formed complex Cu(Nc)$_{2}^{+}$ was continuously measured at a wavelength of 458 nm using a UV-Vis spectrophotometer with a flow cell during the reaction. The kinetic manifold and other instrumentation have been previously described in more detail (42).

The recorded kinetic data, with the frequency of 1 s$^{-1}$, were transferred to a software package, GraphPad Prism Ver. 4.03 for Windows (GraphPad Software, San Diego, CA, USA) for curve fitting, regression analysis and statistics. Details of data processing have been previously described (42).

**Analytical performances**

The precision and accuracy of the method were estimated from recovery studies. Precision was expressed as RSD (%) from three replicates. For recovery (%) calculation known amounts of RSH standards were added to pharmaceutical formulation pre-analysed by the proposed methods. Limit of detection, LOD, was calculated as three standard deviations of a blank divided by the slope of the calibration curve. The influence of possible interfering substances was tested by measuring RSH concentration in synthetic solutions containing RSH ($c = 4.0 \times 10^{-5}$ mol L$^{-1}$) and different concentrations of foreign substances or ions. The tolerable tolerance limit was defined as the concentration of interfering substances that would cause an error of less than ± 5 %.

**RESULTS AND DISCUSSION**

The proposed methods for the determination of RSH are based on the redox reaction (Eq. 1) in which RSH reduces copper(II)-neocuproine complex (Cu(Nc)$_{2}^{2+}$) to a yellow-orange copper(I)-neocuproine complex (Cu(Nc)$_{2}^{+}$), having an absorption maximum at $\lambda = 458$ nm (15):

$$2\text{RSH} + 2\text{Cu(Nc)}_{2}^{2+} \rightleftharpoons \text{RSSR} + 2\text{Cu(Nc)}_{2}^{+} + 2\text{H}^{+} \quad (I)$$
Absorption spectra of the analyte (RSH), reagent (copper(II)-neocuproine) and reaction products (coloured copper(I)-neocuproine) are shown in Supplemental Fig. S1.

As we have previously reported, this redox reaction is very fast and the product Cu(Nc)\textsuperscript{2+} is relatively unstable, which underlines the need for kinetic measurement under dynamic conditions (changes in the concentrations of reactants and products per time) (43).

**Methods optimization**

The optimal pH was tested over the range from 2.0 to 6.0 using the Britton-Robinson buffer solution. Although all RSHs are more stable at lower pH, there was a significant difference between kinetic signals for the three analytes at the observed pH values. The PEN and GSH signals are stable in the pH range 2–3, but at pH 3 the reaction rate is significantly higher. At higher pH values, the signals are unstable. On the other hand, the signals for MPG were unstable at pH = 2 and pH = 6, whereas at pH = 3 the signal was stable (Fig. 1). Therefore, the reaction medium of Britton-Robinson buffer pH 3.0 was selected for further measurements. Our results regarding the optimum pH for this reaction are in accordance with our previous study (43), but do somewhat differ from the article that introduced the copper(II)-neocuproine as a colour reagent (15).

Fig. 1. Absorbance (\(\lambda = 458\) nm) vs. time dependence at different pH values: pH = 2.0, 3.0, 6.0. Experimental conditions: \(c(\text{RSH}) = 4.0 \times 10^{-5}\) mol L\(^{-1}\); \(c(\text{Cu}^{2+}) = 8.0 \times 10^{-5}\) mol L\(^{-1}\); \(c(\text{Nc}) = 1.9 \times 10^{-4}\) mol L\(^{-1}\); temperature 25 °C. The analyte was added 1 minute after the beginning of the experiment. Panels a), b) and c) show the kinetic signal for PEN, MPG and GSH, resp.
The optimum molar ratio of neocuproine and copper(II) in the reaction mixture was tested over the range from 1.0 to 3.5, by maintaining the concentration of copper(II) constant, \(c(Cu^{2+}) = 8.0 \times 10^{-5}\ \text{mol L}^{-1}\). Both the reaction rate and absorbance increased with an increase of the molar ratio, by reaching a constant value at molar ratio 2.4. Therefore, the optimum molar ratio of neocuproine and copper(II) is 2.4 for all analyzed thiol compounds.

The effect of temperature on the reaction kinetics was tested over the range from 5 to 60 °C, by changing the temperature in the reaction vessel. The reaction rate and signal stability remained unaffected over a wide range of temperatures. The laboratory temperature of 25 °C was selected as optimum, so the experiment can be carried out without thermostating the kinetic system during the measurement.

**Kinetics of the reactions**

The absorbance-time curves for the reaction at varying RSH concentrations (6.0 × 10^{-7} to 8.0 × 10^{-5} mol L^{-1}) with fixed analytical concentrations of Cu^{2+} (1.0 × 10^{-4} mol L^{-1}) and neocuproine (2.4 × 10^{-4} mol L^{-1}) were generated (Fig. 2), following the optimum conditions described above.

![Absorbance-time curves for the reaction](image)

**Fig. 2.** Absorbance \((\lambda = 458\ \text{nm})\) vs. time dependence for the considered RSH at optimized experimental conditions: \(c(\text{RSH}) = 6.0 \times 10^{-7}, 8.0 \times 10^{-7}, 1.0 \times 10^{-6}, 2.0 \times 10^{-6}, 4.0 \times 10^{-6}, 6.0 \times 10^{-6}, 8.0 \times 10^{-6}, 1.0 \times 10^{-5}, 2.0 \times 10^{-5}, 4.0 \times 10^{-5}, 6.0 \times 10^{-5}, 8.0 \times 10^{-5}\ \text{mol L}^{-1}; \ c(Cu^{2+}) = 1.0 \times 10^{-4}\ \text{mol L}^{-1}; \ c(\text{Nc}) = 2.4 \times 10^{-4}\ \text{mol L}^{-1}; \ pH = 3.0; \ \text{temperature } 25\ °\text{C}. \ \text{Analyte was added } 1\ \text{minute after the beginning of the experiment. Panels a), b) and c) show the kinetic signal for PEN, MPG and GSH, resp.}
Quantitation methods

**Initial rate method.** – The initial reaction rates ($K$) were calculated from the slopes of absorbance-time curves. Regression analysis using a method of least squares was performed to obtain slope, intercept and coefficient of determination ($R^2$) values, by fitting the data in the following equation:

$$\log K = \log \frac{\Delta A}{\Delta t} = \log k' + n \log c$$  (2)

where $K$ is the reaction rate, $A$ is absorbance, $t$ is the reaction time, $k'$ is the pseudo-first order rate constant, $c$ is the molar concentration of RSH, and $n$ is the order of the reaction. The calculated values are presented in Table I. The calculated values of every regression line slopes were very close to 1.0 (1.033, 1.077 and 1.076 for PEN, MPG and GSH determination, resp.), confirming that the proposed reactions of all these analytes are first-order in respect to analytes concentrations.

**Fixed time method.** – The absorbance of the solution containing different RSH concentrations were recorded at a fixed time. Throughout the optimization part of the fixed time method, various time intervals (30, 60, 120 and 180 s) were observed for the absorbance recording. Calibration plots were created for the tested time periods, for each analyte. Regarding to sensitivity and concentration range, no significant differences were observed for the tested time periods, Therefore, the fixed time of 60 s after addition of analyte was

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Linear range (mol L$^{-1}$)</th>
<th>$\log K = n \log c + \log k'$</th>
<th>$R^2$</th>
<th>LOD (mol L$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Slope (a)$^a$</td>
<td>Intercept (log $k'$)$^a$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PEN</td>
<td>$8.0 \times 10^{-7} - 8.0 \times 10^{-5}$</td>
<td>$1.033 \pm 0.011$</td>
<td>$3.080 \pm 0.035$</td>
<td>0.9981</td>
</tr>
<tr>
<td>MPG</td>
<td>$8.0 \times 10^{-7} - 8.0 \times 10^{-5}$</td>
<td>$1.077 \pm 0.013$</td>
<td>$3.300 \pm 0.038$</td>
<td>0.9996</td>
</tr>
<tr>
<td>GSH</td>
<td>$6.0 \times 10^{-7} - 8.0 \times 10^{-5}$</td>
<td>$1.076 \pm 0.014$</td>
<td>$3.322 \pm 0.040$</td>
<td>0.9976</td>
</tr>
</tbody>
</table>

Experimental conditions: $c$(Cu$^{2+}$) = $1.0 \times 10^{-4}$ mol L$^{-1}$; $c$(Nc) = $2.4 \times 10^{-4}$ mol L$^{-1}$; pH = 3.0; temperature 25 °C.

$^a$ Average of three determinations ± SD.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Linear range (mol L$^{-1}$)</th>
<th>$y = ax + b$</th>
<th>$R^2$</th>
<th>LOD (mol L$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Slope (a)$^a$</td>
<td>Intercept (b)$^a$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PEN</td>
<td>$6.0 \times 10^{-7} - 8.0 \times 10^{-5}$</td>
<td>7186 ± 79</td>
<td>0.0009 ± 1×10$^{-5}$</td>
<td>0.9997</td>
</tr>
<tr>
<td>MPG</td>
<td>$6.0 \times 10^{-7} - 6.0 \times 10^{-5}$</td>
<td>7470 ± 95</td>
<td>−0.0006 ± 9×10$^{-6}$</td>
<td>0.9999</td>
</tr>
<tr>
<td>GSH</td>
<td>$6.0 \times 10^{-7} - 6.0 \times 10^{-5}$</td>
<td>7426 ± 89</td>
<td>−0.0034 ± 4×10$^{-5}$</td>
<td>0.9999</td>
</tr>
</tbody>
</table>

Experimental conditions: $c$(Cu$^{2+}$) = $1.0 \times 10^{-4}$ mol L$^{-1}$; $c$(Nc) = $2.4 \times 10^{-4}$ mol L$^{-1}$; pH = 3.0; temperature 25 °C.

Sampling time was 60 s after adding the analyte and signal sampling, and 120 s after the beginning of the measurement.

$^a$ Average of three determinations ± SD.
selected for all of the proposed methods. The determination coefficients, regression equations, linear range and LOD for each analyte in pre-selected fixed time (120 s of the measurement) are given in Table II.

**Analytical performances**

*Accuracy and precision.* – The results showed that the recovery of the developed kinetic methods processed by both calculational methods were in the range from 98.6 to 102.8 % with RSD < 2 % (Table III). The results supported the accuracy and precision of developed kinetic methods as well as the absence of interference from the excipients in the used samples.

*Selectivity.* – With regard to the calculated tolerance limit, even 500 times higher amount (2.0 × 10⁻² mol L⁻¹) of the tested ionic species (Na⁺, K⁺, NO₃⁻, SO₄²⁻) and organic acids (boric, tartaric and citric acid) did not cause interference with the absorbance signal. Furthermore, 100 times higher concentration of sugar (glucose, fructose, sucrose and lactose) did not interfere as well.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Initial rate method</th>
<th>Fixed time method</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Added (µg mL⁻¹)</td>
<td>Found (µg mL⁻¹)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Metalcaptase</td>
<td>0.0</td>
<td>100.7 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>50.0</td>
<td>151.4 ± 1.0</td>
</tr>
<tr>
<td></td>
<td>100.0</td>
<td>202.6 ± 1.2</td>
</tr>
<tr>
<td></td>
<td>150.0</td>
<td>252.7 ± 1.9</td>
</tr>
<tr>
<td></td>
<td>200.0</td>
<td>303.8 ± 2.1</td>
</tr>
<tr>
<td>Captimer</td>
<td>0.0</td>
<td>101.0 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>50.0</td>
<td>151.4 ± 1.3</td>
</tr>
<tr>
<td></td>
<td>100.0</td>
<td>202.6 ± 1.9</td>
</tr>
<tr>
<td></td>
<td>150.0</td>
<td>253.5 ± 2.0</td>
</tr>
<tr>
<td></td>
<td>200.0</td>
<td>304.7 ± 2.2</td>
</tr>
<tr>
<td>L-glutathione</td>
<td>0.0</td>
<td>100.6 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>50.0</td>
<td>151.3 ± 0.9</td>
</tr>
<tr>
<td></td>
<td>100.0</td>
<td>201.7 ± 1.6</td>
</tr>
<tr>
<td></td>
<td>150.0</td>
<td>254.2 ± 1.9</td>
</tr>
<tr>
<td></td>
<td>200.0</td>
<td>304.4 ± 2.1</td>
</tr>
</tbody>
</table>

*a Average of three determinations ± SD.

b Tablets containing PEN 300 mg.
c Tablets containing MPG 100 mg.
d Capsules containing GSH 50 mg.
It should be noted that the occurrence of interference is expected in the presence of substances that are strong reducing agents, such as ascorbic acid and other RSHs. However, such reducing agents are not normally included in pharmaceutical formulations containing PEN, MPG and GSH. The reported standard potential of Cu$^{II}$/Cu$I$ couple in the solution of neocuproine (0.603 V) (44) specify that only the reducing substances with standard (formal) potential lower than 0.6 V would have the thermodynamic predisposition to interfere with the proposed methods.

Method application

To test the potential of the new methods for the analysis of real samples, both methods were applied to the determination of RSH in commercially available pharmaceutical formulations. Official methods from the British Pharmacopoeia were used for comparison (8). As MPG is an orphan drug and there is no specific assay for MPG described in the British Pharmacopoeia, we used the method described for measurement of acetylcysteine, a thiol compound with the same molecular mass as MPG, for comparison of the methods. There were no statistically significant differences between the values obtained by the official methods and those obtained by the two new approaches (Table IV). This indicates that the new method is not inferior to the official method, in terms of accuracy and precision.

Performance characteristics of the published kinetic spectrophotometric methods for the determination of PEN (36–40) and MPG (41) in pharmaceuticals, and the novel methods, are compared in Table V. As we have previously remarked, we could not find in the literature any kinetic spectrophotometric methods for determination of GSH in pharmaceuticals. Therefore, the presented method would be the first published kinetic spectrophotometric method for the determination of glutathione in pharmaceuticals. The new methods (initial rate and fixed time) have quite a few advantages over previously reported methods: wide linear dynamic concentration range (two decades), higher sensitivity, speed (1 min for fixed time method) and measurement performed in the visible region ($\lambda = 458$ nm) – away from the UV-absorbance of the UV-absorbing interfering excipient materials.

Table IV. Content of RSH in pharmaceutical formulations determined by the new method and the official methods

<table>
<thead>
<tr>
<th>Sample</th>
<th>Initial rate method$^a$ ($m$ (mg))</th>
<th>$t_{\text{calc}}^h$</th>
<th>$F_{\text{calc}}^h$</th>
<th>Fixed time method$^a$ ($m$ (mg))</th>
<th>$t_{\text{calc}}^h$</th>
<th>$F_{\text{calc}}^h$</th>
<th>Official method (8)$^a$ ($m$ (mg))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metalcapta$^b$</td>
<td>301.9 ± 2.5</td>
<td>0.1846</td>
<td>1.254</td>
<td>302.2 ± 2.6</td>
<td>0.3173</td>
<td>1.160</td>
<td>301.5 ± 2.8$^c$</td>
</tr>
<tr>
<td>Captimer$^d$</td>
<td>101.5 ± 1.2</td>
<td>0.3757</td>
<td>1.361</td>
<td>100.9 ± 1.1</td>
<td>0.1946</td>
<td>1.620</td>
<td>101.1 ± 1.4$^e$</td>
</tr>
<tr>
<td>L-glutathionef</td>
<td>51.1 ± 0.8</td>
<td>1.1410</td>
<td>1.306</td>
<td>50.7 ± 0.9</td>
<td>1.6710</td>
<td>1.653</td>
<td>51.8 ± 0.7$^g$</td>
</tr>
</tbody>
</table>

$^a$ Average of three determinations ± SD.
$^b$ Calculated per tablet containing PEN 300 mg. $^c$ Acid-base titration in non-aqueous medium.
$^d$ Calculated per tablet containing MPG 100 mg. $^e$ Redox titration.
$^f$ Calculated per capsule containing GSH 50 mg. $^g$ Redox titration.
$^b$ Tabulated values, $p = 0.05$: $t(4) = 2.776$, $F(2,2) = 19.0$ (degrees of freedom in parentheses).
Table V. Comparison between published kinetic spectrophotometric methods for the determination of RSH in pharmaceuticals and the proposed methods

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Reagent(s)</th>
<th>λ (nm)</th>
<th>Linear range (mol L⁻¹)</th>
<th>LOD (mol L⁻¹)</th>
<th>Kinetic method(s)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>PEN</td>
<td>Fe(III)-phenanthroline and Cu(II)</td>
<td>510</td>
<td>8.0 × 10⁻⁶ – 8.0 × 10⁻⁵</td>
<td>2.5 × 10⁻⁶</td>
<td>Fixed time (5 min)</td>
<td>(40)</td>
</tr>
<tr>
<td>PEN</td>
<td>Sodium azide and iodine</td>
<td>348</td>
<td>6.7 × 10⁻⁸ – 6.7 × 10⁻⁷</td>
<td>6.3 × 10⁻⁹</td>
<td>Fixed time (5 min)</td>
<td>(39)</td>
</tr>
<tr>
<td>PEN</td>
<td>Potassium permanganate</td>
<td>610</td>
<td>1.3 × 10⁻⁵ – 6.7 × 10⁻⁵</td>
<td>1.4 × 10⁻⁶</td>
<td>Fixed time (20 min)</td>
<td>(38)</td>
</tr>
<tr>
<td>PEN</td>
<td>Na₃[Fe(CN)₅(H₂O)]</td>
<td>421</td>
<td>1.0 × 10⁻⁴ – 1.0 × 10⁻⁵</td>
<td>2.1 × 10⁻⁵</td>
<td>Fixed time (5 min)</td>
<td>(37)</td>
</tr>
<tr>
<td>PEN</td>
<td>Hg(II) and [Ru(CN)₅NRS]³⁻</td>
<td>525</td>
<td>2.9 × 10⁻⁶ – 2.7 × 10⁻⁵</td>
<td>3.0 × 10⁻⁷</td>
<td>Fixed time (20 min)</td>
<td>(36)</td>
</tr>
<tr>
<td>PEN</td>
<td>Cu(II) and neocuproine</td>
<td>458</td>
<td>6.0 × 10⁻⁷ – 8.0 × 10⁻⁵</td>
<td>1.4 × 10⁻⁷</td>
<td>Fixed time (1 min) Initial rate</td>
<td>Present work</td>
</tr>
<tr>
<td>MPG</td>
<td>Fe(III) and 2,4,6-tripiridil-s-triazine</td>
<td>593</td>
<td>1.0 × 10⁻⁶ – 1.0 × 10⁻⁴</td>
<td>7.5 × 10⁻⁸</td>
<td>Fixed time (3 min) Initial rate</td>
<td>(41)</td>
</tr>
<tr>
<td>MPG</td>
<td>Cu(II) and neocuproine</td>
<td>458</td>
<td>6.0 × 10⁻⁷ – 8.0 × 10⁻⁵</td>
<td>2.4 × 10⁻⁷</td>
<td>Fixed time (1 min) Initial rate</td>
<td>Present work</td>
</tr>
<tr>
<td>GSH</td>
<td>Cu(II) and neocuproine</td>
<td>458</td>
<td>6.0 × 10⁻⁷ – 8.0 × 10⁻⁵</td>
<td>1.8 × 10⁻⁷</td>
<td>Fixed time (1 min) Initial rate</td>
<td>Present work</td>
</tr>
</tbody>
</table>
CONCLUSIONS

The present study demonstrates the potential application of simple kinetic spectrophotometric methods for assaying of penicillamine, tiopronin and glutathione in pharmaceutical formulations. The new method is based on a redox reaction where the RSH reduces Cu\textsuperscript{II}-neocuproine complex to Cu\textsuperscript{I}-neocuproine complex. It is adequately sensitive and accurate to be used for routine quantification of RSH without expensive reagents and instruments. The advantages of the proposed method over the previously published kinetic spectrophotometric methods include a wide linearity range, higher sensitivity and speed.

Supplementary material available upon request.

REFERENCES


