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ARTICLES

Flow-Injection Spectrophotometric Determination of Methimazole in Pharmaceuticals Using a Charge Transfer Complex Cu(I)–Neocuproine[1]

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Abstract—In this work an accurate, fast and high throughput flow procedure was developed in order to determine methimazole in pharmaceutical formulations. The method is based on reduction of Cu(II) to Cu(I) by methimazole in the presence of 2,9-dimethyl-1,10-phenanthroline (neocuproine), followed by the generating a yellow charge-transfer complex that was spectrophotometrically monitored at 455 nm. The methimazole analytical curve was linear in a concentration range from 4.0×10^{-5} to 1.5×10^{-4} M. The sample throughput was 90 h^{-1} and the relative standard deviation was 0.3% for a methimazole solution. The average recovery was 99%. The developed method is economic due to reduced consumption of samples and reagents. Samples can be analyzed without special pretreatment or using of organic solvents.

Keywords: flow-injection analysis, methimazole, neocuproine, pharmaceutical formulations

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Nowadays, the need has increased to develop efficient analytical methods to determine the compounds accurately, with high celerity and low waste generation [1]. Therefore, the development of automated analytical systems arise as an alternative to this demand [2].

Flow injection analysis (**FIA**) proposed in 1975 by Ruzicka and Hansen is a technique able of aligning various analytical stages in a single analysis platform, providing precision, speed and flexibility [3]. The main features of a FIA is the possibility of automation, considerable decrease in sample and reagent consumption, high sample throughput (normally 50 to 300 samples per hour) which makes it an attractive technique for routine analysis. Compared to batch methods, FIA offers an increased sampling frequency, higher versatility and lower consumption of reagents.

Several procedures are described in the literature employing flow systems to determine the content of the active principle in pharmaceuticals [4–17]. These procedures are developed mainly to identify the possibility of adulteration that may include the replacement of the drug by a neutral preparation and/or chaning the content of the active principle [18]. Thus, to identify adulteration and/or counterfeits, it is necessary to develop methods that can be carried out routinely by controlling laboratories. Among the factors analyzed, regarding the quality of medicines, the dosage of the active ingredient recommended by ANVISA may have a tolerance of ± 10% compared to the labeled content [19].

Methimazole (1-methylimidazole-2-thiol), also known as tapazol, is a drug belonging to the group of thioamides [20], widely used in the treatment of hyperthyroidism, a condition that occurs when there is an increase in the synthesis and release of thyroid hormones [20, 21]. It is a common disease, with a prevalence of 0.2 to 0.5% of the population [22].

Various procedures have been developed for the methimazole quantification. Some procedures involve HPLC with ultraviolet-visible detector [18, 23, 24], gas chromatography–mass spectrometry [25], thin layer chromatography [26], voltammetry [27–31], resonance light scattering spectroscopy [32], chemiluminescence [33], capillary electrophoresis [34], electrochemiluminescence [35], fluorimetry [36] and spectrophotometry [37, 38]. Some of these methods are sophisticated, laborious, and expensive, they consume high quantities of solvents or high purity gases, and require a long experimental time.

As far as we know, only two procedures have been documented in the literature for the determination of methimazole employing flow-injection analysis. Sánchez-Pedreño et al. developed a spectrophotometric method based on the formation of yellow complexes between methimazole and Pd(II) in 0.5 M HCl [12]. Economou et al. developed a method based on the

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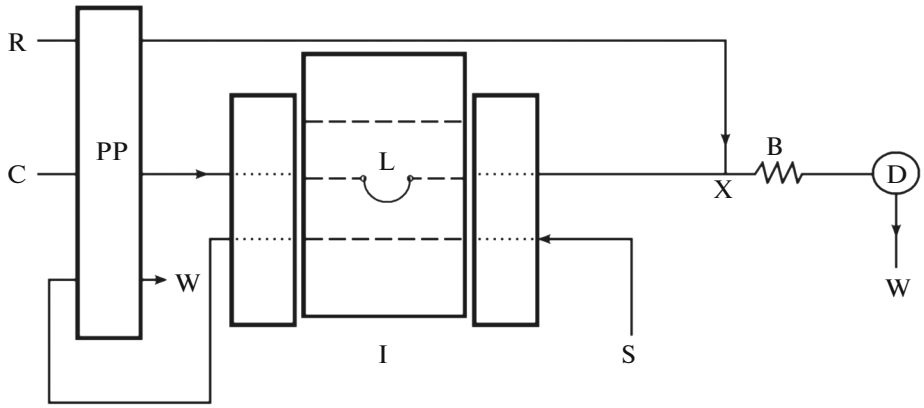


Fig. 1. Schematic diagram of the flow-injection system for spetrophotometric determination of methimazole in pharmaceutical formulations. PP—peristaltic pump, I—injector commutator, S—sample or reference solution, R—reagents (2.0×10^{-3} M neocuproine and 1.0×10^{-3} M Cu(II) solutions, flow rate 1.0 mL/min), C—carrier solution (1.0×10^{-5} M nitric acid, flow rate 2.0 mL/ min), X—confluence point, B—tubular reaction coil (100 cm), L—sampling loop (400 μL), D—spectrophotometer (455 nm) and W—waste.

inhibition caused by methimazole on the Cu(II)-catalyzed chemiluminescence reaction between luminol and H_2O_2 [11].

In this work, it is described a simple, accurate, sensitive, precise and high throughput spectrophotometric procedure for the methimazole determination in pharmaceuticals by using a flow-injection system. In this procedure, methimazole reacts with copper(II) that is reduced to copper(I) that in turn generates a yellow charger-transfer complex with neocuproine absorbing at 455 nm.

EXPERIMENTAL

Apparatus. An 8-channel Gilson Minipuls peristaltic pump with Tygon® pump tubing was used for the propulsion of the solutions. A manual injector-commutator, made of Perspex containing two fixed bars and a sliding central bar, was used for injecting the sample and reference solutions in the flow system [39]. A Femto model 435 spectrophotometer (São Paulo, Brazil) equipped with a glass flow-cell (optical path of 1 cm) was used for the spectrophotometric measurements. The spectrophotometer was interfaced to a microcomputer using software MATLAB 7.9 to enable data acquisition. UV-visible spectra were obtained using a spectrophotometer Hitachi U-2000.

Reagents and solutions. Methimazole (Merck), copper(II) sulphate pentahydrate (Sigma-Aldrich), nitric acid (Vetec) and 2,9-dimethyl-1,10-phenantroline (neocuproine) (Sigma-Aldrich) were used. All chemicals used were of analytical grade and used without any further purification. Water was purified by distillation and deionized.

A stock solution of methimazole 1.0×10^{-3} M was prepared daily. A stock solution of copper(II) 5.0×10^{-3} M was prepared in deionized water. A 5.0×10^{-3} M stock solution of neocuproine was prepared daily in 20% (v/v) ethanol. Working solutions containing copper(II) and neocuproine was obtained by diluting the stock solutions with appropriate volume of 20% ethanol. The stock solution of nitric acid (1.0 M) was prepared by diluting a concentrated solution of HNO_3 (34.9 mL, 65% (w/w)) in deionized water (500 mL). The working solution (1.0×10^{-4} M) was obtained by diluting the stock solution.

Pharmaceutical sample preparation. The samples containing methimazole acquired in the local market (5 and 10 mg per tablet) were analyzed using the developed procedure. For this, five tablets of each sample were weighed and the obtained powder was homogenized with the aid of a mortar and pestle. A mass corresponding to 25 mg of methimazole of each sample was transferred to a 50 mL volumetric flask. After that, deionized water was added to complete this volume. Moreover, the solutions obtained were filtered before analysis. The obtained results were compared with the standard procedure [26].

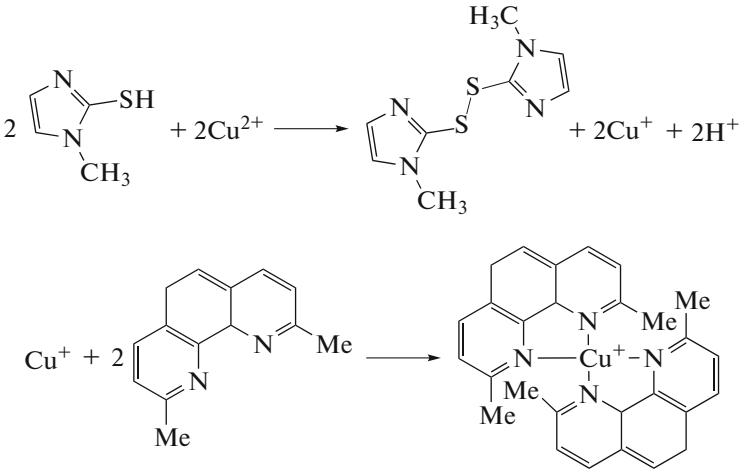
Analysis module. Figure 1 shows the schematic diagram of the flow-injection system with spectrophotometric detection to determine methimazole in pharmaceutical formulations. A simple and robust system was employed to facilitate the use in routine analysis.

When the injector was switched to the injection position, aliquots of 400 μL (reference solutions or samples containing methimazole) were inserted into the carrier solution (nitric acid 1.0×10^{-5} M) at a flow rate of 2.0 mL/min. Subsequently, the aliquot was displaced by the carrier solution to the confluence point (X), which received a copper(II)–neocuproine solution at a flow rate 1.0 mL/min. In the tubular reaction

coil (B) occurred the reduction of copper(II) to copper(I) by methimazole action and subsequent reaction of copper(I) with neocuproine generating a yellow charge-transfer complex which was monitored spectrophotometrically at 455 nm. After maximum signal measurement, the injector commutator was switched back to the initial position starting another cycle. The change of absorbance was proportional to the concentration of the sample/reference solution methimazole injected.

RESULTS AND DISCUSSION

The reduction of Cu(II) in the presence of neocuproine and the subsequent formation of a charge-transfer complex between neocuproine and Cu(I) was the basis for the spectrophotometric determination of methimazole (Scheme 1). In this reaction, the thiol group of methimazole was oxidized to disulfide and Cu(II) was reduced to Cu(I) that generates a highly colored Cu(I)–neocuproine complex showing maximum absorption at 455 nm.

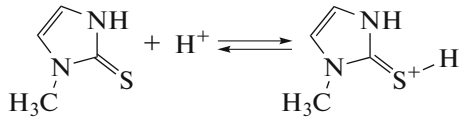


Scheme 1. Reactions of methimazole oxidation by Cu(II) and generation of yellow complex between Cu(I) and neocuproine.

Figure 2 shows the UV-visible absorption spectrum of the complex Cu(I)–neocuproine. The complex was generated adding in a cuvette of 1.0 cm optical path a solution containing 2.0×10^{-3} M Cu(II) and 2.0×10^{-3} M neocuproine, thereafter a methimazole solution 5.0×10^{-3} M was added, resulting in the reduction of Cu(II) to Cu(I) and subsequent formation of the complex Cu(I)–neocuproine. This reaction occurs in a short period of time and is accompanied by a change of color to yellow corresponding to the absorption band at 455 nm.

System optimization. For all studies conducted, univariated method was used, with the purpose of obtain the best relationship between the magnitude of the analytical signal, precision, baseline stability and high sample throughput.

Initially, the carrier solution was selected. Basic solutions were not studied because of the possible precipitation of copper hydroxide. The effect of pH on the indicator reaction is related to the equilibrium thiol–thiolate which is shifted towards the thiolate in basic solutions and towards the thiol acid in acidic solutions. In Scheme 2 it is shown that in the acid medium, the sulfur atom is protonated, inhibiting the thiol group oxidation.



Scheme 2. Protonation of the thiol group that inhibits the oxidation of methimazole molecule.

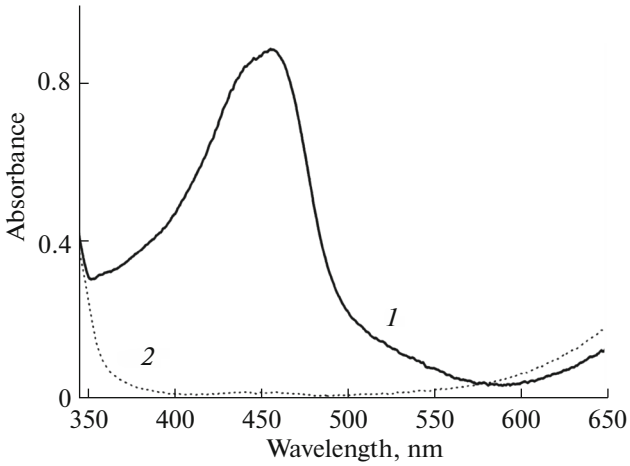


Fig. 2. Absorption spectrum of yellow chromophore Cu(I)–neocuproine ($\lambda_{max} = 455$ nm) (*1*) and blank (solution containing Cu(II) and neocuproine) (*2*).

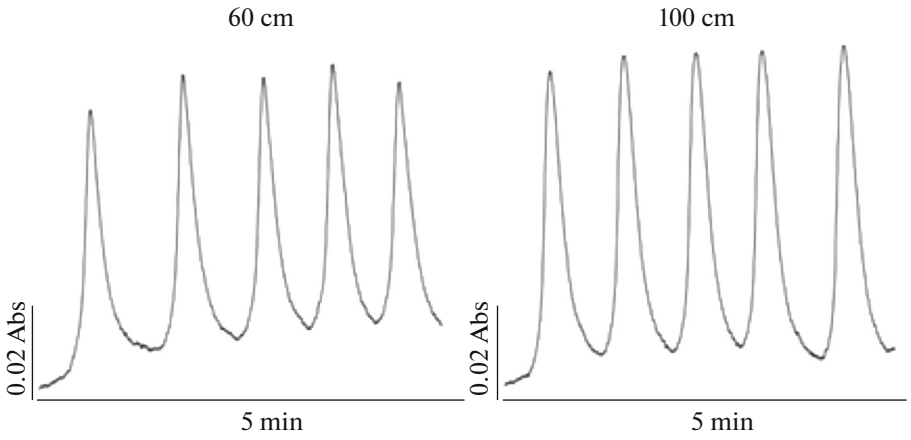


Fig. 3. Stability of baseline employing reactor coil of 60 and 100 cm.

Therefore, the influence of nitric acid in the carrier solution was studied at various concentrations (1.0×10^{-6}, 1.0×10^{-5}, 1.0×10^{-4}, 1.0×10^{-3} and 1.0×10^{-2} M) keeping fixed the following conditions: 1.0 mM methimazole, 0.01 M Cu(II)–neocuproine for both reagents (flow rate 2.0 mL/min); sample loop of 250 μL, 85 cm reactor coil and carrier flow rate of 2.0 mL/min. As expected, there was a decrease in the magnitude of the analytical signal with increasing nitric acid concentration. Thus, to maintain the pH constant, the concentration of nitric acid 1.0×10^{-5} M was selected for further studies.

The concentration of copper(II) was studied between 1.0×10^{-5} and 1.0×10 M. There was an increase in the analytical signal with increasing concentration of copper(II) up to 1.0×10^{-3} M. Taking into account the magnitude of the analytical signal, a concentration of copper(II) of 1.0×10^{-3} M was chosen for further studies.

The influence of the neocuproine concentration on the analytical signal was studied in the concentration range from 1.0×10^{-4} to 5.0×10^{-3} M. The signal increased with the neocuproine concentration up to 2.0×10^{-3} M, and remained practically constant for higher concentrations, which as expected due to the reaction stoichiometry. Thus, a 2.0×10^{-3} M neocuproine solution was chosen for further experiments.

The effect of the sampling loop volume from 100 to 500 μL was investigated. There was an increase in the analytical signal with the increasing of the volumes. Despite 500 μL sample loop showed a higher analytical signal, the volume 400 μL was chosen due to the sampling frequency (65 h^{-1} with 400 μL and 53 h^{-1} for the 500 μL).

The effect of the length of the reactor coil (B) was studied in the range 40–100 cm. Reactor coils 60 and 100 cm showed similar analytical signals. However, the 100 cm reactor coil showed greater stability of the baseline in relation to the 60 cm (Fig. 3), with apparent memory effect between injections, harming the measurement precision. Thus, aiming not only the sample throughput, but the stability of the baseline, the 100 cm reactor coil was chosen for further studies.

The influence of the flow rates of the carrier (C) and reagent stream (R) were examined in the range from 1.0 to 4.0 mL/min. The analytical signal decreased by increasing the flow rate up to 4.0 mL/min, probably due to a shorter residence time of the sample zone in the reactor coil B. Therefore, 2.0 mL/min (carrier stream) was selected, taking into account the better sample throughput in relation to the flow rate of 1.0 mL/min. For the study of reagent the flow rate of 1.0 mL/min was employed to save reagents and obtain higher analytical signals in relation to higher flow rates. Table 1 presents the optimization of the flow-injection parameters studied in this work.

Analytical performance. The analytical curve was linear over the methimazole concentration ranging from 4.0×10^{-5} to 1.5×10^{-4} M ($A = 0.00188 + 2130.6c$; $r = 0.997$, where A is absorbance and c is the concentration of methimazole, M). The method presented a limit of detection (LOD) of 6.0×10^{-6} M (three times the standard deviation of the blank/slope of the analytical curve).

The potential interferences in the determination of methimazole in pharmaceutical formulations were evaluated for excipients normally present in commercial samples. The tested substances were: lactose, magnesium stearate and starch. In these experiments, reference solutions containing 6.0×10^{-4} M methimazole was used with each of the possible interferents in the concentration of 6.0×10^{-3} M. Even with the concentration of excipient being 10 times greater than the drug, the interferences were lower than 1.5%.

Recoveries of methimazole of 96.0–102.0% from two pharmaceutical formulations were obtained using the flow procedure developed. In this study, 9.0, 11.0, and 15.0×10^{-5} M of methimazole were added to each

Table 1. Parameters evaluated to optimize the flow system for determining methimazole in pharmaceutical formulations

Parameter	Evaluated range	Selected value
Reactor coil length, cm	40–100	100
Sample volume, μL	100–500	400
Carrier flow rate, mL/min	1.0–4.0	2.0
Reagent flow rate, mL/min	1.0–4.0	1.0
Nitric acid conc., μM	1.0–10000	10
Neocuproine conc., μM	100–5000	2000
Cu(II) conc., μM	10–1000	1000

Table 2. Determination of methimazole (mg/tablet) in pharmaceutical formulations using the proposed system and the reference method

Sample	Label value	Proposed method*	Reference method*	Error, %**
A	5	5.2 ± 0.4	5.1 ± 0.3	−2.0
B	10	10.2 ± 0.5	10.3 ± 0.1	1.0

* $n = 3$, mean ± standard deviation.

** Relative difference in the results for the proposed and reference.

pharmaceutical product. The obtained recovery results suggested an absence of the matrix effect in the determination of methimazole in these samples.

The precison of the developed procedure was studied by employing standard methimazole solutions at concentration of 1.0×10^{-4} M, obtaining RSD = 0.3%. The sample throughput for the developed procedure was 90 samples per hour. The transient signals are shown in Fig. 4.

The proposed method was applied to the determination of methimazole in pharmaceutical formulations and the results were compared with the labeled value and the reference method (Table 2).

The results obtained by the proposed and reference method are in agreement within a 95% confidence level (*t*-paired test), confirming the accuracy of the proposed flow-injection system.

Table 3 shows the analytical characteristics obtained in this study and in other flow systems developed for the determination of methimazole. The primary advantage of the proposed flow-injection system is better precision with a coefficient of variation of 0.30%. Pedreño et al. employed palladium(II) chloride for the methimazole determination, that is an expensive reagent spent in the amount 4 mg per determination. Economou et al. developed a chemiluminescence method that is potentially more sensitive than the spectrophotometric methods, even so the proposed system presented lower limit of detection, and besides the chemiluminescence method generates 384 mL of waste per hour, being 204 mL more than the proposed system.

CONCLUSIONS

The method proposed for the determination of methimazole is simple, precise and accurate, with reduced consumption of sample/reagent when compared with the flow procedures described in the liter-

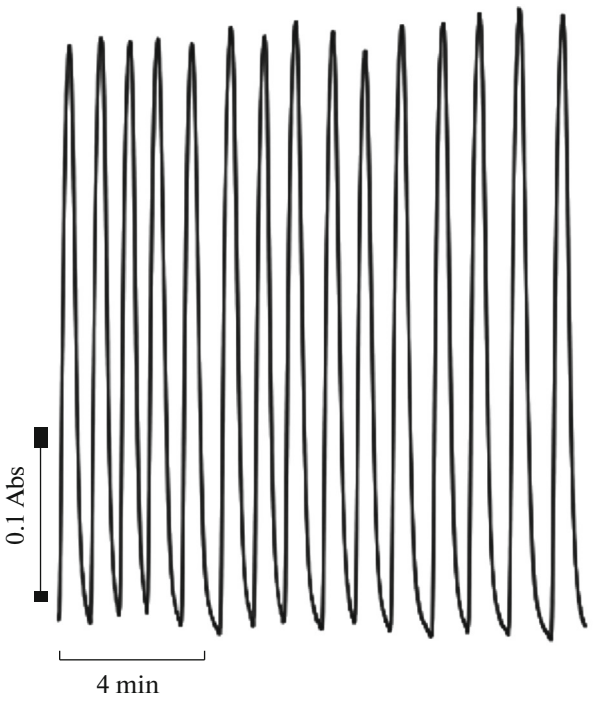


Fig. 4. Repeatability of the analytical signals. Injection volume, 400 μL; methimazole concentration, 1.0×10^{-4} M; flow rate 3.0 mL/min.

Table 3. Comparison of the present method with the published results for methimazole determination employing flow systems

Method	LOD, μM	Linear range, μM	RSD, %	Sampling rate, h^{-1}
Sánchez-Pedreño [12]	3	10–500	0.3	90
Economou [11]	9	17–875	1.9	120
Proposed system	6	40–140	0.3	90

ature. These features make it suitable for routine analysis and quality control of pharmaceutical samples in laboratories. In addition, samples can be analyzed without the need for special handling or the use of any type of organic solvent.

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