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Spectrophotometric determination of cimetidine in pharmaceuticals and urine using batch and flow-injection methods

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Abstract

Two sensitive and fast spectrophotometric methods using batch and flow-injection procedures for the determination of cimetidine (CMT) are proposed. The methods are based on the formation of a green complex between this drug and Cu(II) in acetic/acetate medium of pH 5.9. The calibration graphs resulting from measuring the absorbance at 330 nm are linear over the ranges 2.5×10^{-6}–1.0×10^{-3} and 5×10^{-6}–2.0×10^{-3} M with detection limits of 9.5×10^{-7} and 2.1×10^{-6} for batch and flow-injection methods, respectively. The methods are applied to the routine analysis of CMT in pharmaceuticals and human urine.

Keywords: Spectrophotometric; Flow injection; Cimetidine; Copper (II); Pharmaceuticals; Urine

1. Introduction

Cimetidine [*N*-cyano-*N*′- methyl-*N*″-[2-[(5-methyl-4-imidazol-4-yl) methyl] thio] ethyl] guanidine (CMT) is a molecule largely used in medicine for its protective action on stomach walls in ulcer diseases, due to its histamine H_2 receptor blocking effect. The drug is orally and intravenously administrated and reaches H_2-receptors via the blood-stream [1,2].

The interest in the complexation of CMT with metal ions, in order to understand its pharmacological action has produced an extensive literature [3–6]. This molecule should act as effective ligand towards metal ions, being composed of several groups with a very strong coordination ability [7].

Several methods for the determination of CMT in biological fluids or/and in pharmaceuticals dosage forms have been reported in the literature including titrimetric [8–11], spectrophotometric [12–16], chromatographic [17–25], electrophoretic

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[26–28], polarographic [29,30] and potentiometric [31].

Flow-injection analysis (FI) is characterized by its simplicity, speed, the inexpensive equipment needed and the accuracy of its results. It is an important alternative to other analytical methods, with clear advantages in terms of the short time required for each assay. However, to our knowledge, no FI method has been proposed for the determination of CMT in pharmaceuticals and human urine.

In this paper two, batch and FI, methods using spectrophotometric detection at 330 nm are described for the determination of CMT. The methods are based on the formation of a complex between CMT and copper (II). The proposed methods have been successfully applied to the determination of CMT in pharmaceuticals and human urine.

2. Experimental

2.1. Apparatus

The FI system comprised a Gilson HP4 peristaltic pump with silicone flow tubes of 1.0 mm i.d. (Worthington, OH, USA), an Omnifit injection valve (NY, USA), a Hellma 18 μl flow cell (Jamaica, NY, USA) and a Pyc-Unicam spectrophotometer (Cambridge, UK) as the detector. Poly(tetrafluoroethylene) connecting tubing of 0.5 mm i.d. and various end-fittings and connectors (Omnifit) were used. An ultrasonic bath (Bransonic B5, 55 KH2, 14 W) was also used.

2.2. Reagents

All chemicals were of analytical reagent grade and the solutions were prepared with double-distilled water.

(1) *CMT stock standard solution 10^{-2} M* was prepared by dissolving 0.2523 g of CMT (Sigma, St. Louis, MO) in 100 ml of water. The stock standard solution was stored in a refrigerator at approximately 4 °C and remained stable for at least 1 month. Working standard solutions were prepared by suitable dilution of the stock standard solution.

(2) *Copper (II) acetate solution 10^{-2} M* was prepared by dissolving 1.9967 g of Cu $(CH_3–COO)_2 \cdot H_2O$ (Merck) in 1000 ml of water.

(3) *Acetate/acetic acid buffer (pH 5.9; 0.2 M).*

2.3. Dosage forms of CMT

(1) Tagamet tablets (Smithkline Beecham Lab. Spain): CMT 200 mg and excipients up to total tablet weight; (2) Mansal tablets (Vita Lab. Spain): CMT 200 mg with lactose and other excipients up to total tablet weight, (3) Tagamet ampoules (Smithkline Beecham Lab.): CMT 200 mg and water for injection to 5 ml of solution.

2.4. Recommended procedures for calibration

2.4.1. Batch procedure

To different volumes, 25 μl–2.0 ml of 10^{-3} or 10^{-2} M CMT, 5 ml of 10^{-2} M Cu(II) were added and diluted with acetate/acetic buffer of pH 5.9 to 10 ml in calibrated flasks. After 2 min the absorbance of the Cu(II)–CMT complex formed was measured at 330 nm against water.

2.4.2. FI-procedure

The flow-injection system is shown in Fig. 1. 60 μl aliquots of CMT solutions prepared at different concentrations (5.0×10^{-6}–2.0×10^{-3} M) were injected into an inert carrier stream of acetate/acetic buffer of pH 5.9. The solution of Cu(II) 10^{-2} M was mixed with the carrier stream at the down-stream confluence point. Calibration graphs

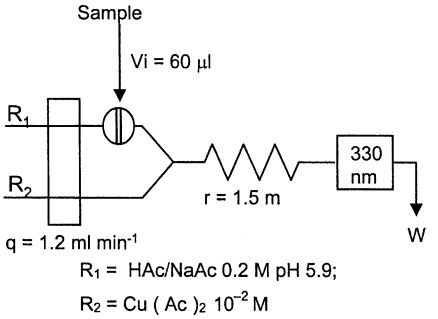

Fig. 1. FI manifold for the determination of CMT.

were prepared by plotting the absorbances of the peak maximum versus CMT concentration.

2.5. Procedure for the assay of dosage forms

The average tablet weight was calculated from the contents of 10 tablets that had been finely powdered and weighed. A portion of this powder, equivalent to 200 mg of CMT, was accurately weighed. The samples were shaken with 25 ml of water. The mixtures were then introduced into an ultrasonic bath for 10 min and diluted with water in a 100 ml calibrated flask. Appropriate aliquots of the solution obtained were centrifuged at 3000 rpm for 10 min and the supernatat was filtered through a Millipore filter. Different volumes, 1.0–2.0 ml, of the filtrate were analyzed in triplicate by recommended batch or FI spectrophotometric procedures.

To validate the methods, aliquots of 1.0 ml of filtered solution of pharmaceutical sample equivalent to 2 mg of CMT, to which different volumes (0.5–1.0 ml) of 10^{-2} M CMT solution were added, were analyzed in triplicate by recommended batch or FI spectrophotometric procedures.

2.6. Procedure for the determination of CMT in urine

Urine samples of 1.0 ml containing different CMT concentrations were mixed with 100 μl of 2 M-NaOH and vortex-mixed with 6 ml ethyl acetate for 10 min. After centrifugation (3000 rpm, 5 min), 5 ml of the organic phase were transferred to a dry test-tube and evaporated to dryness at 35 °C. For the batch procedure, the residue was dissolved in 5 ml acetic/acetate buffer pH 5.9 and 5 ml of 10^{-2} M Cu(II) were added. After 2 min the absorbance was measured at 330 nm. For the FI-procedure the residue was dissolved in 10 ml of water and aliquots of 60 μl were injected into the FI system.

Calibration graphs were previously prepared by adding known amounts of CMT, 50–1000 μl of 10^{-2} M CMT, to 1.0 ml of water and the same procedures described above were applied.

3. Results and discussion

3.1. Preliminary studies

In the bibliography it is reported that CMT forms 1:1 and 2:1. complexes with copper (II) ions in aqueous solutions, with complexation constants of 3.02×10^4 and 2.35×10^4 M^{-1}, respectively [4].

Fig. 2 shows the absorption spectra, all obtained at pH 5.9 (acetic/acetate buffer), of 10^{-3} M Cu(II) (curve 1), 10^{-3} M CMT (curve 2) and 10^{-3} M Cu(II) with 10^{-3} or 2×10^{-3} M CMT (curves 3 and 4). As can be seen, copper (II) has a low absorbance at 620–640 nm, the CMT does not absorb in the range 330–640 nm and the curves 3 and 4 present two absorption maxima, one of high molar absortivity at 330 nm and an other at 620–640 nm, both maxima due to the Cu(II)–CMT complex. The wavelength of 330 nm was selected for following studies.

The influence of pH on the formation of the Cu(II)–CMT complex was studied in the range 3.9–7.0 using acetic acid, acetic/acetate buffers or sodium acetate. It was observed that the absorbance at 330 nm increased when pH increased, while a precipitate was obtained at values above

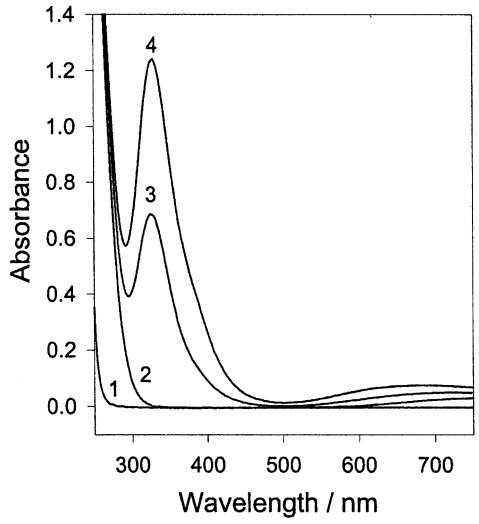

Fig. 2. Absorption spectra in acetic/acetate buffer of pH 5.9 of (1) 10^{-3} M Cu(II); (2) 10^{-3} M CMT; (3) 10^{-3} M Cu(II) and 10^{-3} M CMT (4) 10^{-3} M Cu(II) and 2×10^{-3} M CMT.

pH 6.0. A pH of 5.9 was selected for subsequent studies.

The stoichiometry of the Cu(II)–CMT complex was studied applying the continuous variation method. In our experimental conditions a stoichiometry of 1:2 [Cu(II)]:[CMT] was found with a molar absortivity of 1.42×10^3 l mol^{-1} cm^{-1}.

The stability of the complex was studied for 1 h following the mixture of the reagents. The absorbance of the complex sharply increased up to 2 min and remained constant for at least 1 h.

In this paper, the reaction between Cu(II) and CMT was applied to developing batch and FI spectrophotometric methods for determining CMT.

3.2. Flow systems

Preliminary experiments under continuous-flow conditions were carried out to test the manifold configurations and the approximate ranges of the tested parameters. The design of the manifold selected is shown in Fig. 1. A two-channel FI assembly was adopted, in which the sample was injected into the acetic/acetate buffer of pH 5.9 stream, which was then mixed with a stream of Cu(II). The reagent and the buffer carrier stream were pumped at the same flow rate to achieve effective mixing of the sample and reagent solutions. The Cu(II) reacted with CMT to produce a complex, whose absorbance was measured at 330 nm in the detector previously adjusted to zero with the Cu(II) carrier solution. The presence of the CMT caused an increase in the analytical signal, which was proportional to its concentration.

The use of FI as an alternative to existing methods for CMT determination is dependent on optimization of the system to achieve maximum peak height, with low residence time and minimum dispersion. As a consequence, several experiments were conducted in order to establish the best experimental conditions for operating the FI manifold. All the variables were selected by the univariate method.

Fig. 3 shows the effects of the sample injection volume, reactor length and flow rate on the peak height. The volume of sample injected was varied in the 15–90 μl range by changing the length of the

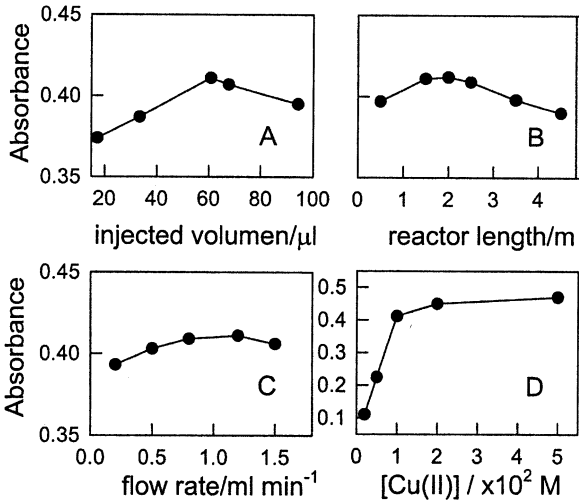

Fig. 3. Effect of the loop size (A); reactor length (B); flow rate (C) and Cu(II) concentration (D), on the peak height in the FI method. Sample injected 10^{-3} M CMT.

sample loop in the injection valve, while the other variables remained fixed (1.5 m reactor length, 1.2 ml min^{-1} flow rate, pH 5.9 (acetic/acetate), 10^{-2} M Cu(II) solution and 10^{-3} M CMT solution injected). The absorbance increased with increasing volume of sample injected up to 60 μl (Fig. 3A) which was selected.

The influence of reactor length was studied from the minimum distance possible between injection valve and detector, 0.5 m, up to 4.5 m in the same experimental conditions selected above. As can be seen from Fig. 3B, maximum absorbance values were obtained at 1.5–2.0 m. A 1.5 m reactor length (inner diameter 0.5 mm) was selected.

The effect of flow rate on peak height was studied over the range 0.2–1.5 ml min^{-1} and in the same experimental conditions. Fig. 3C shows that this variable has little influence on the absorbance in die studied range. A flow rate of 1.2 ml min^{-1} was selected, as a compromise between reproducibility and sampling rate.

According to the results of the preliminary spectrophotometric studies concerning the influence of pH on the absorbance of the Cu(II)–CMT complex, an acetic/acetate buffer of pH 5.9 was used for the FI method.

The influence of the concentration of Cu(II) was studied in the range 10^{-3}–5×10^{-2} M with a

fixed CMT concentration of 10^{-3} M and the same FI variables selected. As can be observed from Fig. 3D, constant and maximum absorbance values were obtained in the concentration range of 1.0×10^{-2}–5×10^{-2} M. A 10^{-2} M Cu(II) concentration was selected, which is sufficient for the total formation of the complex in the range of the calibration graph used for the FI determination of CMT.

The flow systems selected provided a sampling frequency of 60 samples h^{-1}.

3.3. Analytical characteristics of the batch and FI spectrophotometric methods

For the batch and FI methods, the calibration graphs were obtained by the procedures described in Section 2 and a series of standard solutions was analyzed in triplicate to test the linearity. The analytical results obtained are shown in Table 1.

The limit of detection calculated according to the recommendations of IUPAC [32], were ($n = 10$) 9.5×10^{-7} and 2.1×10^{-6} M of CMT for the batch and FI methods, respectively.

The precision of the two methods was tested by analyzing ten replicate samples of 5×10^{-4} M of CMT by the batch and FI spectrophotometric methods. The coefficients of variation were ± 0.5 and $\pm 0.6\%$, respectively.

The between-day precision of the two methods was also determined by obtaining five calibration graphs on randomly selected days during the 15 days that the experiment lasted. The coefficients of variation of the slopes obtained were ± 0.7 and $\pm 1.2\%$ for the batch and FI methods, respectively.

Table 1
Data for the calibration graphs ($n = 12$) for CMT using the proposed methods

Parameter	Batch method	FI method
Linear range (M)	2.5×10^{-6}–1.0×10^{-3}	5.0×10^{-6}–2.0×10^{-3}
Slope (A mol^{-1} l) ±S.E.	1412.3±4.5	398.0±2.6
Intercept ±S.E.	$2.48 \times 10^{-2} \pm 1.8 \times 10^{-3}$	$2.30 \times 10^{-2} \pm 2.2 \times 10^{-3}$
Correlation coefficient	0.9999	0.9996

3.4. Applications

In order of demonstrate the applicability of the proposed methods to the determination of CMT, the methods was applied to the analysis of CMT in various samples of pharmaceutical preparations and human urine.

3.4.1. Pharmaceutical samples

The influence of frequently encountered excipients and additives in pharmaceutical dosage forms of CMT on the proposed methods was studied by adding different amounts of the possible interferents to samples containing 10^{-3} M of CMT. No interference was observed from the presence of lactose, glucose, citrate, saccharose, starch, talc, hydroxypropylmethylcellulose, magnesium stearate, even when mass ratios much greater than that contained in the pharmaceuticals assayed were used.

The two proposed methods where successfully applied to the analysis of different pharmaceutical dosage forms containing CMT and the results are summarized in Table 2. When different pharmaceuticals of CMT were analyzed by the proposed methods, interference from the sample matrix posed no problem. For all the formulations examined the assay results of both methods were in good agreement with the declared content.

The results obtained by the two proposed methods were compared by applying the F-test and the t-test at 95% confidence level. The calculated values for F and t (1.53 and 0.75 for Tagamet tablets, 7.53 and 2.19 for Tagamet ampoules, 1.45 and 0.34 for Mansal tablets), respectively, did not exceed the critical values of

Table 2
Determination of CMT in pharmaceuticals

Sample	Labelled	CMT content/mg $tablet^{-1}$	
		Batch* method	FI* method
Tagamet tablet	200	201.93±1.87	199.92±1.51
Tagamet ampoules	200	209.46±9.13	212.76±1.87
Mansal tablet	200	199.61±4.53	201.86±3.76

*, Mean of five determinations±S.D.

Table 3
Recoveries of CMT from pharmaceuticals

Sample	CMT			
	Batch method		FI method	
	Added[a]	% Recovery[b]	Added[a]	% Recovery[b]
Tagamet tablet	126.2	100.1±3.6	126.2	103.1±3.7
	252.3	95.4±1.6	252.3	99.9±1.0
Tagamet ampoules	126.2	99.3±3.9	126.2	102.4+1.9
	252.3	96.5±2.9	252.3	100.3±2.6
Mansal tablet	126.2	99.0±1.1	126.2	102.2±2.2
	252.3	96.6±1.1	252.3	100.4±0.7

Labelled content of CMT: 200 mg tablet^{-1} or ampoules^{-1}.
[a] mg tablet^{-1} or ampoules^{-1}.
[b] Mean of five determinations±S.D.

$F_{4.4} = 9.60$ and $t = 2.31$ ($n = 5$), confirming that there are no significant differences between the two proposed methods with respect to precision and accuracy in the determination of CMT in pharmaceuticals.

The validity of the two methods was confirmed by applying the standard additions technique to the different pharmaceuticals of CMT analyzed following the procedures described in Section 2. The results obtained are shown in Table 3. In all cases quantitative recoveries between 95.4 and 103.1% were obtained.

3.4.2. Human urine samples

For the determination of CMT in human urine a previous study on the possible interference from the sample matrix was carried out. Several samples from different individuals who had not ingested CMT (blank) were collected and analyzed after appropriate dilution by the procedures described in Sections 2.4.1 and 2.4.2. Absorbance signals at 330 nm were obtained in all the dilutions assayed. For this reason, a previous CMT extraction step, described in Section 2.6, was carried out.

The calibration graphs obtained by applying the batch and FI procedures described in Section 2.6 were linear from 4.2×10^{-5} to 8.0×10^{-4} M CMT, (5.0×10^{-4} to 10^{-2} M CMT in the original urine sample) for both methods. The detection limits were 2.0×10^{-6} M and 4.0×10^{-4} M CMT for batch and FT methods, respectively. A study on the reproducibility of the methods for 4.0×10^{-4} M of CMT added to the same urine samples showed variation coefficients of ±1.5 and ±1.6% ($n = 10$) for batch and FI methods, respectively.

CMT is rapidly and efficiently absorbed after oral administration and is eliminated principally by hepatic metabolism and subsequent urinary excretion. The normal dose for adults is 0.8–2.0 g day^{-1}. In the urine passed during 24 h, between 50 and 80% of the dose administered is excreted as unchanged CMT [33]. The concentration of CMT in this urine was within the range of CMT

Table 4
Determination of CMT in urine samples

Urine	CMT			
	Batch method		FI method	
	Added[a]	% Recovery[b]	Added[a]	% Recovery[b]
1	0.252	95.5±3.4	0.252	97.6±2.7
	0.505	108.4±2.7	0.505	107.6±3.7
	1.26	99.2±4.5	1.26	101.5±1.0
2	0.252	96.4±4.3	0.252	99.6±4.8
	0.505	101.1±3.8	0.505	108.5±1.9
	1.26	100.9±5.9	1.26	103.2±2.6

[a] mg CMT/ml urine.
[b] Mean of five determinations ±S.D.

determination of the batch and FI methods proposed.

The method was validated by applying the standard addition method. Aliquots of 1.0 ml of two different urine samples, to which know amounts (0.252–1.26 mg of CMT had been added were analyzed by the procedures described in Section 2.6. The results obtained are shown in Table 4, in all cases recoveries were in the range 95.5–108.5.

4. Conclusions

The Cu(II)–CMT complex is stable in acetic/acetate buffer of pH 5.9 and has spectrophotometric characteristics suitable for application to the spectrophotometric determination of the drug by batch and FI techniques.

The FI spectrophotometric methods proposed for the determination of CMT in pure, pharmaceutical forms and human urine has the advantages of simplicity, speed, accuracy and the use of inexpensive equipment.

The batch and FI methods are useful for the quality control and routine analysis of CMT in pharmaceuticals since there is no interference from the common excipients that might be found in commercial preparations. There is no significant difference between the two methods with respect to precision and accuracy.

The batch and FI spectrophotometric methods developed for determining CMT allow their determination in human urine samples in the physiological concentration range obtained after the usual therapeutic dose of CMT has been administered.

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