AN OPTIMIZED KINETIC SPECTROPHOTOMETRIC METHOD FOR THE RAPID AND ACCURATE DETERMINATION OF CEFOPERAZONE IN URINE AND TAP WATER SAMPLES

CÉSAR SOTO [a], RENATO SAAVEDRA [b], DAVID CONTRERAS [a], DIEGO P. OYARZÚN [c], AND CRISTIÁN POZA [a]*

[a]*Departamento de Química Analítica e Inorgánica, Facultad de Ciencias Químicas, Universidad de Concepción. Casilla 160-C, Concepción, Chile.*
[b]*Departamento de Física, Facultad de Ciencias Físicas y Matemáticas, Universidad de Concepción. Casilla 160-C, Concepción, Chile.*
[c]*Departamento de Química y Biología, Facultad de Ciencias Naturales, Universidad de Atacama, Avda. Copayapu 485, Copiapó, Chile.*

ABSTRACT

This study presents a new approach for quantifying the antibiotic cefoperazone (CPZ) in spiked urine and tap water samples using an indirect kinetic spectrophotometry method. The method involves the oxidation of CPZ by permanganate (MnO_4^-) in an alkaline media. The progress of the reaction is monitored by tracking the increase in manganate (MnO_4^{2-}) at 610 nm. The optimization of chemical-dependent variables was achieved through the utilization of multivariate statistical methods. The optimized values were 5 minute of reaction time, $KMnO_4$ of $1.11 \cdot 10^{-3}$ mol L^{-1}, and NaOH of 0.27 mol L^{-1}. Under these conditions, calibration curves were constructed. The detection limits obtained in urine and tap water spiked samples were $3.75 \cdot 10^{-7}$ mol L^{-1} and $3.74 \cdot 10^{-7}$ mol L^{-1}, respectively. The results showed that intraday and interday recoveries ranged from 98.25 to 102.7%, indicating acceptable differences between observed and expected values according to the confidence percentage established as a criterion of acceptability; thus, demonstrating repeatability in results as well as satisfactory accuracy for this kinetic analytical method.

Keywords: *Cefoperazone sodium, Kinetic determination, Spectrophotometry*.

1. INTRODUCTION

In recent decades, treatments for infectious diseases have advanced through the development of new, more effective, and less toxic drugs. Cefoperazone (CPZ) is a third-generation semisynthetic cephalosporin commonly used to treat various bacterial infections in humans and animals [1, 2]. It is a polar, water-soluble molecule ($pK_a = 3.38$) with a tetrazolyl moiety that is resistant to beta-lactamase. CPZ works by binding to specific penicillin-binding proteins in the bacterial cell wall, inhibiting wall synthesis. As sodium salt, it is typically administered through intramuscular or intravenous injection. Although the drug is mainly excreted in the bile, urinary excretion primarily by glomerular filtration accounts for up to 30% of a dose unchanged within 12 to 24 hours [3]. As an emerging pollutant, their widespread use has led to pollution of the environment and especially of water bodies. Despite its effectiveness, the widespread use of CPZ has resulted in environmental pollution, particularly in water bodies. Thus, the determination of CPZ is important in various fields such as clinical laboratories, pharmaceutical, and quality control. A HPLC standard method is available by the United States Pharmacopeia [4]. There are several methods for quantifying CPZ in differ ent matrices, UV-vis spectrophotometry [5, 6, 7, 8, 9], HPLC [10, 11, 12, 13], electroanalytical [14, 15, 16], IR spectroscopy [17], electrophoresis [18], and chemiluminescence [17]. The literature on analytical procedures based on kinetics reports a few batch spectrophotometric methods for the quantification of CPZ involving a redox reaction between CPZ and oxidant agents. These kinetic methods exhibit selectivity and low interferences effect as they measure the increase in absorbance over time rather than relying on a single absorbance value. For analyte quantification, the most suitable is evaluated considering, applicability and lineal parameters [19, 20, 21, 22]. This study presents a new approach for quantifying the analyte CPZ in spiked urine and tap water samples using an indirect kinetic spectrophotometric method. The method involves the oxidation of CPZ by $KMnO_4$ in an alkaline media. The progress of the reaction is monitored by tracking the absorbance increase in manganate (MnO_4^{2-}) at 610 nm. The optimization of variables reliant on reaction properties is accomplished by employing multivariate statistics.

2. EXPERIMENTAL

2.1. Chemicals

All chemicals employed in this study were of analytical grade. Stock solutions were prepared by dissolving specific quantities of CPZ ($1.035 \cdot 10^{-3}$ mol L^{-1}, sourced from Sigma-Aldrich), $KMnO_4$ (0.103 mol L^{-1}, obtained from Merck), and NaOH (0.99 mol L^{-1}, also from Merck) in volumetric f lasks. Both the stock solutions and subsequent dilutions were prepared using deionized water.

2.2. Instruments

For absorbance measurements (A), a Perkin Elmer Lambda 35 double beam spectrophotometer equipped with a 10 mm quartz cell was employed. The spectral data were processed using the Perkin Elmer UV Win Lab Data Processor and Viewer 1.00 software. Additionally, an analytical balance, model AS 60/220/C/2 (±0.01 mg precision), and a pH meter, Hanna Edge-HI2020, were utilized.

2.3. Procedures

2.3.1. Factorial Optimization Parameters

The experimental design and analysis of the reaction system were performed using a 2^4 factorial design and response surface methodology. A central composite circumscribed design (CCC) was used to assess the influence of the factors outlined in Table 1. The CPZ concentration was fixed at $4.00 \cdot 10^{-5}$ mol L^{-1}, and the response variable was the absorbance at 610 nm after 5 minutes. The polynomial equation and response surface plots for the reaction were obtained using Modde 7.0® software. The model was statistically validated using ANOVA at a 95% confidence level, with the same software.

Table 1. Coded values for the CCC design.

Factor	-2	-1	0	+1	+2
NaOH /mol L^{-1}	$4.70 \cdot 10^{-3}$	$9.60 \cdot 10^{-3}$	$1.68 \cdot 10^{-2}$	$2.40 \cdot 10^{-2}$	$2.89 \cdot 10^{-2}$
KMnO4 / mol L^{-1}	$9.11 \cdot 10^{-5}$	$3.10 \cdot 10^{-4}$	$6.31 \cdot 10^{-4}$	$9.52 \cdot 10^{-4}$	$1.17 \cdot 10^{-3}$
Time / min	2	5	10	15	18

2.3.2. General Kinetic Procedure

The stock solutions were utilized to create the reaction mixtures, employing a substantial excess of $KMnO_4$ in comparison to CPZ. This approach ensured that pseudo-first order kinetics were maintained throughout all experiments. The absorbance at 610 nm was recorded after 5 minutes of reaction time. The log *v* versus log[CPZ] plots were used to determine the reaction order (n) and the rate constant (k´), as well as the slope changes by derivative analysis. The calibration curves were obtained by using blank solutions and CPZ standards with concentrations ranging from $2.00 \cdot 10^{-6}$ mol L^{-1} to $4.00 \cdot 10^{-5}$ mol L^{-1}. The regression analysis provided the values of the slopes, intercepts, and correlation coefficients.

2.3.3. Initial rate method

Different amounts of the CPZ standard solution were added to calibrated flasks, each one with a volume of 5 mL. Then, 0.75 mL of NaOH and 1.0 mL of $KMnO_4$ were added to each flask. The flasks were filled with distilled water to the mark and mixed well. The solutions were transferred to the spectrophotometric cell and their absorbance was measured at 610 nm over time, from 0 to 35 min, using a reagent blank as a reference. The initial reaction rate (*v*) for each concentration was calculated from the slope of the absorbance-time curve. A calibration curve was obtained by plotting the log *v* versus log C. The regression equation from the graph was used to determine the CPZ quantity.

**Corresponding author email:* cesarsoto@udec.cl

2.3.4. Constant Rate method

From the method in section (2.3.3), the tangents are obtained when the signal of the analyte remains constant at a finite time. The log[CPZ] versus time plots were employed to calculate the constant k, figured as -2.303 times the curve slope. Subsequently, a calibration curve was generated by plotting these k values against their corresponding initial concentrations.

2.3.5. Fixed time method

Aliquots of standard CPZ solution were transferred into a series of 5 mL calibrated volumetric flasks. In each sample, 0.75 mL of NaOH and 1.0 mL of $KMnO_4$ were added, and the volume was then adjusted using deionized water. The samples were mixed well and their absorbance at 610 nm was recorded over time, from 0 to 35 min, every 5 min. A reagent blank was also prepared and measured for reference. The calibration curve was constructed by plotting the absorbance against the concentration of the CPZ. The quantity in each sample was determined using the regression equation.

2.3.6. Procedure for the proposed samples

The water samples were collected from taps in two different laboratories and stored in 500 mL polyethylene bottles without adding any preservatives. Analysis was performed within 5 hours after collection. The samples were filtered with 0.22 µm filters and boiled for 20 minutes to remove suspended solids and dissolved gasses. Then, CPZ standards were spiked into the samples and analyzed as described in the kinetic procedure, except that treated tap water was used to bring the final volume to the mark. A previously reported solid-phase extraction (SPE) method was applied to analyze spiked urine samples [23]. The sample preparation involved the combination of 1 part urine (previously filtered through a 0.22 µm filter), 2 parts CPZ solution ($1.80 \cdot 10^{-2}$ mol L^{-1}), and 1 part buffer solution (acetic acid / sodium acetate 0.1 mol L^{-1}, pH = 4.3). The SPE column (Rec-18 200 mg / 3 mL, flow rate 1 mL min^{-1} Perkin Elmer) was first conditioned with 6 mL of methanol to remove organic compounds and 6 mL of deionized water to remove ionic species. After conditioning, the sample (1.0 mL) was loaded onto the column and washed with 3 mL of HCl 0.1 mol L^{-1} (pH < 4.3). The analyte was then eluted with 3 mL of methanol (flow rate 1 ml L^{-1}). A third of the elution volume was dried under a flow of $N_{2(g)}$ at 40°C and reconstituted in a suitable volume of deionized water to obtain a CPZ standard solution of $5.00 \cdot 10^{-4}$ mol L^{-1}. At the time of determination, aliquots were taken from this solution.

2.3.7. Calibration curve in Proposed Samples

The method described in the section (2.3.6) was used to analyze both tap water and urine samples. However, prior to creating the calibration curves, the samples were diluted with treated tap water and urine respectively. This adjustment was made to ensure that the samples were in the appropriate concentration range for the analysis.

3. RESULTS AND DISCUSSIONS

The spectrophotometric properties of the CPZ-$KMnO_4$ system were studied using a univariate method. The effects of experimental variables such as reaction time (t), concentrations of NaOH and $KMnO_4$ on the colour development and its stability were also examined. The absorbance measurements were carried out using a CPZ concentration of $4.00 \cdot 10^{-4}$ mol L^{-1}. The effect of $KMnO_4$ concentration on the reaction was studied over a range of $3.10 \cdot 10^{-4}$- $2.23 \cdot 10^{-3}$ mol L^{-1}. The results showed a proportional dependence between the oxidant concentration and the reaction rate. The signal had a large increase at concentrations up to $1.27 \cdot 10^{-3}$ mol L^{-1}, but at higher concentrations, a decline in the signal increase was observed (Fig. 1). However, this did not affect the reaction rate as it only depends on the substrate (CPZ) concentration. Therefore, $1.27 \cdot 10^{-3}$ mol L^{-1} was initially selected for further studies. The influence of NaOH concentration on the reaction rate was studied between 0 and $4.55 \cdot 10^{-2}$ mol L^{-1}, and the results showed the influence of the alkalinity of the medium on the oxidation reaction (Fig. 2). The absorbance variations for each NaOH concentration value decreased from $2.40 \cdot 10^{-3}$ mol L^{-1}, so this value was chosen as the most suitable concentration. The effect of the reaction time was studied by measuring the absorbance at increasing time intervals. The measurements were performed every 5 min during a 35 min interval (Fig. 3). The absorbance variations for each reaction time value decreased from 5 min, for longer times the difference in absorbance was negligible. Based on this, t = 5min was selected as the reaction time.

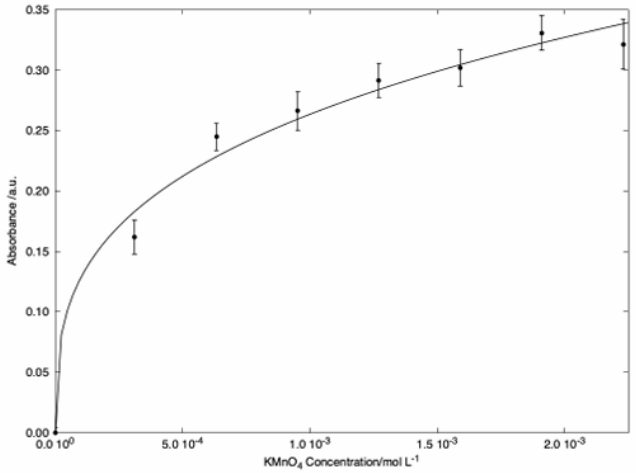

Figure 1. Concentration effect of $KMnO_4$ ($3.1 \cdot 10^{-4}$- $2.23 \cdot 10^{-3}$ mol L^{-1}) on the CPZ-$KMnO_4$ system at 610 nm, using CPZ $1.00 \cdot 10^{-5}$ mol L^{-1}, NaOH $1.68 \cdot 10^{-2}$ mol L^{-1} and t = 5 min.

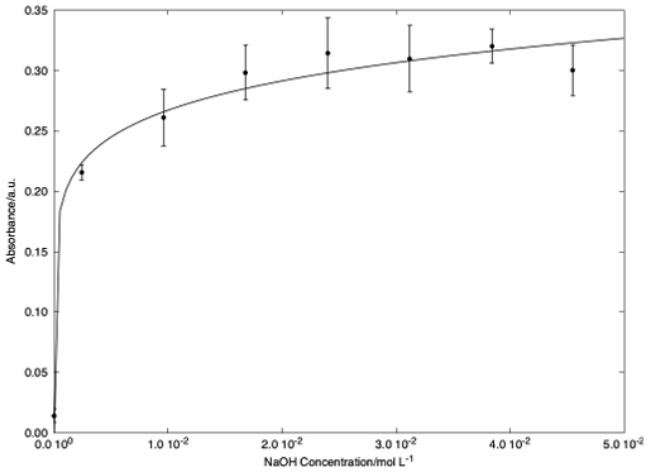

Figure 2. Concentration effect of NaOH (0 and $4.55 \cdot 10^{-2}$ mol L^{-1}) on the CPZ-$KMnO_4$ system at 610nm, using CPZ $1.00 \cdot 10^{-5}$ mol L^{-1}, $KMnO_4$ $1.27 \cdot 10^{-3}$ mol L^{-1} and t = 5 min.

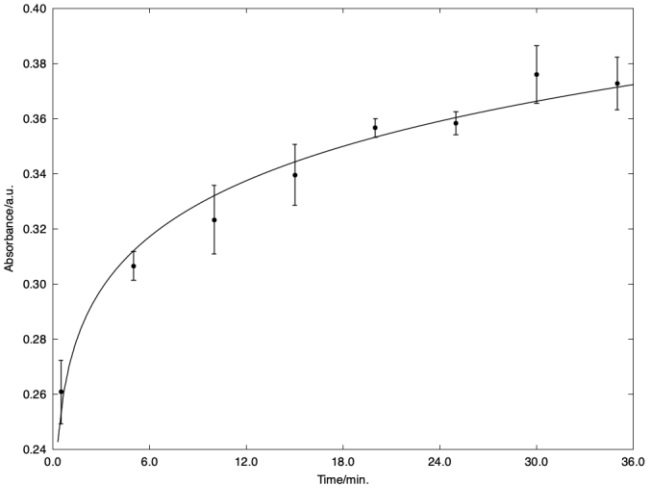

Figure 3. Effect of reaction time (t) on the CPZ-$KMnO_4$ system at 610 nm, using at NaOH $2.40 \cdot 10^{-2}$ mol L^{-1}, CPZ $1.00 \cdot 10^{-5}$ mol L^{-1} and $KMnO_4$ $1.27 \cdot 10^{-3}$ mol L^{-1}.

3.1. Variables Optimization

The optimized experimental variables were determined using the response surface methodology and a circumscribing central composite design (CCC) considering the reaction time, NaOH and $KMnO_4$ concentrations (Table 1). These optimal values obtained were $2.70 \cdot 10^{-2}$ mol L^{-1}, $1.11 \cdot 10^{-2}$ mol L^{-1} and 18.4 min for NaOH, $KMnO_4$ concentrations, and reaction time, respectively (Fig. 4). These values were verified experimentally and then adjusted to $2.70 \cdot 10^{-2}$ mol L^{-1}, $1.11 \cdot 10^{-2}$ mol L^{-1} for NaOH and $KMnO_4$ concentrations and 5 min for reaction time, as a slight increase of 0.02 a.u. was observed between 5 and 18 min (Table 2).

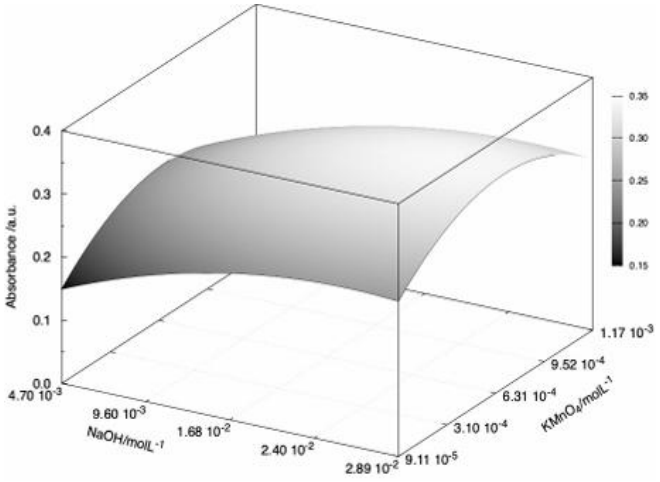

Figure 4. Surface response of the circumscribing central composite design (CCC).

Table 2. Verification of the CCC model.

Value	NaOH/mol L^{-1}	$KMnO_4$/mol L^{-1}	t/min	A/a.u.	A range/a.u.
Foretold	$2.67 \cdot 10^{-2}$	$1.11 \cdot 10^{-3}$	18.24	0.3550	0.3342-0.3759
Obtained	$2.70 \cdot 10^{-2}$	$1.11 \cdot 10^{-3}$	18.24	0.3430	
Foretold	$2.70 \cdot 10^{-2}$	$1.11 \cdot 10^{-3}$	5	0.3236	0.2897-0.3273
Used	$2.70 \cdot 10^{-2}$	$1.11 \cdot 10^{-3}$	5	0.3230	

3.2. Stoichiometry Determination

The stoichiometry of the reaction was determined by applying the logarithmic method [24], by monitoring the absorbance under the optimized conditions. Linear regression plots of Log A versus Log[CPZ] at a constant $KMnO_4$ concentration and Log A versus Log [$KMnO_4$] at a constant CPZ concentration were obtained, with slopes of 0.709 and 0.842 respectively. This indicates a molar ratio of the reaction of approximately 1:1.

3.3. Evaluation of Kinetic studies and quantitation methods

The study of kinetic behaviour of the reaction under pseudo-first-order conditions by using the equation $v = k'[CPZ]^n$. With the plot of log v versus log[CPZ], the numerical value of the order of reaction and the rate constant were found to be n = 0.907 (≈ 1) and $k' = 232.7 s^{-1}$.

The rate law $v = 232.7 s^{-1}[CPZ]^{0.907}$

describes a pseudo-first order reaction and supports the experiments conducted to obtain CPZ concentration. The initial rate, constant rate, and fixed time kinetic methods were tried. The choice of the best method was based on four criteria: applicability, sensitivity, intercept, and coefficient of determination, r^2.

3.3.1. Initial Rate Method

The curves of absorbance A (at 610 nm) versus t are obtained for CPZ concentrations over the range $2.0 \cdot 10^{-6}$ to $4.0 \cdot 10^{-5}$ mol L^{-1} of CPZ. Then, tangents were drawn for each curve with t = 250 s. Afterwards, the respective slopes (k″) were obtained.

The fit equation

$k'' = 14.84[CPZ] + 9.0 \times 10^{-5}$ ($r^2 = 0.9799$)

was obtained from the graph of k″ versus CPZ concentration and it corresponds to a kinetic behaviour of pseudo-zero order. The low slope value indicate poor sensitivity. Consequently, this method was not preferred.

3.3.2. Constant Rate Mehtod

For this method, the plots of Log A (at 610 nm) versus t (0-1800 s) for range of CPZ concentration of $2.0 \cdot 10^{-6}$ to $4.0 \cdot 10^{-5}$ mol L^{-1} were performed, obtaining straight lines, with slope = k′ 2.303 (pseudo-first order). Then, the drug concentration versus k′ was plotted, and the corresponding linear regression equation is

$k' = 0.9514[CPZ] - 0.0002$ ($r^2 = 0.1296$)

The coefficient of determination r^2 and slope values indicate poor linearity and sensitivity, respectively. For this reason, this method was discarded.

3.3.3. Fixed Time

In this method, absorbances (A) measured at 25°C and 610 nm at specific set of fixed time values (0 to 35 minutes). Linear equations and statistical parameters were determined for each time, incorporating a two-tailed Student's t-test with (n − 2) degrees of freedom (Table 3). Assessing the null hypothesis (H_0) - suggesting no correlation between CPZ concentration and absorbance- revealed that all calculated t-student values exceeded the critical value ($t_{calc} > t_{crit}$) for each time, leading to the rejection of the null hypothesis and confirming linearity at every time value. While sensitivity increased with time, a decline in correlation was observed. At 35 minutes, the maximum sensitivity was achieved; however, the increase in absorbance was deemed statistically insignificant. Consequently after careful evaluation, a reaction time of 5 minutes emerged as the optimal choice, providing the best statistical parameters. Hence, the fixed time method was selected for CPZ quantitation due to its superior sensitivity increase.

Table 3. Calibration curves and statistical parameters for different fixed times at 610 nm and CPZ concentration ranging between $2.00 \cdot 10^{-6}$ and $4.00 \cdot 10^{-5}$ mol L^{-1}

Time/min	r^2	Typical Error (10^{-2})	t_{calc}	Intercept (10^{-2})	Slope (10^4)
0	0.9830	4.65	10.71	5.552	1.568
5	0.9741	5.02	12.26	7.887	1.940
10	0.9727	5.72	11.93	8.076	2.149
15	0.9714	6.06	11.65	8.077	2.223
20	0.9727	5.92	11.95	8.391	2.227
25	0.9710	6.21	11.58	8.608	2. 265
30	0.9682	6.50	11.03	9.133	2.257
35	0.9628	7.08	10.18	1.404	2.269

t_{crit} **= 4.3.**

3.4. Calibration

Curve and Analytical Parameters with CPZ Standard Solutions. The analytical parameters were obtained using the optimal experimental conditions and CPZ standard solutions with 30 reagent blanks (without the analyte) (Table 4). The accuracy and precision of the method were evaluated using the recovery rate and Student's t-test at three different CPZ concentrations ($2.00 \cdot 10^{-6}$, $1.00 \cdot 10^{-5}$, $2.00 \cdot 10^{-5}$ mol L^{-1}). After 5 consecutive days of measurement, the results showed that the intraday and interday recoveries were between 98.2% and 102.7%, with a $t_{calc} > t_{crit}$ (Table 5), indicating that there were no significant differences between the observed and expected values based on the established confidence percentage. This indicates that the results are repeatable, and the kinetic analytical method has satisfactory accuracy.

Table 4. Analytical Figures of merit for CPZ quantitation.

Parameters	Standard solutions	Tap water	Urine
Linear Regression	$A=26102.8C + 1.49\cdot10^{-2}$	$A=25659.9C + 2.22\cdot10^{-2}$	$A=25727.8C + 1.85\cdot10^{-2}$
r^2	0.9993	0.9989	0.9988
LD/ mol L^{-1}	$3.50\cdot10^{-7}$	$3.75\cdot10^{-7}$	$3.74\cdot10^{-7}$
LQ/ mol L^{-1}	$1.06\cdot10^{-6}$	$1.14\cdot10^{-6}$	$1.13\cdot10^{-6}$
Linear Rangue / mol L^{-1}	$1.06\cdot10^{-6} – 2.00\cdot10^{-5}$	$1.14\cdot10^{-6} – 2.00\cdot10^{-5}$	$1.13\cdot10^{-6} – 2.00\cdot10^{-5}$
σ (Min. concentration)	$2.77\cdot10^{-3}$	$2.92\cdot10^{-3}$	$2.92\cdot10^{-3}$

LD: Limit of detection = 3σ/slope ; LQ: Limit of quantitation = 10σ/slope

Table 5. Intraday and interday repeatability and reproducibility of the method for CPZ quantitation.

	Standard solution			Tap water			Urine		
	Intraday								
Added 10^{-6} mol L^{-1}	2.00	10.0	20.0	2.00	10.0	20.0	2.00	10.0	20.0
Found 10^{-6} mol L^{-1}	1.96	10.2	19.7	2.04	10.4	19.5	2.03	10.4	19.6
Standar error 10^{-8}	2.17	8.48	6.56	0.77	10.6	24.4	3.73	11.1	24.4
C.L. 10^{-8}	4.58	17.9	13.9	1.63	22.4	51.5	0.79	2.34	5.14
t_{calc}	0.54	0.69	1.66	1.62	1.14	0.74	0.265	1.299	0.615
Recovery %	98.25	101.7	98.37	101.9	103.6	97.3	101.5	104.3	97.75
	Interday								
Added 10^{-6} mol L^{-1}	2.00	10.0	20.0	2.00	10.0	20.0	2.00	10.0	20.0
Found 10^{-6} mol L^{-1}	1.93	10.3	19.8	2.04	10.4	19.6	2.06	10.5	19.7
Standar error 10^{-8}	1.99	5.75	12.7	3.49	8.46	12.6	1.85	8.44	12.6
C.L. 10^{-8}	4.20	12.1	26.7	7.37	17.9	26.6	0.39	1.78	2.65
t_{calc}	1.10	1.57	0.54	0.43	1.67	1.15	1.01	2.13	0.911
Recovery %	96.71	102.7	98.97	102.2	104.2	97.8	102.8	105.4	98.28

C.L.: Confidence limits; n-2 degrees of freedown for intra-day an inter-day repeatability and reproducibility (t_{crit} = 4.3).

3.5. Validation and Application

The method underwent validation and subsequently applied to tap water and urine-spiked samples. Potential interferences such as organic and inorganic matter and dissolved gases were removed from tap water samples. The samples were then spiked with three different concentrations of CPZ ($2.00 \cdot 10^{-6}$; $1.00 \cdot 10^{-5}$; $2.00 \cdot 10^{-5}$ mol L^{-1}) according to the procedures outlined in Section Procedure for the proposed samples. A calibration curve was also created in tap water using CPZ concentrations in the range of $2.00 \cdot 10^{-6}$ to $2.00 \cdot 10^{-5}$ mol L^{-1} and the corresponding blanks. The analytical parameters obtained are summarized in Table 4. To evaluate the accuracy, recovery rates were calculated using the three CPZ concentrations previously mentioned. Measurements were taken over five consecutive days, using 30 blanks, resulting in recovery rates of 97.1-102.9 % and 97.9-103.9 % for intraday and interday assays respectively, indicating satisfactory accuracy (Table 5). The procedure for the proposed samples effectively eliminated any potential interferences. The developed procedure was applied to spiked tap water samples, and the linear regression equation obtained in this study was used for CPZ quantitation. The statistical analysis of the results using Student's t-test and a 95 % confidence level showed that there was no significant difference between the actual and found concentrations (Table 5). For the spiked urine samples, the CPZ calibration curve was prepared over the concentration range of $2.00 \cdot 10^{-6}$ to $2.00 \cdot 10^{-5}$ mol L^{-1} using the respective procedure. The analytical parameters obtained are summarized in Table 4. To evaluate the accuracy, recovery rates were calculated using three CPZ concentrations ($2.00 \cdot 10^{-6}$; $1.00 \cdot 10^{-5}$; $2.00 \cdot 10^{-5}$ mol L^{-1}) prepared with different volumes of spiked urine, which were accurately measured according to the section Kinetic procedure. The measurements for intraday and interday assays were carried out over five consecutive days, using 30 blanks (Table 5), and the recovery rates of the assays were 100.3 101.1% and 100.2- 102.3%, respectively, indicating a satisfactory accuracy. Any potential interferences were eliminated through the application of the procedure for the proposed samples. Based on the provided statements, it appears that the present method offers several advantages over the method described by El-Didamoni. et al [21]. The present method uses a multivariate calibration, shorter reaction time and lower limit of detection are achieved. All these factors make the method a more efficient and effective way to perform the analysis than the previous method described.

CONCLUSIONS

This study presents an optimized and validated indirect kinetic spectrophotometric method for the determination of CPZ. The optimization was achieved using a CCC design and adjusting the reaction time, NaOH, and $KMnO_4$ concentrations to 5 min, $2.70 \cdot 10^{-2}$ mol L^{-1}, and $1.11 \cdot 10^{-2}$ mol L^{-1} respectively. The stoichiometric ratio of the reaction was found to be 1:1 for CPZ:$KMnO_4$. The fixed-time method was found to be the most suitable for kinetic quantitation, with a reaction time of 5 min, as it provided satisfactory analytical parameters. The method is simple, cost-effective, and does not require expensive instruments. The accuracy and reliability of the method were evaluated by conducting several assays on both tap water and urine-spiked samples, and the results were statistically satisfactory with recovery rates and Student's t-test. The method was also shown to effectively eliminate any interference effects from the matrix through the treatment developed for each sample.

CONFLICTS OF INTEREST

The authors state that there are no conflicts of interest related to the publication of this paper.

FUNDING STATEMENT

The authors would like to acknowledge the National Fund for Science and Technology (FONDECYT) project number 1161132 and the University of Concepción VRID 219.021.037-INV for providing financial support for this research.

REFERENCES

1. M. S. Cohen, H. E. Washton, S. F. Barranco, Multicenter clinical trial of cefopera- zone sodium in the united states, The American Journal of Medicine 77 (1, Part 2) (1984) 35 - 41. doi:10.1016/S0002-9343(84)80094-7.
2. H. S. Sader, C. G. Carvalhaes, J. M. Streit, M. Castanheira, R. K. Flamm, Antimi- crobial activity of cefoperazone-sulbactam tested against gram-negative organisms from europe, asia-pacific, and latin america, International Journal of Infectious Dis- eases 91 (2020) 32?37. doi:10.1016/j.ijid.2019.11.006.
3. D. S. Wishart, C. Knox, A. C. Guo, S. Shrivastava, M. Hassanali, P. Stothard, Z. Chang, J. Woolsey, Drugbank: a comprehensive resource for in silico drug dis- covery and exploration, Nucleic Acids Research 34 (suppl. 1) (2006) D668-D672. doi:10.1093/nar/gkj067.
4. USP 29, The United States Pharmacopeia : USP 29: The National Formulary : NF 24 : by Authority of the United States Pharmacopeial Convention, Meeting at Washington, D.C., March 9-13, 2005, Rockville, MD, 2006.
5. L. Chen, O. Sha, W.-J. Zhai, J.-H. Yang, Fading spectrophotometric determina- tion of cefoperazone sodium in injections by Fe3+-sulfosalicylic acid system, Asian Journal of Chemistry 25 (14) (2013) 7918 ? 7920. doi:10.14233/ajchem.2013.14705.
6. V. D. Hoang, N. T. Loan, V. T. Tho, H. M. T. Nguyen, UV spectrophoto- metric simultaneous determination of cefoperazone and sulbactam in pharmaceu- tical formulations by derivative, fourier and wavelet transforms, Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 121 (2014) 704 ? 714. doi:10.1016/j.saa.2013.11.095.
7. R. Ali, H. R. H. Ali, H. A. Batakoushy, S. M. Derayea, M. M. Elsutohy, A reductant colorimetric method for the rapid detection of certain cephalosporins via the pro- duction of gold and silver nanoparticles, Microchemical Journal 146 (2019) 864?871. doi:10.1016/j.microc.2019.02.023.
8. H. A. B. Hassan Refat H. Ali, Ramadan Ali, S. M. Derayea, Spectroscopic analysis and antibacterial evaluation of certain third generation cephalosporins through metal complexation, Analytical Chemistry Letters 7 (4) (2017) 445?457. doi:10.1080/22297928.2017.1366868.
9. Y. Yuan, S. Fu, Q. Xu, J. Yang, X. Hu, S. Liu, The fluorescence and resonance rayleigh scattering spectral study and analytical application of cerium (IV) and ce- foperazone system, Spectrochimica Acta Part A: Molecular and Biomolecular Spec- troscopy 162 (2016) 93 ? 97. doi:10.1016/j.saa.2016.03.001.
10. M. E.-A. Sobhy, E. Mohamed, Hossinny, E. Sadek, H. H. Marwa, HPLC determi- nation of cefepime, cefotaxime and cefoperazone in bulk and dosage forms, Asian Journal of Pharmaceutical Analysis 4 (4) (2014) 168 ? 173.
11. M. F. Al-Hakkani, N. Ahmed, A. A. Abbas, M. H. A. Hassan, Cefoperazone rapidly and sensitive quantitative assessment via a validated RP-HPLC method for different dosage forms, in-use stability, and antimicrobial activities, BMC Chemistry 17 (1) (2023) 72. doi:10.1186/s13065-023-00989-0.
12. R.-H. Wang, L.-M. Cheng, H.-Y. Qu, G.-T. Hao, Z.-Y. Liu, H. Xia, Determina- tion of cefoperazone and sulbactam by LC-MS/MS in plasma and ultrafiltrate of patients undergone continuous renal replacement therapy, Journal of International Pharmaceutical Research 42 (2015) 199?205. doi:10.13220/j.cnki.jipr.2015.02.014.
13. Y.-H. Gao, B.-Q. Li, X.-M. Cheng, A.-C. Liu, J.-G. Tian, Determination of the content of cefoperazone sodium and tazobactam sodium in dog plasma by liquid chromatography-tandem mass spectrometry, Chinese Journal of Pharmaceutical Analysis 25 (11) (2005).
14. P. Norouzi, B. Larijani, F. Faridbod, M. R. Ganjali, Determination of cefoperazone based on nano-composite electrode using coulometric FFT admittance voltammetry, International Journal of Electrochemical Science 8 (5) (2013) 6118?6130.
15. B.Dogan,A.Golcu,M.Dolaz,S.A.Ozkan,Electrochemicalbehaviourofthebacte- ricidal cefoperazone and its selective voltammetric determination in pharmaceutical dosage forms and human serum, Current Pharmaceutical Analysis 5 (2) (2009) 179? 189. doi:10.2174/157341209788172924.
16. S. Billov , R. Kizek, F. Jelen, P. Novotn , Square-wave voltammetric determina- tion of cefoperazone in a bacterial culture, pharmaceutical drug, milk, and urine, Analytical and Bioanalytical Chemistry 377 (2003) 362 ? 369. doi:10.1007/s00216- 003-2109-5.
17. V. D. Hoang, D. T. Huyen, P. H. Phuc, Adsorptive cathodic stripping voltammetric determination of cefoperazone in bulk powder, pharmaceutical dosage forms, and human urine, Journal of Analytical Methods in Chemistry 2013 (Article ID 367914) (2013). doi:10.1155/2013/367914.
18. C. Thongpoon, B. Liawruangrath, S. Liawruangrath, R. A. Wheatley, A. Town- shend, Flow injection chemiluminescence determination of cephalosporins in pharmaceutical preparations using tris (2,2-bipyridyl) ruthenium(II)-potassium permanganate system, Analytica Chimica Acta 553 (1) (2005) 123?133. doi:10.1016/j.aca.2005.07.056.
19. G. A. Saleh, S. R. El-Shaboury, F. A. Mohamed, A. H. Rageh, Kinetic spectropho- tometric determination of certain cephalosporins using oxidized quercetin reagen, Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 73 (5) (2009) 946-954. doi:10.1016/j.saa.2009.05.002.
20. J. Du, H. Li, Sensitive chemiluminescence determination of thirteen cephalosporin antibiotics with luminol-copper(II) reaction, Applied Spectroscopy 64 (10) (2010) 1154- 1159. doi:10.1366/000370210792973613.
21. A. M. El-Didamony, M. Z. Saad, D. S. El-Shaprawy, Direct and indirect spectropho- tometric determination of some selected antibiotics using potassium permanganate, Main Group Chemistry 12 (2013) 139 -152. doi:10.3233/MGC-130096.
22. D. Y. Zhang, L. W. Zheng, Permanganate based chemiluminescence analysis of cefoperazon, in: Measurement Technology and Engineering Researches in Industry, Vol. 333 of Applied Mechanics and Materials, Trans Tech Publications Ltd, 2013, pp. 1807-1810. doi:10.4028/www.scientific.net/AMM.333-335.1807.
23. C. Soto, R. Saavedra, D. Contreras, C. Poza, S. Orellana, G. A. Olivares-Renter a, Flow injection analysis (FIA) with thermal lens spectrometry (TLS) for the rapid determination of cefoperazone in urine and water, Analytical Letters 55 (12) (2022) 1932-1944. doi:10.1080/00032719.2022.2036181.
24. L. G. Hargis, Analytical Chemistry: Principles and Techniques, Englewood Cliffs (N.J.): Prentice-Hall, 1988.