Journal of the Serbian Chemical Society
JSCS@tmf.bg.ac.yu • www.shd.org.yu/JSCS
J. Serb. Chem. Soc. 73 (5) 569–576 (2008) UDC 615.33+615.276+546.723:66.094.3:543.4/.5
JSCS–3739 *Original scientific paper*

An indirect atomic absorption spectrometric determination of ciprofloxacin, amoxycillin and diclofenac sodium in pharmaceutical formulations

MAHMOUD MOHAMED ISSA[1], R'AFAT MAHMOUD NEJEM[1]*, MONZER AL-KHOLY[1], NASER SAID EL-ABADLA[2], RAFIK SOBHI HELLES[1] and AKILA AMIN SALEH[3]

[1]*Analytical Chemistry, Department of Chemistry, Alaqsa University, P. O. Box 4051, Gaza,* [2]*Organic Chemistry, Department of Chemistry, Alaqsa University, Gaza, Palestine and* [3]*Analytical Chemistry, Ain Shams University, Cairo, Egypt*

(Received 23 May, revised 7 December 2007)

Abstract: A highly sensitive indirect atomic absorption spectrophotometric (AAS) method has been developed for the determination of very low concentrations of ciprofloxacin, amoxycillin and diclofenac sodium. The method is based on the oxidation of these drugs with iron(III). The excess of iron(III) was extracted into diethyl ether and then the iron(II) in the aqueous layer was aspirated into an air–acetylene flame and determined by AAS. The linear concentration ranges were 25–400, 50–500 and 60–600 ng ml^{-1} for ciprofloxacin, amoxycillin and diclofenac sodium, respectively. The results were statistically compared with the official method using *t*- and *f*-test at $p < 0.05$. There were insignificant interferences from most of the excipients present. The intra- and inter-day assay coefficients of variation were less than 6.1 % and the recoveries ranged from 95 to 103 %. The method was applied for the analysis of these drug substances in their commercial pharmaceutical formulations.

Keywords: ciprofloxacin; amoxycillin; diclofenac sodium; indirect atomic absorption spectrometry.

INTRODUCTION

Ciprofloxacin, chemically 1,4-dihydro-1-cyclopropyl-6-fluoro-4-oxo-7-(1-piperazinyl)-3-quinolinecarboxylic acid (Fig. 1a), is a quinolone antibiotic drug with a broad spectrum of activity against a variety of gram positive and gram-negative bacteria. It is mainly used to treat respiratory infections (*Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Haemophilus influenzae*), urinary tract infections, for gastrointestinal surgery, typhoid fever, gonorrhoea b (enterotoxigenic strains of *Escherichia coli*), and septicaemia. Ciprofloxacin acts by inhibiting the

* Corresponding author. E-mail: rafatnejem@alaqsa.edu.ps
doi: 10.2298/JSC0805569I

bacterial enzymes DNA gyrase. Amoxycillin (Fig. 1b) is the only phenolic penicillin used as an antibacterial drug. It is a moderate-spectrum β-lactam antibiotic used to treat bacterial infections caused by susceptible microorganisms. Amoxycillin is susceptible to degradation by β-lactamase-producing bacteria and so may be administered together with clavulanic acid to decrease its susceptibility. Diclofenac sodium is described as the salt of 2-[(2,6-dichlorophenyl)amino]benzene acetic acid (Fig. 1c). It is a non-steroidal anti-inflammatory drug of the cyclo--oxygenase (COX) inhibitor type. It is used for the treatment of rheumatoid arthritis, osteoarthritis, and ankylosing spondylitis, and for a variety of non-rheumatic inflammatory conditions.

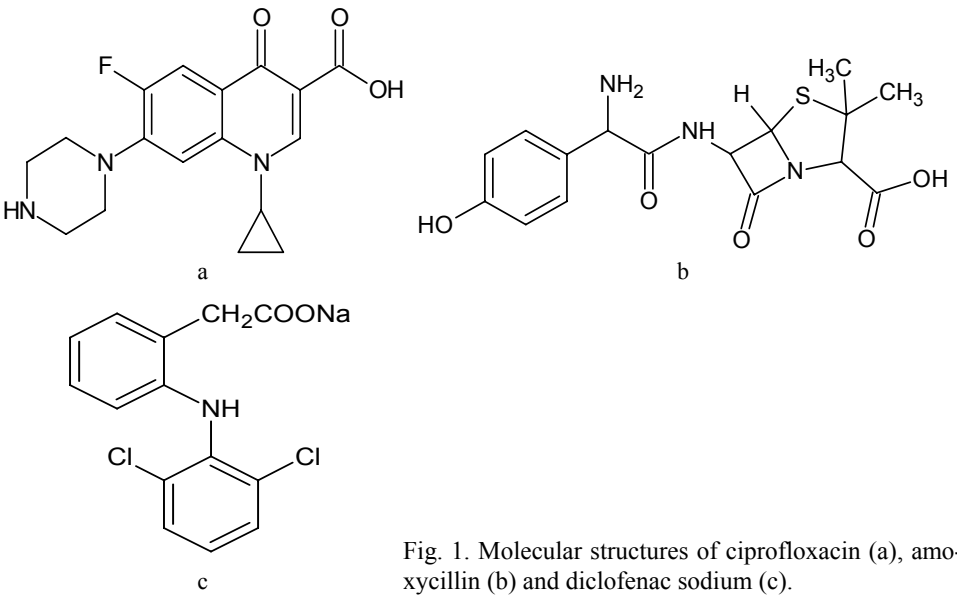

Fig. 1. Molecular structures of ciprofloxacin (a), amoxycillin (b) and diclofenac sodium (c).

Several methods have been reported for the quantitative analysis of the cited drugs, such as spectrophotometry,[1–4] flow injection,[5] fluorimetry,[6] titrimetry,[7,8] electrophoresis,[9] microbiology,[10,11] HPLC[12,13] and AAS[14–16] methods.

The reduction process of iron(III) to iron(II) by certain drugs, such as paracetamol,[17] diclofenac,[18] salbutamol sulphate,[19] captopril,[20] amoxycillin[21] and ciprofloxacin,[21] was used as the basis for their quantification based on a colorimetric method.

The present work aims to develop a highly sensitive, simple and rapid atomic absorption spectrometric method for application in quality control analysis. The proposed method is based on the reduction of iron(III) by the investigated drugs (ciprofloxacin, amoxycillin and diclofenac sodium). The excess of iron(III) was extracted into diethyl ether[22] and then iron(II) in aqueous layer was determined by AAS. The reaction method is simple since it is a single step process.

EXPERIMENTAL

Apparatus

The atomic absorption measurements were performed using a Perkin-Elmer AAS, Model A Analyst 100 spectrophotometer equipped with an iron hollow-cathode lamp under the following conditions: wavelength, 302.1 nm; lamp current, 30 mA; slit width, 0.2 nm; air/acetylene ratio, 3.5/1.5. A UV/Vis spectrophotometer (Spectro 23 Labomed Inc. USA) with quartz cells was used for all spectrophotometric measurements.

Chemicals and solutions

Analytical grade ciprofloxacin, amoxycillin and diclofenac sodium standards were obtained from the Middle East Pharmaceutical and Cosmetics Laboratories, Palestine. Various pharmaceutical formulations of ciprofloxacin, amoxycillin and diclofenac sodium were obtained commercially. These formulations contained only the investigated drug and were not in combination with other drugs.

Ferric sulphate was purchased from Sigma. Hydrochloric acid (32 %) was supplied by BDH and diethyl ether by Carlo Erba. Solutions of 2.60×10^{-5} M ciprofloxacin (10 μg ml^{-1}), 2.38×10^{-5} M amoxycillin (10 μg ml^{-1}) and 3.14×10^{-5} M diclofenac sodium (10 μg ml^{-1}) were prepared in 0.10 M HCl. A 1.00×10^{-4} M ferric sulphate solution (40 μg ml^{-1}) was also prepared in 0.10 M HCl. Standard solutions were prepared by serial dilution. All the employed chemicals were of analytical grade. Deionised water was used for the preparation of all solutions.

Procedure

Six 2.5 ml standard solutions of ciprofloxacin (25–400 ng ml^{-1}), amoxycillin (50–500 ng ml^{-1}) or diclofenac sodium (60–600 ng ml^{-1}) were pipetted into a series of 10 ml volumetric flasks. To each flask, 1.5 ml of 1.0×10^{-5} M ferric sulphate was added. The mixtures were heated using a boiling water bath for 10 min (100 °C). After cooling, 4.0 ml of 12 M HCl was added and the excess iron(III) was extracted with three portions of 10 ml of diethyl ether using a separatory funnel. Then the aqueous layer containing iron(II) was aspirated into an air–acetylene flame. The absorbance of iron(II) was measured at 302.1 nm, and the iron concentration was determined from a previously constructed calibration curve.

RESULTS AND DISCUSSION

It was found that ciprofloxacin, amoxycillin and diclofenac sodium were very weak reducing agents at room temperature. The oxidation of these compounds was non-quantitative, slow and time-consuming. However, on heating at 100 °C using a boiling water bath, the given drugs immediately reduced iron(III) to iron(II) in amounts which corresponded to the concentration of the drugs. The amount of iron(II) was determined by AAS.

Response characteristics

The equations of the calibrations describing the relation between drug concentration and atomic absorbance measurements obtained for ciprofloxacin, amoxycillin and diclofenac sodium are summarized as:

Ciprofloxacin:	$A = 1.510c - 0.007$	($R = 0.9991$)
Amoxycillin:	$A = 1.021c - 0.005$	($R = 0.9986$)
Diclofenac sodium:	$A = 0.9832c - 0.0003$	($R = 0.9998$)

where A and c are the absorbance and concentration (in μg ml^{-1}) of the drug, respectively. R is the correlation coefficient. The linear concentration ranges were 25–400 ng ml^{-1} for ciprofloxacin, 50–500 ng ml^{-1} for amoxycillin and 60–600 ng ml^{-1} for diclofenac sodium, based on AAS measurements. The proposed method exhibited a high sensitivity for the three drugs, which are 3.1, 4.4 and 4.5 ng ml^{-1} for ciprofloxacin, amoxycillin and diclofenac sodium, respectively.

Comparison of the proposed and official method using the *t*-test and *f*-test ($p < 0.05$) showed the high accuracy and precision of the AAS method. The results obtained by the proposed and official method[23] are summarized in Table I.

TABLE I. Assay of commercial tablet formulations by the AAS method and the official method[23]

Drug	Official method[23]			Proposed AAS method		
	Found	*t*-Test[a]	*f*-Test[b]	Found	*t*-Test[a]	*f*-Test[b]
Ciprofloxacin, 500 mg/tablet	498.3±2.8	1.66	2.6	496.7±3.7	1.32	2.2
Amoxycillin, 500 mg/capsule	499.6±4.2	0.64	2.9	502.4±4.4	1.78	3.1
Diclofenac, 100 mg/tablet	100.2±2.9	1.05	2.3	101.5±3.1	0.78	1.7

[a]$t_{tab}(n = 5) = 2.776$; [b]$f_{tab}\,(5,5) = 6.390$

The intraday, interday precisions and recoveries were tested (Table II). These data indicate that the method was reproducible within and between days. The mean percentage recovery ranged from 95 to 103 % (*RSD* < 6.1 %).

TABLE II. Intraday and interday assay of ciprofloxacin, amoxycillin and diclofenac sodium

Drug	c / μg ml^{-1}	Intraday assay			Interday assay		
		Found μg ml^{-1}	Recovery %	RSD %	Found μg ml^{-1}	Recovery %	RSD %
Ciprofloxacin	0.10	0.103±0.006	103.0	5.8	0.098±0.005	98.0	5.1
	0.40	0.39±0.01	97.5	2.6	0.406±0.01	101.5	2.2
Amoxycillin	0.20	0.19±0.01	95.0	4.7	0.196±0.01	98.0	5.1
	0.50	0.48±0.02	96.0	4.2	0.490±0.03	98.0	6.1
Diclofenac	0.24	0.235±0.01	97.9	4.7	0.238±0.01	99.2	5.5
sodium	0.60	0.61±0.03	101.7	5.2	0.590±0.03	98.3	5.8

The effects of the presence of common excipients (dextrose, glucose, saccharine sodium, starch, talc and magnesium stearate) were tested (Table III). There were no significant interferences due to the presence of any of these excipients.

Selecting the optimum solvent for an efficient extraction

Several organic solvents were tested to extract Fe(III) in the presence of Fe(II), *i.e.*, 1,2-dichloroethane, chloroform and diethyl ether. To select the suitable solvent, atomic absorbance values caused by the presence of Fe(II) and Fe(III) were measured after extraction with each of the three different organic solvents. The most suitable solvent for the extraction of only Fe(III) in the presence of Fe(II) was diethyl ether. The amount of extracted Fe(III) metal depen-

ded on the concentration of the acid. The maximum extraction value was attained with ≈ 6 M HCl, where approximately 99.99 % of the iron(III) was extracted.

TABLE III. Recovery of ciprofloxacin, amoxycillin and diclofenac sodium in the presence of common excipients (10 μg ml^{-1}) using the proposed method

Interfering material	Drug recovery, %		
	Ciprofloxacin	Amoxycillin	Diclofenac sodium
Sucrose	99.9±1.7	103.0±1.1	100.4±2.2
Glucose	101.0±2.2	101.0±1.8	101.0±1.4
Saccharin sodium	98.5±1.2	99.6±2.1	102.0±1.7
Starch	102.0±1.6	100.2±1.3	99.20±2.1
Talc	99.8±2.0	99.6±2.1	100.1±1.7
Magnesium stearate	97.2±2.8	100.5±1.7	100.8±2.2

Effect of temperature

The effect of the temperature was investigated by heating the reaction mixtures of these drugs in a water bath at different temperatures for 10 min. The absorbance was found to increase with increasing reaction temperature. The maximum absorbance was observed at 100 °C, as shown in Fig. 2. The time of heating is important to ensure complete reaction. Different heating time intervals were investigated at a constant temperature (100 °C); 10 min was found to be the optimal interval time to achieve complete reaction, as is shown in Fig. 3.

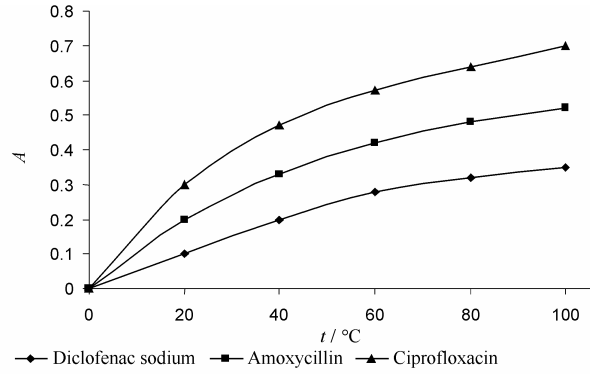

Fig. 2. Effect of temperature on the oxidation reactions of the drugs, ciprofloxacin 0.4 μg ml^{-1}, amoxycillin 0.5 μg ml^{-1}, and diclofenac sodium 0.3 μg ml^{-1}.

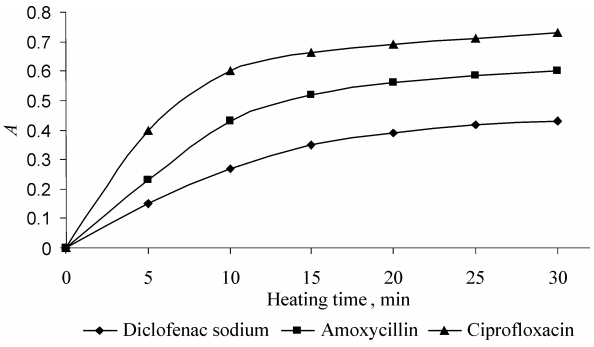

Fig. 3. Effect of heating time on the oxidation reactions of the drugs, ciprofloxacin 0.4 μg ml^{-1}, amoxycillin 0.5 μg ml^{-1}, and diclofenac sodium 0.3 μg ml^{-1}.

Effect of pH

The effect of pH on the response of the oxidation reactions was determined by recording the absorption of ciprofloxacin (400 ng ml^{-1}), amoxycillin (500 ng ml^{-1}) and diclofenac sodium (300 ng ml^{-1}) at different pH values. The absorption *vs.* pH graph, Fig. 4, showed the absorbance was almost independent of pH in the range 1.0–2.5 for ciprofloxacin, amoxycillin and diclofenac sodium.

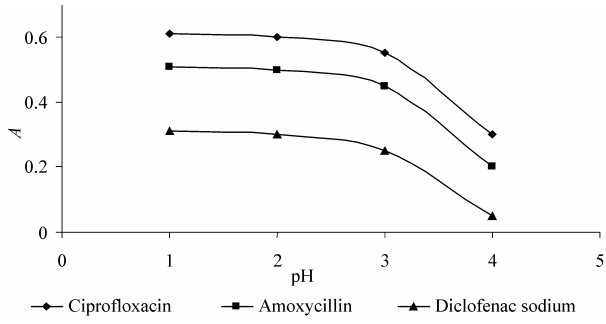

Fig. 4. Effect of pH on the oxidation reactions of the drugs, ciprofloxacin 0.4 μg ml^{-1}, amoxycillin 0.5 μg ml^{-1}, and diclofenac sodium 0.3 μg ml^{-1}.

Iron(III)/Drug mole ratio

For the stoichiometric relation of the oxidation reaction of the drugs, different amounts of iron(III), 0.2–2.5 ml aliquots (1.0×10^{-5} M ferric sulphate) were added to 2.5 ml aliquots of solutions of the drugs, 1.19×10^{-6} M ciprofloxacin, 1.04×10^{-6} M amoxycillin and 1.88×10^{-6} M diclofenac sodium. The maximum absorbance was attained with a five-fold, ten-fold and two-fold amount of iron(III) for ciprofloxacin, amoxycillin and diclofenac sodium, respectively (Fig. 5). Thus, 1.5 ml of a 1×10^{-6} M ferric sulphate solution was employed to achieve a constant and maximum absorbance for the three drugs.

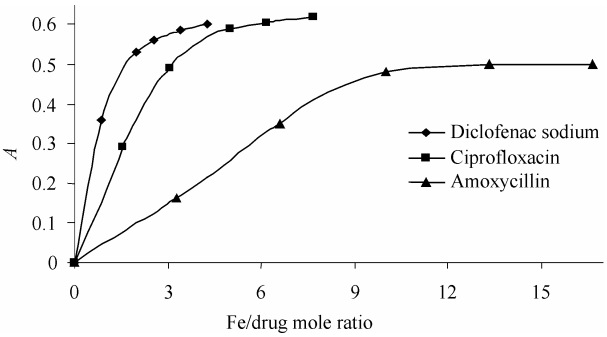

Fig. 5. Effect of iron(III) concentration on the drugs, ciprofloxacin 0.4 μg ml^{-1}, amoxycillin 0.5 μg ml^{-1}, and diclofenac sodium 0.3 μg ml^{-1}.

CONCLUSIONS

The AAS method is highly sensitive and, moreover, it can be used for routine analysis of the investigated drugs in raw materials and pharmaceutical formulations. The method is simple and rapid since it is a single step process. The

statistical parameters and the recovery tests data clearly indicate the reproducibility and accuracy of the method. The results demonstrate that the method has an equivalent accuracy and precision as the official methods, as found from the *t*- and *f*-tests.

И З В О Д

ИНДИРЕКТНО ОДРЕЂИВАЊЕ ЦИПРОФЛОКСАЦИНА, АМОКСИЦИЛИНА И НАТРИЈУМ-ДИКЛОФЕНАКА У ФАРМАЦЕУТСКИМ ФОРМУЛАЦИЈАМА АТОМСКОМ АПСОРПЦИОНОМ СПЕКТРОМЕТРИЈОМ

MAHMOUD MOHAMED ISSA[1], R'AFAT MAHMOUD NEJEM[1], MONZER AL-KHOLY[1], NASER SAID EL-ABADLA[2], RAFIK SOBHI HELLES[1] и AKILA AMIN SALEH[3]

[1]Analytical Chemistry, [2]Organic Chemistry, Department of Chemistry, Alaqsa University, Gaza, Palestine и [3]Analytical Chemistry, Ain Shams University, Cairo, Egypt

Развијена је високоосетљива индеректна атомска апсорпциона спектрометријска метода (AAS) за одређивање веома ниских концентрација ципрофлоксацина, амоксицилина и натријум-диклофенака. Метода се заснива на оксидацији лекова помоћу Fe(III). После уклањања вишка Fe(III) диетил-етром, Fe(II) се по аспирирању водене фазе у пламену ваздух–ацетилен одређује помоћу AAS. Линеарност је потврђена у опсегу концентрација 25–400 $ng \cdot ml^{-1}$ за ципрофлоксацин, 50–500 $ng \cdot ml^{-1}$ за амоксицилин и 60–600 $ng \cdot ml^{-1}$ за натријум-диклофенак. Резултати су упоређени са официналном методом применом *t*- и *f*-теста за $p < 0.05$. Нису утврђене значајне интерференције са најчешће коришћеним ексципијенсима. Коефицијент варијације је у свим случајевима мањи од 6,1 %, а проценат приноса у опсегу од 95 до 103 %. Метода је примењена у анализи поменутих лековитих супстанци у комерцијалним фармацеутским препаратима.

(Примљено 23. маја, ревидирано 7. децембра 2007)

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