Differential Pulse Polarographic Study of Amoxicillin and Ciprofloxacin and its Determination in Pharmaceuticals

Research Article

Salam A.H. Al-Ameri[1*], Najlah M.H. Al-Waeli[2]

Department of chemistry, College of Science, Al-Mustansiriyah University, Baghdad, Iraq.

Abstract

A differential pulse Polarographic method have been developed and validated for the direct determination of amoxicillin and ciprofloxacin in pharmaceuticals. The results show that the optimal analysis conditions for amoxicillin and ciprofloxacin was; the water as a best solvent at neutral medium, pH 7 , while for ciprofloxacin was at acidic medium, pH 4, with Briton-Robinson buffer solution and 3M KCl as a supporting electrolyte also 4mm^3 mercury drop size at 25°C.

Amoxicillin showed two peaks, the first one at - 0.25V and the other one at -0.83V, while the ciprofloxacin appears one peak at the applied potential -1.41V.

A standard calibration plot of amoxicillin and ciprofloxacin was prepared in the range between 0.05- 0.525μg.ml^{-1}. The accuracy and precision of the method for the determination of both analyte was tested. The results of laboratory amoxicillin samples showed the SD was 0.08 and RSD% did not exceed 0.13%, while the results of laboratory ciprofloxacin samples illustrate the SD was 0.26 and the RSD% did not exceed 0.4%.

The actual value of the peak potential E_p and the number of electrons required for the reduction for amoxicillin and ciprofloxacin was calculated using Ilkovic -Heyrovsky equation, the results confirm the first peak potential E_p was -0.832V with 2 electron used and the second E_p was -0.249V with 1 electron used, while the value of the ciprofloxacin peak potential E_p was -1.38 V with 2 electrons required.

This method effectively applied in commercial amoxicillin and ciprofloxacin pharmaceuticals.
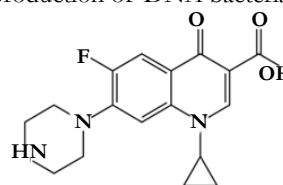
Keywords: Amoxicillin; Ciprofloxacin; DPP; Analysis.

Introduction

Antibiotics are a substance inhibits or kills the growth of bacteria [1] and belongs to a broader range of anti-vehicle microbiology group, used to treat infections caused by micro, including fungi and parasites organisms. The term "antibiotic" drafted by Waxman in 1942 to describe any substance produced by microorganisms counteracts the growth of other micro-organisms. Amoxicillin is an antibiotic of the penicillin group, it is one of the most commonly used antibiotics, addresses many of the bacterial infections by turning off the disease-causing bacteria growth, Figure below [2, 3].
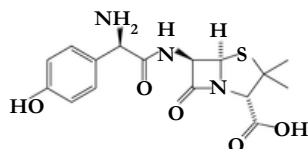
Several methods were use for the determination of amoxicillin in tablets or urine includes spectrophotometic [4-6], chromatography [7, 8], LC/MS [9] and electrochemical methods [10]. Most of the reported methods undergo disadvantages, such as difficult procedure and expensive instruments requirement and low detection sensitivity.

Ciprofloxacin is antibacterial belongs to a family of largely effectual spectrum florokonyolon against the most Gram-negative and positive and is also effective against anaerobic bacteria and prevents the reproduction of DNA bacterial, Figure below [11, 12].

Salam A.H. Al-Ameri, Najlah M.H. Al-Waeli (2016) Differential pulse polarographic study of amoxicillin and ciprofloxacin and its determination in pharmaceuticals. *Int J Bioanal Methods Bioequival Stud.* 3(1), 47-54.

47

A simple DPP method for the determination of ciprofloxacin hydrochloride and Tinidazole from tablets has been developed, these components produced waves at- 1.30 V and -0.38 V respectively in a solution of pH 6.5 [13].

Ciprofloxacin was determined individually by non-aqueous titration [14], UV-spectrophotometry and colorimetry [15-18], HPLC [19-24], capillary zone electrophoresis [25] Fluorescence Spectrodensitometry [26].

The purposes of this study were suggested and develop a simple and sensitive method for the qualitative and quantitative determination of amoxicillin and ciprofloxacin antibiotics in pure state and in commercial pharmaceuticals.

Materials and Methods

Apparatus

All measurements were conducted in a way differential pulse Polarographic, Using Polarography device 797VA Computrace-Metrohm, Herisau, Switzerland, The electrode assembly consist of the dropping mercury electrode, DME as working electrode, Ag/AgCl as reference electrode and a platinum auxiliary electrode, pH meter, four digit sensitive balance, Water bath, electric heater, Micropipette, Nitrogen gas with 99.999% purity, Glassware varied, Thermometer.

Materials

All the material and solvents used of high-purity(A.R. grade) purchased and obtained from Fluka and BDH and no further purification was need, water employed was de-ionized water, a standard samples for amoxicillin and ciprofloxacin supplied from in of Samarra drugs industries (SDI), Iraq, amoxicillin as Pulmoxyl, 500 mg and ciprofloxacin, 500mg commercially available from Julfar and ROX pharma Companies.

Preparation of samples

A 500 µg. ml^{-1} concentration standard solution of amoxicillin and ciprofloxacin drugs was prepared by dissolving the exact 25 mg weight of standard material in 50 ml water. Amoxicillin and ciprofloxacin in commercial pharmaceutical was also prepared as 500 µg. ml^{-1} concentrations based on the initial concentration of theirs active ingredient.

Analysis

Amoxicillin showed two peaks, the first one at applied potential -0.83V and the other at -0.25V, Figure-1, while the ciprofloxacin appears only one peak at the applied potential -1.41V, Figure-2 at the optimal experimental conditions. A 1–10.5 ml volume of the amoxicillin or ciprofloxacin solutions were analysis using Polarography device 797VA Computrace- Metrohm under the optimal experimental conditions found during this work. A standard calibration graph for amoxicillin and ciprofloxacin (Figure-3 and 4) in the concentration range 0.05 to 0.525 µg. ml^{-1} were prepared using the Method of Least Squares, M.L.S [27], and used to determine the amounts of amoxicillin or ciprofloxacin.

The regression equation was utilized for the calculation of unknown concentration in pharmaceuticals samples. The validity of the regression equation was testing by analysis laboratory made samples. Beers law was valid within the concentration range of amoxicillin or ciprofloxacin calculate.

General polarographic procedure

An a volume of amoxicillin or ciprofloxacin was transferred to a polarographic cell and diluted with de-ionized water, 2 ml of Britton-Robinson buffer was added at pH 7 for amoxicillin and pH 4 for ciprofloxacin with 0.5 ml of KCl as supporting electrolyte, the final cell volume with all other additions was equal to 20 ml, degassed with high purity nitrogen for 5 minutes to purge the oxygen. The polarograms were recorded at least twice from –0.0 to –1.8 mV and the mg amoxicillin or ciprofloxacin in the sample solutions were calculated from calibration plots.

Results and Discussion

The results show that the optimal analysis conditions for amoxicillin and ciprofloxacin was; the water as a best solvent at neutral medium, pH 7, while for ciprofloxacin was at acidic medium, pH 4, with Briton- Robinson buffer solution and 3M KCl as a supporting electrolyte also 4mm^3 mercury drop size at 25°C. Tables 1 and 2.

Amoxicillin showed two peaks, the first one at -0.83V while the second at -0.25V, Figure 1. It can be suggested a mechanism for the electrochemical reduction for those two peaks which show the reduction of carbonyl groups to hydroxyl, Figure 5.

While the ciprofloxacin appears only one peak at the applied potential -1.41V at the optimal conditions, Figure 2, this peak may be return to the reduction of the lone carbonyl to hydroxyl group, Figure 6.

Number of transferred electrons and the value of $E_{1/2}$ (or E_p)

The actual number of transferred electrons in a reversible electrode process and the actual value of $E_{1/2}$ (or E_p) was calculated using Heyrovsky–Ilkovic equation which explains the cathodes reduction wave as reversible process at 25°C [28].

$$E_{applied} = E_{1/2} - (0.0591/n) \, Log \, (i/i_d\text{-}i)$$

This equation defines the relationship between diffusion current and applied potential for a reversible reaction involving the formation of a soluble product. Number of electrons (n) can be establish from the plot of log $(i/i_d – i)$ against applied voltage (E) at set species concentrations. For a reversible process, (n) appear to be a correct number, whereas, an incomplete number for (n) represented an irreversible process. Number of required (n) for the reduction of amoxicillin and ciprofloxacin was calculated. A straight line obtained demonstrated the amoxicillin results, the actual first peak voltage E_p was -0.832V and 2 electrons was used whereas the second peak voltage E_p was -0.249V with 1 electron used, while the ciprofloxacin straight line obtained shows the actual peak voltage E_p was -1.38 V with 2 electrons required for the reduction, Figures -7, 8 and 9.

Salam A.H. Al-Ameri, Najlah M.H. Al-Waeli (2016) Differential pulse polarographic study of amoxicillin and ciprofloxacin and its determination in pharmaceuticals. *Int J Bioanal Methods Bioequival Stud.* 3(1), 47-54.

Figure 1. Amoxicillin polarogram at special concentrations.
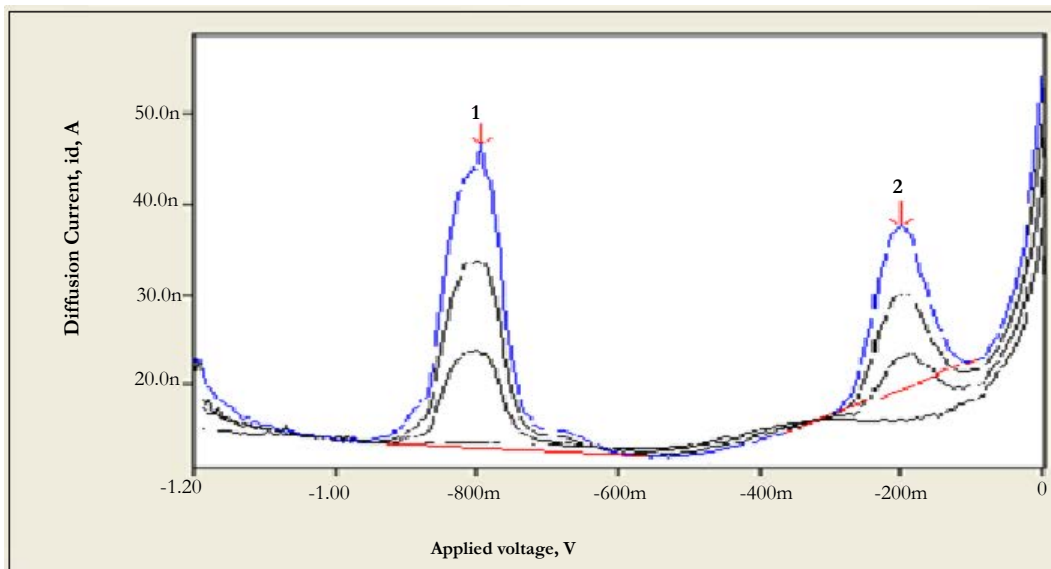

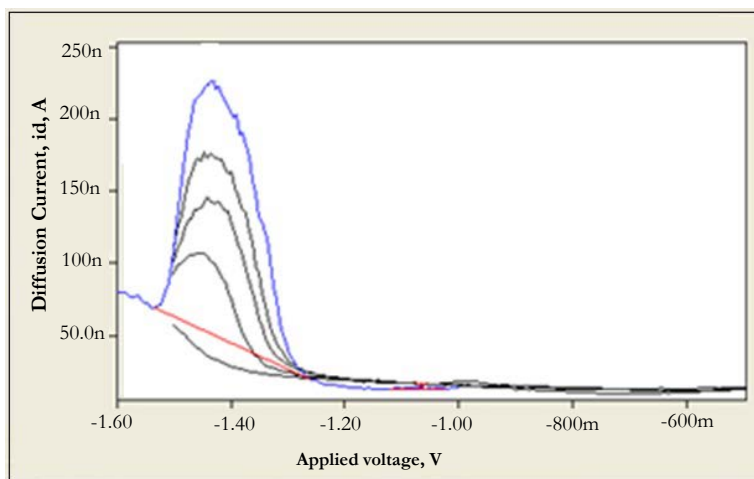**Figure 2. Ciprofloxacin polarogram at special concentrations.**
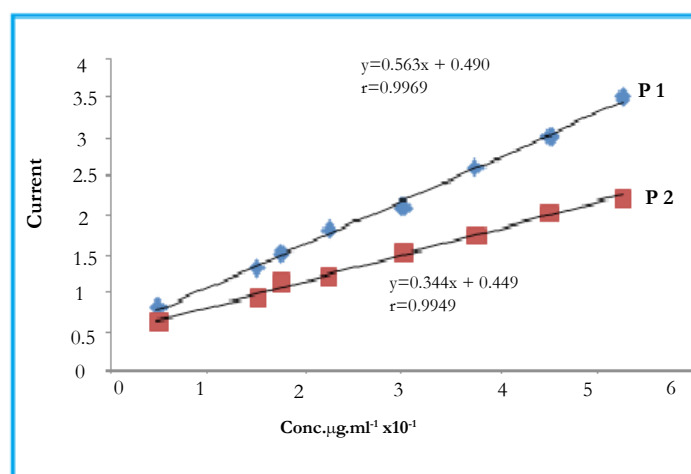

Figure 3. Standard calibration graph for amoxicillin.
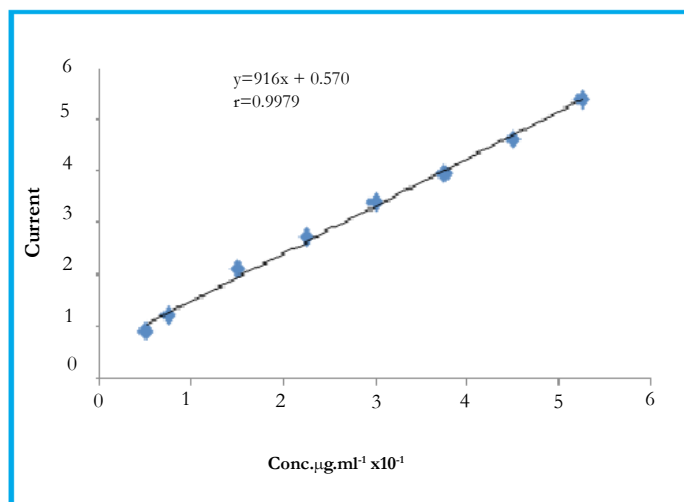
Figure 4. Standard calibration graph for ciprofloxacin.

Table 1. Optimal analytical conditions for Amoxicillin analysis.

Appropriate conditions	Range	Experimental condition
B-R buffer	Britton – Robinson (B-R) buffer Phosphate buffer Carbonate buffer	Buffer
7	2,4,7,9	pH
KCl	LiCl, KCl	Supporting Electrolyte
Water	Water -methanol –acetonitrile	Solvent
4	1,2,3,4	mercury drop size
25 °C	20,25,30,35, 40 °C	Temperature effect

Table 2. Optimal analytical conditions for ciprofloxacin analysis.

Appropriate condition	Range	Experimental condition
B-R buffer	Britton – Robinson (B-R) buffer Phosphate buffer Carbonate buffer	Buffer
4	2,4,7,9	pH
KCl	KCl, LiCl	Supporting Electrolyte
Water	Water , methanol , acetonitrile	Solvent
4	1,2,3,4	mercury drop size
25 °C	20,25,30,35,40 °C	Temperature effect

Figure 5. Suggested reduction mechanism for Amoxicillin reduction.
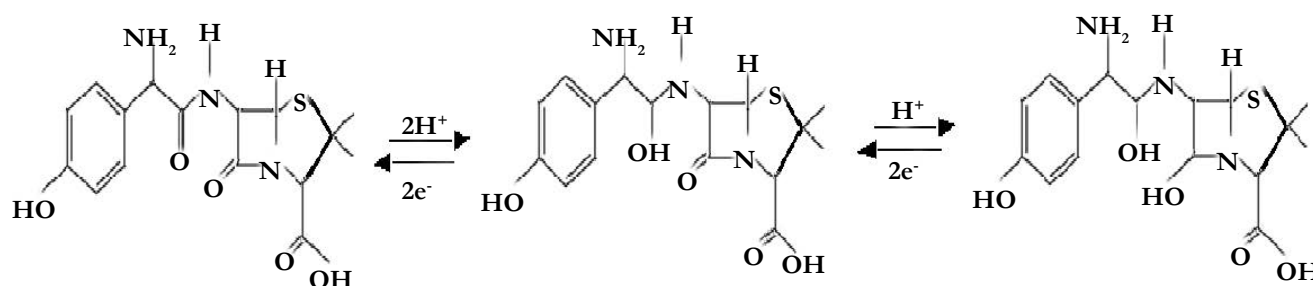
Salam A.H. Al-Ameri, Najlah M.H. Al-Waeli (2016) Differential pulse polarographic study of amoxicillin and ciprofloxacin and its determination in pharmaceuticals. *Int J Bioanal Methods Bioequival Stud*. 3(1), 47-54.

Figure 6. Suggested reduction mechanism for ciprofloxacin.
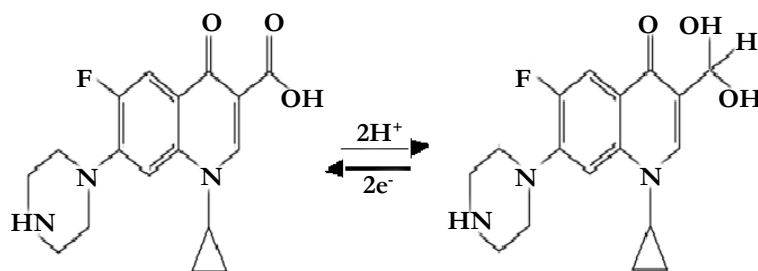
Figure 7. Effect of E applied on the log (i/id- i) variation using Heyrovsky-Ilkovic equation at 3μg.ml^{-1} amoxicillin concentration, P1.
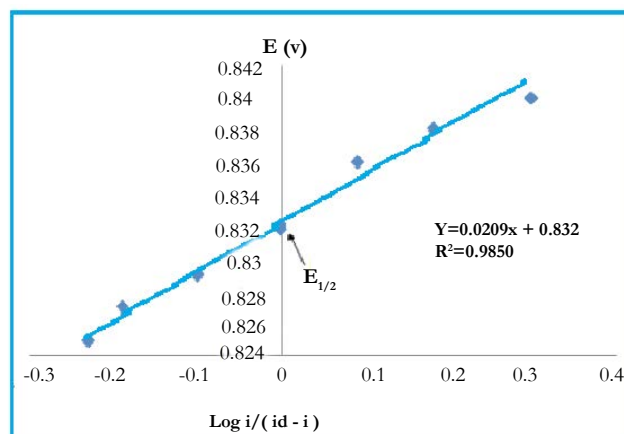
Figure 8. Effect of E applied on the log (i/id- i) variation using Heyrovsky-Ilkovic equation at 3μg.ml^{-1} amoxicillin concentration, P2.
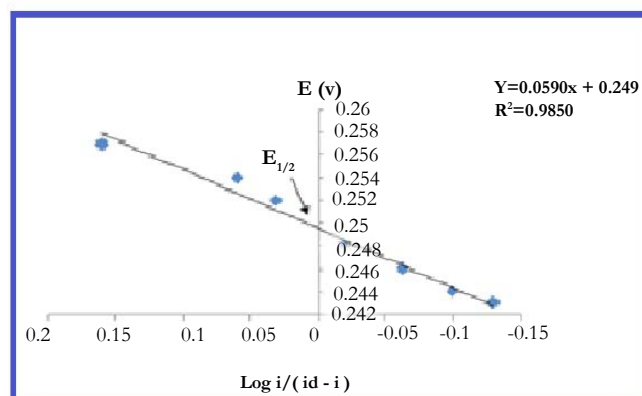
Figure 9. Effect of E applied on the log (i/id- i) variation using Heyrovsky-Ilkovic equation at 3μg.ml^{-1} ciprofloxacin concentration.

Salam A.H. Al-Ameri, Najlah M.H. Al-Waeli (2016) Differential pulse polarographic study of amoxicillin and ciprofloxacin and its determination in pharmaceuticals. *Int J Bioanal Methods Bioequival Stud*. 3(1), 47-54.
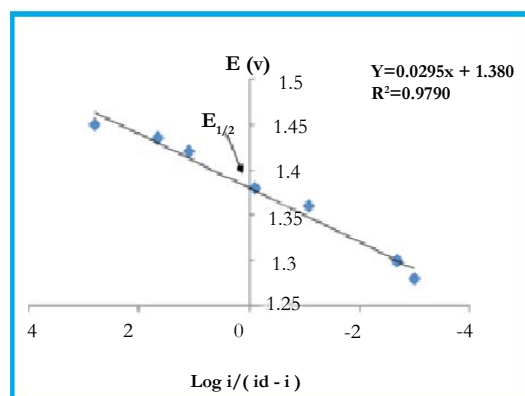
Table 3. Analysis of synthetic made amoxicillin sample.

RSD%	SD	%R.E	A.E	Rec. %	Measured conc. µg/ml	Measured Current, nA	Primary Conc. µg/ml
0.13	0.08	-0.22	-0.13	99.78	59.68	2.17	60 (other conc 0.3 µg.ml-1 may be deleted)
		0.02	0.01	99.95	59.82	2.174	
		-0.03	-0.02	99.89	59.79	2.173	
		0.13	0.08	100.06	59.89	2.176	
		0.06	0.04	100	59.85	2.175	
				av. =99.94	av. = 59.81		

Table 4. Analysis of synthetic made ciprofloxacin sample.

RSD%	SD	%R.E	A.E	Rec. %	Measured conc. µg/ml	Measured Current, µA	Primary Conce. µg/ml
0.4%	0.26					3.297	60
		-0.02	-0.01	99.9	59.54	3.298	
		0.02	0.01	100	59.56	3.299	
		0.05	0.03	100	59.58	3.296	
		0.02	0.01	99.9	59.51	3.297	
		-0.07	-0.04	99.9	59.54		
				99.9	59.55	av.=3.296	

Table 5. Analysis of commercial pharmaceuticals amoxicillin.

Pulmoxyl Capsules, 500mg Amoxicillin						
RSD%	SD	Rec. %	Found in Drug mg	Measured Conc. µg/ml	Measured Current	Initial conc. µg/ml
0.05	0.26	99.94	498.5	89.73	3.016	90 (other conc 0.45 µg.ml-1 may be deleted)
		99.9	498.3	89.7	3.015	
		100	498.7	89.8	3.018	
		99.8	498.1	89.66	3.014	
		100	498.7	89.8	3.018	
		99.9	av.=498.46	av.=89.78	av.=3.016	

Salam A.H. Al-Ameri, Najlah M.H. Al-Waeli (2016) Differential pulse polarographic study of amoxicillin and ciprofloxacin and its determination in pharmaceuticals. *Int J Bioanal Methods Bioequival Stud.* 3(1), 47-54.

Table 6. Analysis of commercial pharmaceuticals ciprofloxacin.

Ciprofloxacin, 500 mg						
Initial conc. μg.ml^{-1}	Measured Current, nA	Measured Conc., μg/ml	Found in Drug	Rec. %	SD	RSD%
90	4.607	88.14	489.66	99.9	0.46	0.09%
	4.605	88.1	489.44	99.8		
	4.599	87.97	488.72	100		
	4.608	88.17	489.83	100		
	4.608	88.17	489.83	100		
	av.=4.605	88.11	489.5	99.96		

Limit of detection and limit of quantification

The Limit of detection (LOD) and limit of quantification (LOQ) for the amoxicillin and ciprofloxacin was calculated using signal to noise ratio (S/N) of 3.3 and 10 respectively [27], it was found equal to 0.04 and 0.12 μg. ml^{-1} for amoxicillin and 0.02 and 0.06, μg. ml^{-1} for ciprofloxacin respectively.

Accuracy and Precision

The accuracy and precision of the method for the determination of amoxicillin and ciprofloxacin was tested. A 60 μg.ml^{-1} synthetic amoxicillin and ciprofloxacin samples was prepared and analysis, the results shows absolute errors between -0.02 to 0.08 and relative errors ranging from -0.03 to 0.13 % with 0.08 SD and %RSD not exceed ± 0.13 for amoxicillin, Table-3.

While ciprofloxacin analysis results shows absolute errors between -0.04 to 0.03 and relative errors within the range of - 0.07 to 0.05% with 0.26 SD and %RSD no more than 0.4, Table-4.

The developed DPP method was utilized for the determination of amoxicillin and ciprofloxacin in commercial pharmaceuticals, the results shows that the actual amoxicillin amounts in pulmoxyl capsules, 500mg in the range 498.1 – 498.7 mg which is actually equal to the amount installed in the original product, where's ciprofloxacin amounts in ciprofloxacin 500 drug, shows their amounts between 488.72 – 489.83 mg which is negligible less than the amount installed in the original product, Tables-5 and 6.

Conclusion

In the practical applications of this method several advantage found, first; using DPP which give sensitive and selective determination of these antibiotics, second, the developed methods proved to be rapid since the analysis of each sample required few minutes.

References

[1]. News Ghana (2015) "Utilizing antibiotics agents effectively will preserve present day medication".

[2]. Amoxicillin (2015) *The American Society of Health-System Pharmacists*.

[3]. 2004 GlaxoSmithKline. Amoxil (amoxicillin) capsules, chewable tablets, and powder for oral suspension prescribing information. Research Triangle Park, NC.

[4]. H Salem, GA Saleh (2002) Selective spectrophotometric determination of phenolic β-lactam antibiotics. Journal of Pharmaceutical and Biomedical Analysis 28(6): 1205–1213.

[5]. MQ Al-Abachi, H Haddi, AM Al-Abachi (2005) Spectrophotometric determination of amoxicillin by reaction with N, N dimethyl aniline and potassium hexacyanoferrate(ll). Anal. Chim. Acta 554(1): 184-189.

[6]. Pasamontes, MP Callao (2003) Determination of amoxicillin in pharmaceuticals using sequential injection analysis (SIA). Evaluation of the presence of interferents using multivariate curve resolution. Analytica Chimica Acta 485(2): 195–204.

[7]. A. Aghazadeh, G Kazemifard (2001) J. Pharm Biomed Anal 25: 325.

[8]. JID Wibawa, D Fowkes, PN Shaw, DA Barrett, J Chromatogr B (2002) Anal Technol Biomed Life Sci 774: 141.

[9]. A Wen, T Hang, S Chen, Z L Ding, Y Tian *et.al* (2008) Simultaneous determination of amoxicillin and ambroxol in human plasma by LC-MS/MS validation and application to pharmacokinetic study. J Pharm Biomed Anal 48(3): 829.

[10]. B Rezaei, S Damiri (2009) Elecrtoanalysis 21: 1577.

[11]. Pradeep KB, Riyaz AD, Krishna S P (2012) Conducting polymer film based electrochemical sensor for the determination of amoxicillin in micellar media. Sensors and actuators b chemical 176: 307-314.

[12]. Zuhair AK, Noora SM (2014) An Eco-Friendly Method for Extraction and Determination of Ciprofloxacin in Blood Serum and Pharmaceuticals. Science Journal of Analytical Chemistry 2(5): 47-54.

[13]. Salvi VS, Sathe PA, Rege PV (2010) Determination of Tinidazole and ciprofloxacin hydrochloride in single formulation tablet using Differential Pulse Polarography 1(3): 110.

[14]. Kilic E, Koseoglu F, Akay MA (1994) Non-aqueous titrimetric assay of selected antibiotics using tetra-N-butylammonium hydroxide as titrant. J Pharm Biomed Anal 12(3): 347-352.

[15]. Patel SA, Patel NM, Patel MM (2006) Simultaneous spectrophotometric estimation of ciprofloxacin and ornidazole in tablets. Indian J Pharm Sci 68(5): 665-667.

[16]. Amin AS (2000) Spectrophotometric micro determination of some important aminoquinoline antimalarials as ion-pair complex. Mikrochim Acta 134(3-4):133-138

[17]. Abdel-Gawad FM, Issa YM, Fahmy HM, Hussein M (1998) Spectrophotometric determination of ciprofloxacin in pure form and in tablets through charge-transfer complexation reactions. Mikrochim. Acta. 130(1-2): 35-40.

[18]. Rizk M, Balal F, Ibrahim F, Ahmed S, Sheribah ZA (2001) Derivative Spectrophotometric Analysis of 4-Quinolone Antibacterial in Formulations and Spiked Biological Fluids by their Cu (II) Complexes. J AOAC Int 84(2): 368-375.

[19]. Gomez DG, Anunciacion Espinosa-Mansilla, Arsenio Munoz de la Pena, Canada FC (2006) HPLC determination of ciprofloxacin, cloxacillin and ibuprofen drugs in human urine samples. J Pharm Sci 29(13): 1969-1976.

[20]. Thoppil SO, Amin PD (2000) Stability indicating reversed-phase liquid chromatographic determination of ciprofloxacin as bulk drug and in phar-

Salam A.H. Al-Ameri, Najlah M.H. Al-Waeli (2016) Differential pulse polarographic study of amoxicillin and ciprofloxacin and its determination in pharmaceuticals. *Int J Bioanal Methods Bioequival Stud.* 3(1), 47-54.

53

maceutical formulations. J Pharm Biomed Anal 22(4): 699-703.

[21]. Witte BD, Dewulf J, Demeestere K, Ruyck MD, Langenhove HV (2007) Critical points in the analysis of ciprofloxacin by high-performance liquid chromatography. J Chromatogr A 1140(1): 126-130.

[22]. Kassab NJ, Singh AK, Hackmam ERMK, Santoro MIRM (2005) Quantitative determination of ciprofloxacin and norfloxacin in pharmaceutical preparations by high performance liquid chromatography. Brazilian J Pharm Sci 41(4), 507-513.

[23]. Shervington LA, Abba M, Hussain B, Donnelly J (2005) Simultaneous separation and determination of five quinolone antibotics using isocratic reversed-phase HPLC: Application to stability studies on an ofloxacin tablet formulation. J Pharm Biomed Anal 39(3-4): 769-775.

[24]. Novakovic J, Nesmerak K, Nova H, Filka K, (2001) An HPLC method for the determination and the purity control of ciprofloxacin HCl in coated tablets. J Pharm Biomed Anal 25(5): 957-964.

[25]. Michalska K, Pajchel G, Tyski S, (2004) Determination of ciprofloxacin and related impurities by capillary zone electrophoresis. J Chromatogr A 1051(1): 267-272.

[26]. Wang PL, Feng YL, Chen LA (1997) Simultaneous Determination of Trace norfloxacin, pefloxacin, and ciprofloxacin by TLC–Fluorescence Spectrodensitometry. Microchem J 56(2): 229-235.

[27]. James N. Miller, Jane C. Miller (2005) Statistics and Chemometrics for Analytical Chemistry(5th edtn), Person Education Limited, Edinburgh Gate, Harlow, England.

[28]. Al-Rufaie E, Hussain M.A. (2014) Study of interaction between Vitamin C and Nickel (II) using a Polarographic Methods. Iraqi Journal of Science 55(3A): 878-885.

Salam A.H. Al-Ameri, Najlah M.H. Al-Waeli (2016) Differential pulse polarographic study of amoxicillin and ciprofloxacin and its determination in pharmaceuticals. *Int J Bioanal Methods Bioequival Stud.* 3(1), 47-54.

54