Article

a special issue for the scientific conference held by the Department of Chemistry- College of Education for Girls/University of Kufa and in cooperation with Hilla University College, under the title (**5'th Postgraduate Students Annual Conference**) **(PSAC2024)**, which held for Wednesday, **24/4/2024.**

Application of Cloud Point Extraction of Amoxicillin Determination using Liquid Ion Exchange method Compared with HPLC in Pharmaceuticals

Ruqayah Jasim Mohammed and Safa Majeed Hameed

[1,2] Department of Chemistry, Faculty of Education for Women, University of Kufa, Al-Najaf, 54001, Iraq

Emails: [1]rugayaj.alsunbali@student.uokufa.edu.iq

[2]Safaa.alhassani@uokufa.edu.iq

Abstract

A direct and compassionate approach has been examined to quantify Amoxicillin (AMX) in pharmaceuticals. This method involves a liquid ion exchange process that relies on forming an ion-pair association complex between AMX and Co (II) as a chloroanion complex by the presence of hydrochloric acid and the non-ionic surfactant Triton X-100. The highest wavelength recorded was 357nm. An analysis was undertaken on the impact of the optimal conditions on the separation process, specifically at a temperature of 95°C. After a heating duration of 15 min., the Co(II) concentration was determined to be 100µg/mL, with an optimal TritonX-100 surfactant volume of 0.5mL. Furthermore, an investigation was conducted to analyze the impact of various organic solvents with differing dielectric constants on the dissolution of the Cloud Point Layer. This study aimed to elucidate the most likely structure of the extracted complex was 1:1 [AMX]+; HCoCl4-. The linear calibration curve spanned the concentration range of 5-1000 µg/mL, exhibiting a limit of detection (LOD) of 4.790 µg/mL and a limit of quantification (LOQ) of 14.515 µg/mL. The analysis of Amoxicillin in pharmaceutical samples was conducted using

the CPE-ILE method compared to HPLC, aiming to enhance the precision of the CPE-ILE method for determining Amoxicillin.

Keywords: Amoxicillin, Cloud Point Extraction, Cobalt, Liquid Ion Exchange

Introduction

Amoxicillin AMX, the scientific name (2s.5r.6r)-6- [(2r)-2-amino-2-(4-hydrowyphenyl)-acetyl] amino-three, 3-dimethyl-7-oxo-four-thia-l-azabicyclo [3.2.0] heptane-2-carboxylic acid, (Figure 1). It is freely soluble in water. The molecular formula is $C_{16}H_{19}N_3O_5S$ and molar mass is 419.45 g/mol. The structure of amoxicillin is shown in Figure 1 [1,2].

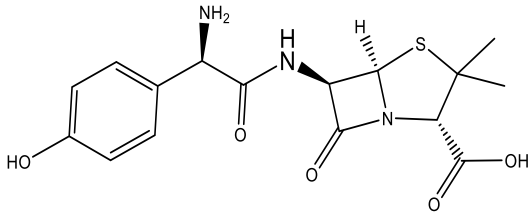


Figure 1. Structure of amoxicillin

Amoxicillin is a semi-synthetic antibiotic that belongs to the penicillin family. Amoxicillin is effective against gram-positive and gram-negative microorganisms. It is found as amoxicillin trihydrate in drugs[3]. Amoxicillin is used to treat many different types of infection caused by bacteria, such as tonsillitis, bronchitis, pneumonia, gonorrhoea, and infections of the ear, nose, throat, skin, or urinary tract[4]. Several methods for AMX determination in pharmaceutical formulations and biological fluids have been reported including HPLC[5-7], chemiluminescence[8,9], spectrofluorimetric[10], and flow-injection analysis[11-13], spectrophotometric method[14-16], Degradation reaction[17], Electrochemical analysis[18], Adsorption method[19], nanoparticles applications [20], iodometric method [21], This study described a pioneering approach for the spectrophotometric quantification of AMX by employing Co(II) as a chloroanion to produce an ion pair association complex in an HCl medium. Subsequently, the suggested methodology was compared to the HPLC technique and determined appropriate for assessing AMX in pharmaceuticals.

Experimental

- **Equipments:** a biochem double beam UV-Vis spectrophotometer model (Biochrom libra S60) from the UK, an HPLC-column C18 (Skam 2012) from Germany, and an Electrostatic water bath from Germany were used.
- **Preparation of solutions:** involved standard Co(II) solution (1mg/mL) by dissolving 0.249g of $CoCl_2.6H_2O$ (Merck 99.8%) in 100 mL distilled water, 1% TritonX-100 (Alpha

Chemika) and Amoxicillin (100µg/mL) by dissolving 0.01g (Merck 99.96%) in 100 mL distilled water, any other working solutions prepared by serial dilution with distilled water.

Pharmaceutical Samples preparation: It encompassed various forms of AMX products, such as capsules, injections, and oral suspension.

AMX capsules: Ten capsules of AMX were weighed and ground into a fine powder; the content of one capsule (0.573 g), equivalent to 500 mg of AMX, was dissolved in an appropriate amount of distilled water with continued shaking and filtered to dispose of the insoluble particles. The filtrate solution was carried into a 100 mL volumetric flask and diluted with deionized water [22].

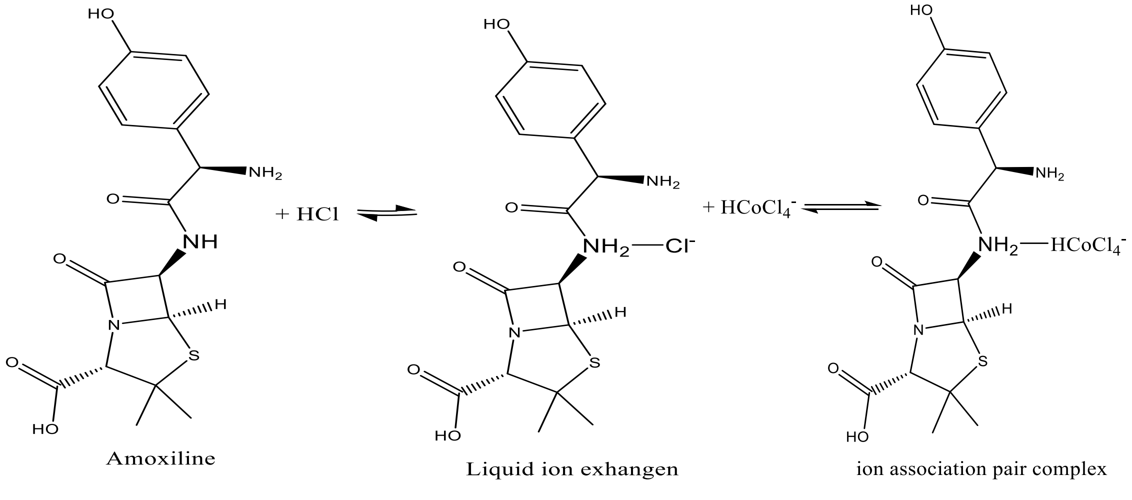
AMX injection: Dissolved 1.0g of injection powder in 10ml of deionized water, carried into a 100 mL deionized water in volumetric flask. [23]

AMX oral-suspension: An accurate volume equivalent to 250 mg of AMX oral suspension was dissolved in 100mL deionized water [24].

- **General Procedure of CPE-ILE:** Created 10mL aqueous solutions containing Amoxicillin (100µg/mL) and Co(II) (100µg/mL) in HCl media. An appropriate amount of 1% TritonX-100, a non-ionic surfactant, was added, and the solutions were in an electrostatic water bath for a suitable duration at the correct temperature. Then, separated the cloud point layer from the aqueous solution and dissolved it in 5mL of ethanol. The absorbance measurement was conducted using ethanol as a reference at the maximum absorption wavelength (λmax).

Results and Discussion

The mechanism by which liquid ion exchange occurs to create ion pair association complexes.



The process of determining the maximum wavelength

The absorbance of the ion association complex solution was measured in the wavelength range of 200-500nm. The resulting spectrum is shown in Figure 1, with the maximum absorbance occurring at a wavelength of 357nm.

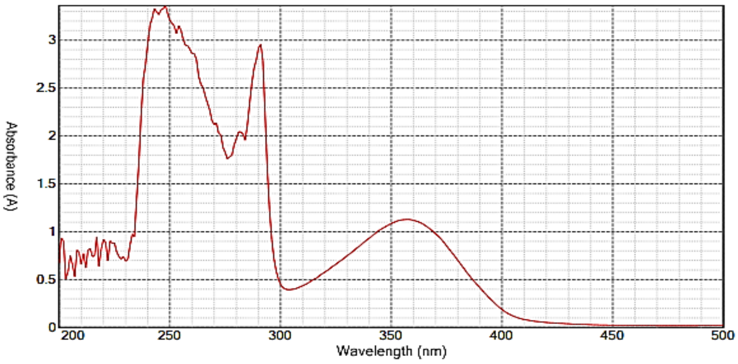


Figure 1. UV-Vis. Spectrum for ion association complex between AMX and $HCoCl_4^-$

Optimising the Experimental Conditions

Concentration of Hydrochloric Acid

The influence of varying hydrochloric acid concentrations within the range of 0.1 to 1.0M was examined using the general procedure. The results are depicted in Figure 2.

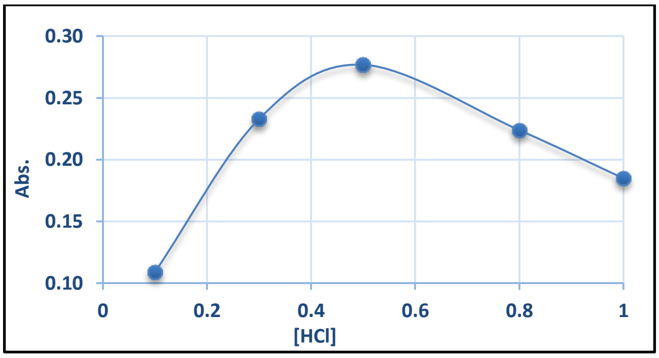


Figure 2. HCl effect on the absorbance of ion association complex

The ideal concentration of HCl is 0.5 M, as it allows the formation of a liquid ion exchanger of Amoxicillin and chloroanion complex of Co(II) according to Scheme (1). Concentrations that are lower or higher than the optimal value will decrease the efficiency of extracting the Amoxicillin complex to CPL, leading to a reduction in the concentration of the liquid ion exchanger and chloroanion complex of Co(II) ions, as dictated by the mass action law. This, in turn, will cause a decrease in the speed of the ion exchange equilibrium[25].

Role of Temperature

The temperature plays a crucial effect in the creation of CPL. A study was conducted to extract amoxicillin at temperatures ranging from (70-95)°C, following the general procedure to better understand its significance. The findings are shown in Figure 3.

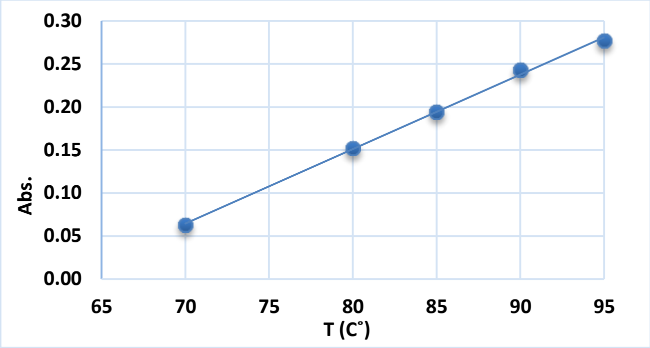


Figure 3. The impact of temperature on the absorbance of ion association complexes

The results showed that the optimum temperature (95°C) will provide the necessary energy to aggregation the micelles of surfactant, remove water molecules, and form a CPL layer with ideal characteristics for the quantitative extraction of the ion pair complex of the Amoxicillin[26].

Impact of Heating Time

Amoxicillin was extracted using the general procedure at various times of heating. The findings illustrated in (Figure 4)

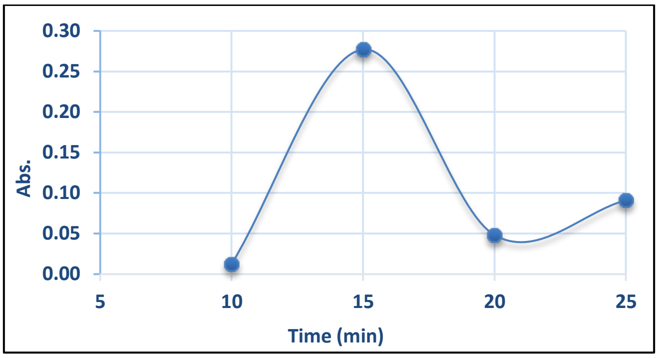


Figure 4. The change in absorbance of the ion association complex concerning heating time.

From the results, the optimal time for amoxicillin is (15min), which represents the kinetic aspect of the extraction process and the formation of the CPL layer with the highest density and smallest size, and with high hydrophobic properties for extracting the ion pair complex of AMX [27].

Influence of Co(II) Concentration

Amoxicillin was extracted in varying Co(II) ions concentrations in a 10mL aqueous solution with 0.5M HCl. Based on the general procedure, the results accorded with those depicted in Figure 5.

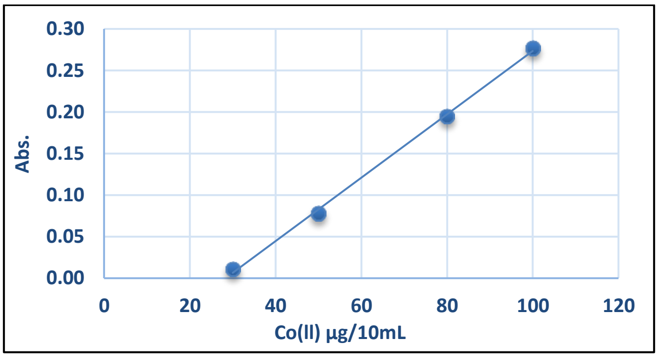


Figure 5. Role of varying Co(II) concentration on the formation of ion association complex

The results show that the concentration of 100µg/10mL is the optimal concentration for the Co(II) ion. This indicates the formation of ion pair complex with the highest stability, and the speed of formation equilibrium of the complex is greater than the speed of dissociation equilibrium, while concentrations less than the optimal value do not achieve sufficient stability for the ion pair complex extracted by CPL[28].

Influence of Surfactant Volume

Using general procedure, amoxicillin was extracted from 10mL aqueous solutions under ideal conditions in varying concentrations of TritonX-100. The results are depicted in Figure 6.

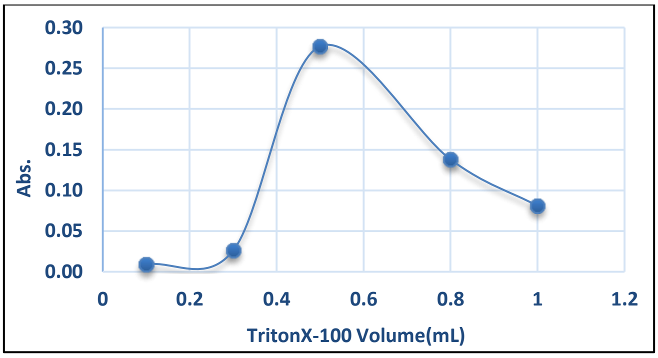


Figure 6. Impact of surfactant volume

According to the results, the ideal volume of TritonX-100 was 0.5 mL, representing the critical concentration, or CMC. of the surface active substance Triton X-100، which gives, at the appropriate temperature, the best assembly process for micelles of the Triton X-100 to give the best CPL with good specifications, that are able to separate and extract the ion pair complex, volumes less than the optimal do not achieve the (CMC), meaning that the

CPL formed by these volumes does not have ideal specifications, so its efficiency in the separation and extraction process is unfavorable volumes larger than the optimum value cause great repulsion of the micelles, causing them to spread in the aqueous solution [29].

Influence of Solvent

Extracted amoxicillin under ideal conditions from 10 mL aqueous solutions, following the general procedure; the CPL was subsequently dissolved in various organic solvents; The dielectric constant's effect (ε_r), which can be expressed in relation to Gati and Szalay [30] as shown in equation 1.

$$\Delta\tilde{V} = \left[(a-b)\left(n^2 - 2\frac{1}{n^2} + 1\right)\right] + b\left(\frac{D-1}{D+1}\right) \ldots\ldots\ldots (1)$$

The results were as in (Table 1):

Table (1): Maximum wave length and dielectric constant of organic solvent

Solvent	λmax (nm)	Dielectric constant(ε_r)	(D-1/D+1)
Benzene	286	2.28	0.390
Chloroform	294	4.80	0.655
Dichloromethane	292	8.93	0.799
1-Butanol	272	17.1	0.890
2-Propanol	237	18.3	0.896
Ethanol	357	24.3	0.921
Methanol	272	32.6	0.940

When Plotted λmax against (D-1)/(D+1), the results in Figure7:

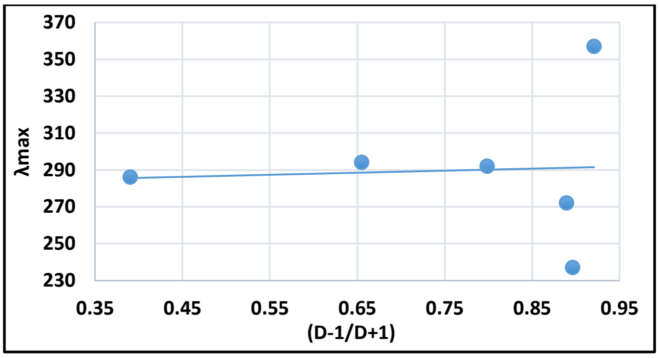


Figure 7. Relation between λmax and dielectric constant of organic solvent

The concept of Stoichiometry

Slope analysis was performed under ideal conditions to determine the more likely structure of the ion pair association complex extracted into CPL as in the general produced the results shown in Figures 8 and 9, which indicate the more likely structure of the complex that was extracted:

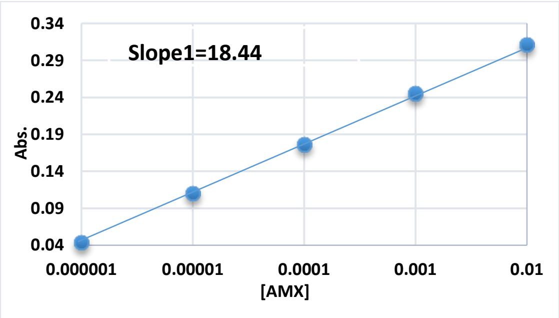


Figure 8. Abs.= f[AMX]

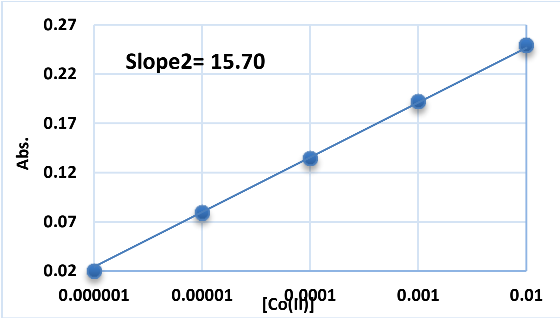


Figure 9. Abs.= f[Co(II)]

$$Slope\ ratio = \frac{Slope1\ (AMX)}{Slope2\ (Co(II))} = \frac{18.44}{15.70} = 1.174$$

The two straight lines in Figures 9 and 10 have a slope ratio value of 1:1, $[AMX]^{+}$; $HCoCl_4^{-}$, which indicates that this is more likely the structure of the ion pair association complex that was extracted into the CPL of AMX and Co(II).

Calibration Curve Construction by CPE-LIE

Combining cloud point extraction and liquid ion exchange is a compassionate and selective approach to spectrophotometrically determining Amoxicillin. This method utilizes Co(II) as a chloroanion complex. Using an general procedure under optimal conditions, we constructed a calibration curve at the wavelength of maximum absorption (λmax) of 357nm. The results are shown in Figure 10.

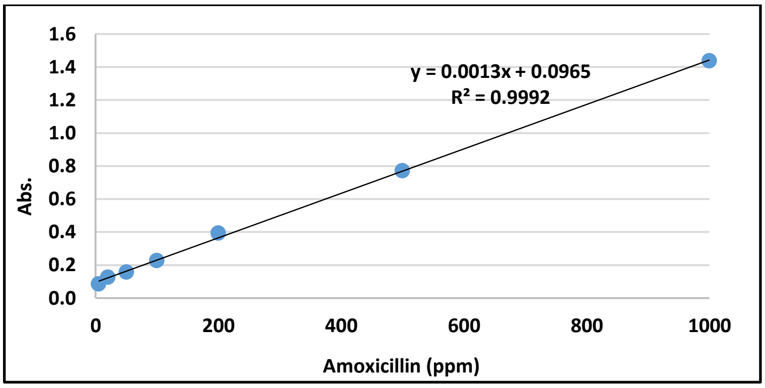


Figure 10. Calibration curve of Amoxicillin using CPE - liquid ion exchange method

Table (2): Parameters for the determination of Amoxicillin by CPE method

Parameter	Co (II)
λ_{max} (nm)	357
Bear's law obey (ppm)	5-1000
Molar absorptivity ($L.mol^{-1}.cm^{-1}$)	0.174×10^{3}
Limit of Detection(μgmL^{-1})	4.790
Limit of Quantity (μgmL^{-1})	14.515

HPLC analysis

HPLC analysis used for determination of AMX in pharmaceuticals to compare with CPE-LIE method, analysis program as below, also the chromatograms of AMX standard and samples shown as in Figures (11→14):

Table(3): Chomatogratographic paraetes used for the determination of amoxicillin

Parameter	Optimized HPLC conditions
Column	C18
Flow rate	1mL/min.
UV	230nm
mobile phase	Distilled water:Acetonitrile 80:20
Add	0.1 acetic acid pH~4.5

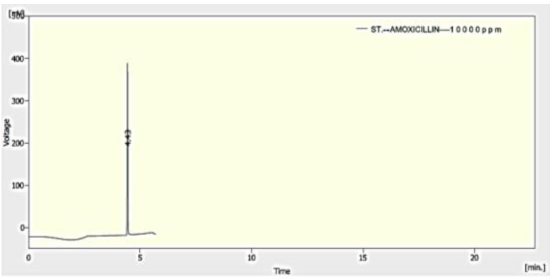


Figure 11. Chromatogram of AMX Standard 1000ppm

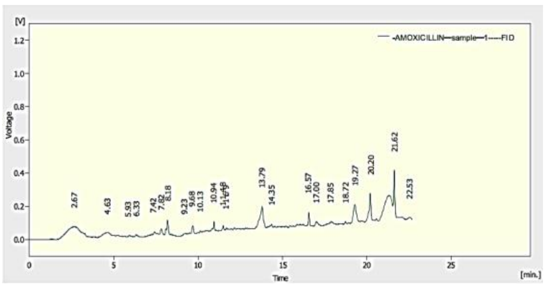


Figure 12. Chromatogram of Amitron (Injection)

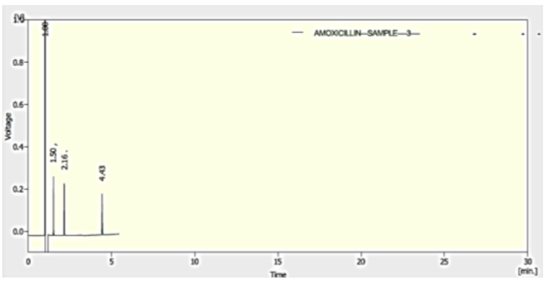


Figure 13. Chromatogram of Neomox (Oral suspension)

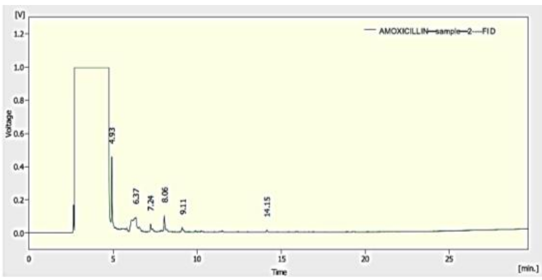


Figure 14. Chromatogram of Labmox (Capsul)

Table (4): Determination of Amoxicillin by two methods CPE-ILE method and HPLC method

Name	Company	Dose	Label claim taken (mg/g)	Measured Concentration ±SD		%Recovery	
				UV method	HPLC method	UV method	HPLC method
Neomox (Oral suspension)	NEOPHARMA	250µg/5ml	0.05	0.0472	0.0437	94.40	87.4
Labmox (Capsul)	LABORATE	500mg	500	458.85	445.027	91.77	89.01
Amitron (Injection)	LOP	500mg	500	464.23	445.500	92.90	89.10

References

[1] Marcel, S., Tito, U., Ines, I., & Pierre, N. J. (2018). Validation of HPLC-UV method for determination of amoxicillin Trihydrate in capsule. Annals of Advances in Chemistry, 2, 055-072.

[2] Jalal, M. T. (2023). Spectrophotometric Determination of Amoxicillin Trihydrate in Pharmaceutical Preparation. HIV Nursing, 23(2), 140-147.

[3] Adem, A. S. A. N., & SEDDIQ, N. (2022). A Simple Spectrophotometric Determination of Amoxicillin in Drug samples. Journal of the Turkish Chemical Society Section A: Chemistry, 9(2), 423-432.

[4] Abbas, A. S., Al-Khafaji, Y., & Al-Khafaji, H. A. H. (2018). Cloud point extraction as a procedure of separation and pre-concentration for cupper (II) determination using spectrofluorometric techniques. Journal of Pharmaceutical Sciences and Research, 10(7), 1748-1752.

[5] Almalki, A. H., Hussein, E. A., Naguib, I. A., Abdelaleem, E. A., Zaazaa, H. E., & Abdallah, F. F. (2021). Development and validation of ecofriendly HPLC-MS method for quantitative assay of amoxicillin, dicloxacillin, and their official impurity in pure and dosage forms. Journal of Analytical Methods in Chemistry.

[6] Becze, A., Resz, M. A., Ilea, A., & Cadar, O. (2022). A Validated HPLC Multichannel DAD Method for the Simultaneous Determination of Amoxicillin and Doxycycline in Pharmaceutical Formulations and Wastewater Samples. Applied Sciences, 12(19), 9789.

[7] Gebretsadik, H., Kahsay, G., Eticha, T., & Gebretsadikan, T. (2023). A validated new RP-HPLC method for simultaneous determination of amoxicillin, ampicillin

and cloxacillin in pharmaceutical formulations. Acta Chromatographica, 35(2), 193-203.
[8] Pauter, K., Szultka-Młyńska, M., & Buszewski, B. (2020). Determination and identification of antibiotic drugs and bacterial strains in biological samples. Molecules, 25(11), 2556.
[9] Salehian, S., Sohrabi, M. R., & Davallo, M. (2021). Rapid and simple spectrophotometric method using feedforward backpropagation and radial basis function neural networks for the simultaneous determination of amoxicillin and clavulanic acid in commercial tablet and human blood serum. Optik, 247, 167908.
[10] Riezk, A., Wilson, R. C., Rawson, T. M., Vasikasin, V., Arkel, P., Ferris, T. J., ... & Holmes, A. H. (2023). A rapid, simple, high-performance liquid chromatography method for the clinical measurement of beta-lactam antibiotics in serum and interstitial fluid. Analytical Methods, 15(6), 829-836.
[11] Abood, N. K., & Hassana, M. J. M. (2021). Spectrophotometric determination of sulfadoxine drug use cloud point and flow injection methods in pharmaceutical formulations. Egyptian Journal of Chemistry, 64(6), 2913-2924.
[12] Halko, R., Hagarová, I., & Andruch, V. (2023). Innovative approaches in cloud-point extraction. Journal of Chromatography A, 1701, 464053.
[13] Fathi, S. A., Othman, N. S., & AL-Taee, A. T. (2023). Indirect Spectrophotometric Method for Determination of Methyldopa in Pure and Pharmaceutical Formulation. Biomedicine and Chemical Sciences, 2(2), 149-154.
[14] Sharma, D. K., Sood, S., & Raj, P. (2019). Spectrophotometric determination of amoxicillin, ampicillin, cefalexin and cefadroxil in pharmaceutical formulations, biological fluids and spiked water samples. Analytical Chemistry Letters, 9(3), 345-361.
[15] Sabbar Falih, M., Abbas, R. F., Mahdi, N. I., Abood, N. K., & Hassan, M. J. M. (2023). FIA-spectrophotometric method for the determination of amoxicillin in pharmaceuticals; application of AES, GAPI, and AGREE greenness assessment tools. MethodsX, 11, 102437.
[16] Mohammad, M., Sliman, F., & Barakat, H. (2023). Development and validation of derivative UV spectrophotometric method for simultaneous determination of amoxicillin and clavulanic acid in tablets (Augmentin 1000mg). Research Journal of Pharmacy and Technology, 16(6), 2795-2800.
[17] Dias, I. M., Mourão, L. C., Andrade, L. A., Souza, G. B., Viana, J. C., Oliveira, S. B., & Alonso, C. G. (2023). Degradation of antibiotic amoxicillin from pharmaceutical industry wastewater into a continuous flow reactor using supercritical water gasification. Water Research, 234, 119826.

[18] Hrioua, A., Loudiki, A., Farahi, A., Bakasse, M., Lahrich, S., Saqrane, S., & El Mhammedi, M. A. (2021). Recent advances in electrochemical sensors for amoxicillin detection in biological and environmental samples. Bioelectrochemistry, 137, 107687.

[19] Mojiri, A., Vakili, M., Farraji, H., & Aziz, S. Q. (2019). Combined ozone oxidation process and adsorption methods for the removal of acetaminophen and amoxicillin from aqueous solution; kinetic and optimisation. Environmental Technology & Innovation, 15, 100404.

[20] Caetano, P., Simões, N. M., Pinto, P. S., Fernandez-Outon, L. E., Albuquerque, A. S., Macedo, W. A., & Ardisson, J. D. (2020). Application of nickel ferrite nanoparticles in adsorption of amoxicillin antibiotic. Journal of the Brazilian Chemical Society, 31, 2452-2461.

[21] Сердюкова, Ю. Ю., Колісник, О. В., Томаровська, Т. О., Полуян, С. М., Шовкова, З. В., & Погосян, О. Г. (2022). Validation of Amoxicillin iodometric procedure in quantitative analysis of pure substance and medical preparation. Актуальні питання фармацевтичної і медичної науки та практики, 15(1), 19-24.

[22] Khammas, Z. A., & Abdulkareem, H. M. (2016). A new visible spectrophotometric approach for mutual determination of amoxicillin and metoclopramide hydrochloride in pharmaceuticals after cloud point extraction. *Science*, *4*(5), 66-76.

[23] Mahmood, H. A. A., Sabah, R., & Abood, N. K. (2023). Spectrophotometric Determination of Amoxicillin Using New Organic Reagent via Different Analytical Methods. Indonesian Journal of Chemistry, 23 (6), 1609 - 1617.

[24] Aljeboree, A. M., & Alshirifi, A. N. (2019, September). Colorimetric determination of Amoxicillin using 4-Aminoantipyrine and the effects of different parameters. In Journal of Physics: Conference Series (Vol. 1294, No. 5, p. 052067). IOP Publishing.

[25] Safa Majeed Hameed, Sahar Aqeel Hussain, Alaa J. AL-Khkany. Extraction for Mo(VI), W(VI) and Mn(VII) as Oxyanions by Incorporation Cloud Point with Liquid Ion Exchange Methods. Research Journal of Pharmacy and Technology. 2022; 15(8):3685-9. doi: 10.52711/0974-360X.2022.00618

[26] Mortada, W. I. (2020). Recent developments and applications of cloud point extraction: A critical revie Microchemical Journal, 157, 105055.

[27] Jawad, S. K., & Ridha, R. K. (2019). Cloud point extraction coupled with liquid ion exchange for separation and determination Mn (VII) in real samples. Research Journal of Pharmacy and Technology, 12(10), 4861-4866.

[28] Gürkan, R., & Kartal Temel, N. (2020). Manganese sensitised-indirect determination of melamine in milk-based samples by flame atomic absorption spectrometry coupled with ultrasound assisted-cloud point extraction. International Journal of Environmental Analytical Chemistry, 100(2), 152-174.
[29] Kojro, G., & Wroczyński, P. (2020). Cloud point extraction in the determination of drugs in biological matrices. Journal of chromatographic science, 58(2), 151-162.
[30] Mutar, M. M., & Ali, H. M. (2021, November). Preparation, Characterization and Analytical Studies of novel Azo dyes and Diazo dyes. In Journal of Physics: Conference Series (Vol. 2063, No. 1, p. 012023). IOP Publishing.