Abhath Journal of Basic and Applied Sciences., 2(1) (2023) 23-28

Abhath Journal of Basic and Applied Sciences

Website: https://ojs.abhath-ye.com/index.php/OJSRJBAS/about

doi: https://doi.org/10.59846/ajobas.v2i1.444

Research paper, Short communication, Review, Technical paper

Green Spectrophotometric Determination of Amoxicillin Anti-Biotic Drug in Pure Form and Pharmaceuticals Using Ninhydrin

Nagib Qarah[1*], Ezzouhra El-Maaiden[2], Basavaiah Kanakapura[3]

[1]Department of Chemistry, Faculty of Education-Zabid, Hodeidah University, Hodeidah, Yemen.

[2]Department of Chemistry, University of Hassan 1[er], Settat, Morocco

[3]Department of Chemistry, Manasagangothri, University of Mysore, Mysore-570 006, Karnataka, India.

***Corresponding author E-mail:** nagib2015qarah@gmail.com*

Received: 11 June 2023. Accepted: 01 July 2023. Published: 05 July 2023.

Abstract

An inexpensive, accurate, and simple green spectrophotometric assay was developed and validated for the determination of the antibiotic drug, Amoxicillin (AMX), in pure drug and tablet using ninhydrin (NIH). The method relied on the condensation reaction between the drug and Ninhydrin reagent (NIH) in the present $NaHCO_3$, and then the colored product was measured at 420 nm. The colored product absorbance that was measured was related to the concentration of AMX. Different experimental conditions of assay were studied, and optimized. Beer's law for the method is obeyed over the concentration ranges30-180 µg mL^{-1}. The value of molar absorptivity was 1.76×10^3 L mol^{-1} cm^1. The values of limits of detection (LOD) and quantification (LOQ) were 0.9 and 2.73µg mL^{-1}. The method was validated according to ICH guidelines. The relative standard deviation (%RSD) intraday and inter-day values for precisions were found to be 1.53 to 2.62%, whereas the values of respective relative error (%RE) for accuracy were best than 2.25%. Robustness and ruggedness were tested, and the values of %RSD in both instances were within acceptable limits. Also, placebo blank and synthetic mixture analysis was applied for method selectivity. The results showed that there is no interference resulting from co-formulated materials in the developed assay, which was successfully applied for AMX determination in tablets and capsules with reliable, satisfactory results, and indicated that the developed assay was as accurate and precise as the official methods. Hence, the developed assay can be used in pharmaceutical laboratories for quality analysis.

Keywords: *Amoxicillin, Ninhydrin, Condensation reaction, Spectrophotometry, Pharmaceuticals.*

1. Introduction

Amoxicillin (AMOX) is in the penicillin group of antibiotics, It is used to treat diseases resulting from bacterial infections, such as infections of the respiratory tract, as well as to treat infections of the middle ear, tonsillitis and throat, urinary tract, and skin. According to IUPAC, Amoxicillin known as (2S,5R,6R)-6-[[(2R)-2-amino-2-(4-hydroxyphenyl) acetyl] amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0] heptane-2-carboxylic acid **[1]**, with the empirical formula $C_{16}H_{19}N_3O_5S$, that corresponding to a molecular weight of 365.41 (Figure 1). It is soluble in water, and alcohol such as ethanol, and methanol, while it is insoluble in hexane, benzene, and acetone. Officially, the drug described HPLC method in the United States Pharmacopoeia (USP) with column (4-mm × 25-cm; 10-µm packing L1) as stationary phase and monobasic potassium phosphate buffer, pH 5± 0.1, acetonitrile and Diluent (buffer) (1:24) as mobile phase with gradient elution at a flow rate of 1.5 mL min^{-1} with UV- detection at 230 nm **[2]**. Also, the drug described a similar HPLC method in British Pharmacopoeia (BP) with column (4.6-mm × 25-cm); octadecyl silyl silica gel for chromatography R (5 µm) as stationary phase and potassium dihydrogen phosphate buffer, pH 5. The mobile phase consists of two parts, acetonitrile and buffer solution pH 5 (1:99 v/v) as mobile phase A; and acetonitrile and buffer solution pH 5 (20:80 v/v) as mobile phase B, with a ratio of (8:92 v/v; A:B). A flow rate of 1.0 mL min^{-1} and UV- detection at 254 nm [1]. In addition to the official BP and USP methods, AMOX has been reported in bulk and formulations in multiple reported assays, including chromatography techniques, such as UPLC, HPLC-DAD, RP-HPLC, HPLC-MS/MS, HPLC, LC-MS, LC[3-14], electrochemical techniques [15-19],Raman, near-infrared and FTIR spectroscopy [20-22], Spectrofluorimetric techniques [23-26],

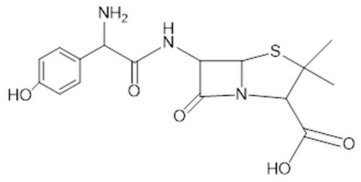

Figure 1. Chemical structure of amoxicillin (AMX).

UV spectrophotometry [27-30], and spectrophotometric techniques serving various chromogenic agents reagent such as iodate-iodide mixture [31], potassium permanganate [32-35], Cerium ammonium nitrate [36], Sulphanilamides [37], bromocresol green [38,39]. potassium iodate [40], Folin-Ciocalteu [41], 2,4-dinitrophenylhydrazine [42], p-N, N-dimethyl phenylenediamine and sulphanilamide [43], and o-nitroaniline [44].

The last reported spectrophotometric methods for AMX are not green or simple, which require difficult experimental conditions, such as a pre-hydrolysis step at elevated temperature, scrupulously anhydrous medium, liquid-liquid extraction step, and too many reagents. As well as the literature survey presented above indicates no spectrophotometric reported for the determination of AMX drug in formulations relied on condensation reaction using ninhydrin reagent. Also, the literature on the analytical applications of ninhydrin revealed that this reagent has been widely used for the quantitative analysis of many pharmaceutical formulations drugs such as

famotidine [45], Baclofen [46], Pregabalin [47,48], cefaclor [49], glucosamine [50], heptaminol [51], acyclovir [52], Cephalexin [53] Memantine Hydrochloride [54], and hydrazine and hydroxylamine [55], to mention few. This prompted the author to use ninhydrin as a condensation agent to develop a green and simple spectrophotometric assay for amoxicillin determination in pure drug and dosage forms. The assay has relied on the formation of colored products following the reaction between AMX and ninhydrin dye, in the presence of sodium bicarbonate; and the colored species being measured at 420 nm.

2. Experimental

2.1. Apparatus

For absorbance measurements, a UV-Visible spectrophotometer Systronics model 166 with matched 1-cm quartz cells (Gujarat, India) was used.

2.2. Materials and methods

All chemicals used were of analytical reagent grade and distilled water was used throughout the study. Tablets (Perssmox) and capsules (Bigmex) both containing 250 mg AMX, were purchased from local commercial stores. A standard stock AMX solution (300 g ml^{-1}) AMX was prepared by dissolving accurately weighed 30 mg of the pure drug (AstraZeneca, Bangalore, India) in water and diluting to volume with the same solvent in a 100 mL calibrated flask. Ninhydrin (0.5%) (Merck, Mumbai, India) was prepared in water. Sodium bicarbonate (Saturated solution), was prepared by placing approximately 25 g of the chemical (Loba Chemie, Mumbai, India) in a hundred ml of distilled water and stirring for 20 minutes, and filtering.

2.3. General procedures

2.3.1. Preparation of the standard graph

Into a series of 10 ml calibrated flasks, different aliquots of 300 g ml^{-1} AMX solution equivalent to the concentrations in the range 10-200 g ml^{-1} were accurately transferred, and the volume was completed to 6 ml with distilled water and added 2 ml of 0.5% ninhydrin followed by 1.5 ml of a saturated solution of $NaHCO_3$ to each flask and completed volume to 10 ml with distilled water. Used a boiling water bath to keep the flasks for five minutes. The flasks were cooled, and the absorbance was measured at 420 nm against the reagent blank prepared. Using the regression equation derived using Beer's law data, the concentration of the unknown was computed.

2.3.2. Procedure for formulations

An amount of tablet or capsule powder equivalent to 30 mg of the sample of AMX was shaken with about 50 mL of water in a glass-stoppered bottle. The solution product was filtered, and the filtrate was collected in a 100 ml calibrated flask and diluted to the mark with water to obtain a solution of 300 µg mL^{-1} AMX and mixed well. Five mL aliquots of tablet/capsule extract were analyzed in five replicates as described under section 2.3.1.

2.3.3. Procedure for placebo and synthetic mixture analyses

A placebo blank that consists of a mixture of talc (10 mg), magnesium stearate (10 mg), Starch (10 mg), lactose (10 mg), and methylcellulose (5 mg) was mixed well, ten mg of it was dissolved and extracted as the same in the section 2.3.2. Five milliliters of the extract were taken for analysis as described in general procedures. 30 mg of pure drug was added to 10 mg of the last placebo, mixed well, and followed the steps in section 2.3.2.

3. Results and discussion

3.1. Chemistry and absorption spectra

Ninhydrin forms colored products with amino groups, which served as the basis for the quantitative determination of compounds that contain primary amino groups [44-55]. All these methods used different organic solvents such as Dimethyl sulfoxide (DMSO) and Dimethylformamide (DMF). One of the most important advantages of this study is the use of an aqueous medium, unlike previous studies that used organic solutions that are not associated with the environment. In the present study we found that in the presence of a saturated solution of $NaHCO_3$, ninhydrin produces a yellow-colored product with AMX. The reaction took place in a water bath for 5 minutes, because the reaction between the drug and the reagent is very slow at room temperature. The colored species product was measured at 420 nm against the reagent blank (Figure 2).

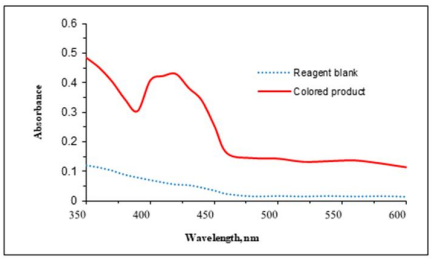

Figure 2. Absorption spectra of (——) Colored product; (.....) Reagent blank.

3.2. Optimization of reaction conditions

The reaction of NIH with AMX is needed to $NaHCO_3$ and heating to get a new product. The concentration of NIH, volume of $NaHCO_3$, and boiling time conditions were studied and optimized. We found through experiments that 1.5 mL of a saturated $NaHCO_3$, and 2 mL of NIH reagent solutions in a volume of 10 mL, were ideal as they gave an ideal yellow-colored reaction product between the drug and NIH, and absorbs at 420 nm (Figures 3 and 4).

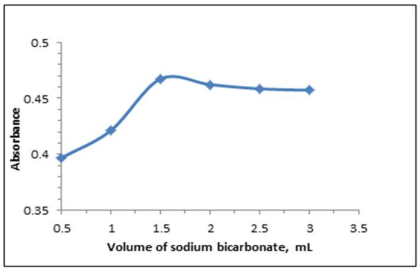

Figure 3. Effect of $NaHCO_3$. (AMX: 90 µg mL^{-1}).

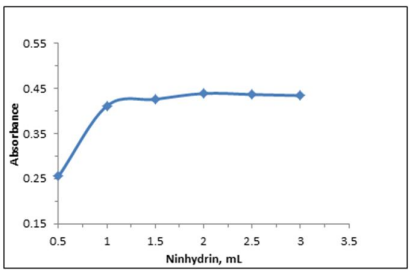

Figure 4. Effect of ninhydrin concentration (AMX: 90 µg mL^{-1}).

A contact time in a water bath of 5 min was found to be optimum for the completely developed yellow color product and was stable for 60 min thereafter.

4. Method validation

After the experimental variables were optimized for optimum conditions for yellow color formation, the developed method was validated using a standard solution of AMX by investigation of several analytical parameters such as sensitivity, accuracy and precision, and others.

4.1. Linearity and Sensitivity

A standard calibration graph for AMX determination was plotted between the concentration versus absorbance. The concentration range was found to be 30-180 µg mL^{-1} (Figure 5**).** The molar absorptivity, Sandell sensitivity, limits of detection (LOD) and quantification (LOQ) values, and the regression analysis of Beer's law were computed and summarized in Table 1.

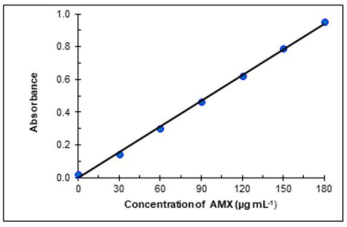

Figure 5. Calibration curve for AMX determination through condensation with ninhydrin.

4.2. Selectivity

The absorbance of the extract of the placebo blank, which was analyzed under the developed method, was equal to the reagent blank reading, which indicated the selectivity of the proposed assay. Also, when subjected to the synthetic mixture to be analyzed under the proposed method, the results shown in Table 2 indicate accepted recoveries percent, as well as indicate no interference by additives or excipients with the proposed method.

Table 1: Regression parameters and sensitivity

Parameter	Value
λ_{max}, nm	420
Color stability, min	60
Linear range, µg mL^{-1}	30-180
Sandell sensitivity*, µg cm^{-2}	0.2079
Molar absorptivity (ε), l mol^{-1} cm^{-1}	1.76×10^{3}
Limit of detection (LOD), µg mL^{-1}	0.9
Limit of quantification (LOQ), µg mL^{-1}	2.73
Regression equation	**Y= -0.0212 + 0.0054 X**
Intercept (a)	-0.0212
Slope (b)	0.0054
The standard deviation of a (S_b)	9.98×10^{-2}
The standard deviation of b (S_m)	0.00056
Regression coefficient (r)	0.9999

Table 2. Results of analysis of a synthetic mixture

AMX concentration, µg mL^{-1}	Percent recovery±SD (n=5)
40	103.6±2.58
80	98.76±1.85
120	101.3±1.76

4.3. Precision and accuracy

The precision of the proposed method as indicated by the percent of relative standard deviation (%RSD), and the accuracy by percent relative error (%RE), both were evaluated using three concentration levels (Table 3) with good results for precision and accuracy.

Table 3: Results of intra-day and inter-day accuracy and precision study

AMX taken µg mL^{-1}	Intra-day accuracy and precision (n=7)			Inter-day accuracy and precision (n=5)		
	AMX found[a] µg mL^{-1}	RSD %	RE %	AMX found µg mL^{-1}	RSD %	RE%
50	50.88	1.53	1.76	50.98	2.43	1.96
100	102.00	2.24	1.98	101.8	1.89	1.85
150	153.40	2.62	2.25	153.1	2.16	2.04

[a]Mean value of seven determinations.

4.4. Robustness and ruggedness

For the evaluation of method robustness, some parameters such as the amount of ninhydrin reagent and heating time were slightly varied; the effect of these changes on the absorbance of colored systems was studied. Method ruggedness was demonstrated by having the analysis done by three analysts using the same instrument, and also by a single analyst performing analysis on three different instruments in the same laboratory. The intermediate precision values (%RSD) of this study were in the range of 0.78-2.16% (Table 4).

4.5. Application to dosage forms analysis

The proposed and official USP methods [2] were applied to amoxicillin determination AMX in tablets/capsules for comparison. The results indicate a good compatibility between the developed method and the USP method, the accuracy of the method is evident through the t-test and F-test values indicated in Table 5.

Table 4: Method robustness and ruggedness results

AMX taken (µg mL^{-1})	Robustness		Ruggedness	
	Parameters altered		Inter-analysts' (%RSD) (n=3)	Inter-instruments'(%RSD) (n=3)
	*Heating time	Volume of Ninhydrin		
40	2.46	0.98	1.95	1.39
80	1.62	1.06	0.78	2.16
120	2.22	0.95	1.74	1.25

*In this study, the heating time were: 5 and 5±1 min. volume of ninhydrin were: 2.0 and 2.0±0.25 mL.

Table 5: Results comparison of formulations analysis by proposed method and USP method

Formulation analyzed	Nominal amount	Found* (% of nominal amount ± SD)	
		Official USP assay	Proposed assay
[a]Perssmox tablets	250 mg	98.46±1.63	97.18±2.55 t = 0.95 F= 2.45
[b]Bigmex capsules	250 mg	101.7±1.55	102.1±1.85 t = 0.37 F= 1.42

*Mean value of five determinations.

Tabulated t-value =2.77for four degrees and 95% confidence level.Tabulated F-value =6.39 for four degrees and 95% confidence level. [a](Polaris Health Care, India), [b](Astra Zeneca, Bangalore, India) both containing 250 mg AMX,

4.6. Application to dosage forms analysis

The proposed and official USP methods [2] were applied to amoxicillin determination AMX in tablets/capsules for comparison. The results indicate a good compatibility between the developed method and the USP method, the accuracy of the method is evident through the t-test and F-test values indicated in Table 5.

4.7. Accuracy by recovery study

The accuracy of the developed method was confirmed using a recovery experiment and following the procedure of the standard addition method. Pure AMX at 50, 100, and 150% of the level found in the tablet/capsule was added to a known amount of AMX in the tablet/capsule powder and the total amount was estimated by the proposed method. (Table 6). The results indicated that no interference by co-formulated substances with the assay.

Table 6: Results of the recovery experiment

Formulation studied	AMX in formulations µg mL^{-1}	Pure AMX Added µg mL^{-1}	Total found µg mL^{-1}	Pure AMX recorded (Percent±SD*)
Perssmox tablets (250 mg)	58.31	30	85.07	96.33±2.16
	58.31	60	116.74	98.67±1.98
	58.31	90	150.53	101.5±1.78

*Mean value of three determinations

5. Conclusion

The use of ninhydrin presents a novel analytical method for the spectrophotometric assay of AMX. The method is simple to perform compared to reported methods, as it used a single available reagent in addition to the few steps to complete the reaction, unlike the previous multi-step methods and reagents. Also, the proposed method seldom uses organic solvent or extraction processes and suffers from the disadvantages of elevated temperature and longer reaction time. As well as the wide range applied by the method led to the possibility of determining the drug at different concentration levels. In addition to these advantages, the developed method used cheap and available chemicals and simple devices, which made the method suitable for use in quality control laboratories, especially in developing countries. which cannot afford expensive techniques such as chromatography (HPLC).

Data Availability

No data were used to support this study.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

N. Qarah wishes to express his thanks to Hodeidah University,Yemen for supporting research.

How to Cite : Qarah et al. (2023). Green Spectrophotometric Determination of Amoxicillin Anti-Biotic Drug in Pure Form and Pharmaceuticals Using Ninhydrin, *Abhath Journal of Basic and Applied Sciences*, 2(1), 23-28.

REFERENCES

[1] British Pharmacopoeia Commission Office, Medicines and Healthcare Products Regulatory Agency, volume I, page. 171-173, 1 January 2022, London, UK. http://www.webofpharma.com/

[2] United States Pharmacopeia (2023). *USP Monographs, Amoxicillin*. USP-NF. Rockville, MD: United States Pharmacopeia. https://doi.org/10.31003/USPNF_M4100_06_01

[3] **Raju C, Deepak K, Mithun K (2016)** Quantitative determination of amoxicillin from formulated dosage form by reversed phase high performance liquid chromatography separation technique and a new method validation. *Asian J Pharm Clin Res* 9(4): 308-311.

[4] **Nasir Uddin M, Das S, Khan SH, Shill SK, Bhuiyan HR, Karim R (2016)** Simultaneous determination of amoxicillin and chloramphenicol and their drug interaction study by the validated UPLC method. *J Taibah Univ Sci* 10(5): 755–765. https://doi.org/10.1016/j.jtusci.2015.11.005 .

[5] **Sabry SM, Abdel-Hay MH, Belal TS, Mahgoub AA (2015)** Development and validation of HPLC-DAD method for the simultaneous determination of amoxicillin, metronidazole and rabeprazole sodium. Application to spiked simulated intestinal fluid samples. *Ann Pharm Fr* 73(5): 351–360. https://doi.org/10.1016/j.pharma.2015.04.008.

[6] **Sahoo NK, Sahu M, Algarsamy V, Srividya B, Sahoo CK (2016)** Validation of Assay Indicating Method Development of Amoxicillin in Bulk and One of Its Marketed Dosage Form by RP-HPLC. *Ann Chromatogr Sep Tech* 2(1): 1014-1018. https://www.jsmcentral.org/sm-chromatography/fulltext_acst-v2-1014.pdf.

[7] **Zhongxiao P, Jie T (2008)** Improved method for determination of amoxicillin capsules by HPLC. *Chin Pharm Affairs* 22(8): 682-683. http://caod.oriprobe.com/order.htm?id=16146748&ftext=base

[8] **Huang M, Yuan Y, Qian W, Zhang M (2013)** Simultaneous determination of amoxicillin and clavulanate in amoxicillin and clavulanate potassium tablets by UPLC-UV and its application in dissolution profile study in domestic tablets. *Chinese J pharm Anal* 33(12): 2171-2175. https://www.ingentaconnect.com/content/jpa/cjpa/2013/00000033/00000012/art00029.

[9] Durga MRT, Narendra S **(2010)** Development and Validation of Stability Indicating HPLC Method for Simultaneous Estimation of Amoxicillin and Clavulanic Acid in Injection. *Amer J Anal Chem* 1: 95-101. https://doi.org/10.4236/ajac.2010.13013.

[10] **Claudio RFC, Maria IRA, Nelson RAF (2012)** Determination of amoxicillin in human plasma using cephalexin as an internal standard and HPLC-MS/MS for the application of bioequivalence studies. *Int Res J Pharm Pharmacol* 2(12): 299-305. Microsoft Word - Cardoso et al pdf.doc (interesjournals.org).

[11] **Dorota K, Anna G (2012)** HPLC analysis of amoxicillin using AccQ-Fluor reagent for pre-column derivatization. *Pol J Environ Stud* 21(1): 139-143. http://www.pjoes.com/pdf-88734-22593?filename=HPLC%20Analysis%20of.pdf.

[12] **Khuroo AH, Monif T, Verma PRP, Gurule S (2008)** Simple, Economical, and Reproducible LC-MS Method for the Determination of Amoxicillin in Human Plasma and its Application to a Pharmacokinetic Study. *J Chrom Sci* 46(10):854-861, https://doi.org/10.1093/chromsci/46.10.854.

[13] **Li Y, Gou H, Yang X, Wang Z (2008)** RP-HPLC determination of amoxicillin. *Phys Test Chem Anal Part B Chem Anal* 44(3), 216-217, 220.

[14] **Basavaiah K, Tharpa K, Prasad NR, Hiriyanna SG, Vinay KB (2009)** Determination of amoxycillin in pharmaceutical forms by visible spectrophotometry and HPLC. *Indian J Chem Technol* 16(3): 265-271. https://nopr.niscpr.res.in/handle/123456789/5930.

[15] **Hatamie A, Khan A, Golabi M, Turner AP, Beni V, Mak WC, Nur O (2015)** Zinc oxide nanostructure-modifed textile and its application to biosensing, photocatalysis, and as antibacterial material. *Langmuir* 31(39):10913–10921.https://doi.org/10.1021/acs.langmuir.5b02341.

[16] **Fatma A (2016)** Electrochemical Determination of Amoxicillin on a Poly(Acridine Orange) Modified Glassy Carbon Electrode. *Anal Lett* 49(9): 1366-1378. https://doi.org/10.1080/00032719.2015.1101602.

[17] **Kumar BP, Ahmed DR, Sadashiv PK (2013)** Conducting polymer film based electrochemical sensor for the determination of amoxicillin in micellar media. Sens Actuators B 176: 307-314. https://doi.org/10.1016/j.snb.2012.09.007.

[18] **Masoud F, Reza HM, Ali KM, Pourya B, Nader T, Hadis B (2011)** Voltammetric determination of amoxicillin at the electrochemical sensor ferrocenedicarboxylic acid multi wall carbon nanotubes paste electrode. *Int. J. Electrochem. Sci* 6(5): 1355-1366.

[19] **Behzad R, Sajjad D (2009)** Electrochemistry and adsorptive stripping voltammetric determination of amoxicillin on a multiwalled carbon nanotubes modified glassy carbon electrode. *Electroanalysis* 21(14): 1577-1586. https://doi.org/10.1002/elan.200804571.

[20] **Saidkarimova NB, Yunuskhodjaev AN (2019)** Nondestructive determination of the ampicillin and amoxicillin tablets by Raman spectroscopy. world J Pharm Med Res 5(11): 05-08.

[21] **Maurício AMS, Marcus HF, Jez WBB, Marcelo MS (2012)** Development and analytical validation of a multivariate calibration method for determination of amoxicillin in suspension formulations by near infrared spectroscopy. *Talanta* 89: 342–351. https://doi.org/10.1016/j.talanta.2011.12.039.

[22] **Stefany F, Alexander Z, Eliane GT, Herida RNS (2018)** FTIR Spectrophotometry as a Green Tool for Quantitative Analysis of Drugs: Practical Application to Amoxicillin. *J Chem* Volume 2018, Article ID 3920810. https://doi.org/10.1155/2018/3920810.

[23] **Pawar SP, Walekar LS, Gunjal DB, Dalavi DK, Gore AH, Anbhule PV, Kolekar GB (2017)** Fluorescence-based sensor for selective and sensitive detection of amoxicillin (Amox) in aqueous medium: application to pharmaceutical and biomedical analysis. *Luminescence* 32(6):918–923. https://doi.org/10.1002.

[24] **Saini A, Singh J, Kaur R, Singh N, Kaur N (2015)** Fluoremetric determination of amoxicillin drug in aqueous medium using hybrid framework of organic-inorganic nanoparticles. *Sens Actuators B Chem* 209: 524-529. https://doi.org/10.1016/j.snb.2014.12.018.

[25] **Hui-nan T, Wei-dong G, Mou-sheng L, Ya-ling Y (2011)** beta-cyclodextrin enhanced fluorimetric method for determination of amoxicillin in dosage. *Chem Res Chin Univ*27(2): 190-192. https://hero.epa.gov/hero/index.cfm/reference/details/reference_id/5945932.

[26] **Hong J, Mo F, Hua Z (2010)** Fluorophotometric determination of amoxicillin. *Phys Test Chem Anal B Chem Anal* 46(12): 1447-1449.

[27] **Kemal U, Murat PI, Elif K, Feyyaz O (2008)** Spectrophotometric determination of amoxicillin in pharmaceutical formulations. *Turk J Pharm Sci* 5(1): 1-16. https://www.turkjps.org/archives/archive-detail/article-preview/spectrophotometrc-determnaton-of-amoxclln-n-pharma/12535.

[28] **Theerasak R, Praneet O, Tanasait N, Choedchai S, Suthep W, Vithaya M** (2010) A simple, sensitive and green bienzymatic UV-spectrophotometric assay of amoxicillin formulations. *Enzyme Microb Technol* 46(3-4): 292-296. https://doi.org/10.1016/j.enzmictec.2009.11.011.

[29] **Anusha N, Uday BK** (2014) Spectrophotometric Determination of Amoxycillin in Different Formulations. Asian J Sci Res 7(3): 335-340. https://doi.org/10.3923/ajsr.2014.335.340.

[30] **Maya SE, Eman D, Mohammed RE, Mostafa AS (2016)** UV Spectrophotometric methods for the simultaneous determination of amoxicillin and flucloxacillin. *Az J Pharm Sci* 54(1): 174-187. https://doi.org/10.21608/ajps.2018.6642.

[31] **Qarah NAS, Abdulrahman SAM, Algethami FK, Basavaiahd K, El-Maaiden E (2020)** New applications for amoxicillin determination in pure form and pharmaceuticals based on iodate-iodide mixture: titrimetry and spectroscopy studies. *Quim Nova* 43(1): 44-49, 2020. http://dx.doi.org/10.21577/0100-4042.20170455.

[32] **Omed IH (2015)** Simple Indirect Spectrophotometric Determination of Amoxicillin in Pharmaceutical Preparations. *J Nat Sci Res* 5(16): 142-146. https://core.ac.uk/download/pdf/234656080.pdf. kmno4

[33] **Abdul Majeed KA, Shahla JS (2019)** Determination of Amoxicillin in Pharmaceutical preparations by spectrophotometric and flow Injection-activated chemiluminescence methods. *Tikrit J Pharm Sci* 14(1): 63-79. https://tjphs.tu.edu.iq/index.php/j/article/view/353.

[34] **Al-kadumi AS, Sahar RF, Mohammed AA, Luma AM (2019)** Colorimetric Determination of Amoxicillin in Pure and some Pharmaceutical Formulations Via Reaction with Potassium Permanganate as Oxidant Reagent. *Int J Pharm Qual Assur* 10(2); 222-228. https://impactfactor.org/PDF/IJPQA/10/IJPQA,Vol10,Issue2,Article2.pdf.

[35] **El-Didamony AM, Saad MZ, El-Shaprawy DS (2013)** Direct and indirect spectrophotometric determination of some selected antibiotics using potassium permanganate. *Main Group Chem* 12(2): 139-152. https://doi.org/10.3233/MGC-130096.

[36] **Ameera HH, Sura KI, Saadiyah AD, Amal HM (2019)** Indirect Spectrophotometric Determination of Amoxicillin by oxidative with Cerium ammonium nitrate using Arsenazo III as a reagent. *J Pharm Sci Res* 11(4): 1617-1620. http://www.e-journals.in/PDF/V8N2/289-294.pdf.

[37] **Oksana K, Olha K, Petro R (2020)** Development and Validation of the Simple and Sensitive Spectrophotometric Method of Amoxicillin Determination in Tablets using Sulphanilamides. Acta Chim. Slov. 2020, 67(1), 23–35. https://doi.org/10.17344/acsi.2019.5041.

[38] **Asan A, Seddiq N (2022)** A Simple Spectrophotometric Determination of Amoxicillin in Drug Samples. *J Turk Chem Soc Sect A: Chem* 9(2): 423-432. https://doi.org/10.18596/jotcsa.978686.

[39] **Keskar MR, Jugade RM (2014)** A new spectrophotometric method for determination of amoxicillin using bromocresol green. *World J Pharm Pharm Sci* 3(2): 1340-1348. https://www.wjpps.com/Wjpps_controller/abstract_id/824.

[40] **Chaudhary S, Ali SK, Singh YP (2010)** Spectrophotometric and titrimetric methods for the determination of gentamycin and amoxicillin. *Orient J Chem* 26(1): 75-80. http://www.orientjchem.org/?p=11518.

[41] **Abdullah SA, Nafisur R, Fakhrul I (2004)** Spectrophotometric determination of ampicillin, amoxycillin, and carbenicillin using folin-ciocalteu phenol reagent. *J Anal Chem* 59(2): 119–123. https://doi.org/10.1023/B:JANC.0000014736.59554.5c.

[42] **Nagaraja P, Shrestha AK (2010)** Spectrophotometric method for the determination of drugs containing phenol group by using 2,4-dinitrophenylhydrazine. *E-J Chem* 7(2): 395-402. https://doi.org/10.1155/2010/328061.

[43] **Chirapa KS, Babu NP, Divakar TE (2014)** Indirect visible spectrophotometric method for the determination of penicillins with periodate, p-N,N-dimethylphenylenediamine and sulphanilamide. *Rasayan J Chem* 7(2): 170-176. https://www.rasayanjournal.co.in/vol-7/issue_2/26_Vol.7(2),%20170-176,%202014,%20RJC-1114.pdf.

[44] **Freitas SKB, Lins DSV, Araujo AN, Montenegro MC, Reis BF, Paim APS (2011)** A multicommuted flow analysis method for the photometric determination of amoxicillin in pharmaceutical formulations using a diazo coupling reaction. J Braz Chem Soc 22(2): 279-285. https://doi.org/10.1590/S0103-50532011000200013.

[45] **Nafisur R, Mohammad K (2003)** Application of ninhydrin to spectrophotometric determination of famotidine in drug formulations. Il Farmaco 58(10):1045-1050. https://doi.org/10.1016/S0014-827X(03)00184-8.

[46] **Eyed JH, Fereshteh P, Ehsan M, Mohsen A (2010)** Colorimetric Determination of Baclofen with Ninhydrin Reagent and Compare with HPLC Method in Tablet. *Asian J Chem* 22(1): 522-526. https://www.researchgate.net/publication/268435509.

[47] **Memoona R, Talib H, Muhammad S, Mosab A, Amjad H, Nasir A, Nadeem IB (2022)** Precise and sensitive ambient temperature based analytical colorimetric method for Pregabalin. *Braz J Pharm Sci* 58: e201048. http://dx.doi.org/10.1590/s2175-97902022e201048.

[48] **Alka B, Prateek G (2011)** A novel method for spectrophotometric determination of pregabalin in pure form and in capsules. *Chem Cent J* 5(1):59. http://journal.chemistrycentral.com/content/5/1/59.

[49] **Asad R, Abdul Subhan I, Shabbir A (2009)** Development and application of spectrophotometric method for the determination of cefaclor in pharmaceutical formulations. *Quím Nova* 32(5): 1180-1183.https://doi.org/10.1590/S0100-40422009000500018.

[50] **Yunqi W, Munir H, Reza F (2005)** Development of a simple analytical methodology for determination of glucosamine release from modified release matrix tablets. *J Pharm Biomed Anal* 38(2): 263-269. https://doi.org/10.1016/j.jpba.2005.01.001.

[51] **Mahmoud AO, Dalia MN, Monica EH (2018)** Utility of ninhydrin reagent for spectrofluorimetric determination of heptaminol in human plasma. *Luminescence* 33(6): 1107-1112. https://doi.org/10.1002/bio.3516.

[52] **Ukpe A, Johnson O (2015)** Spectrophotometric determination of acyclovir after its reaction with ninhydrin and ascorbic acid. *J Appl Pharm Sci* 5(4): 65-69. https://doi.org/10.7324/JAPS.2015.50411.

[53] **Mario RD, Celina MM, María DT (2013)** Validation of Ninhydrin Quantitative Method for Cephalexin Generic Tablets. *Curr Pharm Anal* 2013, 9(1): 13-19. http://dx.doi.org/10.2174/157341213804806142.

[54] **Omara H (2017)** Spectrophotometric Determination of Alzheimer's Drug, Memantine Hydrochloride in Biological Samples Using Ninhydrin and Ferric Chloride. *IOSR J Appl Chem* 10(2): 52-59. https://doi.org/10.9790/5736-1002015259.

[55] **Khaled E, Mohamed AE, Mohamed H, Hend G (2022)** Applications of ninhydrin: spectrophotometric determination of hydrazine and hydroxylamine. *Malaysian J Anal Sci* 26(4): 855-866. https://mjas.analis.com.my/mjas/v26_n4/pdf/Khaled_26_4_16.pdf