DocID: https://connectjournals.com/03896.2020.20.4139

Biochem. Cell. Arch. Vol. 20, Supplement 2, pp. 4139-4145, 2020 | www.connectjournals.com/bca | *ISSN 0972-5075*

INDIRECT COLORIMETRIC METHOD FOR DETERMINATION OF AMPICILLIN IN BULK AND SOME PHARMACEUTICAL PREPARATIONS VIA OXIDATION WITH POTASSIUM PERMANGANATE

Abbas Shebeeb Hasan Al-Kadumi[1], Iqbal S. Mohammed[2]* and Dhuha H. Fadhil[3]

[1]College of Science, Al-Karkh University, Iraq.
[2]College of Education for Pure Sciences, University of Diyala, Iraq.
[3]College of Science, Al-Nahrain University, Iraq.
*e-mail : ikbalsalman696@gmail.com

(Received 1 May 2020, Revised 7 July 2020, Accepted 12 July 2020)

ABSTRACT : We proposed two rapid, simple, accurate and precise methods were described for determination of Ampicillin in pure and pharmaceutical formulations. In first method, they were based on the measuring emission of potassium ion at 722 nm by flame photometric technique and absorbance determination of green colored solution at 610 nm for manganite ion formed via reaction of ampicillin with potassium permanganate oxidant agent in basic medium in the second colorimetric method. The optimum working conditions investigated for methods. The Beer's law plot shows a good obeying in the concentration ranging (5-80, 5-90 ì g/ml) for colorimetric and flame photometric methods, respectively. The detection limits and relative standard deviations were (2.265,1.839 ì g/ml) (2.147,1.645) for colorimetric absorption method and (2.903,2.271 ì g/ml) (2.149,2.522) for the flame photometric emission method for capsules and suspensions, respectively. These methods were successfully applied in determination of ampicillin in two formulation studied. The obtained results give good agreement with the label claim and no interference from the commonly encountered additives and expectancies observed.

Key words : Ampicillin, potassium permanganate, colorimetry, flame photometry, oxidation, determination.

INTRODUCTION

One of class of antibiotic drugs that is called Penicillin. It is semi-synthetic penicillin with abroad-spectrum that is good effective in the treatment of both types gram-positive and negative bacterial infections produced via *Streptococcus*, *Haemophilus influenzae*, *Bacillus anthracis*, *Escherichia coli* and *Neisseria gonorrhoeae*. Ampicillin is used in the treatment of upper respiratory urinary tract infections, otitis media in children, and genital and tract infections**.** The systematic nomenclature (4-Thia-1-azabicyclo [3,2,0] heptane-2-carboxylic acid -6- (2-amino-2-phenylacetamido) -3.3-dimethyl-7-oxo), molecular formula $C_{16}H_{19}N_3O_4S.3H_2O$, the Percent of Composition: H 5.48%, C 55.00%, N 12.02%, S 9.18%, O 18.32%, the molecular weight of 403.5 with a white crystalline powder, melts in the range between (198-200°C) and the solubility in water is slightly, dissolves in dilute alkaline and acids solutions as a result of the presence of primary amine and carboxyl groups, the Fig. 1 shows that. However, it is insoluble in ether, alcohols and in fatt oils (British Pharmacopeia, 2007; William Martindale-James Martindale The extra pharmacopeia, 1996).

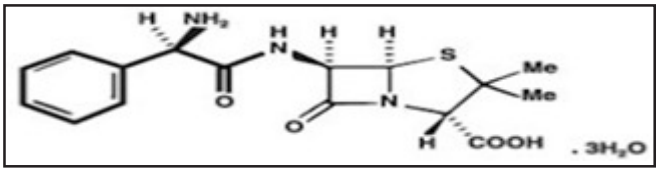

Fig. 1 : Chemical structure of Ampicillin trihydrate.

Several analytical methods reported were used for determination of Ampicillin in different matrixes such as derivative titermetric method (Ejele *et al*, 2008), titermetry with other methods (Resmi *et al*, 2018), NMR (Moon *et al*, 1981), FTIR (Soledad *et al*, 2017), new fractional wavelet approach (Erdal-Dumitru, 2006), fluoroimmunoassay (Bacigalupo *et al*, 2008), univariate and multivariate spectrophotometric methods (Khalid *et al*, 2018), spectrophotometric methods (Mohamed, 2001; Abdelrahman, 2015; Rašiæ, 2013; Yan *et al*, 2013; Mariam, 2006; Smith *et al*, 1967; Akanni, 1992; Issa and Amin, 1993; Peter *et al*, 1973; Ivana *et al*, 2013; Akanni, Ayim, 1992), voltammetery (Issam, Sundus, 2016; Mohd *et al*, 2013), derivative spectroscopy (Audumbar *et al*, 2018), chromatography (Wenhong *et al*, 1997; Gabriela *et al*, 2013; Ticiano *et al*, 1993; Barot *et al*, 2009; Shireesha-Anusha, 2017; Nelis *et al*, 1992; Tushar *et al*, 2009;

Ghoulipour *et al*, 2011; Madhukara *et al*, 2011), HPLC-MS (Suzanne *et al*, 2015; Straub *et al*, 1993) and capillary chromatography (Rade, 2009). The aim of this work is to use the precise and accurate colorimetric and flame photometric emission methods for the determination of Ampicillin content in pure and some drug formulation in Iraq to comparison information of these products with standard method or other official methods.

MATERIALS AND METHODS

Instruments and Equipment

1- Varian Gary 100 UV-Vis spectrophotometer double-beam.

2- Jenway PFP7 / UK: Flame emission spectrophotometer.

3- DENVER Instrument Max 220 gm, d=.0001g : Analytical balance.

4- Hermle Laborti, 6000rpm: Centrifuge.

5- BS-11 Lab companion, Jero Tech, CE :Water Bath temperature- controller.

5- Jenway 3020pH: pH/mV.

Chemicals

Ampicillin trihydrate standard material, Ampicillin 250 mg and Ampicillin 250 mg powder for suspension formulations were obtained from the State Company for Drug Industries and Medical Appliances (Samara-IRAQ-SDI). All other chemicals and reagents of analytical grade were supplied from Fisher, Fluka and BDH Companies.

Preparation of solutions

1. About 0.1g of Ampicillin trihydrate standard material was dissolving in 100 ml distilled water for preparing stock solution of Ampicillin 1000 $\mu g.ml^{-1}$.Subsequent dilution of stock solution to prepare other standard solutions.

2. Total of 10ml of stock solution was diluting to 100 ml distilled water in volumetric flask to prepare Ampicillin solution 100 µg/ml, dilution of this solution to prepare 50 µg/ml for recorded UV-Vis spectrum.

3. Diluting Ampicillin solution 100 $\mu g.ml^{-1}$ to prepare standard solutions for calibration curve**.**

4. Dissolving 0.158 g of Potassium permanganate analytical reagent in 100 ml volumetric flask to prepare 0.01 mol/ml.

5. A 0.5 mol/ml Sodium hydroxide was prepared by dissolving 2.0 grams of pure material in 100 ml DW.

6. Hydrochloric acid 0.1 $mol.l^{-1}$, diluting 1.17ml of 36%, specific gravity 1.19 to 100 ml.

Ampicillin capsules procedure

Ten capsules (250 mg) were emptied and the contents of mix well. The powdered capsules equivalent to 10 mg of Ampicillin were weighed quantity, transferred, dissolved with distilled water and made up to the mark of 100 ml volumetric flask. The contents of the flask was stirred with magnetic bar for 10 min., then 10 ml of this solution transferred to 100 ml flask and complete with distilled water to mark, pipit 5ml from last solution and proceed as described under "Recommended Procedure".

Ampicillin suspension procedure

Dissolve five containers of Ampicillin suspension (250 mg) in 100 ml warm distilled water. An accurately transfer two ml of solution into a 5 ml test tube, 3.0 ml of NaOH 0.5 mol/ml solution was add and then centrifuged for five minutes at rate 3000 rpm. Washing the residue with alkaline solution at least three portions, then was transferred quantitatively into 100 ml volumetric flask, after the complete dissolving in HCl 0.6 mol/ml solution, distilled water used for diluted to the mark, checking in water bath for 10 min at 50^0C. then transferred 10 ml of this solution into 100 ml volumetric, the volume was complete to mark with distilled water, pipet five ml from last solution and proceed (Recommended Procedure).

Recommended procedures

Standard solutions volumes of Ampicillin trihydrate covers the working concentration ranging between 5.0-100.0 ìg/ml, were transferred to ten 25 ml volumetric flasks, 3.0 ml of 0.01 mol/l potassium permanganate were added, followed with 3.0 ml of 0.5 mol/l sodium hydroxide and shake well, then complete to the mark with DW and allow the reaction mixture to standing for four minetus. In molecular absorption spectrophotometry first procedure, the absorbance of the menganate ion at 610 nm were measured viruses a simultaneously prepared blank solution. The absorbance values against the final concentration solutions in ìg/ml were plotting obtainably the calibration graph. The second procedure involves using flame photometric emission to measure the intensity of potassium emission at 766 nm.

RESULTS

The molecular absorption spectra of Ampicillin shows two bands small band at 235 nm and broad band at 275 nm as in Fig. 2, three absorption peaks at 510, 530 and 550 nanometer for potassium permanganate solution in basic medium shows in Fig. 3. After adding aqueous solution of Ampicillin to potassium permanganate solution in basic medium it causes changes in the molecular absorption band spectrum of potassium permanganate, with new characteristic bands at 610 nm in Fig. 4.

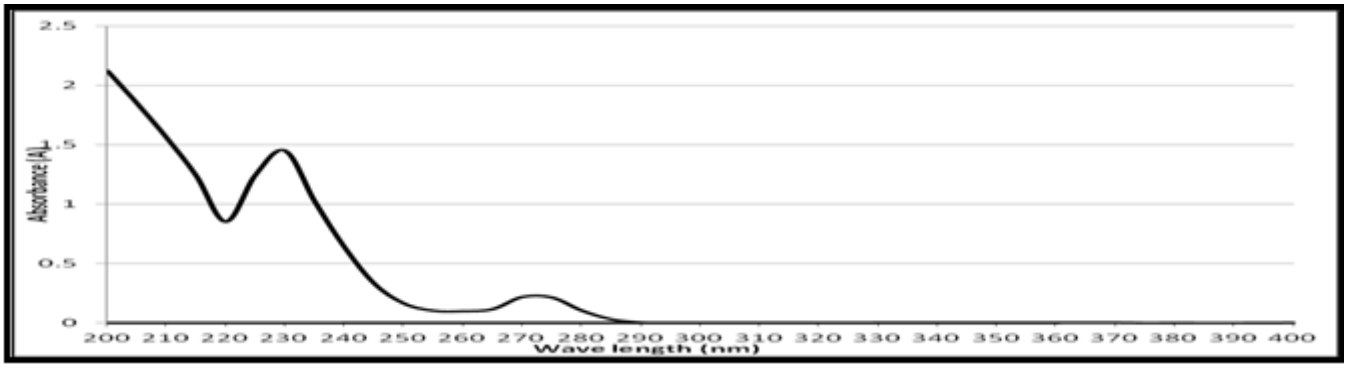

Fig. 2 : The molecular absorption spectrum of 50 µg/ml Ampicillin solution.

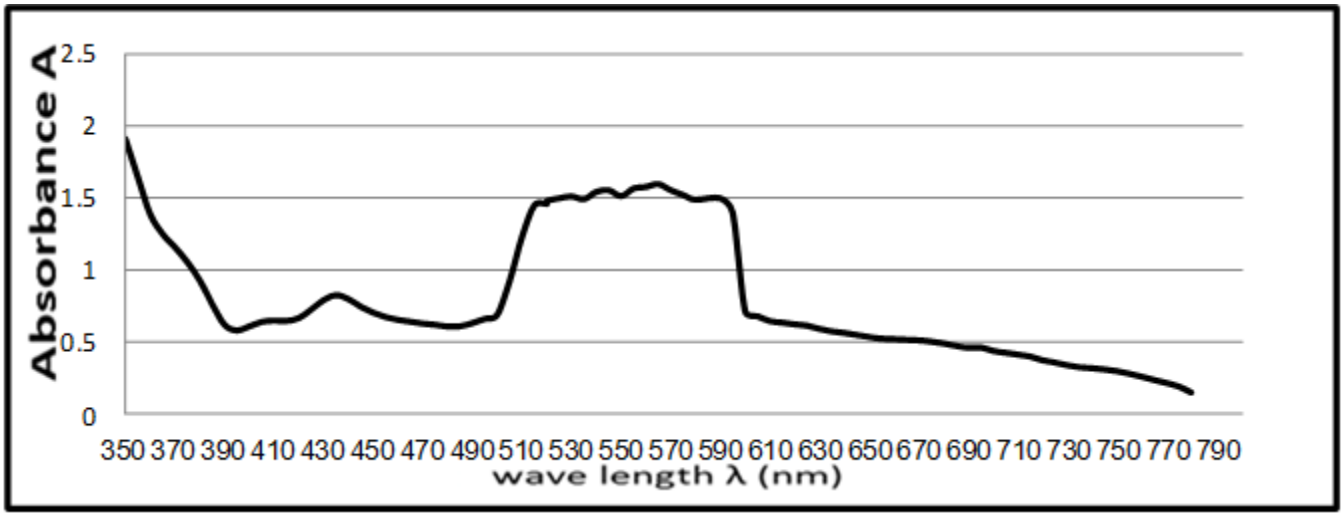

Fig. 3 : Molecular spectrum of 0.01 mol/ml $KMnO_4$ in alkaline medium.

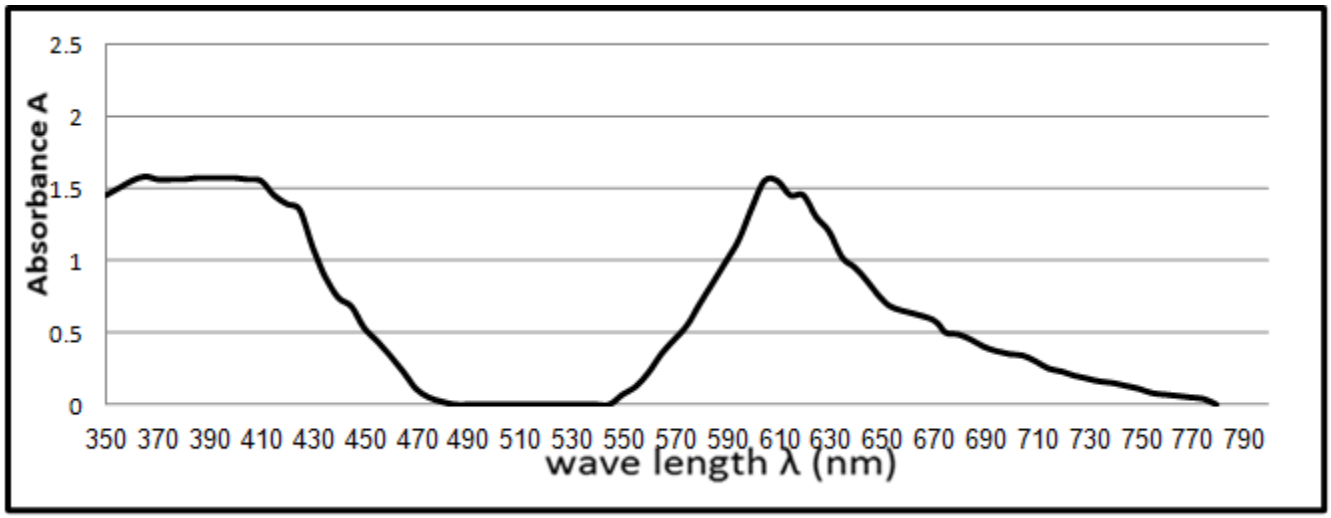

Fig. 4 : Molecular spectrum of 0.01 mol/ml $KMnO_4$ with 50 ($\mu g.ml^{-1}$) Ampicillin in alkaline medium.

Optimization of variables

The colored product posses spectrophotometric properties as well as the different experimental conditions effect on development of the color and stability of its were studied and optimized carefully. Any factors was changing individually, while the others variables were kept constant. These conditions includes concentration of the oxidant reagents,reaction media,temperature and time consumed in reaction.

1. Effect of pH : In this study, the pH effect involves reading absorbance of manganite resulting from reaction of Ampicillin with potassium permanganate in many reaction media by adding 0.1 mol/ml HCl or 0.1 mol/ml NaOH solution, the optimum pH was close to 10.

2. Effect of NaOH concentration : Many solutions

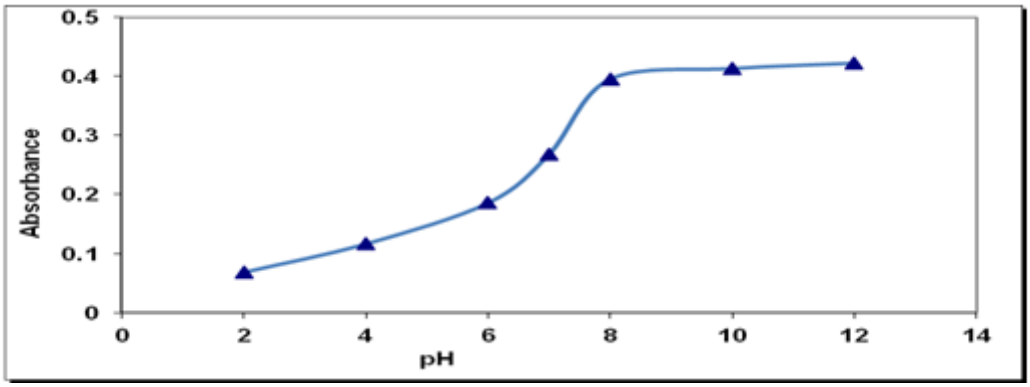

Fig. 5 : Relation between pH and absorbance of manganite ion.

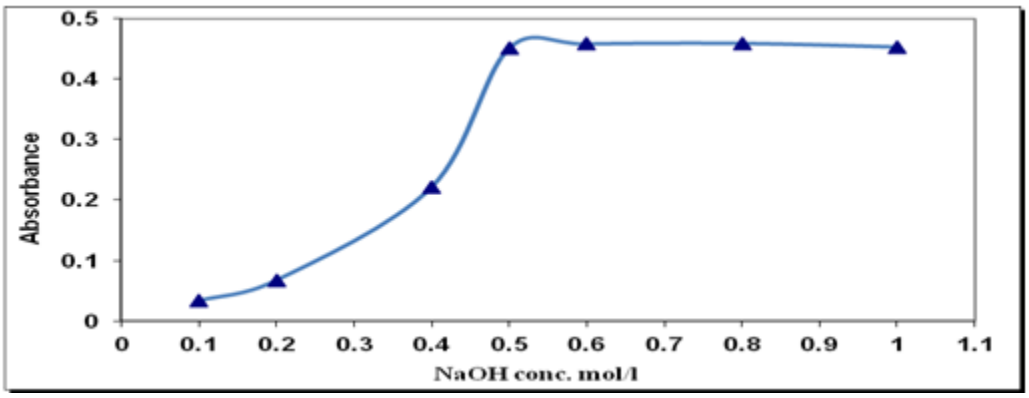

Fig. 6 : Relation between Sodium hydroxide and absorbance of manganite ion.

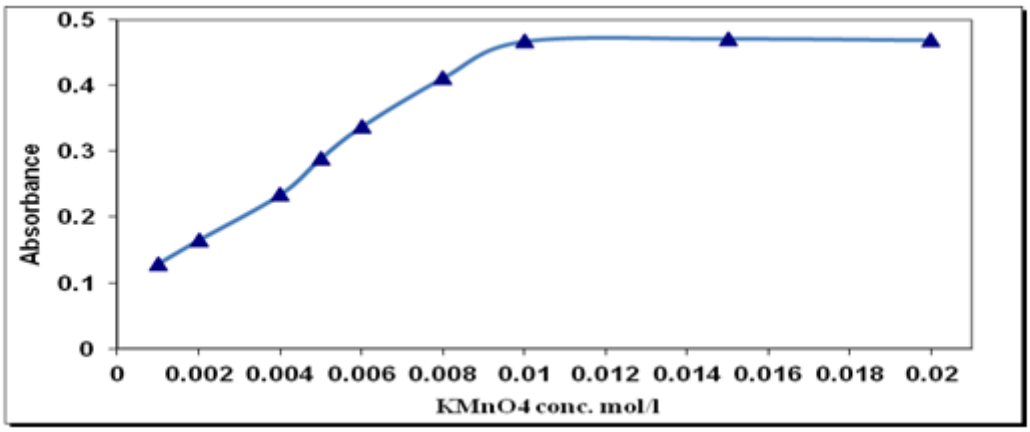

Fig. 7 : Relationship between potassium permanganate and absorbance of manganite ion.

of sodium hydroxide with different concentrations were prepared, and the absorbance for it after adding to solution containing Ampicillin and potassium permanganate were measured, 0.5 mol/ml NaOH considered optimal value for this study.

3. Effect of $KMnO_4$ concentration : Many solutions with different concentrations of potassium permanganate were prepared. After mixing with ampicillin and sodium hydroxide, the absorbance measured, the 0.01 mol/ml $KMnO_4$ concentration considered preferred value.

4. Effect of reaction temperature : The reaction temperature effect studied with reading absorbance of manganite ion in many temperature, we obtained the 25-30ºC optimal temperatures.

5. Effect of reaction time : The time reaction effect involves recording absorbance of manganite ion at different time, four minutes considers the optimum time for reaction.

Recoveries calculation

Table 1 shows the recoveries calculated for the determination of Ampicillin by applied two proposed methods, Molecular Absorption Spectrophotometry (visible) and flame photometric emission using three standard solution with different concentrations, the absorbance were readied three times for any solutions

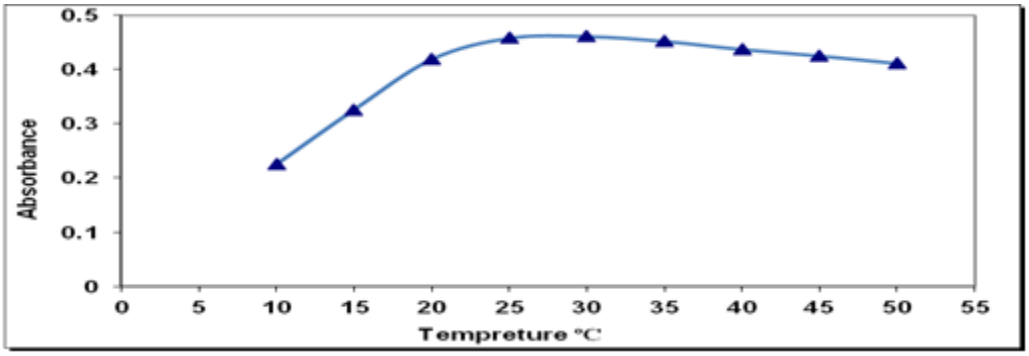

Fig. 8 : Relationship between reaction temperature and absorbance of manganite ion.

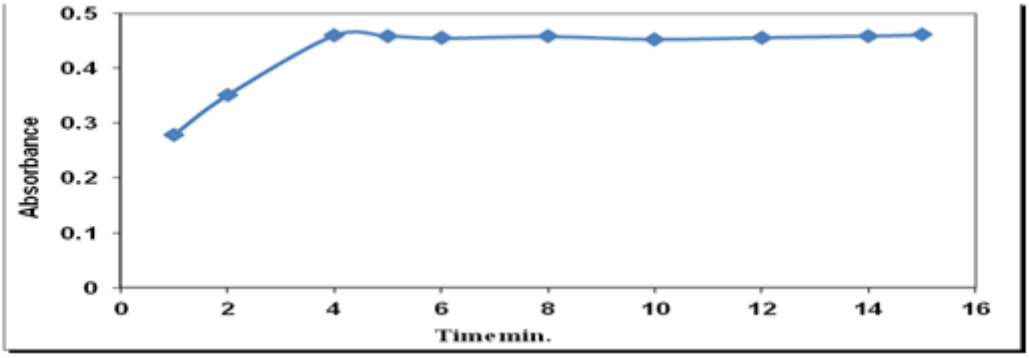

Fig. 9 : Relationship between reaction time and absorbance of manganite ion.

Table 1: The calculated recoveries for the determination of Ampicillin by applying two methods.

Molecular Absorption (Colorimetry)					
Taken (µg/ml)	**Found (µg/ml)**	**Recovery (%)**		**Error (%)**	**RSD (n=3)**
10	9. 797	97.970	Mean =97.536	-2.030	1.217
20	19.469	97. 345		-2. 655	3.161
30	29.188	97.293		-2.707	3.627
Flame Atomic Emission (Flame Photometry)					
Taken (µg/ml)	**Found (µg/ml)**	**Recovery (%)**		**Error (%)**	**RSD (n=3)**
10	9.771	97. 710	Mean =97.373	-2.290	1.706
20	19.459	97.295		-2.705	3.312
30	29.135	97.116		-2.884	3.785

Table 2 : Analytical data for Ampicillin determination by colorimetry.

Preparation type	Found (µg/ml)	Linearity (µg/ml)	Regression equation	Correlation coefficient	Recovery% (Rec.%)	Detection Limit DL (µg/ml)	RSD%	Relative Error (RE%)
Capsules (250mg)	243.597	5-80	Y=0.0076 X+0.0554	0.9954	97.438%	1.839	1.645	2.562
Suspension (250mg)	243.224	5-80	Y=0.0076 X+0.0554	0.9954	97.289%	2.265	2.147	2.711

Table 3 : The analytical data for determination of Ampicillin by flame photometric emission.

Preparation type	Found (µg/ml)	Linearity (µg/ml)	Regression equation	Correlation coefficient	Recovery% (Rec.%)	Detection Limit DL (µg/ml)	RSD%	Relative Error (RE%)
Capsules (250mg)	243.327	5-90	Y=0.8896 X+12.66	0.9958	97.331%	2.271	2.149	2.669
Suspension (250mg)	242.929	5-90	Y=0.8896 X+12.66	0.9958	97.171%	2.903	2.522	2.829

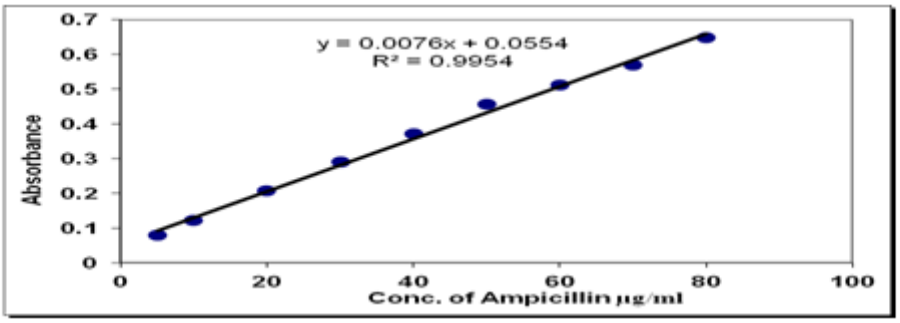

Fig. 10 : Colorimetric calibration graph of Ampicillin-$KMnO_4$ product in basic medium.

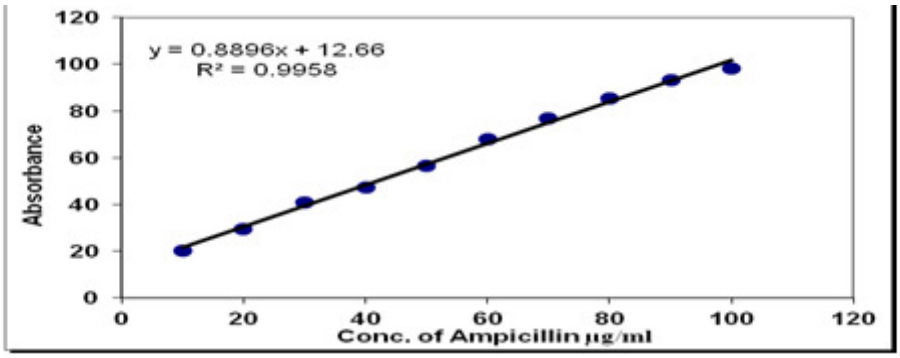

Fig. 11 : Flame Photometric calibration graph of Ampicillin-$KMnO_4$ product in basic medium.

that used.

Constructing calibration curves : Figs. 10, 11 demonstrated normal calibration graphs for the determination of Ampicillin in two methods by plotting the concentrations and its absorbances.

DISCUSSION

The optimum conditions of concentrations for oxidant reagent $KMnO_4$ and reaction medium sodium hydroxide were studies in this work were (0.01 mol/ml, 0.5 mol/ml), respectively after increase concentrations of $KMnO_4$ and NaOH, the absorbance remaining constant in first state but decreased in second state.

When potassium permanganate reacting with Ampicillin in alkaline medium the appearance of one broad peak at 610 nm for manganite ion, the three original peaks of permanganates at (510, 530, 550 nanometer) were disappeared with changing purple to blue color (Daniel, 2010; Holler *et al*, 2007).

$$MnO_4^- + OH^- + \longleftrightarrow e^- \; MnO_4^{-2} \quad E = +0.564 \text{ V}$$

In the reaction of a strong oxidant reagent potassium permanganate with Ampicillin, it ether oxidized sulfur atom to sulfoxide group, and with carbonyl â-lactam ring to carboxyl group (Madhukara *et al*, 2011) or primary aliphatic amine to form an oxide amine (Morrison-Boyd, 2000). We applieds new colorimetric method in determination of Ampicillin in pure and its drug formulations, the concentration of Ampicillin in dosage form obtained was very closely for the value mentioned (labeled) on marketed Ampicillin capsule and suspension (243.597, 243.224µg/ml), respectively in molecular absorption (colorimetry) and in Flame Atomic Emission Photometric spectrophotometry (243.327, 242.929 µg/ml).

CONCLUSION

The proposed study involved simple, fast, precise and accurate methods for the determination of Ampicillin in pure and dosage form by molecular absorption spectroscopy via oxidation reduction reaction in solutions. They obtained law percentage errors, detection limits and good linearity.

REFERENCES

Abdelrahman (2015) Three Spectrophotometric Methods for Simultaneous Determination of Ampicillin and Dicloxacillin in Presence of Their Major Impurity 6-Aminopenicillanic Acid. *Austin J. Anal. Pharm. Chem.* **2**(5), 1-7.

Akanni A O and Ayim J S (1992) Determination of ampicillin in the presence of cloxacillin. *J. Pharm. Biomed. Anal.* **10**(1), 43-47.

Audumbar Digambar Mali, Ritesh Bathe and Ashpak Tamboli (2015) Estimation of ampicillin trihydrate in bulk and formulation by first order derivative area under curve UV-Spectrophotometric methods. *Int. J. Adv. Scientific Res.* **1**(05), 239-243.

Bacigalupo M A, Meroni G and Secundo F (2008) Time-resolved fluoroimmunoassay for quantitative determination of ampicillin in cow milk samples with different fat contents. *Talanta* **77**, 126-130.

Barot Tushar G, Patidar, Kanhaiyalal, Kshartri Nayan and Vyas Niranjan (2009) Development and Validation of LC Method for the Determination of Ampicillin and Dicloxacillin in Pharmaceutical Formulation Using an Experimental Design. *E-J. Chem*. **6** (4), 955-964.

British Pharmacopeia (2007) on CD-Rom. V.3.

Daniel C Harris (2010) *Quantitative Chemical Analysis*. 8th edition, p 352, W. H. Freeman and Company, New York.

Ejele A, Anyanwu N and Anunuso C (2008) Iodometric determination of ampicillin in proprietary capsules. *Int. J. Nat. Appl. Sci.* **4**(4).

Erdal Dinc and Dumitru Baleanu (2006) A new fractional wavelet approach for the simultaneous determination of ampicillin sodium and sulbactam sodium in a binary mixture. *Spectrochimica Acta Part A* **63**, 631–638.

Gabriela Coelho Miguel, Eduardo Adilson Orlando and Valéria Colnaghi Simionato (2013) Quantification of beta-lactam antibiotics in veterinary drugs: amoxicillin and ampicillin by high performance liquid chromatography. *Quim. Nova* **36**(8), 214-1221.

Ghoulipour V Related information, Shokri M Related information and Waqif-Husain S Related information (2011) Determination of ampicillin and amoxicillin by high-performance thin-layer chromatography. *Acta Chromatographica* **23**(3), 483-498.

Holler Skooge and Crouch (2007) *Instrumental Chemical Analysis*. 6th edition, International Student Edition.

Issa Y M and Amin A S (1993) Spectrophotometric Determination of Ampicillin with Some Nitro Compounds. *Analy. Lett.* **26**(11), 2397-2407.

Issam A Latif and Sundus H Merza (2016) Nanoscience and Nanotechnology. Fabrication of Functionalize Reduce Graphene Oxide and Its Application in Ampicillin Detection. *Nanoscience and Nanotechnology* **6**(2), 24-33.

Ivana Rašiæ Mišiæ, Gordana Miletiæ, Sne•ana Mitiæ, Milan Mitiæ, and Emilija Pecev-Marinkoviæ(2013) A Simple Method for the Ampicillin Determination in Pharmaceuticals and Human Urine. *Chem. Pharm. Bull*. **61**(9), 913–919.

Khalid Abdel-Salam M Attia, Omar Abdel-Aziz, Nancy Magdy and Ghada F Mohamed (2018) Determination of Flucloxacillin-sodium in Binary Mixture with Ampicillin-trihydrate Using Univariate and Multivariate Spectrophotometric Methods: A Comparative Study. *J. Adv. Pharm. Res.* **2**(3), 180-190.

Madhukara A Badgujar and Kiran V Mangaonkar (2011) Simultaneous Estimation of Ampicillin Sodium and Sulbactam Sodium in Injectable Dosage Form by High Performance Liquid Chromatography. *Oriental J. Chem.* **27**(4), 1659-1664.

Mariam Yacoub Yousif (2006) Determination of ampicillin trihydrate alone and in presence of some metals by drevative spectrophotometric method. *M.Sc. Theseis.* College of Science, Al-nahrien Unevercity.

Mohd Dzul Hakim Wirzal, Abdull Rahim Mohd Yusoff, Jiri Zima and Jiri Barek (2013) Degradation of Ampicillin and Penicillin G using Anodic Oxidation. *Int. J. Electrochem. Sci*. **8**, 8978 – 8988.

Mohamed G G (2001) Spectrophotometric determination of ampicillin, dicluxacillin, flucloxacillin and amoxicillin antibiotic drugs: ion-pair formation with molybdenum and thiocyanate. *J. Pharm. Biomed. Anal.* **24**(4), 561-567.

Moon Hee Shin, Man Ki Park, Chang Hwa Yu and Jung Kap Choi (1981) Determination of ampicillin and cloxacillin mixture by NMR. *Arch. Pharm. Res.* **4**(1), 9-17.

Morrison R and Boyd R (2000) *Organic Chemistry*. 6th edition, New York.

Nelis H J, Vandenbranden T J, Verhaeghe B, Dekruifm A, Mattheeuws D and Deleenheer A P (1992) Liquid Chromatographic Determination of Ampicillin in Bovine and Dog Plasma by Using a Tandem Solid-Phase Extraction Method. *Antimicrobial Agents and Cheomotherapy* **36**(8), 1606-1610.

Peter M Monteleone, Michael K Vasiljev and Joseph Bomstein (1973) Spectrophotometric determination of ampicillin in presence of hetacillin. *J. Pharmaceut. Sci.* **62**(11), 1830-1833.https://doi.org/10.1002/jps.2600621120

Rade Injac, Nina Koèevar and Borut Štrukelj (2009) Optimized Method for Determination of Amoxicillin, Ampicillin, Sulfamethoxazole, and Sulfacetamide in Animal Feed by Micellar Electrokinetic Capillary Chromatography and Comparison with High-Performance Liquid Chromatography. *Croat. Chem. Acta* **82**(3), 685–694.

Rašiæ Mišiæ I, Miletiæ G, Mitiæ S, Mitiæ M and Pecev-Marinkoviæ E (2013) A simple method for the ampicillin determination in pharmaceuticals and human urine. *Chem. Pharm. Bull.* (Tokyo) **61**(9), 913-919.

Resmi Mustarichie, Sandra Megantara and Dolih Gozali (2018) Comparison of Ampicillin Determination in Pharmaceutical Preparations with Iodometry Titration, HPLC, And Diffusion Methods. *Res. J. Pharmaceut., Biol. Chem. Sci.* **9**(1), 515-523.

Shireesha Battula and Anusha Shivaraj (2017) RP-HPLC Method for the Simultaneous Estimation of Ampicillin and Cloxacillin in Capsule Dosage Form. *Int. J. Appl. Pharmaceut. Sci. and Res.* **2**(3), 93-98.

Smith J W G, de Grey G E and PatelIssue V J (1967) The spectrophotometric determination of ampicillin. *Analyst* **92**, 247-252.

Soledad-Rodríguez B, Pilar Fernández-Hernando and Durand-Alegría J S (2017) Effective determination of ampicillin in cow milk using a molecularly imprinted polymer as sorbent for sample preconcentration. *Food Chem.* **224**(1), 432-438.

Straub R and Voyksner R (1993) Determination of penicillin G, ampicillin, amoxicillin, cloxacillin and cephapirin by high-performance liquid chromatography-electrospray mass spectrometry. J. *Chromatography* **647**(1), 167-181.

Suzanne L Parker, Syamhanin Adnan, Jenny L Ordóñez Meija, David L Paterson, Jeffrey Lipman, Jason A Roberts and Steven C Wallis (2015) An UHPLC–MS/MS method for the simultaneous determination of ampicillin and sulbactam in human plasma and urine. *Bioanalysis* **7**(18), 2311-2319.

Ticiano Gomes do Nascimento, Cícero Flávio Soares Aragão, Francinalva Dantas de Medeiros, Eduardo de Jesus Oliveira and Rui Oliveira Macêdo (1993) Validation of a Method for Determination of Ampicillin in Human Plasma using LC–DAD. *J. Chromatographic Sci.* **47**, 749-755.

Tushar G Barot, Kanhiyalal Patidar, Nayan Kshartri and Nirajan Vyas (2009) Development and Validation of LC Method for the Determination of Ampicillin and Dicloxacillin in Pharmaceutical Formulation Using an Experimental Design. *E-J. Chem.* **6**(4), 955-964.

Wenhong Luo, Eugene B Hansen, Catharina Y W Ang, Joanna Deck, James P Freeman and Harold C Thompson (1997) Simultaneous Determination of Amoxicillin and Ampicillin in Bovine Milk by HPLC with Fluorescence Detection. *J. Agric. Food Chem.* **45**(4), 1264–1268.

William Martindale and James E F Reynolds (1996) *Martindale The extra pharmacopeia*, 31 ed.

Yan Zhang B R, Yunling Wua and Quanmin Lia (2013) indirect determination of ampicillin sodium with sodium nitrate-ammonium thiocyanate-water system by extraction-flotation of cuprous thiocyanate. *J. Analytical Chem.* **68**(5), 417–422.