Open Access
Baghdad Science Journal **Vol. 13(2s(Supplement))2016**
The 2nd National Conference of Chemistry

DOI: http://dx.doi.org/10.21123/bsj.2016.13.2.2NCC.0480

Flame Atomic Emission and Colorimetric Methods for the Determination of Cephalexin Monohydrate in Pharmaceutical Preparations

Abbas S. Hasan Al-kahdimy ***Mohammed Abdullah Ahmed***

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

Received 14/9/2015
Accepted 20/12/2015

This work is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International Licens

E-mail: gladiatorman85@yahoo.com

Abstract:

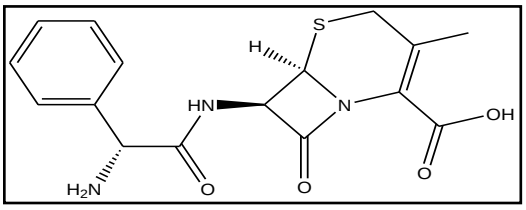
We propose two simple, rapid, and convenient spectrophotometric methods which are described for the determination of cephalexin in bulk and its pharmaceutical preparations. They are based on the measurement of the flame atomic emission of potassium ion (in the first method) and colorimetric determination of the green colored solution at 610 nm formed after the reaction of cephalexin with potassium permanganate as an oxidant agent (in the second method) in basic medium. The working conditions of the methods are investigated and optimized. Beer's law plot shows a good correlation in the concentration range of 5-40µg ml^{-1}. The detection limits are 2.573,2.814 µg ml-1 for the flame emission photometric method and 1.844,2.016 µg ml-1 for colorimetric methods for capsules and suspensions respectively.The methods are successfully applied to the determination of cephalexin in capsules and suspensions, and the obtained results are in good agreement with the label claim. No interference is observed from the commonly encountered additives and excipients.

Key words: Cephalexin ,FlameAtomic Emission ,Colorimetry

Introduction:

Cephalexin can be defined as one of the semisynthetic derivatives of cephalosporin which has an antibacterial activity against gram-positive and gram-negative bacteria. It is also an active cephalosporin portrayed by an expansive range of antibiotic activity, frail capacity to bonding with blood protein, without metabolites, low toxicity and to be quickly retained after oral administration bringing on a high serum levels and urine concentration. In this manner, the cephalexin is extensively utilized for clinical chemotherapy [1].Cephalexin chemically naming -5-thia-1-aza-bicyclo [4.2.0] octa-2-ene-2- carboxylic acid, 7-[2-mino-2-phenyl acetamido] -3-methyle-8-oxo ,is a white or almost white, crystalline powder , molecularly

weighted 365.4 g/mol. It is soluble in water, particularly insoluble in alcohol and ether pKa 5.2, 7.3, UV Maxima 260 nm. Molecular formula: $C_{16}H_{17}N_3O_4S$: Percent Composition: C 55.32%, H 4.93%, N 12.10%, O 18.42%, S 9.23 %[2,3].



Scheme (1): Chemical Structure Formula of Cephalexin

According to literature surveys, there are different analytical methods reported for the determination of cephalexin. They include spectrophotometricaly such as acetylation of cephalexin with acetic anhydride in aqueous solution at PH 11.5 and measurement at 335 nm[4].Another method is based on the charge transfer reaction between cephalexin and quinalizarin in dimethylsulfoxide medium spectrophotometricaly [5].Another method involves the reaction of cephalexin with 2-cyanoacetamide in the presence of 33% ammonia solution; the formed fluorescent product exhibits maximum fluorescence intensity at λ 439 nm, after excitation at λ 339 nm[6].Cephalexin is Determined in bulk drug and pharmaceutical formulations by measuring absorbance at 261and 257 nm[7]. Two methods for the estimation of cephalexin in tablets: the first method is based on the reaction of cephalexin with Folin-Ciocalteu reagent, giving a blue colorchromogen, which shows maximum absorbance at 753 nm ,while the second method is based on the estimation of cephalexinat 263 nm [8].

Cephalexin is Determined in tablet by reacting with ninhydrin and the blue color chromogen is measurred absorbance at 576 nm [9].the drug is detrmined in pure and in dosage form.the method is based on the formation of charge transfer complex using chloranilic acid as acceptor in non-aqueous solvent. the complex show maximum absorption of 520 nm [10].Also,it is determined after its oxidation by persulfate in an alkaline medieum.the maximum absorbance is measured at 350 nm [11]. paracetamol and cephalexin in mixture are determined Simultan [12].Cephalexin polymorphs commercial medicines are analyzed using X-ray powder diffraction (XRPD), Fourier transform infrared spectroscopy (FTIR) and solid state 13C NMR spectroscopy[13].Indirect spectrophotometric method is used for the determinaion of cephalexin in pharmaceutical product without pretreatment.the method is based on the fading effect of the cephalexin on developed colored product results by bromination reaction of NBS with p-anisidine in acidic medium, the vanished colored species is measured subsequently at 522 nm[14].Cephalexin is determined Using UV-Vis spectroscopy.The method is based on the reaction of cephalexin with Fe III to form a chlating complex at PH 2 and also is determined indirectly Flame Atomic Absorption Spectrophotometery FAAS [15]. Amoxicillin and cephalexin are determined individually and simultaneouslly depending on the first and second derivative techniques[16].,Simultaneous determination of vancomycin and cephalexin in human plasma by using HPLC-DAD with second-order calibration algorithms[17].Cephalexin is determined in pharmaceutical dosage forms performing an isocratic separation on an Enable C18G column (250 mm × 4.6 mm i.d., 5 μm) using methanol:0.01 M TBAHS (50:50, v/v) as the mobile phase at a flow rate of 1.0 ml/min

[18].Liquid chromatographic determination in human plasma and urine involves microporefiltration of urine specimens and methanol extraction of plasma samples followed by HPLC separation on a bonded reverse phase column utilizing a mobile phase of methanol water containing acetic acid, the absorbance is measured at 254 nm [19] Cephalosporines are determined by TLC on stannic oxide layers using citrate and borate buffers as mobile phases[20]. High Performance Thin Layer liquid Chromatographic (HPTLC) in human plasma separation is achieved on the aluminum backed layer of silicagel 60F254 by using (Toluene: Methanol: Triethylamine) (6:4:0.1 v/v/v) as mobile phase [21].

Simultaneous determination (RP-HPLC) of Cephalexin and Carbocisteine from capsules, using 0.025 M monobasic sodium phosphate: acetonitrile (87:13, v/v) as a mobile phase, and a C8 Shodex column as the stationary phase, detection is carried out by using a UV detector at 210 nm [22].After precolumn derivatization Cephalexin is derived in human plasma , with 9-fluorenylmethyl chloroformate in borate buffer (5 mM, pH 8.5) for 15 min at 25°C, the derivative is chromatographed on an XDB-C18 column with water-acetonitrile (10:90, v/v) as amobile phase at a flow rate of 1.0 ml/min. The fluorescence excitation and emission wavelengths are 268 nm and 314 nm, respectively[23].Dual Electrospray ionization, multistage tandem mass spectrometry LC-MS are used to identify Amoxicillin, Ampicillin and Cephalexin, Ion detection is performed by using Quadrupole -Time of Flight coupled with dual ESI ion source and identified corresponding ions[24].

Amoxicillin and ampicillin are Determined of by complexation with Au(III) and Hg(II) ions in bulk and pharmaceutical preparations using UV-Vis. spectrophotometry, atomic absorption, and HPLC techniques, at PH 4 and (2-4) for Au(III) and Hg(II)complexes respectively [25].The heated glassy carbon electrode (HGCE) is used to hydrolyze and detect the cephalexin without oil/water bath setup. The cephalexin could be rapid hydrolyzed in 15min by HGCE, and the good electro activity could be found in the hydrolysate of cephalexin, the determination of cephalexin by detecting degradation products using square wave voltammetry SWV [26)].

Materials and Methods:

Instruments and Equipment:-

1-Flame emission spectrophotometer (JenwayPFP7 / UK) is used for absorbance measurements.

2- Double-beam UV-Visible spectrophotometer: Varian Gary 100 UV-Vis spectrophotometer.

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

4- Centrifuge**5-** Water bath

Chemicals:-

Cephalexin monohydratestandard material, cephalexin 250mg and cephalexin 255 mg powder fo rsuspension formulations are supplied from the State Company for Drug Industries and Medical Appliances (Samara-IRAQ-SDI).All other chemicals and reagents of analytical grade are obtained from Fisher, Fluka and BDH Companies.

Preparation of Solutions:-

1- Stock solution of cephalexin 1000 μgml^{-1} is prepared by dissolving 0.1 g of Cephalexin monohydrate standard material in 100 ml distilled water distilled water. Other standard solutions are prepared by subsequent dilution of stock solution.

2- Cephalexinsolution 100 μgml^{-1} is prepared by diluting 10 ml of Stock solution to 100 ml DW in volumetric flask, this solution is used for recorded UV-Vis spectrum.

3- Standard solutions for calibration curve ere prepared by diluting cephalexinsolution100 μgml^{-1} to (5-40 μgml^{-1})**.**

4-Potassium permanganate 0.01 M, diluting 10 ml of 0.1 M Potassium permanganate standard solution to 100 ml.

5- Sodium hydroxide 0.5 M, is prepared by dissolving 2.0 g of pure NaOH in 100 ml distilled water.

Procedure for Cephalexin Capsules:-

Empty the contents of 10 capsules (250 mg), and mix well. Transfer a weighed quantity of the powdered capsules equivalent to 10 mg of cephalexin into 100 ml volumetric flask and make up to the mark with distilled water. The content of the flask is stirred magnetically for 10 minutes, then transfer 10 ml of this solution into 100 ml volumetric flask, complete to mark with distilled water,pipet 5mlfrom last solution and proceed as described under "Recommended Procedure".

Procedure for Cephalexin Suspension:-

Five containers of cephalexin suspension (250 mg)after dissolving in 100 ml warm distilled water are mixed. An accurately 2ml of this solution transferred into a 5 ml test tube, added 3.0 ml of 0.5 M NaOH and then centrifuged at arate 4000 rpm for five minutes. The residue is washed at least three portions with alkaline solution, then was quantitatively transferred into 100 ml volumetric flask and after the complete dissolution in 0.6M HCl, diluted to the mark with distilled water, checking in water bath at 60 C^o for 10 min. then transferred 10 ml of this solution into 100 ml volumetric flask, complete to mark with distilled water, pipet 5 ml from last solution and proceed as described under "Recommended Procedure".

Recomended Procedures:-

Transfer aliquot volumes of cephalexin standard solution covering the working concentration ranged from 2.0 to 60.0 $\mu g\ ml^{-1}$ into 25 ml volumetric flasks; add 3.0 ml of 0.01 $moll^{-1}$ potassium permanganate followed by 3.0 ml of 0.5 $moll^{-1}$NaOH and shake well, then make up to the mark with water. Allow the reaction mixture to stand for 20 min. In first procedure molecular absorption spectrophotometry, measure the absorbance of the resulting solution at 610 nm against a reagent blank prepared simultaneously. Plot the values of the absorbance against the final concentration in $\mu g\ ml^{-1}$ to get the calibration curve. Alternatively, derive the corresponding regression equation.The flame emission photometry second procedure involves measure the intensity of potassium emission at 766 nm.

Results and Discussion:

The molecular absorption spectra of cephalexin show two bands small band at 210 nmand broad band at 260 nm fig. 1, KMnO4 in basic medium shows an absorption broub bands at 510, 530 and 550 nm fig. 2. The addition of aqueous solution of tetracycline to $KMnO_4$ solution in basic mediumcauses a change in the absorption spectrum of $KMnO_4$, with new characteristic bands at 610 nm Fig. 3.

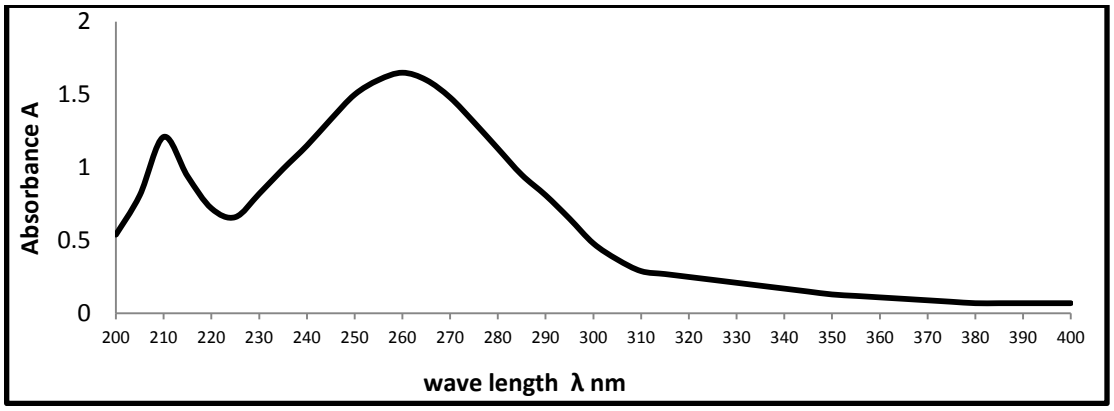


Fig. (1):Molecular Spectrum of 50 µg/ml Cephalexin solution.

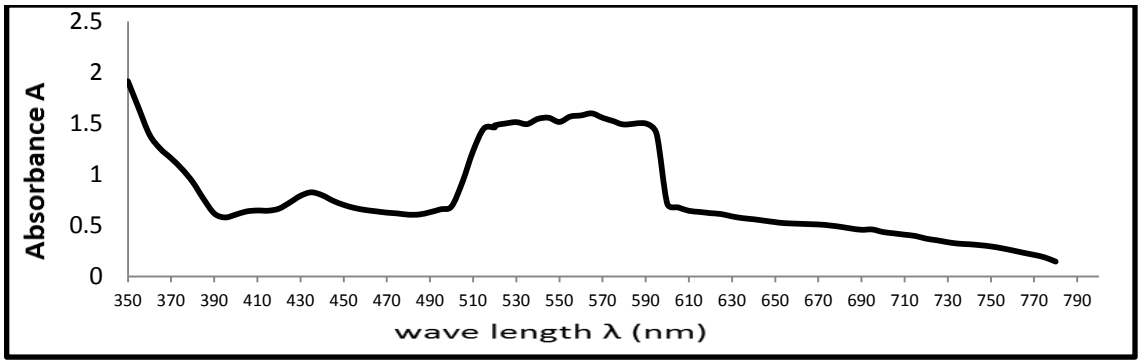


Fig. (2):Molecular Spectrum of $KMnO_4$in Alkaline Medium.

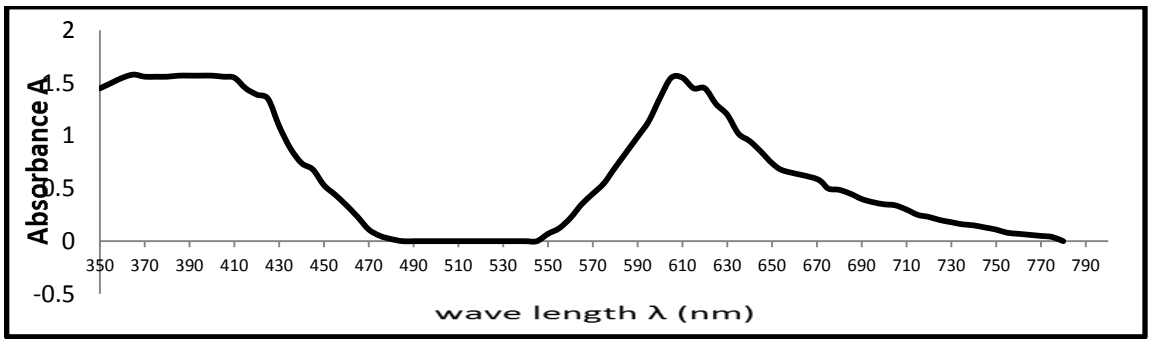


Fig. (3):Molecular Spectrum of $KMnO_4$with Cephalexin in Alkaline Medium.

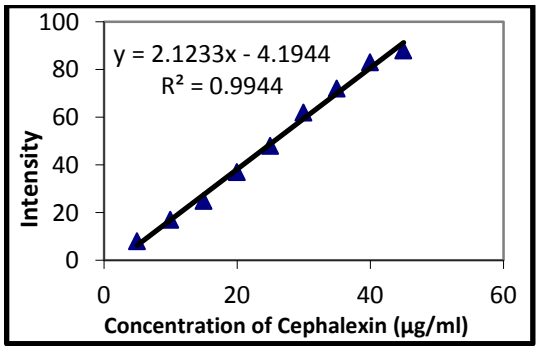


Fig. (3): Calibration Curvefor Cephalexin with $KMnO_4$in Alkaline Medium by Flame Atomic Emission Photometry.

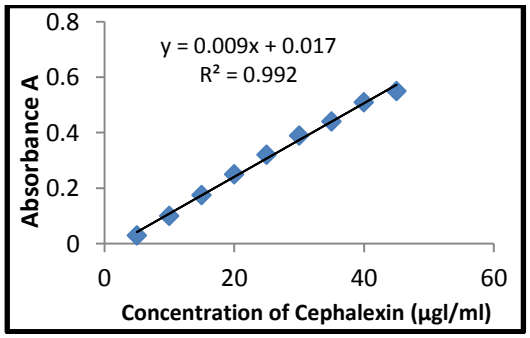


Fig. (4): Calibration Curvefor Cephalexin with $KMnO_4$in Alkaline Medium by Molecular Absorption Spectrophotometry.

Optimization of Variables:-

The spectrophotometric properties of the colored product as well as the different experimental parameters affecting the color development and its stability are carefully studied and optimized. Such factors are changed individually while the others are kept constant. These factors include concentration of the reagents ($KMnO_4$ and NaOH) and time of reaction.

1-Effect of $KMnO_4$ Concentration:-

After measuring absorbance of many solutions with different concentrations, It is found that 0.01 M $KMnO_4$ is considered an optimal value.

Table (1):Relationship between Potassium Permanganate Concentrations and Absorbance

$KMnO_4$Concentration(Mol/L)	**10^{-4}**	**$5X10^{-4}$**	**10^{-3}**	**$5X10^{-3}$**	**10^{-2}**	**$5X10^{-2}$**	**10^{-1}**	**$5x10^{-1}$**	**1**
Absorbance	**0.13**	**0.15**	**0.18**	**0.20**	**0.23**	**0.23**	**0.23**	**0.23**	**0.23**

2-Effect of NaOH Concentration:-

With preparing many solutions with different concentrations, and measuring the absorbance for it after reacting with cephalexin, 0.5 M NaOH considered an optimal value for this study.

Table (2):Relationship between Sodium Hydroxide Concentrations and Absorbance

NaOHConcentration(Mol/L)	**10^{-2}**	**$5X10^{-2}$**	**10^{-1}**	**$5X10^{-1}$**	**1**	**1.5**	**2**
Absorbance	0.20	0.22	0.25	0.27	0.24	0.21	0.19

Table (3): The Analytical data for Determination of Cephalexin by Flame Atomic Emission Photometry.

Formula-tion type	linearity (µg/ml)	Regression equation	correlation coefficient	Recovery%(Rec.%)	Detection Limit (DL) (µg/ml)	RSD%	Relative Error (RE%)
Capsules (250mg)	5-40	Y=0.009 X+0.017	0.992	97.76%	1.844	1.645	2.240
suspension (250mg)	5-40	Y=0.009 X+0.017	0.992	96.98%	2.016	1.932	3.020

Table (4): The Analytical data for Determination of Cephalexin by Calorimetry.

Formula-tion type	linearity (µg/ml)	Regression equation	correlation coefficient	Recovery%(Rec.%)	Detection Limit (DL) (µg/ml)	RSD%	Relative Error (RE%)
Capsules (250mg)	5-40	Y=2.1233 X-4.1944	0.9944	98.05%	2.573	2.137	1.950
suspension (250mg)	5-40	Y=2.1233 X-4.1944	0.9944	97.89%	2.814	2.498	2.110

Conclusion:-

The optimum conditions of concentrations for oxidant reagent $KMnO_4$ and reaction medium sodium hydroxide studied in this work are (0.01 M,0.5 M) respectively, after increasing concentrations of $KMnO_4$ and NaOH,the absorbance remains constant in first state but decreases in the second.

When cephalexin reacts with $KMnO_4$ in basic medium, the three bands (510, 530, 550 nm) are disappear with the appearance of one peak at 610 nm with changing the purple color of permanganates to blue for manganite ion [27-29].

$$MnO_4^{-1} + OH^- + e \longleftrightarrow MnO_4^{-2} \quad E = +0.564V$$

Potassium permanganate is considered strong oxidant reagent in reaction of cephalexin , it ether oxidizes sulfur atom in cephalosporins ring to sulfoxide group [13], and with carbonyl β-lactam ring to carboxyl group [29], or primary aliphaticamine to form an oxide amine[11].We apply a new colorimetric method for the determination of cephalexin in bulk and its pharmaceutical preparations we obtain the concentration of cephalexin in dosage form which is close for the value recorded (labeled) on the cephalexin capsule and suspension (245.125, 244.725 µg/ml) respectively in Flame Atomic Emission Photometry, and in molecular absorption spectrophotometry (2.440,242.450 µg/ml).

References:

[1] Geo, F.; Janet, S. and Stephan, A. 2004. Medical microbiology, 23 ed, MC Graw Hill.

[2]British Pharmacopeia, 2007. onCD-Rom, V3, 470-472.

[3]Martindale, W.; Reynolds, J. E. F.;and Royal Pharmaceutical Society of Great Britain. 1996. *Martindale: The extra pharmacopoeia*. London: Royal Pharmaceutical Society.

[4]Alwarthan, A. A.; Abdel Fattah, S. and Zahran N. M. 1992. Spectrophotometric determination of cephalexin in dosage forms with imidazole reagent,Talanta, 39 (6) :703 – 707.

[5]Carlos E. R.; Vanessa, G. K.; Ricardo, J. 2010. Spectrophotometric determination of cephalexin in pharmaceutical formulations exploring its charge transfer reaction with quinalizarin, Quim. Nova, 33 (4): 914-919 Sao Paulo.

[6] Dalia, R. and El-Wasseef. D. R. 2007. Spectrofluorometric Determination of Cephalexin in Pharmaceutical Preparations and Spiked Human Urine Using 2-Cyanoacetamide, Spectroscopy Letters: AIJFRC40: (6):797-809.

[7]Venkata, G.; Sravani, S.; Mohammed, B.; Madhu, M.; Sreenivasulu Munna and Gopinath, C., 2013. Development and Validation of UV-Spectrophotometric Method for Determination of Cephalexin, AJRC; May, 6 (5):6.

[8]Patel, S. A., Patel, N. M., and Patel, M. M. 2006, Spectrophotometric methods for the estimation of Cephalexin in tablet dosage forms, 68 (2) : 278-280.

[9]Patel, S. A. and Patel, N. J. 2011. Spectrophotometric determination of cephalexin using ninhydrin reagent in tablet dosage form, IRJP, 2(9): 123-126.

[10]Attama, A. A.; Nnamani, P. O. and Agbo A, N. 2006. Development of Alternative Assay Technique for Cephalexin by Charge Transfer Interaction of the Donor: Acceptor Type with Chloranilic Acid, TCPJ, 58, 11-18.

[11]Murad, I. H.; Eyad, S. M. and Rasheed, M. A. Q. 1998. spectrophotometric determination of selected cephalosporins,Acta polonaise Pharmaceutica-Drug Research,55(2):87-91.

[12]-Khaleda, H.; Firyal, W. A. and Asraa, A. 2010. Simultaneous determination of paracetamol and cephalexin binary mixtures by using derivative spectrophotometry and H-point standard addition methods, JCE .1,43-59.

[13]Danial, L. M.; Deaguir, A. S., Leandro B., Monica R. C. and Anedr, L. 2011. evaluation of polymorphs in cephalexin medicines by ^{13}C solid state NMR, IJPPS. 3(3): 293-298.

[14]Rebwar, O. Hassan, 2013. Indirect Spectrophotometric Determination ofCephalexin in Pharmaceutical Formulations, Chemical Science Transactions, 2(4): 1110-1117.

[15]Amera, A. 2009. Determination of Cefalexin by Direct UV- Vis Spectrophotometer and Indirect Flame Atomic Absorption Technique, Iraqi J Pharm Sci,18 (1): 50-56.

[16]Sarmad, B. 2009. First- and Second-Order Derivative Spectrophotometry for Individual and Simultaneous Determination of amoxicillin and cephalexin, NJoC, 34, 260-269.

[17]Le-Qian, H.; Chun-Ling, Y.; Ya-Hui, D. and Zhi-Peng, Z. 2012. Simultaneous and Direct Determination of Vancomycin and Cephalexin in Human Plasma by Using HPLC-DAD Coupled with Second-Order Calibration Algorithms), JoAMC, 2012. 8.

[18]Sagar Suman, P.; Bera, V. V.; Ravi, K.; Rabisankar, D. and Ganeswar Mohanta, 2013. Determination of Cephalexin Monohydrate in Pharmaceutical Dosage Form by Stability-Indicating RP-UFLC and UV Spectroscopic Methods), ScientiaPharmaceutica.

[19]Terumichni, A.; Haginakak, U. N.; Iyoshiy, A. and Tovozo, U. high speed liquid chromatoghraphy determination of cephalexin in human plasma and urine, JoA, 8:769-775.

[20]NabiS, A.; Laiq, E. and Islam A. 2004. selective separation and determination of cephalosporins by TLC on stannic oxide layers, ACTA Chromatographea,14: 92-101.

[21]Nandan, V.;Prashant, U., Mrinalini, C. and Ashwini, R. 2013. Development and Validation of HPTLC Method for Estimation of Cephalexin in Human Plasma, IJoRPBS, 4 (4):Oct–Dec, 1126-1123.

[22] Argekar, A. P.; Raj, S. V. and Kapadia, S. U., 1997. Simultaneous Determination of Cephalexin and Carbocisteine from Capsules by Reverse Phase High Performance Liquid Chromatography RP– HPLC Analytical Letters, 30 (4): 821-831.

[23]Meng, X. P. 2009; Liquid Chromatographic Analysis of Cephalexin in Human Plasma by Fluorescence Detection of the 9-Fluorenylmethyl Chloroformate Derivative, Analytical Letters, 42, (12): 1844-1854.

[24]Bose, K. S. C. K.; Tulam, V. R.; Chinta R, R.; Shriram, S.; Dubey, P. K. and Murali, P. M. 2012. Qualitative Analysis of Amoxicillin, Ampicillin, Cephalexin by QuadrupoleaTime of Flight (LCMS) Using Electrospray Ionization, IJoCTR, Jul-Sep, 4, (3) 1151.

[25] Abdulghani, A. J.; Jasim, H. H. and Hassan, A. S. 2012. Determination of β-lactam Antibiotics in Pharmaceutical Preparations by Uv-visible Spectrophotometry Atomic Absorption and High Performance Liquid Chromatography, 2(3): 1-11.

[26] Yiting, C.; Lu, H. and Qi, L. 2012. Rapid hydrolysis and Electrochemical Detection of Cephalexin at a Heated Glassy Carbon Electrode, IJoES, 7:7948-7959.

[27] Holler, Skooge and Crouch, 2007. instrumental chemical analysis 6th edition, 1nternational student addetion.

[28] Daniel, C. 2010. Quantitative Chemical Analysis, 8th edition, P 352, W. H. Freeman and Company, New York.

[29] Karpova, S. P. 2014. Quantitative determination of amoxicillin trihydrate in medical forms using kinetic method, JoCPR, 6(4):1120-1125.

التقدير بطريقتي الانبعاث الذري اللهبي واللوني للسيفالكسين احادي الماء في مستحضراته الصيدلانية

عباس شبيب حسن الكاظمي **محمد عبد الله احمد**

قسم الكيمياء- كلية العلوم –الجامعة المستنصرية

الخلاصة:

تم اقترحنا طريقتان طيفيتان امتازت كل منهما بالبساطة والسرعة والملائمة لتقدير عقار السيفالكسين كمادة نقية او في مستحضراته الصيدلانية ،استندت الطريقتان على اكسدة العقار بمحلول برمنغنات البوتاسيوم في الوسط القاعدي ،ومن ثم قياس شدة الانبعاث الذري اللهبي لايون البوتاسيوم في الطريقة الاولى ،و قياس الامتصاصية في المنطقة المرئية للون الاخضر المتكون عند الطول الموجي 610 نانومتر في الطريقة الثانية.

وبعد تحديد ظروف العمل المثلى، من خلال العلاقة البيانية بين شدة الانبعاث او الامتصاصية والتركيز ،وجد ان مدى الخطية لمطاوعة قانون بير-لامبرت في حدود التراكيز 5-40 مايكروغرام مل$^{-1}$. ان حدود الكشف كانت 2.573 و 2.814 مايكروغرام مل$^{-1}$ لطريقة الانبعاث الطيفي اللهبي الاولى و 1,844 و 2.016 مايكروغرام مل$^{-1}$ لطريقة الامتصاص في المنطقة المرئية.طبقت الطريقتان بنجاح لتقدير عقار السيفالكسين في نماذجهة النقية او مستحضراته الصيدلانية، من خلال النتائج المستحصلة والخاصة بقيم الاسترجاعية المئوية اظهرت الطريقتان ان لاتأثير للمتداخلات على عملية القياس.

الكلمات المفتاحية:- السيفالكسين ، الانبعاث الذري اللهبي ، اللوني