مجلة العلوم البحثة والتطبيقية
Journal of Pure & Applied Sciences
www.Suj.sebhau.edu.ly ISSN 2521-9200
Received 12/05/2019 Revised 16/08/2019 Published online 11/12/2019

Spectrophotometric method for the determination of Colchicine in pure and pharmaceutical forms (A Kinetic Study)

*Suad, K. Omar [a], Noreldin, S.Y. Abdolla [b], Firuze, F. Boujwod [c], and Yaman, M. Maan Rajab[a]
[a] Chemistry Department, Faculty of Science, Omar Al-Mukhtar University, El-Bieda, Libya
[b] Chemistry Department, Faculty of Science, Tobrouk University, Tobrouk, Libya
[c] Chemistry Department, Faculty of Science, Omar Al- Mukhtar University, Darna, Libya
* Corresponding author: suad.khalifa@omu.edu.ly

Abstract Simple, rapid spectrophotometric method for the determination of colchicine in pure as well as in dosage form is described. This method was based on oxidation of colchicine by alkaline potassium permanganate at room temperature. Under the optimized reaction conditions, Beer's law correlating the absorbance with colchicine concentration was obeyed in the range of 4-20 µgml^{-1}. The absorbance was measured at λ_{max} 433, 524, and 610 nm. The calibration graph was linear over these concentrations. The limits of detection were 2.33, 5.83 and 2.86 µgml^{-1}, respectively. The stoichiometry of the reaction was studied. The method was successfully applied to the determination of colchicine in its pharmaceutical tablets, with high-quality accuracy and precisions.
Keywords: Colchicines, , Kinetic, Oxidation, Permanganate, Spectrophotometer.

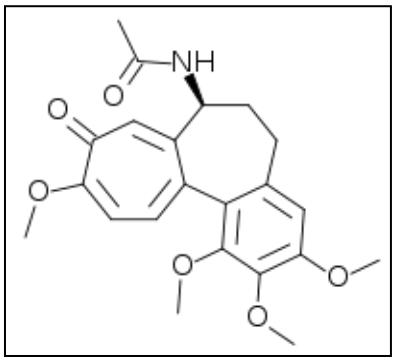
طريقة طيفية لتقدير الكولشيسين في الصورة النقية والمنتجات الدوائية (دراسة حركية التفاعل)

*سعاد خليفةعمر[1] و نورالدين اسويكر يحي[2] و فيروز فتح الله بوجود[3] و إيمان محمد معن[1]
[1] قسم الكيمياء– كلية العلوم ،جامعة عمر المختار–البيضاء،ليبيا
[2] قسم الكيمياء– كلية العلوم–جامعة طبرق، ليبيا
[3] قسم الكيمياء–كلية العلوم–جامعة عمر المختار، ليبيا
*المراسلة: suad.khalifa@omu.edu.ly

الملخص طريقة طيفية بسيطة وسريعة لتقدير الكولشيسين في الصورة النقية وفي المنتجات الدوائية، تعتمد الطريقة على أكسدة الكولشيسين بواسطة برمنجنات البوتاسيوم القاعدية عند درجة حرارة الغرفة. تم تطبيق قانون بيير–لامبرت لدراسة العلاقة بين تراكيز الكولشيسين والامتصاصية تحت الظروف المثلى للتفاعل، حيث تم قياس الامتصاصية عند الأطوال الموجية (λ_{max}) 433 ، 524، 610 نانوميتر لمدى من تراكيز الكولشيسين (4 – 20 ملجم/مل). ومن تم تقدير حدود الكشف (LOD) وكانت 2.33، 5.83، 2.86 ملجم/مل على التوالي. اوضحت الدراسه ان هذه الطريقة ذي كفاءة عالية من الدقة وصحة النتائج المتحصل عليها إذ تم تطبيقها على المنتجات الدوائية الكولشيسين بنجاح.
الكلمات الافتتاحية: الكولشيسين، حركية، اكسدة، برمنجنات، القياس الطيفي.

Introduction

Colchicine is a naturally alkaloid used in human and veterinary medication, which has been used as an antibiotic agent in cancer studies involving cell cultures[1]. It is a phenyl ethyl isoquinoline derived alkaloid, and it is a poisonous, lipid-soluble alkaloid with a unique 7-memberd aromatic tropolone ring [2], the molecular formula $C_{22}H_{25}NO_6$, with IUPAC name N-[(7S)-1, 2, 3, 10-tetramethoxy-9-oxo 5, 6, 7, 9 tetrahydro-benzo[a]heptalen-7-yl] acetamide. Its As shown in **scheme 1**. Colchicine is used for the alleviation of the inflammatory agent and also for decreasing pain and gouts[3]. It is generally classified as an anti-inflammatory agent, and is therefore therapeutic in familial Mediterranean fever, Behcet's disease, pericarditis, coronary artery disease and other inflammatory and fibrotic conditions [4].



Scheme 1: Structure of Colchicine

A review of the literature indicated that numerous techniques have been applied for the analysis of colchicine in biological specimens and/or pharmaceutical formulations, including chromatography [2, 3, 5-16], electrochemistry and voltammetric techniques [1, 17-24]. Potassium

permanganate is commonly used as an oxidizing agent for many organic compounds in various media, and is considered to be the most powerful multi-electron oxidant utilized in kinetic studies in neutral, alkaline, and acid media. The mechanism of oxidation by this eco-friendly oxidant depend not only on the reduction, but also on the reaction medium. During permanganate oxidation, Mn (VII) species in permanganate is reduced to a variety of oxidation states in different media[25]. Only a few spectrophotometric methods have been reported to determine colchicine have been reported in the literature. Singh et. al.(2004), developed a method involves the oxidation of colchicine by Fe(III) chloride and subsequent complexation of iron (II) with 1, 10-phenanthroline forming a red coloured complex having maximum absorbance at 510 nm [26]. Arpna and Patial (2012) introduced a method to analyze colchicine in phosphate buffer saline pH 6.4. The maximum absorbance λmax selected was 353.8 nm[27]. In 2012 Daneshfar and co-workers utilised a method based on the reaction of the colchicine with quaternary ammonium salt in aqueous solution [28]. When Sravya's group tested colchicine for dissolution in water, methanol, ethanol, 0.1M hydrochloric acid solution and 0.1M sodium hydroxide solution, and it scanned in UV range of between 200 and 400 nm, showed maximum aborbance at 246 nm [29]. The present study describes a simple, sensitive and economic analytical method for the estimation of Colchicine spectrophotometrically in bulk and pharmaceutical formulations. The method is based on the oxidation of colchicine by alkaline potassium permanganate as the oxidizing reagent, at room temperature for a fixed time, followed by measuring a decrease in absorbance at 524 nm. In terms of sensitivity, color stability, optimization condition, and accuracy and precision.

Material and Method:

Apparatus: The absorbance was measured on a DU 800 spectrophotometer (Beckman Coulter, USA) with one cm glass cells.

Chemical material and reagent: All chemicals and solvents used were of either pharmaceutical or analytical reagent grade, colchicine was supplied gratis by Omar Al-Mukhtar University, Colchicine ($C_{22}H_{25}NO_6$) Fluka, Biochemical, 97.0% (HPLC), M.wt.:399.45 $gmol^{-1}$, potassium permanganate ($KMnO_4$), BDH Chemicals Ltd Poole England, 99%, M.wt.:158.03 $gmol^{-1}$, potassium hydroxide (KOH), Scharlau Chemie S.A., 85%, M.wt. 56.11 $gmol^{-1}$.

Preparation of solution: All chemicals used were of analytical grade and solutions were made in distilled water.

Potassium permanganate: An accurately weighed quantity of $KMnO_4$ (3.16mg) was transferred into a 200 *ml* volumetric flask, dissolved and diluted up to mark with distillated water.

Potassium hydroxide: A solution was prepared by dissolving the calculated amount of potassium hydroxide into an appropriate volume of water making the solutions always 1M.

Standard stock solution of colchicine: the solutions was prepared by dissolving 0.399 mg of pure colchicine in water in a 100 *ml* measuring flask. Further dilution was preformed to obtain a final concentration of 0.01M solution.

Colchicine in pharmaceutical formulations: Pharmaceutical formulations obtained from a local pharmacy in Al-Beyda city, were used for analysis, Colchicine opocalcium one mg (Mayoly Spindler, France), colchicine opocalcium one mg (Cooper Pharma, Casablanca), Colchicine tablets 500 □g (El Nasr Pharmaceutical chemical, Egypt).

Procedure: Construction of calibration graphs and colchicine standard solution:

A fixed amount of $KMnO_4$ (100 □*l* of 0.1 M), was added to each standard flask to degrade in KOH (1 *ml* of 1M) for 15 min., then a liquots of 4-20 □*g* ml^{-1} of colchicine standard test solution were pipetted into these series of standard 10 *ml* volumetric flasks, and then diluted to 10 *ml* volume with distilled water at room temperature. After mixing, the solution was tacked for the optimum time of 30min. for complete reaction and stability of color. It was then transferred to a spectrophotometric cell, the decrease in absorbance was recorded as a function of time, at 524 nm, and the absorbance increase of the colored manganate ions was measured at 610 nm. Calibration graphs were prepared by plotting the absorbance values versus concentration of colchicine. The unknown concentration was read from the respective calibration graph and deduced from the regression equation using Beer's law data.

Procedure for pharmaceutical form: An average weight tablet was dissolved in water and the volume was filled to 50 *ml* with distilled water. The solution was shaken well and filtered as necessary. The procedure was completed following the described procedure in standard solution.

Kinetic Measurements: Kinetic runs were carried out under pseudo-second order conditions where [colchicine] < MnO_4^-, at room temperature. The process of the reaction in basic media was followed by observing the decrease in the absorbance of permanganate ion, as a function of time, at λ = 433 nm. These measurements were performed on a DU 800 one-beam spectrophotometer. The observed-second order rate constants (k_{obs}) were calculated as the gradients of ln(absorbance) time plots, which were straight for about 80-95% of the reactions completion. The orders of the oxidation reaction with respect to the reactant concentration were determined from the plots of ln (rate) versus ln (Conc.) by varying the concentrations of colchicine while keeping all others constant.

Results:

Time-Resolved Spectra:

Sets of the concentration of potassium permanganate in one *ml* of hydroxide ion (1M) were kept for 15 min. at room temperature in a 10 *ml* measuring flask. The absorbance spectrum of aqueous potassium permanganate solution in alkaline media exhibited absorption bands at 524, 535, and 307 nm as shown in **figure 1** and the

average molar absorptivity (ε) of standard permanganate in alkaline medium is estimated to value 9.23×10^{-8} $mol.l^{-1}cm^{-1}$ at 524 nm. The addition of the studied drugs to this solution produced new characteristic bands at 344, 433, and 610 nm in 1M potassium hydroxide solution as shown in **figure 2**. The band at 610 nm is due to formation of manganate ion MnO_4^{-2}, while at 433 and 344 nm absorbance at crest or through spectrum in alkaline medium is due to formation of an oxidized form of colchicine. The absorbance at bands 307, 344, 433 and 610 nm increases with time, while at 524 nm the absorbance decreases where the actual concentration of alkaline permanganate after oxidation with colchicine is estimated with reference to molar absorptivity of standard permanganate.

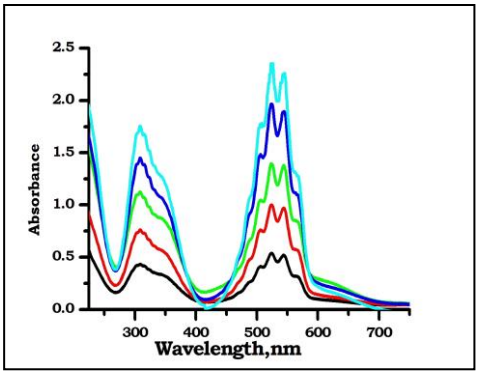


Fig.1: Absorption spectra of standard permanganate ion solution in alkaline medium

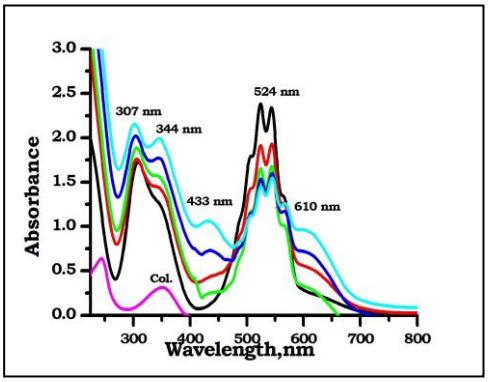


Fig. 2: Absorption spectra of Colchicine in the presence of one mM $KMnO_4$ and 100 mM KOH

Stoichiometry and reaction mechanism:

The molar ratio between permanganate and colchicine to complete the oxidation reaction is determined by the Slope Ratio method. In this method, two series of solution are prepared. In the first series, various amounts of colchicine are added to a large excess of permanganate solution, while in the second series different quantities of permanganate solution are added to a large excess of Colchicine. The absorbance of the solution in each series is measured at 433 nm and plotted versus the concentration of the variable component. The ratio of the slopes of the two straight lines, the molar absorptivities, determines the molar ratio of the two components. The slope ratio method was applied to establish the stoichiometry molar ratio of colchicine to $KMnO_4$ in basic medium and was found to be nearly 4:1 (COLC:$KMnO_4$) as given in **table 1** and shown in **figure 3**.

Table 1: Calculation of Molar ratio of Colchicine to permanganate ion by the slope ratio method.

ε_{433} Colchicine	1.224×10^{-7}
ε_{433} $KMnO_4$	2.994×10^{-8}
Molar Ratio (COLC: $KMnO_4$)	4.09

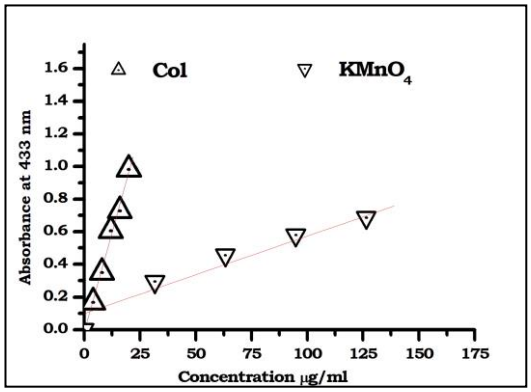
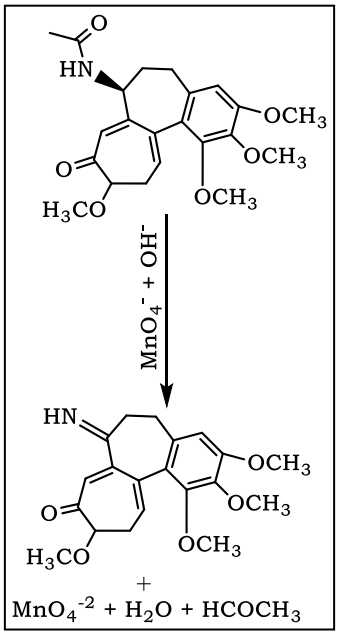


Fig. 3: Slope ratio method graphs for the Colchicine-$KMnO_4$ reaction in alkaline medium

Colchicine was found to be susceptible to oxidation with alkaline $KMnO_4$producing a green color peaking at 610 nm, then dark brown precipitate of MnO_2 after the complete consumption of permanganate ion. Therefore, the reaction is proposed as shown in **scheme 2**. The amine group in colchicine converted to primary ketimine group by manganate ion, Mn(VI), precedes the oxidation step forming acetone and colchicine derivative. Therefore, this reaction mechanism is proposed on the basis of the literature background [21, 22]. It has been established that, in a strongly alkaline medium, the stable reduction product of permanganate ion is managanate ion MnO_4^{-2} [30, 31].

$$MnO_4^- + 4\ OH^- \rightarrow MnO_4^{-2} + 2H_2O + O_2$$



Scheme 2: Represents the reaction Colchicine and alkaline permanganate ion.

Effect of permanganate concentration:

The absorbance increases significantly with an increase in the concentration of potassium permanganate as shown in **figure 4**. Maximum absorbance was obtained when 100*µl* of 0.1 M of alkaline potassium permanganate was used in a 10 *ml* measuring flask equivalent to one mM final concentration containing 4-20 *µgml*$^{-1}$ of colchicine.

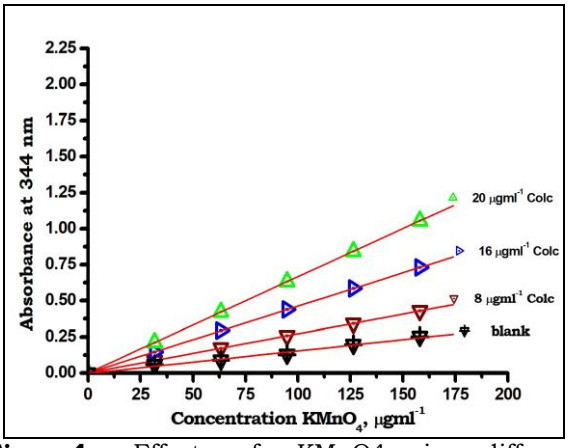


Fig. 4: Effect of KMnO4 in different concentrations of Colchicine

Effect of alkali concentration:

Maximum absorption was obtained when one *ml* of 1M of KOH was used. Over this volume no change in absorbance could be detected.

Validation of the proposed method:

Calibration curve was constructed covering the concentration range 4-20 *µg ml*$^{-1}$ for colchicine in alkaline medium as shown in **figure 5**.

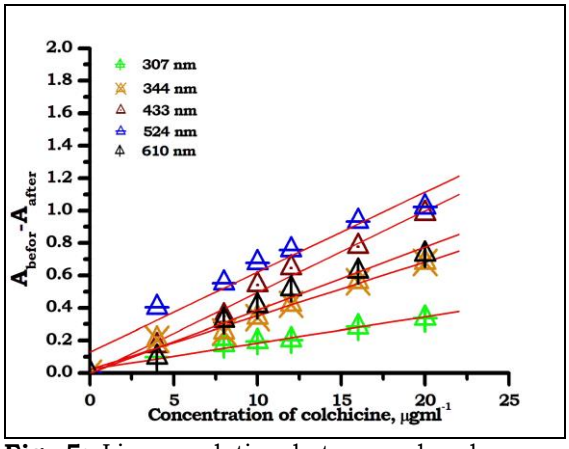


Fig. 5: Linear relation between absorbance and Concentration of Colchicine

The limit of detection (LOD) was calculated based on the standard deviation of correlation coefficient $S_{y/x}$ and the slope of the calibration curve. The limit of detection in µg/ml was expressed [32] as: LOD = 3 $S_{y/x}$ /Slope. whilest, The limit of quantitation (LOQ) was calculated based on the standard deviation of intercept and slope of the calibration curve. In this method, the limit of quantitation in *µg ml*$^{-1}$ is expressed [32] as: LOQ = 10 $S_{y/x}$/slope. Both, LOD and LOQ results summarized in **table 3** and indicating good sensitivity of the proposed method.

Table 2: Regression and Analytical parameters of Colchicine in alkaline permanganate.

Parameter	Wavelength, nm				
	307	344	433	524	610
Molar absorptivity *mol. l*$^{-1}$ *cm*$^{-1}$	1.36 ×10^{-6}	8.07 ×10^{-8}	1.21 ×10^{-7}	1.23 ×10^{-7}	9.50 ×10^{-8}
Con., range mM	0.01-0.05				
gml^{-1}	4-20				
Regression equation, Y*					
Intercept (a)	0.022	0.044	-0.018	0.114	–0.009
Slope (b)	0.016	0.032	0.048	0.049	0.038
Correlation coefficient					
R	0.99	0.99	0.99	0.97	0.99
$S_{y/x}$	0.017	0.035	0.034	0.087	0.032
LOD, *µg ml*$^{-1}$	3.53	3.59	2.33	5.83	2.86
LOQ, *µg ml*$^{-1}$	10.70	10.89	7.06	17.69	8.68

*Y = a ± b X, where Y is the absorbance and X, concentration in *µg ml*$^{-1}$

Kinetics of the reaction:

Under the optimum, the absorbance time curve of investigating colchicine with alkaline $KMnO_4$ reagent was constructed as shown in **figure 6**. The initial rate of the reaction was determined from the slope of the tangents of the absorbance time curve. The order of the reaction with respect to colchicine was determined by studying the variation of time with reaction absorbance at different concentrations of colchicine using relative excess amount of potassium permanganate and potassium hydroxide (pseudo order reaction). The graphs obtained, depicted in **figure 7,** clearly indicate that reaction rate obeys the following general equation:

$$\text{Rate of Reaction} = \frac{\Delta A}{\Delta t} = k\, C^n$$

Where A is the absorbance at 433 nm, t is the measureing time, k is the pseudo-order rate constant, C is the molar concentration of colchicine and n is the order of the reaction, the natural logarithmic form of the above equation is written as follows:

$$\ln \text{ rate} = \ln \left(\frac{\Delta A}{\Delta t}\right) = \ln k + n \ln C$$

Plotting the natural logarithm of initial rate of the reaction versus the natural logarithm of the molar concentration of investigating colchicine was found to be second order (n >1) in alkaline potassium permanganate.

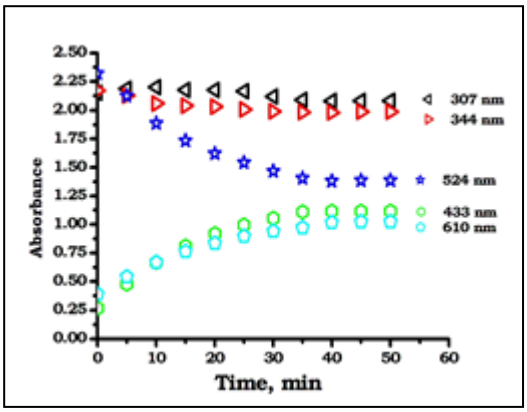


Fig. 6: Absorbance versus time graph for the reaction between one mM alkaline permanganate and 20 µg ml^{-1}Colchicine.

Linear regression of ln (Rate) versus ln C least square method yielded the following calibration equation:

$$\ln (Rate) = 0.571 - 1.803 \ln C$$

With correlation coefficient R = 0.996, k = 0.28 $M^{-1}s^{-1}$. Therefore, pseudo-second order condition was obtained with respect to concentration of colchicine in excess amounts of alkaline $KMnO_4$ as shown in **figure 7**.

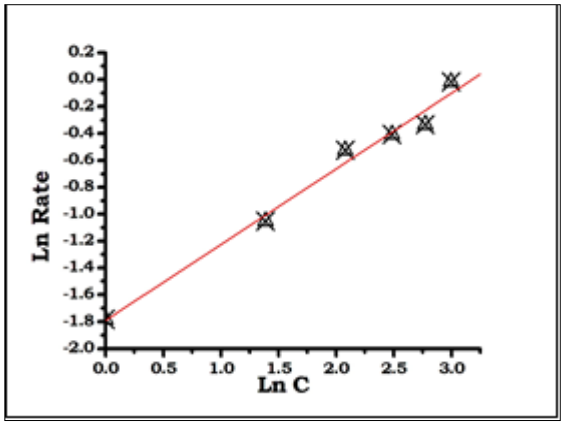


Fig. 7: plot ln (Rate) of the reaction for 4-20 µgml^{-1} Colchicine in alkaline potassium permanganate at room temperature.

Application of pharmaceutical dosage forms

The fixed time method of the proposed spectrophotometric method for determination of investigating colchicine has been tested on commercial pharmaceutical dosage forms. The concentration of determination of colchicine was computed from its responding regression equations, with respect to accuracy and precision as shown in **table 3**, to ensurethat there was no interference of other additives present in the studied formulations.

Table 3: Spectrophotometric determination of the studied compound in pharmaceutical preparation by the proposed method.

Tablet	Content Label Claim (mg)	[a]Recovery Mean%	[b]SD%	[c]RSD %
Colchicine, (Egypt)	0.5	91.36	12.2	13.36
Colchicine, (France)	1	97.48	3.5	3.6
Colchicine, (Casablanca)	1	95.91	5.7	6.02

[a]Average of triplicate measurements; [b] Standard Deviation and [c] Relative Standard Deviation

Discussion:

Through the reaction the valence of manganese changes and intermediate ions have been suggested as participating oxidants, but which species have the main role as potential oxidants depends upon the nature of the substrate and the pH of the medium [33] Oxidation reactions are very important in organic synthesis and,among the different oxidizing agents, permanganate ion is used in the oxidation of many organic compounds in neutral, alkaline and acidic media. The mechanism of oxidation reactions by permanganate ion is managed by pH of the medium. During oxidation by permanganate, it is evident that the Mn (VII) in permanganate is reduced to different oxidation states in acid, alkaline and neutral media. In an acid media, permanganate ion (MnO_4^-) can exist in a number of different forms, $HMnO_4$, $H_2MnO_4^+$, $HMnO_3$, and Mn_2O_7 depending on the nature of the reductant [34]. The oxidation of organic substrate by potassium permanganate depends on the pH of the medium. The hepta-valent manganese changes to Mn (VI) in alkaline medium while in neutral and acidic media the permanganate is further reduced ultimately formingMn(II) [31]. In a strongly alkaline medium, the stable reduction product of permanganate is manganate ion, MnO_4^{2-}. MnO_2 appears only after a long time, i.e. after the complete consumption of MnO_4^- [35].

Conclusion

The initial rate and fixed time methods can be easily applied for investigating colchicine in pure and dosage forms. The proposed, fixed time method is sensitive enough to enable determination of lower amounts of the drug, which makes its application in routine quality control advantageous. Finally, this method is useful for improving selectivity, and avoiding the interference of colored and/or turbidity background of samples because it measures the increase in absorbencies with time against blank treated similarly.

References

[1]- Wen, W., Tan, Yumei,Xiong, Huayu,Wang, Shengfu,, Voltammetric and spectroscopic investigations of the interaction between colchicine and bovine serum albumin. Int. J. Electrochem. Sci, 2010. **5**: p. 232-241.

[2]- Gowda, B. G., High-Performance liquid chromatographic determination of colchicine in pharmaceutical formulations and biological fluids. Int. J Pharm Pharm Sci, 2014. **6**: p. 335-337.

[3]- Chandrethiya, G. D., P.K. Shelat, and M.N. Zaveri, RP-HPLC method development and validation for estimation of colchicine in gelatin nanoparticles formulation. Int J Pharm Pharm Sci, 2013. **5**(1): p. 228-231.

[4]- Leung, Y. Y., L. L. Y. Hui, and V. B. Kraus. Colchicine—Update on mechanisms of action

and therapeutic uses. in Seminars in arthritis and rheumatism. 2015. Elsevier.

[5]- Sarg T. M, El-Domiaty M. M, Bishr M. M, Salama O. M, El-DomiatyGindy AR. Thin-layer chromatographic scanner, spectrophotometric and high-performance liquid chromatographic methods for the determination of colchicine. Analyst. 1989;114(5):575-8.

[6]- Tracqui A, Kintz P, Ludes B, Rouge C, Douibi H, Mangin P. High-performance liquid chromatography coupled to ion spray mass spectrometry for the determination of colchicine at ppb levels in human biofluids. Journal of Chromatography B: Biomedical Sciences and Applications. 1996 Jan 26;675(2):235-42.

[7]- Alali, F., K. Tawaha, and R. M. Qasaymeh, Determination of colchicine in Colchicum steveni and C. hierosolymitanum (Colchicaceae): Comparison between two analytical methods. Phytochemical Analysis: An International Journal of Plant Chemical and Biochemical Techniques, 2004. **15**(1): p. 27-29.

[8]- Bodoki E, Oprean R, Vlase L, Tamas M, Sandulescu R. Fast determination of colchicine by TLC-densitometry from pharmaceuticals and vegetal extracts. Journal of pharmaceutical and biomedical analysis. 2005 Apr 29;37(5):971-7.

[9]- Hamscher G, Priess B, Nau H, Panariti E. Determination of colchicine residues in sheep serum and milk using high-performance liquid chromatography combined with electrospray ionization ion trap tandem mass spectrometry. Analytical chemistry. 2005 Apr 15;77(8):2421-5.

[10]- Nikhil S, Praveen P, Darshan D, Kamlesh D. A validated HPTLC method for determination of colchicine in Gloriosa superb collected from different regions of India. Journal of Pharmacy Research. 2012;5(7):3858-9.

[11]- Nirmala, K. and R. R. Raju, A novel method development for validation and detection of colchicine drug by RP-HPLC. Rasayan J Chem, 2012. 5: p. 106-11.

[12]- Maslarska, V. and I. Pencheva, Determination of colchicine content in drug by RF-HPLC. Int. J. Adv. Pharm. Bio. Chem., 2014. 3: p. 116-119.

[13]- Turk, W. and S. Olimat, Determination of Colchicine Contents in Different Jordanian Colchicum spp. Jordan Journal of Pharmaceutical Sciences, 2015. 8(2).

[14]- Abdulbaqi I. M, Darwis Y. U., Khan NA, Assi R. A., Loh G. O. A simple (HPLC–UV) method for the quantification of colchicine in bulk and ethosomal gel nano-formulation and its validation. Int J Pharm Pharm Sci. 2017;9(7):72-8.

[15]- Joshi S. A, Jalalpure S. S., Kempwade A. A., Peram M. R., Development and validation of HPLC method to determine colchicine in pharmaceutical formulations and its application for analysis of solid lipid nanoparticles. Current Pharmaceutical Analysis. 2018 Jan 1;14(1):76-83.

[16]- Rajasree, B. and M. A., Mukthinuthalapati, Analytical Methods for the Determination of Anti-Gout Drugs. Acta Scientific Pharmaceutical Sciences, 2019. 3(4).

[17]- Aboul Kasim, E., Voltammetric behavior of the anti-inflammatory alkaloid colchicine at a glassy carbon electrode and a hanging mercury electrode and its determination at ppb levels. Analytical letters, 2002. 35(12): p. 1987-2004.

[18]- Zhang, H., Electrochemistry and voltammetric determination of colchicine using an acetylene black-dihexadecyl hydrogen phosphate composite film modified glassy carbon electrode. Bioelectrochemistry, 2006. 68(2): p. 197-201.

[19]- Bodoki, E., R. Săndulescu, and L. Roman, Method validation in quantitative electrochemical analysis of colchicine using glassy carbon electrode. Open Chemistry, 2007. 5(3): p. 766-778.

[20]- Bodoki E, Laschi S, Palchetti I, Săndulescu R, Mascini M. Electrochemical behavior of colchicine using graphite-based screen-printed electrodes. Talanta. 2008 Jul 15;76(2):288-94.

[21]- Wang F, Zhou J., Liu Y., Wu S., Song G., Ye B. Electrochemical oxidation behavior of colchicine on a graphene oxide-Nafion composite film modified glassy carbon electrode. Analyst. 2011;136(19):3943-9.

[22]- 22. Bodoki, E., L., Vlase, and R. Săndulescu, Mechanistic study of colchicine's reduction behavior. Electrochemistry Communications, 2015. 56: p. 51-55.

[23]- Stanković D. M., Švorc Ľ, Mariano JF, Ortner A, Kalcher K. Electrochemical determination of natural drug colchicine in pharmaceuticals and human serum sample and its interaction with DNA. Electroanalysis. 2017 Oct;29(10):2276-81.

[24]- Moreira D. A., Oliveira F. M., Pimentel D. M., Guedes T. J., Luz R, Damos F. S., Pereira AC, da Silva RA, dos Santos WT. Determination of colchicine in pharmaceutical formulations and urine by multiple-pulse amperometric detection in an FIA system using boron-doped diamond electrode. Journal of the Brazilian Chemical Society. 2018 Sep;29(9):1796-802.

[25]- Fawzy A, El Guesmi N, Ali H. M., Abdallah M. Oxidation of Tryptophan by Permanganate Ion in Acid, Neutral and Alkaline Media: A Comparative Kinetic and Mechanistic Study. 2018.

[26]- Singh, D., B., Srivastava, and A. Sahu U., Spectrophotometric determination of colchicine using iron (III) chloride and 1, 10-phenanthroline. Journal of the Indian Chemical Society, 2004. 81(2): p. 171-173.

[27]- Patial, A. and P. Verma, Development of UV spectrophotometric method for estimation of colchicine in phosphate buffer saline pH 6.4. Int Res J Pharm, 2012. 3: p. 87-9.

[28]- Daneshfar, A., F. Ansari, and S. Hasanvandi, Spectrophotometric determination of colchicine by phase transfer catalyst. Oriental Journal of Chemistry, 2012. 28(1): p. 89.

[29]- Sravya A., S. P. A., Abdul Sadaf, Ravi Teja Reddy A., Sireesha D., Vaudha Bakshi, Development and validation of UV spectrophotometric methodfor estimation of colchicine in bulkand pharmaceutical dosage forms. World Journal of Pharmaceutical Research, 2019. 8(3): p. 933-938.

[30]- Fawzy, A., S. Ashour, and M. Musleh, Kinetics and Mechanism of Oxidation of l-Histidine by Permanganate Ions in Sulfuric Acid Medium. International Journal of Chemical Kinetics, 2014. 46(7): p. 370-381.

[31]- Jain, A., G. Tazwar, and V. Devra, Kinetics and mechanism of permanganate oxidation of nalidixic acid in aqueous alkaline medium. Journal of Applied Pharmaceutical Science, 2017. 7(01): p. 135-143.

[32]- Miller, J.C. and J.N. Miller, Basic statistical methods for analytical chemistry. Part I. Statistics of repeated measurements. A review. Analyst, 1988. 113(9): p. 1351-1356.

[33]- Rahman, N., N. Khan, and S. Azmi, Kinetic spectrophotometric method for the determination of silymarin in pharmaceutical formulations using potassium permanganate as oxidant. Die Pharmazie-An International Journal of Pharmaceutical Sciences, 2004. 59(2): p. 112-116.

[34]- Fawzy, A., Ishaq A. Zaafarany, Fahd A. Tirkistani, Basim H. Asghar., Oxidative Degradation of Atropine Drug by Permanganate Ion in Perchloric and Sulfuric Acid Solutions: A Comparative Kinetic Study. Advances in Biochemistry, 2016. 4(5): p. 58-65.

[35]- Simandi L. I., Jaky M, Savage C. R, Schelly Z. A. Kinetics and mechanism of the permanganate ion oxidation of sulfite in alkaline solutions. The nature of short-lived intermediates. Journal of the American Chemical Society. 1985 Jul;107(14):4220-4.