Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2014, 6(7): 2752-2758

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

New kinetic-spectrophotometric method for microgram determination of doxycycline hyclate in aqueous formulations, tablets and capsules

R. D. Kaushik[1]*, Payal Rathi[1], Manila[1], Jaspal Singh[1] and Rajendra Yadav[2]

[1]Department of Chemistry, Gurukul Kangri University, Haridwar(U.K.), India
[2] Department of Pharmaceutical Sciences, Gurukul Kangri University, Haridwar (U.K.), India

---

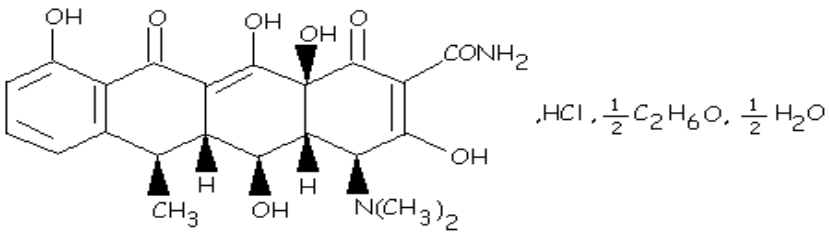
ABSTRACT

The Mn^{II} catalysed oxidation of Doxycycline Hyclate(DCH) by periodate ion in aqueous medium was followed by monitoring the increase in the absorbance of reaction intermediate and made the basis for developing newer method for microgram estimation of DCH. The reaction is first order with respect to substrate and oxidant each. The best fit conditions developed for a new and simple kinetic-spectrophotometric method for microgram determination of DCH in the range 51.30 µg/mL – 615.54 µg/mL are, $[NaIO_4]$ = 1.0 × 10^{-5} mol dm^{-3}, $[Mn^{II}]$ = 1.0 × 10^{-3} mol dm^{-3}, Temperature = 35.0 ± 0.1°C, pH = 5.0 and λmax = 486 nm. The characteristics of various calibration curves, percentage recovery, effect of excipients, correlation coefficient and comparison with other reported methods are presented.

Keywords: Kinetic-spectrophotometric method, Microgram Estimation, Doxycycline Hyclate, Periodate ion, Aqueous formulations, capsules, Tablets.

INTRODUCTION

Doxycycline hyclate(DCH) is 4*S*,4a*R*,5*S*,5a*R*,6*R*,12a*S*)-4-dimethylamino-1,4,4a,5,5a,6,11,12a-octa-hydro-3,5,10,12,12a-penta hydroxy-6-methyl-1,11-dioxonaphthacene-2-carboxamide hydrochloride hemi-ethanoate hemihydrate with molecular formula $C_{22}H_{24}N_2O_8$,HCl, ½ C_2H_6O), ½ H_2O, molecular weight 512.95 and structure given below [1]. It is a synthetic analogue of oxytetracycline which provides certain pharmacokinetic advantages over tetracycline being more active against some organisms. It is well known for its antibacterial action today and as a choice for pelvic inflammatory diseases, renal impairment [2] and prophylaxis of travellers' diarrhea [3-6].



Doxycycline Hyclate

Not many methods are available for estimation of doxycycline. Doxycycline is estimated in raw form, tablets, capsules and injections by employing microbial assays and HPLC [7-23]. No periodate oxidation based kinetic-spectrophotometric method has been reported so far for its estimation.

Substrates having amino group, like aromatic amines, are already reported to get oxidized by periodate ion [24-36]. Therefore, the presence of amino group in DCH makes it susceptible for attack by periodate. We have already explored this reaction and have reported the kinetics of periodate oxidation of DCH in aqueous medium [37]. Keeping in view the time consuming pretreatments, involvement of high cost and requirement of costly equipments for the estimation of doxycycline by the reported methods [7-23], and in continuation to our report on the kinetic study of periodate oxidation of DCH, it was considered necessary to utilize the results of kinetics of periodate oxidation of doxycycline hyclate, to arrive at the optimum conditions required for its estimation in aqueous medium in micrograms. However, Mn^{II} was employed as a catalyst for improving the rate of reaction. As a result, this communication proposes a newer, simpler and cost effective kinetic-spectrphotometric method for the estimation of this drug in aqueous formulations, capsules and tablets.

EXPERIMENTAL SECTION

Reagents

Sodium metaperiodate (CDH, AnalaR grade), DCH of Ranbaxy make was used in pure form. To avoid the photooxidation the drug solutions were kept in dark bottles and fresh solutions were prepared as soon as they started getting coloured. Manganese sulphate monohydrate and all other chemicals of (CDH AnalaR) analytical reagent/ guaranteed reagent grade were used after redistillation/ recrystallization. Triply distilled water was used for preparation of the solutions. The pH of the reaction system was measured on Systronics digital pH meter. The pH of reaction mixtures was maintained by using Thiel, Schultz and Coch buffer [38] (in which different volumes of 0.05 M oxalic acid + 0.02 M boric acid + 0.05 M succinic acid + 0.05 M sodium sulfate + 0.05 M borax were used to maintain the pH values of reaction mixtures).

Recommended Kinetic-spectrophotometric procedure

Reaction between doxycycline hyclate (DCH) and periodate ion resulted in the development of dark brown colour which remained unchanged for 72 hours. The reaction was studied in a spectrophotometric cell and initiated by adding temperature equilibrated $NaIO_4$ solution of known concentration to the reaction mixture containing the DCH, and buffer and maintained at 35 ± 0.1^0C. The progress of the reaction was followed by recording the absorbance on Shimadzu double beam spectrophotometer (UV-Pharmaspec-1700), at 486 nm, i.e., the λ_{max} of the reaction mixture. λ_{max} was not found to change with change in time under experimental conditions (Fig.1). Desired temperature was maintained with the help of a high precision thermostatic control.

To improve the reaction rate the Mn^{II} was used as catalyst for the reaction under consideration. Following are the finally worked out conditions for running the kinetic sets: [DCH] = 51.30-615.54 µg/mL, $[NaIO_4] = 1.0 \times 10^{-5}$ mol dm^{-3}, $[Mn^{II}] = 1.0\times10^{-3}$ mol dm^{-3}, Temperature = 35.0 ± 0.1°C, pH = 5.0 and λmax = 486 nm.

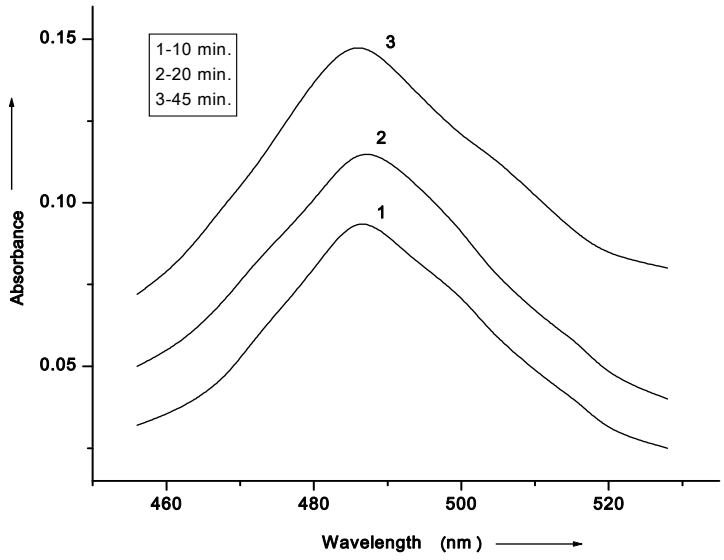


Fig. 1. UV-VIS Scan at different time for for periodate oxidation of Doxycycline Hyclate

A definite volume of stock solution of DCH in water was mixed with calculated volume of the stock solution of buffer and stirred a little with the help of the pipette. This mixture and stock solution of $NaIO_4$ were then clamped in a thermostat at 35 ± 0.1^oC. After 25 minutes, a required amount of the periodate solution was added to the mixture

and stirred to start the reaction. All additions were made in amounts calculated for maintaining the concentrations of different reagents as mentioned above. Different sets were prepared in a similar manner varying the [DCH]. Aliquots were withdrawn from the reaction mixture after repeated intervals of 30 seconds and the absorbance was recorded on a double beam spectrophotometer. The absorbance vs time plots were then made for different sets. The initial rates $[(dA/dt)_{30}]$ were evaluated after 30 seconds from the start of the reaction by applying plane mirror method on the absorbance vs time plots. The pseudo first order rate constants (k_1) were found by Guggenheim's method.

Using the method of least squares, linear calibration curves were obtained in terms of type 'A', type 'B', type 'C', type 'D', type 'E', type 'F' , type 'G', type 'H', type 'I'and type 'J' plot i.e. A_{30} or A_{60} or A_{90} or A_{120} or A_{150} or A_{180} or A_{210} or A_{240} or initial rate or k_1 vs [DCH] plots respectively (where A_{30}, A_{60}, A_{90}, A_{120}, A_{150}, A_{180}, A_{210} and A_{240} are the absorbance values after 30, 60, 90, 120, 150, 180, 210 and 240 seconds from the start of reaction respectively) (Fig. 2, 3).

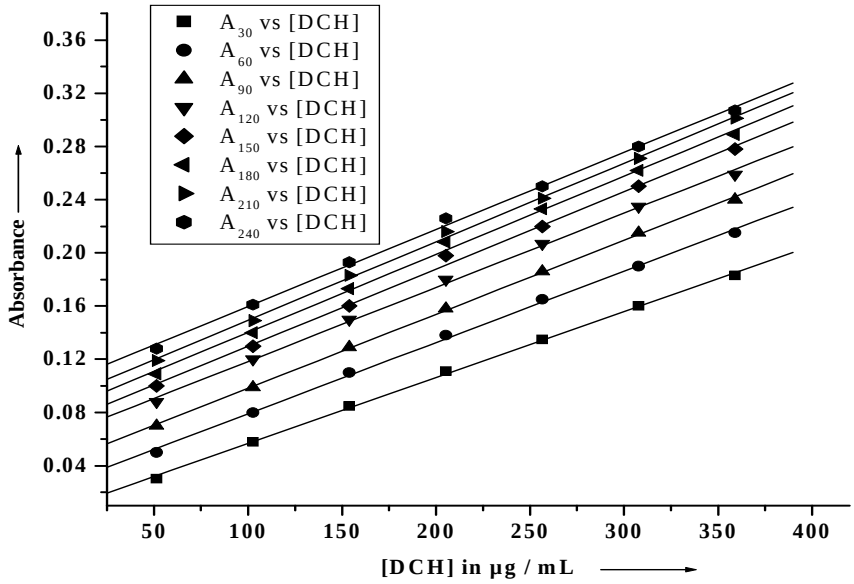


Fig. 2: Calibration curves in terms of absorbance vs [DCH] plots at $[NaIO_4] \times 10^5$ = 1.0 mol dm^{-3}, $[Mn^{II}] \times 10^3$ = 1.0 mol dm^{-3}, Temp. = 35 ± 0.1°C, pH = 5.0, λ_{max} = 486 nm

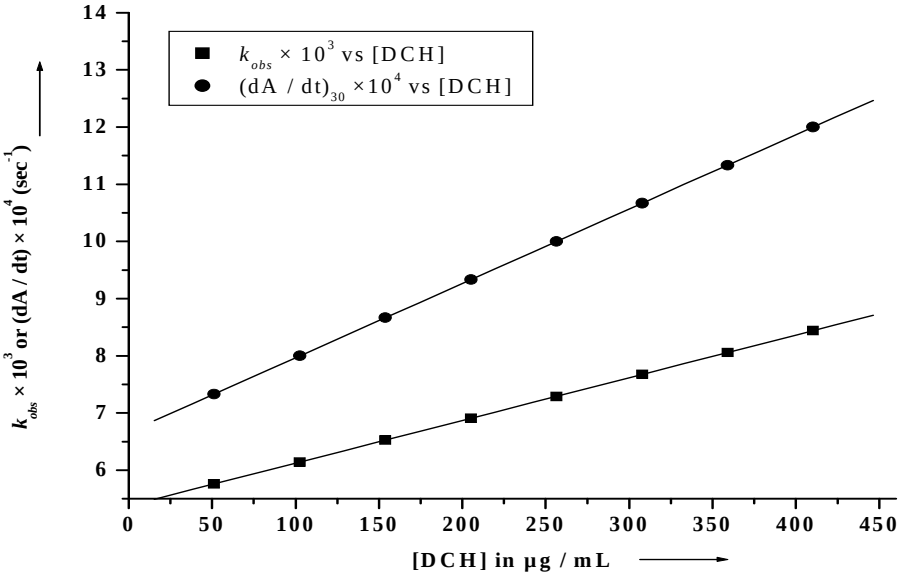


Fig. 3: Calibration curves in terms of initial rate or pseudo first order rate constant vs [MDP] plots at at $[NaIO_4] \times 10^5$ = 1.0 mol dm^{-3}, $[Mn^{II}] \times 10^3$ = 1.0 mol dm^{-3}, Temp. = 35 ± 0.1°C, pH = 5.0, λ_{max} = 486 nm

RESULTS

Validity of Beer's law and other characteristics of the method

The range of [DCH] in which the Beer's law is obeyed, Sandell's sensitivity, correlation coefficient and the coefficient of determination, LOD, LOQ, value of 't' (at 0.01 significance level), relative standard deviation and % error for various calibration curves are given in Table 1 and 2. The characteristics of calibration curves were evaluated in the form of equations of straight line as follows:

$A_{30} = 9.4242 \times 10^{-3} + 4.8186 \times 10^{-4}$ [DCH] (1)

$A_{60} = 29.5152 \times 10^{-3} + 5.1233 \times 10^{-4}$ [DCH] (2)

$A_{90} = 47.3485 \times 10^{-3} + 5.2882 \times 10^{-4}$ [DCH] (3)

$A_{120} = 65.5909 \times 10^{-3} + 5.4034 \times 10^{-4}$ [DCH] (4)

$A_{150} = 78.1515 \times 10^{-3} + 5.4566 \times 10^{-4}$ [DCH] (5)

$A_{180} = 89.0606 \times 10^{-3} + 5.4593 \times 10^{-4}$ [DCH] (6)

$A_{210} = 98.0909 \times 10^{-3} + 5.4709 \times 10^{-4}$ [DCH] (7)

$A_{240} = 107.5758 \times 10^{-3} + 5.4763 \times 10^{-4}$ [DCH] (8)

$(dA/dt)_{30} = 66.666 \times 10^{-5} + 1.30 \times 10^{-6}$ [DCH] (9)

$k_1 = 5.3736 \times 10^{-3} + 7.48 \times 10^{-6}$ [DCH] (10)

In Eq. (1) to (8), the slopes and intercept are in absorbance units. μg^{-1}. cm^3 and absorbance units respectively. For Eq. (9) and (10), the values of slope and intercepts are in absorbance units. μg^{-1}. cm^3. sec^{-1} and absorbance units. sec^{-1} respectively. The [DCH] are in μg/ml.

Table 1: Characteristics of different Calibration curves for estimation of DCH

Parameter	'A' plot	'B' plot	'C' plot	'D' plot	'E' plot
Beer's law limits (μg/ml)	51.30-615.54	51.30-615.54	51.30-615.54	51.30-615.54	51.30-615.54
Sandell's sensitivity (μg. cm^{-2})	2.1	2.0	1.9	1.9	1.8
Slope x 10^4 absorbance units. $\mu g^{-1}.cm^3$ (from regression equation)	4.82	5.12	5.29	5.40	5.46
Intercept x 10^3 (abs. units)(from regression equation)	9.42	29.52	47.35	65.59	78.15
Limit of Detection	5.92	4.76	3.23	5.89	5.39
Limit of Quantification	17.9	14.4	9.78	17.8	16.3
Correlation coefficient (r)	0.9997	0.9995	0.9995	0.9996	0.9990
Coefficient of determination (r^2)	0.9994	0.9990	0.9990	0.9992	0.9980
't' (at 0.01 significance level)	6.6096	7.3204	7.9181	8.5153	8.9188
Relative Standard deviation (%) (aqueous formulations)	1.8759×10^{-4}	4.1947×10^{-4}	3.5095×10^{-4}	2.6529×10^{-4}	4.3994×10^{-4}
Recovery (%)	99.79	99.47	99.61	99.72	99.53
Relative Standard deviation (%) (DOXYTAS Tablets)	1.6461×10^{-3}	1.9675×10^{-3}	2.0635×10^{-3}	2.7185×10^{-3}	2.9868×10^{-3}
Recovery (%)	96.88	96.84	97.05	96.45	96.33
Relative Standard deviation (%) (CODOX Capsules)	1.3721×10^{-3}	1.5583×10^{-3}	1.7497×10^{-3}	3.0480×10^{-3}	2.8910×10^{-3}
Recovery (%)	97.40	97.54	97.52	96.03	96.47

Effect of interferring ions

The method may be used in presence of the ions like Na^+, K^+, NO_2^-, ClO_4^{-2}, NO_3^-, and SO_4^{-2} as they do not interfere significantly in present case. It was observed that upto 100 μg/ml of Cl^-, PO_4^{-3}, SCN^-, NO_3^-, CO_3^-, CH_3COO^-, Ca^{++}, Mg^{++}, Na^+, K^+ and Zn^{++} do not interfere. Upto 50 μg/ml of Cu^{++} and Fe^{++} do not interfere.

However, the metals like Ag, As, B, Co, Cd, Cr, Hg, Mo, Ni, Pb, Sb, Se, and U are expected to interfere in this method. Cu^{++} and Fe^{++} interfere if present in amounts greater than 50 μg/ml. Therefore, a pretreatment is required for separating/ precipitating/ masking these ions before undertaking the proposed method. For this purpose, H_2S may be passed in presence of 0.3 M H^+ solution, followed by filtration and boiling off H_2S. After it, a dilute alkaline solution of α-nitroso-β-naphthol should be added and again the solution should be filtered [39]. Thereafter, the solution should be neutralized and the present method be applied. Fe may be removed by precipitation using basic

formate method [40-41]. In absence of the above given interferrants, the proposed method may successfully be used for the determination of microgram quantities of DCH in aqueous medium.

Table 2: Characteristics of different Calibration curves for estimation of DCH

Parameter	'F' plot	'G' plot	'H' plot	'I' Plot	'J' plot
Beer's law limits (µg/ml)	51.30-615.54	51.30-615.54	51.30-615.54	51.30-615.54	51.30-615.54
Sandell's sensitivity(µg. cm^{-2})	1.8	1.8	1.8	---	---
Slope x 10^4 absorbance units. μg^{-1}. cm^3 (from regression equation)	5.46	5.47	5.48	1.30×10^{-2} s^{-1}	7.48×10^{-2} s^{-1}
Intercept x 10^3 (abs. units)(from regression equation)	89.06	98.09	107.58	66.67×10^{-2}	5.37
Limit of Detection	3.83	4.24	5.78	3.50	3.64
Limit of Quantification	11.6	12.8	17.5	10.6	11.03
Correlation coefficient (r)	0.9988	0.9989	0.9992	0.9999	0.9999
Coefficient of determination (r^2)	0.9976	0.9978	0.9984	0.9999	0.9999
't' (at 0.01 significance level)	9.2895	9.5929	9.9141	15.8482	19.657
Relative Standard deviation (%) (Aqueous formulations)	4.5950×10^{-4}	5.1374×10^{-4}	5.6277×10^{-4}	0.1008	0.1018
Recovery (%)	99.49	99.45	99.40	97.34	96.67
Relative Standard deviation (%) (DOXYTAS Tablets)	2.7793×10^{-3}	2.6761×10^{-3}	2.1348×10^{-3}	0.1008	0.1139
Recovery (%)	96.76	96.99	97.68	97.34	95.83
Relative Standard deviation (%) (CODOX Capsules)	3.2247×10^{-3}	2.7982×10^{-3}	2.9152×10^{-3}	9.0200×10^{-2}	0.1018
Recovery (%)	96.25	96.86	96.85	97.88	96.67

Effect of excipients

In order to examine the applicability of the proposed method to pharmaceutical analysis, the effect of various excipients was studied. It was found that glucose, fructose, saccharose, starch, polyethylene glycol, sodium chloride and sodium sulfite do not interfere and when these excipients were added 1 to 10 times the concentration of DCH, the recovery of DCH by using the proposed method was between 96 – 105%.

Starch, talc, lactose, and magnesium stearate do not interfere in the proposed method.

Proposed method for determination of DCH* in *aqueous formulations

Dilute the sample with buffer solution in order to obtain a concentration of DCH between the range worked out for the proposed method and carry out the procedure described.

Proposed method for determination of DCH* in *tablets and capsules

Crush the tablet in mortar, dissolve one tablet in buffer solutions in order to obtain a concentration in the range worked out for the proposed method. The capsule should be opened and the drug should be treated similarly as in case of tablet. By using mechanical shaker, the powder is completely disintegrated and the solution is clarified by passing it through Whatman filter paper no. 1, rejecting the first 10 ml and then appropriate dilutions are to be made. After it, the proposed method can be applied.

DISCUSSION

The proposed method was tested for many water samples containing known amounts of DCH in the range of the detection limits reported above. The results were found to be reproducible with reasonable standard deviation and low range of errors as calculated from six determinations (Table 1 and 2).

A change in absorbance by 0.001 unit is expected on changing the concentration of DCH by 1.8–2.1 µg/ml. Further, a change in concentration by 0.1 mg/ml will change the rate of reaction by 0.0078 absorbance units/minute. In addition, the value of k_1 will change by 0.00045 in 1 minute on changing [DCH] by 1 µg/ml i.e. a change sufficient enough to be observed easily. The range of determination (51.30 - 615.54 µg/ml) is also considerably low and these are good for the general determination of DCH. The correlation coefficient (r) is in the range 0.9988 to 0.9999 which indicates the high precision involved in the determination and almost perfect correlation of the data. The value of coefficient of determination (r^2) suggests that 99.76% to 99.99% change in the value of A_{30} or A_{60} or A_{90} or A_{120} or A_{150} or A_{180} or A_{210} or A_{240} or initial rate or k_1 is caused by DCH and the rest 0.01% to 0.24% is the effect of unknown factors.

The value of 't' as calculated for the calibration curves, are in the range 6.6096 to 19.6570 which are much higher than the tabulated critical value at 0.01 significance level. This suggests that there are less than 1% chances of error in drawing conclusions. The standard deviation is within reasonable limits. Percentage recovery on the basis of six parellel determinations is 96.67% to 99.79% in solutions, 95.83% to 97.68% in Doxytas tablets and 96.03% to 97.88% in Codox capsules. Overall, the 'G' and 'H' plots can be said to be the best calibration curve suited very well for estimation of DCH.

Table 3: Comparison with other reported methods

S.N.	Range of Detection	Reference	LOD	LOQ	Comment
1	Not Reported	9, 10	Not Reported	20 ng/ml	HPLC- UV based method for Blood Serum. Complicated sample Preparation
2	Not Reported	11	15 ng/ml	Not Reported	HPLC- UV based method for Blood plasma. Complicated sample Preparation
3	Not Reported	12	< 10 ng/ml	Not Reported	HPLC- UV based method for Blood Serum, Urine (cow). Complicated sample Preparation.
4	Not Reported	13	25 ng/ml	Not Reported	HPLC- UV based method for Blood Serum , Urine. Complicated sample Preparation
5	Not Reported	14	50 ng/ml	Not Reported	HPLC- UV based method for Blood Serum and Urine. Complicated sample Preparation
6	10 - 100µg/ml	15	Not Reported	Not Reported	HPLC- UV based method for bulk, tablets and capsules Formulation
7	Not Reported	16	Not Reported	Not Reported	HPLC- UV based method for cell suspension. Complicated sample Preparation
8	Not Reported	17	0.05 ppm	Not Reported	HPLC- UV based method for Food (Honey). Complicated sample Preparation
9	Not Reported	18	0.1 ppm	Not Reported	HPLC- UV based method for Food (Honey). Complicated sample Preparation
10	Not Reported	19	Not Reported	Not Reported	HPLC- UV based method for injections, saline, water, stability – indicating Formulations
11	Not Reported	20	Not Reported	Not Reported	HPLC- UV based method for Ointments. Complicated sample Preparation
12	Not Reported	21	1.15 ng/ml	2.22ng/ml	HPLC- UV based method for Milk. Complicated sample Preparation
13	Not Reported	22	Not Reported	30 ng/g	HPLC- UV based method for kidney tissue. Complicated sample Preparation
14	Not Reported	23	< 5 -10 ng/g	Not Reported	HPLC- UV based method for cow, muscle, renal cortex, renal medulla, liver, lung tissue. Complicated sample Preparation

The value of slope of the calibration curves and Sandell's sensitivity indicated that the sensitivity of the method is not very good in comparison with the other methods reported for estimation of DCH [9-13]. However, as the DCH amount is to be estimated in tablets, capsules and other aqueous formulations in which it is present in the range 100 mg per tablet or capsules, the sensitivity in micrograms is sufficient enough for all practical purposes. Keeping this in view, it is not necessary to have an ultra micro analytical quantitative method for its determination. On the contrary, a method workable in a higher concentration range is needed so that preparation of solutions etc becomes simple. A comparison of the proposed methods with other reported methods has been given in Table 3. It is encouraging that the proposed method is better than many of the reported ones as far as the simplicity, lesser facilities required for applying the method, interference of ions or excipients, validity of Beer's law, detection limits, correlation of data and reproducibility of results is concerned for the general estimation of DCH in aqueous formulations, capsules and tablets especially in mg. Further the simplicity involved in the procedure and the low cost of determination go in favour of the proposed method.

CONCLUSION

A new, simple and cost effective kinetic-spectrophotometric method for microgram determination of doxycycline hyclate(DCH) in aqueous formulations, capsules and tablets based on its oxidation by periodate has been developed. The best fit conditions developed for determination of DCH in the range 51.30 µg/mL – 615.54 µg/mL are, $[NaIO_4] = 1.0 \times 10^{-5}$ mol dm^{-3}, $[Mn^{II}] = 1.0 \times 10^{-3}$ mol dm^{-3}, Temperature = 35.0 ± 0.1°C, pH = 5.0 and λmax = 486 nm. MnII has been used as a catalyst. The calibration curves were developed in terms of absorbance at different time or initial rate or pseudo first order rate constant versus concentration of DCH. The characteristics of various calibration curves, percentage recovery, effect of excipients, correlation coefficient and comparison with other reported methods show that the developed method is better in terms of simplicity and for all practical purposes for determination of DCH in tablets, capsules and other aqueous formulations.

Acknowledgements
The authors are thankful to the Department of Chemistry, Gurukul Kangri University for providing laboratory etc. facilities for undertaking this work.

REFERENCES

[1] CR Craig, RE Stitzel. Modern Pharmacology, 3rd edn., Little Brown and Co., Boston, **1990**.
[2] American Medical Association, Department of Drugs, Division of Drugs and Toxicology. AMA Drug Evaluations 1992, AMA, Chicago, **1992**, 1372.

[3] DA Sack; DC Kaminsky; RB Sack; JN Itotia; RR Arthur; AZ Kapikian; E Orskov; K Orskov, *N. Eng. J. Med.*, **1978**, 298, 758.
[4] RB Sack; JL Froehlich; A Zulich; DS Hidi; AZ Kapikian; F Orskov; K Orskov; HB Greenberg, *Gastroenterology*, **1979**, 76, 1368.
[5] TK Satterwhite; HL Dupont, *Med. Clin. North. Am.*, **1983**, 67, 203.
[6] Editorial, Traveller's Diarrhea, *J. Am. Med. Assoc*., **1985**, 253, 2700.
[7] The controller of publications, Delhi, on behalf of Government of India, Ministry of health and family welfare, Indian Pharmacopoeia, **1996**, 1, 274, 477.
[8] Secretary of United States pharmacopoeial convention, The United States Pharmacopoeia, Twinbrook Parkway, Rockville, Meryland -20852, 23rd review, **1995**, 1160
[9] G Lunn, NR Schmuff. HPLC Methods for Pharmaceutical Analysis, John Wiley & Sons INC., N. York, **1997**, 534
[10] I Gastearena; MC Dios-Vieitez; E Segura; MM Goni; MJ Renedo; D Fos, *Chromatographia*, **1993**, 35, 524
[11] F Kramer-Horaczynska, *J. Chromatogr. Sci*., **1991**, 29, 107
[12] J L Riond; KM Hedeen; K Tyczkowska; J E Riviera, *J. Pharm. Sci.*, **1989**, 78, 44
[13] ME Sheridan; GS Clarke, *J. Chromatogr.*, **1988**, 434, 253.
[14] AP De Leenheer; HJCF Nelis, *J.Pharm.Sci.*,**1979**, 68, 999
[15] PD Bryan; J T Stewart, *J. Pharm. Biomed. Anal.*, **1994**, 12, 675
[16] A Kersten; C Poitschek; S Rauch; E Aberer, *Antimicrob. Agents Chemother*., **1995**, 39, 1127
[17] H Oka; Y Ikai; N Kawamura; K Uno; M Yamada; K Harada; M Uchiyama; H Asukabe; Y mori; M Suzuki, *J. Chromatogr.*, **1987**, 389, 417
[18] H Oka; Y Ikai; N Kawamura; K Uno; M Yamada; K Harada; M Suzuki, *J. Chromatogr.*, **1987**, 400, 253
[19] ML Stiles; Jr LV Allen; SJ Prince, *Am. J. Health-Syst. Pharm.*, **1995**, 52, 70
[20] H Lingeman; HA Van Munster; JH Beynen; WJ Underberg; A Hulshoff, *J. Chromatogr.*, **1986**, 352, 261
[21] MC Carson, *J. AOAC Int.*, **1993**, 76, 329
[22] JM Degroodt; BW de Bukanski; S Srebrnik, *J. Liq. Chromatogr.*, **1993**, 16, 3515
[23] JL Riond; KM Hedeen; K Tyczkowska; JE Riviere, *J.Pharm.Sci.*, **1989**, 78, 44
[24] RD Kaushik; R Kumari; T Kumar; P Singh, *Asian J. Chem.*, **2010**, 22, 7959
[25] RD Kaushik; R Malik; A Kumar, *J. Indian Chem. Soc*., **2010**, 87, 317
[26] RD Kaushik; RK Arya; S Kumar, *Asian J. Chem*., **2000**, 12, 1229
[27] RD Kaushik; SD Oswal; D Singh, *Asian J. Chem*., **2000**, 12, 1129
[28] RD Kaushik; V Kumar; S Kuma, *Asian J. Chem*., **1999**, 11, 633
[29] RD Kaushik; R Malik; R Agarwal; J Singh, *Res. J. Pharm. Biol. Chem. Sci*., **2014**, 5(3), 1644
[30] RD Kaushik; D Kumar; A Kumar; A Kumar, *J. Indian Chem. Soc*., **2010**, 87, 811
[31] RD Kaushik; R Malik; T Kumar; P Singh, *Oxid. Commun*., **2012**, 35, 316
[32] RD Kaushik; M Kaur; R Malik; A Kumar, *Int. J. Chem. Sci*., **2010**, 8, 1379
[33] RD Kaushik; P Sundriyal; P Tyagi; P Singh, J Singh, *Int. J. Chem. Sci*., **2014**, 12(2), 600
[34] RD Kaushik; A Kumar; T Kumar; P Singh, *React. Kinet. Mech. Cat*., **2010**, 101(1), 13
[35] RD Kaushik; Shashi; Amrita; S Devi, *Asian J. Chem*., **2004**, 16, 818
[36] J Singh; R Malik; O Singh; Sushma; RD Kaushik, *Int. J. Chem. Sci*., **2014**, 12(2), 445
[37] RD Kaushik; R Yadav; MM Tiwari; P. Rathi; J Singh, *Der Pharmacia Lettre*, 2014; 6(4): In Press
[38] HTS Britton. Hydrogen ions, D. Von Nostrand Co., **1956**, 354.
[39] L Meites. Handbook of Analytical Chemistry, Mc Graw-Hill book Co., INC, New York, **1963**, 3-4
[40] PD Biswas; K De, *J. Indian Chem. Soc.*, **2003**, 80**,** 195
[41] AI Vogel. A Text Book of Quantitative Inorganic Analysis, Longmanns Green, London, **1961**