International Journal of ChemTech Research
CODEN(USA): IJCRGG ISSN : 0974-4290
Vol.2, No.1, pp 584-591, Jan-Mar 2010

Non-aqueous Titrimetric Assay of Doxycycline Hyclate in Pharmaceutical Preparations

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ABSTRACT: Doxycycline is a member of the tetracycline derivation and has a wide range of antibacterial activity. Two simple, cost-effective, accurate and precise direct titrmetric methods to the determination of doxycycline hyclate (DOX) in pharmaceutical preparations have been developed and validated. The methods are based on the titration of DOX with 0.01 N acetous perchloric acid in the presence of mercuric acetate in glacial acetic acid using either crystal violet as indicator in visual end point detection (method A) or by using combined glass electrode in potentiometric end point detection (method B). The methods were applicable over the range of 4.0 - 40 mg DOX. The validation of the methods yielded good results that included precision (RSD < 3 % for intra- and inter-day precision), and accuracy (RE ≤ 2.68 %). It was also found that the excipients in the commercial tablet preparation did not interfere with the assay and the results were compared with the official HPLC method.
KEY WORDS: Doxycycline, Titrimetry, Assay, Non-aqueous, Pharmaceuticals.

INTRODUCTION

Doxycycline hyclate (DOX), (4S,4aR,5S, 5aR,6R, 12aS)-4-(dimethylamino)-3,5,10,12,12a-penta hydroxy-6-methyl-1,11-dioxo-1,4,4a,5,5a,6, 11, 12a-octahydrotetracene-2-carboxamide mono hydro chloride, compound with ethyl alcohol (2:1), monohydrate, is a semisynthetic tetracycline antibiotic derived from oxytetracycline with a broad spectrum of activity against a wide range of gram-positive and gram-negative pathogens[1,2]. DOX is frequently used to treat chronic prostatitis, sinusitis, syphilis, chlamydia, pelvic inflammatory disease, acne, rosacea, and Rickettsial infections.

For the determination of DOX, liquid chromatography (LC) has been recommended in some pharmacopoeias[3-5]. Several methods have been proposed for the determination of DOX both in pharmaceutical preparations and biological samples. These include spectrophotometry[6-12], fluorimetry[13], phosphorimetry[14], thin-layer chromatography[15], liquid chromatography[16-34], sequential injection chromatography[35], LC-MS[36], micellar electrokinetic capillary chromato graphy[37,38], flow injection analysis[39], capillary electrophoresis[40],doxycycline-optosensors[41,42], doxycycline selective membrane electrodes[43] and bioassay[44]. A fast thin layer chromatography-fluorescence scanning densitometry (TLC-F)[45] has been developed for the determination of DOX in honey, serum and urine samples. DOX has also been determined in milk and milk powder[46] by using HPLC.

The chromatographic techniques although, specific, most of the described methods are time consuming and require multistage extraction procedures. Equally, spectrophotometric and fluorimetric procedures take long reaction time for full color development, and some times require prior extraction of the colored product. In

addition, most of the described procedures require expensive instrumental setup.

No titrimetric procedure has ever been reported for the determination of DOX in pharmaceuticals although the technique is very simple and easily adoptable to determine the drug content in milligram level in the quality control laboratories across the developing countries where modern and expensive instruments are not available.

In this piece of work, the titration of DOX in acetic acid medium with acetous perchloric acid with either visual end point detection using crystal violet as indicator or potentiometric end point detection employing modified glass electrode-saturated calomel electrode. The methods were successfully applied to the formulations containing DOX and the results were highly encouraging.

MATERIALS AND METHODS

Apparatus

A Metrohm Swiss made Tiamo 809 and 803 potentiometer provided with a combined glass-SCE electrode system was used for potentiometric titration. The KCl of the salt bridge was replaced with saturated solution of KCl in glacial acetic acid.

Reagents and Solutions

All chemicals used were of analytical reagent grade. All solutions are made in glacial acetic acid (S. D. Fine Chem, Mumbai, India) unless mentioned otherwise.

Perchloric Acid (0.01 M): The stock solution of (~0.1 M) perchloric acid (S. D. Fine Chem, Mumbai, India) was diluted appropriately with glacial acetic acid to get a working solution of 0.01 M perchloric acid and standardized with pure potassium hydrogen phthalate and crystal violet as indicator[47].

Crystal violet indicator (0.1 %): Prepared by dissolving 50 mg of dye (S. D. Fine Chem, Mumbai, India) in 50 mL of glacial acetic acid.

Mercuric acetate solution (5 %): Five gram of the pure $Hg(OAc)_2$ (Merck) was dissolved in 100 mL of glacial acetic acid, filtered and used.

Standard drug solution

Stock standard solution containing 4 mg/mL drug was prepared by dissolving the 400 mg of DOX (Lotus Pharma Ltd, Bangalore) in glacial acetic acid.

General Procedures

Visual Titration (Method A)

An aliquot of the drug solution containing 4.0-40.0 mg of DOX was measured accurately and transferred into a clean and dry 100 mL titration flask and the total volume was brought to 10 mL with glacial acetic acid. Then, 2 mL of 5 % $Hg(OAc)_2$ was added, the content was mixed and after 2 min, two drops of crystal violet indicator were added and titrated with standard 0.01 M perchloric acid to a blue colour end point.

A blank titration was performed in the same manner without DOX, and the necessary volume corrections were made.

The amount of the drug in the measured aliquot was calculated from the formula:

$$Amount(mg) = \frac{VM_wR}{n}$$

where V = volume of perchloric acid required, mL; M_w = relative molecular mass of the drug; and R = molarity of the perchloric acid and n = number of moles of perchloric acid reacting with each mole of DOX.

Potentiometric Titration (Method B)

An aliquot of the standard drug solution equivalent to 4.0-40.0 mg of DOX was measured accurately and transfered into a clean and dry 100 mL beaker and the solution was diluted to 25 mL by adding glacial acetic acid followed by the addition of 2 mL of 5 % $Hg(OAc)_2$. The combined glass-SCE (modified) system was dipped in the solution. The contents were stirred magnetically and the titrant (0.01 M $HClO_4$) was added from a microburette. Near the equivalence point, titrant was added in 0.05 mL increments. After each addition of titrant, the solution was stirred magnetically for 30 s and the steady potential was noted. The addition of titrant was continued until there was no significant change in potential on further addition of titrant. The equivalence point was determined by applying the graphical method. The amount of the drug in the measured aliquot was calculated as described under visual titration.

Procedure for Formulations

Two brands of tablets, namely, DOX 100 (Dr. Reddy's Lab) and Doxy 100 (Micro Labs Ltd) were used in the investigation.

Twenty tablets were weighed and ground into a fine powder. An amount of powder equivalent to 400 mg DOX was weighed accurately and transferred into a 250 mL round bottomed (RB) flask and sonicated for 5 min with 100 mL of methanol. The solution was filtered through Whatmann No. 42 filter paper and the filtrate was collected in a 250 mL RB flask. Then, methanol was evaporated at 40 – 45^{o} C under the stream of nitrogen. The resulting residue was dissolved in glacial acetic acid and transferred into 100 mL volumetric flask and the volume was brought to 100 mL with glacial acetic acid. A suitable aliquot was next subjected to analysis by applying the general procedures as described earlier.

RESULTS AND DISCUSSION

The reaction between DOX in non-aqueous medium and acetic acid is an acid-base reaction where the strong acid can donate a proton to nitrogen of the amino group of the drug molecule[48].

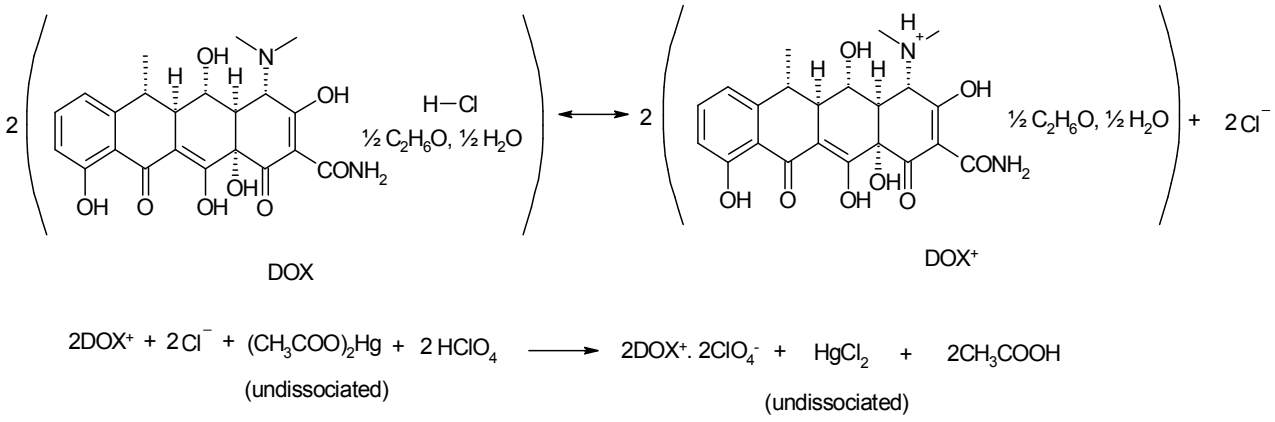
In the presence of perchloric acid, acetic acid will accept a proton:

$$CH_3COOH + HClO_4 \longleftrightarrow CH_3COOH_2^+ + ClO_4^-$$

$$2CH_3COOH_2^+ + 2CH_3COO^- \longleftrightarrow 4CH_3COOH$$

The $CH_3COOH_2^+$ can very readily give up its proton to react with a base, so basic properties of a base is enhanced and hence, titration between weak base and perchloric acid can often be accurately carried out using acetic acid as solvent.

Since, DOX is a hydrochloride, which is too weakly basic to react quantitatively with acetous perchloric acid. Addition of mercuric acetate (which is undissociated in acetic acid solution) to a halide salt replaces the halide ion by an equivalent quantity of acetate ion, which is a strong base in acetic acid as shown in the scheme given below:



Scheme. Possible way of the neutralization reaction.

The enhanced basicity of DOX in acetic acid medium is due to non-lavelling effect of acetic acid and the determination of DOX is very easier. The procedures involve the titration of DOX with perchloric acid with visual and potentiometric end point detection. Crystal violet gave satisfactory end point for the concentrations of analyte and titrant employed. A steep rise in the potential was observed at the equivalence point with potentiometric end point detection (figure 1). With both methods of equivalence point detection, a reaction stoichiometry of 1:1 (drug:titrant) was obtained which served as the basis for calculation. Using 0.01 M perchloric acid, 4.0-40.0 mg of DOX was conveniently determined. The relationship between the drug amount and the titration end point was examined. The linearity between two parameters is apparent from the correlation coefficients of 0.9965 and 0.9986 obtained by the method of least squares for visual and potentiometric methods, respectively. From this it is implied that the reaction between DOX and perchloric acid proceeds stoichiometrically in the ratio 1:1 in the range studied.

METHOD OPTIMISATION

In both the methods, the optimum amount of mercuric acetate required was studied by varying its amount and keeping the drug amount constant followed by the measurement of the stoichometric amount of drug found in each case. It was found that, a 2 mL of 5 % $Hg(OAc)_2$ was sufficient for complete replacement of chloride in drug by acetate and the same amount was fixed through out the investigation. A contact time of 2 min was essential after the addition of mercury(II) acetate.

METHOD VALIDATION

Intra-day and inter-day accuracy and precision

The precision of the methods was evaluated in terms of intermediate precision (intra-day and inter-day). Three different amounts of DOX within the range of study in each method were analysed in seven and five replicates in method A and method B, respectively, during the same day (intra-day precision) and five consecutive days (inter-day precision). For inter-day precision, each day analysis was performed in triplicate and pooled-standard deviation was calculated. The RSD values of intra-day and inter-day studies for DOX showed that the precision of the methods was good (table 1). The accuracy of the methods was determined by the percent mean deviation from known concentration, and results are presented in table 1.

Robustness and ruggedness of the methods

The robustness of the methods was evaluated by making small incremental changes in volume of $Hg(OAc)_2$ and standing time after adding $Hg(OAc)_2$, and the effect of the changes was studied by recording the volumes of $HClO_4$ required to titrate three different amounts separately. The changes had negligible influence on the results as revealed by small intermediate precision values expressed as % RSD ($\leq$ 2.09 %). The results are shown in table 2.

Method ruggedness was expressed as the RSD of the same procedure applied by four different analysts as well as using four different burettes. The inter-analysts RSD were within 2.36 % whereas the inter-burettes RSD for the same DOX amounts was less than about 2.63 % suggesting that the developed method was rugged. The results are shown in table 2.

Application

The described titrimetric procedures were successfully applied for the determination of DOX in its pharmaceutical formulations (DOX 100 and DOXY 100). The obtained results (table 3) were statistically compared with the official BP method[5]. The method consisted that the determination of DOX by liquid chromatography with UV detection. The results obtained by the proposed methods agree well with those of reference method and with the label claim. The results were also compared statistically by a Student's t-test for accuracy and by a variance F-test for precision[49] with those of the reference method at 95 % confidence level as summarized in table 3. The results showed that the calculated t-and F-values did not exceed the tabulated values inferring that proposed methods are as accurate and precise as the reference method.

Recovery Study

Accuracy and the reliability of the methods were further ascertained by performing recovery experiments. To a fixed amount of drug in formulation (pre-analysed): pure drug at three different levels was added, and the total was found by the proposed methods. Each test was repeated three times. The results compiled in table 4 show that recoveries were in the range from 98.56 to 103.6 % indicating that commonly added excipients to tablets did not interfere in the determination.

CONCLUSION

The non-aqueous titrimetric methods proposed for the determination of doxycycline hyclate in pure and pharmaceutical dosage forms has the advantages of simplicity, speed, accuracy and precision and the use of inexpensive equipments compared to many reported techniques. The methods are useful for the quality control and routine analysis of doxycycline since there is no interference from the common excipients that might be found in commercial tablet dosage form.

Table 1. Intra-day and inter-day accuracy and precision data.

Method	DOX taken, mg	Intra-day accuracy and precision			Inter-day accuracy and precision		
		DOX found, mg	RE, %	RSD, %	DOX found, mg	RE, %	RSD, %
Visual titrimetry, (n=7)	8.00	8.09	1.13	1.95	8.13	1.63	2.85
	24.0	23.99	0.04	0.56	24.30	1.25	1.25
	40.0	40.10	0.25	1.02	41.07	2.68	0.99
Potentiometric titrimetry (n=5)	8.00	8.06	0.75	0.98	8.09	1.13	2.36
	24.0	24.09	0.38	0.99	24.25	1.04	1.20
	40.0	40.08	0.20	1.06	40.92	2.3	0.89

RE.relative error, RSD. relative standard deviation.

Table 2. Results of robustness and ruggedness studies (% RSD)

Method	DOX taken, mg	Robustness	Ruggedness		
		Change in mL of $Hg(OAc)_2$*	Change in standing time**, s	Inter-analysts (%RSD), (n=4)	Inter-instruments (%RSD), (n=4)
Visual titrimetry	10	2.09	1.98	2.36	2.63
	20	1.66	1.56	1.36	1.56
	30	1.03	0.98	1.13	1.23
Potentiometric titrimetry	10	1.56	1.86	1.86	1.85
	20	1.26	1.35	1.30	1.36
	30	0.86	0.89	1.10	1.08

*The volume of $Hg(OAc)_2$ varied were 1.8, 2.0 and 2.2 mL.
**Standing times employed were 90, 120 and 150 s.

Table 3. Results of assay in tablets and comparison with official method.

Brand name	Label claim, mg/tablet	Found* (Percent of label claim ± SD)		
		Official method	Proposed methods	
			Visual titrimetry	Potentiometric titrimetry
DOX 100	100	99.06±1.23	101.3±1.95 **t**=2.23 **F**=2.51	100.6±1.06 **t**=2.13 **F**=1.35
DOXY 100	100	101.6±0.89	103.6±1.40 **t**=2.76 **F**=2.47	102.3±0.86 **t**=1.26 **F**=1.07

*Average of five determinations.
Tabulated t value at the 95% confidence level is 2.77.
Tabulated F value at the 95% confidence level is 6.39.

Table 4. Results of recovery study using standard addition method.

	Visual titrimetry				**Potentiometric titrimetry**			
Tablet studied	DOX in tablet extract, mg	Pure DOX added, mg	Total DOX found, mg	Pure DOX recovered (Percent±SD*)	DOX in tablet extract, mg	Pure DOX added, mg	Total DOX found, mg	Pure DOX recovered (Percent±SD*)
DOX 100	10.13	5.0	15.07	98.76±1.36	10.06	5.0	15.07	100.16±1.23
	10.13	10.0	20.29	101.6±1.30	10.06	10.0	20.02	99.56±1.53
	10.13	15.0	25.19	100.4±0.99	10.06	15.0	24.84	98.56±0.56
DOXY 100	10.36	5.0	15.54	103.6±2.56	10.23	5.0	15.35	102.3±1.66
	10.36	10.0	20.54	101.8±1.89	10.23	10.0	20.59	103.6±2.6
	10.36	15.0	25.36	99.98±0.56	10.23	15.0	25.37	100.9±1.50

*Mean value of three determination.

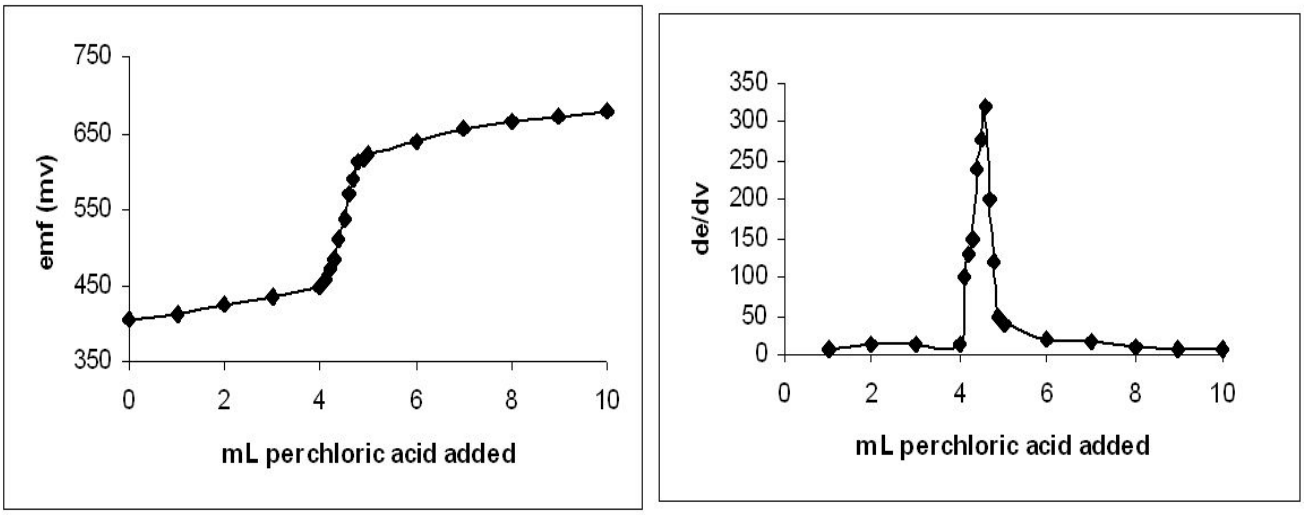


Figure 1. Potentiometric titration curves for 20 mg DOX *Vs* 0.01 M $HClO_4$.

ACKNOWLEDGEMENT

Authors thank Lotus Pharama Ltd, Bangalore, India, for gifting pure DOX. Three of the authors (PJR, KBV and NRP) thank the authorities of the University of Mysore, Mysore, for permission and facilities. One of the authors (PJR) is indebted to the Jubilant Organosys Ltd, Mysore, India, for their kind permission to pursue Ph.D. degree programme. NRP is grateful to the University Grants Commission, New Delhi, India, for awarding a Meritorious Research Fellowship.

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