A Novel Visible Spectrophotometric Validated Determination for the Assay of Doxycycline Hyclate in Pure and Pharmaceutical Samples

SUNITHA R MANGALA*[1], SHIVAPRASAD K HALAPPA[1], VIRUPAXAPPA S BETEGERI[2] and LATHA M SHIVASHANKAR[2]

[1] Department of Chemistry, VSK University, Ballari, Karnataka, (INDIA)
[2] Department of Chemistry, GM Institute of Technology, Davangere, Karnataka, (INDIA)

Abstract:

A new introduced and sensitive method for the assay of Doxycycline hyclate has been proposed in pure and pharmaceutical samples. The current method is based on the reaction between Doxycycline hyclate and Ethylene diamine tetraacetic acid (EDTA) with ammonia which leads to the formation of a yellow colored complex having a λ_{max} =444nm. The reaction conditions were optimized to obtain the complex of high sensitivity and longer stability. Under these optimum conditions, the absorbance of the complex were found to increase the linearly with increase in concentration of the drug, which was verified with correlation coefficient value .The system obeyed Beer's law in the concentration range and Sandell's sensitivity. The purpose of this review article is to present into the analyte in pharmaceutical preparations were in agreement with those of the obtained from a comparison method, as revealed by statistical analysis of the reported results using Student t-test and the variance F-test.

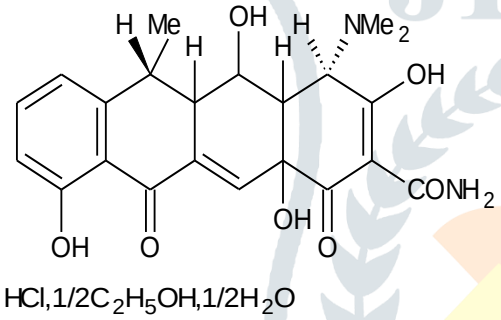
Keywords: Ammonia, Doxycyline, EDTA and spectrophotometry.

1. INTRODUCTION:

The molecular formulae of Doxycycline hyclate is C22H24N2O8,HCl,½C2H6O,½H2O.The systematic IUPAC name is Hydrochloride hemiethanol hemihydrate of (4S,4aR,5S,5aR,6R,12aS)-4- (dimethylamino)-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-1,4,4a,5,5a,6,11, 12a-octahydrotetracene-2-carboxamide. Doxycycline was discovered in 1967 and has undergone extensive investigation, both for its antimicrobial property as well as the effects on the physiology of higher organisms [1]. The synthetic pathway of DOX involve MTC as an intermediate, during this process 6-epidoxycycline (EDOX) can be formed as a side product. DOX is a semisynthetic broad spectrum tetracycline antibiotic, widely used in veterinary medicine and as an animal feed supplement to prevent diseases [2]. The literature contains several methods for the determination of DOX in pharmaceutical dosage forms, including liquid chromatography,sequential injection chromatography[3] and capillary electrophoresis [4]. The determination od Doxycycline is done for both in pharmaceutical preparations and biological samples, such as fluorimetry [5], phosphorimetry[6],thin layer chromatography [7], liquid chromatography [8–25]. spectrophotometric [26] electrochemiluminescent, [27] voltammetric[28], electro capillary chromatography [29] and titrimetric methods [30]. Besides, kinetic spectrophotometry using Cu(II)/H2O2 [31] and multivariate calibration

method [32] have also been reported by different workers. The determination includes FIA-spectrophotometry with copper carbonate [33] and spectrophotometry based on colour reactions with thorium(IV) [34], sodium cobaltnitrite [35] and uranyl acetate [36]. Folin-Ciocalteu reagent (F-C) is widely used in the area of plant biology for analysis of polyphenols [37] and for the determination of many phenolic compounds in pharmaceuticals [38,39] based on Folin-Ciocalteu reagent reduction. The present study describes the spectrophotometric determination of Doxycycline hyclate based on the reaction of the drug and EDTA with ammonia.

Fig1: Structure of Doxycycline Hyclate



2. EXPERIMENTAL

2.1 Apparatus

All absorbance measurements were conducted with a double beam LABMAN – UV-1200 spectrophotometer provided with quartz cells having thickness around 1-cm. During all spectrophotometric measurements, temperature controller was used to maintain the temperature. Systronics pH meter were used for accurate pH determinations.

2.2 Materials and reagents

All the reagents and solvents used were of analytical reagent grade and the solutions were prepared with double distilled water, sample of Doxycycline hyclate was bountifully supplied by Lotus Pharma Ltd, India. Standard EDTA solution of 0.01 M is prepared by dissolving 3.722 g of analytical reagent grade ethylene-diamine tetra acetic acid disodium salt in sufficient volume of distilled water and dilute the solution to 1 L. Ammonia solution is prepared by measuring 42.5 ml into a 100 ml volumetric flask, add sufficient distilled water to mark. The pH of the solution was adjusted to an appropriate value with the aid of a pH meter.

2.3 Preparations of standard solution

Stock solution of Doxycycline hyclate was prepared by dissolving 0.044g in 100 ml of double distilled water. Various aliquots of standard solutions of Metformin hydrochloride (100µg/ml) were diluted to get standard solutions across the range of 1-25µg/ml.

2.4.1 Recommended procedure

After a systematic and exhaustive study on various parameters involved in the formation of coloured products (as described under results and discussion), the following procedure was suggested for the assay of the selected Doxycycline hyclate drug. Different aliquots of (0.2, 0.5, 1.0, ... ,4.0 ml) standard Metformin hydrochloride solution(100 µg ml^{-1}) were accurately transferred into a series of 100 ml calibration flasks using a micro burette, to this 1ml of EDTA, 2.0ml of ammonia were added and shaken well for about 2minutes. The total volume was made up to 100ml with distilled water. An aliquot of the solutions was quickly

transferred into a quartz cell and then it was held in the cell chamber (kept at 25^0C) of the spectrophotometer.

2.4.2 Preparation of Sample Solutions:

Average weight of Doxycycline hyclate tablets of each brand was calculated. Then the tablets were grinded to fine powder with the help of mortar and pestle. Then, powder containing 10mg Doxycycline hyclate was dissolved in water, shaken for about 10 minutes and filtered through filter paper. The filtered solution was further diluted to make the final concentration of working sample equivalent to 100% of target concentration (100µg/ml). Suitable aliquot was then subjected to analysis using the procedure described under method 2.4.1

2.5 Determination of λ_{max}:

Standard solution containing 100µg/ml of Metformin hydrochloride was scanned using water as blank in the range of 200-800 nm to determine the wavelength of maximum absorption (λ_{max}) of the drugs. Metformin hydrochloride showed absorbance maxima at 444 nm.

2.6 Development of Equation for Assay:

The amount of Doxycycline hyclate per tablet and respective potency (%) in the marketed brands were determined using the following equations.

Amount of (mg) per tablet,

$$Z=\frac{A}{As}\times\frac{Ws}{100\times10}\times\frac{100\times10}{W}\times Wt\times\frac{P}{100}$$

$$\frac{Z}{Wc}\times100\%=\frac{A}{As}\times\frac{W}{100\times10}\times\frac{100\times10}{W}\times Wt\times\frac{P}{100}\times\frac{100}{Wc}\%$$

Amount of Doxycycline hyclate (mg) per tablet, Potency; (%) = Where, A= absorbance of sample solution; As= absorbance of reference standard solution; Ws= weight of reference DOX powder (mg); W = weight of generic powder sample (mg); Wt= average weight of tablet (mg); Wc= weight of drug claimed per tablet (mg), P = potency of reference Metformin hydrochloride powder.

3. Result and discussions

Ethylene diamine tetra acetic acid, which acts as a chelating agent that reacts with metal ion of the drug to form a stable colored complex. During the course of the reaction, the phenolic character of the C_{10} –OH group is utilized here. This method involves the addition of 0.01 M EDTA and NH_3 to Doxycycline hyclate solution, the mixture is shaken well for about 3 minutes, which results in production of an yellow colour that can be measured at 444nm. These analytical aspects have been successfully utilized to develop sensitive spectrophotometric methods for the assay of Doxycycline hyclate in pure and pharmaceutical formulations. Calibration graph of the drugs are given below in fig2.

Fig2. The absorption spectrum of EDTA which showed an maximum absorption band at 444nm.

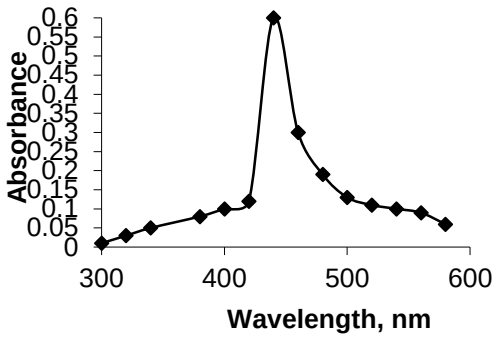
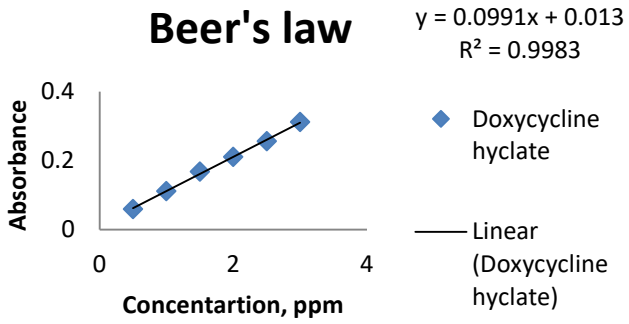


Fig 3. Calibration graph of Doxycycline hyclate



4. Fixation of optimum condition for the formation of coloured species

4.1 Absorption Spectrum

The reaction of Doxycycline hyclate with EDTA in the presence of ammonia yields a stable yellow coloured complex which was studied by measuring the absorbance of a series of solutions which exhibited an absorption band at 444nm

4.2 Effect of temperature

It is observed that, at the room temperature i.e. 25^0C lower or higher temperature gives the accurate results. The other reason for maintaining the room temperature is that the precipitation takes place at higher temperature and hence it was marked to carry out all the spectral measurements at room temperature.

4.3 Effect of reagent

In order to acquire the maximum stability and sensitivity, the reagent was studied. It was noticed that an optimum volume of 1.0ml of the reagent was adequate for maximum absorbance and stability. Below these volumes of reagents, low absorbance was observed and besides they required long time or completion of the reaction. However, the excess of reagent had no consequence on maximum absorbance and on the stability of the coloured species.

4.4 Accuracy and Precision

The accuracy and precision of the methods were studied by analyzing solutions containing known amount of Doxycycline hyclate lies within Beer's law range. The relative standard deviation (RSD) did not exceed 2% which reveals the high reproducibility of the results and precision of the method. This good level of precision was appropriate for quality control analysis of Doxycycline hyclate of its pharmaceutical tablets.

4.5 Analytical features

The solution containing dissimilar amounts of Doxycycline hyclate, the same amount of reagent and concentrations of drugs in the optimum conditions were used to work on the validity of Beer's law in each method. It was noticed that Beer's law was valid in the concentration ranges of 33.1-331 µg/ml for Doxycycline hyclate. The Sandell's sensitivity, linear range, intercept range, LOD and LOQ values were calculated) are calculated as per the ICH guidelines.

5. Conclusion

From an analytical opinion, it is concluded that the outlined procedure permits for the determination of Doxycycline hyclate in pure and pharmaceutical dosage forms. The great advantage is that the results obtained are reproducible. Unlike HPLC, gas chromatography and spectroflourometery, the UV-Visible spectrophotometer is simple, sensitive, stable, inexpensive with sensible accuracy and precision. The results also certainly recommend the utility of the proposed method for the analysis of the drug. Additionally, the reported method is free from the interference by various excipients. Consequently, the official method would be securely suggested for regular quality control in pharmaceutical industries.

Table 6.1 Analytical parameters of Doxycycline hyclate with reagent:

Parameters	**Values**
λ_{max}	444
Intercept	0.04
Slope	0.10
Regression coefficient(r)	0.9983
LOD	0.02
LOQ	0.26
Sandell's Sensitivity	0.014
Regression equation	Y=a+bx
Confidence level	95%

6.2 Analytical of Doxycycline hyclate in pharmaceutical formulations:

DRUG	Labeled	Found (X± RSD)		
		Proposed method	Reference method	
Atridox	50mg/Tab	49 ± 0.84 t= 0.92, F=1.49	49± 0.6	
Doxteric	200mg/Tab	198 ± 0.6 t= 0.42, F=1.01	198.27±0.4	
Monodox	100mg/Tab	97.1±0.77 t= 0.11, F=0.58	100.2±0.44	

References:

1. D. T. Dennis, Ingles by T.V.Henderson, D. A. Bartlett, J. G. Ascher, M. S. Eitzen, E. Lillibridge, 2001, *Jama*, *285*(21), 2763-2773.
2. J. Fiori, G. Grassigli, P. Filippi, R. Gotti, V.Cavrini, *J. Pharm. Biomed. Anal.* 2004, 37:979-985.
3. D. Satinsky, L. M. L. Dos Santos, H. Sklenáøová, P. Solich, M. C. B. S. M. Montenegro, A. N. Araújo, *Talanta* 68**,** 2005, 214–218.
4. A. Van Schepdael, R. Kibaya, E. Roets, J. Hoogmartens, *Chromatographia* 41**,**1995, 367–369.
5. F. Salinas, A. Munoz de la Pena, M.I. Duran, *Anal. Lett.*, 1990, vol. 23, p.863.
6. H.Z. Xie, C.A. Dong, W.J Jin, Y.S. Wei, C.S. Liu, S.S. Zhang, B.L.Zhou, *Anal. Chim. Acta*, 1996, vol. 319, p. 239.
7. W. Naidong, S. Greelen, E. Roets, J. Hoogmartens, *J. Pharm. Biomed. Anal.*, 1990, vol. 8, p. 891.

8. P.D. Bryan, J.T. Stewart, *J. Pharm. Biomed. Anal.*, 1994, vol. 12, p. 675.
9. S.Croubeks, H. Vermeersch, P. De Backeer, M.D.F. Ssantos, J.P.Remo, C.van Peteyhem, *J. Chromatogr. B*, 1998, vol. 708, p. 145.
10. D. Farin, G. Piva, I. Gozlan, R. Kitzes, *Chromatographia*, 1998, vol. 47, p. 547.
11. N. Ruz, M. Zabala, K M.G. ramer, M.A.Campanero, M.C. Dios-Viéitez, M.J. Blanco-Príeto, *J. Chromatog.*, 2004, vol. 1031, p. 295.
12. J.L.Riond, K.M.Hedeen, K. Tyczkowska, J.E.Riviere, *J. Pharm. Sci.*, 1989, vol. 78, p. 44.
13. I. Gastearena, M.C. Dios-Vieitez, E. Segura, M.M. Goni, M.J. Renedo, D. Fos, *Chromatographia*, 1993, vol. 35, p. 524.
14. A Gajda, A. Posyniak, K. Pietruszka, *Bull. Vet. Inst. Pulawy*, 2008, vol. 52, p. 417.
15. A. Zarghi, A. Kebriaeezadeh, R. Ahmadkhaniha, *Boll. Chim. Farm.*, 2001, vol. 140, p. 112.
16. A.L. Cinquina, F. Longo, G. Anastasi, L.Giannetti, R. Cozzani, *J Chromatogr. A*, 2003, vol. 987, p. 227.
17. J. Klimes, M. Dohnalová, J Sedlácek, *J. Chromatogr. A*, 1999, vol. 846, p. 181.
18. H.J. Nelis, A.P. De Leenheer, *Clin. Chim. Acta*, 1980, vol. 103, p. 209.
19. J.M. Prévosto, B. Béraud, V. Cheminel, Y. Gaillard, C. Mounier, J.F Chaulet, *Ann. Biol. Clin. (Paris)*, 1995, vol. 53, p. 29.
20. M.D.Santos, H.Vermeersch, J.P. Remon, M.Schelkens, P.De Backer, R. Ducatelle, F.Haesebrouck, *J. Chromatogr. B*, 1996, vol. 682, p. 301.
21. S.S.Mitic, Z.M.Gordana, A.K.Danijela, D.C.Naskovic-Djokic, B.A.Biljana, D.R Ivana, *Serb. Chem. Soc.*, 2008, vol. 73, p. 665.
22. K. Dihuidi, M.J.Kucharski, E.Roets, J.Hoogmartens, H.Vanderhaeghe, *J. Chromatogr. A*, 1985, vol. 325, p. 413.
23. J.Hoogmartens, N.H.Khan, H.Vanderhaeghe, M.Oosterbaan, G.L.Veld-Tulp, W Plugge, D.Mialanne, R.A.Melamed, *J. Pharm. Biomed. Anal.*, 1989, vol. 7, p. 601.
24. L.Monser, F.Darghouth, *J. Pharm. Biomed. Anal.*, 2000, vol. 23, p. 353.
25. P.Seth, A.Stamm, *Drug Dev. Ind. Pharm.*, 1986, vol. 12, p. 1469.
26. I.Choma, K.A.Pilorz, *J. Liq. Chromatogr. Rel. Tech.*, 2004, vol. 27, p. 2143.
27. D. Satinsky, M.L.D.Lucia, H.Santos, P.Sklenarova, M.Solich, B.S.M.Conceicao, M.C.B.S.M.Montenegro, N.A.Alberto, *Talanta*, 2005, vol. 68, p. 214.
28. V.Sreeran, A.V.D.Nagendrakumar, M.Madhu, *chemical science transactions*, vol. 4, no. 1, pp. 69-74, 2015.
29. H.M.Lofty, M. A.Hegha, E.H.Mohamed, *spectrochimica Acta* , vol. 153, pp.321-332, 2016.
30. Li S, Li J, Q.Lin, X.Wei, *Analyst J*, vol.140, pp. 4702-4707, 2015.
31. *J. of pharmaceutical and biomedical analysis*, vol. 84, pp. 263-268, 2013.
32. R.Injac, J.Kac, B.Strukyelj, *Analytical and Bio analytical chemistry*, vol. 387, no. 2, pp. .695-701, 2007.

33. S.M.Sunaric, S.S.Mitic, G.Z.Miletic, A.N.Pavlovic, D.Naskovic-Djokic, *J. Anal. Chem.*, 2009, vol. 64, p. 231.
34. A.F.Espinosa-Mansilla, F.Salinas, I.De Orbe Paya, *Anal. Chim. Acta*, 1995, vol. 313, p. 103.
35. L. Lopez Paz, J. M. Calatayud, *J. Pharm. Biomed. Anal.* 11 (1993) 1093–1098.
36. Y. S. Chandra, V. S. Rao, P. S. R. Murthy, Y. Siva Chandra and V. Suryanarayana Rao, *Indian J.Pharm. Sci.* 58 (1996) 157–159.
37. M. S. Mahrous, M. M. Abdel-Khalek, *Talanta* 31 (1984) 289–291.
38. U. Saha, A. K. Sen, T. K. Das, S. K. Bhowal, *Talanta* 37 (1990) 1193–1196.
39. N. P. Sadler, H. Jacobs, *Talanta* 42 (1995) 1385–1388.