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# QUANTIFICATION OF DOXYCYCLINE HYCLATE IN TABLETS BY ULTRAVIOLET SPECTROPHOTOMETRIC METHOD

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**Abstract-** Doxycycline hyclate is a broad-spectrum antibiotic, used as both human and veterinary medicine. This work proposed the development and validation of an ultraviolet spectrophotometric method for the determination of doxycycline hyclate in tablets. The method was completely validated according to the International Conference on Harmonization guidelines, showing linearity, accuracy, precision, selectivity and robustness. In the concentration range of 6-21 mg $L^{-1}$ it was linear with correlation coefficients greater than 0.9997 and limits of detection and quantification of 0.12 and 0.37 mg $L^{-1}$, respectively. The validated method is suitable and very helpful to the routine quality control of doxycycline hyclate, since it does not use polluting reagents, it is fast and cost effective.

**Keywords-** doxycycline hyclate, quality control, tablets, ultraviolet spectrophotometric, method validation

## Introduction

The oxytetracycline is a natural product produced by *Streptomyces rimosus*. Tetracycline is a semisynthetic derivative of chlortetracycline. Metacycline, doxycycline and minocycline are all semisynthetic derivatives of a strain of *S. aureofaciens* [1].

The synthetic pathway of doxycycline involves metacicline as an intermediate, during this process 6-epidoxycycline can be formed as a side product. Doxycycline is a spectrum broad semisynthetic antibiotic, widely used in veterinary medicine and as an animal feed supplement to prevent diseases [2].

Doxycycline hyclate has the molecular formula $C_{22}H_{24}N_2O_8$ HCl $0.5C_2H_5OH$ 0.5 $H_2O$ and molecular weight 512.94 g $mol^{-1}$. It is the hydrochloride hemiethanol hemihydrate of doxycycline. Doxycycline hyclate is much more soluble than doxycycline monohydrate, which is one of the main reasons for its more frequent use in pharmaceutical samples [3].

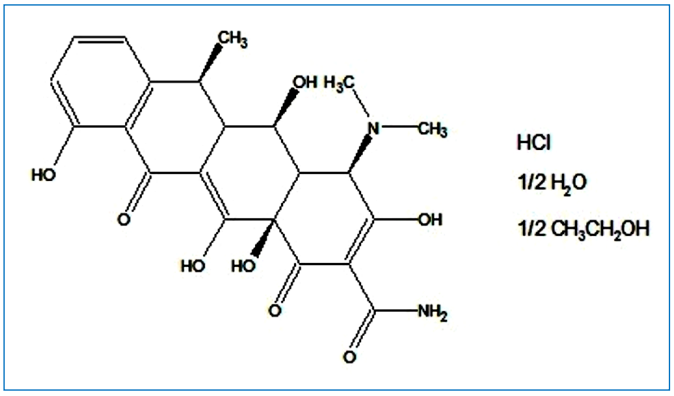


**Fig. 1-** Chemical structure of doxycycline hyclate CAS 24390-14-5. The doxycycline hyclate burn without melting at 201°C [4]. pKas values of doxycycline hyclate are: 3.02 ± 0.3 pKa1; 7.97 ± 0.15 pKa2; 9.15 ± 0.3 pKa3 [5].

The doxycycline hyclate became more interesting by having different mechanism of action of other tetracycline. Tetracycline inhibit bacterial protein synthesis through their link to the bacterial 30S ribosome, impeding access of aminoacyl-tRNA acceptor site in the mRNA-ribosome complex [1]. However, doxycycline hyclate has been studied as an inhibitor of matrix metalloproteinases, an action unrelated to its effects on bacterial protein synthesis [1,6].

Gastrointestinal irritation and perturbation of intestinal bacterial flora occur less frequently with doxycycline than with the more hydrophilic drugs, which must be given in higher doses for absorption [7]. As doxycycline is not interfered by food uptake is possible to improve the tolerability to administer the drug with food [1].

Doxycycline is more active than others tetracyclines against many bacterial species including *Streptococcus pyogenes*, *Nocardia spp.*, enterococci and several anaerobic [8]. Doxycycline is preferred to other tetracyclines in the treatment of specific infections because of its fairly reliable absorption and its less frequent dosage. It is used to treat chronic prostatitis, sinusitis, syphilis, chlamdyia, and pelvic inflammatory disease [3]. It has action against protozoa and must be administered in combination with quinine in the management of chloroquine resistant *Plasmodium falciparum* [8].

Doxycycline is also used for malignant effusions in solution form Yellin [9] which occur when there is an increase in the amount of biological fluids, generally associated with malignancies and lymphomas of the lung, breast and ovary [8].

The literature described different methods for quantitative determination of doxycycline in milk [5,10,11] and biological samples [3,5,12-14]. These techniques include high performance liquid chromatography (HPLC) with ultraviolet (UV) spectrophotometric detection.

In pharmaceuticals products, analytical procedures have been reported for the analysis of doxycycline hyclate using HPLC with UV spectrophotometric detection. However, HPLC techniques for routine analysis are often time consuming and costly. Moreover, the described spectrophotometric method for determination of doxycycline hyclate requires the use of toxic solvents as methanol [15].

For an alternative to existing method, the aim of this study was to develop, validate and apply a low cost, fast and simple UV spectrophotometric method for quantitative routine determination of doxycycline hyclate in pharmaceuticals products.

## Experimental

### Equipments

The equipments used were Shimadzu UV mini - 1240 (Kyoto, Japan); ultrasonic bath Unique USC2800A (São Paulo, Brazil); analytical balance Kern 410 (Kern, Germany); water purification system Millipore (Bedford, EUA) and liquid chromatograph system Waters 1525 (California, USA) connected to a UV/Visible Waters 2487 and an injector fitted Rheodyne Breeze 7725i with a 20 µL loop.

### Chemicals and Reagents

Hydrochloric acid Qhemis (Brazil), acetonitrile J.T. Baker (USA), trifluoroacetic acid Sigma Aldrich (Germany), Milli Q water Millipore (EUA) were used to prepare the solutions for the UV spectrophotometric and HPLC methods. Doxycycline hyclate standard (assigned purity 97.10% lot 0900002795) and tablets (Doxitrat tablets 80 mg) were kindly supplied by União Química Indústria Farmacêutica, Pouso Alegre, Brazil. Diluent was $1x10^{-2}$ mol $L^{-1}$ of hydrochloric acid. Stock standard solution equivalent to 100 mg $L^{-1}$ doxycycline hyclate was prepared by dissolving an accurately weighed amount of pure drug in the diluent solution. The placebo mixtures were prepared in the laboratory by mixing appropriate amounts (commonly used in tablets) of following pharmaceutical grade excipients: hydroxypropylmethylcellulose, polyethylenoglycol, mannitol, talc, starch, microcrystalline cellulose, croscamelose and magnesium stearate.

### UV Spectrophotometric Method

UV spectra of reference and sample solutions were recorded in 1 cm quartz cells. The absorbance values obtained in the spectra were obtained at 268 nm for quantification of Doxitrat tablets 80 mg. The spectrophotometric measurements were recorded by using $1x10^{-2}$ mol $L^{-1}$ of hydrochloric acid solution as a blank solution.

### HPLC Method

The chromatograms of reference and sample solutions were obtained on a Luna CN column particle of 5 µm (250 mm x 4.6 mm) and pore 10A° (Phenomenex, USA). The mobile-phase consisted of water + 0.1% trifluoroacetic acid (TFA): ACN + 0.1% TFA (60:40, v/v), flow rate 1 ml/min, injection volume 20 µL, using a UV-VIS detector at 360 nm and at room temperature [16].

### Preparation of Solutions

### Stock and Working Standard Solutions

Stock standard solution containing 100 mg $L^{-1}$ of doxycycline hyclate was prepared by accurately weighing 10.3 mg of doxycycline hyclate reference substance into a 100 mL volumetric flask and complete with $1x10^{-2}$ mol $L^{-1}$ of hydrochloric acid solution. Working standard solutions were prepared immediately before use by suitable dilutions of the corresponding stock solutions to appropriate concentration levels by using $1x10^{-2}$ mol $L^{-1}$ of hydrochloric acid solution as diluent.

### Sample Solutions

Twenty tablets of Doxitrat 80 mg were used. The tablets were weighed and totally pulverized. The mass equivalent to one tablet doxycycline hyclate was weighed into a 100 mL volumetric flask and complete with $1x10^{-2}$ mol $L^{-1}$ of hydrochloric acid solution. Appropriate dilutions were made into the range of calibration curve by using the same solvent.

### Method Validation

Method validation was performed according to International Conference on Harmonization (ICH) specifications [17] for linearity, selectivity, accuracy, precision, robustness, detection limit and quantitation limit.

### Linearity

Linearity was evaluated by regression analysis of doxycycline hyclate standard solutions at six concentration points in triplicate ranging from 6 to 21 mg $L^{-1}$ prepared on three consecutive days (n = 3). The values are reported as the mean ± S.D. of the calibration curves. The data were analyzed at 268 nm. Correlation coefficient and. analysis of variance (ANOVA) were calculated and presented.

### Selectivity

Selectivity was evaluated by analysis of the spectra of placebo solutions and the doxycycline hyclate working standard solution at the concentration of 15 mg $L^{-1}$. The placebo solutions of Doxitrat 80 mg containing the same composition as the pharmaceutical formulation were prepared for this study.

### Accuracy

The method accuracy was determined by measuring the reference standard recovery in triplicate at three levels from 80 to 120% of the method concentration (15 mg $L^{-1}$), according to ICH recommendations. A standard stock solution containing 100 mg $L^{-1}$ of doxycycline hyclate was prepared in $1x10^{-2}$ mol $L^{-1}$ of hydrochloric acid. Aliquots of 0.6, 0.9 and 1.2 mL of this standard solution (concentrations of 6.0, 9.0 and 12.0 mg $L^{-1}$, respectively) were individually added to 0.6 mL of sample solutions at 100 mg $L^{-1}$ (concentration of 6.0 mg $L^{-1}$), in volumetric flasks of 10 mL. The final concentrations were 12.0, 15.0 and 18.0 mg $L^{-1}$, which correspond to 80, 100 and 120% of the target concentration, respectively. The mean recoveries were expressed in terms of percent recovery of the tablets (Doxitrat 80 mg) by the assay and the respective relative standard deviation (R.S.D.) were determined.

### Precision

Precision was evaluated with respect to both repeatability and intermediate precision. Repeatability was evaluated by examining doxycycline hyclate work standard solutions at the same concentration and during the same day. Intermediate precision was studied by repeating the assays on two different days by two analysts. Six replicates at a concentration of 15 mg $L^{-1}$ were prepared and assayed. The data were analyzed at 268 nm. The percentages of relative standard deviation (R.S.D.) of the analytical responses were calculated.

### Robustness

The robustness was evaluated by analyzing data after changing the wavelength doxycycline hyclate working standard solutions at the concentration of 15 mg $L^{-1}$ were used in these experiments.

### Limits of Detection and Quantification

The limit of detection (LOD) and the limit of quantification (LOQ) of

the methods were obtained from the Eq-1 and Eq-2:

$$LOD = 3\ (S.D./a) \quad (1)$$

$$LOQ = 10(S.D./a) \quad (2)$$

where S.D. is intersection standard deviation and *a* is the mean slope, obtained from calibration curves of the linearity study.

### Assay of Pharmaceutical Products

The validated UV spectrophotometric method was applied for doxycycline hyclate quantitation in tablets (Doxitrat tablets 80 mg). The results were obtained by comparison of the sample spectrophotometric measurements (n=6) with those obtained from doxycycline hyclate standard solutions (n=6) at the same concentration levels.

### Comparison of UV and HPLC Methods

The results obtained in this study were compared with those by HPLC. The methods were compared through the F test (Snedecor) of homogeneity of variance and t (Student), which indicates whether there is a significant difference between these methods at a 5% significance level.

## Results and Discussion

### Method Development

The reported method for the determination of doxycycline hyclate is time consuming and requires the use of toxic solvent as methanol. In this paper, a non-toxic solvent was chosen in order to obtain a low cost, simple and environmentally friendly spectrophotometric method for quantification of doxycycline hyclate in tablets. Zero-order UV spectrum of doxycycline hyclate in $1x10^{-2}$ mol $L^{-1}$ of hydrochloric acid showed maximum drug absorption at 268 nm. No significant interference from the tablet excipients was verified in the region of doxycycline hyclate absorption spectrum, so the analytical use of zero-order spectrophotometry.

### Method Validation

After identifying derivative order and the wavelength of maximum absorption (268 nm for Doxitrat tablets 80 mg), the analytical method was validated according to ICH recommendations [16].

### Linearity

The analytical curves, generated on three consecutive days (n=3) by plotting the mean absorbance values of spectra at 268 nm against concentration yielded correlation coefficients greater than 0.9997. Additionally, the data were validated by means of analysis of variance [Table-1], which showed significant linear regression (Fcalculated > Fcritical, P = 5%) and no significant lack of fit (Fcalculated < Fcritical, P = 5%).

**Table 1-** Linearity parameters for the determination of doxycycline hyclate[a] and summary of ANOVA

| Parameter | 268 nm |
|---|---|
| Linearity range (mg $L^{-1}$) | 06-21 |
| Slope | 0.0387 ± 0.0005 |
| Intercept | -0.0253 ± 0.0014 |
| Correlation coefficient (r) | 0.9997 ± 0.0001 |
| Regression | 963.64 (4.75) |
| Lack of fit | 0.01 (3.26) |

*[a]Values are reported as mean ± S.D. of three calibration curves generated on three consecutive days (n = 3).*

### Selectivity

The spectra analyses show that formulation excipients of the pharmaceutical tablet product Doxitrat 80 mg did not interfere significantly in the spectrophotometric method [Fig-2].

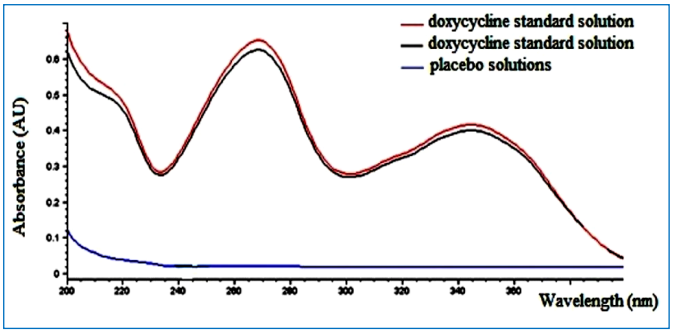


**Fig. 2-** Overlap of the spectra of placebo solution, doxycycline hyclate standard solution and doxycycline hyclate sample solution at 15 mg $L^{-1}$ in $1x10^{-2}$ mol $L^{-1}$ of hydrochloric acid.

### Accuracy

The accuracy of the method was confirmed by determining the average recoveries from the samples by applying the standard addition method. As shown in [Table-2], the mean recovery percentages of product Doxitrat 80 mg was in accordance with fixed limits of 98.0 to 102.0%, indicating the suitability of the developed method in quantifying the concentration of doxycycline hyclate in pharmaceutical tablets.

**Table 2-** Method accuracy results for doxycycline hyclate tablets

| Samples at 6 mg L-1 | Reference standard concentration (mg L-1) | | R.S.D. (%) n=3 | Recovery (%) | Mean Recovery (%) |
|---|---|---|---|---|---|
| | Added | Found | | | |
| Doxitrat 80mg (268 nm) | 6.0 | 5.92 | 1.41 | 98.67 | 99.02 |
| | 9.0 | 8.96 | 1.03 | 99.56 | |
| | 12.0 | 11.86 | 0.32 | 98.83 | |

### Precision

Repeatability (intra-day precision) of the analytical method was found to be reliable based on % R.S.D. (< 2%). Intermediate precision (inter-day precision) was demonstrated on different days by two analysts. The %R.S.D. values were less than 2%, confirming that the method is sufficiently precise [Table-3].

**Table 3-** Method precision results for doxycycline hyclate tablets

| Wave length | Level | Absorbances | | | | | | R.S.D (%) |
|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | |
| 268 nm | A | 0.55 | 0.55 | 0.55 | 0.54 | 0.55 | 0.55 | 0.2 (n=6) |
| | B | 0.54 | 0.54 | 0.55 | 0.54 | 0.54 | 0.55 | 0.4 (n=12) |
| | | 0.55 | 0.55 | 0.55 | 0.54 | 0.55 | 0.55 | |

*A = Repeatability, B = Intermediated precision*

### Robustness

**Table 4-** Robustness test results

| Test | Wavelength (nm) | | | |
|---|---|---|---|---|
| | 266 | 268 | 268 | 270 |
| Fcal | 8.35 | | 1.83 | |
| Ftab | 19 | | 19 | |
| *t*cal | 2.64 | | 2.07 | |
| *t*tab | 2.78 | | 2.78 | |

The results obtained in robustness test are shown in [Table-4]. The influence of variation in 2 units of wavelength up and down to the

working wavelength (268 nm) was evaluated statistically. The robustness was confirmed by F test (Snedecor) homogeneity of variance and *t* (Student) to compare the mean, which showed Fcalculated < Fcritical, P = 5% and *t*calculated < *t*critical, P = 5%. Thus, the mean are equivalent.

### Limits of Detection and Quantification

LOD and LOQ values were found to be respectively 0.123 and 0.371 mg $L^{-1}$ for Doxitrat tablets 80 mg (268 nm). The values are close to zero which indicate the sensitivity of the method.

### Assay of pharmaceutical products

The validated method was applied for determination of doxycycline hyclate in tablets. Samples from Doxitrat 80 mg tablet were analyzed. The results are shown in [Table-5].

**Table 5-** Assay of doxycycline hyclate in pharmaceutical tablets samples

| Day | Content of doxycycline hyclate[a] (mg $L^{-1}$) | (%) | Mean content | R.S.D.(%) |
|---|---|---|---|---|
| 1 | 16.65 | 111.01 | | |
| 2 | 16.58 | 110.56 | 110.68 | 0.26 |
| 3 | 16.57 | 110.47 | | |

[a]*Each value corresponds the mean of six determinations.*

### Comparison of UV and HPLC Methods

The results obtained in this study were comparable with those obtained by HPLC. For the UV spectrophotometric method, the content of doxycycline hyclate found was 111.34% and for HPLC method, 110.07%.

Comparative analysis of the two methods was not statistically significant for a significance level of 5% [Table-6].

**Table 6-** F test (Snedecor) and t (Student) to analyze the results of two different methods for the quantification of doxycycline hyclate in tablets

| Tests | Methods |
|---|---|
| | UV spectrophotometric HPLC |
| Fcal | 2.67 |
| Ftab | 19 |
| P value | 0.27 |
| tcal | 1.89 |
| ttab | 2.78 |
| P value | 0.13 |

The quantification of medicaments by methods such as UV spectrophotometry and HPLC are widely used in the department of Quality Control. Despite HPLC be the method of choice for the analysis of doxycycline hyclate in tablets, UV spectrophotometric method is simpler, faster and it has low cost. The liquid chromatograph is more expensive than a UV spectrophotometer, in this analysis is not necessary the preparation of mobile phase and the beginning of the work is faster because it is not necessary to acclimatize the column, for example.

### Conclusions

In this work, an analytical UV spectrophotometric method was successful developed for quantitative determination of doxycycline hyclate in tablets. Its advantages over other existing method are its simplicity, fastness, inexpensive conditions and it does not use polluting reagents.

The results indicated that the UV spectrophotometric method presents linearity, adequate detection and quantification limits, selectivity, precision, accuracy and robustness. Therefore, the validated method can be applied in routine analysis of doxycycline hyclate.

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This paper is dedicated to School of Pharmaceutical Sciences on the occasion of its 90th anniversary.

**Conflicts of Interest:** None declared.

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