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A rapid and reliable determination of doxycycline hyclate by HPLC with UV detection in pharmaceutical samples

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Abstract: An accurate, sensitive and reproducible high performance liquid chromatographic (HPLC) method for the quantification of doxycycline hyclate in pharmaceutical samples has been developed and validated. The drug and the standard were eluted from a Lichrosorb RP-8 (250 mm×4.6 mm, 10 μm particle size) at 20 °C with a mobile phase consisting of methanol, acetonitrile and 0.010 M aqueous solution of oxalic acid (2:3:5, v/v/v). The flow rate was 1.25 ml min^{-1}. A UV detector set at 350 nm was used to monitor the effluent. Each analysis required no longer than 4 min. The limits of detection and quantification were 1.15 and 3.84 μg ml^{-1}, respectively. Recoveries for different concentrations ranged from 99.58 to 101.93 %.

Keywords: high performance liquid chromatography; doxycycline hyclate; ultraviolet detection.

INTRODUCTION

Doxycycline ($C_{22}H_{24}N_2O_8 \cdot H_2O$, molecular mass 462.5 g mol^{-1}, CAS number: 17086-28-1), is the monohydrate of (4*S*,4a*R*,5*S*,5a*R*,6*R*,12a*S*)-4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxonaphthacene-2-carboxamide, a substance obtained from oxytetracycline or metacycline or by other means.[1] It is a broad spectrum anti-bacterial tetracycline derivative with a wide range of activity against gram positive and gram negative organisms, including *Spirochetes, Actinomyces* sp., and *Mycoplasma* sp.[2] It is the drug of choice in the treatment of sexually transmitted diseases.[3] Doxycycline is preferred to other tetracyclines in the treatment of specific infections because of its fairly reliable absorption and its long half-life, which permits less frequent dosage. Doxycycline hyclate ($C_{22}H_{24}N_2O_8 \cdot HCl \cdot 0.5C_2H_5OH \cdot 0.5\ H_2O$, molecular

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mass 512.94 g mol^{-1}, CAS number: 24390-14-5) is the hydrochloride hemiethanol hemihydrate of doxycycline. The synonym for doxycycline hyclate is doxycycline hydrochloride. Doxycycline hyclate is much more soluble than doxycycline monohydrate, which is one of the main reasons for it more frequent use in pharmaceutical samples. One of the broadly used and important veterinary pharmaceuticals is Tiadox powder. It is a complex powder with doxycycline hyclate as the main component.

Various methods for determination of doxycycline *in vitro* and *in vivo* have been reported. These include microbiology,[4] fluorimetry,[5] TLC-fluorescence scanning densitometry[6] and HPLC[7–17] for the determination of doxycycline in biological materials. HPLC was also applied for the determination of doxycycline in pharmaceutical formulations.[18] Various chromatographic methods have also been reported for the determination of doxycycline in human tissues[19] and foods.[20,21] Derivative spectrophotometry (pharmaceuticals, urine and honey)[22] and sequential injection chromatography (SIC) methods (pharmaceutical preparations)[23] for the determination of doxycycline have also been developed. Reviewing literature data, it was concluded that there are a few methods reported for the determination of doxycycline hyclate in pharmaceutical samples (spectrophotometric methods[24–26] and HPLC methods[27,28]). The standard procedure is given in the British Pharmacopoeia.[29]

HPLC offers several advantages over other techniques, including minimal sample manipulation before chromatography, rapid analysis and the simultaneous analysis of multiple compounds with good specificity, precision and accuracy. For this reason, a fast, simple method for the determination of doxycycline hyclate in the mentioned veterinary powder (Tiadox powder) was developed and is presented in this paper. The proposed method is reproducible, reliable and permits more samples to be analyzed in a short period of time.

EXPERIMENTAL

Reagents and chemicals

Doxycycline was obtained from PromoChem, Teddington, United Kingdom. All the employed solvents were of HPLC grade, while other chemicals were of spectroscopic grade and were obtained from Merck (Darmstadt, Germany). The Pharmaceutical Industry "Actavis", Leskovac, supplied the Tiadox powder. It consists of various ingredients, among them doxycycline hyclate (10.0 g doxycycline hyclate per 100 g of Tiadox powder) represents the only active component.

HPLC apparatus and conditions

The HPLC system consisted of an Agilent 1100 series isocratic LC system, an Agilent 1100 series variable wavelength detector SL and a Hewlett Packard 1000 data system (Agilent Technologies, Inc., Santa Clara, CA, USA). The detector wavelength was set at 350 nm. The mobile phase consisted of methanol:acetonitrile:0.010 M oxalic acid (2:3:5, v/v/v). Routine degassing of the mobile phase was performed by passing it through a 0.45 μm membrane filter (Millipore, Bedford, MA, USA). The mobile phase was pumped isocratically at a flow rate of 1.25 ml min^{-1} at 20 °C. The injection volume was 50 μl.

Stock solutions

Doxycycline hydrochloride (25 mg) was dissolved in 25 ml of mobile phase. The working solution was obtained by dilution of 1.0 ml of the stock solution to 10 ml with mobile phase.

Assay procedure

Tiadox powder (250 mg) was dissolved in 25 ml of mobile phase. This solution (1.0 ml) was diluted to 10 ml with mobile phase.

RESULTS

Assay validation

A high performance liquid chromatographic method for the quantification of doxycycline hyclate concentrations in a pharmaceutical sample (Tiadox powder) was developed. The active ingredients were monitored by measurement of the peak area of both doxycycline hyclate from Tiadox powder and the standard, and the ratio of the peak areas was calculated. The specificity of the chromatographic method was determined by the screening of a placebo solution and the assay solution. The placebo solution was prepared in the same manner as the investigated solution but without doxycycline hyclate. Typical chromatograms of the standard and assay solution are presented in Figs. 1 and 2, respectively.

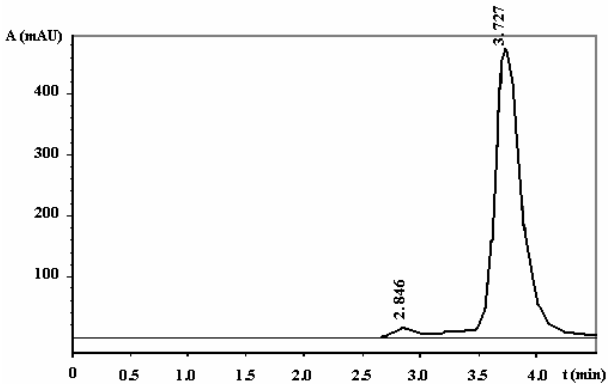

Fig. 1. Representative chromatogram of a standard solution of doxycycline hyclate.

Linearity

The linearity was checked on samples of standard doxycycline hydrochloride at five different concentrations (25.2–252.0 μg ml^{-1}).

A regression curve was constructed:

$$y = 77498x - 72.455, \text{ with } R^2 = 0.9997$$

where x represents concentration in μg ml^{-1}, y represents the HPLC peak area, which was automatically measured by an integrator of the HPLC instrument, and R is the correlation coefficient. The calculations were performed on a personal computer using the Microsoft Excel program (Version 2003, Microsoft Co., Redmond, USA, 2003).

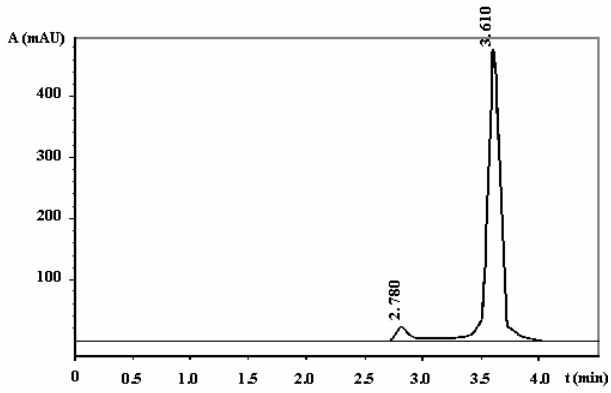

Fig. 2. Representative chromatogram of an assay solution – – Tiadox powder.

Accuracy

The accuracy of the method was checked by determining recovery values. Series of solution were made containing 80, 100 and 120 % of doxycycline hyclate regarding the declared content. The results are presented in Table I.

TABLE I. Accuracy of the method

Concentration g/100g	Recovery %	*N*	x_{sr}	*SD*	*RSD* %
8.00	101.7	3	101.5	0.1819	0.1809
	101.4				
	101.4				
10.00	101.8	3	100.5	1.108	1.102
	100.2				
	99.6				
12.00	100.8	3	101.6	0.6264	0.6167
	101.9				
	101.9				

Limit of detection and quantification

The limit of detection (*LOD*) and quantification (*LOQ*) were calculated according to the following formula:

$$LOD = 3S_{do}/b_{sr} = 1.15 \text{ μg ml}^{-1}$$

$$LOQ = 10S_{do}/b_{sr} = 3.84 \text{ μg ml}^{-1}$$

where S_{do} is the standard deviation of the response and b_{sr} is the mean value of the slope of the calibration curve constructed during the linearity determination.

Precision

The precision was determined by measuring five sample probes under the same experimental conditions. The obtained results are given in Table II, together with the calculated values of their standard deviation, *SD*, and relative standard deviation, *RSD*.

TABLE II. Precision of the method

N	Content of doxycycline hyclate in Tiadox powder, g/100g	x_{sr}	*SD*	RSD %
5	9.98 9.71 9.81 9.78 9.82	9.82	0.0992	1.01

The method is precise since $RSD < RSD_{max}$. RSD_{max} is 2 %, which represents the maximum allowed value of the *RSD* for HPLC methods according to the Pharmacopoeias.

DISCUSSION

A comparison of the proposed method with other methods is given in Table III. The results obtained by the proposed method have an *RSD* of 1.01 %, better than that reported by Šatínský *et al.*[22], Salinas *et al.*[23] and Lopez-Paz and Martinez Calatayud.[24] The first two methods[22,23] have relatively high *RSD* values, higher than RSD_{max}. This suggests that the proposed method is more precise than some of the previously published methods. The *LOD* values[22,23] were also a little higher than in the method described in this paper. Mahrous and Abdel-Khalek[25] described a long pre-treatment of the drug through mixing with acetic acid and sodium cobalt nitrite, then boiling the mixture, followed by cooling. In addition, the same author[26] described a determination employing ammonium vanadate. On the other hand, Hoogmartens *et al.*[27] revealed a method for the determination of doxycycline hyclate by HPLC using poly(styrene–divinylbenzene) packing materials. Estimates for the repeatability and reproducibility of the method, expressed as relative standard deviations of the results of the determination of doxycycline hyclate, were found to be 0.90 and 1.20 %, respectively. The authors[27] used a complex mobile phase, consisting of 2-methyl-2-propanol, 0.2 M potassium hydrogen phosphate buffer, tetrabutylammonium hydrogen sulfate and 0.10 M Na_2EDTA. Using this mobile phase system, the analysis time is about 20 min, which is 5 times longer, compared to the method proposed in this paper.

TABLE III. Comparison of the proposed method with other methods for the determination of doxycycline hyclate in pharmaceutical preparations

Ref.	Concentration range, μg ml^{-1}	*RSD*[a] %	Recovery %	*LOD* μg ml^{-1}	Comments, conditions
This paper	25.20–252.00	1.01	100.2	1.15	HPLC, UV detection
22	2.00–100.00	5.05	99.3 102.8	2.00	Sequential injection chromatography
23	–	0.78–5.00[b]	95	0.6-3.00[b]	Derivative spectrophotometry
24	10.00–80.00	1.40[d]	2.3[c]	–	FIA-spectrophotometry

Table III. Continued

Ref.	Concentration range, μg ml^{-1}	*RSD*[a] %	Recovery %	*LOD* μg ml^{-1}	Comments, conditions
25	10.00–30.00	–	100.3	–	Spectrophotometry
26	20.00–100.00	–	99.6	–	Spectrophotometry
28	–	0.1–1.7[e]	92–107[e]	–	HPLC, UV detection

[a]*RSD* values were taken and compared for concentrations of ≈ 10 μg ml^{-1} of doxycycline; [b]ranges of *RSD* and *LOD* values at different first-derivative signals; [c]relative error; [d]*RSD* for samples containing 2.5 μg ml^{-1} of doxycycline; [e]recovery range for different pharmaceuticals

CONCLUSION

The proposed HPLC method provides a rapid, sensitive, accurate, and reproducible determination of doxycycline hyclate in pharmaceutical samples, without sample derivatization.

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И З В О Д

БРЗО И ПОУЗДАНО ОДРЕЂИВАЊЕ ДОКСИЦИКЛИН-ХИКЛАТА МЕТОДОМ HPLC СА UV ДЕТЕКЦИЈОМ У ФАРМАЦЕУТСКИМ УЗОРЦИМА

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Тачна, осетљива и репродуктивна метода високо ефективне течне хроматографије (HPLC) за квантификацију доксициклин-хиклата у фармацеутским узорцима је развијена и потврђена. Лек и стандард су елуирани са колоне Lichrosorb RP-8 (250 mm×4,6 mm, величине честице од 10 μm) на 20 ºC са мобилном фазом која се састојала од метанола, ацетонитрила и 0,010 M воденог раствора оксалне киселине (2:3:5, v/v/v). Брзина протока је била 1,25 ml min^{-1}. За праћење ефлуента коришћен је UV детектор подешен на 350 nm. Свака анализа је трајала не дуже од 4 минута. Граница детекције је 1,15, док је граница квантификације 3,84 μg ml^{-1}. Ефикасност за различите концентрације се креће од 99,6 до 101,9 %.

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