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QUANTITATIVE ESTIMATION AND SEPARATION OF DOXYCYCLINE HCl AND ITS RELATED PRODUCTS.

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ABSTRACT

An HPLC method is presented for the separation of doxycycline HCl from its analogs. The method, employing on line U.V.detection allows separation and quantitative estimation of doxycycline HCl when its analogs are present upto 1% w/w. By this method doxycycline HCl powder and tablets were analysed.

INTRODUCTION

Doxycycline HCl, on degradation can be converted to one or more of the following products: oxytetracycline HCl, tetracycline HCl, methacycline HCl and 6-epidoxycycline HCl. These products may even occur as impurities during manufacturing of doxycycline HCl(1-2). The existing fluorimetric and microbiological procedures have good sensitivity but are unable to differentiate doxycycline HCl from its analogs (3-5). Various TLC methods can be used for separation (6-8), but quantitative estimation by densitometer has large variation. Some papers have reported HPLC use in determination of tetracycline and tetracycline analogs (9-10). An HPLC method was standardised to separate doxycycline HCl and its degradation products, keeping in view to estimate quantitatively doxycycline HCl in pharmaceutical preparations and to limit the impurities.

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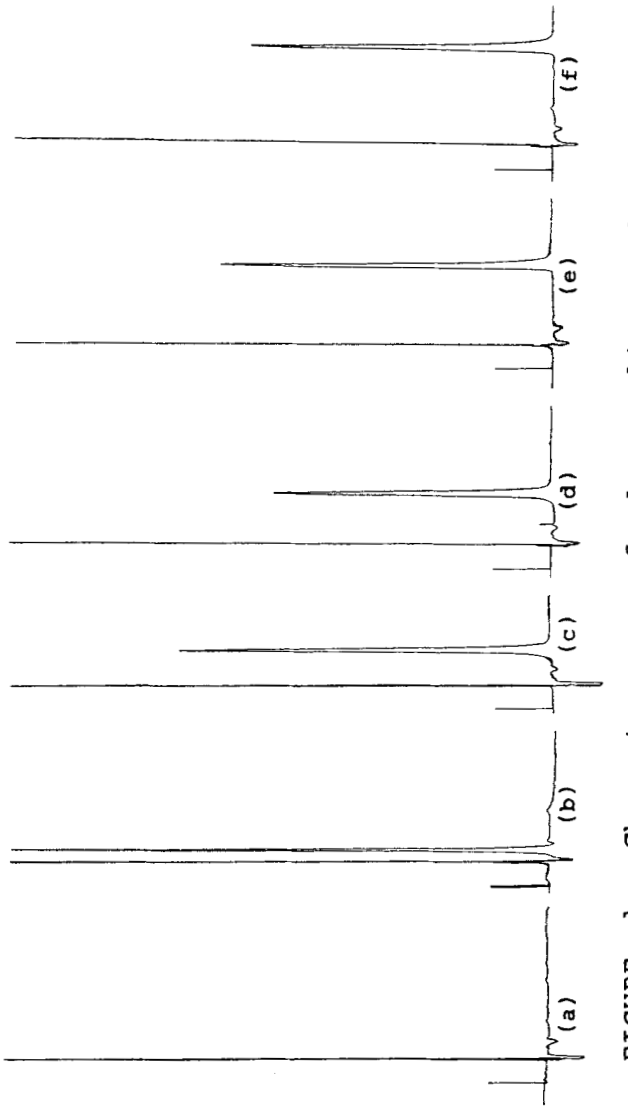

FIGURE 1. Chromatograms of doxycycline HCl and its analogs. Key: (a) Blank; (b) oxytetracycline HCl; (c) doxycycline HCl; (d) tetracycline HCl; (e) methacycline HCl; (f) 6-epidoxycycline HCl.

MATERIALS USED

Doxycycline HCl, methacycline HCl and 6-epidoxycycline HCl were chemical reference substances (c), tetracycline HCl, oxytetracycline HCl as A/R products (d). HPLC apparatus with variable wavelength detector (e). A Nucleosil analytical column (f). All solvents used were chromatography grade.

OPERATING CONDITIONS

MOBILE PHASE:	Tetrahydrofuran	720 ml
	Dimethylformamide	100 ml
	Acetic acid	160 ml
	Distilled Water	20 ml
	EDTA disodium	15 mg
DETECTION:	Wave length	350 nm
COLUMN:	SS 250/6/4 NUCLEOSIL 10 CN	
pH OF SOLVENT:	3.65	
FLOW RATE:	2.0 ml/min	
VOLUME OF INJECTION:	20 microliters	
SENSITIVITY:	0.02	
SOLVENT OF DRUG:	Acidic Methyl Alcohol(1%HCl)	

EXPERIMENTAL

A blank of acidic methyl alcohol(1%HCl) was injected (Fig.1a). Each of the following products were accurately weighted and dissolved in acidic methyl alcohol (1% HCl): doxycycline HCl, oxytetracycline HCl, tetracycline HCl, methacycline HCl and 6-epidoxycycline HCl. Necessary dilutions were made and the final dilutions of each were injected separately (Fig.1b-f). All the products were mixed together and diluted further. This was injected to see the separation (Fig.3a). Four standard solutions of doxycycline HCl, of concentrations between 0.004 mg/ml and 0.016 mg/ml were prepared and injected (Fig.2a-d).

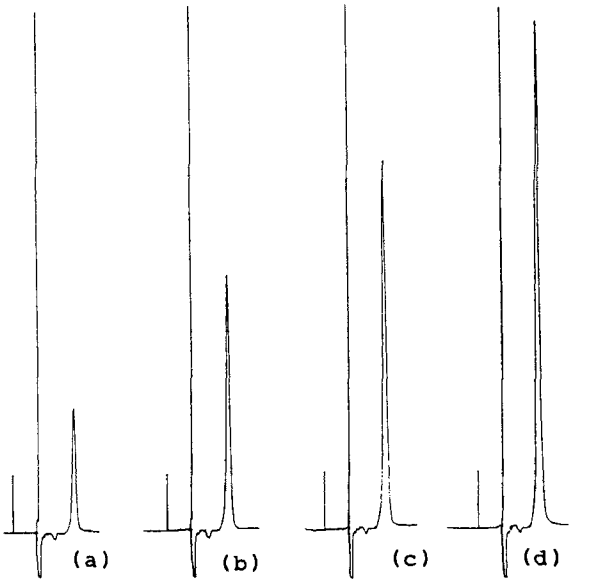

FIGURE 2. Chromatograms of doxycycline HCl standard solutions. Key: (a) conc. 0.004 mg/ml; (b) conc. 0.008 mg/ml; (c) conc. 0.012 mg/ml; (d) conc. 0.016 mg/ml

The areas under the peaks were integrated and a regression was made with area as function of concentration. Each standard solution was injected three times.

Two solutions of known concentrations were prepared with doxycycline HCl powder (a) and analysed in HPLC.

A solution containing the following:

Doxycycline HCl	0.0288 mg/ml
Oxytetracycline HCl	0.00064 mg/ml
Tetracycline HCl	0.00064 mg/ml
Methacycline HCl	0.00064 mg/ml
6-Epidoxycycline HCl	0.00064 mg/ml

was made and analysed by detection at 0.005 sensitivity (Fig.3b).

Doxycycline HCl tablets (b) were analysed for content of doxycycline HCl and to see degradation products. One whole tablet was weighted, crushed and taken in 250 ml volumetric flask. 200 ml of acidified methyl alcohol was added and the flask was kept in ultrasonic bath for 1 hr. After cooling, the volume was made up

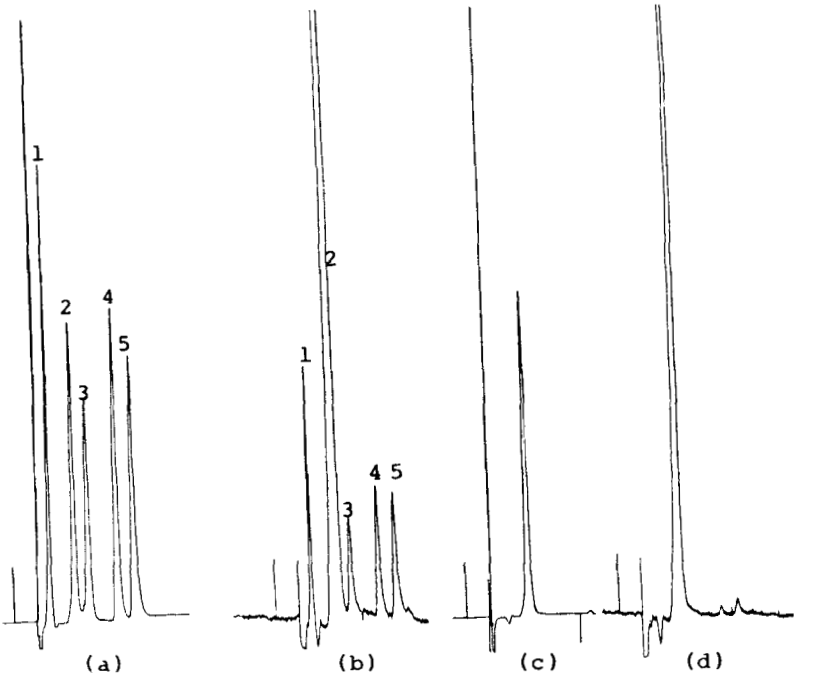

FIGURE 3. Numbers 1-5 represent peaks of: 1,oxytetracycline HCl; 2,doxycycline HCl; 3,tetracycline HCl; 4,methacycline HCl; 5,6-epidoxycycline HCl. Key: (a) chromatogram at sensitivity 0.02 of mixture of all products in equal proportion;(b) chromatogram at senstivity 0.005 of mixture containing 91.8% doxycycline HCl and 2.04% of the other analogs;(c) doxycycline HCl tablets at sensitivity 0.02;(d) doxycycline HCl at sensitivity 0.005.

and necessary dilution was made. The final dilution was analysed, once at sensitivity 0.02 and again at sensitivity 0.005 (Fig.3c-d). This was repeated three times.

RESULTS AND DISCUSSION

A linear regression (n=4) of areas under peaks vs concentration had coefficient of correlation = 0.9996. A clear separation of all products could be obtained and the retention times of products in mixture corresponded exactly to those, obtained after individual determination.

The retention times of the products were:

Oxytetracycline HCl	2.4 minutes
Doxycycline HCl	4.2 minutes
Tetracycline HCl	5.2 minutes
Methacycline HCl	7.2 minutes
6-Epidoxycycline HCl	8.4 minutes

The three tablets (b) contained 97.79 mg,99.12 mg and 103.11 mg of doxycycline HCl. The tablets contained less than 0.5% of 6-epidoxycycline HCl as impurity.
The doxycycline HCl powder (a) was found to contain 99.73% pure drug.
Degradation products could be quantitatively measured when present upto 2% w/w of doxycycline HCl, by detection at 0.005 sensitivity. The detection of analogs was possible even when present upto 1% w/w.
An increase of water proportion from 2 to 4% in mobile phase decreased the retention times of the products. This resulted in overlaping of doxycycline HCl and tetracycline HCl peaks. Change of dimethylformamide proportion from 100 to 130 and proportional decrease of tetrahydrofuran, increased the retention times of methacycline HCl and 6-epidoxycycline HCl, without effecting the elution of the other analogs.

FOOT NOTES

(a) Danish Powder and Tabletting Factory LTD, Copenhagen, Denmark
(b) DOMS Laboratories, Courbevoie, France.
(c) British Pharmacopoeia Comission Laboratory, Stanmore, U.K.
(d) Sigma Laboratories
(e) Waters Associates
(f) Société Francaise Chromatocolonne.

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