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Assay of Oxytetracycline by High Performance Liquid Chromatography

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High Performance Liquid Chromatography을 이용한 Oxytetracycline의 분석

기기분석과

강은미 · 박건용 · 손병목 · 김주형

=초 록=

캅셀중의 oxytetracycline 분석은 u-Bondapak C_{18} reverse phased column을 이용하여 50% methanol containing 0.05 M sodium-1-hexanesulfonate을 첨가한 이동상 및 365 nm 파장의 HPLC system에서 retention time 7.85 그리고 recovery rate 99.48%로 biological assay보다 신속하며 instrumental analysis에 따른 정확한 결과를 얻을 수 있었으므로 품질관리에 이용할 수 있을 것으로 생각된다.

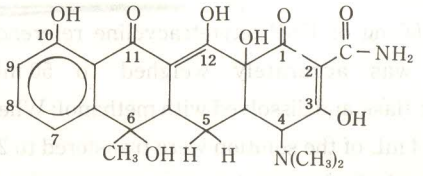
INTRODUCTION

Oxytetracycline is a synthetic antibiotics in tetracycline which is characterized by $C_{22}H_{24}N_2O_9$ and has 460.44 molecular size.

Tetracyclines have a common structure of 1,4,4α, 5α,6,11,12-octahydrophenacene (Table 1)[1]. These chemical substances generally have wide activities in human and animal pathogens by the mechanism of protein synthesis inhibition, particulary, have effects in variable infections such as bacterial and amebaoidal diarrheas, actinomycosis, and staphylococcus.

The characteristics of oxytetracycline are a yellow chrystalline powder, odourless and soluble in dilute acids, alkalis and ethanol, but insoluble in ether. The commercialized oxytetracycline preparations have been produced injections, capsules, tablets and troches as a single componant or in combination with others.

Table 1. Structural formulas of the tetracyclines.



Congener	Substituent(s)	position(s)
Chlortetracycline	-Cl	(7)
Oxytetracycline	-OH, -H	(5)
Demeclocycline	-OH, -H; -Cl	(6; 7)
Minocycline	-H, -H; -$N(CH_3)_2$	(6; 7)
Doxycycline	-OH, -H; -CH_3, -H	(5; 6)

The quality control of assays of these products have been officialized by antibiotics criteria formulary[2], USP[3] and BP[4]. These assays have been processed as biological tests: spectrophotometer, turbidimetric and cylinder-plate assays. However, the methods mentioned as above are not always available because of problems on the method which are not only management of bacterial strain for assay, experimental technique difficulties and particulary, long experimental time, but also the applications of body fluids such as serum and urine to human and animal tested.

Therefore, authors suggested that instrumental analysis method such as the high performance liquid chromatography (abbreviated as HPLC) was utilized as a good determination on the assay of oxytetracycline rapidly and accurately.

MATERIALS AND METHODS

Reference standard and sample preparation.

Reference standard* solutions were prepared at appropriate concentrations depending on the expected drug level in the linearity of standard calibration curve by dissolving and diluting the USP oxytetracycline reference standard.

Sample** was prepared by extracting a suitable quantity of capsule contents with methanol to obtain a solution cotaining about 240 μg of oxytetracycline per mL.

Calibration curve of USP oxytetracycline reference standard

About 100 mg of USP oxytetracycline reference standard was accurately weighed to 50 mL volumetric flask and dissolved with methanol; When 1, 2, 3 and 4 mL of the solution were transfered to 25 mL volumetric flasks, respectively and were diluted with methanol to concentrations of about 80, 160, 240 and 320 μg of oxytetracycline per mL, these met the requirement of the linearlity of standard calibration curve.

Recovery rate of oxytetracycline

Recovery rate was obtained from the theoretical amount versus sample which prepared from the combination of equvalent volume (1 : 1) of the above prepared standard and sample solution. And the data was achieved from the replications of five times.

*Referece standard: USP Oxytetracycline reference standard was obtained from U.S.P.C., INC. ROCKVILLE, MD.

**Sample: Capsule preparation contained oxytetracycline 500 mg.

All other chemicals were American Chemical Society reagent grade or equivalent except that chromatographic grade solvents were used for all chromatography and were filtered through a 0.45 μm membranfilter before use.

Chromatographic equipment

Chromatography was performed using a component chromatographic system composed of a Waters model 6000A solvent delivery system (Waters associates, Milford, Mass), a Waters 244 model U6K injector fitted with a fixed 100 μl loop, 440 UV detector and 730 data module.

The separation was performed on μ-Bondapak C_{18} column (300 mm×3.94 mm ID) packed with 10-μm O.D.S. bonded phase (Waters associates).

Other instruments for extraction and degasing were used a ultrasonic bath (Branson 8200).

Two mobile phases were consisted of well shaken mixtures of 50% metanol containing 0.05 M sodium 1

Table 2. Protocol of HPLC Conditions for Oxytetracycline Analysis.

Model	Waters 244 (U6K injector, 6000A solvent delivery system 440 UV detector, 730 data module)
Column	μ-Bondapak column (300 mm×3.94 mm I.D.)
Detector	365 nm
Attenuation	0.1 aufs.
Mobile phase	50%-Methanol containing 0.05 M sodium 1-hexane sulfonate (I) 30%-Acetonitrile containing 0.1 M citric acid (II)
Frow rate	0.9 mL/min. (2000 psi.)
Simple size	10 μL

-hexane sulfonate (ion-pair dhromatography; 0.94 g plus HAc 20 mL) or 30% acetonitrile containing 0.1 M citric acid.

RESULTS AND DISCUSSION

The HPLC system was used to determine oxytetracycline using a working hypothesis that for good resolution the tetracycline molecule must be in the form of a salt, the ionization characteristics of the tetracyclines were investigated by Knox *et al*[5] Thus, the amine function of the oxytetracycline molecule allowed the authors to design a HPLC system that operates between pH 3 and 6 gives for a good separation profile. With this theoretical background, the conditions HPLC system authors used, were shown in (Table 2).

The designs of mobile phase: The second solvent system was shown in Protocol was suggested by Nelis *et al*[6]. and was applied at these HPLC system authors used as a little modified condition for oxytetracycline determination. Alternately, the first solvent system was shown in Protocol suggested by authors was performed using an ion pairing reagent, in relation with the basic structure of oxytetracycline amine function. And uses of some differential methanol concentrations (30, 40, 50, 60 and 70%) in the mobile phases were necessary to achieve a good resolution and to complete the analyse in reasonable time. As a result, it was observed that reducing the nonpolar component of the mobile phase generally increased the retention times and improved separation.

Therefore, the efficient mobile phase system was designed as 50% methanol containing 0.05 M sodium -1-hexanesulfonate.

All assays on this study were analyzed under relative standard deviation (abbreviated as RSD) of less than 2% obtained from five replicated injections of standard solution (RSD: 0.93).

An assay for capsulated oxytetracycline: Suitable separations of the compound were achieved using the chromatographic condition mentioned in Protocol.

Retention time data and a typical chromatogram demonstrating determiniation or identification for oxytetracycline were studied in two mobile phases which were shown in Fig. 1 and Fig. 2. Thereby, according to prediction both mobile phase I and II were shown high absorption values in the condition of Protocol suggested and then, the mobile phase I was only used in this study because of the design that the mobile II was proposed by Nelis *et al*.

Linearity and precision of analysis: The linearity of the HPLC response for this study was calculated by a linear regression analysis of data for a series of

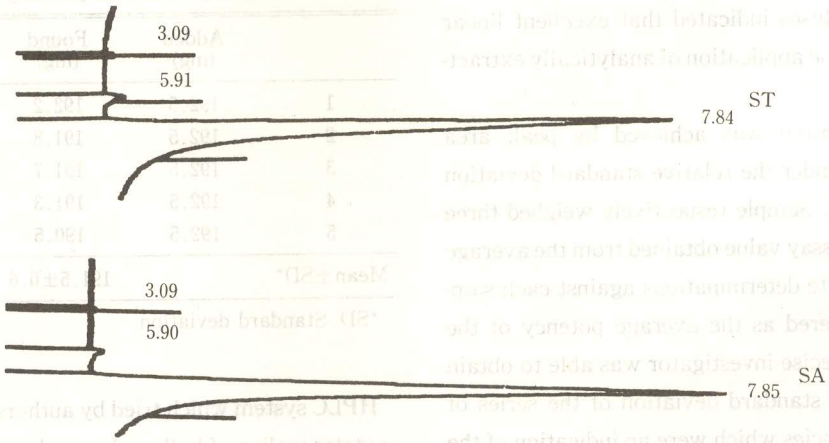


Fig. 1. Chromatogram of standard and sample applied with mobilephase I.

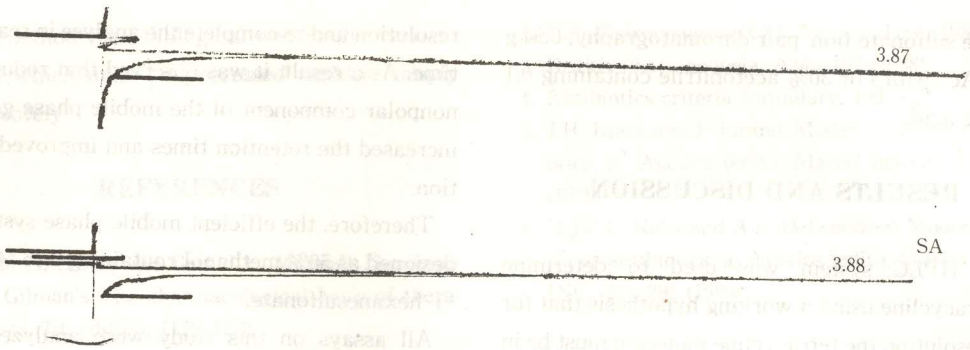


Fig. 2. Chromatogram of standard and sample applied with mobilephase II.

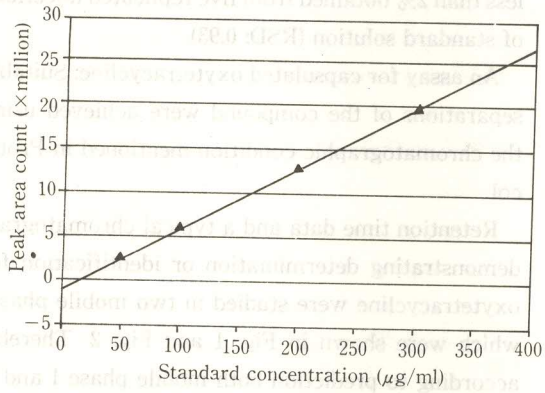


Fig. 3. Standard calibration curve.

standard weight injections from regression equation. Thus, y=69567x−1132961 and correlation coefficient was 0.9997. The scatter plot for the line of USP oxytetracycline reference standard solution was presented in Fig. 3. Therefore, from above parameters for the lines and correlation coefficients for the regression analyses indicated that excellent linear response over the application of analytically extracted samples.

The quantitation was achieved by peak area measurement under the relative standard deviation of less than 2%. Sample respectively weighed three times and the assay value obtained from the average of three replicate determinations against each sample was considered as the average potency of the sample. The precise investigator was able to obtain a value for the standard deviation of the series of individual potencies which were an indication of the sample 1, sample 2 and sample 3 (Table 2). It was considered that the extraction procedure or experimental error to test the recovery rate thus, this sample contained 191.50+0.64 mg in the found amount equivalent to 99.48+0.30% of recovery rates against 192.52 mg as thioretical amount with five replicated injections (Table 3).

Table 2. Assay results of oxytetracycline.

	Potency (μg per mg)	Percent of the labeled amount
name	mean±SD*	mean±SD
sample 1	902.43±14.74	99.57±1.60
sample 2	891.60± 6.05	98.33±0.68
sample 3	904.40±30.34	99.83±3.37
sum	899.40± 6.89	99.23±0.81

*SD, Standard deviation.

Table 3. Recovery rates of oxytetracycline in sample.

	Added (mg)	Found (mg)	Recovery rate (%)
1	192.5	192.2	99.8
2	192.5	191.8	99.6
3	192.5	191.7	99.6
4	192.5	191.3	99.4
5	192.5	190.5	99.0
Mean±SD*		191.5±0.6	99.5±0.3

*SD, Standard deviation.

HPLC system which tried by authors' schedule for oxytetracycline of bulk and capsulated preparation, from the above experimental result, it was shown as

a good method than biological test in the view to analysis which must be processed rapidly, simply and accuately.

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