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SPECTROPHOTOMETRIC DETERMINATION OF TETRACYCLINES USING 4-AMINOPHENAZONE AND POTASSIUM IODATE

Elisha Divakar Tella[1*], Mohammad Taherunnisa[1], Garikipati. K. Deepthi[2], Ben Manuel Choragudi[3] and Beulah Ranjani Choragudi[1]

[1]D epartment of Chemistry Noble College(Autonomous) Machilipatnam, A.P.
[2]Arutha sai Institute of Science & Technology, Paritala, Kanchincharla
[3]Sujay Bio-Tech Pvt. Ltd., Jawahar Auto Nagar, Vijayawada.
[*]E-mail : divakartella@gmail.com

ABSTRACT

The oxidative coupling of the proposed method is simple, rapid and sensitive with reasonable precision and accuracy. The precision of the method was found by analyzing a set of eight solutions, each containing a final concentration value approximately in the middle of the Beer's law range. The percent relative standard deviation in method is presented in table-2. The accuracy of the method was determined by taking different known amounts (with in Beer's law limits) of the drug and analyzing them by proposed method. The results are given in table – 3. In the determination of tetracyclines the excipients usually present in formulations (glucose, starch, sodium hexa phosphate and some vitamins) and the other antibiotics such as cycloserine, streptomycin, lidocaine or penicillins did not interfere.

Keywords: Tetra cyclones, Spectrophotometer, 4-Aminophenazone, Potassium iodate.

INTRODUCTION

The purple-red coloured antipyrine dye formed on the condensation of 4-aminoantipyrine or amidopyrine with phenols in the presence of an alkaline oxidizing agent[1] has been used extensively for the determination of trace amounts of phenols[2-8]. The sensitivity is greatly increased of course with little hypsochromic shift by extracting the reaction product with organic solvents. The red aqueous reaction mixtures are less stable (30 min) when compared to organic solvent extracts (3 hrs).

Antipyrine dye formation has been utilized for the estimation of phenol[9-11], catechol[12], resorcinol[13-14], hydroquinone[14], 1-naphthol[13], 2-naphthol[13], cresols[14], guaiacol[15], thymol[13] and salicylamide[13]. The antipyrine dyes formed with catechol and resorcinol are sufficiently acidic and so they are not extractable from the aqueous phase with chloroform. Rosenblatt et al took advantage of these properties to determine small amounts of guaiacol in the presence of catechol[4]. Miyamoto et al estimated phenolic compounds with 4-aminoantipyrine and hydrogen peroxide in the presence of peroxidase enzyme at pH 7.25[5-16].

In this section the author has incorporated his investigations regarding the spectrophotometric determination of tetracyclines hydrochloride (TH), oxytetracycline hydrochloride (OTH), chlortetracycline hydrochloride (CTH), doxycycline hydrochloride (DH) and demeclocycline hydrochloride (DCH) based on the formation of green coloured species with 4-aminophenazone and Potassium iodate at pH 7.0. The author has carried out a systematic study for the assay of the above mentioned antibiotics in bulk samples and in dosage forms. The results are incorporated in this section.

The authors have carried out for the first time micro determination of tetracylines using 4-Aminophenazone and potassium iodate at pH 7.0.

EXPERIMENTAL

Preparation of reagents

4-aminophenazone

0.03M of recrystallised 4-amino-phenazone (BDH, LR) was prepared by dissolving 600 mg in 100 ml of methanol, water mixture (1:3).

Potassium iodate

Analytical grade Potassium iodate was prepared by dissolving 200 mg in 100 ml of distilled water.

Tetracyclines (1mg/ml)

This solution was prepared by dissolving 100mg of the I.P. grade tetracycline (tetracycline hydrochloride, chlortetracycline hydrochloride, oxytetracycline, hydrochloride or doxycycline hydrochloride) in 100ml of distilled water.

Buffer solution

It was prepared by mixing 6.1ml potassium dihydrogen phosphate and 38.8ml of disodium hydrogen phosphate.All the other chemical reagents were of analytical grade.

Instrumentation

Spectral and absorbance measurements were made on shimadzu double beam spectrophotometer UV-140 with matched 1cm quartz cells and pH measurements were carried out using on Systronics pH meter 335.

In order to obtain the optimum conditions for the determination of tetracyclines with the reagent, 4-aminophenazone (4-APZ) – IO_3^- , the effects of possible variables were studied and the results are summarized below.

Absorption spectra

The absorption spectrum of the coloured species formed on mixing tetracycline with 4-APZ – IO_3^- in appropriate pH medium exhibiting maximum absorbance was scanned in the wavelength region 400 – 700 nm against reagent blank and the results are graphically represented in Fig.1. The λ max was 600nm. Under the experimental conditions, IO_3^- with 4-APZ or tetracycline has practically no or low absorbance in this region.

Procedure

Aliquots of sample solution (0.5 to 3.5 ml) were transferred into a series of 15 ml volumetric flasks containing 9.0 ml of buffer solution. To each, 1 ml of 4-APZ and 1.0 ml of IO_3^- solutions were added and made upto the mark with distilled water. The flasks were heated in water bath at 70^0C for 2 min in the case of TH, OTH, CTH and 5 min for DH and DCH. After cooling under tap water the absorbance of green colour was measured at 600 nm against a reagent blank. The standard graph was then plotted over a range of 5-31 µg/ml of overall solution.

For dosage forms

Twenty tablets were weighed and powdered. A quantity of tablet or capsule powder equivalent to 100 mg of tetracycline was dissolved in water and filtered. The filtrate was made upto 100 ml with water. Aliquots of the solution were analysed by following the procedure given above.

For biological fluids

A 1.0 ml of human urine containing known amount of the antibiotic was taken and protein was precipitated with trichloroacetic acid (0.5 ml of 5% solution). To the clear centrifugate few drops of concentrated sodium hydroxide solution were added to adjust the pH to 7.0. Then the proposed procedure was followed.

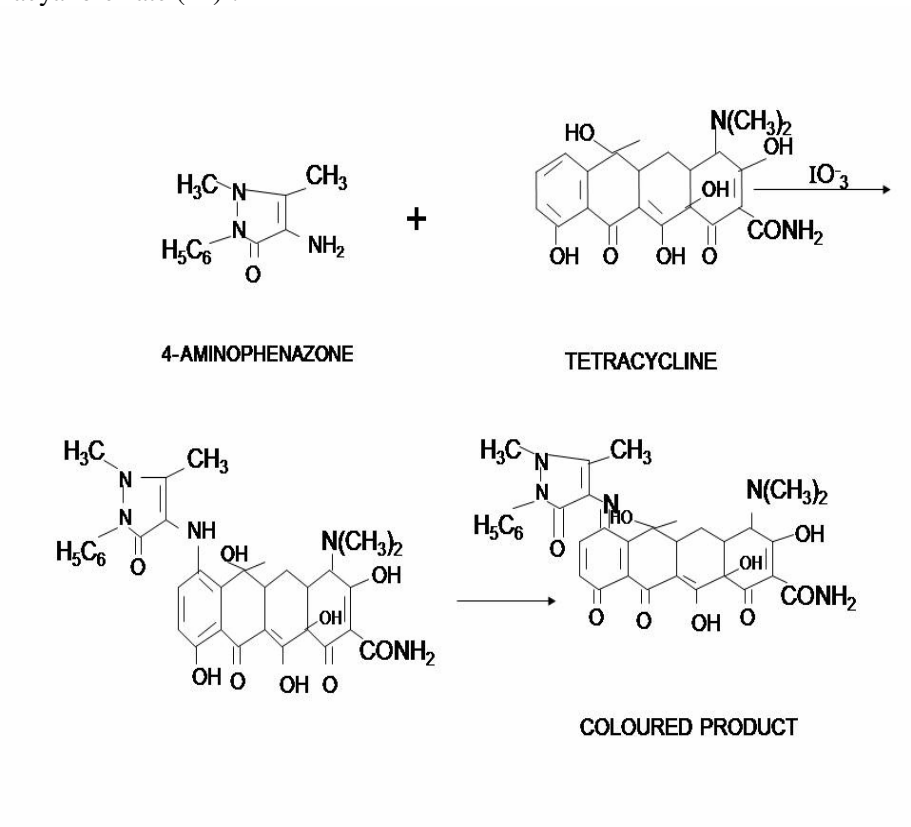
RESULTS AND DISCUSSION

The results incorporated in Tables 1 – 4 clearly suggest that the method using the reagent 4-APZ – IO_3^- is simple, rapid, sensitive and useful for routine determination of tetracyclines in bulk samples and dosage forms with reasonable precision and accuracy.

Chemistry involved

A green colour was developed for tetracyclines (possesses phenolic hydroxyl in which p- position was vacant) by treatment with 4-APZ in the presence of IO_3^- under slight alkaline conditions. Although the structure of the coloured species has not been established, the formation of oxidative coupling product

may be postulated taking analogy of the coupling reaction between 4-APZ and phenol in the presence of potassium hexacyanoferrate (III)[9].



Scheme-1

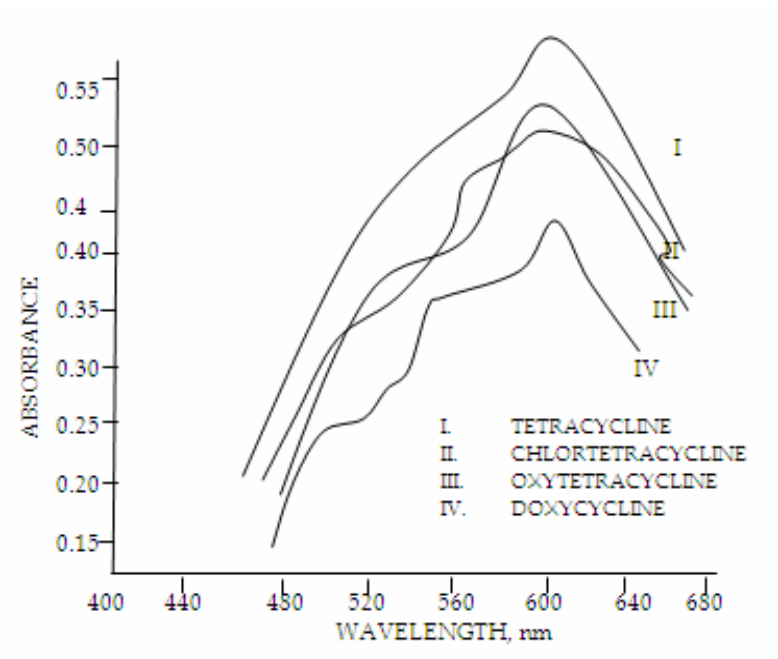


Fig.-1: Absorption spectra of Tetracyclines

Table-1: Optical Characteristics

Parameter	Tetracycline hydrochloride	Chlortetracycline hydrochloride	Oxytetracycline hydrochloride	Doxycycline hydrochloride
Concentration range (µg/ml) (C)	5-31	7-31	7-31	8-31
Regression equation*	A=0.0031 + 0.0099 C	A=0.0024 + 0.0090 C	A=0.0027 + 0.0089 C	A=0.0054 + 0.0065 C
Correlation coefficient	0.9987	0.9994	0.9989	0.9986
Molar absorptivity (1. $mole^{-1}cm^{-1}$)	7.22 x 10^3	7.04 x 10^3	6.86 x 10^3	5.12 x 10^3
Sandell's sensitivity (µg/cm^2/0.001 absorbance unit)	0.0652	0.0728	0.0712	0.0931
Optimum photometric range (µg/ml)	6.4 – 30.4	9.3 – 30.0	9.3 – 30.0	13.6 – 31.0

*Found in this work; It must be determined independently by users of the method.

Table-2: Precision of the Method

Antibiotic	% RSD	Percent range of error Confidence limit	
		0.05 level	0.01 level
Tetracycline hydrochloride	0.50	± 0.62	± 1.14
Chlortetracycline hydrochloride	1.19	± 1.36	± 1.82
Oxytetracycline hydrochloride	1.13	± 1.38	± 1.75
Doxycycline hydrochloride	1.16	± 1.43	± 1.96

Table-3: Accuracy of the Method

Antibiotic	Amount of antibiotic (µg)		% error
	Taken	Found	
Tetracycline hydrochloride	500	490.1	1.98
Chlortetracycline hydrochloride	500	492.0	1.60
Oxytetracycline hydrochloride	500	494.0	1.20
Doxycycline hydrochloride	500	490.1	1.98

Table-4: Assay of formulations and % recovery data*

Sample	Labelled amount (mg)	Amount found (mg)		% Recovery (proposed method)
		Proposed method	Reported method	
Tetracycline (Capsule)	250	247.2	246.4	98.9
Tetracycline (Tablet)	500	495.7	493.9	99.14
Tetracycline (Capsule)	150	148.3	150.1	98.9
Oxytetracycline (Capsule)	150	147.5	146.0	98.3
Oxytetracycline (Injection)	50mg/ml	98.7	98.6	98.7
Doxycycline (Capsule)	100	99.2	98.6	99.2
Tetracycline (Syrup)	25mg/ml	97.7	97.3	97.7
Urine**				98.4

* Each result is an average of three determinations
** After adding 5 mg of drug.

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