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Determination of Some β- lactam Antibiotics

Ethylene Blue Method.

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Abstract

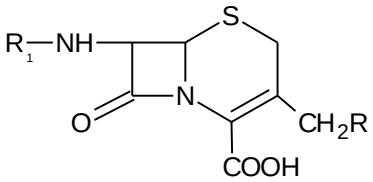
All Cephalosprins studied gave hydrogen sulphide on hydrolysis in 0.5M NaOH solution. Aspectrophotometric method based on the conversion of sulphide formed to ethylene blue was developed. The method is selective for cephalosprins as penicillins don't give sulphide under these practical conditions. Determination of cephalosprins in penicillin sample was found to be possible, the detection limit being 1-2 µg/g of penicillin V.

INTRODUCTION

All β- Lactam antibiotics are derivatives of bicyclic ring system. Both penicillin and cephalosporins have a 4-membered lactam ring which has amino group attached by a cycle group as aside chain [1]. The difference between penicillins and cephalosporins comes from the fact that the penicillins lactam ring is attached to a five membered thiazolidine ring, and a six membered dihydrothiazine ring in the case of cephalosporines [2] . The structure of cephalosporins and penicillins are given in table 1 and table 2.

Table 1

Structures of Some Cephalosporins



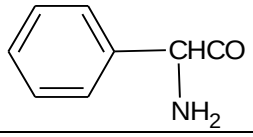
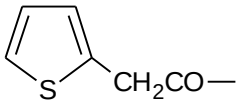
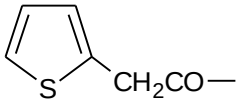
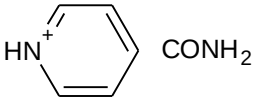
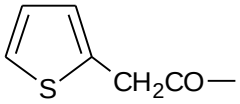
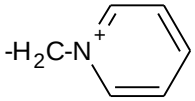
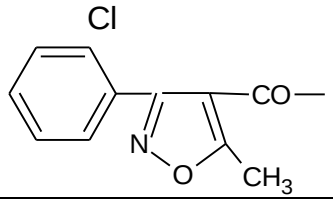
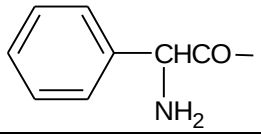
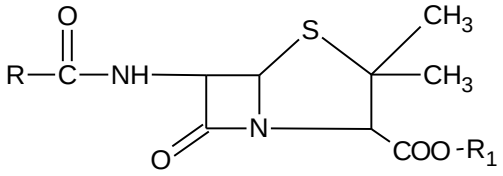
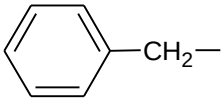
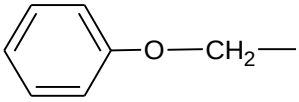
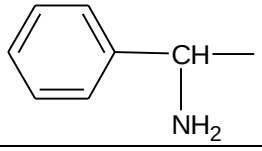
Compound	R_1	R
Cephalexin		-H
		- $OCOCH_3$
Cephalonium		
7-ACA	H-	- $OCOCH_3$
Cephaloridine		
Cephoxazole		$-CH_2OCOCH_3$
Cefaclor		-Cl

Table 2

Structures of Some Penicillins



Compound	R	R_1
Penicillin G		K, Na, Benzathine Procaine
Penicillin V		H,K, Benzathine
Ampicillin		H,Na

The β-Lactam ring of penicillin and cephalosporin is readily susceptiable to attack by variety of nucleophiles. Isolated β- lactams are considerably less reactive than penicillin lactam ring this due to resonance stablization between nitrogen atom and carbonyl function [3].

Penicillins have been widely used in veterinary medicine for more than 30 years and today still form the most important group of antibiotics [4]. The presence of double bond in cephalosporin ring system lead to more extensive cleavage on hydrolysis of the molecule.

Several methods have been reported for the quantitative determination of cephalosporins. These include fluorimetric, polarographic and spectrophotometric methods [5].

Penicillins were observed to cause more allergic reaction than with any other group of drugs; hence penicillin allergy was thought of as a major clinical problem. Because of structural difference between penicillins and cephalosporins , there was some hope that cephalosporins might prove to be non allergic[6].

Chemical methods used for the assay of β- lactam antibiotics are based on determining the product formed after hydrolysis of β-lactam ring.

The methods include iodometric procedure [7-8], and hydroxylamine procedure as described by Ford [9] is based upon the fact that penicillin reacts rapidly with hydroxylamine to give hydroxamic acid which formed colored complex with ferric ion that can be determined colorimetrically. [10]

Colorimetric detection of penicillins and cephalosporins on paper chromatography was used by Thomas. [11] Hishta[12] et al converted several penicillins to their trimethylesters and resolved most of them on gas chromatography.

Many papers have appeared describing high performance liquid chromatography application to β-lactam antibiotics.

Expermintal and Result:

Apparatus: spectrophotometric studies were made with perkin-Elmer spectrometer, Model 550s UV/ visible.

Samples: sample of cephalexin, cephalolthin, cephaloridine, cephalonium and cephoxazole were obtained from Glaxo Operation (UK) Ltd.Sample of cephaclor was obtained from lilly Research Center Ltd.

Solutions:

- N,N diethyl-p- pheylenediamine sulphate 0.01M prepared in 1M H_2SO_4.
- 1M H_2SO_4.

- 0.25M Ammonium iron (iii) sulphate prepared in 0.5 M H_2SO_4.
- Stock solutions of all cephalosporins(100ppm) were prepared in
- 0.5 M NaOH by heating on a water bath to different temperatures.

Experimental techniques:

The following sets of experiments were carried out in order to optimize the experimental conditions to produce the maximum color intensity.

Effect of N,N-diethyl-p-phenylenediaminesulphate concentration:

Into six 50 cm^3 volumetric flasks containing 2 cm^3 cephalexin solutions (100ppm), 5 cm^3 of N.N- diethyl-p-phenylenediamine sulphate of different concentrations were added, followed by 2 cm^3 of ammonium iron (iii) sulphate. The flasks were shaken for 30s allow to stand for 2 mints, diluted to volume with distilled water and absorbances were measured at 667nm. The results are shown in table 1.

Table (1)

Molarity of the reagent used $\times 10^{-3}$	3	5	7	9	10	30
Absorbance	0.122	0.178	0.216	0.327	0.398	0.451*

The optimum concentration of the N.N-diethyl-p-phenylendiamine sulphate was found to be 0.01M.

*reddish colour is produced.

The effect of sulphuric acid concentration:

The experiment was repeated as before except that the reagent (0.01M) N.N-diethyl-p-phenylenediaminesulphate was prepared in different sulphuric acid concentration from 0.20 - 2.0M. The results are shown in table2.

Table 2:

Molarity of H_2SO_4	0.2	0.4	0.6	0.8	1.0	2
Absorbance at 667nm	0.185	0.243	0.308	0.329	0.399	0.308

The optimum concentration of the acid was found to be 1.0M solution.

The effect of time of mixing:

The effect of time mixing was studied at room temperature at the optimum reagents concentration.The results are shown in table3.

Table 3:

Time/ mints	0.50	1.00	1.50	2.00	2.50	3.00
Absorbance	1.009	1.009	1.008	1.007	1.008	1.009

The optimum of mixing was found to be 0.50 min.

The effect of time of heating on the formation of hydrogen sulphide for various cephalosporins.

0.01g of each cephalosporin was dissolved in 0.5M NaOH in 100cm^3calibrated flask and diluted to the mark with 0. 5M NaOH.

The flask was heated in boiling water bath. The procedure of formation of ethylene blue was then applied after every 10 min and the absorbance was measured at 667nm and the suitable times of heating recorded for each cephalosporin. The results are given in table 4.

Table 4: Suitable time of heating selected for each cephalosporins:

compound	Recommended time of heating/ min
cephalexin	30
cephaloridine	60
cefaclor	60
cephalothin	60
cephaloninm	60
cephoxazole	60

The effect of penicillin on calibration curve.

Into five clean 50 cm^3calibareted flasks 0.01g of penicillin (V) samples were transferred, by means of pipette 2, 4, 6, 8, 10 cm^3 of cephalosporin solution (100 ppm), were added, the flasks were heated in a boiling water bath for a length of time appropriate for the particular cephalosporin to produce a maximum yield of sulphide. The absorbances were measured and the results are shown in tables 5-10.

Table 5:

Volume of Cephalexin(100ppm) used/cm^3	2.00	4.00	6.00	8.00	10.00
Absorbance at 667nm	0.404	0.826	1.183	1.493	1.482
Absorbance at 667nm after addition of penicillin	0.405	0.826	1.1814	1.497	1.489

Table 6:

Volume of cafaclor (1000ppm) used /cm^3	2.00	4.00	6.00	8.00	10.00
Absorbance at 667nm	0.300	0.558	0822	1.019	1.300
Absorbance at 667nm after addition of penicillin	0.300	0.555	0.820	1.020	1.302

Table 7:

Volume of Cephaloridine (100ppm) used/ml	2.00	4.00	6.00	8.00	10.00
Absorbance at 667nm	0.169	0.322	0.494	0.654	0.185
Absorbance at 667nm after addation of penicillin	0.170	0.324	0.497	0.657	0.817

Table 8:

Volume of Cephalonium(100ppm) used/ml	2.00	4.00	6.00	8.00	10.00
Absorbance at 667nm	0.134	0.258	0.385	0.509	0.629
Absorbance at 667nm after addation of penicillin	0.133	0.259	0.388	0.509	0.627

Table 9:

Volume ofcephalonium (100ppm) used/cm^3	2.50	5.00	7.50	10.00	12.50
Absorbance at 667nm	0.166	0.310	0.450	0.591	0.730
Absorbance at 667nm after addition of penicillin	0.165	0.321	0.455	0.601	0.732

Table 10:

Volume of Cephoxazole (100ppm) used/cm^3	2.50	5.00	7.50	10.00	12.50
Absorbance at 667nm	0.193	0.364	0.537	0.701	0.869
Absorbance at 667nm after addition of penicillin	0.194	0.360	0.537	0.703	0.866

Discussion:

The recommened producure for the determination of cephalosporins as ethylene blue is very simple and compares favourably with ultraviolet spectrophotometric method based on the imidazole reaction of cephalosporins and penicilins in the presence of mercury (ii) as reported by Bungaard et al and as that of methylne blue reported by Abdalla et al.[14-15]

This method reported here has the advantage of using the visible region and being selective for cephalosporins in the presence of penicilins. But lack behind the imidazole method for stability measurement, as some degradation product could also give hydrogen sulphide for example diketopiperazine derivative.

In addition of utilizing the ethylene blue method for the determination of cephalosprins and cephalosporin's in the presence of penicilin V the procedure developed could prove useful in the elucidating the degradation mechanisms of cephalosporins in alkaline solution.

The recommended procedure for the determination of cephalosprins and cephalosporins in the presence of penicilin V is highly satisfactory. The recent method however is selective for cephalosprins in presence of equal concentration of penicilin V, uses of visible region rather than the ultraviolet region where lots of degradation products absorb and does not require the use of mercury (ii) which is an environmental hazard.

The method will not distinguish between cephalosprins and other sulphide producing impurities in the penicilin samples, but it should be possible to distinguish trace of inorganic sulphide from cephalosporins and other sulphide impurities, if this was felt to be necessary by just adding weakly acidic solution of penicilin sample before the addition of 0. 5M NaOH.

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