RJC

RASĀYAN *J. Chem.*
Vol. 7 | No.2 |170 - 176| April - June| 2014
ISSN: 0974-1496 | e-ISSN: 0976-0083 | CODEN: RJCABP
http://www.rasayanjournal.com
http://www.rasayanjournal.co.in

INDIRECT VISIBLE SPECTROPHOTOMETRIC METHOD FOR THE DETERMINATION OF PENICILLINS WITH PERIODATE, p-N, N-DIMETHYLPHENYLENEDIAMINE AND SULPHANILAMIDE

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ABSTRACT

The acid hydrolyzed product of penicillins (penicillamine) is oxidized with sodium metaperiodate. The iodate, so formed is determined at pH 3.0 with p-N, N-dimethylphenylenediamine (DMPD) and sulphanilamide masking the excess periodate with sodium molybdate. The absorbance of the p-N, N-dimehylbenzoquinonemonoimine – sulphanilamide charge-transfer complex is measured at 520nm. This 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 this method is presented in table-4.5. 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-4.4. In the determination of penicillins the excipients usually present in formulations (glucose, starch, sodium hexa phosphate) and the other antibiotics did not interfere.

Keywords: Spectrophotometer, Penicillins, DMPD, sodium metaperiodate, sulphanilamide, sodium molybdate, Buffer pH 3.0.

INTRODUCTION

Aqueous solution of Sodium metaperiodate at pH 4.0 or below is the most suitable one as the oxidant.[1] Periodic acid oxidation [2-11] is applicable to compounds having two hydroxyl groups or a hydroxyl and an amino group or thiol group and an amino group attached to adjacent carbon atoms and is characterized by the cleavage of the carbon-carbon bond, as illustrated by the following equations.

$$RCH(OH)CH(OH)R^1 + HIO_4 \longrightarrow RCHO + R^1CHO + H_2O + HIO_3$$
$$RCH(OH)CH(NH_2)R^1 + HIO_4 \longrightarrow RCHO + R^1CHO + NH_3 + HIO_3$$
$$RCH(NH_2)CH(SH)R^1 + HIO_4 \longrightarrow RCHO + R^1CHO + (NH_4)_2S + HIO_3$$

The methods of analysis which are used to determine polyhydroxy compounds by oxidation with periodate can be divided in to eight main groups as follows-

i. Determination of the amount of periodate consumed
ii. Determination of iodate formed
iii. Determination of the formic acid produced by cleavage of α, β, γ –triols and related structures. Other acids are also occasionally determined.
iv. Determination of formaldehyde produced from the terminal primary alcohol groups of compounds containing the 1, 2 – diol structure.
v. Determination of acetaldehyde
vi. Determination of ammonia and amines produced by periodate oxidation of α-aminoalcohols.

vii. Determination of carbon-dioxide
viii. Methods based on combinations of two or more of the above groups

The author describes the Indirect visible spectrophotometric determination of sodium metaperiodate consumed during oxidation of the penicillins. The iodate, so formed is determined at pH 2.8 ± 0.2 with DMPD and sulphanilamide masking the excess periodate with sodium molybdate. The absorbance of the purple red colored p-N-N-dimethylbenzoquinonemonoimine- sulphanilamide charge-transfer complex is measured at 520 nm.
Chloramphenicol (after reduction), Dihdrostreptomycin, and Framycetin were estimated by indirect visible spectrophotometer by using periodated and metol[12]. DMPD (p-N-methylaminophenol as sulphate) and potassium iodate[13] has been found to be valuable reagent for the determination of primary aromatic amines**.**

EXPERIMENTAL

Preparation of reagents

DMPD Solution (0.05%): It was freshly prepared by dissolving 50 mg of the Analytical grade substance in 100 ml of water.

Sodim meta periodate Solution (0.2%): It was prepared by dissolving 200 mg of the analytical grade reagent in 100ml water.

Sodium Iodate solution ($9.3X10^{-3}$M): It was prepared by dissolving 185 mg of sodium Iodate in 100 ml of water.

Sodium molybdate solution ($4.9X10^{-2}$M): It was prepared by dissolving 1.0 grm of sodium molybdate in 100 ml of water.

Sulphanilamide solution ($1.2X10^{-2}$M): It was prepared by dissolving 200 mg of recrystallised sulphanilamide in 100 ml of water.

Buffer solution (pH3.0): It was prepared by mixing 50 ml potassium acid phthalate (0.2M) and 40.8 ml of hydrochloric acid (0.1M) and 109.2ml of water.

Penicillins: Stock solutions of I.P grade penicillins (potassium penicillin G, potassium penicillin V, sodium methicillin, Sodium amoxicillin, and sodium cloxacillin and sodium ampicillin) were prepared by dissolving 100mg of the substance in 100ml of water. Working solutions were prepared by appropriate dilution of stock solution to 200µg/ml.

All the other chemical reagents were of analytical grade.

Instrumentation

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

Absorbance curve

The absorbance curve of iodate in the presence of appropriate reagents was scanned on a spectrophotometer in the range 400-600 nm against the reagent blank. However, the maximum characteristic absorption is obtained at 520nm. The results were graphically represented in Fig-1. Whereas the periodate in the presence of molybdate, DMPD and sulphanilamide have practically no absorption in this region. Prepared the calibration graph similarly (Fig.-2).

Optical characteristics - adherence to beer's law

In order to know the Beer's law limits of the proposed method, the absorbances of a series of solutions containing varying amounts of penicillins and specified concentrations of the remaining as given in the procedure in a total volume of 25ml were measured at 520nm against a reagent blank. The linearity of plots between absorbance and the concentration of penicillins shows that the system obeys Beer's Law (Fig.-3).

The Beer's law limits, regression equation, correlation coefficient, molar absorptivity, sandell's sensitivity, optimum photometric range were calculated and recorded in Table-3.

Procedure

To a 25ml standard flasks containing 0.2 – 2.0 ml of penicillin solutions 1.0ml of 1M Hydrochloric acid was added and heated for 1hour. Then 1.0ml of 1M Sodium hydroxide solution and 1.0ml of sodium metaperiodate solution were added and maintained the pH in the range 7.0 – 7.5. Then 1.5ml of sodium molybdate solution was added. After 10 min 15ml of pH 3.0 buffer and 1.5ml of DMPD solutions were added. After 2 min 1.5ml of sulphanilamide solution was added and heated for 2 min at 70°C. Cooled, made upto the mark with distilled water, and measured the absorbance at 520 nm against a reagent blank. Prepared the calibration graph similarly (Fig.-2).

For dosage forms

A known quantity of sample equivalent to 100mg of the penicillin was taken, dissolved it in 100ml of water, filtered and diluted accurately to 250ml. Diluted the solution as necessary and determined the penicillin content by recommended procedure.

1. Accuracy of the method

The accuracy of the method was determined by taking aliquots containing known quantities of each penicillin and estimated them by proposed method and the results were tabulated in Table-4.

2. Interference studies

In the determination of penicillins the excipients usually present in formulations (glucose, starch, sodium hexa phosphate) and the other antibiotics did not interfere.

3. Analysis of pharmaceutical preparations and recovery experiments

In order to find out the suitability of the proposed method, the pharmaceutical preparations were analyzed by the proposed and reported methods. Further, recovery experiments were conducted by adding a known amount of the drug to previously analyzed formulation and the total content of the drug was determined by the proposed method. The results were tabulated in Table-6.

RESULTS AND DISCUSSION

Aqueous solution of Sodium metaperiodate at pH 4.0 or below is the most suitable one as the oxidant. Periodic acid oxidation is applicable to compounds having two hydroxyl groups or a hydroxyl and an amino group or thiol group and an amino group attached to adjacent carbon atoms and are characterized by the cleavage of the carbon-carbon bond.

Comparison of the results incorporated in Tables-3 to 6 reveal that the proposed method is rapid, sensitive and simple with reasonable precision and accuracy. Sensitivity of the method is better than many of the methods.

Chemistry involved

Sodium metaperiodate can oxidize the hydrolyzed product of penicillins. The iodate so formed can further oxidize DMPD into p-N-N-dimethylbenzoquinonemonoimine. This can form a purple red p-N-N-dimethylbenzoquinonemonoimine- sulphanilamide charge-transfer complex with sulphanilamide at pH 3.0. This can be measured at 520 nm.

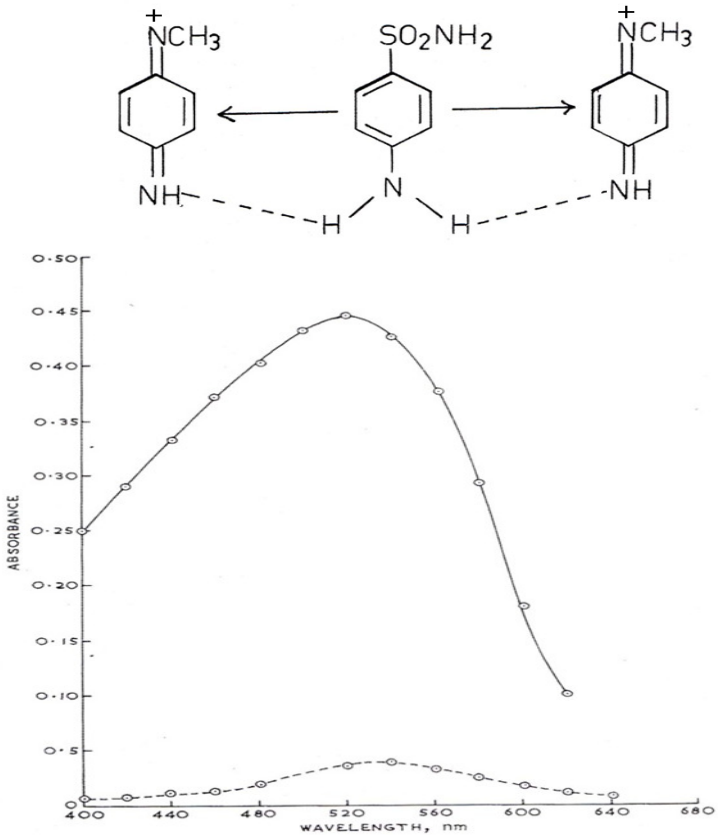

Fig.-1: Absorption Spectra of Iodate- DMPD-Sulphanilamide System

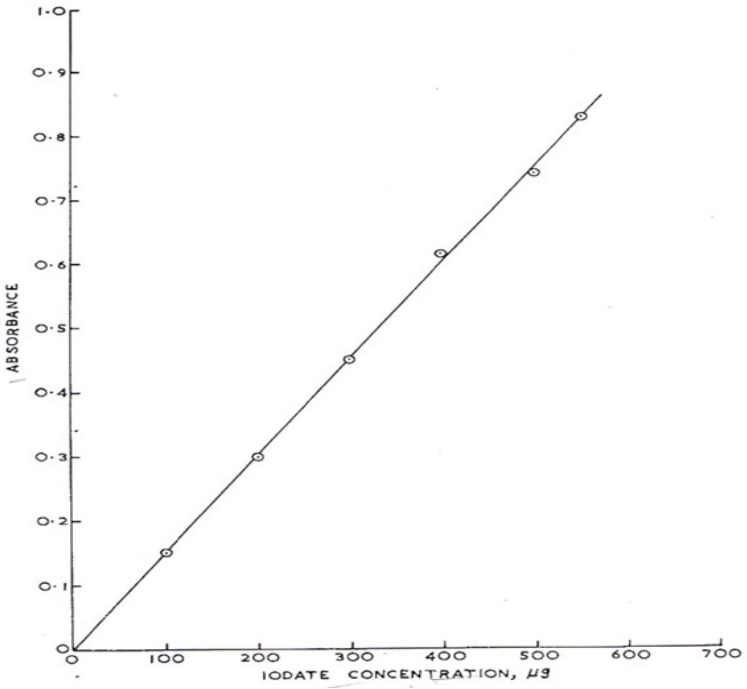

Fig.-2: Adherence to Beer's Law of Iodate- DMPD-Sulphanilamide System

Table-1: Optimum conditions of the method

	Optimum Range	Conditions in Procedure
Sodium metaperiodate	0.8 – 1.1 ml	1.0 ml
Temperature for oxidation	25 – 35 °C	28±2 °C
Time for oxidation	1 – 5 min	2 min
pH Range	6.5 – 7.5	7.0
Sodium molybdate	1.5 – 2.5 ml	1.5 ml
DMPD	1.25 – 1.75 ml	1.5 ml
Sulphanilamide	1.25 – 1.75 ml	1.5 ml
Temperature and time necessary for maximum color development		70°C for 2min

Table-2: DMPD and Sulphanilamide

Iodate Taken (µg)	Iodate obtained (µg)	Iodate + Masked periodate (µg)
200	201.0	201.6
300	301.8	302.2
400	398.8	399.7
500	502.0	502.9

Table-3: Optical Characteristics

Antibiotic	Concentration Range (µg/ml) (c)	Regression equation	Correlation coefficient	Molar absorptivity ($1.mole^{-1}cm^{-1}$)	Sandell's sensitivity (µg/ml)	Optimum Photometric Range (µg/ml)
Potassium penicillin G	2-14	A=0.587-0.0399C	0.9987	$1.49x10^4$	0.0250	3.2-12.7
Sodium amoxicillin	2-14	A=0.525-0.0355C	0.9996	$1.43\ x10^4$	0.0276	4.0-12.9
Sodium Cloxacillin	2-14	A=0.529-0.0339C	0.9991	$1.60x10^4$	0.0296	4.0-12.6
Potassium penicillin V	2-14	A=0.527-0.0342C	0.9994	$1.40x10^4$	0.0286	4.0-9.2
Methicillin	2-14	A=0.534-0.0332C	0.9988	$1.30x10^4$	0.0302	3.8-6.0
Sodium ampicillin	2-14	A=0.519-0.0345C	0.9993	$1.30x10^4$	0.0286	4.0-12.6

Table-4: Accuracy of the method

Amount of Penicillin (µg)			
Antibiotic	Taken	Found	%error
Potassium penicillin G	500	492.8	1.44
Sodium amoxicillin	500	489.7	2.46
Sodium Cloxacillin	500	494.5	4.10
Potassium penicillin V	500	488.9	2.22
Methicillin	500	491.5	1.70
Sodium ampicillin	500	490.4	1.92

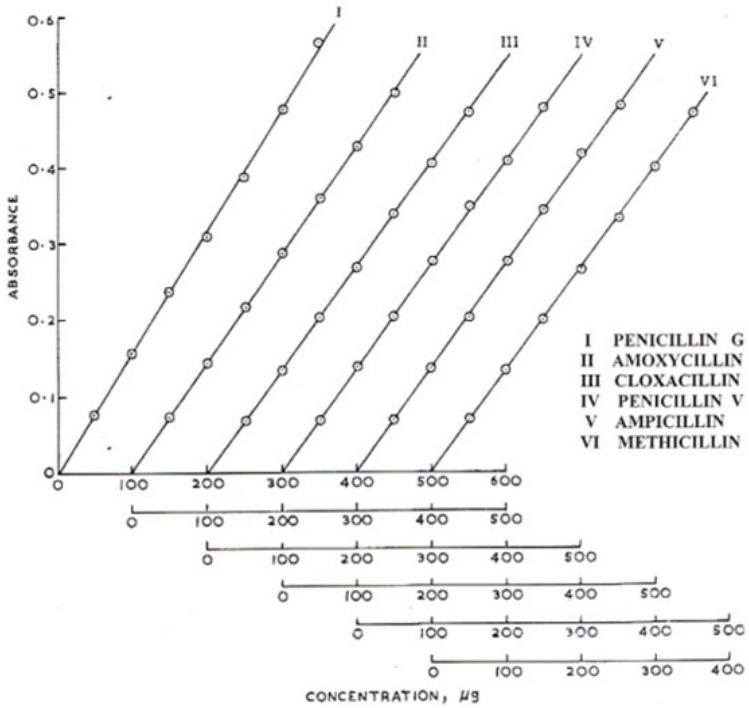

Fig.-3: Beer's Law Plots of Penicillins

Table-5: Precision of the method

		Percentage of error Confidence limit	
Antibiotic	%RSD	0.05 level	0.01 level
Potassium penicillin G	1.24	±1.31	±2.23
Sodium amoxicillin	1.51	±1.67	±2.46
Sodium Cloxacillin	1.22	±1.29	±1.91
Potassium penicillin V	1.65	±1.72	±2.62
Methicillin	1.73	±1.94	±2.98
Sodium ampicillin	1.44	±1.59	±2.35

Table-6: Analysis of dosage forms by proposed and reference methods and % recovery data

Drug	Labeled amount (mg)	Amount found (mg)		%recovery (Proposed method)
		Proposed method	Reported method	
Potassium penicillin G Powder	100	99.1	97.3	99.1
Sodicilin injection (HAL)	100	98.8	97.2	98.8
Sodium amoxicillin powder	100	100.6	99.2	100.6
Amoxicillin capsule (Biddle Sawyer)	100	100.1	99.9	100.1
Commixing (Suspension) (Cevoe pharma)	100	99.8	99.3	99.8
Sodium cloxacillin powder	100	101.2	98.1	101.2
Cloxacillin capsule (Biochem)	100	100.9	98.9	100.9
Cloxacillin injection (Biochem)	100	100.7	99.0	100.7
Sodium ampicillin powder	100	100.9	98.9	100.9
Ampisyn capsule (Cipla)	100	101.3	99.3	101.3
Ampisyn syrup (Cipla)	100	98.1	96.7	98.1
Average of 6 determinations, value based on nominal content.				

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[RJC-1114/2014]