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Spectrophotometric and titrimetric methods for the determination of gentamycin and amoxycillin

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ABSTRACT

This paper describes the Spectrophotometric and titrimetric methods for the determination of gentamycin and amoxycillin. The use of potassium iodate reagent for direct titrimetric method for the determination of some selected antibiotic has been described. The end point was characterized by a sharp change in color from colorless to deep red followed by spectrophotometric measurements.

Key words: Spectrophotometric and titrimetric methods, gentamycin, amoxycillin.

INTRODUCTION

Several methods have been reported fro the quantitative determination of Antibiotics. Spectrophotometric methods still enjoy its publicity and it is very widely used for the determination of Antibiotics. Issopoulos[1-2] has reported two papers for the determination of Antibiotics spectrophotometrically at 700 nm by using molybdophsphoric acid as an oxidizing agent and the mean correlation coefficient was < 0.9998. Another spectrophotometric method has been proposed[3]. The drug was boiled with ammonium vanadate solution is sulphuric acid medium for 10 minutes and the absorbance of the color developed was measured at 750 nm. The proposed method was successfully applied to the determination of Antibiotics either in pure form or in pharmaceutical preparations. The same authors[4] have discussed the used of ammonium molybdate in the colorimetric assay of Antibiotics. The method was based on the blue color formed by the reaction of Antibiotics with ammonium molybdate. Indirect spectrophotometric method for the determination of Antibiotic has also been reported[5]. In this method, the Antibiotic are estimated by oxidizing them under neutral or slightly acidic conditions, after alkaline hydrolysis, by means of a known and excessive quantity of iodine solution. Antibiotic have also been determined spectrophotometrically[6] by alkaline degradation to hydrogen sulfide and resulted to the formation of violet color. Moreover, the charge-transfer reaction of cefalexin with p-benzaquinone and tetrachloro benzaquinone was investigated[7]. Another spectrophotometric method[8], depends on condensation of acetaldehyde, vanillin or p-dimethylaminobenzaldehyde with α-free thienyl moiety of antibiotics. Another colorimetric method[9] for the investigation of β-lactam antibiotics in pharmaceutical preparations was developed. The principle of the method was the reduction, under the optimum conditions, of the Fe(III) to Fe(II) by the cephalosporin assayed and then the Fe(II) resulted was reacted with o-phenanthroline to form the well highly stable orange-red colored complex. λ_{max} of the complex benzaquinone formed was 510nm. Antibiotics have also been determined in capsules and tablets spectrophotometrically by measuring the absorbance at 465 nm after reaction with copper-pyridine[10]. The recoveries were > 100% Alwarthan and co-workers[11] have developed a procedure for measuring small amounts of antibiotics in pure samples as well as in formulation. The method has based on forming a vis-absorbing compounds with N,N-diethyl-p-phenylenediamine sulfate after the hydrolysis of antibiotics in sodium

hydroxide solution to give hydrogen sulfide. Furthermore, abdalla[12] has reported a selective spectrophotometric method for the determination of some antibiotics in pharmaceutical preparation. The method was based on the hydrolysis of the antibiotics in NaOH solution to produce H_2S and reaction of the sulfide formed with N,N-diethyl-P-Phenylenediamine to give ethylene blue. By as simple colorimetric reaction, antibiotics were differentiated from penicillins by hydrolysis and identification of the H_2S formed by methylene blue. Another spectrophotometric method[14] described the reaction of antibiotics with paramolybdate benzaquinone anion and 0.5M H_2SO_4 and the measurement of the absorbance of the blue colored solution at 810 nm. The recoveries and relative standard deviations were 96.7 – 104.7 and 0.60 – 2.8%, respectively. A new reagent, haematoxyline-chloramine-T in the presence of phosphate buffer at pH 7.0 was proposed for the spectrophotometric determination of antibiotics[15]. Antibiotics were hydrolysed with 5MHCl and subsequent treatment with oxidized haematoxyline. The resulting color exhibited λ_{max} at 555nm. Korany et al. [16-17] have utilized derivative spectrophotometry for the determination of certain antibiotics with a greater sensitivity. In the first paper, they proposed the assay of some antibiotics and their alkali-induced degradation products and in subsequent paper they determined their acid-induced degradation products. In addition, many other spectrophotometric methods for the analysis of antibiotics have been traced in the literature[18-27].

Many analytical method for the determination of antibiotics in pure form and pharmaceutical preparations have been reported in the literature. These methods include, fluorimetry[28-29], titrimetry[30-31], polarography[32-34] and flow injection analysis[35-36]. Moreover, chromatography (HPLC), thin layer chromatography (TLC) and liquid chromatography (LC) have been widely used[37-45].

In this chapter, the use of potassium iodate reagent for direct titrimetric method for the determination of some selected antibiotic has been described. The end point was characterized by a sharp change in color from colorless to deep red followed by spectrophotometric measurements.

EXPERIMENTAL

Reagents and Apparatus

Freshly prepared, double distilled water was used throughout. Carbon tetrachloride and other reagents were of analytical grade obtained from BDH, England.

Systionic-105 spectrophotometer was used for absorbance measurements thermostatically controlled water bath model NSW-133, was also used.

Antibiotics

Gentamycin (Orizolin, 125mg from Alidae, India) Amoxycillin (Deffor, 250mg from Ranbaxy, India) and Cefadroxil (Cefadrox, 500mg from Aristo, India). These compounds were chosen as an example Antibiotics in common usage. Table 3.1 shows the structure of the Antibiotics studied.

Solutions

Standard solutions of antibiotics. These were prepared freshly, as required, by dissolving the appropriate amount of each antibiotic in water to provide 1 mg/ml solutions. The standard solutions must be protected from the direct contact with light 0.1ml 1^{-1} sodium hydroxide, 1.0 mol 1^{-1} potassium iodate were prepared.

Procedure

To aliquots containing (1.0 – 5.0 ml) of Gentamycin sodium, Amoxycillin and Cefadroxil, 2.0 ml of 0.1 mol 1^{-1} Sodium hydroxide was added. These solutions were mixed by shaking and placed for 10-15 minutes in a water bath thermo stated at 80°C. After completion of the heat treatment, the reaction mixtures were cooled to room temperature. Then, 0.3ml of 1.0 mol 1^{-1} hydrochloric acid and 5ml Carbon tetrachloride were added. The mixtures were titrated with 0.01 mol 1^{-1} potassium iodate with continuous vigorous shaking. The color of carbon tetrachloride layer changed from colorless to deep red. The end point was taken as the first permanent colorization of the carbon tetrachloride layer. The blank titrations were also performed under the same set of conditions. Blank titrations were negligible. To the same reaction mixture, excess amount of iodate was added (0.5ml) of each flask, where the intensity

of red colour was increased. The organic layer was then separated (using 50ml separating funnel) and dried over anhydrous sodium sulphate. The absorbance was measured at λ_{max} 250nm against carbon tetrachloride.

Determination of injection solution

For the determination of Antibiotics in dry injection solutions, an appropriate amount of each antibiotic was dissolved in water to provide 1mg/ml solution and recommended procedure was applied. The presence of other substances caused no significant interference in the determination of the antibiotics.

Determination of Capsules

An amount of capsule equivalent to about 50mg of the antibiotic was weighed and dissolved in sufficient amount of distilled water. The solution was shaken for thirty minutes. The residue was filtered off on whatman No. 1 filter paper and washed with water. The filtrate diluted upto the mark in a 50ml volumetric flask. The general procedure was applied with no modification and the presence of excipients in the capsules (such as lactose, fructose, glucose, sucrose or starch) caused no interference in the determination and process of separation was not required.

RESULTS AND DISCUSSION

The hydrolysis of β- lactam ring, which is the common feature for antibiotic and penicillins, has achieved by the sodium hydroxide addition, where the product formed or the sequences cleavage has been varied from one researcher to another. Grekas *et al.* [46] have reported the formation of H_2S and Yang and co-workers[47] have claimed the sulfuret formation, but, Abdalla[12] and Thiele *et al.* [13] have reported the sulfide formation, whereas several degradation and cleavage sequences of the cephalosporins under various conditions have been discussed[48]. Therefore, the hydrolytic cleavage of the β- lactam ring by using sodium hydroxide leads to the appearance of a free thiol group, which depends on hydroxide ion concentration. The addition of hydrochloric acid, acidifies the mixture and lowers the pH form 13 to 2 and provides an appropriate conditions for the reduction of iodate to iodine. The liberated iodine has been utilized for the direct titrimetric and indirect spectrophotometric determination of some selected cephalosporins. The absorption spectra of the reaction products are shown in Fig. 3.1. The proposed reaction mechanism in presented in Fig. 3.2.

Study of the proposed experimental conditions

The proposed method has been successfully applied to the determination of antibiotic in pharmaceutical preparations. The results of the titrimetric as well as the spectrophotometric procedure are listed in Table 3.2.

The effect of different variables such as temperature, time and reagents volume have been

Table 3.2: Results obtained for the determination of antibiotics by proposed methods and compared with reference method

Sample	Proposed method % Recovery ± S.D.*	Reference method % Recovery ± S. D.*	Reference No.
Cefazolin Sodium injection (Alidac)	99.4 ± 0.17 (a) 103.3 ± 0.1 (b)	102.3 ± 1.86	[1] **
Cefaclor Capsules (Ranbaxy)	95.6 ± 0.21 (a)	102.8 ± 1.38	[1]**
Cafadroxil Capsules (Ranbaxy)	99.6 ± 0.47 (a) 102.6 ± 0.08 (b)	100.3 ± 1.74	[2]**

* Standard deviation of five determinations.
(a) Results obtained by titrimetric method
(b) Results obtained by indirect spectrophotometric method.
**VIS – Spectrophotometric method.

Table 3.3: Determination of antibiotics by standard addition technique using spectrophotometric method

Sample	Authentic Added (Mg)	Claimed Added (Mg)	Total Added (Mg)	Total Found (Mg)	% Recovery
Cefazolin	100.0	50.0	150.0	151.0	100.6
Sodium	100.0	100.0	200.0	203.0	101.5
Orizolin	200.0	50.0	250.0	246.0	98.4
(Alidac)	200.0	100.0	300.0	296.0	98.6
		Mean Recovery = 99.77			
Cefadroxil	100.0	50.0	150.0	148.0	98.6
Cefadrox	100.0	100.0	200.0	190.0	95.0
	200.0	50.0	250.0	248.0	99.2
	200.0	100.0	300.0	293.0	97.6
		Mean Recovery = 97.60			

Table 3.4: Maximum amount tolerance of excipients for the determination of Antibiotics

Common excipient	Maximum amount tolerance (Mg)
Fructose	36.0
Lactose	34.0
Glucose	54.0
Sucrose	34.2
Starch	0.49

evaluated to permit selection of the most advantageous technique. It is observe that the optimum reaction temperature is 80°C – 58°C (Fig. 3.3), lower or higher temperature gives inaccurate results, and the reaction time for complete hydrolysis of ®- lactam ring is 10-15 minutes (Fig. 3.4. Fig. 3.5 and 3.6 shows the effect of NaOh and CHI, respectively. While studying the stoichiometry, it is observed that l mol of cephalosporin reacts with 2 mol of potassium iodate for Cefazolin sodium and cefadroxil and 3 mol of potassium iodate for cefaclor. Beer's law for all the drug is obeyed over the concentration range 1-5 mg/ml (Figs. 3.7, 3.8 and 3.9 respectively).

In order to determine the validity of the recommended procedure, standard addition technique has been applied to solutions containing four different concentration of the antibiotics studied by adding the standard solution to the solution pharmaceuticals (studied samples). Therefore, the results obtained are given in Table 3.3.

The linearity of the calibration graphs is apparent from the correlation coefficient values which are equal to 0.9999 for cefazolin sodium and 1.0000 for both cefadroxil and cefaclor. Furthermore, the regression line equitation, y= m x + b, is used to calculate the unknown concentrations, slope and intercept. Where, b= intercept, m = slope, x = concentration. Therefore, for Cefacolin sodium y = 0.212x–0.321; for cefadroxil, y =- 0.247x – 0.378 and for cefaclor, y = 0.168x – 0.244.

Effect of common excipients

The influence of common excipients has been examined by the proposed methods. Table 3.4 shows the preliminary studies of interferences, where the amount of maximum tolerance is indicated.

The present method has several advantages over other existing methods. The method described, offers a one step titration followed by spectrophotometric determination. Therefore, the proposed methods are simple, precise, accurate and suitable for routine analysis of antibiotics in drug control laboratories.

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