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In Depth Analysis of Unique Antibiotic Drugs and Theirpharmaceutical Treatments

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Abstract:*The current study depicts that it is observed the even the slight presence of easily oxidizable substance like thio-urea, ascorbic acid, hydrazine; alcohols etc. interfere in the estimation. In such case higher recovery is obtained because the compound reacts with the reagent. Therefore, the presence of such substances was avoided. Excipients like starch, calcium carbonate, sodium carbonate, cellulose, magnesium tri-silicate, tri-calcium phosphate and gum acacia if present in the pharmaceutical preparations do not interfere in the estimation.*

Background*: Antibiotic drugs are chemical substances derivable from a mold or bacterium that can kill microorganisms and cure bacterial infections; "when antibiotics were first discovered they were called wonder drugs" The first antibiotic was penicillin. Such penicillin-related antibiotics as ampicillin, amoxicillin and benzylpenicilllin are widely used today to treat a variety of infections – these antibiotics have been used around for a long time. There are several different types of modern antibiotics and they are only available with a doctor's prescription in industrialized countries.*

Materials and Methods*: An aliquot containing 5mg of the sample was taken in a l00mL stoppered conical flask and 5mL of 0.02NNCS reagent, prepared in hydrochloric acid and 5mL of 4N hydrochloric acid was added to it. The reaction mixture was shaken thoroughly and allowed to react for 15minutes at room temperature (25-300C). After the reaction is over 5mL of 5% potassium iodide was added to it. Contents were shaken thoroughly and allowed to react for a minute. The unconsumed NCS was determined iodometrically. A blank experiment was also run under identical conditions using all the reagents except the sample.*

Results*: Amilcacin sulphate is an amino substituted carbohydrate derivative collectively called as amino sugars. It contains two amino substituted carbohydrate rings and a six membered cyclohexane ring bound though oxide link with both glycoside rings. Hexane ring has an amino substituted aliphatic side chain ending with primary amine group, contains a keto group and secondary alcoholic group. Amikacin consumes three moles of NCS reagent. The structure of compound is very complex and there are many positions which may affected by NCS reagent. Yet some less hindered hydroxyl group may be oxidized like a primary hydroxy group of ring B, Secondary hydroxyl group at ring A and a secondary hydroxy group in side chain at ring A in to keto group. On the basis of available literature, a possible course of reaction is postulated as below. In any one of the reaction products predicted for above compound no authentic proof has been given. I have not been able to located intermediate and the final reaction product. All the reaction is hypothetical based on stoichiometry and the reactions of the reagent.*

Conclusion:*The current study depicts that it is observed the even the slight presence of easily oxidizable substance like thio-urea, ascorbic acid, hydrazine; alcohols etc. interfere in the estimation. In such case higher recovery is obtained because the compound reacts with the reagent. Therefore, the presence of such substances was avoided. Excipients like starch, calcium carbonate, sodium carbonate, cellulose, magnesium tri-silicate, tri-calcium phosphate and gum acacia if present in the pharmaceutical preparations do not interfere in the estimation.*

Key Word*: antibiotics, drugs, pharmaceutical treatments*

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I. Introduction

Antibiotic drugs are chemical substances derivable from a mold or bacterium that can kill microorganisms and cure bacterial infections; "when antibiotics were first discovered they were called wonder drugs" The first antibiotic was penicillin. Such penicillin-related antibiotics as ampicillin, amoxicillin and benzylpenicilllin are widely used today to treat a variety of infections – these antibiotics have been used around for a long time. There are several different types of modern antibiotics and they are only available with a doctor's prescription in industrialized countries.

Antibiotics are used to treat infections caused by bacteria. Bacteria are microscopic organisms, some of which may cause illness. The word bacterium is the plural of bacterium. Such illnesses as syphilis, tuberculosis,

salmonella, and some forms of meningitisare caused by bacteria. Some bacteria are harmless, while others are good for us. Before bacteria can multiply and cause symptoms, the body's immune system can usually destroy them. We have special white blood cells that attack harmful bacteria. Even if symptoms do occur, our immune system can usually cope and fight off the infection. There are occasions, however, when it is all too much and some help is needed from antibiotics.

Antibiotic resistance is a serious and growing phenomenon in contemporary medicine and has emerged as one of the pre-eminent public health concerns of the 21st century; particularly as it pertains to pathogenic organisms (the term is especially relevant to organisms which cause disease in humans). In the simplest cases, drug resistant organisms may have acquired resistance to first-line antibiotics, thereby necessitating the use of second-line agents. Typically, a first-line agent is selected on the basis of several factors including safety, availability and cost; a second-line agent is usually broader in spectrum, has a less favourable risk-benefit profile and is more expensive or, in dire circumstances, be locally unavailable. In the case of some MDR pathogens, resistance to second and even third-line antibiotics is thus sequentially acquired; a case quintessentially illustrated by Staphylococcus aureus in some nosocomial settings. Some pathogens, such as Pseudomonas aeruginosa, also possess a high level of intrinsic resistance.

It may take the form of a spontaneous or induced genetic mutation or the acquisition of resistance genes from other bacterial species by horizontal gene transfer via conjugation, transduction, or transformation. Many antibiotic resistance genes reside on transmissible plasmids, facilitating their transfer. Exposure to an antibiotic naturally selects for the survival of the organisms with the genes for resistance. In this way, a gene for antibiotic resistance may readily spread through an ecosystem of bacteria. Antibiotic-resistance plasmids frequently contain genes conferring resistance to several different antibiotics. This is not the case for Mycobacterium tuberculosis, the bacteria that causes Tuberculosis, since evidence is lacking for whether these bacteria have plasmids. Also M. tuberculosis lack the opportunity to interact with other bacteria in order to share plasmids.

Survey of literature reveals that N-chlorosuccinimide (NCS) in acidic medium has been used for quantitative estimation of some antimalarials (Quinine, Amodiaquine, Santoquine, Cloroquine etc.) diuretics e.g. frusemide, chlorothiazide, and other organic compounds like phenols, carboxylic acids etc. Till now it has not been used for the determination of antibiotic drugs like lamivudine, stavudine, Zidovudine, aciclovir and Amikacin. This initiated me to undertake the present study. Available pharmaceutical preparations of afore said drugs were also analyzed by proposed method. Effects of various variables such as temperature, acid concentration, reagent concentration and reaction time were studied. Simple and convenient method has been described for the micro scale determination of mentioned drugs in pure forn and in their pharmaceutical preparations. In every case standard deviation (SD), relative standard deviation (RSD) or coeflicient of variation (CV) and percentage error has been calculated.

For testing the quantitative validity of reaction, lamivudine was taken as the test sample. Different amounts of sample l-5mg were allowed to react with varying concentrations of N-chlorosuccinimide (NCS) at room temperature (25-300C) for different reaction time. The unconsumed NCS was back titrated iodometrically. A blank experiment Was also run under identical conditions using all the reagents except the sample. The difference in the volumes of sodium thiosulphate consumed for blank and actual experiments was used to calculate the amount of the sample present in a particular experiment. The Stoichiometry of the reaction was established for each drug sample and a possible course of reaction was also suggested. On the basis of reaction conditions developed for lamivudine, the determination of other compounds in pure form and in their pharmaceutical preparation were done

II. Material and Methods

An aliquot containing 5mg of the sample was taken in a l00mL stoppered conical flask and 5mL of 0.02NNCS reagent, prepared in hydrochloric acid and 5mL of 4N hydrochloric acid was added to it. The reaction mixture was shaken thoroughly and allowed to react for 15minutes at room temperature (25-300C). After the reaction is over 5mL of 5% potassium iodide was added to it. Contents were shaken thoroughly and allowed to react for a minute. The unconsumed NCS was determined iodometrically. A blank experiment was also run under identical conditions using all the reagents except the sample. The amount of NCS consumed for the sample was calculated with the difference in the volumes of sodium thiosulphate solution for blank and the actual experiments. The recovery of the sample was calculated with the amount of NCS consumed for the sample. For every sample percentage error, standard deviation and relative standard deviation were calculated.To evaluate the authenticity of the method recovery experiments were also carried out by standard drug addition method. For such experiments a known amount of the pure drug taken and varying amounts of the pharmaceutical preparations of that compound are added and the total amount of the sample was find out with titration and calculations.

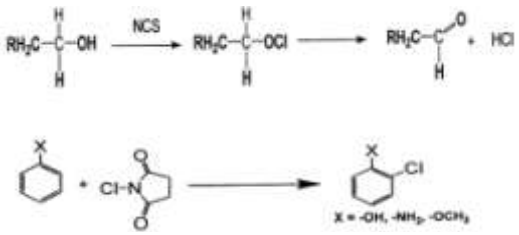
III. Resultand Discussion

Stochiometric ratios of NCS reagent and antibiotic drugs such as lamivudine (1:1), stavudine (1:2), zidovudine (1:2), aciclovir (1:1) and amikacin sulphate (1:3) in pure form and in their pharmaceutical preparations has been mentioned. This ratio remains constant even under varying reaction conditions i.e., change in reaction time, concentration of reagent and reaction temperature etc. As described in the study of variations of the reaction, a particular reaction time was needed for completion of the reaction and for concordant and accurate results. It varies from one compound to another. At a reaction time lesser than the described, inaccurate results are obtained because of incomplete reaction. The increase in reaction time does not change percentage recovery of the sample because the reaction is completed at a recommended reaction time.

The use of hydrochloric acid as a proper reaction medium has also been studied. Hydrochloric acid gives quantitative and stochiometric results with lamivudine. The same results were obtained in the case of other samples. Reaction was also canied out in the absence of hydrochloric acid. In this case, it was found that the reaction is slow and the percentage error is very high. So it was observed that a proper reaction medium is very necessary for the accurate results. After variation in the concentration of volume of hydrochloric acid, it was observed that the use of 5mL of 4N hydrochloric acid was necessary for suitable reaction medium. NCS is the main active agent, which reacts with antibiotic drugs. As indicated that 5mL of 0.02NNCS was sufficient for all the samples for accurate results. Reaction was also carried out at lower and higher concentration at variable volumes of NCS. In this case, it was observed that the concentration and volume other than the prescribed under reaction conditions gives lesser recovery because of insufficient reagent. Higher concentration and volume do not give any improvement over the results. Therefore, prescribed concentration and volume of the NCS reagent was used. The effect of temperature has also been studied. It was observed that results improve with increase in reaction time. The best recovery was obtained at room temperature (25-30^{0}C). An increase in the reaction temperature above 25-30^{0}C gives inaccurate results. It happens due to the decomposition of reagent at higher temperature. At a lower temperature upto5^{0}C it was observed that the reaction is very slow and needs more reaction time. It gives higher percentage error.

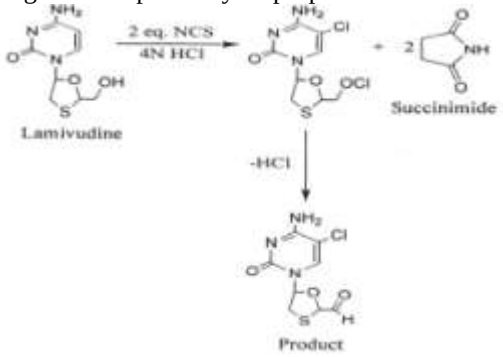
Possible course of reaction

On the basis of oxidation pattern of these compounds and literature available[35-64] following course of reaction may be suggested for the reactions of NCS with each antibiotic drugs.Asdescribed[60-64&37] in of first chapter the oxidation of primary and secondary alcohols with NCS reagent gives rise to carbonyl group. Similarly, if there is an activating group in benzene ring the chlorination takes place at proper position.

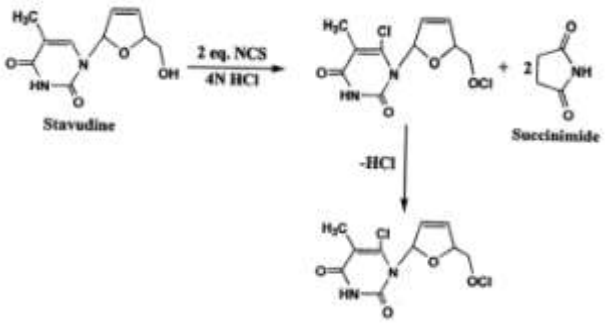


On the same basis it can be postulated that all antibiotic drugs, depending on their structure get oxidized or and chlorinated to given corresponding product.

Lamivudine is a pyrimidine analogue antibiotic having a five membered heterocyclic ring attached at position-1 through nitrogen of pyrimidine nucleus. The five membered heterocyclic ring contains a primary alcoholic group in side chain at position-2. According to the nature of NCS the primary alcoholic group may get oxidized to keto group. In the pyrimidine ring the only active position is at C-5, which gets chlorinated. On the basis of these assumptions following reaction path may be proposed.

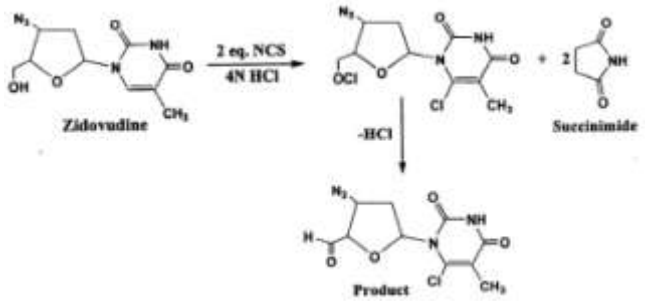


Stavudine is having a substituted furan ring attached at N to the pyrimidine nucleus. Furan ring has got a aliphatic side chain attached at position-5. Side chain at furan ring has a primary hydroxyl group, which may get oxidized to an aldehyde group. In the pyrimidine nucleus the only vacant and active position is at C-5. The molecule consumes two moles of NCS reagent therefore it can be postulated the C-5 get chlorinated and primary alcoholic group of furan side chain get oxidizes to carbonyl group. On this basis a possible path of reaction may be suggested as below:

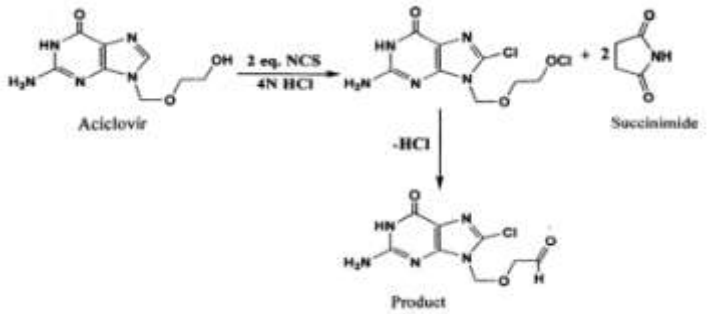


Zidovudine is also a pyrimidine derivative. It contains substituted furan ring at N-l which is further substituted by an azide group at C-4 and an aliphatic side chain at C-5. The azide group is resistant to NCS reaction while primary hydroxy group gets oxidized to an aldehyde group. Pyrimidine ring is also highly substituted by two keto group and a methyl group, the other available and active position is C-6 because it is near methyl group, which is an electron donating group.

The compound consumes two moles of NCS to complete the reaction. On the basis of above assumption it can be thought that the hydroxyl group gets converted to carbonyl group and pyrimidine ring gets chlorinated at C-6 position. On this basis a possible course of reaction may be given as below:

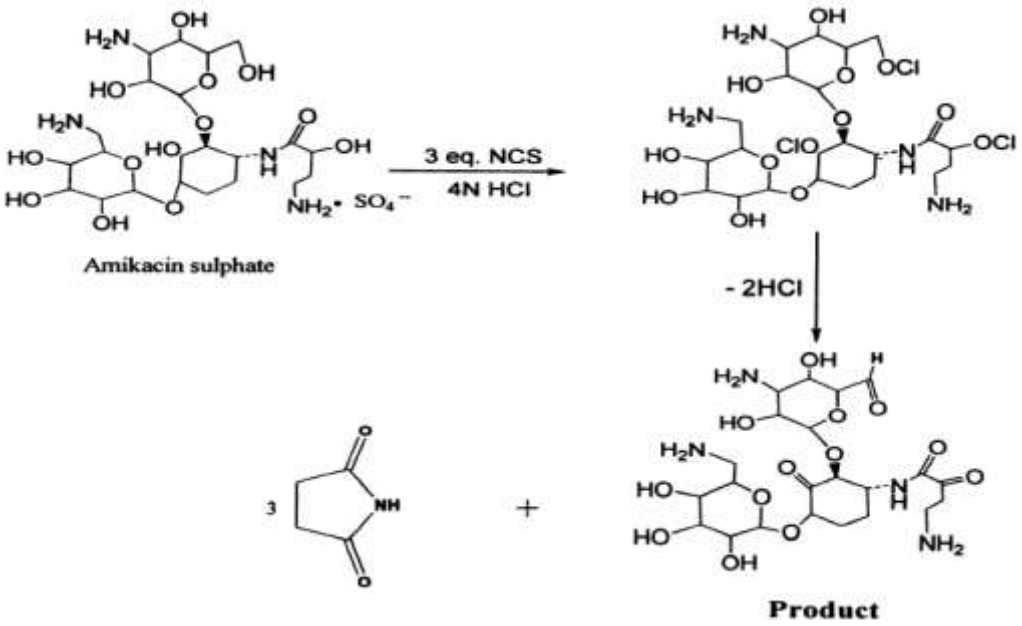


Aciclovir is an analogous of purine base. It contains an oxide linkage which ends with a primary alcoholic group. The aciclovir molecule consumes two moles of NCS reagent out of which one molecule oxidizes primary -OH group present in side chain in to an aldehyde group while second mole of NCS reagent may give chlorinated product. In whole of the molecule only available active position is C-7. There is only possibility that the chlorine atom attaches to that position. On this basis it can be assumed that the reaction product is formed in the following way:



Amilcacin sulphate is an amino substituted carbohydrate derivative collectively called as amino sugars. It contains two amino substituted carbohydrate rings and a six membered cyclohexane ring bound though oxide

link with both glycoside rings. Hexane ring has an amino substituted aliphatic side chain ending with primary amine group, contains a keto group and secondary alcoholic group. Amikacin consumes three moles of NCS reagent. The structure of compound is very complex and there are many positions which may affected by NCS reagent. Yet some less hindered hydroxyl group may be oxidized like a primary hydroxy group of ring B, Secondary hydroxyl group at ring A and a secondary hydroxy group in side chain at ring A in to keto group. On the basis of available literature a possible course of reaction is postulated as below:



In any one of the reaction products predicted for above compound no authentic proof has been given. I have not been able to located intermediate and the final reaction product. All the reaction are hypothetical based on stoichiometry and the reactions of the reagent.

IV. Conclusion

The current study depicts that it is observed the even the slight presence of easily oxidizable substance like thio-urea, ascorbic acid, hydrazine; alcohols etc. interfere in the estimation. In such case higher recovery is obtained because the compound reacts with the reagent. Therefore the presence of such substances was avoided. Excipients like starch, calcium carbonate, sodium carbonate, cellulose, magnesium tri-silicate, tri-calcium phosphate and gum acacia if present in the pharmaceutical preparations do not interfere in the estimation.

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