SUMMARY

The chemistry of β-lactam antibiotics is discussed. The β-lactam ring is susceptible to nucleophiles, electrophiles and oxidizing agents. Various degradation reactions are discussed. The initial hydrolytic product is penicilloic acid which degrades further depending on the reaction conditions. The presence of sulfur atom, carboxylic group and an appropriate side chain is essential for the biological activity. β-Lactam ring should be intact, because the opening of this ring results into a considerable loss in activity. Structure activity relationship observed among β-lactam antibiotics is discussed. The study provides an excellent example how molecular modifications of naturally occurring active constituent can be converted into clinically useful drug.

Various physico-chemical and biological methods for the determination of β-lactam antibiotics are surveyed.

In the present work, three reagents (acetylacetone-formaldehyde, ferric and sodium nitrite) for the estimation of β-lactam antibiotics are suggested.
(1) **Acetylacetone-formaldehyde method:**

The method is based on the reaction between primary amino group present in side chain of amino β-lactam antibiotics with acetylacetone-formaldehyde reagent. The yellow colored chromophore (probably N-substituted 1,4-dihydrolutidine) having \( \lambda_{\text{max}} \) at 400 nm is formed. The reaction is specific for amino β-lactam antibiotics.

Ampicillin, Amoxicillin, Cephalexin, Cephradine and 6-Aminopenicillanic acid are analyzed by the proposed method. Regression analysis using the method of least squares was done for the slope \( b \), intercept \( Y \) and correlation coefficient \( r \) values. Separate determinations at different concentration levels were carried out for each drug to assess the reproducibility. The coefficient of variation was less than 2% indicating good reproducibility.

To prove the validity and applicability of the proposed method, synthetic mixtures were prepared with different proportions of amino- and non-amino β-lactam antibiotics. They were analyzed for amino β-lactam antibiotic using the proposed procedure. The results obtained were both precise and accurate.
Commercial pharmaceutical preparations of drug (Capsules, Injections, Paediatric Drops, Oral Suspensions, Tablets, etc.) in single dosage forms and in combination with other drugs were assayed using the proposed method and the official method. The two methods gave concordant results.

The results obtained show the high reliability, sensitivity and reproducibility of the proposed method which does not require prior extraction or separation of the drug.

(2) Ferric reagent method:

A sensitive and rapid method for the determination of \( \beta \)-lactam antibiotics is described. Iron (III) is quantitatively reduced by \( \beta \)-lactam antibiotics to iron (II) which forms an orange colored complex with 1,10-phenanthroline having maximum absorption at 510 nm. However, the sensitivity is generally very low. Therefore, the alkali hydrolysed sample of \( \beta \)-lactam antibiotic was acidified and then treated similarly with ferric reagent. The sensitivity increased to a greater extent.

The reaction carried out at 37\( ^\circ \) in acetate buffer (pH 5.0) can be applied to determine hydrolyzed \( \beta \)-lactam antibiotics in presence of unhydrolyzed \( \beta \)-lactam antibiotics. The procedure
can be employed in stability indicating assay of some of these antibiotics.

When this reaction was conducted at \(100^\circ\) in phthalate buffer (pH 3.0), the sensitivity of the test has increased to about 20 fold.

The \(\beta\)-lactam antibiotics such as ampicillin, amoxycillin, cephalaxin, cephadine, cloxacillin, carbenicillin, cephaloridine and 6-aminopenicillanic acid were hydrolyzed and analyzed by reaction with ferric reagent at \(100^\circ\) in presence of phthalate buffer (pH 3.0). The calibration graph passed through origin and was linear upto 4 mcg per ml of drug. For each case, the slope of the straight line was reproducible. The precision of the procedure was checked by calculating the relative standard deviation of 10 replicate determinations of a sample.

Results for recoveries of \(\beta\)-lactam antibiotics are given. A student's t-test based on the Associate Referee's counts showed no significant difference (\(P > 0.05\)) between the mean percentage recoveries for official and proposed method.

The proposed method is employed for the determination of tablet, capsule and injections of \(\beta\)-lactam antibiotics.
Comparison of the proposed method with official methods showed good agreement. However, the paediatric drops and the oral suspensions cannot be assayed by the proposed procedure.

Investigations on the influence of frequently encountered excipients and additives on assay of β-lactam antibiotics were carried out. Results showed the absence of interference from starch, talc, magnesium stearate and dicalcium phosphate. However, the sweetening agent like sucrose affects the intensity of the color.

The merits of the present method are that it is not very time consuming, yet it is very sensitive and dependable for handling numerous samples in routine analysis of pharmaceutical formulations. Comparison of the proposed method with the official method revealed that the present method is simple and conservative of reagents. Because of its high sensitivity, the proposed method might find application not only in quality control of β-lactam antibiotic bulk powder, tablet, capsule and injection, but also in therapeutic monitoring of the drug as well as in pharmacokinetic studies.
(3) **Nitrite method:**

The method involves reaction between hydrolysed β-lactam antibiotics and sodium nitrite in acidic medium. The excess of nitrite is reacted with procaine. The diazonium salt formed is coupled with N-1-(naphthyl)ethylenediamine dihydrochloride to give a violet colored solution having $\lambda_{\text{max}}$ at 545 nm.

Under the conditions of the proposed method, a plot of β-lactam antibiotic versus absorption followed Beer's law within a concentration range of 0-4 mcg per ml of reaction mixture. To account for difference in spectrophotometers for the preparation of the calibration curve, a standard was always run simultaneously with the sample. The recoveries obtained from authentic samples have demonstrated a high degree of accuracy and precision of the proposed method as indicated by t-test.

Contents of ampicillin, amoxycillin, cephalexin, cephradine, cloxacillin, carbenicillin, cephaloridine and 6-amino-penicillanic acid in raw material, tablet, capsule, injection, paediatric drops and oral suspensions estimated by the proposed method agree well with the declared amounts of the drug and are comparable with results obtained by official methods.
This procedure was more selective than others because officially recommended excipients such as starch, dicalcium phosphate, talc, magnesium stearate, lactose and sucrose in pharmaceutical dosage forms do not generally release nitrite ions. Furthermore, this method is highly sensitive and allows dilution of the sample to an extent where the possibility of interference by various undesirable compounds is reduced. It can be used as a control method for production lots.

The proposed method could be used for the determination of ampicillin and cephalexin in presence of different concentrations of probenecid or lactobacillus sporogenes upto the ration 1:2. The hydrolysed solutions of these drugs were analyzed by the proposed method. Recovery of the drugs were comparable with those obtained by official methods. Therefore, various probenecid hydrolysed products or lactobacillus sporogenes have no effect on the determination of the β-lactam antibiotic by the proposed procedure.

The proposed method is selective for the determination of β-lactam antibiotics. Bromhexine hydrochloride having primary amino group on aromatic ring was found to interfere in the assay of ampicillin and amoxycillin.
All the three procedures do not require costly reagents or instrumentation. The reactions are carried out under ambient conditions.