Automated Spectrophotometric Assay of Cefazolin

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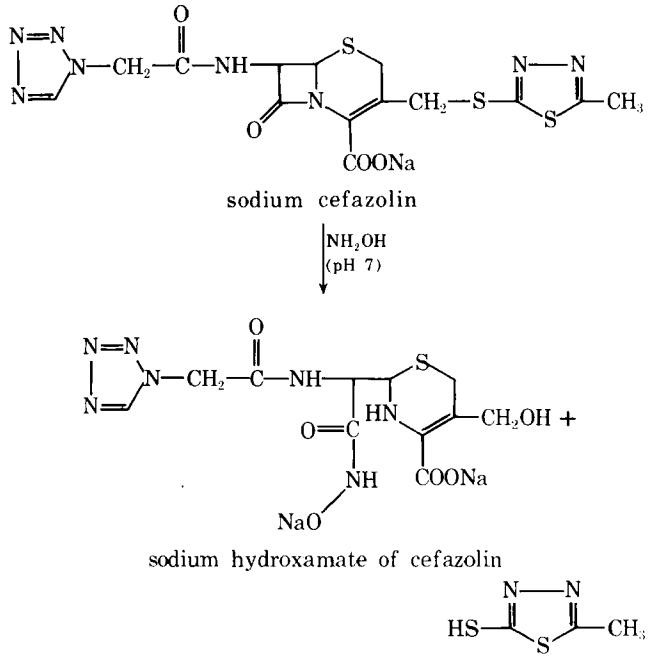
Abstract □ An automated, stability-indicating, UV spectrophotometric assay for cefazolin is presented. The method employs a reaction with hydroxylamine and derives its stability-indicating power through comparison of reacted and unreacted aliquots of the sample. A double-probe sampling procedure is used. Good agreement with microbiological assays is obtained, and the coefficient of variation is about 1%.

Keyphrases □ Cefazolin—automated spectrophotometric assay, stability indicating, double-probe sampling procedure □ Automated methods—spectrophotometric assay of cefazolin, stability indicating, double-probe sampling procedure □ Spectrophotometry, automated—assay of cefazolin, stability indicating, double-probe sampling procedure

Cefazolin sodium, a potent new cephalosporin, is used parenterally and exhibits a broad spectrum of antibiotic activity (1–3). It is a certifiable antibiotic, and two methods of analysis have been approved by the Food and Drug Administration for certification. One method is an agar diffusion microbiological procedure (4), and the other is a manual colorimetric hydroxylamine procedure (4).

While such methods are adequate for certification, a more rapid method is desirable for production and stability studies where the number of samples becomes large. For this reason, an automated assay procedure that is both rapid and stability indicating was developed. The method employs the reaction between cefazolin and hydroxylamine to derive its stability-indicating characteristics. Hydroxylamine reacts with the β-lactam function of all cephalosporins and penicillins in an alkaline medium to produce a corresponding hydroxamic acid salt (5) (Scheme I).



Scheme I

In the case of cefazolin, the UV absorption spectra of the intact cephalosporin and its corresponding hydroxamic acid are different (Fig. 1). This difference makes it possible to assay for intact cefazolin in the presence of breakdown products or other UV-absorbing background materials which do not react with hydroxylamine. The procedure is to make a stock solution of the sample to be tested and then remove two identical aliquots. One aliquot is diluted to a suitable concentration for spectrophotometry, while the other is first reacted with hydroxylamine and then diluted.

The absorbance of both solutions is measured at 271 nm, and the difference is calculated. It is this absorbance difference that is proportional to the con-

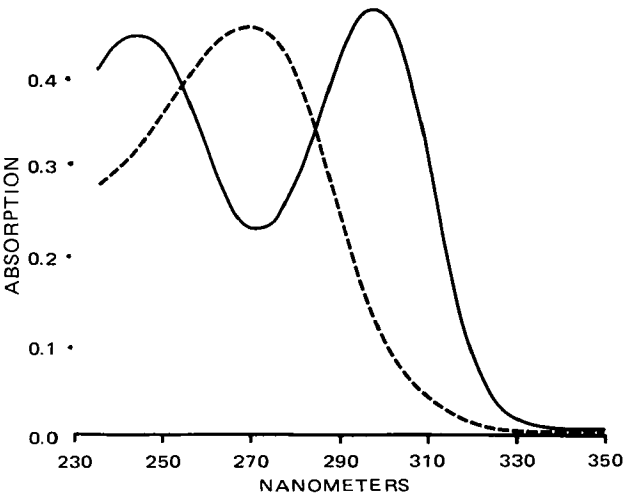


Figure 1—*Absorption spectra of cefazolin (- - -) and its hydroxamic acid salt (—).*

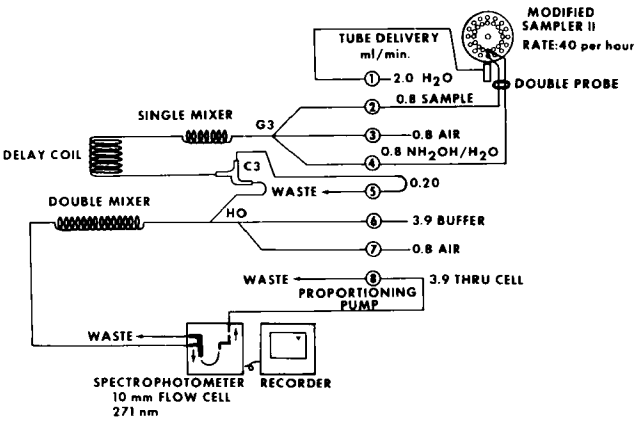


Figure 2—*Flow diagram of automated spectrophotometric assay for cefazolin.*

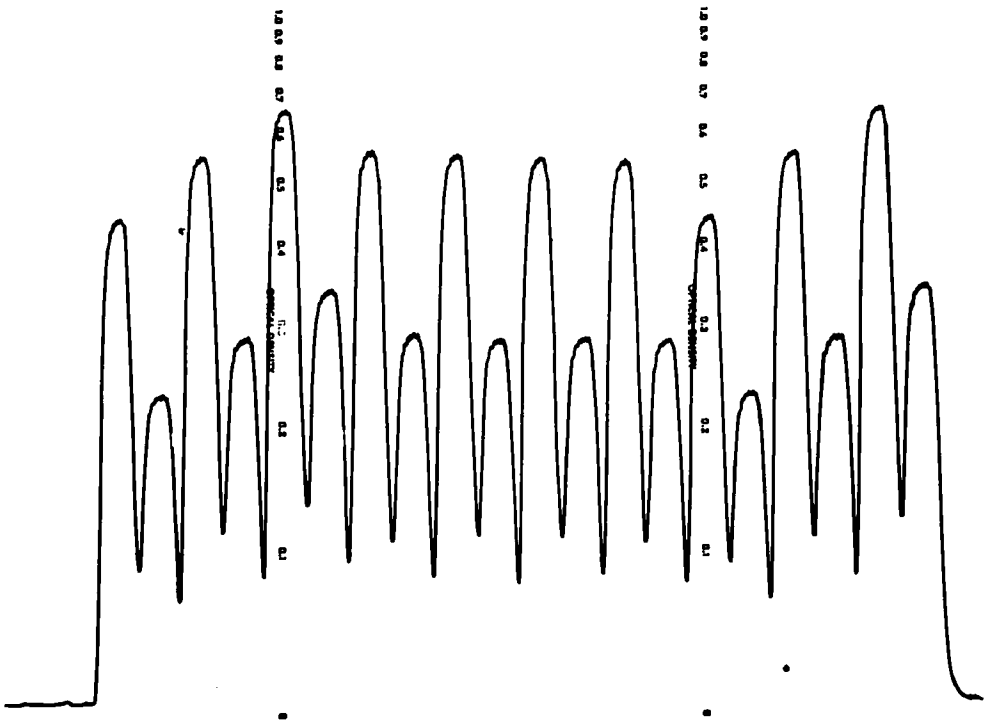


Figure 3—*Typical recording showing four samples preceded and followed by three standards.*

centration of intact β-lactam, cefazolin. The actual concentration is calculated by comparison with a reference standard run in the same manner.

EXPERIMENTAL

Reagents—The following were used:

1. A 0.5 *M* sodium bicarbonate aqueous solution.
2. Buffer, pH 4—Mix equal volumes of aqueous 0.1 *M* sodium acetate and 0.1 *M* acetic acid and adjust to pH 4.0.
3. Alkaline sodium acetate solution—Dissolve 34.6 g of sodium hydroxide and 4.1 g of sodium acetate in water and dilute to 1000 ml.
4. Hydroxylamine reagent—Mix 100 ml of aqueous 5 *M* hydroxylamine hydrochloride and 500 ml of alkaline sodium acetate solutions.

Standard Solutions—*Stock Standard Solution*—Weigh accurately about 100 mg of cefazolin reference standard and transfer to a 100-ml volumetric flask. Add 10 ml of 0.5 *M* sodium bicarbonate solution and swirl to dissolve the cefazolin. Dilute to volume with water and mix well.

Working Standard Solutions—Transfer 20, 25, and 30 ml of stock standard solution to separate 50-ml volumetric flasks. Dilute to volume with water and mix well.

Sample Preparation—Weigh accurately a portion of sample expected to contain about 100 mg of cefazolin and transfer to a 200-ml volumetric flask. Add 10 ml of 0.5 *M* sodium bicarbonate solution and swirl to dissolve the cefazolin. Dilute to volume with water and mix well.

Instrumentation—The following were used: a standard, continuous-flow, analyzer proportioning pump[1] with a manifold platter conforming to the flow diagram shown in Fig. 2 (6); a UV spectrophotometer[2] equipped with a low-volume flow cell[3]; strip-chart recorder suitable for recording the output of the spectrophotometer as a function of time; and a sampler modified to allow the installation and use of two solution pick-up probes, with its turntable drilled to accommodate two standard 2-ml sample cups side by side at each position.

The modification of the sampler allows the solution to be aspirated from two side-by-side cups simultaneously. Thus, two identical aliquots of sample can be aspirated into the analytical system, with one being reacted with the simultaneously aspirated hydroxylamine and the other simply being mixed with a volume of water equivalent to that of the hydroxylamine. This principle has utility in other situations where an unreacted sample blank is required. It has been applied in this laboratory to the iodometric procedure for cephalosporins as well.

This approach, first used by Strandjord and Clayson (7) for enzyme assays, is superior to the commonly used blank procedures which either make a separate run without one reagent for the blank or set up a parallel duplicate system devoid of one reagent. In the first method, instrumental drift or changes in pumping characteristics of the manifold tubes may introduce errors; the second method requires perfect matching of two systems, which is quite difficult to achieve. The double-probe technique overcomes both these problems, since sample and blank run through the exact same system side by side, virtually eliminating any effect of drift.

Procedure—A reagent baseline is established with the reagents flowing according to the flow diagram of Fig. 2. The sample cups are arranged in two concentric circles: an outer circle and an inner circle. Two cups of each cefazolin-containing solution, either standard or sample, are placed in neighboring positions in the outer circle of cups. A cup containing hydroxylamine reagent is placed in the position in the inner circle alongside one of the pair of cefazolin-containing cups; alongside the other is placed a cup of water. Thus, at one sampler position, sample solution plus hydroxylamine reagent is aspirated into the analytical system while another aliquot of the sample solution plus water is aspirated at the next position.

This procedure produces two peaks for each sample (or standard); one has been reacted with hydroxylamine and one has not. The difference between absorbances represented by these two peaks is proportional to the concentration of intact cefazolin in the solution. A typical recording is shown in Fig. 3. The concentration of the sample solution may be determined by plotting the absorbance difference *versus* concentration of the standards and reading the sample concentration from this graph.

[1] Technicon AutoAnalyzer, Technicon Instruments, Tarrytown, N.Y.
[2] Hitachi-Perkin-Elmer-Coleman 111, Arthur H. Thomas, Philadelphia, Pa.
[3] Arthur H. Thomas microflow cell, Arthur H. Thomas, Philadelphia, Pa.

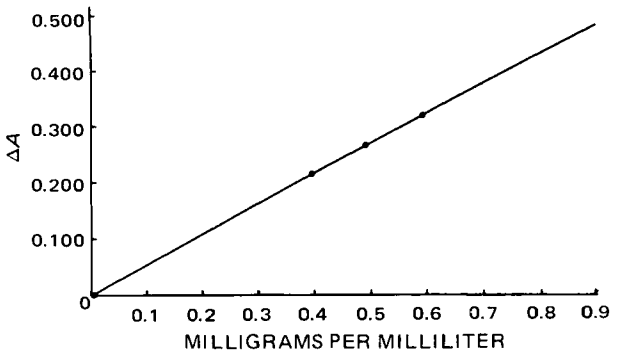


Figure 4—*Relationship between absorbance difference and cefazolin concentration.*

Table I—Replicate Determinations on a Single Sample

Milligram of Cefazolin per Milliliter	Milligram of Cefazolin per Milliliter
0.493	0.503
0.505	0.501
0.500	0.502
0.503	0.501
0.505	0.491

Mean = 0.500
SD = 0.0047
CV = 0.94%

Table II—Day-to-Day Variation

Sample	Percent Purity		
	Day 1	Day 2	Difference
1	78.5	80.0	1.5
2	90.3	90.4	0.1
3	66.4	66.5	0.1
4	67.7	68.7	1.0
5	93.5	94.0	0.5

Mean difference = 0.64
SD = 0.59
CV = 0.74%

Table III—Comparison between Automated Chemical and Microbiological Procedures

Lot	Percent Purity		
	Chemical	Microbiological	Difference
A	94.3	92.7	1.6
B	99.5	99.4	0.1
C	93.5	93.3	0.2
D	94.6	94.7	0.1
E	94.4	95.4	1.0
F	94.0	95.2	1.2
Mean	95.1	95.1	0.7

Alternatively, since such a graph is linear (Fig. 4), an average response factor may be calculated from the standards and used to calculate sample concentration. If the automated system is equipped with an automatic peak detection device, the calculations can be done directly by computer. This mode of operation has been used successfully in this laboratory.

RESULTS AND DISCUSSION

As can be seen from Fig. 2, the method is extremely simple and requires only two reagents: the alkaline hydroxylamine reagent and the acidic buffer used to quench the reaction. The only critical part of the assay is the time of reaction between the cefazolin and the alkaline hydroxylamine. An experiment was carried out to determine the optimum reaction time, and the result was a reaction time of 6 min (Fig. 5). This time is achieved by a delay coil consisting of 10.5 turns of 2-mm i.d. glass tubing wound with a 14-cm diameter.

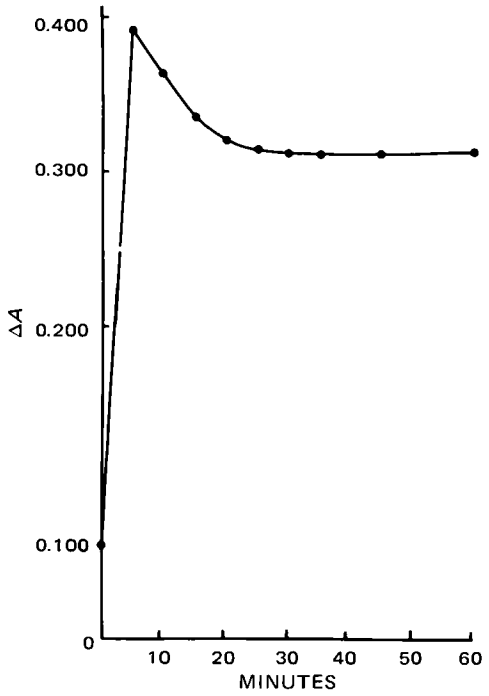


Figure 5—*Absorbance difference* versus *time of reaction between cefazolin and hydroxylamine.*

Table I shows the results of 10 successive determinations of the same sample solution run at one time. The standard deviation is 0.0047 mg/ml or 0.94%. Table II shows the results obtained by repeating five samples on two different days; the coefficient of variation of 0.74% is the same order of magnitude as that obtained on a single day. Table III shows a comparison of results obtained by this procedure with those of a microbiological procedure (8). These results show excellent agreement, as indicated by the mean difference of 0.7%.

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