Rapid, Precise, Turbidometric Assay for Low Levels of Ampicillin in Serum After Single-Dose Oral Administration

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A turbidometric assay is described for the quantitative measurement of ampicillin in serum. Standard curves prepared with known concentrations of ampicillin in serum exhibited acceptable linearity over a concentration range of approximately 0.2 to 1.8 \( \mu g/ml \). Data are presented to show the excellent precision of the assay and the application of the assay to clinical studies. The advantages of this method over other procedures are discussed. Because of the questionable stability of ampicillin, samples containing known concentrations of ampicillin in serum were assayed after storage for various lengths of time. Serum samples maintained in the frozen state until the time of assay exhibited approximately 12\% degradation after 7 days, whereas those samples which were subjected to repeated thawing and refreezing exhibited approximately 25\% degradation after the same time interval.

The evaluation of serum antibiotic levels is of considerable importance in view of the recently demonstrated non-equivalence of certain marketed pharmaceutical preparations, e.g., tetracycline (3), oxytetracycline (6), chloramphenicol (8), and ampicillin (17). The assay described herein was developed to precisely and rapidly quantitate serum ampicillin levels obtained after the oral administration of a single 250-mg capsule (available from 16 different distributors) to 16 healthy volunteers during a 16-week cross-over study. The questionable stability of ampicillin in biological samples (16) necessitated the development of an assay method suitable for the rapid analysis of a large number of samples.

Several methods for the quantitative assay of ampicillin (\( \alpha \)-aminobenzylpenicillin) in aqueous solution have been reported. These include ultraviolet spectrophotometry (1, 7), colorimetric (10) and iodometric (13) techniques, and a spectrophotometric assay based on the hydrolysis of ampicillin to \( \alpha \)-aminobenzylpenicillic acid (20). These chemical methods are not sufficiently susceptible to measure ampicillin in biological fluids at low concentrations. Automated microbiological procedures for the assay of ampicillin have also been reported (9, 14). However, such techniques do not appear to exhibit the degree of susceptibility or precision necessary to quantitate serum levels after the ingestion of a single 250-mg capsule.

To date, procedures utilizing cup-plate microbiological assay (4, 19) and fluorometry (15) have been the only non-isotopic methods available to quantitatively measure ampicillin at the low concentrations found in urine and serum after therapeutic doses of the drug. However, ampicillin metabolites appear to interfere with determination of ampicillin by this fluorometric procedure (15). Problems inherent with cup-plate microbiological assay systems include a 10 to 20\% variability in precision, long incubation periods, the somewhat complicated procedures for calculating and plotting data, and the need for sample dilution to narrow ranges of concentration required for zonal inhibition. This latter problem is magnified when the drug is being assayed in samples of biological fluids.

Because of these limitations, we have developed a turbidometric assay for ampicillin in serum. With this assay, the entire range of serum concentrations observed after a single oral dose of 250 mg can be determined directly, without dilution and with a high degree of precision.

MATERIALS AND METHODS

General procedures. Several organisms were tested for susceptibility. The microorganism selected was \textit{Staphylococcus aureus} (Baptist Memorial Hospital, Memphis, Tenn., 331-036) maintained on tryptic soy agar (Difco) slants. Fresh slants were prepared biweekly and stored at 4\C. The purity of the cultures was checked at weekly intervals. Overnight subcultures were prepared with the microorganism in broth (Difco antibiotic medium no. 3) yielding 20 to 25\%
light transmittance at 550 nm. The assay medium was prepared by diluting the subculture 1:200 in the same medium. The assays were carried out in round cuvettes with an internal diameter of 1.2 cm. Light transmittance was measured spectrophotometrically (Bausch-Lomb Spectronic 20). In all instances, the tubes were incubated at 37 C for 5 h or until 20 to 25% light transmittance was achieved at 550 nm in ampicillin-free control tubes.

Preparation of standard curve. Known concentrations of anhydrous ampicillin (Ayerst Laboratories, N.Y.) were diluted in normal pooled serum (Moni-
trol I-X Dade Div., American Hospital Supply Co.) to provide concentrations of standards within previously defined limits as determined from a 250-mg oral dose pilot study. Standard curves were prepared using triplicate 0.25-ml samples of five known concentrations of ampicillin and 4.75 ml of inoculated broth (Difco antibiotic medium no. 3). In those samples where the ampicillin serum levels were sufficiently high, so as to be beyond the linear portion of the prepared standard curve, a new standard curve was prepared using triplicate 0.1-ml samples of five known concentrations of ampicillin and 4.9 ml of inoculated broth. The standard curves were obtained by plotting concentration of ampicillin against percent inhibition of growth.

Assay of unknown samples. Venous blood samples were obtained in vacutainers (Becton-Dickinson and Co., Rutherford, N.J.) for the analysis of ampicillin levels after the oral administration of a 250-mg ampicillin capsule to volunteers. The blood samples were allowed to clot and the serum was separated within 30 min after collection and stored at -10 C until assayed. On the day of assay, the samples were thawed and triplicate 0.25-ml portions were placed directly into cuvettes together with 4.75 ml of inoculated broth. In those samples which had high levels of ampicillin not encompassed by the linear portion of the prepared standard curve, triplicate 0.1-ml portions of serum were analyzed. As stated previously, a separate standard curve was prepared for these samples.

Stability of ampicillin in serum samples. Several studies have been reported on the stability of ampicillin in solution (5, 8, 11, 12). It appears that the stability depends upon solvent, pH, temperature, and ampicillin concentration. There is a lack of data in the literature on the stability of ampicillin in serum, although Jusko (16) has recently indicated that plasma samples containing ampicillin should be assayed within 2 days of collection "to prevent appreciable deterioration of ampicillin which occurs even in the frozen state."

Stability studies were conducted on serum samples prepared to contain 1 µg of ampicillin per ml. The samples were divided into two groups which were stored at -10 C. Samples from the first group were thawed only once, just prior to their assay. The second group was thawed, assayed, and again frozen, with the cycle being repeated at each assay time over a period of 14 days.

RESULTS AND DISCUSSION

The precision of the turbidometric assay was evaluated by preparing standard curves on each of 3 days. Six replicate samples were prepared for each of the five ampicillin concentrations ranging from 0.2 to 2.4 µg/ml of serum.

Figure 1 shows the standard curves obtained from the turbidometric assay of these serum samples. The standard curve exhibited acceptable linearity over a concentration range of approximately 0.2 to 1.8 µg/ml. The vertical bars represent the values of the 95% confidence limits at each concentration. The application of Bartlett's test indicated that the variances were homogeneous. The data were subjected to a two-way analysis of variance to test for differences between days and between concentrations. As expected, the results indicate highly significant differences (P < 0.001) between the concentrations assayed and between days of assay. This observed day-to-day variability necessitates the preparation of a standard curve, concomitantly with the assay of unknown samples, each day. The application of the assay to clinical studies is shown in Table 1. This representative data shows ampicillin serum levels after the oral administration of a single 250-mg capsule to a normal human volunteer.

The results of the ampicillin serum stability study, using spiked samples, are shown in Fig.

![Figure 1](http://aac.asm.org/) Standard curves for the inhibition of growth of *S. aureus* by known concentrations of ampicillin. Each point represents the mean of six replicate tubes at each concentration. The vertical bars represent 95% confidence limits at each concentration.
The serum samples, which were maintained in the frozen state until the time of assay, exhibited approximately 12% degradation of the ampicillin after 7 days of storage. In contrast, the samples which were subjected to multiple analyses, requiring thawing and subsequent refreezing, exhibited approximately 26% degradation after the same time interval. The results of the study indicated that ampicillin serum samples could be stored at -10°C for up to 5 days prior to assay with approximately 10% loss of activity.

These data indicate the turbidometric assay exhibits suitable susceptibility and excellent reproducibility for relatively low ampicillin serum levels. In addition, the 5-h incubation time is significantly less than required for other microbiological assay methods. Further, small volumes of serum (as low as 0.1 ml) can be assayed directly without the need for extensive dilution.

**TABLE 1. Serum ampicillin levels in a human subject after a 250-mg oral dose**

<table>
<thead>
<tr>
<th>Time after dose administration (h)</th>
<th>Serum ampicillin (µg/ml)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.00</td>
</tr>
<tr>
<td>1</td>
<td>2.35 ± 0.03</td>
</tr>
<tr>
<td>2</td>
<td>2.43 ± 0.00</td>
</tr>
<tr>
<td>3</td>
<td>1.69 ± 0.01</td>
</tr>
<tr>
<td>4</td>
<td>1.02 ± 0.02</td>
</tr>
<tr>
<td>6</td>
<td>0.35 ± 0.04</td>
</tr>
<tr>
<td>8</td>
<td>0.18 ± 0.05</td>
</tr>
</tbody>
</table>

*Mean of triplicate analyses ± standard error.

**FIG. 2. Effect of storage at -10°C on ampicillin concentration in frozen serum samples. Symbols: ● serum samples which were repeatedly thawed and refrozen; O, samples that were continuously frozen until the time of assay.**

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**LITERATURE CITED**


