# Oligomeric substances in ampicillin preparations

## A comparison of Penbritin, Famicillin and Petercillin

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### Summary

An investigation into the presence of potentially harmful oligomers in formulations of ampicillin for parenteral administration available in the RSA was undertaken by means of high-pressure liquid chromatography. Significant differences were found to exist between formulations.


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It is known that ampicillin sodium in aqueous solution undergoes a self-aminolytic degradation reaction to produce a dimer, followed by the formation of oligomers of higher molecular weight.[1-3] Since these oligomerisation products of ampicillin have been reported to possess strong antigenic properties in animals,[4-7] it has been recommended that their presence in clinically used ampicillin preparations be limited to the lowest possible level.[2]

During *in vivo* ampicillin-gentamicin interaction studies undertaken by our group, it was observed during high-pressure liquid chromatographic (HPLC) analysis that, in addition to the parent ampicillin, various other oligomeric peaks were also present. These complicated interpretation of the results of the interaction studies in animals. Since ampicillin dimer is the major initial oligomer peak it was decided to determine the ratio of peak areas of ampicillin to dimer in solutions of Famicillin, Penbritin and Petercillin. Freshly prepared solutions were compared immediately after reconstitution, as well as over a time course of 8 hours to investigate possible differences.

## Methods

Vials containing 500 mg and 250 mg ampicillin from each of four different batches of Famicillin and each of three different batches of Penbritin and Petercillin were examined.

HPLC grade distilled water (2,5 ml or 1,25 ml) was added to to each vial and the contents dissolved by shaking vigorously for 3 minutes. Immediately after this a 100 $\mu$l aliquot of the solution was made up to 1 ml with water and vortexed. From this solution 2 $\mu$l was taken, and added to 250 $\mu$l water and 375 $\mu$l solvent diluent within 30 seconds and vortexed. From this final mixture 500 $\mu$l, containing approximately 32 $\mu$g ampicillin, was drawn up in a HPLC syringe and injected onto the extraction column of a Hewlett-Packard 1090 L liquid chromatograph. The apparatus was equipped with an autosampler and a manual injection valve system and was coupled to a Hewlett-Packard 3392 reporting integrator. Separation of components was effected on a Whatman Partesil 5 ODS-C8 25 cm column at 50°C, using an eluent consisting of a phosphate buffer, in a gradient from 10% to 70%, and an acetonitrile/isopropanol mixture, over a time course of 20 minutes. Flow rate was maintained at 1,5 ml/min and components in the column effluent were detected photometrically at 230 nm by means of the built-in photometric detector of the liquid chromatograph. Prazepam (100 ng) was used as internal standard to monitor the consistency of the HPLC method.

Time course studies were also performed on a sample from each of the batches of Famicillin, Penbritin and Petercillin. Procedures followed were exactly the same as described above except that aliquots of the initial mixture of the ampicillin of 500 mg/2,5 ml or 250 mg/1,25 ml were removed and analysed at 3 and 30 minutes and at 1, 1,5, 2, 3, 4, 6 and 8 hours, respectively.

In order to positively identify ampicillin and ampicillin dimer in the solutions, a split-effluent technique was used and samples eluted from the HPLC column were collected and freeze-dried. Chloroform extraction of these residues yielded samples which were analysed by probe-insertion mass spectrometry and by UV spectroscopy. In addition, the antibacterial activity in cultures of *Escherichia coli* of each of these two substances was determined.

Student's *t*-test was used in the statistical analysis of the results.

## Results

Peaks A, B and C in Fig. 1 indicate ampicillin, ampicillin dimer and prazepam internal standard respectively. Peaks occurring at retention times of 3,3 minutes and less are solvent and injection peaks and are method-related, as are those occurring after peak C. Other peaks are probably due to higher oligomers and impurities.

Table I gives information on the batch numbers and the expiry dates of the ampicillin products analysed.

**TABLE I. PRODUCT INFORMATION ON SAMPLES ANALYSED**

| Product | Batch No. | Expiry date |
|---|---|---|
| | 899106 | 1/89 |
| | 802116 | 12/88 |
| Famicillin | 801116 | 1/89 |
| | 807116 | 1/89 |
| | 102632 | 4/92 |
| Penbritin | 102817 | 5/92 |
| | 102971 | 9/92 |
| | 201391 | 12/89 |
| Petercillin | 201624 | 5/90 |
| | 201045 | 3/89 |

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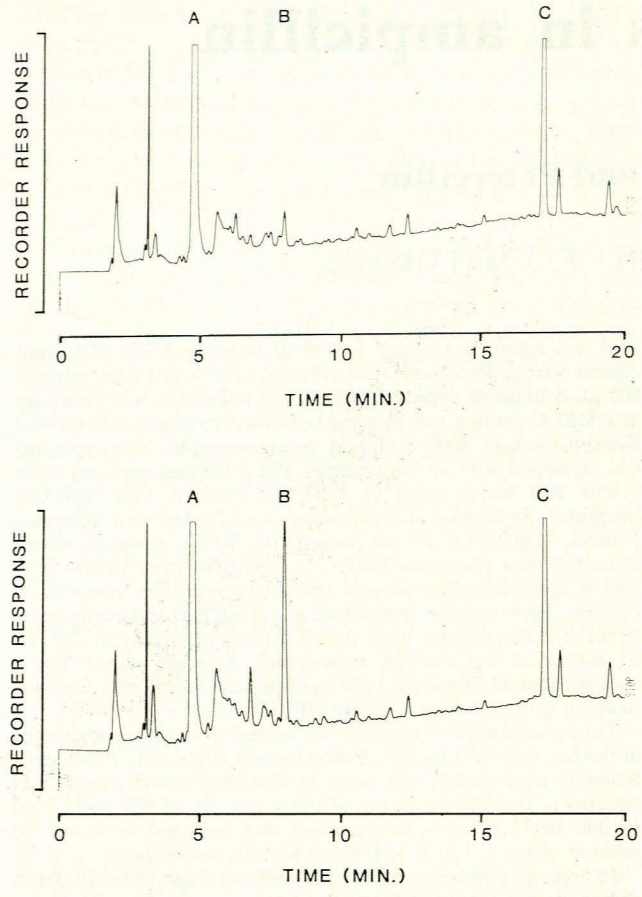


**Fig. 1.** Top: **Representative high-pressure liquid chromatogram obtained for freshly prepared Penbritin. Peak A = ampicillin, peak B = ampicillin dimer and peak C = internal standard (prazepam).** Bottom: **Representative high-pressure liquid chromatogram obtained for freshly prepared Famicillin. Peak A = ampicillin, peak B = ampicillin dimer and peak C = internal standard (prazepam).**

Average ratios of areas under the curves A and B (Fig. 1) determined 3 minutes after reconstitution for the various batches of ampicillin (Table I) are shown in Fig. 2. While no significant differences could be shown between these ratios for Petercillin and Penbritin both these differed significantly ($P < 0{,}0005$) from the ratio A/B for Famicillin.

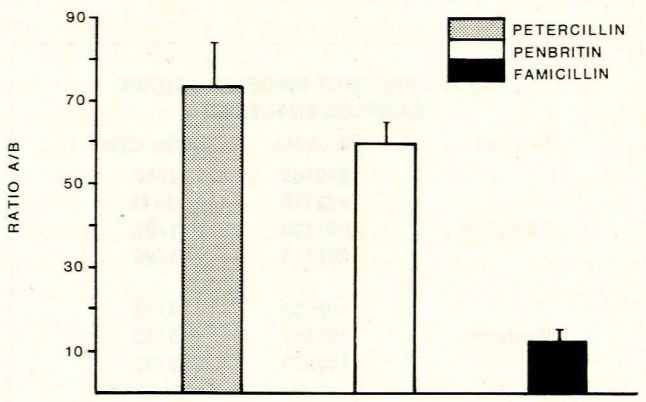


**Fig. 2. Average ratio A/B determined 3 minutes after reconstitution for various batches of Petercillin ($N = 3$), Penbritin ($N = 3$) and Famicillin ($N = 4$), where A = area under the curve of ampicillin and B = area under the curve for ampicillin dimer.**

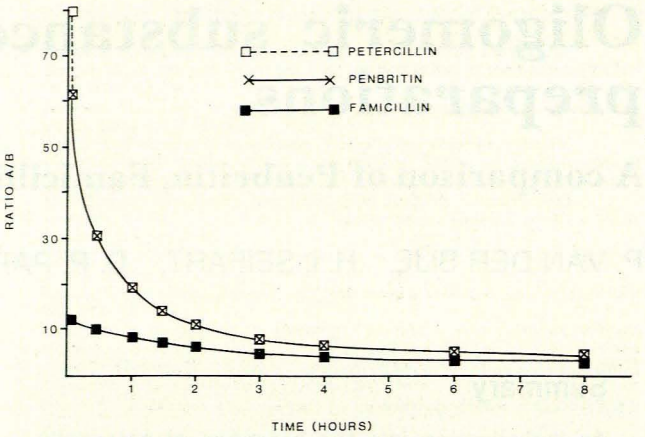


**Fig. 3. Curves showing the decreases in the ratio A/B with time for a randomly selected sample of Petercillin (batch No. 201045), Penbritin (batch No. 102971) and Famicillin (batch No. 802116), where A = area under the curve for ampicillin and B = area under the curve for ampicillin dimer.**

Fig. 3. shows the decreases found in the ratios A/B over a period of 8 hours after reconstitution of samples, taken from batches of Petercillin, Penbritin and Famicillin.

Material collected by the split-effluent HPLC technique from peaks A and B (Fig. 1) and analysed by UV spectroscopy and mass spectrometry confirmed that peak A corresponded to ampicillin and peak B to ampicillin dimer. While ampicillin showed the usual zone of inhibition of growth of *E. coli* in cultures, no such inhibition could be observed for the ampicillin dimer.

## Discussion

Since oligomer formation in aqueous solution of ampicillin (10% w/v) is rapid, samples for HPLC analysis for oligomeric substances should be injected within 5 minutes of dissolution.[2] In our study 20% (w/v) aqueous solutions of ampicillin were used and only 3 minutes were accordingly allowed for the dissolution step as it is evident from Fig. 3 that dimers form quickly at these concentrations.

It is evident from Fig. 2 that 3 minutes after dissolution, Famicillin contained a ratio of ampicillin to ampicillin dimer at least 5 times lower than that of Penbritin or Petercillin. Similarly, the ratio of ampicillin to its dimer was twice as high in Penbritin and Petercillin relative to Famicillin for the first 30 minutes after reconstitution of a vial taken randomly from each of the batches of the three different ampicillin formulations.

It has been clearly shown in the present study that the content of potentially harmful oligomeric substances in generically equivalent parenteral ampicillins available in the RSA may vary considerably. Although no limits have as yet been placed on the content of these substances it would seem prudent to do so.

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# AIDS and South Africa — towards a comprehensive strategy

## Part I. The world-wide experience

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### Summary

In this, the first of a three-part series of articles in which we propose steps towards a comprehensive strategy for the control of HIV infection, we consider briefly the world-wide experience with the HIV epidemic. Our objective is to highlight the problems and controversial issues which are pertinent to strategies for the control of HIV infection. We focus on problems of case-definition, differences between 'African' and 'Western' AIDS and the implications for South Africa, and problems with sensitivity and specificity of tests used at present, particularly in the context of false positivity in a community with a low prevalence of HIV infection. We consider some of the ethical issues, including the need for adequate counselling, the need for informed consent before testing, and the centrality of confidentiality, particularly in the context of possible victimisation and neglect of HIV-positive individuals. Differences between 'notification' and 'reporting' are emphasised. Recommendations are made regarding these problems.

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The objective of these three articles is to focus on the controversies and issues that are central to an effective comprehensive strategy for the control of HIV infection and AIDS in South Africa and to make recommendations based on our deliberations and reading. These articles are not a review of reports about AIDS but rather a synthesis of those reports in a quest for some of the answers to successful control. For example, the clinical spectrum of HIV infection and its treatment are not considered because they have received adequate attention elsewhere.[1-3] Furthermore, little attention is given to the detailed but limited epidemiological information available, and hence health service planning, which has an adequate database as a prerequisite,[4,5] is not discussed.

This article has been prepared by a large group, but the authorship does not yet adequately reflect the spectrum of opinion that needs to be involved in the formulation of an AIDS strategy in South Africa. We have made recommendations largely based on consensus, but there were occasions when one or other member of the group would allow a recommendation to be made despite strong personal opinion against it. This exercise in seeking the common path has further convinced us of the need for a multidisciplinary AIDS group to meet the brief spelled out in the latter pages of these articles.