# PAPER CHROMATOGRAPHY OF PENICILLIN V

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Received July 4, 1955

The use of paper chromatography for the separation of penicillins is not new but so far as is known has not been applied previously to the examination of penicillin V. The method has been used qualitatively only, in order to demonstrate the position of penicillin V when a mixture of penicillins is chromatographed and to confirm the work described elsewhere[1,2].

## Experimental

### *Chromatographic Method*

A method of descending chromatography based on those of Goodall and Levi[3] and Glister and Grainger[4] was used. Modifications included the replacement of paper strips by sheets which simplified the technique, and gave a better comparison of samples than can be obtained on separate strips, where the distance run varies even in the same tank. Water-saturated A.R. diethyl ether was used as the developing solvent and particular attention was paid to maintaining the water-ether equilibrium. The wet ether was kept at the temperature of development for several hours before use. Glass tanks 12 in. × 8 in. × 18 in. deep with vapour-tight glass lids were used for development. Layers of water and ether were kept at the bottom of each tank and, for the purpose of maintaining the equilibrium, unbuffered filter papers were suspended from the top of the tank, close to the walls and dipping into the water layer. Halfway through the equilibration period mentioned below, 20 ml. of wet ether was added to each of these papers. The tanks were thermostatically controlled at 24 to 25° C.

Sheets of Whatman No. 4 filter paper 19 in. × 7½ in. were used on which 4 (or 5) 2·5 μl. spots of the penicillin solutions were run. The sheets were soaked in 2·5 per cent. w/v *p*H 6·6 phosphate buffer and dried. A capillary pipette was used for applying the spots to the dried buffered sheets and, after allowing the spots to dry, an equilibration period of half an hour was given before development for 3 to 4 hours in the tanks.

After removal from the tanks the sheets were dried and "biographed" in the following way. The sheets were placed on the surface of large assay plates and left for 10 minutes; the assay plates consist of glass plates 22 in. × 15 in. × 3/16 in. with aluminium frames 18½ in. × 12 in.; they were prepared as described by Goodey, Reed and Stephens[2] except that double quantities of medium inoculated with *B. subtilis* 288 were used.

The normal inoculum was twice that used for the cavity-plate assay, but when the very small zones shown in the chromatograph illustrated in Figure 2 were expected, one eighth of this amount was used in order to increase the zone size. The plates were incubated overnight at 37° C.

Methylene blue prints were taken as a record of the inhibition zones. This was done by flooding the surface with a 1·0 per cent aqueous solution of methylene blue (containing 1·0 per cent. phenol to kill the test organism), washing off the surplus stain after a minute or so, blotting with Whatman No. 1 filter paper by quickly smoothing a sheet over the surface, and finally taking several prints in a similar manner, but leaving the paper in contact with the surface until the dye had been taken up sufficiently to give a clear print. The methylene blue prints were photographed for the illustrations used in this paper.

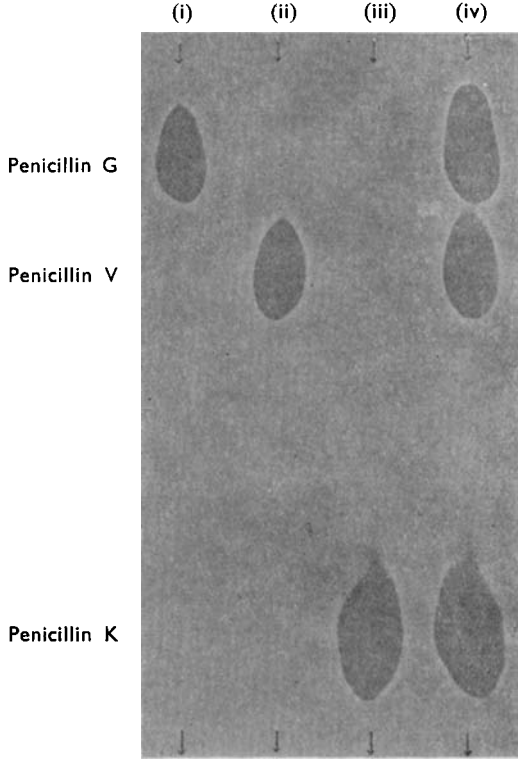


FIG. 1. The identification of penicillin V in a mixture. (i) Penicillin G. (ii) Penicillin V. (iii) Penicillin K. (iv) Mixtures of penicillins G, V and K.

*Materials.* Penicillin G (benzyl penicillin) A.S.C. III sodium salt which has been assigned a potency of 1682 units per mg. Penicillin V (phenoxymethyl penicillin free acid) samples A, B, C, D and E as described elsewhere[1]. The purified sample E was used for all work except where otherwise stated. Penicillin K (*n*-heptyl penicillin) ammonium salt prepared in our laboratories, to which we have assigned a potency of 995 units per mg. against *B. subtilis*.

*Application.* The identification of penicillin V in a mixture is demonstrated by Figure 1. Penicillins G, V and K were chromatographed both together and separately. Chromatographs of penicillin V samples A, B, C, D and E are illustrated in Figure 2. Samples A, B, C and D were shown to contain an unknown impurity which was not present in the purified sample E. The spots each contained the same weight of material.

Samples D and E were "biographed" also on plates inoculated with *B. subtilis* strains ATCC 6633 and ICI No. Pen. D/C8, *Staph, aureus* strains 209P, Oxford H and NCIB 8244 and *Sarcina lutea* strain NCIB 8553 and were found to give the same picture in each case.

Confirmation of the stability of penicillin V compared with G in the presence of acid is given by Figure 3 which shows a chromatograph of solutions 1, 3 and 5 assayed on Day 2 by Goodey, Reed and Stephens[2]. This chromatograph clearly demonstrates that the penicillin remaining after treatment is mainly V, although on this particular day there was a small amount of G as well. The zone sizes in the illustration are not strictly comparable, as the solutions used, even before treatment, contained different amounts of the penicillins. The solutions used for preparing the spots were as follows (i) was a mixture of penicillin G, 800 units per ml. (476 $\mu$g./ml.) and penicillin V, 476 $\mu$g./ml. (ii) was penicillin G, initially 350 units per ml. (208 $\mu$g./ml.), acid treated (iii) was penicillin V, initially 208 $\mu$g/ml., acid treated, and (iv) was a mixture of penicillin G, initially 175 units per ml. (104 $\mu$g./ml.) and penicillin V, 104 $\mu$g./ml., acid treated. The fact that penicillin G was present after acid treatment of the mixture, and in an amount of the same order as in the solution of acid treated G alone, supported the assumption made for calculating the penicillin V remaining in the mixtures given in Table V, of the paper by Goodey, Reed and Stephens[2].

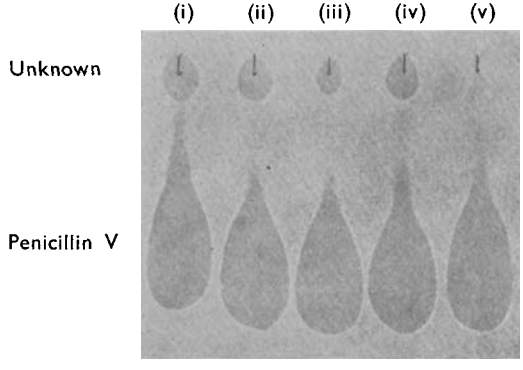


FIG. 2. The purification of penicillin V. (i) Sample A. (ii) Sample B. (iii) Sample C. (iv) Sample D. (v) Sample E.

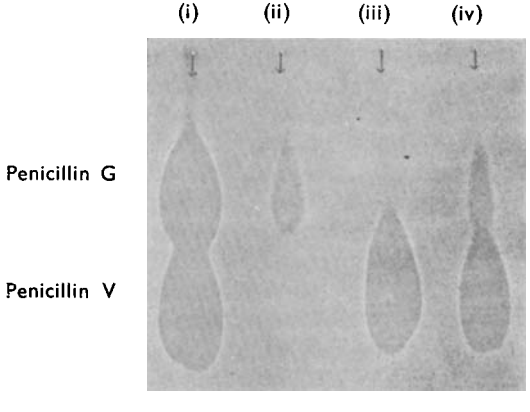


FIG. 3. Stability of penicillin V compared with penicillin G in the presence of acid. (i) Untreated mixture of G and V. (ii) Acid treated G, solution 2. (iii) Acid treated V, solution 3. (iv) Acid treated mixture of G and V, solution 5.

## Discussion

Penicillin V has been shown to move faster than G on a chromatograph of a mixture of the two, taking the position normally attributed to F($\Delta^2$ pentenylpenicillin); it may well be that V and F chromatographed side by side would show a slight difference in position, but no specimen of pure F was available to investigate this.

The unknown impurity in the cruder penicillin V samples A, B, C and D is thought to be another acid stable penicillin since, like V, it remained after the acid treatment described by Goodey *et al.*[2] and was inactivated by penicillinase. It is hoped to extract enough of this material to make more tests.

## Summary

1. The use of chromatography as a qualitative method of identifying penicillin V in a mixture is demonstrated.

2. Stages in purification of penicillin V are illustrated.

3. The much greater stability of penicillin V as compared with penicillin G in the presence of acid is confirmed by chromatography.

We wish to thank Miss P. Greenwell for technical assistance and Mr. F. Fox for preparation of the photographs.

## References

1. Parker, Cox and Richards, *J. Pharm. Pharmacol.*, 1955, **7**, 683.
2. Goodey, Reed and Stephens, *ibid.*, 1955, **7**, 692.
3. Goodall and Levi, *Analyst*, 1947, **72**, 274.
4. Glister and Grainger, *ibid.*, 1950, **75**, 310.

## DISCUSSION

The three papers were presented by DR. G. PARKER.

MR. G. SYKES (Nottingham) asked the authors whether it might not be found helpful to use the penicillinase method of assay described at the 1952 Conference.

DR. G. E. FOSTER (Dartford) pointed out that the authors suggested that penicillin V was more stable to acid than penicillin G, and suggested it would be more suitable for oral administration. Had any experiments been carried out to assess the rate of absorption of the penicillin *in vivo?*

DR. F. HARTLEY (London) asked whether the authors could explain the alleged relative stability of the compound towards acid. It was difficult to see what could cause the stability of the lactam ring portion which would be broken on acid hydrolysis. Clearly it could not be solely the function of solubility.

DR. G. PARKER, in reply, said with regard to the absorption of penicillin V, he could not quote exact figures, but it had been given to a large number of volunteers and it compared very favourably indeed with penicillin G both as regards rate of absorption and blood levels obtained.

He had no suggestion as to why penicillin V should be more stable than penicillin G. The theory had been put forward in one paper that it was due to hydrogen bonding.

MR. R. GOODEY added that the penicillinase method of assay had not been tried.