

## Colorimetric Estimation of Vitamin D

SIR,—The well-known colorimetric procedure using antimony trichloride-acetyl chloride reagent was used by us for the estimation of calciferol in pharmaceutical preparations, on the lines described by Dewitt and Sullivan.<sup>1</sup> We observed that better results were obtained by dissolving antimony trichloride as well as vitamin D in chloroform, which has been used as a solvent of choice by Stross and Brealey<sup>2</sup> also.

Published literature on the method indicates that the concentration of acetyl chloride in the reagent is not critical and can be varied between 2 to 5 per cent. We observed that a concentration range of 2.5 to 3.0 per cent of acetyl chloride in the reagent was advantageous. If the concentration of acetyl chloride was less than 2.5 per cent, turbidity appeared during the experiment, while if the concentration was more than 3 per cent, the fall in the optical density of the colour was very rapid, giving erroneous results.

Dewitt and Sullivan have recommended that the initial reading of the optical density should be taken at 30 seconds and subsequent readings at one minute intervals up to 6½ minutes, while Stross and Brealey read the optical density only once, at 1½ or 2 minutes. We observed that reading the optical density at 30 second intervals up to 6 minutes is advantageous, firstly because it gives a smoother curve from which the optical density at zero time can be more correctly extrapolated and, secondly, because it is not necessary to purify the oily solutions of calciferol by chromatography as recommended by Stross and Brealey since the interfering sterols, as observed by us, Dewitt and Sullivan and also by Zimmerli and others,<sup>3</sup> affect the colour only after 4 minutes and thus there is no significant change in the value of the optical density at zero time.

The procedure modified as above has been successfully employed for the estimation of vitamin "D" contents of tablets, oily solutions of vitamin D and injection of calcium with vitamin D.

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## REFERENCES

1. Dewitt and Sullivan, *Industr. Engng Chem. (Anal.)*, 1946, **18**, 117.
2. Stross and Brealey, *J. Pharm. and Pharmacol.*, 1955, **7**, 739.
3. Zimmerli, Nield and Russel, *J. biol. Chem.*, 1943, **148**, 245.