Gas-liquid chromatography (GC) has been very successfully applied to the analysis of a number of different groups of biologically important compounds. In particular, the analysis of the multiplicity of metabolites of secreted steroid hormones found in human urine has been greatly simplified by the introduction of GC (1). Using conventional packed columns (2) and wall-coated capillary columns (3), 'profiles' of varying degrees of complexity have been produced. Plasma steroids have also been analysed by GC using electron capture detection (4) and more recently using gas chromatography-mass spectrometry (GC-MS, 5).

Vitamin D and its hydroxylated metabolites are secosteroids and as such might be expected to behave like steroid hormones. They should therefore be susceptible to analysis by GC using similar methods to those already used for steroids. Vitamin D and its metabolites, like the steroids, are relatively non-volatile compounds and require GC oven temperatures of the order of 200–350°C for separation. There are many parallels between the problems associated with the GC analysis of steroid hormones and vitamin D, which is perhaps not surprising when one considers their common chemical origin. Both groups of steroids can be analysed by GC without formation of derivatives although considerable peak broadening occurs, probably indicating adsorption into the 'inert' support of the column. The choice of suitable derivatives can enhance separation and improve peak shape. C21-corticosteroids containing a 17-hydroxyl group are thermally labile and unless suitable derivatives are formed prior to GC, the high oven temperatures give rise to side chain cleavage, producing C19 steroids. In a similar fashion, vitamin D and its metabolites undergo thermal changes during GC. Injection of both derivatised and underivatised vitamin D onto a GC column gives rise to closure of the B ring, producing two isomers, pyro- (9αCH₃, 10βH) and isopyro- (9βCH₃, 10βH) calciferols.
The formulae of these two isomers and the mechanism of formation are illustrated in Figure 1. Thus the injection of a single vitamin D metabolite into a GC column gives rise to two peaks, which are usually formed in a constant proportion (pyro:isopyro::2:1). Similar cyclisation occurs in the test tube and the proportion of the isomers to each other remains constant at all temperatures above 125°C (6).

Those involved in GC prefer to use systems in which single compounds give rise to single peaks, particularly if a 'profile' is required. The presence of more than one peak from a single compound can give rise to considerable problems of interpretation. For this reason, many efforts have been made to find derivatives of vitamin D and its metabolites which would resist thermal cyclisation during GC analysis and enable one metabolite to give rise to one peak. The only derivatives which have been successfully used in this context so far are the isotachysterols. Many