Quantitative in Situ Thin-Layer Chromatography of Ergocalciferol in Multivitamin Tablets

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Summary
A TLC method is reported for the determination of ergocalciferol in multivitamin tablets. Silica Gel HF₅₄ is used as sorbent and cyclohexane-ethyl acetate as mobile phase. The concentration of the separated vitamin is determined by direct UV absorption scanning of the vitamin spot on the chromatogram. Results are reliable with samples containing between 0.05 and 3.0 μg of ergocalciferol. For comparison purposes ergocalciferol determination was carried out after TLC separation with the spectrocolorimetric antimony trichloride-acetyl chloride method.

Due to the small amount of ergocalciferol present in multivitamin tablets in relation to other lipophilic and hydrophilic vitamins and tablet excipients preliminary extensive cleanup procedures are required for analysis of this vitamin by standard spectrocolorimetric and ultraviolet absorption methods [1-16]. The present study was aimed to develop a fast, simple and at the same time more sensitive method for the routine control of the ergocalciferol content in multivitamin tablets. The proposed method involves extraction of the lipophilic vitamins from the tablets, TLC separation and direct absorption scanning of the ergocalciferol spot on the chromatogram. A determination in duplicate by a skilled analyst does not take more than 3-4 hours.

For comparison purposes the spectrocolorimetric antimony trichloride-acetyl chloride method after TLC separation was used [17, 18]. Both determinations, the spectrocolorimetric and the absorption scanning method, were carried out from the same chloroform table extract. However, for the determination of ergocalciferol by the proposed direct scanning method, due to its higher sensitivity, the extraction of lipophilic vitamins from the tablets can be carried out with a smaller quantity of the sample: instead of an aliquot containing 2000 I.U. (50 μg) of ergocalciferol one can take an aliquot corresponding to only 1000 I.U. (25 μg).

Experimental
Extraction of lipophilic vitamins. To an accurately weighed portion of 20 pulverised tablets equivalent to 2000 I.U = 50 μg of ergocalciferol in a beaker were added 20 cm³ of a 2 % ammonia solution previously warmed to 60 °C. The beaker was heated on a water bath under swirling until the sample was completely dispersed. After cooling the content was quantitatively transferred into a separator, by rinsing with 40 cm³ of ethanol, then 40 cm³ of water was added and the vitamin was extracted three times with 100 cm³ portions of petrol ether [30-40 °C]. If emulsion was formed it was removed by adding a few drops of ethanol. After separation of the layers the ether layer was gently washed with about 100 cm³ of water and the extract filtered through 2-3 g of anhydric sodium sulfate in a folded filter paper. The solvent was evaporated to dryness in a rotavi vacuum evaporator (Rotavapor R, Büchi, Flawil, Switzerland) heating the flask in a water bath at a temperature not exceeding 40 °C and the residue was dissolved in 2 cm³ of chloroform. This chloroform sample extract was used for the further steps of the determination.

TLC separation and direct absorption scanning. By means of a Hamilton microsyringe 100 mm³ (= 2.5 μg of ergocalciferol) of the chloroform sample extract and 100 mm³ of a standard solution containing 12.5 mg Vitamin D₂ USP Reference standard (40,000,000 I.U. in 1 g) in 500 cm³ chloroform were applied separately along the starting line of a precoated 20 × 20 cm plate Silica Gel HF₅₄, layer thickness 0.25 mm E. Merck AG, Darmstadt.
(German Federal Republic) as horizontal 3 cm streaks. The chromatogram was run immediately with 75 : 25 cyclohexane-ethyl acetate (18 cm) in a previously saturated chamber. The vitamin spots were located in the UV254 light and the absorption of the spots scanned at 270 nm with a Carl Zeiss (Oberkochen, German Federal Republic) Model PMQ II Opton Chromatogram Spectrophotometer, equipped with a Varian Model A-25 recorder. The control of spot uniformity was carried out by dividing the spot into three segments and measuring every segment separately. The concentration of ergocalciferol in the sample was calculated from the obtained mean values of the sample spot relative to that of the standard spot; peak area values were obtained by multiplying peak height with its width at half height.

TLC separation and spectrocolorimetric measurement. One cm³ of the chloroform sample extract (= 25 mg of ergocalciferol) and 50 mm³ of a standard solution containing 25 mg of Vitamin D₂ USP Reference Standard in 50 cm³ of chloroform were applied as streaks along the starting line and the chromatogram run as described above. Silica Gel HF254 coated 20 X 20 cm plates with a layer thickness of 0.5 mm and activated 1 hour at 105 °C were used. The vitamin zones were located under UV254 light and the vitamin extracted by mechanical shaking for 15 minutes with 10 cm³ of chloroform. Each extract was filtered through anhydric sodium sulfate (2–3 g) in folded filter paper. Two cm³ of each of the filtrates were pipetted into 50 cm³ Erlenmayer flasks and 1 cm³ chloroform and 5 cm³ of the color reagent (20 % SbCl₃ solution containing 2 % acetic anhydride) were added. After 1 minute the absorbancies were measured at the 500 nm maximum with the Zeiss spectrophotometer in a 2 cm glass cell against a reagent blank. The correction for possible by-absorption products in the sample (mainly vitamin A) was performed by the addition of one cm³ of the inhibitor solution (1:1 chloroform-acetic anhydride and 5 cm³ of the color reagent to 2 cm³ of the sample chloroform extract. The absorbancy was measured as described for the sample and the obtained extinction value subtracted from that of the sample. The vitamin concentration in the tablets was calculated relative to the standard solution. Both methods were carried out protected from direct light.

Results and Discussion

Since various solvent systems are known to date for the TLC separation of lipophilic vitamin mixtures, no systematic investigation was necessary. However, some of the known systems were rechecked, such as 1:1 cyclohexane-ether, 88 : 2 : 10 n-hexane-methanol-acetone and 75 : 25 cyclohexane-ethylacetate. The latter system proved to be most suitable. The separation was better in the direct scanning procedure than in the spectrocolorimetric procedure since due to the considerably higher sensitivity of the method a tenfold smaller volume of the sample extract was applied into the sorbent layer. Moreover, the regular form of the longitudinal spot was convenient for in situ scanning. Owing to the sensitivity of the apparatus used for scanning the minimum quantity of the vitamin necessary for a reliable determination was found to be 0.05 mg. Since the solutions were applied manually i.e. by means of a microsyringe, special precaution had to be taken to the way of application. In order to control the uniformity of vitamin concentration in the spot on the chromatogram every spot was divided into three segments and the uniformity checked as described in the procedure.

Although fluorescent sorbent layers were used, in situ scanning was carried out by absorption and not by fluorescence quenching since the base line obtained with the latter technique exhibited a rather strong noise. A secondary UV filter of 250–360 nm was used to cut off exciting radiation below 250 nm and above 360 nm to exclude any possible interference of the fluorescent indicator in the sorbent.