

TLC ANALYSIS OF PHARMACEUTICAL PREPARATIONS CONTAINING VITAMINS A, D AND E

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Background

Vitamins are basic to human health and their determination gained increased significance in several areas of analytical chemistry such as pharmaceutical, clinical and food applications.

A large number of methods have been developed for quantifying vitamins content in pharmaceuticals.

Most of the fat-soluble vitamins present in pharmaceutical preparations or in natural products are accompanied by a number of closely related compounds. This explains why the chromatographic methods are so frequently used in analysis of these compounds.

Aim

The paper presents research results regarding the establishing of the optimal conditions for the determination of fat-soluble vitamins A, D₂ and E in multivitamin pharmaceutical products, by using the TLC method. TLC is almost ideally suited for these compounds because of its simplicity, speed, selectivity and sensitivity.

Materials and methods

- Standards: vitamins A, D₂ and E (purchased from Merck; stock solutions: 410.8 mg vitamin A, 52 mg vitamin D₂ and 391 mg vitamin E in chloroform at 10 mL volumetric flask)
- Fixed phase: TLC plastic sheets silica gel 60F₂₅₄
- Mobile phase: *system 1* - hexane/ether (9:1, v:v)
system 2 - benzene/chloroform (1:1, v:v)
- Examination: UV 254 nm, by using a Vilber-Lourmat system, equipped with a black-white video camera, software BioProfil ver. 2.0.

Results and discussions

Vitamins A and D₂ have been assayed in the system 1 (chromatogram presented in Fig. 1); 2µL have been spotted from each compound and their 1:1 mixture (the concentration of the solutions were 10.27 mg/mL for vitamin A and 1.3 mg/mL for vitamin D₂). The corresponding R_f obtained is presented in Table 1.

Table 1. R_f obtained for the vitamins

	Vitamin A	Vitamin D ₂	Vitamin E
System 1	0.463	0.065	-
System 2	-	0.186	0.703

Fig. 1 Chromatogram for vitamins A, D₂ and mixture (M₁) in the system 1



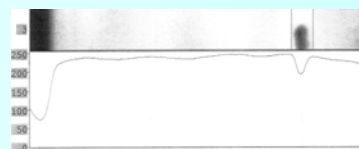
Fig. 2 Chromatogram for vitamins D₂, E and mixture (M₂) in the system 2



Vitamins D₂ and E have been assayed in the system 2 (Fig. 2). 2 µL have been spotted from each compound and their mixture (the concentration of the solutions were 1.3 mg/mL for vitamin D₂ and 9.77 mg/mL for vitamin E). The corresponding R_f obtained is presented in Table 1.

A semi quantitative assay of the vitamins was performed via the blacking-out curves obtained for different amounts spotted (Fig. 3).

Fig. 3 Blacking-out curve for Vitamin D₂ 1.8 µg



The regression lines obtained for the three substances is plotted in Figs. 4. and 5.

For vitamin A, a second order polynomial regression curve was obtained (R-square = 1) – figure 4 and for vitamins D₂ and E a regression line was obtained (R=0.98994, and 0.98191, respectively) – figure 5.

As little as 5 µg, 0.5 µg and 5 µg of A, D₂ and E vitamins, respectively, can be assayed.

Fig. 4. Polynomial regression for vitamin A quantification

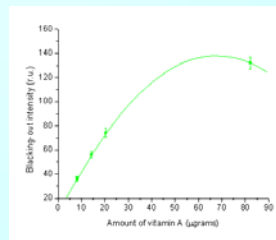
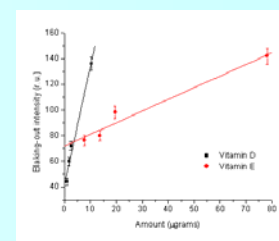


Fig. 5. Linear regression for vitamins D₂ and E quantification



Conclusions

- TLC methods for qualitative and semiquantitative assay of vitamins A, D₂ and E have been developed (fixed phase: silica gel 60F₂₅₄, mobile phases: hexane/ether (9:1, v:v) for vitamin A and benzene/chloroform (1:1, v:v) for vitamins D₂ and E.

- The semiquantitative assay of vitamin A in the conditions given above resulted in a 2-order polynomial regression curve in the field 5-85 µg.

- The semiquantitative assay of vitamins D₂ and E in the conditions given above resulted in a linear regression curves in the fields: 0.5-10.5 µg for vitamin D₂ and 5-80 µg for vitamin E.

References

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