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DETERMINATION OF VITAMIN D_2 FROM FISH OIL AND METHOD VALIDATION

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Abstract: *This paper presents analysis method in UV of vitamin D_2 from fish oil (Biofarm pharmaceutical product). Validation of spectrophotometric method for quantitative determination of vitamin D_2 in fish oil was performed through parameters: precision (repeatability and reproducibility), selectivity, linearity, detection limit, quantification limit, range. Pharmaceutical product such as fish oil from Biofarm was used. Vitamin D_2 was analyzed in chloroform solution in UV, with a maximum at 274 nm.*

1. Introduction

A large number of spectrophotometric methods [1-5] have been developed for quantifying vitamin D_2 content in pharmaceuticals because this vitamin is basic to human health and its determination gained increased significance in several areas of analytical chemistry such as pharmaceutical, clinical and food applications.

The paper presents results regarding the possibility to determine fat-soluble vitamin D_2 in fish oil using the UV/Vis spectrophotometry as quantification method.

2. Materials and methods

As standard vitamin D_2 purchased from Merck, chloroform stock solution of 1.04 mg/mL for spectrophotometric determination has been used. As pharmaceutical product, *fish oil* from Biofarm was used; chloroform stock solutions of 10 capsules (2.6942 g) for spectrophotometric determination have realized. Diluted solutions at different concentrations were obtained.

Investigations were carried out with UV spectrophotometer Cary 100 Bio Varian.

3. Results and discussions

The UV absorption spectra were recorded for vitamin D_2 solutions and *fish oil* in chloroform (Figure1.) in following conditions:

- spectral domain: 250-500 nm,
- the correction of basis line was made before every experiment with chloroform. [6,7]

The dosage curve for standard solutions was realised (Figure2.). Analysing by linear regression the values of absorbance obtained in the case of maximum 274 nm, a calibration graph with the next equation has been obtained:

$$A_{274} = - 0{,}00147 + 0{,}04525 \cdot [D_2] \qquad (1)$$

where $[D_2]$ = the concentration of vitamin D_2 (μg/mL).

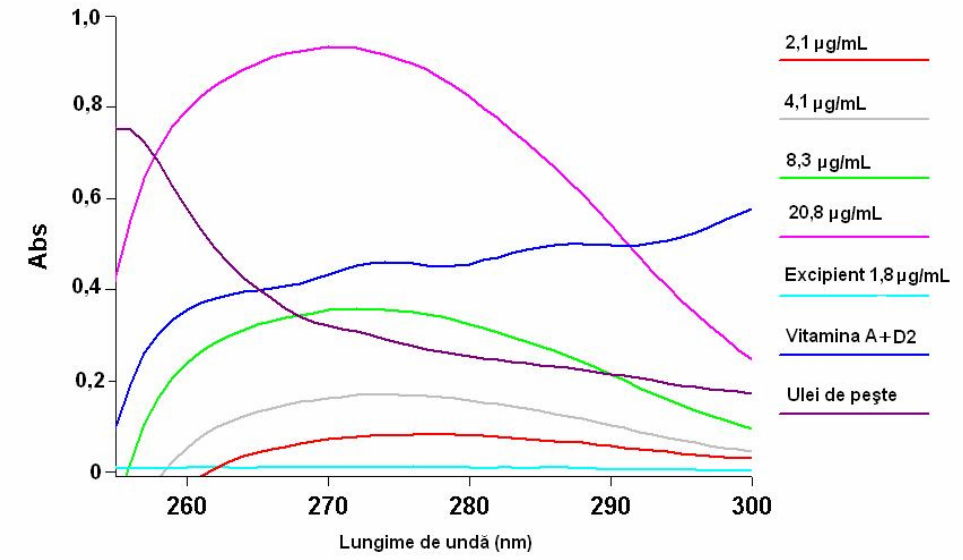

Figure 1. UV Spectra for standard solutions of vitamin D_2 in domain 2.1 – 20.8 µg/mL, for excipient solutions (sun flower oil) and for fish oil (pharmaceutical product)

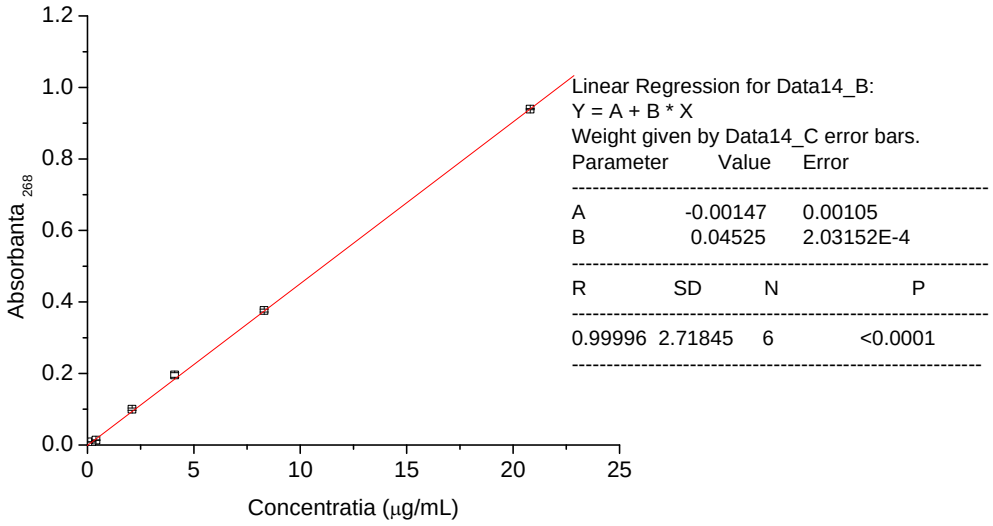

Figure 2. Calibration graph obtained for maximum 274 nm.

Linearity domain, detection limit (DL) and quantitation limit (QL) were calculated in concordance with validation guide [8]:

$$DL = \frac{3.3 \cdot \sigma}{S} = 0.076 \text{ µg/mL},$$

(2)

$$QL = \frac{10 \cdot \sigma}{S} = 0.232 \text{ µg/mL},$$

(3)

where σ = the standard deviation of the response
S = the slope of the calibration curve.

In this range of concentration, the calibration graph is linear (correlation coefficient 0.99996). The repeatability of the method (RSD) is 2% and the recoverie of added vitamin is 99.4%. The stability in time for standard solutions and samples was determined (Figure3.) and the conclusion is that these solutions aren't stable in time and for realising of quantitative determination fresh solutions must be preparated.

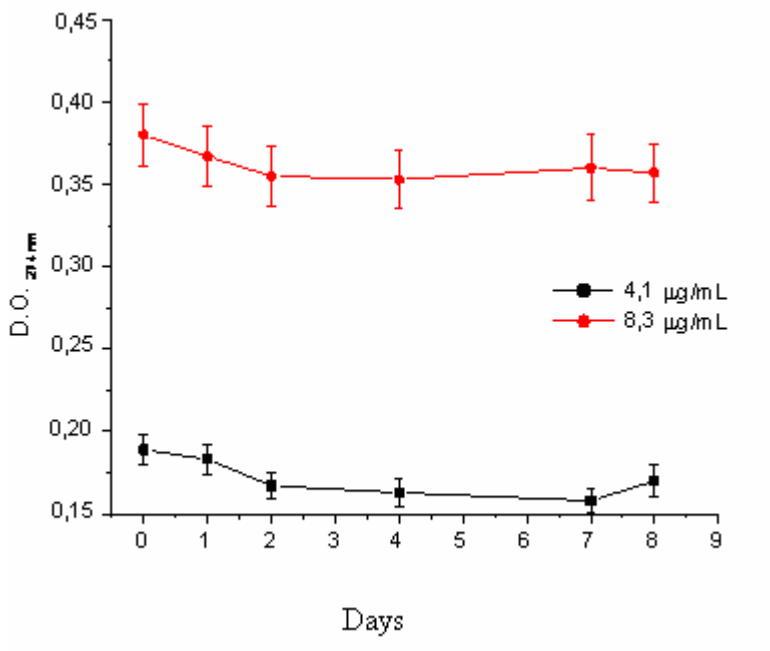

Figure 3. The stability of vitamin D_2 solutions in chloroform

4. Conclusions

The tested method was suitable for routine analysis of vitamin D_2 in multivitamin preparations and *fish oil* with a range of concentrations, which is widespread for the preparation used. Precision and accuracy of this method is similar to the literature values for other methods of the vitamin D_2 determination. The method, which was proposed for the vitamin D_2 determination, allows a good quantification of this.

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