Thus, we believe that the methods that use environmentally aggressive catalysts (sulfuric acid, ammonium sulfate, sulfuric chloride, polyfluoroalkanesulfonic acid) allow obtaining ethyl 4-nitrobenzoate with a higher yield. However, methods using ultrasound, microwaves, and catalysts of ultra-dispersed natural zeolites are environmentally and financially more profitable and easier to perform.

REFERENCES

1. Papenfuhs, T., Hess, R. and Fuss, A. Process for the Preparation of Alkyl Nitrobenzoates. US 5087725A, 2010. https://patents.google.com/patent/US5087725A/en
2. Ramishvili, T., Tsitsishvili, V. and Bukia, T. Preparation of Ethyl 4-Nitrobenzoate Using Ultradispersed Natural Zeolite Catalysts, Ultrasound and Microwave Irradiation. Advances in Chemical Engineering and Science, 11, 2021, 251-262. https://doi.org/10.4236/aces.2021.114016
3. Raymond M. David Ph.D., DABT, Richard H. McKee Ph.D., DABT, John H. Butala MS, DABT, Robert A. Barter Ph.D., DABT, Martin Kayser MD.Esters of Aromatic Mono-, Di-, and Tricarboxylic Acids, Aromatic Diacids and Di-, Tri-, Or Polyalcohols, 2001. https://doi.org/10.1002/0471435139.tox080

T. Linko, L. Sydorova, O. Posudiievska

IDENTIFICATION AND QUANTIFICATION OF THE TOTAL CONTENT OF VITAMIN D (D_2+D_3) BY IMMUNOENZYMATIC METHODS

Immunological analysis for free vitamin D:

The test refers to an immunological assay and to the analysis of a blood sample or blood components for presence of free vitamin D, including vitamin D metabolites, 25-hydroxyvitamin D or 1,25-dihydroxyvitamin D, where free vitamin D is a circulating, unbound fraction of vitamin D [2].

The method includes the following stages:

(a) adding an immobilized binding protein or antibody to 25-(OH)-vitamin D in the sample;

(b) mixing the sample with a diluent, whereby the diluent contains from 0.1% to 0.25% fluoroalkyl surfactant;

(c) incubating the sample for a period of time sufficient to ensure binding of the required amount of vitamin D to the binding protein;

(d) removing unbound serum and serum components by washing;

(e) performing an analysis of immobilized binding proteins or antibodies containing the seized vitamin D, which is associated with them, by the method of competitive binding, using a labeled compound of vitamin D;

(f) determining the concentration of the labeled compound of vitamin D associated with the binding protein [2].

The study refers to the use of fluoroalkyl surfactants, mostly – of perfluorocarboxylate surfactants, predominantly – of perfluorooctanoic acid as a substance for increasing the solubility of vitamin D in an immunological assay for the determination of free vitamin D [2].

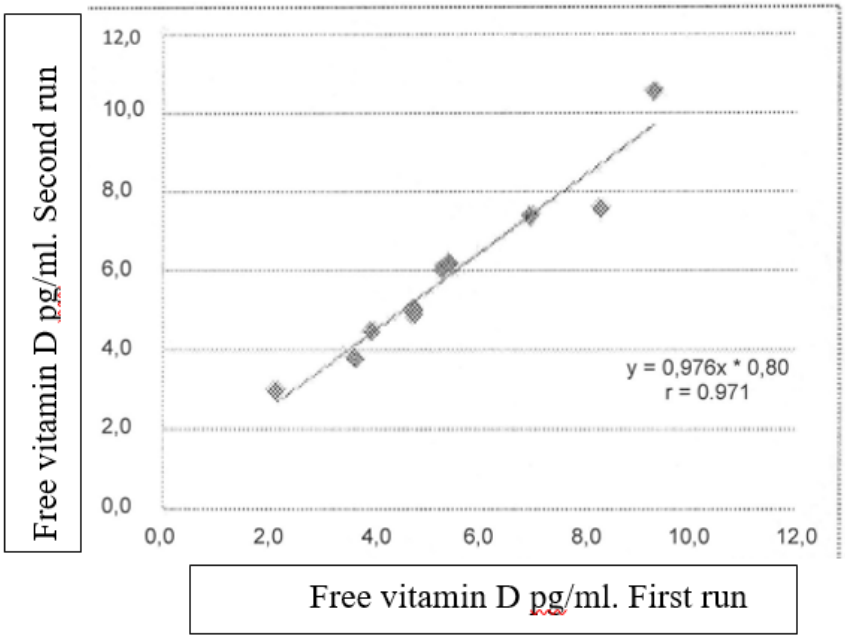

Fig. 1 – Correlation between the concentration of free vitamin D in the first and repeated analysis

The graph shows the correlation between the concentration of free vitamin D in the first and repeated analysis. The line indicates the results of repeated immunoextraction using 0 samples with different levels of free vitamin D. The slope of the regression line is not significantly different from "one".

This study provides a method for analyzing a fragment of vitamin D in a sample that involves [1]:

a) contact with a sample containing or being suspected of containing a fragment of vitamin D with a buffer capable of dissociating the moiety of vitamin D from its binding protein and/or an acidic pH buffer, and at least two monoclonal antibodies attached, for instance, separately to the surface, wherein one monoclonal antibody, or the first antibody, has a specific binding affinity for the fragment of vitamin D, and the other antibody, or the second antibody, has a specific binding affinity for the complex created between the first antibody and vitamin D;

b) evaluation of the binding between the above-mentioned specific antibodies and the fragment of vitamin D to determine the presence, absence and/or amount of the fragment of vitamin D in the sample.

The advantage of the method for determining free vitamin D (including vitamin D metabolites), according to the proposed method, is that it provides a format of analysis that can be automatized. In this way, the type of analysis performed in the study differs significantly from any previously existing assay of free vitamin D.

A binding protein for 25-OH-vitamin D is added to the assay. Binding proteins, such as antibodies, for vitamin D are known in this field of technology and are widely used in existing immunoassays for vitamin D. These antibodies, as well as other binding proteins, can also be used in this method. For example, instead of an antibody, its fragment can be applied to vitamin D, which was obtained by phage display technology [3].

REFERENCES

1. Harmonization of serum 25-hydroxycalciferol assay results from high-performance liquid chromatography, enzyme immunoassay, radioimmunoassay, and immunochemiluminescence systems: A multicenter study/ B. Nikooyeh [et. al.] // *JCLA Journal of Clinical Laboratory Analysis*. – 2017. – Vol. 31, N 6. – Access mode: doi: 10.1002/jcla.22117
2. Pat. EP 3491392 B1, G01N 33/82 (2006.01). Methods and compositions for the analysis of vitamin D / Yuan Chong-Shen, Saida Fakhri Ben (US); applicant and patent owner Witte, Weller & Partner PatentanwältembBPostfach 10 54 62 70047 Stuttgart (DE) – № 17755585.1; filed 28.07.2017; published 31.03.2021
3. Pat. WO 2011/122948 A1, G01N 33/82 (2006.01). Immunoassay for free vitamin D/ Michaël Franciscus Wilhelmus Cornelis (NL), George Henry (US), Franciscus Maria Fnna (NL), Leon Maria Jacobus Wilhelmus (NL) ; applicant FUTURE DIAGNOSTICS B.V. [NL/NL]; Nieuweweg 172, NL – 6603 BT Wijchen (NL) – № PCT/NL 2011/050219; filed 01.04.2011; published 06.10.2011