# OPTIMIZATION AND VALIDATION OF AN UHPSFC METHOD FOR THE QUALITY CONTROL OF VITAMIN D3 RAW MATERIAL

Bertyl Andri[a], Pierre Lebrun[ab], Amandine Dispas[a], Régis Klinkenberg[c], Bruno Streel[c], Roland Marini Djang'eing'a [a], Philippe Hubert[a]

[a] University of Liège (ULg), CIRM, Laboratory of Analytical Chemistry, 15 Avenue Hippocrate, B36, B-4000 Liège, Belgium [b] Arlenda s.a., B-4000 Liège, Belgium. [c]Galephar Research Center M/F, 39 Rue du parc industriel, B-6900 Marche en Famenne, Belgium

Nowadays, cholecalciferol or vitamin D3 arouses an increasing interest among the scientific community. Indeed, it is known to be involved in many health benefits such as supporting the bone health, immune system and lowering occurrence of chronic diseases [1]. However, despite these advantages, a worldwide deficiency is currently recognized for the large extent of the population - adults and children comprised- due to the small exogenous intakes and the low endogenous synthesis. Therefore, to obtain satisfying levels of vitamin D, supplementation with medicines or pharmaceuticals is necessary. In order to ensure the quality of these products, it is mandatory to proceed to quality control of the raw material used for their manufacturing. Among the panel of tests proposed by pharmacopeias, Normal Phase Liquid Chromatography (NPLC) is used to assess the purity of cholecalciferol. However, given the large consumption of toxic solvents and the long runtimes usually required, alternatives to such analytical technique may be appreciated and considered for intensive work.
In the context of green analytical chemistry, Supercritical Fluid Chromatography (SFC) is often suggested as an alternative to NPLC [2]. Indeed, modern SFC provides fast, efficient and green separations [3]. So, given the current interests devoted to vitamin D3 and green analytical chemistry, the quantitative performances of a modern UHPSFC method were challenged on a real-life case study: the Quality Control (QC) of vitamin D3 as a raw material.
A rapid and green UHPSFC method was optimized thanks to the Design of Experiment–Design Space (DoE-DS) methodology. Robust method with a high quality separation of the compounds of interest in 2 minutes was obtained using a gradient of ethanol as co-solvent of the carbon dioxide. The analytical method was then fully validated according to the total error approach, demonstrating the compliance of the method to the specifications of U.S. Pharmacopeia (USP: 97.0 – 103.0%) and European Pharmacopeia (EP: 97.0-102.0%) for an interval of [50 – 150%] of the target concentration. In order to allow quantification of impurities with vitamin D3 as an external standard in SFC-UV, correction factors were determined and confirmed during method validation. Thus, accurate quantification of impurities was demonstrated at the specified levels (0.1 and 1.0% of the main) in a 70.0 – 130.0% dosing range. This work demonstrates the validity of an UHPSFC method for the QC of vitamin D3. Therefore, the present study clearly supports the interest of the switch to a greener and faster alternative to NPLC in the pharmaceutical industry.

**[1]** M. Wacker, M.F. Holick, Vitamin D-effects on skeletal and extraskeletal health and the need for supplementation, Nutrients. 5 (2013) 111–148. http://dx.doi.org/10.3390/nu5010111
**[2]** L. Taylor, Past, Current, and Future Directions in Supercritical Fluid Chromatography, LC GC North America. 31 (2013) s44–s49.
**[3]** A. Tarafder, Metamorphosis of supercritical fluid chromatography to SFC: An Overview, TrAC Trends in Analytical Chemistry. (2016) in press. http://dx.doi.org/10.1016/j.trac.2016.01.002