

Title: **Comparison of different extraction methods for the analysis of vitamin D and vitamin D metabolites in liver samples**

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Vitamin D (VD) deficiency is related to several diseases, so it is crucial to correctly detect and quantify its concentration in the human body. One of the most challenging parts in the analysis of VD in biological samples is to extract the compounds, due to the complexity of these kinds of samples, as well as the low levels of concentration at which VD analogues are present in it, that required the application of very sensitive and selective techniques. Moreover, the amount of sample will not be unlimited, so miniaturized selective extraction techniques must be considered. Despite the existing methodologies for VD analysis, the existing methods for its analysis in biological samples should be improved. Also, to the best of our knowledge, there is no method that allows determining all the different VD compounds at the same time and some of the compounds have never been detected yet.

The aim of this study is to compare different extraction methods to find the most suitable and the greenest option for the analysis of VD its metabolites in liver samples. Finally, the parameters that can affect the extraction efficiency will be determined, studied and optimized.

Different methods were tested and the expected results were not obtained, especially because of the low extraction yield achieves for some of evaluated compounds including the ester metabolites and VD₂ and VD₃. On account of that, a backward step was done and some experiments were performed to study the viability of VD compounds extraction by using different solvents. Classical Folch (i.e., a liquid-liquid extraction method using a mixture of chloroform with

methanol and water), 2-propanol, acetone and acetonitrile were found as the most suitable options for extracting the target substances.

Moreover, some gravimetric experiments were done in order to study how much fat was extracted from the liver alongside the VD compounds depending on the solvent used. This study was carried out taking into account the hypothesis that the more fat is extracted, the more analytes akin to fat would be extracted (these kinds of analytes were the ones that showed the worst results when extracting VD from liver samples). These experiments indicated that mixture of chloroform and methanol and 2-propanol are the best options for analyzing the desired compounds, as with these two solvents the highest amount of fat was extracted.

More experiments should be done in order to find the most reachable approach to extract these compounds from the liver samples. However this study constitute the first step to recognize those parameter that have a remarkable effect on the extraction of vitamin analogues from such complex matrices.