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Quantitative analysis of 25 Hydroxy vitamin D by LC/MS-assay standardization surprises.

Eduard Rogatsky
University of Iceland, Iceland

Abstract

Vitamin D deficiency is a widespread clinical problem and has been associated with many adverse health outcomes. Analysis of Vitamin D2 (ergocalciferol) and D3 (cholecalciferol) and their major metabolites 25(OH)D2 and 25(OH)D3 has become a high priority topic in clinical analysis. Currently a variety of LC/MS methods have been developed to support vitamin D analysis. These LC/MS methods utilize different transitions, ionization modes, sample preparation strategies, mobile phases and columns. In LC/MS analysis of 25 OH Vitamin D, dehydration (water loss) is the major side reaction. Comparing acetonitrile to methanol, which are typically used as mobile phases for LC separation, acetonitrile does not support hydrogen bond formation; therefore, proton-induced water elimination in-source becomes a major side-reaction, especially given the low pH of the mobile phase and positive mode electrospray and APCI ionization. MeOH, in contrast, supports hydrogen bond formation with the 25(OH)D2 and 25(OH)D3 hydroxyl groups. This efficiently "shields" most of hydroxyl groups by hydrogen bonding, and protects against protonation and resultant water elimination. We found that quantitation of the 25(OH)D from its [M+H]+, "intact" precursor ion, is temperature invariant. In contrast, quantitation using the in-source dehydrated precursor (parent) ion, leads to increased sensitivity with a rise in temperature, due to its better ionization efficiency at higher temperatures. Since droplets evaporation region can vary with mass spectrometer hardware design, ratio between intact [M+H]+ and dehydrated precursor can be unpredictable. We also noticed that degree of dehydration is concentrationdependent. Chromatographic separation between analyte and its deuterated internal standard might cause different levels of analyte and internal standard dehydration and resulted in quantitative error.

Biography:

Rogatsky serves as the Editor-in-Chief for the Journal of Chromatography and Separation Techniques (OMICS publishing group). During the last 10 years (from 2005) he has published over 30 scientific papers in per-reviewed journals (mostly as the first author) and has presented over 50 posters and lectures. Overall, he has made more than a hundred scientific presentations and publications. Eduard completed his M.Sc. in physical chemistry at Belarus State University (former USSR) in 1990. He completed his PhD in bioanalytical chemistry (Bar-Ilan University, Israel) in 1998. At the end of 1999, he started his post-doctorate at Albert Einstein College of Medicine and became a faculty member since 2001 and was a mass spectrometry director at the Biomarker Analytical Resource Core. From October 2015 Dr. Rogatsky is a supervisor of the Chemical Threat Laboratory in the Division of Environmental Health Sciences at Wadsworth Center, Albany NY, USA and continues to be an Adjunct Professor at Albert Einstein College of Medicine.

Speaker Publications:

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