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Aspects of electrospray ionization of 25–hydroxy vitamin D lessons learned

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 actual temperature of droplets can vary with mass spectrometer hardware, flow rate, and mobile phase composition, fluctuations of these factors may contribute additional variability to the assay.

Biography

Rogatsky is a senior faculty member at Albert Einstein College of Medicine (NY, Bronx) and director of mass spectrometry at Biomarker Analytical Resource Core as part of the Harold and Muriel Block Institute for Clinical and Translational Research at Einstein and Montefiore . He has worked in the field of chromatography more than 20 years. Since 2001 his work has been within the service of the field of clinical mass spectrometry. During last 10 years (from 2004) Dr Rogatsky published 25 scientific papers in per-reviewed journals (mostly as the first author) and presented over 50 posters and lectures. Currently Dr Rogatsky serve as the Editor-in-Chief for the Journal of Chromatography and Separation Techniques (OMICS publishing group). Eduard Rogatsky completed his M.Sc (physical chemistry) in Belarus State University (former USSR) in 1990. In 1998 has completed PhD in bioanalytical chemistry (Bar-Ilan University, Israel). At the end of 1999 he started post-doctorate at Albert Einstein College of medicine and since 2001 joined faculty.

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