Evaluation of LC-MS/MS Scrambling Ratios for Deuterium-Labeled Vitamin D Metabolites, Steroids and Other Compounds of Clinical Significance

Abstract

Introduction and Objective: 25-Hydroxyvitamin D is a key indicator of vitamin D status, but the development of methods to quantify deuterium-labeled compounds of clinical interest is required. The use of stable isotope labeled internal standards (IS) is considered the gold standard to correct for matrix effects, and it is an essential tool for improving the accuracy and precision of quantitation. However, full labeled internal standards are not always available or feasible. Thus, smaller, partially labeled IS may be used in a combination with a deuterium-scrambled detection. This manuscript examines the effects of measuring deuterium-labeled vitamin D metabolites with stable isotope-labeled internal standards of the same family (13C3 vs. 13C5) or of different LC-MS systems (tandem quadrupole vs. quadrupole time-of-flight). The goal is to provide a general framework for normalizing IS signals of different compounds, in different matrices, and with different IS labeling.

Methods and Procedures

LC/MS Systems

LC/MS systems used were: Agilent 1100 HPLC-6410 triple quad, Waters Xevo G2 Q-Tof, and SCIEX Triple Quadrupole. The instruments were run in both positive and negative ionization modes to optimize ionization efficiency and selectivity for the compounds of interest.

Comparison of Scrambling of 25-Hydroxyvitamin D2 and D3 at Different Conditions

The comparison of scrambling was conducted in different conditions: 100 µg/mL Testosterone-13C3, 254 µg/mL 5α-Testosterone, 100 µg/mL 5α-Testosterone-13C3, and 100 µg/mL Progesterone-13C3. The results showed that the scrambling was consistent between different conditions, and no matrix effects were observed.

Selection of Transitions Greatly Impacts Scrambling

Selection of transitions is critical for controlling scrambling. The study found that some transitions were less affected by scrambling than others, indicating the importance of selecting appropriate transitions for accurate quantitation.

INVESTIGATION OF TESTOSTERONE SCRAMBLING

Testosterone Scrambling Kinetics

The study investigated the kinetics of testosterone scrambling, revealing that testosterone scrambling was highly dependent on the matrix and the instrument used. The results showed that testosterone scrambling was more pronounced in the positive ionization mode compared to the negative mode.

CONCLUSIONS

- Scrambling was observed on both the Agilent 6410 triple quadrupole and the Waters Xevo G2 Q-Tof, and in some cases was very pronounced.
- For a specific transition, scrambling ratios were consistent between solvent and serum. No matrix effects on scrambling.
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