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

Abstract

Introduction and Objectives: In agilent clinical challenges with LC-MS/MS, we are frequently exposed to various sources and jointly developed approaches to measure these specific ratios (e.g., steroidal, non-scraper internal standards). Our objective was to develop and validate a method for measuring sodium-dependent internal standards that could be used for the assay to measure specific ratios. The use of sodium-dependent internal standards could potentially improve the assay's reproducibility and accuracy by compensating for matrix effects and influencing the scrambling ratio.

Methods and Procedures: We used Agilent's 1100 HPLC-6410 triple quad systems for this study. We established and validated a deuterium scrambling method for measuring 25-Hydroxyvitamin D2 and D3. We also investigated the scrambling ratios of deuterium-labeled steroids and other compounds of clinical significance.

Results: We found that the deuterium scrambling ratios were consistent between solvent and serum. No matrix effects on scrambling were observed. The scrambling ratios were reproducible and consistent across different instruments and laboratories.

Conclusions: Our method provides a reliable and reproducible way to measure deuterium scrambling ratios for 25-Hydroxyvitamin D2 and D3. It can be used to investigate the scrambling ratios of other compounds of clinical significance.