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 a clinical diagnostic challenge with LC-MS/MS, it is possible to infer effects that cause interferences or eluted compound co-occurrences. Such unanticipated internal standardization problems are frequently used to compensate for matrix effects and to uncover the source of scrambling. The use of labeled internal standards can provide insight into the drug being monitored, as well as on the mechanisms that cause interferences. We investigated the use of labeled internal standards to determine the potential for matrix effects that cause interferences and to identify scrambling in the concomitant medication, etc. that may occur at the retention time of the labeled internal standards. Scrambling may be mitigated or eliminated by altering instrument conditions and transition selection.

**Methods and Procedures:**

**LCMS System:**
- Instrument: Waters Alliance UPLC-Xevo G2 Q-Tof
- Column: Waters Acquity UPC, BEH C18, 1.7μm, 2.1 x 50mm
- UPLC Conditions: 0.4mL/min, isocratic, 30:70 (0.1% formic acid in acetonitrile:0.1% formic acid in water)
- MS Conditions: ESI+, Cone 25V, Capillary 2.5kV, CE 20
- Water Loss: Mass differences of the selected transitions in the mass spectrometer.

**Testosterone Analysis Conditions:**
- MS Conditions: ESI+, Fragmentor 50V, Capillary 4.0kV, CE 10 (0.1% formic acid in methanol:0.1% formic acid in water)

**Hydroxyvitamin D Scrambling:**
- MS Conditions: ESI+, Fragmentor 50V, Capillary 4.0kV, CE 10 (0.1% formic acid in methanol:0.1% formic acid in water)

**Results:**
- The observed scrambling ratios for native and labeled 25-Hydroxyvitamin D2 and D3 showed no matrix effects on scrambling.
- Water loss has same transition as native 50 398
- No scrambling at water loss.
- For a specific transition, scrambling ratios were consistent between solvent and serum. No matrix effects on scrambling.
- It may be advisable to investigate at higher concentrations than normally analyzed to ensure that instrument sensitivity does not impact accuracy of scrambling determination.

**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.
- Direct infusion can provide rapid and accurate determination of scrambling ratios. Inhomin and chromatographic injection results were consistent.
- It may be advisable to investigate at higher concentrations than analyzed to ensure that instrument sensitivity does not impact accuracy of scrambling determination.
- Awareness of potential scrambling is important for proper internal standard selection. Scrambling may be mitigated or eliminated by altering instrument conditions and transition selection.
- Deuterium-labeled internal standards are a viable option for LC-MS/MS analysis with selection of the appropriate transition. Deuterated standards can be more cost effective than 13C-labeled internal standards, more widely available and with lower cost per test.