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

**Abstract**

Introduction and Objective: In an agmatine clinical challenge with 25-Hydroxyvitamin D as a marker for effects that cause an increase in dietary vitamin D intake, reliable quantitation of serum levels of vitamin D metabolites is critical for the analysis of clinical studies. Complications in the use of deuterated internal standards were seen from hydrocarbon scrambling in the calibration of the selected transitions or in the ion source. In this study, we examined deuterated labeled 25-Hydroxyvitamin D, testosterone, and other compounds of clinical significance by LC-MS/MS at multiple transitions. We investigated the effect of the scrambling ratio on the ability of 25-Hydroxyvitamin D to serve as a biomarker for vitamin D deficiency, and we determined whether scrambling and detection was possible in the clinical standard.

**Methods and Procedures**

**LCMS System**

Instrument: Waters Alliance UPLC, TQ Symmetry 2, 5µm, 2.5 x 100mm
d6-

Column: Waters Acquity UPLC, BEH C18, 1.7µm, 2.1 x 50mm

**Testosterone Analysis Conditions:**

Column: Phenomenex Kinetex, C18, 3µm, 2.1 x 50mm

Instrument: Agilent 1100 HPLC-6410 triple quad MS Conditions: ESI+, Cone 25V, Capillary 2.5kV, CE 20

25-Hydroxyvitamin D Analysis Conditions:

Column: Waters Acquity UPLC, BEH C18, 1.7µm, 2.1 x 50mm

Instrument: Agilent 1100 HPLC-6410 triple quad MS Conditions: ESI+, Cone 25V, Capillary 2.5kV, CE 20

Testosterone Analysis Conditions:

Column: Phenomenex Kinetex, C18, 3µm, 2.1 x 50mm

Instrument: Agilent 1100 HPLC-6410 triple quad MS Conditions: ESI+, Cone 25V, Capillary 2.5kV, CE 20

LCMS System 2:

Instrument: Agilent 1100 HPLC, Q-Tof single quad MS

Column: Phenomenex Kinetex, C18, 3µm, 2.1 x 50mm

**Testosterone Chromatograms on Xevo G2**

**Testosterone Chromatograms on 6410**

**Investigation of Testosterone Scrambling**

Note: No scrambling at major transitions. Can be problem if compounds are not well resolved chromatographically.

**Testosterone Scrambling on Xevo G2**

**Testosterone Scrambling on 6410**

**Conclusion:**

- Scrambling was observed on both the Agilent 6410 triple quadrupole and the Waters Symmetry 2, Q-Tof, and in some cases was very pronounced.
- For 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. Influenza and chromatographic injection results were consistent.
- It may be advisable to investigate at higher concentrations than normally analyzed to ensure that instrument sensitivity does not impact accuracy of scrambling determination.

**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 house cost per test. 13C-labeled internal standards are most effective when deuterium scrambling issues can be resolved.**