Selection of Internal Standards for LC-MS/MS Applications

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Internal standards are utilized across a wide range of mass spectrometry applications including therapeutic drug monitoring, newborn screening, endocrinology, and pain management testing. Internal standards are used to improve the accuracy of quantitation of analytes in complex matrices by normalizing for differences in extraction, chromatography, ionization and detection between samples.

Critical considerations in selection of a suitable internal standard include label placement, isotopic distribution and purity, impact of natural isotope abundance, cross talk and scrambling, and desired dynamic range of the method. The optimal internal standard has chromatographic retention identical to the analyte of interest; adequate mass differentiation; and retention of the mass differential in the daughter ion. Numerous examples will be presented to illustrate selection criteria.

Ionization and fragmentation of the analyte are impacted by the mode of ionization and the mass spectrometry technique. Traditional GC/MS platforms used relatively strong electron impact ionization whereas LC/MSMS platforms use softer electrospray and chemical ionization. Zolpidem-d_7 is suitable for both GCMS and LCMS applications whereas Zolpidem-d_6 is not suitable for GCMS applications due to loss of label during electron impact ionization and fragmentation.

Understanding the mass contribution of natural abundance of isotopes to the analyte mass distribution is important in selection of mass and ions to be monitored. Halogenated compounds have characteristic mass distributions based on relative abundance of chlorine and bromine. Due to the relative abundance of chlorine 35 vs. 37, improper mass selection can lead to interferences from the internal standard. Benzodiazepines and their metabolites are good examples of the influence of isotope distribution on mass selection.

Deuterium scrambling has been observed due to collisional activation in the mass spectrometer. Scrambling can be mitigated and controlled by proper selection of ionization parameters and transitions to be monitored. 25-Hydroxyvitamin D metabolites internal standards are available labeled on the side chain (d_6) as well as the backbone (d_3). We have observed scrambling in both of the labeled internal standards depending on platform and mode of ionization thus requiring mitigation by careful selection of ionization parameters.

Deuterium labeled internal standards are widely used in both GCMS and LCMS applications. Recent advances in LCMS chromatography systems and increased sensitivity requirements have led to the need and development of numerous ^{13}C-labeled internal standards for select applications. ^{13}C-analogs do not resolve chromatographically however are limited by availability and cost. Testosterone-d_3 (D-ring labeled) is suitable for use in quantitation of male testosterone ranges but may not be suitable for quantitation of low levels of testosterone due to loss of label in the daughter ion. Deuterium label placement on other parts of the steroid ring system is often introduced by chemical exchange in which the deuterium atoms are susceptible to loss back to hydrogen in protic solvents. Although more challenging to synthesize and more costly to obtain, select A-ring ^{13}C-labeled steroids offer greater sensitivity for quantitation of low steroid hormone levels.

Internal standard selection is a balance between method sensitivity requirements and cost considerations. Internal standard selection requires understanding of analyte fragmentation, method sensitivity and dynamic range requirements, and optimization of ionization parameters.