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THE USE OF STABLE-ISOTOPE-LABELED (SIL) INTERNAL STANDARDS TO COMPENSATE FOR MATRIX EFFECTS: KEY CONSIDERATIONS

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INTRODUCTION

The high sensitivity and selectivity of LC/MS/MS has made this technology the predominant analytical technique in trace analysis, regardless of the analyte(s) and matrix. However, LC/MS/MS is susceptible to matrix effects. Residual matrix components are a significant source of imprecision in quantitative analyses. Matrix effects result from co-eluting matrix components that affect the ionization of the target analyte, resulting either in ion suppression, or, in some cases, ion enhancement. Matrix effects can be highly variable and can be difficult to control or predict.

Most researchers now include an evaluation of matrix effects as part of method development. If the level of matrix effects is determined to be unacceptable, many researchers use combinations of sample preparation techniques such as solid-phase extraction (SPE) with optimization of chromatographic parameters to mitigate matrix effects [1]. For many researchers, the use of an internal standard, often a stable-isotope-labeled (SIL) analog of the analyte is used to compensate for the alteration in signal [2].

In the majority of quantitative analyses, the use of a SIL internal standard is the norm and is recommended when feasible. This SIL analog should behave nearly the same way as the analyte of interest – i.e. have the same extraction efficiency from the matrix as the analyte and co-elute chromatographically. In this way, the SIL internal standard compensates for inefficiencies/losses in the extraction and sample preparation steps, as well as for any matrix effects in the MS, as summarized in the FDA/AAPS Crystal City III Workshop/Conference Report [3]. In the majority of applications, this is true.

The ability of an SIL internal standard to compensate for matrix effects is dependent upon its co-elution with the unlabeled compound, however, this does not always happen. When the two do not co-elute on a reversed-phase column, it is typically due to the deuterium isotope effect. The deuterium isotope effect is thought to be caused by changes in lipophilicity of the molecule when hydrogen is replaced with deuterium. In the example of carvedilol in plasma [4], the analyte-to-internal standard ratio changed between two lots of commercially available human plasma. The slight retention time difference between the two resulted in a different degree of ion suppression between the analyte and its isotopically labeled internal standard.

Both Wang [4] and Jemal [5] have demonstrated that the matrix effects experienced by the analyte and its SIL internal standard can differ by 26% or more. This observation has been reported in both plasma and urine [4, 5].

Not only have different retention times been observed for the analyte and SIL internal standard [6, 7] by multiple researchers, but researchers have also observed different extraction recoveries for analytes and their SIL internal standards. Weiling reported a 35% difference in extraction recovery between haloperidol and deuterated haloperidol [7].

Problems have also been reported with the stability (due to exchange with hydrogen) of deuterium labeled internal standards in water [8] which have precluded use of the deuterated IS. This was also observed to a lesser extent in plasma. A 28% increase in the non-labeled compound was observed after incubating plasma with the deuterated compound for an hour. This again, would render the SIL internal standard not suitable for use in a quantitative method.

Researchers have investigated ion suppression with analytes and their corresponding SIL internal standards [9]. It was found, that for the nine compounds studied, all co-eluting SIL internal standards and analytes suppress each others ionization when ESI was used. When APCI was chosen, the pairs actually enhanced each others ionization. In addition, the suppression of the D0 analytes increased as the concentration of D3-IS increased. "The extent of the suppression in each drug-IS pair was concentration dependent in a non-linear fashion" [9]. The degree of suppression between analyte and SIL internal standard is compound dependent as well.

It is also critical to verify the purity of an SIL internal standard, as any non-labeled impurity can adversely affect quantitation and lead to artificially high concentrations of analyte.

Mohammed Jemal [5] states that, under certain conditions (particularly when higher degrees of matrix effects are present) "the use of a stable isotope analog internal standard does not, contrary to conventional thinking, guarantee the constancy of the analyte/internal response ratio, which is a prerequisite for a rugged bioanalytical method."

While we do advocate the use of SIL internal standards for quantitative analyses, it is important to be aware of some of the pitfalls of solely relying on the IS to compensate for matrix effects and other method inefficiencies. It is equally important to fully characterize the behavior of the SIL internal standard along with the analyte(s) of interest.

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