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Letter to the Editor

Stable isotope dilution techniques

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Sir,

An editorial statement that reports of quantitative methods based upon mass spectrometric measurements for drugs, drug metabolites, endogenous metabolites, environmental contaminants and the like, which employ chromatographic inlet (LC, GC, CE, etc.) of extracts will be more, or perhaps only, acceptable for publishing if stable isotope dilution techniques are employed when it is possible to do so. Simply using "similar" compounds as internal standards introduces differential recoveries that change with minor uncontrollable changes in sample preparation technique, chromatographic characteristics that change with column age, selective losses that result in the complete failure of a carrier effect at very low levels, and ion-source ionizing efficiencies that change with progressive source contamination. These add together to introduce errors that can only be detected and corrected with great difficulty or expense and, therefore, are usually ignored. Using a stable isotope-labelled analog of an analyte avoids these problems, as the analyte and the internal standard are virtually indistinguishable chemically, chromatographically, and in the ionization process, but are separately measurable in the mass analyzer.

Using compounds that are only chemically or structurally related to the analyte as internal standards can yield data of questionable value. For example, assay of methylmalonic acid in human urine has been reported using ethylmalonic acid added as the internal standard [1]. Ethylmalonic acid is biosynthesized in the normal human by carboxylation of butyryl-CoA giving rise to normal urinary levels that are several times those for methylmalonic acid. Use of 2-[methyl-$^{2}H_{3}$]methylmalonic acid [2] in this instance is more appropriate.

Many commercial sources of compounds labelled with deuterium and heavy stable isotopes of carbon, nitrogen and oxygen now exist. Many analytes of interest may be purchased ready-made, and when these are not available, they may be synthesized in house from labelled starting materials. Among the important considerations in selecting a stable isotope-labelled analog for use as an internal standard is that the labelling atom(s) be refractory to back exchange in the isolation and measurement protocol, and that it be available in a precisely known purity. The latter must often be determined by a 'reverse' stable isotope dilution technique, that is, measurement of an only approximately known concentration of the labelled standard relative to a pure sample of the analyte used as the reference. Deuterium is often troublesome if it is substituted in a labile position and subject to back exchange in an aqueous medium at high or low pH. For example, all the hydrogens are easily exchangeable in succinylacetone, a metabolite of tyrosine elevated in hereditary tyrosinemia.

Problems associated with stable isotope dilution techniques are relatively very much more minor. Deuterium-labelled analogs have gas chromatographic retention times relative to the unlabelled compound that are inversely related to the degree of deuterium substitution, and may amount to as much as 1–2%; this effect is much less pronounced for

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carbon, nitrogen and oxygen. Differences in ionization efficiency between labelled and unlabelled are very small, and become unmeasurable when the labelling site is remote from the charge centre. While ion fragmentation is sensitive to the kinetic isotope effect, it becomes less than a measurable problem when isotopes of carbon, nitrogen or oxygen are employed, and when the labelling site is distant from the fragmenting bond in the case of deuterium labelling.

The advantages of using stable isotope dilution techniques are significant; in devising an analytical protocol involving an internal standard with mass spectrometric measurement, one should carefully consider one based upon this technique.

References

[1] E.J. Norman, H.K. Berry and M.D. Denton, Biomed. Mass Spectrom., 6 (1979) 546.
[2] J.A. Montgomery and O.A. Mamer, Methods Enzymol., 166, (1988) 47.