Choosing an Internal Standard

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When creating a new method for quantitation, the choice of the correct internal standard (IS) can improve the accuracy and precision of the method. The proper internal standard should be chemically similar to the compound(s) that you are analyzing, but is not expected to be naturally present in your sample. It is best to choose compounds that have the same functional groups, boiling points, and activity as your target compounds.

Two examples

If you are using MS, then it is common to use a deuterated analog of your compound of interest i.e. Amphetamine & Amphetamine d-5.

If you are working with a non-MS detector, the deuterated internal standards would coelute with your analyte of interest and cause problems with quantitation. In this case, you would try to use a compound that is somewhat chemically similar, but would not be found in your sample, i.e. if your target is tri-chlorophenol, you might use a tri-bromophenol or a di-chlorophenol internal standard.

Any time you choose in internal standard, you have to validate that it does correct for small amounts of variation within the analytical process. The best quantitation is achieved when with an internal standard for each one of your target compounds, but in many cases this is impractical because of the number of analytes in your mix and/or the cost of each internal standard.

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