# A Case Against Using Internal Standards for EPA Method TO-15 Involving Toxic Compounds in Ambient Air

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## INTRODUCTION

Gas chromatography is a primary tool to quantitate organics in many sample matrices. By examining unknown samples under identical conditions as standards, their ratio of detector responses will yield concentration for the unknown. Unfortunately, some experimental conditions may not be identical between standards and samples. By adding an artificial component to all samples and standards, performance of the sampling process can be monitored and corrected mathematically. These added components are internal standards.

Internal standards are employed in a number of standard methods[1] to provide corrections to experimental inaccuracies that can occur in a measurement, and provide a judgment of the overall performance of the experiment. By adding in a known quantity of compound meeting specific requirements, its response can be used in the final computations to correct for systematic errors that degrade results for unknowns.

For example, appropriate internal standards are added into a sample matrix just prior to solvent-solvent extraction of pesticides in foods. Subtle variations in extraction efficiencies, such as temperature and pH of the matrix, are likely to affect both analytes and internal standards. Deviations in expected results with internal standards are then mathematically applied to the final results.

Another situation involves purging volatile analytes from water, where purging efficiencies can be impacted by matrix pH, temperature and atmospheric pressure. Again, responses for internal standards can be monitored and corrections applied for these perturbations.

A third application entails changes in injection volume when introducing a sample into a gas chromatograph. When an internal standard is added to the sample volume, a doubling of the injection volume, for example, will double the responses for both the analytes and for the internal standards. However, the ratio of concentrations for internal standards and unknowns remains constant. Thus a change in volume is compensated during the final calculations when including appropriate computations with internal standards.

The whole chromatographic process can be stable for very short periods, but, unfortunately, could vary from run to run, especially over the long term. Proper use of internal standards can bring results into compliance by checking on these variations and then correcting results for the change, especially with mass spectrometers that tend to wander around in sensitivities.

Measuring toxic compounds by EPA Method TO-15 is a whole-sample measurement and does not involve any solvent extraction, nor purge from the matrix. Therefore, these two applications for internal standards do not apply to measurement of analytes by this method. Typically with the TO-15 method, internal standards are added into the process just after the sample is trapped and thus do not monitor the sample loading process and sample volume. The only check remaining is performance of the chromatograph and detector.

## DISCUSSION

### Propagation of Errors

The mathematics for computations with internal standards becomes:

$$\left[analyte_i\right] = \frac{area_i \times \left[IS\right]}{RRF_i \times area_{IS\ in\ sample}}$$

where

$$RRF_i \equiv \frac{area\ \ STD_i \times \left[IS\right]}{\left[analyte\ \ \ STD_i\right] \times area_{IS\ in\ std}}$$

Since all four areas have statistical errors from detector uncertainties, the relative standard deviation for the analyte concentration is impacted directly by the relative standard deviation of all four by the following:[2]

$$RSD\ of\ \left[analyte_i\right] = \sqrt{\left(\frac{a}{A}\right)^2 + \left(\frac{b}{B}\right)^2 + \left(\frac{c}{D}\right)^2 + \left(\frac{d}{D}\right)^2}$$

where ***A*** is *area*$_i$, ***a*** is standard deviation of *area*$_i$, ***B*** is *area*$_{IS\ in\ sample}$, ***b*** is standard deviation of *area*$_{IS\ in\ sample}$, ***C*** is *areaSTD*$_i$, ***c*** is standard deviation of *areaSTD*$_i$, ***D*** is *area*$_{IS\ in\ sample}$, ***d*** is standard deviation of *area* $_{IS\ in\ std}$. If relative standard deviations for all areas are tentatively assigned a typical experimental value of 10%, then the uncertainty for the analyte concentration degrades to 20%.

With external standard computation, the formula simplifies to:

$$\left[analyte_i\right] = \frac{area_i}{RF_i}$$

where

$$RF_i \equiv \frac{area\ \ \ STD_i}{\left[analyte\ \ \ \ STD_i\right]}$$

And the uncertainty for the analyte concentration becomes:

$$RSD\ of\ \left[analyte_i\right] = \sqrt{\left(\frac{a}{A}\right)^2 + \left(\frac{c}{D}\right)^2}$$

Again, if the relative deviations are assigned a typical value 10%, the RSD for the ratio degrades to only 14%.

### Mandates for Choice of Internal Standards

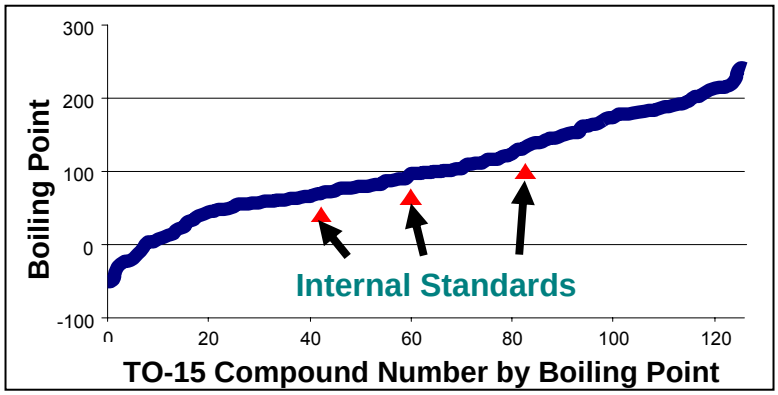
Internal standard cannot impact the measurement of any target analyte. The following are guidelines for ensuring that addition of internal standards does not alter the accuracy of the final results:

1. not present in samples
2. chemically and physically similar to related analytes
3. high purity, especially of targets
4. perform similar chromatography to assigned analytes
5. chromatographically separated from targets, or possess unique MS ions
6. not interfere in or be interfered with by any analyte or matrix component to enable identifying ions to be picked

***1. Internal standard must not be present in samples*** - The target list for Method TO-15 is extensive, with typically more than 64 components being measured. Finding an internal standard not on the list can be a problem. Some choices can be deuterated, such as chlorobenzene-d5, but most of the light components have either no hydrogen atoms to exchange for deuterium, such as dichlorodifluoromethane (Freon 12), or not enough differences in mass to distinguish them in a mass spectrometer. As a result, we have very limited choices for assigning internal standards that meet the mandated criteria. The recommended choices as listed in TO-15 are bromochloromethane, chlorobenzene-d5 and 1,4 diflurobenzene.

***2. Internal Standard must be chemically and physically similar to related analytes*** - Figure 1 illustrates the boiling point range for common analytes and the usual TO-15 internal standards denoted. Forty-one analytes have much lower boiling points than the first internal

**Figure 1. Mandated Internal Standards for TO-15 do not match boiling points for many of the target analytes in Method TO-15.**

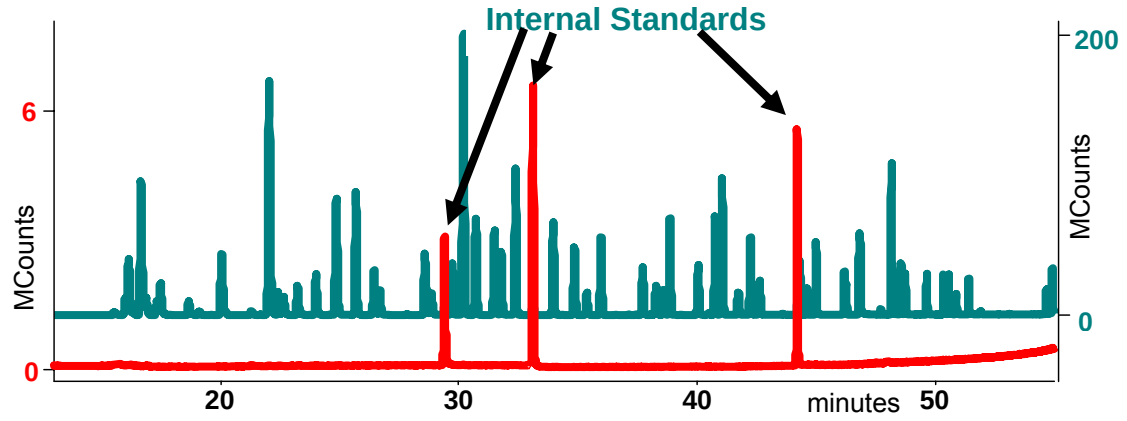



standard, and 45 have much higher ones than the last internal standard. These low and high target compounds are not fully monitored by assigned internal standards during the trapping and desorption processes due to their significant differences in boiling points. Serious problems with any trapping process are failures to be either adsorptive or cool enough to fully trap all analytes, especially the light boilers, such as Freons, or sufficiently hot enough to fully desorb all analytes, such the heavy ones, including naphthalene and hexachlorobutadiene. With no internal standards close to these analytes, a poor performing trap is not identified by the usual internal standards.

***3. Internal standards must be pure, especially of target compounds*** - High performance TO-15 systems are now capable of measuring target analytes from 1 ppt V/V to nearly 100 ppb V/V - a linear range of 100,000. To meet the purity mandate for internal standards, their starting materials must be pure to greater that 1:10,000, or <0.01%, for internal standard concentrations of 10 ppb V/V. Since many of these standards end up being chemically related to analytes, invariably some of these analytes show up as impurities in the internal standard mix and will then lead to elevated reported concentrations, especially at the lowest levels.

***4. Problems in chromatographic separations are similar to the boiling point issue*** - Figure 2 features typical total reconstructed ion chromatograms of common target analytes with internal standards overlaid. Thirty-five percent of target compounds elute prior to the first internal standard - Bromochloromethane, and 27% come off the column after the last - Chlorobenzene-d5. Any degradation of these compounds is not monitored through the assigned internal standards. If the cryo-focus trap temperature fails to start cool enough to ensnare the light ends, or if the column is not hot enough to elute the late ones, then these results will be in error and not correctable by internal standards. Internal standards can remain intact, but the problem analytes could be grossly impacted and their results are then in significant error.

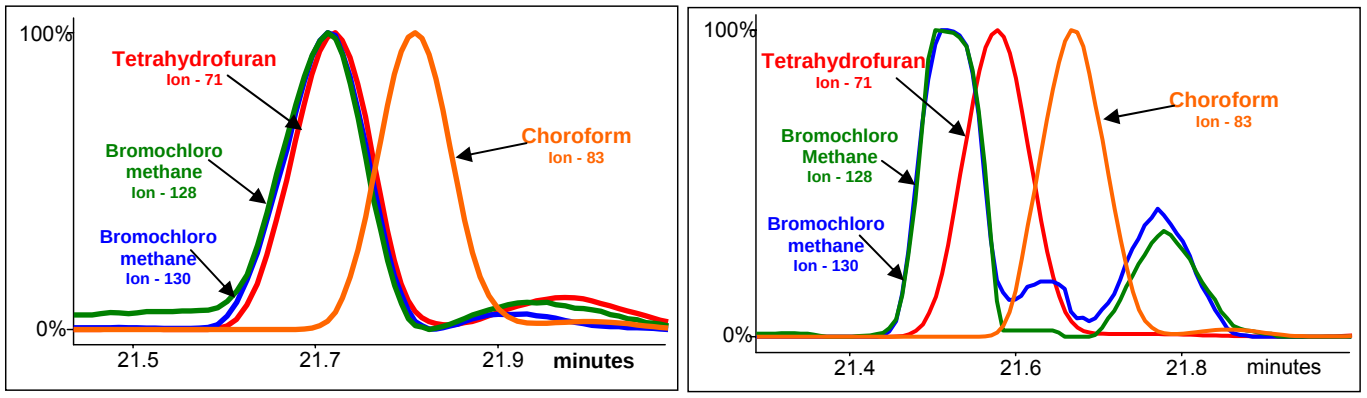
**Figure 2. Many target analytes elute before the first internal standard and after the last one.**




***5. Internal Standards must be chromatographically separated from targets, or possess unique MS ions*** - The three internal standards listed in Method TO-15 have been carefully evaluated to ensure that none of their ions match with nearby target analytes under most chromatographic conditions. Radical departure from operating parameters listed in the method, such as alternate column choice, could invoke issues with overlapping ions in analytes and internal standards and must be rigorously validated. Any overlap can yield false positive results for target analytes, or improper areas for internal standards with consequential errors in the applied corrections.

***6. Internal Standards must not interfere in or be interfered with by any analyte or matrix component, to enable identifying ions to be properly picked -*** The ionization process at the source of mass spectrometers can effect unintended changes in the ions generated, especially with ion traps holding on to the ions until appropriately ejected to the detector. This process can alter the mass spectrum as the peaks elute from the column, especially when huge concentrations are involved. Figure 3 illustrates a situation where two compounds coelute, with the dominant analyte altering the spectrum of a lesser ion.

**Figure 3. Coelution of a major peak and an internal standard can lead to severe distortion of the minor peak. The example on the left shows no distortion when concentration are below 10 ppbV. However, when the major peak - Tetrahydrofuran - elutes right on top of Bromochloromethane, as shown on the right, the internal standard is split into multiple peaks, with significantly reduced areas and the peak retention time varies. Peaks are normalized for easier visual comparisons.**




Judicious selection of operating parameters for the mass spectrometer, particularly maximum ion time and target RIC, can reduce or eliminate the effect, resulting in enhanced linearity over a wider concentration range, especially at the upper concentration end.

## Stability of High Performance GCMS

The only remaining application of Internal Standards is corrections for systematic errors occurring with the chromatographic process and the detector responses. Method TO-15 allows a variation in daily check standards of ±30% from the most recent calibration series.[3] The Lotus Consulting Ultra Trace Toxics System (Lotus Consulting, Long Beach, CA), based on the Varian 4000 GCMS (Varian Inc., Walnut Creek, CA), is easily consistent enough to meet this criterion readily without implementing internal standard corrections. Figure 4 illustrates one typical analyte - dichlorodifluoromethane - run as a daily check sample over a 15 day period. Table I lists results for several other analytes and internal standards employed over the same 15 day interval. These raw areas are definitely within the precision requirement of the method.

**Figure 4. Control Chart for DiF DiCl Methane in Daily Checks over 15 days**

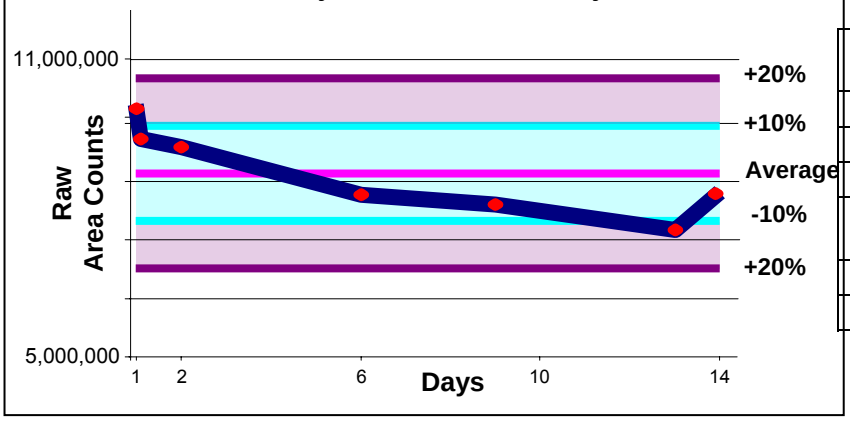



**Table 1. Variation of Raw Area Counts for Representative Analytes and Internal Standards**

| Analyte | Variation over 14 days |
|---|---|
| Dichlorodiflurormethane | ±9% |
| Toluene | ±15% |
| HexaChloroButadiene | ±5% |
| Bromochloromethane (IS) | ±10% |
| 14 Difluorobenzene (IS) | ±7% |
| Chlorobenzene-d5 (IS) | ±8% |

## SUMMARY

Internal standards are not appropriate for use with USEPA Method TO-15 because they do not provide adequate monitoring, or corrections for many of the target analytes. Internal standards listed in the method do not match chemical, physical, and chromatographic characteristics with every analyte. Errors in sample loading and sample trapping are not monitored, since the addition usually occurs ***after*** the sample is loaded into the first adsorbent trap.

The insertion of the uncertainty for internal standard areas into the computations for the final result severely impacts the combined confidence in the quality of the final answer if results are strictly limited by random measurement error. Simplifying the calculation to an uncomplicated ratio of sample and standard areas reduces the propagation of errors.

Commercial internal standards may not be pure enough to cover the wide dynamic range of high performance mass spectrometers, and could add detectable artifacts to the quantitation of many target analytes, especially when measuring low ambient levels. False positives could be reported.

Addition of internal standard into the complex soup of all target compounds can greatly complicate the chromatographic process. Extreme precautions must be exercised to keep all compounds properly separated, and to avoid spectral interferences that could occur, notably for related analytes.

High performance GCMS systems are stable enough over the long term to easily meet the quality check criterion listed for the method ***without*** internal standards. The elimination of the added check simplifies the chromatographic process. By deleting the mandate for internal standards, the hardware becomes simpler and the calculations are more straightforward and easier to understand.

## References


1. Typically examples are:

   - USEPA Method 8081B "Organochlorine Pesticides by Gas Chromatography", www.epa.gov/epawaste/ hazard/testmethods/sw846/pdfs/8081b.pdf.

   - USEPA Method 8270D, "Semivolatile Organic Compounds by Gas Chromatography/ Mass Spectrometer", www.epa.gov/epawaste/ hazard/testmethods/sw846/pdfs/8570d.pdf.

   - USEPA Compendium "Method TO-15 Determination of Volatile Organic Compounds (VOCs) in Air Collected in Specially-prepared Canisters and Analyzed by Gas Chromatography/Mass Spectrometry (GC/MS)", www.epa.gov/ttn/amtic/files/ambient/airtox/to-15r.pdf.

2. Waser, J., *Quantitative Chemistry*; W A Benjamin; New York, 1964, p.373.

3. *Op Cit.*, USEPA Compendium Method TO-15, page 15-26.



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