

INTERNAL STANDARD CALIBRATION



DEFINITION

Internal standard calibration involves the comparison of the instrument responses from the target compounds in the sample to the responses of reference standards added to the sample or sample extract before injection.

Ref: SW846, 8000C, Section 11.4.3, Revision 3, March 2003



The response of the target compound is normalized to the response of the reference standard. This reference standard is called an internal standard because it is contained within the aliquot of the sample or sample extract that is actually injected into the instrumentation.

*Ref: SW846, 8000C, Section 11.4.3,
Revision 3, March 2003*



A constant amount of the internal standard is added to all samples or extracts. That same amount of the internal standard is also included in each of the calibration standards.

***Ref: SW846, 8000C, Section 11.4.3, Revision 3,
March 2003***



The ratio of the peak area (or height) of the target compound in the sample or sample extract to the peak area (or height) of the internal standard in the sample or sample extract is compared to a similar ratio derived for each calibration standard.

*Ref: SW846, 8000C, Section 11.4.3, Revision 3,
March 2003*



This ratio is termed the response factor (RF) or relative response factor (RRF), indicating that the target compound response is calculated relative to that of the internal standard.

Ref: SW846, 8000C, Section 11.4.3, Revision 3, March 2003



Response Factor Equation

$$RF = ((A_x)(C_{is})) / ((A_{is})(C_x))$$

Where:

- A_x = Area of the compound
- C_x = Concentration of the compound
- A_{is} = Area of the internal standard
- C_{is} = Concentration of the internal standard



SELECTION OF INTERNAL STANDARDS

- Internal standards that are similar in analytical behavior to the compounds of interest, and not expected to be found in the samples
- The analyst needs to demonstrate that the measurement of the internal standard is not affected by target analytes, surrogates, or by matrix interferences
- This is not as useful for GC and HPLC methods with non-MS detectors, unless the internal standards could be separated from target compounds chromatographically.

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Benefits

- Accounts for routine variation in the response of the chromatographic system
- Accounts for the variations in the exact volume of sample or sample extract introduced into the chromatographic system

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Benefits

The retention times of the target compound and the internal standard may be used to calculate the relative retention time (RRT) of the target compound and can then be used to compensate for small retention time shifts

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March 2003*



Disadvantage

The principal disadvantage to internal standard calibration is that the internal standards must be compounds that are not found in the samples to be analyzed and they must produce an unambiguous response on the chromatographic detector system.

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March 2003*

