APPLICATIONS TECHNICAL NOTE

Internal Standards – What are they? How do I choose, use, and benefit from them?

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What is an Internal Standard?

An Internal Standard (IS) is a chemical substance which is added at the same concentration to all samples throughout a quantitative analysis. It is a commonly used and effective technique in chromatography and spectroscopy.

Results are calculated not by using the peak area of the target analyte but by using the peak area ratio which is calculated as follows:

$$\text{Peak Area Ratio} = \frac{\text{Peak area of analyte}}{\text{Peak area of IS}}$$

This means that variations in the sampling process, or during introduction into the analyser can be accounted for as the internal standard will be affected in the same way as the target analyte(s), with the peak area ratio helping to adjust for these variations and minimise their effects on the results.

Using an internal standard is a powerful tool for minimising the effects of random and systematic errors during analysis, helping to improve the precision of results and reduce the need for repeat measurements.

Choosing an Internal Standard

The main criteria for choosing an internal standard is based on resolution – the IS should not be present within the sample matrix or interfere with any other compounds present within the sample. Ideally a compound which is similar in nature to the target analyte(s) would be chosen, as this is likely to behave in a very similar way, giving a similar retention time, peak shape, and response.

For example it is very common in Gas Chromatography Mass Spectrometry (GC-MS) methods for the deuterated form of the target analyte to be used.

Using an Internal Standard

An internal standard should ideally be introduced to all samples at the same concentration throughout the analysis and at a concentration that is similar to that of the target analyte(s).

Adding an IS at an early stage of sample preparation can account for variations caused by this process and help alleviate the effects of challenging sample prep.

For complex analysis with a large number of components, multiple internal standards can be used to calculate analyte concentrations throughout the method. This is especially useful when target analytes are at varied concentrations and differ structurally from each other.

Results Comparison

In order to demonstrate the effectiveness of using an IS, 2 separate sets of method precision samples were prepared using Eugenol technical grade active ingredient (TGAI).

Hexadecane was chosen as the internal standard for this analysis, due to its good resolution, peak shape and relative response to Eugenol.

Each set of method precision samples were made up of 5 independent aliquots of Eugenol at a concentration of 0.5 mg/mL. One set was made to volume using Acetonitrile only, and the other set was made to volume using an internal standard spiking solution (0.5 mg/mL Hexadecane in Acetonitrile).

The samples were ran on a SCION 8500 GC equipped with flame ionization detector (FID), SCION-5 GC column, and SCION Focus GC liner.

Tables 1 and 2 show the results from the 2 sets of method precision samples.

Table 1 RSD of 5 method precision samples without internal standard

Injection No.	Peak Area of Eugenol (µV/min)
1	2811.5
2	2801.9
3	2816.7
4	2777.3
5	2800.3
RSD (%)	**0.48**

Table 2 RSD of 5 method precision samples with internal standard

Injection No.	Peak Area of Eugenol (µV/min)	Peak Area of IS (µV/min)	Peak Area Ratio
1	2694.8	3795.8	0.7099
2	2668.1	3760.1	0.7096
3	2659.6	3750.3	0.7092
4	2630.8	3702.9	0.7105
5	2603.4	3658.8	0.7115
RSD (%)	-	-	**0.11**

The results show that using an IS improves the repeatability of results by a factor of 4.4.

This calculation was performed on a stable active ingredient present at >98% in the sample matrix and with simple preparation steps. Using an IS can be even more crucial for complex sample matrices requiring convoluted sample preparation and for challenging trace impurity analysis.