Hints for the Capillary Chromatographer

Quantitative Analysis

(This is Part 1 of a two part series on Quantitative Analysis. Part 2, in November's issue, will cover external standard and area percent techniques.)

PART 1: Internal Standard Technique

This quantitative technique requires the use of analyte specific calibration standards, plus the addition of internal and surrogate standards. It offers the highest quantitative accuracy compared to other techniques. It is commonly used for many environmental monitoring methods as well as forensic and clinical analyses.

When should the internal standard technique be used?

1) If each analyte has a unique detector response.
2) If detector response varies slightly over time.
3) If analyte retention times vary slightly from run-to-run.
4) If injection size varies slightly over time.

What are the differences between internal and surrogate standards?

Internal standards, as we will be defining them, are the standards that are added to the sample just prior to analysis. The same internal standards are also added to the calibration standards. Surrogate standards are added to the sample at the beginning of the sample preparation sequence. They are used to determine matrix effects and recoveries of target compounds during sample work-up.

What are the criteria in selecting internal and surrogate standards?

1) They should be chemically similar to the target compounds.
2) They must chromatograph similarly to the target compounds under the same run conditions.
3) They can be integrated consistently as a single entity either in the standard or unknown.

When performing analyses with routine GC detectors, the internal and surrogate standards should be completely resolved from all other analytes and interferences in the sample. When performing GC/MS anabses, these standards are typically deuterium or 13C labeled analogs of the target compounds. Differentiating target compounds from labeled analogs can be accomplished by using quantitation ions based on their different molecular weights. Selection of the most appropriate internal and surrogate standards is critical to obtaining accurate quantitative results.

How many internal and surrogate standards should be used?

When target compounds encompass a wide range of boiling points, molecular weight discrimination**can occur. Therefore, it** is advisable to select multiple internal and surrogate standards which encompass a wide boiling point range and elute from the chromatographic column at various retention times (early, middle, late). If the sample contains multiple chemical classes (i.e. acids, bases, etc.) then the internal and surrogate standards chosen should also reflect the differences in functional groups.

If the sample is relatively simple in composition and the boiling point distribution is narrow, then single internal and surrogate standards will suffice. They should be tihosen to elute near the mid-point of the analysis. The internal standard should be spiked into the unknown samples just prior to the GC analysis. The surrogate standards are spiked into the unknown samples prior to sample work-up. Both the internal and surrogate standards should be spiked into the calibration standards.

How are internal standards used for quantitation?

Calibration standards, which contain all the target compounds, are prepared at various concentration levels to bracket the working range of the detector. Each calibration standard is spiked with the identical amount of internal and surrogate standards. The calibration standards are analyzed in series (low concentration to high concentration) and the resultant retention times and peak areas are recorded for each analyte and the surrogate standards to calibrate the instrument.

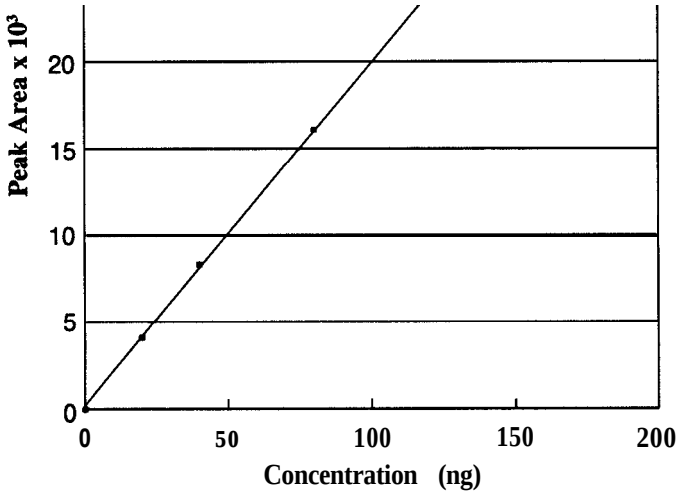
Relative retention times (RRTs) and relative responses factors can then be calculated for each target compound and the surrogate standards. Relative rqtention time windows are established for each compound and used to verify compound identity in the unknown sample. In the case of GC/MS, the mass spectra of each compound is compared to library spectra to confirm identity. Relative retention times are more accurate since they take into account slight shifts in absolute retention time that may occur from run-to-run. Figure 1 shows the calculation for RRT.

Figure 1

$$\text{Relative Retention Time (RRT)} = \frac{\text{Retention time (compound)}}{\text{Retention time (IS)}}$$

Responses or calibration curves are constructed for each compound using the peak area response plotted on the y-axis and the amount of the analyte injected on the x-axis (Figure 2). The linearity of the calibration curve will indicate whether the analyte responds proportionally to concentration over the range of operation or if the compound shows adsorptive effects at low concentration levels.

Figure 2 - Typical Calibration Curve



As an alternative to generating individual calibration curves for each compound, the analyst can calculate relative response factors (RRF). This is performed by taking into account the concentrations of the analytes and the internal standards. The calculations for RRFs are shown in Figure 3. If the amount of internal standard added to the calibration standards and samples are identical, the internal standard's concentration does not have to be included in any of the calculations. If the amount of internal standard added to the calibration standards varies with the analyte's concentration, then the variation must be accounted for in the calculations. If the compound exhibits linear response over the concentration range analyzed, a plot of concentration (x-axis) versus RRF (y-axis) will result in a relatively flat line (Figure 4). The RRFs can then be used to calculate the concentration of target compounds in samples (Figure 5).

Figure 3

$$\text{Relative Response Factors (RRF} = \frac{\text{Area (compound)} \times \text{Conc (IS)}}{\text{Area(IS)} \times \text{Conc. (compound)}}$$

Figure 4 - Relative Response Curve

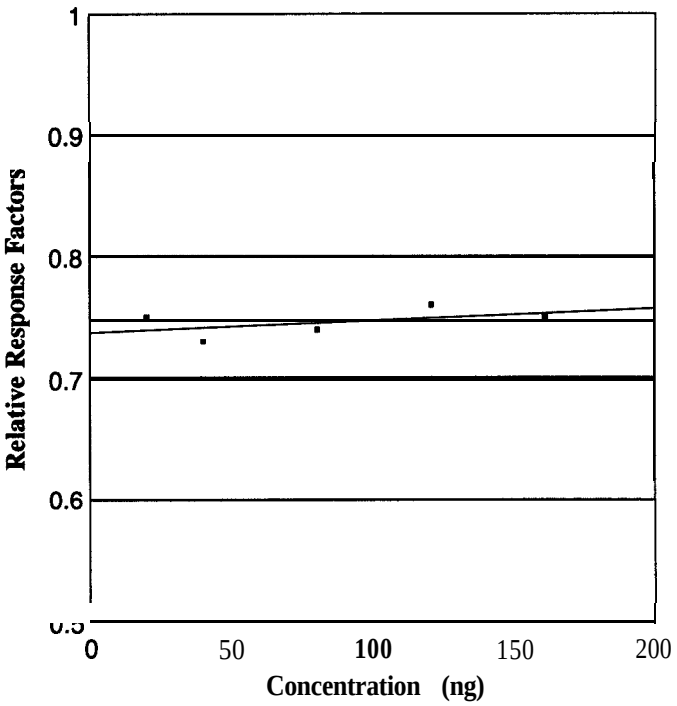


Figure 5

$$\text{Concentration} = \frac{\text{Area (unknown)} \times \text{Cont. (IS)}}{\text{Area (IS)} \times \text{RRF}}$$

How often is re-calibration necessary?

While the internal standard technique offers superior quantitative results, it is also advisable to run a continuing calibration check at regular intervals to determine if detector response or adsorption is affecting compound response factors. After a specified length of time (-once per 8 hours) or number of unknown samples (-10-25 samples), a mid-point calibration standard is analyzed. The RRFs are calculated and compared to the values obtained in the initial calibration of the instrument. If significant differences are noted, corrective action should be taken. Always recalibrate after performing routine maintenance procedures such as changing inlet sleeves, reinstalling or changing the column, or resetting the flow rates. While the internal standard technique offers a compensation mechanism for small changes in retention time, detector response or injection size, it does not eliminate the need for routine maintenance on the inlet system, detector, and column.

The method used for quantitation should be chosen based on sample type and the requirements of the analysis. The internal standard technique offers most accurate quantitation, since it allows for minor variation in instrument response and injection size. The major drawback to this technique is the requirement of extensive calibration.