Hints for the Capillary Chromatographer

Quantitative Analysis

(This is Part 2 of our series on Quantitative Analysis covering external standard and area percent techniques. Part 1, which appeared in the September issue, covered internal standard techniques.)

PART 2: External Standard Technique

This quantitative technique requires the use of analyte specific calibration standards. Samples analyzed using this technique. cover a broad application spectrum. One of the most common applications of the external standard technique is in pesticide residue determinations.

When can the external standard technique be used?

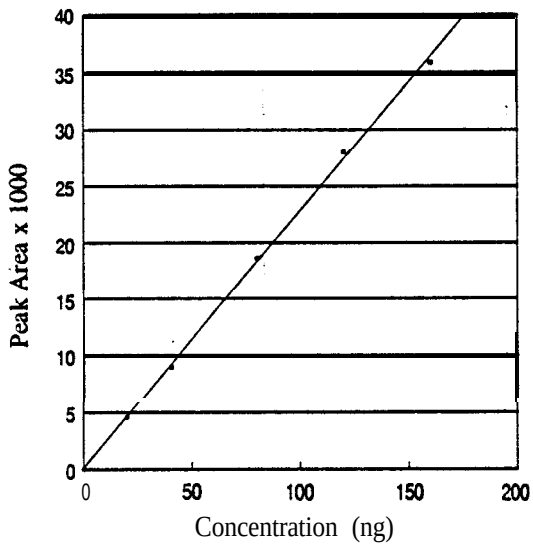
1 If each analyte has a unique detector response.
2) If detector response will not vary during some specific length of time.
3) If retention times remain constant.
4 If injection size can be precisely controlled.

How are external standards used for quantitation?

Prior to sample analysis, calibration standards are prepared for each target compound. These calibration standards are prepared at several different concentration levels which bracket the expected range that target compounds will appear in the sample. For semi-quantitative work, a single point calibration may be performed. For quantitative work, a five point calibration is typically used. The calibration standards are then analyzed in series from lowest to highest concentration. The resultant peak areas for each standard analyte are determined and a retention time window is established. This data is used later for comparison to unknown peaks obtained during sample analysis.

Data obtained from the analysis of calibration standards may be generated by the same two techniques used for the internal standard method. One way to use the external standard technique uses a calibration curve for each compound. The calibration curve is generated by plotting the peak area on the y-axis and the concentration injected on the x-axis. Figure 1 shows a linear calibration curve. After the initial calibration curve has been prepared, analyte linearity can be determined for each analyte over the expected concentration ranges. If a non-linear response is observed, corrective action should be taken. A non-linear response is commonly seen at the extremes in concentration. At low concentrations, non-linearity typically occurs from analyte adsorption or breakdown. At high concentrations, non-linearity typically occurs from detector or column overload. Quantitation should not be performed at concentrations where non-linear response is observed, or erroneous results will occur. Responses for each sample target compound are then compared to the standard calibration curve and the concentration of the analyte is determined by reading the intercept point on the x-axis. This amount of analyte is then used to back calculate the concentration of the analyte in the actual volume of sample.

Figure 1 - Typical Calibration Curve



The second way to use the external standard technique involves the use of response factors for each target compound. Response factors are calculated by dividing the peak area of the calibration standard by the concentration injected. The response factors can be compared for each analyte at various concentrations to determine if they are uniform over the calibration range. A linear plot of response factors (y-axis) versus concentration (x-axis) will be indicated by a flat line. Figure 2 shows a linear plot of response factors. If the response factors are uniform over the calibration range, an average response factor may be calculated and used for the concentration range. Unknown samples are then analyzed, and the resulting peak areas of the target compounds are multiplied by the average response factor to determine analyte concentration in the sample. Figure 3 shows the calculation to determine sample analyte concentrations. The retention time of the target compound must lie within the retention time window of the calibration standard.

Figure 2 - Response Factor Plot

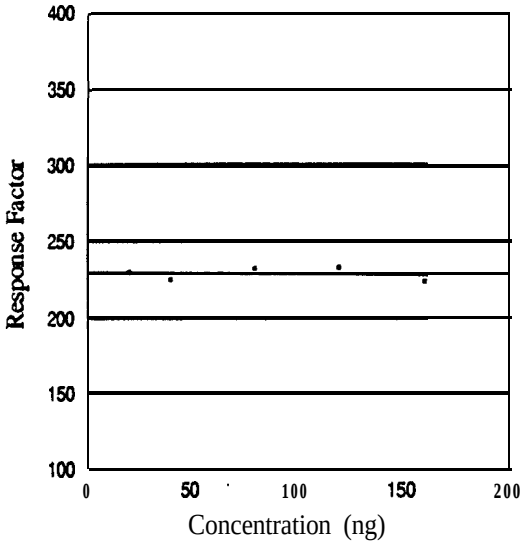


Figure 3

$$\text{Concentration} = \frac{\text{Area (unknown)}}{\text{RF}}$$

What problems are encountered using external standards? Generating a calibration curve or response factor corrects for the varying response of each analyte. However, there is no mechanism to compensate for instrument condition variations (detector response, injection size, inlet performance) after the initial calibration runs have been made. One way to determine if responses have changed involves analyzing a "continuing check sample". A continuing check sample is a calibration standard prepared at a median concentration level. The check sample is analyzed at a regular time interval to monitor the performance of the system. The frequency of the check sample analysis is largely dependent upon the instrument and samples being analyzed. Extremely dirty samples can contaminate the inlet, detector, or column and may require frequent analysis of the continuing check sample. The peak areas or response factors from the check sample are compared to the values obtained in the initial calibration sequence to monitor instrument response changes. A large change in response necessitates generating a new calibration curve.

Another problem that decreases accuracy of the external standards method is inconsistent injection techniques. The problem is minimized by using automated techniques (i.e. autosamplers) instead of manual injection techniques, but it can still be a major source of error. Changes in room temperature can create variations in the solvent volume, directly effecting the absolute amount being injected into the instrument.

The external standard method also suffers from the problem of shifting retention times. When the initial calibration is established, absolute retention times and/or retention time windows are also established f- Chromalytic Technoilogy Pty Ltd Fax : +61 3 9761 1169il adtarget compound. Over extended time periods, instrument variation in oven temperature, column flow rate, or sample matrix effects on the column can cause changes in the retention times of compounds. Comparison of the sample analyte retention times may fall outside the windows previously. established. One solution is the establishment of large retention time windows which can lead to misidentification of closely eluting analytes.

To improve the accuracy of analyses done by external standard techniques, confirmation can be done on a second column of different polarity. Dual column confiiation increases both qualitative and quantitative data.

Area Percent Technique

This technique does not require the use of calibration standards. A sample is analyzed and the area of each component in the sample is measured. The total area of all in the mixture is then used to calculate the relative percent composition of each component in the mixture.

This technique is useful only if the relative percent composition of each compound within the mixture is required. This form of quantitative analysis has been used for analysis of natural products such as fatty acid methyl esters and flavor components where impurity concentrations may be monitored in respect to major components. It is also useful for complex hydrocarbon mixtures in petrochemical products.

The **area** percent technique is also routinely employed is in the determination of compound purity. The assumption is made that the impurities will respond similarly to the main component. It is assumed that the impurities can be volatilized and analyzed under the same conditions as the compound of interest.

When should the area **percent technique be** used?

1) If ALL compounds in the sample respond with equal intensity.
2) If standards for all target compounds are not readily available.

Although no calibration standards are required for the area percent technique, it is advisable that a standard of each compound of interest be prepared from pure material at the same concentration. The absolute response obtained for each individual compound can be used to confii that all responses are equal. These standards can also be used to establish retention times and windows.

The quantitation method employed should be chosen based on sample type and the requirements of the analysis. The internal standard technique offers the most accurate quantitation, but requires extensive calibration. The external standard also requires extensive calibration, but consistent retention times and injection volumes are necessary for accurate identification and quantitation. The area percent technique requires less calibration, but is only effective when all target compounds exhibit similar detector response.