Quantitative Analysis Practice – Internal Standards

Quantitative gas chromatography is almost always accomplished using an internal standard. For example, one method for the analysis of the chlorinated pesticide lindane (γ-1,2,3,4,5,6-hexachlorocyclohexane, also known as γ-BHC) in water uses the related compound β-BHC as an internal standard. Here is how it is done. A stock solution of exactly 1000–ppm lindane in iso-octane is prepared. This solution is then diluted twice to create a working standard solution by first diluting a 100–μL portion to 10 mL with iso-octane and then diluting a 1–mL portion of the resulting solution to 10 mL with iso-octane.

A set of calibration standards containing both lindane and the internal standard are prepared from the working standard solution and a 20–ppm solution of β–BHC by using the information shown below:

mL diluted to 10.00–mL using iso-octane		Peak Areas	
lindane	β-BHC	Lindane	β-BHC
0.00	0.500	0	5515
2.00	0.500	878	5486
4.00	0.500	1757	5493
6.00	0.500	2727	5682
8.00	0.500	3403	5317
sample extracted into iso-octane		1558	5567

A 40.00–mL sample of water collected from a local stream is spiked with 0.100–mL of a 20.00 ppm solution of β-BHC. A 2.00–mL portion of iso-octane is added to the sample and the lindane and β-BHC completely extracted into the iso-octane by a liquid-liquid extraction. Injecting 1–μL portions of each standard and the sample into a GC equipped with an ECD detector gives the peak areas for lindane and β–BHC listed in the table shown above.

Working with this information, calculate the concentrations of lindane and β–BHC in each standard and prepare a internal standardization calibration curve. Finally, determine the concentration of lindane in the sample of stream water.

Begin by preparing a calibration curve with the ratio $Area_{lindane}/A_{BHC}$ on the y-axis and the ratio [lindane]/[BHC] on the x-axis. The concentration of BHC in each standard is 1.00 ppm (a dilution of 0.500 mL of a 20.00 ppm solution to a final volume of 10.00 mL). The stock solution of 1000.0 ppm lindane is diluted twice to give a 1.00 ppm working standard, which in turn is diluted in preparing the standard solutions; the second standard, for example, is 0.200 ppm lindane. The calibration curve is linear with a calibration equation of

$$Area_{lindane}/A_{BHC} = 0.8004([lindane]/[BHC]) - 0.0003$$

The area ratio for the sample is 0.2799, which, when substituted into the calibration equation, gives the concentration ratio as 0.3489, which is also the mass ratio (since both analyte and internal standard are in the same total volume). The mass of added internal standard is 0.1 mL × 20.00 ppm, or 2 μg. The mass of lindane, therefore, is 0.3489×2 or 0.6978 μg. This is the mass of lindane in the 2.00-mL portion of iso-octane, all of which originally was in the 40.00-mL sample of water. The concentration in the water sample, therefore, is 0.6978 μg/40.00 mL or 0.0175 ppm lindane.