Agilent Technologies

Innovating the HP Way

Negative Concentrations

Consider the following data for an Internal Standard and an analyte

Int Std Conc	Int Std Area	Analyte Conc	Analyte Area	Amount Ratio	Response Ratio
50	12,438	200	207,463	4	16.6798
50	12,228	150	176,723	3	14.4523
50	12,248	100	133,503	2	10.8999
50	13,418	50	68,567	1	5.1100
50	12,112	20	16,974	0.4	1.4014

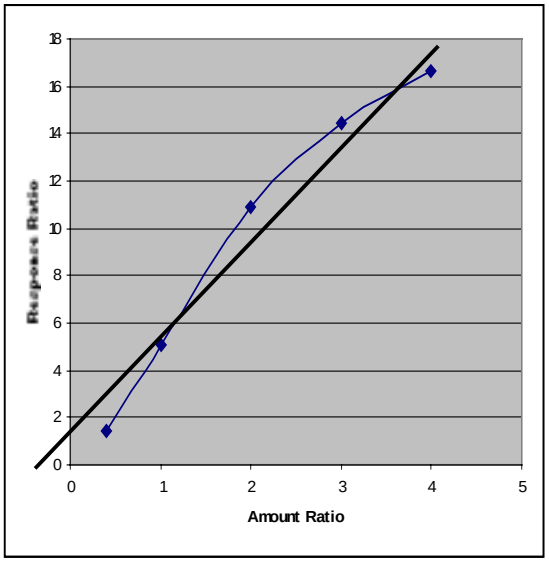
The following calibration curve fits can be used and give the following values:

Curve Fit	Linear Term	Quadratic Term	Constant Term	RF %RSD	Coeff of Determination
Avg. RF	4.61	0	0	16.9	-
Linear Regression	4.29	0	0.788		0.964
Linear Regr Force (0,0)	4.56	0	0		0.964
Quadratic Regression	7.84	-0.811	-1.71		0.999
Quadratic Regr Force (0,0)	6.14	-0.475	0		0.999

When used against a sample giving a small analyte area, the curve fits give the following concentrations:

Curve Fit	Int Std Area	Analyte Area	Concentration Reported
Avg. RF	11,103	61	0.06
Linear Regression	11,103	61	**-9.12**
Linear Reg Force (0,0)	11,103	61	0.06
Quadratic Regression	11,103	61	11.17
Quadratic Regr Force (0,0)	11,103	61	0.04

When the Raw data are plotted, you observe the calibration graph to the right. To determine concentration you first calculate the Response Ratio (area unknown / area of Int Std), draw a horizontal line from the Y axis to where it touches the calibration curve, then drop down to the Amount ratio axis and read the amount ratio. The concentration is then the amount of internal standard times the calculated amount ratio.



The Linear Regression (black line) gives a positive Response Ratio intercept when the Amount Ratio is zero. At very low Response Ratios, the amount ratio is negative – thus causing the reported concentration to be negative.

Forcing the zero point forces the Response Ratio intercept to occur at an Amount Ratio of zero, thus there can never be an occasion of small response Ratios giving a negative Amount Ratio.

The reported concentrations vary quite a bit. The –9.12 value from the linear regression is due to the y=0 giving a negative value of Amount Ratio.

If you extend the blue line (an approximation of the quadratic curve fit) to very low values of Response Ratio, you see that the value remains positive, as such this particular curve should not give a negative concentration.

Which reported concentration is correct? Intuition demands that the value be positive yet small. When 20 PPM of the analyte was injected, the area was 16,974. In the unknown sample, the area of the analyte was 61. A simple approximation using External Standard calculations is 61 * (20/16,974) = 0.018 PPM. You as the analyst must decide which curve fit to use. The choice for large areas of the analyte in the unknown sample may be a different curve fit from small areas of the analyte in the unknown. In this example, the simplest solution is to use the Average Response factor. The most accurate solution for an area of 61 is to use the Quadratic Regression forcing a 0,0 intercept.

Please note due to variations in calibration data, you may NOT be able to use the curve fit required by the method.