6. Quality Management

6.2 Limit of detection (LOD) and of quantification (LOQ)

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6.2.1 Degrees of freedom

The number of *degrees of freedom* mainly depends on the number of available information (A).

(A) f = n-v-m

f: degrees of freedom
n: number of independent observations (measured values)
v: number of independent variables (for calibration 1)
m: number of values which affect calculation of the parameters (e.g. for mean 1)

The degree of freedom is the number of parameters of the system, which can be changed without changing the parameter. For example, a mean of 3 measured values has 2 degrees of freedom because 2 observations could change without changing the mean. The parameter is the concentration of the component or analyte, which has to be determined.

6.2.2 Limit of detection and of quantification

The ***limit of detection*** (LOD) is the critical concentration of the analyte, which can distinguish between blank values and measured values. The LOD depends on the quality of the blank values or the calibration line (Fig. 6.2-1 and 6.2-2). It is the decision limit for the existence of the analyte.

- An analyte is detected **qualitatively** or exists, if it is higher than the critical value of the measured value (higher than the sum of the blank values and its uncertainty (with the degree of scatter)).
- The LOD assesses the analysis.

The ***limit of quantification*** (LOQ) is that concentration of the analyte, at which the relative uncertainty of the result takes predefined values. The uncertainty of the result results from the (two-sided) prediction range, the level of confidence and the associated analyte concentration. It describes the efficiency of the quantitative procedure.

- An analyte can be **quantified**, if its concentration can be determined with a relative uncertainty of result, which means that the measured value is higher than the LOQ. The LOQ provides information about feasibility of a quantitative analysis.

The LOD and LOQ do not hold true alone, but always for a certain sample material (water, soils), a certain method of analysis and a fixed level of confidence.
Further information about LOD and LOQ can be found in DIN 32465 (2008).

6.2.3 Flash estimate of LOD and LOQ from blank values and calibration line

The **blank sample** is a sample, which does not contain the analyte, but matches the samples apart from it. Since such sample is rarely existing or cannot be produced, a sample with the lowest possible concentration of the analyte and a matrix similar to sample is used (salinity, pH and suchlike).
The **blank value** is the arithmetic mean of the blank samples. The **blank value method** is the direct determination of the LOD and LOQ. The **number of measurements (blank sample)** determines den safety factor Φ and **should be ≥ 10.**
The **blank value** is determined by the concentration of the analyte (in µmol l^{-1}) by a calibration function, without being part of the LOD und LOQ. The LOD is a multiple of the standard deviation of the procedure (C, blank value method), whereby

(B) $s = F \times S_L$

(C) $LOD = \Phi_{n,\alpha} \times s$

Φ: factor from Tab. 6.2-1
n: number of measurements
α: significance level (for one-side error)
s_L: standard deviation of extinction
s: standard deviation of blank value (in µmol l^{-1})
F: increase of calibration line (x = ext., y = conc.)

Table 6.2-1 Factors $\Phi_{n,\alpha}$ for estimation of blank value method (n: number of measurements, α: significance level, whereby 0.05 correspondents to a 5 % error probability). The commonly used significance level and the for the estimation suited number of measurement values are shaded in grey.

n	**$\Phi_{n,0.05}$**	**$\Phi_{n,0.025}$**	**$\Phi_{n,0.01}$**	**$\Phi_{n,0.005}$**
4	2.6	3.6	5.1	6.5
5	2.3	3.0	4.1	5.0
6	2.2	2.8	3.6	4.4
7	2.1	2.6	3.4	4.0
8	2.0	2.5	3.2	3.7
9	2.0	2.4	3.1	3.5
10	1.9	2.4	3.0	3.4
11	1.9	2.3	2.9	3.3
12	1.9	2.3	2.8	3.2

The LOQ can be calculated approximately from the LOD (D)

(D) $LOQ \approx k \cdot LOD$ $k = 3\,f$ for n = 10 measurements and 33.3% relative uncertainty of results

An additional safety factor considers the increase in standard uncertainty by spreading of the calibration lines (E).

(E) $LOD = 1.2 \times \Phi_{n,\alpha} \times s$

s: standard deviation of procedure (as concentration, e.g. µmol l^{-1})

1.2: safety factor

The measured values of the blank samples have to be distributed normally and the variances have to be distributed homogenously. The blank value method should be preferred, if the above-mentioned prerequisites are fulfilled (blank sample same matrix, free from analyte).

The LOD and LOQ can also be directly calculated from the calibration line, if no suited blank samples are available.
The number of measured values of the calibration line should also be ≥ 10. That could be 10 different concentrations (of an equidistant dilution series), but also replicates of less dilution levels.

In suitable programs (not Excel, but Origin) the 95 % confidence band can be plotted around the calibration line (here concentration = x and extinction = y) (Fig. 6.2-1). Draw the point of intersection of the upper 95 % confidence band with the y axis parallel to the x axis to the calibration line. Let fall a perpendicular onto the x axis. The point of intersection with the x axis is the LOD (Fig. 6.2-2).

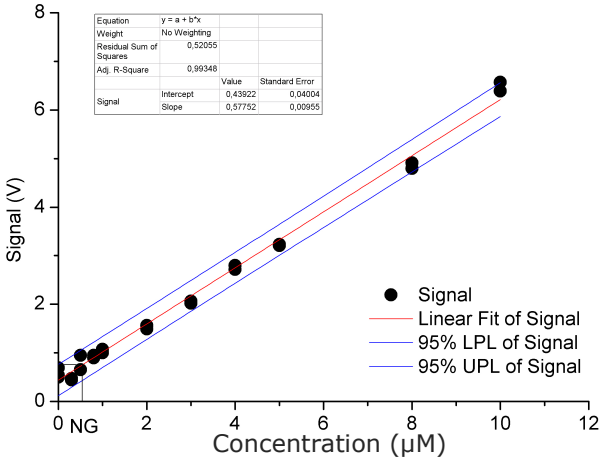

Fig. 6.2-1 Calibration line with 95 % confidence band (Origin)

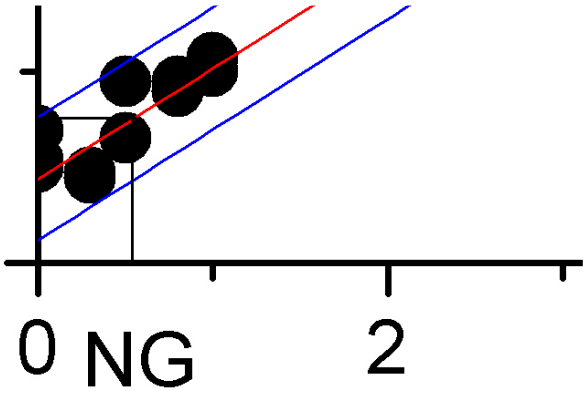

Fig. 6.2-2 Zoom into the area, in which the LOD = 0.54 µmol l^{-1} was graphically deduced.

6.2.4 Calibration line

- It is calibrated with an analyte, which has typically the same or similar occurrence to them in the sample. Its solubility (in the appropriate matrix and the given conditions) has to be taken into consideration.
- A very good straight line can be created, if from a stock solution with the highest measurable concentration (upper limit of the measurement range) the lower concentrations are made in a ***geometrical dilution series.*** A geometrical dilution series can be created by repeated mixing of the same volumes (1+1, from the diluted solution 1+1 again, Fig. 6.2-3).

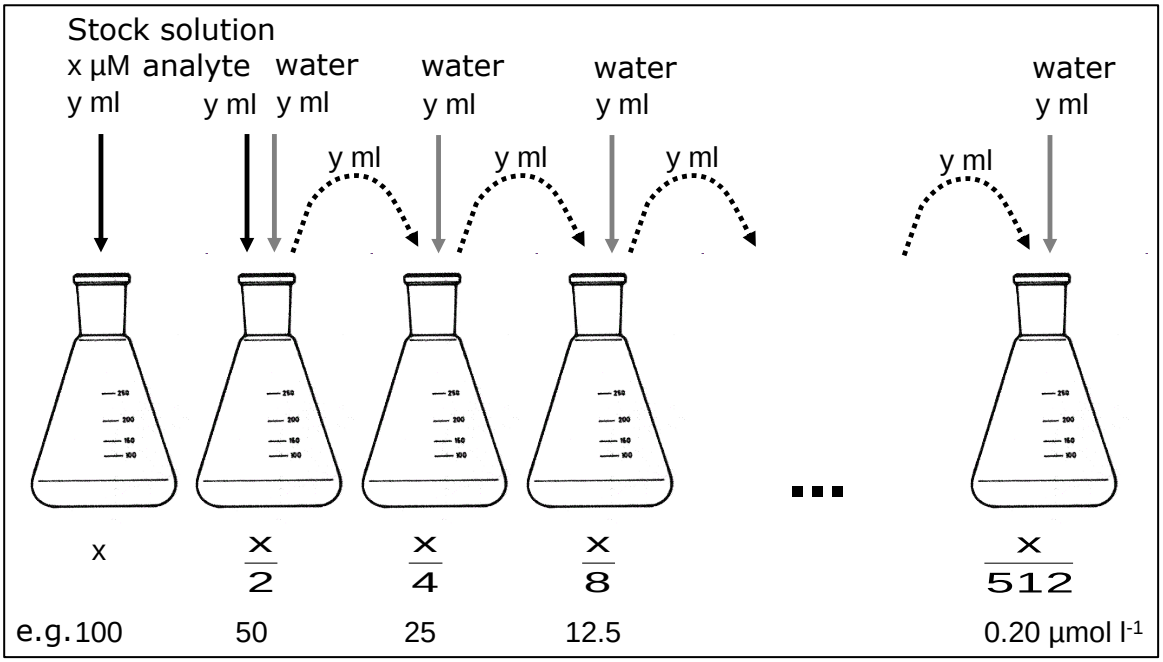

Fig. 6.2-3 Scheme of a geometrical dilution series. x: concentration of analyte in the stock solution, y: sample volume for measurement, from the last sample vessel half of the mixture has to be discarded.

Equidistant dilution series are common in chemical analytics (Fig. 6.2-4). The upper limit of the measurement range should be higher than the probably highest sample concentration to ensure that all measured values can be calculated by interpolation (all measured values are in the calibrated range). The lower limit of the measurement range should be near the blank values.

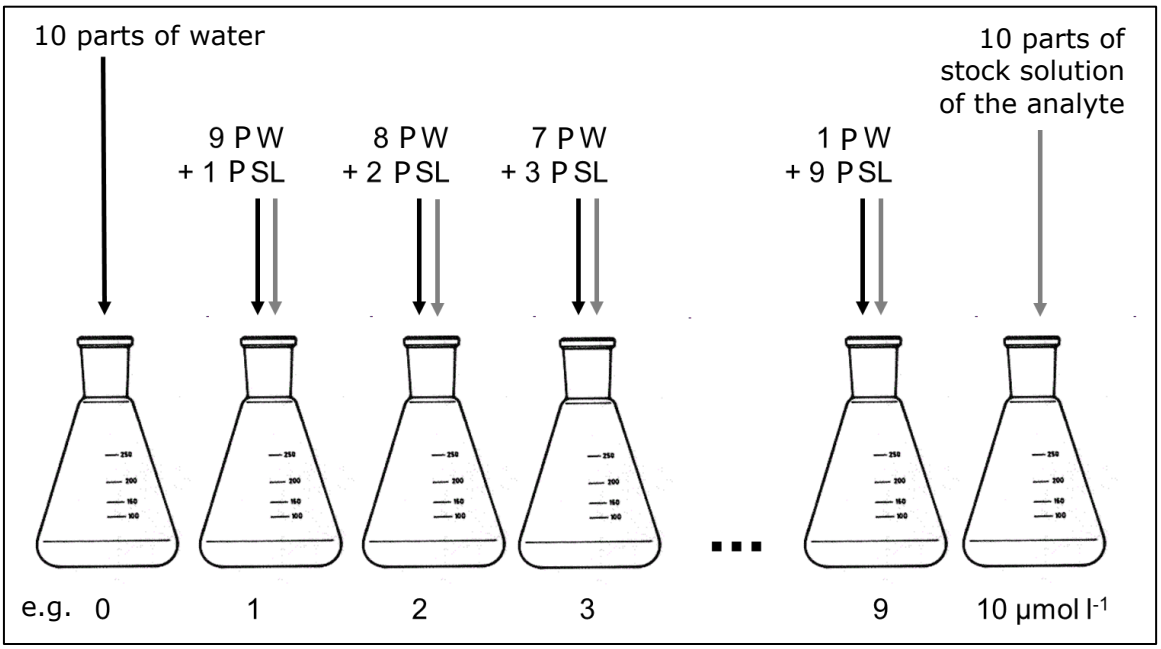

Fig. 6.2-4 Scheme of an equidistant dilution series. P: parts, W: ultra-pure water, SL: stock solution of the analyte.

Pragmatically, this can be done in the following way:

- Determine the volume of the calibrant like it is for the samples. That means 25 ml for phosphate and nitrite. The 17.5 ml for ammonium are not suitable. Therefore, produce 20 or 25 ml for each calibrant. Either increase the amounts of reagents proportionally or discard the surplus volume of each calibrant
- The volume of one part is 1/10 of it, e.g. here 2.5 ml.
- Add 25 ml ultra-pure water (10 parts, 0 µmol l^{-1}) and subsequently 25 ml analyte (10 parts, 10 µmol l^{-1}), measured by measuring cylinders.
- Afterwards, pipette the necessary parts in the other 8 vessels. No shortcuts! Really pipette 9 x 2.5 ml.
- Add in reverse order the stock solution, shake well!

Since most methods have a blank value correction (reagent blank value), the blank values have to be subtracted from measured values of the **calibration line** before graphic representation and calculation of the function. With 10 calibrants the coefficient of determination should be $R^2 > 0{,}995$.
For practical reasons (calculated function can directly be used for calculation of concentration), the dependent variable (extinction) is on the x axis and the independent variable (concentration) on the y axis (Fig. 6.2-5 and 6.2-6). Subsequently, the **increase** is a **conversion factor F** from extinction to concentration.
The absolute term of the straight-line equation is commonly not used for calculation of concentration. This term should be nearly zero and is, therefore, negligible. If this term is systematically > 0, there is no analyte-free blank value (in the narrow sense ultra-pure water). This absolute term can be omitted also in this case. If the point of intersection with the ordinate is regularly < 0, there is a problem with too high uncertainty of the position of the calibration lines.

This calibration has to be created and documented for each instrument, for all sample matrices (e.g. liquid solutions of different salinity) and *each reagent charge*.

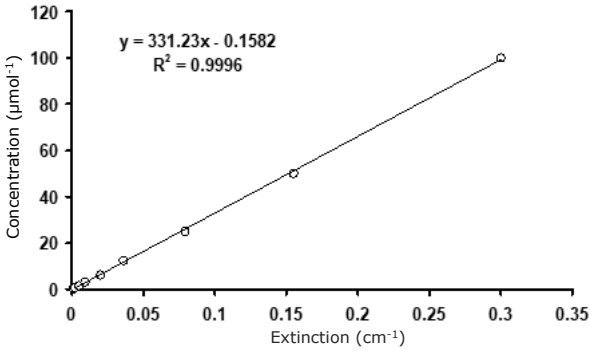

Fig. 6.2-5 Geometric calibration line with factor F = 331 and coefficient of determination $R^2 > 0.995$

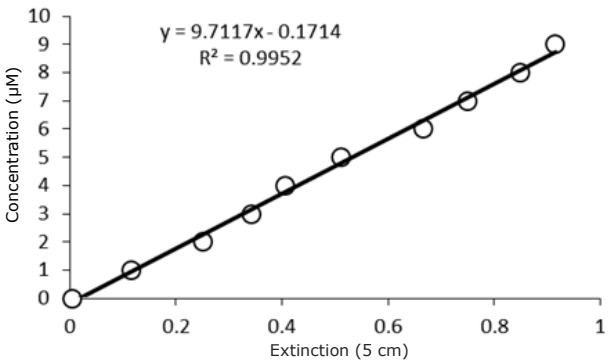

Fig. 6.2-6 Equidistant calibration line for ammonium (at 630 nm) with factor F = 9.7 and coefficient of determination $R^2 > 0.995$

6.2.5 Quality management

Besides, 10 calibration values and 10 blank values for calibration and LOD, several blank values have to be measured for the *monitoring of reagents, the ultra-pure waters and cleanliness of vessels* at each measurement day. Per measurement series (simultaneously with samples with reagents) 1 blank value is sufficient. Alternatively, (low sample number) at least 3 should be set per measurement day. If the values are low, they are inserted in the **blank value – target map** (Fig. 6.2-7).

If blank values increase significantly with time, a new charge of reagents ("Shelf Life" out-of-date) has to be prepared. If necessary, the purity of ultra-pure waters has to be controlled as well. Reagents of the ammonium and phosphate determination are very sensitive, for example if ultra-pure waters is stored for a longer time, it can absorb ammonium from the atmosphere and retain it.

Per measurement series at least 1 suited *standard value for monitoring of function of reagents and reaction conditions* (e.g. temperature, incubation period), but also for troubleshooting should be inserted (e.g. cuvette length, wave length at measurement, zero position of the photometer). For displaying the standards, a **set point – target map** is a suited instrument (Fig. 6.2-8). This standard is an analyte of medium concentration. Best suited are standards with a certificate (independent verification of analyte, very expensive) or at least out of the same stock solution as the calibrant. If these additional conditions are met, the standard can check the accuracy of measurements.
Suited standards contain at least the analyte. If in an analytical method the analyte still has to be released from different compounds by the digestion or extraction, at least one further compound in the standard

should be a compound from which the analyte is still released during the analytical procedure. This compound should not directly react with the reagents.

The reproducibility of samples (= *precision*) can be monitored in so-called **range-target maps** (Fig. 6.2-9). A range is the absolute value of the difference of two measured values divided by their mean (equation G).

(G) $$SW = \frac{x_{R1} - x_{R2}}{\bar{x}_R} \times 100\ \%$$

SW: range of a measurement

x_{R1}: value of first measurement (replicate 1)

x_{R2}: value of second measurement (replicate 2)

x_R: mean of both measurements

For 3 and more replicates, the standard deviation can be normalised to the mean. The range can be given in percent. The target map is especially important, if not each sample can be measured in replicates.

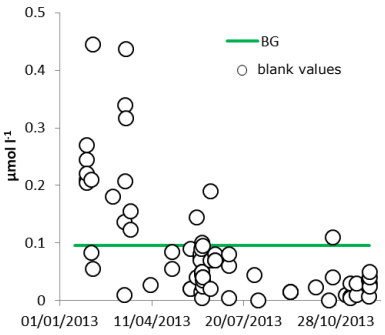

Fig. 6.2-7 Blank value - target map of all phosphate measurements (µmol l^{-1}) in 2013. LOQ: limit of quantification

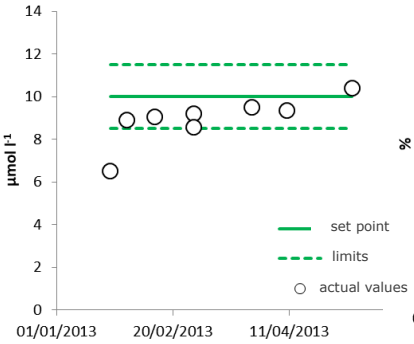

Fig. 6.2-8 Set point - target map of all phosphate measurements (µmol l^{-1}) in 2013. set point: standard concentration, limits: ± 15 % of set point

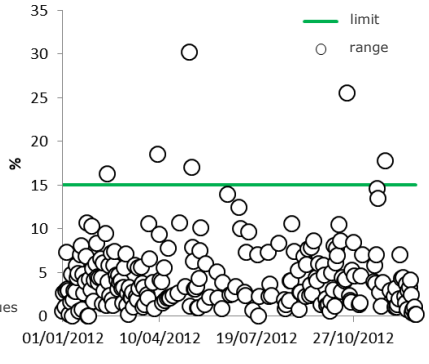

Fig. 6.2-9 Range - target map with an upper limit of 15 %

The precision of repeated measurement of standards and/or samples is called **standard uncertainty**. The precision of standards is calculated by the standard deviation, which is normalised to the mean, of all in the sample period measured standards (equation H). The precision of measurement of samples is calculated by the range as mean (equation I). In the material and method part the combined standard uncertainty is shown (as far as measured) (equation J).

(H) $$V_{KS} = \frac{s_{St}}{\bar{x}_{St}} \cdot 100\%$$

V_{KS}: confidence interval or standard uncertainty of all standards (set point) in the measurement period
s_{St}: standard deviation of all standards
x_{St}: mean of all standards

(I) $$V_{KP} = \bar{x}_{SW}$$

V_{KP}: confidence interval or standard uncertainty of all samples in the measurement period
X_{SW}: mean of all ranges

(J) $$V_K = \sqrt{V_{KS}^2 + V_{KP}^2}$$

V_K: combined standard uncertainty of all samples and standards in the measurement period

References

DIN 32465 (2008) Chemical analysis - Decision limit, detection limit and determination limit under repeatability conditions - Terms, methods, evaluation

Wellmitz & Gluschke (2005) Leitlinie zur Methodenvalidierung. UBA-Bericht 01/05

For citation: Zimmer D, Baumann K, Berthold M, Schumann R (*insert year of download*): Handbook on the Selection of Methods for Digestion and Determination of Total Phosphorus in Environmental Samples. Version 1.0. DOI: 10.12754/misc-2020-0001