3 Implementation and validation of analysis methods

3.1 Preface

When implementing new methods basically three cases can be differentiated:
- Implementation of official methods (internationally approved, validated methods, e.g. methods of the “§ 64 LFGB” / German Food, Feed and Commodities Act, the Analytica EBC or the MEBAK)
- Modification of already existing methods
- Implementation of new methods that require comprehensive validation.

In trace analysis the approach differs from the other methods like content determinations, physical measurements etc.
In addition, content determinations can be differentiated in calibrateable methods, at which specific reference substances are available for addition tests and such, for which that is not possible, e.g. foam measurement or viscosity determination.

3.2 Terms, Definitions

Work area
The work area is defined as concentration area of an analyte in an analysis solution, in which the test procedure provides reliable results in constant accuracy.

Calibration function
Functional correlation between measurable dimension and concentration to be determined.

The regression analysis provides a calibration function ($y = a + bx$ in case of a linear first degree function) with the following characteristics:

- Slope *b* (measure for the sensitivity of a method)
- Axis intercept *a*
- Residual standard deviation (dispersion of the measured data around the regression line)
- Method depending standard deviation (absolute accuracy measure)
- Method depending coefficient of variation (relative accuracy measure)

Sensitivity
The sensitivity specifies, to what extent the signal of the system to be measured varies when changing one concentration value.

Recovery rate
The recovery rate equals the quotient of recovered and added quantity multiplied by 100 (specification in percent). If a recovery rate of 100 % is determined, the method is free of deviations.

Accuracy
Accuracy is a qualitative term for the extent of approximation of test results on a reference value, e.g. the “true value”. Accuracy is a superordinate term for precision and trueness.

Precision
Precision is a qualitative term for the extent of the mutual approximation of test results independent from another in multiple applications of a defined test method under predefined conditions. A distinction is drawn between repeatability and reproducibility.

Repeatability
Repeatability is the qualitative term for the extent of mutual approximation of test results under repetitive conditions. They exist when under identical conditions (same person in charge, same equipment and same laboratory) within short intervals a predefined analysis method is carried out several times using identical samples.

Reproducibility
The reproducibility is a qualitative term for the extent of mutual approximation of test results under comparative conditions. They exist, if measurement results are obtained using a defined analysis method using identical samples at varying intervals under different conditions (different persons in charge, different equipment, and various laboratories)

Trueness
Trueness is a qualitative term for the extent of approximation of the expected value of the test result on the reference value, whereas this can be the true or proper value.

Detection limit
The detection limit (DL) is the lowest content of an analyte in a sample that can be detected with high predefined probability.

Limit of quantitation
The limit of quantitation (LOQ) corresponds with the lowest analyte content that can be quantified with a predefined precision.

Specificity
The analytical specificity is the ability of a test method to solely detect the sought analyte, whereas other elements present in the sample do not interfere with the test result.

The MANDEL goodness- of- fit- test
Calculatory check of the calibration data on linearity under consideration of the residual standard deviation as well as the variances of the linear calibration function and the second-degree calibration function.

Outlier test by residual analysis
Calculatory check of the calibration data on outliers under consideration of the residual standard deviations.

3.3 Implementation of new methods: approach

	New methods		**Modification of existing methods**			**official methods**		
	Content determina-tion	Trace analy-sis	Content determination		Trace analysis	Content determination		Trace analy-sis
			ca	nca		ca	nca	
Specificity	x	x			x			
Linearity	x	x	x		x			x
Trueness	x	x	x					
Precision	x	x	x	x	x	x	x	
Recovery	x	x			x	x		
Limit of quantitation		x			x			x
Detection limit		x						
Robustness	x	x						

ca = calibrateable method
nca = non calibrateable method

3.4 Monitoring/Quality assurance

For monitoring the quality of analysis methods various instruments can be used.

Interlaboratory tests/comparison analysis
On one hand they shall show systematic deviations; on the other hand they shall convey security for involved employees. The results of the interlaboratory tests are being evaluated and respective measures are being deduced.

Control charts

The principle of control charts is an optical illustration of measured data taking as basis

- Quality objectives (specified value of the results of control samples)
- Quality bounds

The latter are differentiated between warning limits, whose individual exceedance is being tolerated, and control or action limits, whose exceedance causes measures.

Control charts are mainly used there, where the analysis precision and the trueness of a result is of very high importance, e.g. criteria of sales analysis, that form the basis for billing.

Control samples

Various categories of control samples can be used:

- Blank tests that definitely do not contain the sought analyte
- Reference materials with defined concentrations of individual analytes, e.g. CRM-samples (certified reference materials)
- Reference materials with defined properties, e.g. calibration oil for calibration of the viscometer or standard malt for calibrating the malt Friabilimeter.

3.5 Mathematical Basics

3.5.1 Regression analysis

Auxiliary quantities

$$Q_{xx} = \sum x_i^2 - \frac{1}{N} \bullet (\sum x_i)^2 \qquad Q_{yy} = \sum y_i^2 - \frac{1}{N} \bullet (\sum y_i)^2$$

$$Q_{xy} = \sum (x_i \bullet y_i) - \left(\frac{1}{N} \sum x_i \bullet \sum y_i \right) \qquad Q_{x^3} = \sum x_i^3 - \left(\frac{1}{N} \sum x_i \bullet \sum x_i^2 \right)$$

$$Q_{x^4} = \sum x_i^4 - \left(\frac{1}{N} (\sum x_i^2)^2 \right)$$

$$Q_{x^2y} = \sum (x_i^2 \bullet y_i) - \left(\frac{1}{N} (\sum y_i \bullet \sum x_i^2) \right)$$

$$\bar{x} = \frac{1}{N} \sum x_i \qquad \bar{y} = \frac{1}{N} \sum y_i$$

First degree regression

Slope $$b = \frac{Q_{xy}}{Q_{xx}}$$

Axis intercept $$a = \bar{y} - b \bullet \bar{x}$$

Correlation coefficient $$r = \frac{\sum (x_i y_i) - \frac{1}{N} \left(\sum x_i \right) \left(\sum y_i \right)}{\sqrt{\left[\sum x_i^2 - \frac{1}{N} \left(\sum x_i \right)^2 \right] \left[\sum y_i^2 - \frac{1}{N} \left(\sum y_i \right)^2 \right]}}$$

Residual standard deviation $$s_y = \sqrt{\frac{1}{N-2} \left(Q_{yy} - \frac{Q_{xy}^2}{Q_{xx}} \right)}$$

Method standard deviation $$s_{xo} = \frac{s_y}{b}$$

Method coefficient of variation $$V_{xo} = \frac{s_{xo}}{\bar{x}} \bullet 100\%$$

Lower limit of work area $$x_p = 2 \bullet s_{xo} \bullet t \sqrt{\frac{1}{N} + 1 + \frac{(y_p - \bar{y})^2}{b^2 \bullet Q_{xx}}}$$

Second degree regression

Axis intercept $$a = \bar{y} - b \bullet \bar{x} - \frac{c}{N} \sum x_i^2$$

Coefficient x $$b = \frac{Q_{xy} - c \bullet Q_{x^3}}{Q_{xx}}$$

Coefficient x^2 $$c = \frac{Q_{xy} \bullet Q_{x^3} - Q_{x^2y} \bullet Q_{xx}}{(Q_{x^3})^2 - Q_{xx} \bullet Q_{x^4}}$$

Residual standard deviation $$s_y = \sqrt{\frac{1}{N-3} \bullet \left(\sum y_i^2 - a \bullet \sum y_i - b \bullet \sum x_i y_i - c \bullet \sum x_i^2 y_i \right)}$$

Sensitivity $$E = b + 2 \bullet c \bullet \bar{x}$$

Method standard deviation $$s_{xo} = \frac{s_y}{E}$$

3.5.2 Linearity test: MANDEL adaptation test

s_{y_1} Residual standard deviation for first degree calibration function

s_{y_2} Residual standard deviation for second degree calibration function

$$DS^2 = (N-2)s_{y_1}^2 - (N-3)s_{y_2}^2$$ Degree of freedom f = 1

Test value $$PW = \frac{DS^2}{s_{y_2}^2}$$

Comparison with table value $F\ (f_1 = 1,\ f_2 = N-3,\ P = 99\ \%)$

If PW $\leq$ F, then no significantly better adaptation is obtained by the second degree calibration function. The calibration function is linear.

3.5.3 Outlier test via F-test of residual variances

As a basic principle, calibration data must be outlier-free. The residual analysis can be used for detection of outliers. For this purpose at first the calibration line is calculated via residual standard deviation $s_{y_{A1}}$ based on all pairs of variates. After elimination of a suspect outlier pair of the data a new calibration line is calculated with the residual variance $s_{y_{A2}}$. The verification is conducted with the F-test.

$s_{y_{A1}}$ Residual standard deviation for calibration function using all values

$s_{y_{A2}}$ Residual standard deviation for calibration function without suspect outlier value

Test value $$PW = \frac{(N_{A1} - 2)s^2_{y_{A1}} - (N_{A2} - 2)s^2_{y_{A2}}}{s^2_{y_{A2}}}$$

Comparison with table value $F\ (f_1 = 1,\ f_2 = N_{A2} - 2,\ P = 95\,\%)$

If PW < F, then no outlier exists.

3.6 Literature

1. W. Funk, V. Dammann und G. Donnewert, Qualitätssicherung in der Analytischen Chemie, VCH Verlagsgesellschaft mbH, Weinheim, Germany 1992

t-table

f	t (P = 95 %)	t (P = 99 %)
1	12.706	63.657
2	4.303	9.925
3	3.182	5.841
4	2.776	4.604
5	2.571	4.032
6	2.447	3.707
7	2.365	3.499
8	2.306	3.355
9	2.262	3.250
10	2.228	3.169
11	2.201	3.106
12	2.179	3.055
13	2.160	3.016
14	2.145	2.977
15	2.131	2.947

f = degree of freedom

F-table for f1 = 1

f2	F (P = 95 %)	F (P = 99 %)
1	161.4	4052
2	18.51	98.50
3	10.13	34.12
4	7.71	21.20
5	6.61	16.26
6	5.99	13.75
7	5.59	12.25
8	5.32	11.26
9	5.12	10.56
10	4.96	10.04
11	4.84	9.65
12	4.75	9.33
13	4.67	9.07
14	4.60	8.86
15	4.54	8.68

f1, f2 = degrees of freedom

FOR Method validation

Type of sample: _____

Validation on: _____

Method: _____

Statistic characteristics

Calibration function	linear	☐
	second degree polynomial	☐
	others	☐
Precision (2.3)	r: ……	
	R: ……	
Recovery	Additive: …… …….. %	
	Additive: …… …….. %	
Limit of quantitation	……...	
Measuring range	……	

Approved: ……..

Date/Signature ……..

1. Specificity

		Yes	No	Date/Signature
1.1.	Analysis of sample solvent	☐	☐	
1.2.	Analysis of contaminations/degradation products	☐	☐	
1.3.	Comparison of peak form, retention time and UV-spectrum of standard and sample	☐	☐	
1.4		☐	☐	

2. Precision

		Yes	No	Date/Signature
2.1.	Precision of device: 6 times determination of standard	☐	☐	
2.2.	Precision of device: 6 times determination of sample	☐	☐	
2.3.	Precision of method: 6 times reconditioning of homogenous pattern	☐	☐	
2.4.		☐	☐	

3. Trueness/Recovery

		Yes	No	Date/Signature
3.1.	Recovery, additive equivalent to 1 × QL determination times….	☐	☐	
3.2.	Recovery, additive equivalent to 10 × QL determination times……	☐	☐	
3.3.	Recovery, additive equivalent to ……. determination times……	☐	☐	
3.4.	Analysis of certified reference material, CRM……. determination times……	☐	☐	
3.5	Comparison of various extraction techniques	☐	☐	
3.6.		☐	☐	

4. Linearity

		Yes	No	Date/Signature
4.1.	Linearity standard: 5 concentrations between …….. and ……..……	☐	☐	
4.2.	Linearity sample preparation: 5 concentrations between ……..…. and ……..……	☐	☐	
4.3.		☐	☐	

5. Measuring range

		Yes	No	Date/Signature
5.1.		☐	☐	

6. Detection limit

		Yes	No	Date/Signature
6.1.	Detection limit (Signal/noise 3:1): ……...	☐	☐	

7. Limit of quantitation

		Yes	No	Date/Signature
7.1.	Limit of quantitation (Signal/noise 10:1): …….	☐	☐	

8. Robustness

		Yes	No	Date/Signature
8.1.		☐	☐	
8.2.		☐	☐	
8.3.		☐	☐	

9. System qualifying examination

		Yes	No	Date/Signature
9.1.	Retention time of ……	☐	☐	
9.2..	Injection precision of ……	☐	☐	
9.3.	Analysis of blank test ……	☐	☐	
9.4.	 ……	☐	☐	
9.5.	 ……	☐	☐	