HBC. HOCHSCHULE BIBERACH UNIVERSITY OF APPLIED SCIENCES

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School of Advanced Professional Studies

STUDIENBRIEF

Bioanalytical Methods – Basics and Advanced

Modul 4.3

Im Studiengang Biopharmazeutisch-Medizintechnische Wissenschaften (Master of Science)

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Modulinhalte

Modulnummer	4.3
Modultitel	**Bioanalytical Methods – Basics and Advanced**
Modulkürzel	BMB
Studiengang	Biopharmazeutisch-Medizintechnische Wissenschaften (M.Sc.)
Ort der Veranstaltung	Universität Ulm
Modulverantwortlichkeit	**Prof. Dr. Boris Mizaikoff**
Lehrende	Prof. Dr. Boris Mizaikoff
Voraussetzungen	---
Verwertbarkeit	Das Modul ist im Masterstudiengang Biopharmazeutisch-Medizintechnische Wissenschaften, aber auch für andere naturwissenschaftliche Studiengänge, vor allem im Bereich der Biophysik, Biochemie, Biopharmazie und Biotechnologie anwendbar.
Semester (empfohlen)	2
Max. Teilnehmerzahl	25
Art der Veranstaltung	☐Präsenzveranstaltung(en) ☐Präsenzveranstaltung(en) mit E-Learning-Elementen ☐Präsenzveranstaltung(en) im Labor mit E-Learning-Elementen ☒reine E-Learning-Veranstaltung(en)
Veranstaltungssprache	☐Deutsch, ☒Englisch, ☐Weitere, nämlich:
ECTS-Credits	6 Credits
Prüfungsform und –umfang	☐Klausur, ☐Referat, ☐Kolloquium, ☐Posterpräsentation, ☐Podiumsdiskussion, ☐Mündliche Einzel-/ Gruppenprüfungen, ☒Essay, ☐Forumsbeitrag, ☒Übungen, ☐Wissenschaftspraktische Tätigkeit, ☐Bachelor- und Masterarbeit ☐Haus-/ Seminararbeit, ☐Einzel-/Gruppenpräsentation, ☐Portfolio, ☐Protokoll, ☐Projektarbeit, ☐Lerntagebuch/ Lernjournale <u>Umfang der Prüfung:</u> Die Teilnahme an den Übungen ist Voraussetzung für die schriftliche Ausarbeitung (Essay). Prüfungssprache wird mit Studierenden gemeinsam festgelegt.
Lernziele	**Fachkompetenz** Die Studierenden können bioanalytische Methoden und Verfahren (inkl. Chemo-/Biosensoren) grundlegend erklären.

	Die Studierenden können verschiedene Anwendungsgebiete identifizieren. Die Studierenden können analytische Ergebnisse bewerten. Die Studierenden können Methoden zur Strukturaufklärung, bildgebende Verfahren, sowie weitere fortschrittliche Methoden erklären. Die Studierenden erkennen den fachlichen Zusammenhang zwischen bioanalytischen Methoden und verschiedenen Anwendungsgebieten. **Methodenkompetenz** Die Studierenden verfügen über die Fertigkeit bioanalytische Fragestellungen zu analysieren und lösen zu können. Die Studierenden können selbstständig eine Datenanalyse durchführen. **Selbst- und Sozialkompetenz** Lernbereitschaft und Belastbarkeit helfen den Studierenden Anwendungsaufgaben zu analysieren und Lösungen zu erörtern.
Lehrinhalte	**Basics:** - Grundlagen und Kenngrößen der Analytischen Chemie - Probenvorbereitung (Zellaufschluss, Fällung, Zentrifugation, Dialyse, Filtration, Extraktion, Gelfiltration, Präzipitation) - Spektroskopische Methoden (Wechselwirkung Licht-Materie, UV-Vis-, Fluoreszenz-, IR-, Raman-, SPR-Spektroskopie, FRET) - Elektrophoretische Verfahren (Wanderung geladener Teilchen in elektrischem Feld, Gel-, Zonen-, Disk-, Kapillarelektrophorese, SDS-PAGE, nativ, isoelektrische Fokussierung, Elektroblotting, 2D) - Chromatographische Trennmethoden (Verteilung zwischen mobiler und stationärer Phase, RP, HIC, HILIC, IEXC, SEC, AC) - Massenspektrometrie (Trennung von Ionen, MALDI, ESI, TOF, Quadrupol, Ionenfalle, SEV, Nachweis, Identifizierung) - Assays (Prinzip, Enzym-, Immuno-Assays) - Chemo- und Biosensoren (Aufbau, elektrochemisch, optisch, radiochemisch) - Weitere Methoden (DNA Sequenzierung, PCR) **Advanced:** - Methoden zur Strukturaufklärung (CD-, NMR-Spektroskopie, Röntgenstrukturanalyse, SAXS, Sequenzanalyse, MS) - Bildgebende Verfahren (Licht-, Fluoreszenz-, Elektronen-, Raster-sondenmikroskopie, Probenpräparation)

	- Kopplungs- und Hochdurchsatzverfahren: LC-MS, MS-MS, Sensorarrays, etc. - Miniaturisierte Chemo- und Biosensoren - Lab-on-a-chip - Weitere Methoden (Ultrazentrifugation, Mikrokalorimetrie, etc.)
Literatur	- F. Lottspeich, J. W. Engels: Bioanalytik, 3. Auflage, Springer Spektrum, 2012 - S. R. Mikkelsen, E. Cortón: Bioanalytical Chemistry, Wiley-Interscience, 2004 - M. H. Gey, Instrumentelle Analytik und Bioanalytik, Springer Berlin Heidelberg, Berlin, Heidelberg, 2. Auflage, 2008. - Cammann, Instrumentelle Analytische Chemie, Spektrum Akademischer Verlag, Heidelberg, 1. Auflage, 2010. - M. Hesse, H. Meier and B. Zeeh, Spektroskopische Methoden in der organischen Chemie, Georg Thieme Verlag, Stuttgart, 7th edn., (2005). - D. A. Skoog, D. M. West, F. J. Holler and S. R. Crouch, Fundamentals of Analytical Chemistry, Cengage Learning, Brooks/Cole, 9th edn., (2014). - Skoog, F. J. Holler and S. R. Crouch, in Principles of Instrumental Analysis, Cambridge University Press, Cambridge, (2007), vol. 9.

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Chapter 1: Spectroscopic methods

Chapter 2: Imaging methods

Chapter 3: Combined and high-throughput technologies

Chapter 4: Miniaturization and biosensors – an overview

1

Basics of analytical chemistry

1.1 Introduction

The concept of *chemical analysis* was first developed by R. Boyle (1627 – 1691). However, analytical chemistry was readily used before, e. g. by Paracelsius (1493 – 1541) for water analysis. Even alchemy can be viewed as the allocation of materials into individual compounds. First *quantitative experiments* were executed by Antoine Lavoisier, who is known as the "father" of analytical chemistry. In 1801, the subject of analytical chemistry was first mentioned in a textbook leading to the establishment of **Analytical Chemistry** as an independent scientific discipline.

Fig. 1.1: Antoine Lavoisier (Public Domain, details on wikimedia commons)

Nowadays, analytical chemistry is used in many different areas ranging from clinical tests for blood or saliva samples to monitoring of critical parameters in food analysis. Likewise, concepts derived from analytical chemistry are essential in biomedical analysis, environmental analysis, and quality monitoring in production processes. Additionally, analytical tools are also used in security and forensic scenarios, and for the investigation of ancient art or archaeological artifacts, as well as for counterfeit screening.

1.2 Definition

In general, analytical chemistry combines and embraces several scientific disciplines. Accordingly, analytical chemistry deals with a variety of challenges including, e. g., guidelines for inter-laboratory comparisons or sample (pre)treatment (i. e., sample collection, preparation, storage, and handling). Additionally, methods and critical data evaluation routines are defined and optimized for maximizing efficiency and accuracy. Analytical methods can be classified into **classical analytics** and **instrumental analytics**, whereby the classical methods include separation or extraction of

different components via so-called **wet-chemical methods**. This includes techniques like titration, gravimetry, extraction, distillation, filtration or chemical pulping.

Since the 20th century **instrumental methods** became increasingly important, and the technological progress along with advancements in computers and software have enabled the detection and determination of increasingly smaller compounds or traces of analytes within complex matrices. Nowadays, modern analytical methods deal with challenges including miniaturization (i. e., detection at micro- and nano-scales, but also devices that are small (e. g., on-chip) yet remain manageable), combination of techniques (i. e., so-called "hyphenated-techniques"), on-line detection scenarios, increasingly smart and reliable analytical tools (i. e., control systems and automation of processes), and the optimization of qualitative and quantitative results toward more precision, accuracy, reliability, and repeatability.

Different types of instrumental methods have been defined, which can be divided into several groups. The physical and chemical parameters used for the characterization and determination of analytical species are divided into radiation, electrical properties, mass and charge of the analytes, thermal or kinetic characteristics, radioactivity, etc. resulting in different demands and in consequence different instrumental methods. Hence, analytical chemistry is not just aimed towards the detection of analyte species, but also with the optimized application of physical stimuli followed by evaluation of the obtained response of the sample, i. e., analytical information and data. Analytical methods can also be divided with respect to the collected information. There are four "W-questions", which can be answered by analytical methods:

What's in the sample?	→	Qualitative analysis
What's the amount of analyte?	→	Quantitative analysis
Where is the analyte located?	→	Surface or distribution analysis
What's the structure of the sample?	→	Structural/chemical composition of the analytes

1.3 Criteria for selecting analytical methods

There are numerical criteria for selecting suitable analytical methods, and for assessing the obtained analytical result to its trueness and accuracy. In the following, some statistical descriptors are briefly explained, which are commonly used in analytical chemistry.

1.3.1 Population vs. sample

The **population** represents the entire sample unit (e. g., an entire lake, river, etc.). The entire population is therefore usually not available for evaluation due to restrictions in time, cost and appropriate effort. Hence, a so-called **sample** is collected, which should be a representative amount of the unknown species representing the entire system. By definition, statistical terms, which pertain to the entire population are written in *Greek letters*, and terms describing a sample are written in *Latin letters*.

	population	sample
Mean value	$\mu = \lim_{N\to\infty} \frac{\sum_{i=1}^{N} x_i}{N}$	$\bar{x} = \frac{\sum_{i=1}^{N} x_i}{N}$
Standard deviation	$\sigma = \sqrt{\lim_{N\to\infty} \frac{\sum_{i=1}^{N}(x_i-\mu)^2}{N}}$	$s = \sqrt{\frac{\sum_{i=1}^{N}(x_i-\bar{x})^2}{N-1}}$
Variance	$\sigma_t^2 = \sigma_1^2 + \sigma_2^2 + ... + \sigma_n^2$	$s_t^2 = s_1^2 + s_2^2 + ... + s_n^2$
Relative standard deviation	$RSD = \frac{\sigma}{\mu} \times 10^z$	$RSD = \frac{s}{\bar{x}} \times 10^z$
Coefficient of Variation (z=2)	$CV = \frac{\sigma}{\mu} \times 100\ \%$	$CV = \frac{s}{\bar{x}} \times 100\ \%$

With: number of measurement (N), the signal value (x_i, with $i = 1 - N$) and $z = \frac{(x-\mu)}{\sigma}$.

Fig. 1.2: Population vs. sample

1.3.2 Trueness vs. precision vs. accuracy

The **trueness** of a result describes the level of agreement of the measured mean value with the *true value*. This value is in general unknown, however, the goal remains to get as close as possible to the true value. Therefore, every systematic error has to be eliminated. This can be realized by using *reference materials* and performing a method or instrumental *validation*. By repeating the measurement ≥ 30 times and taking the average, one may statistically assume normally distributed values close to the true value (in most cases; not always), if there is no systematic error. It should be noted again that meaningful average values are only obtained, if the underlying data points are normally distributed.

The **precision** describes the coincidence of individual data points, and the reproducibility of the obtained results using the same method (i. e., same technique, same instrumental set-up, etc.). In other words, the scattering of the data points around the average value is described using the absolute/relative standard deviation, variance, and the coefficient of variation. As a statistical parameter, precision can be divided into *reproducibility* of a result investigated by different laboratories, *repeatability* of consecutive measurements, and repeated measurements over a period of time (*intermediate precision*).

The **accuracy** describes the correctness of the result and combines trueness with the precision of an experimental value. It comprises all errors within the method or the system.

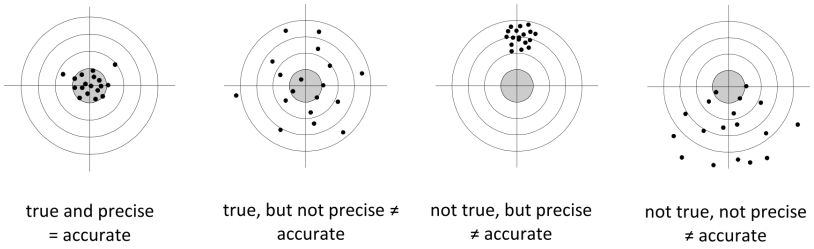

Fig. 1.3: Trueness vs. precision vs. accuracy

1.3.3 Differences in errors

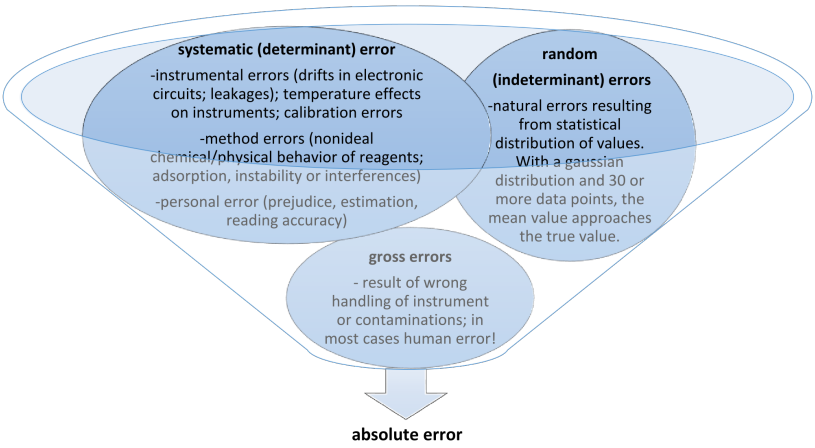

Fig. 1.4: Differences in errors

In an experiment with a large number of individual data points and the assumption that no systematic errors occur, the distribution of the values is safely assumed as "normal" (i. e., Gaussian). This implies the symmetrical distribution of values around the mean value, which has the maximum frequency of data points. Additionally, the behavior of the data points should show an exponential decrease with increase of the deviation from the mean value. This can be expressed by a *normal Gaussian distribution*.

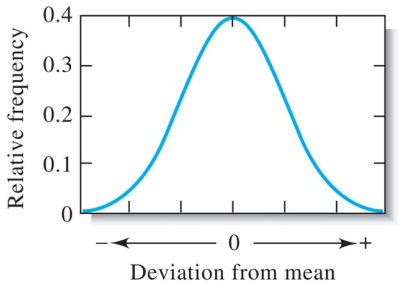

Fig. 1.5: Normal Gaussian distribution (ISBN: 9780495558286)

In general, a set of data is normally distributed – especially if a large number of data points is collected – and the sample can be viewed as a population. In most experiments, it is not possible however to repeat a measurement that frequently. Therefore, the mean value and a data range can be estimated and assuming that with a sufficiently high probability the true value is within this region. This is called the **confidence interval P**. The interval that is not taken into account, is called the **significance level α**. The calculation of an appropriate interval is done using the *t-test* (or student distribution).

It should be noted that next to a normal distribution, there are also other possibilities for data distributions, e. g., *Bernoulli* distribution, *Poisson* distribution, etc.

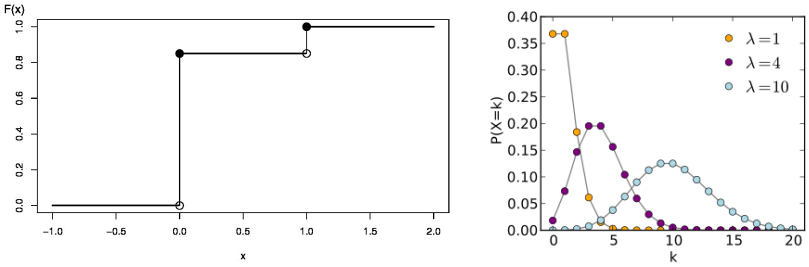

Fig. 1.6: Bernoulli distribution (left, https://media.wsb.wisc.edu/data/act_sci/Frees/ActMathII/InterestRateRisk/InterestRateRisk04Nov2012/bernoulli_distribution.html) and Poisson distribution (right, by Skbkekas, Poisson pmf, CC BY 3.0)

Hence, it is obvious that the mean value ± the standard deviation is not always the most suitable representation of the experimental values. Another possibility to characterize the measurement is the *median*. This is the value, which is in the middle of all data, i. e., divides all data points into 50 % to the left and right of the median. This method is more resistant towards outliers. The *mode* of a data set is the most frequently occurring value within all determined values, and is also rather insensitive to outliers.

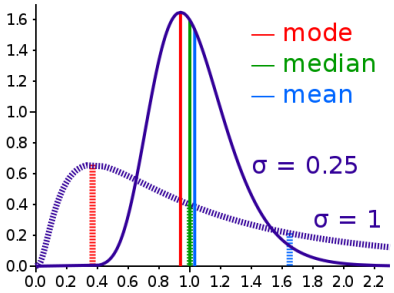

Fig. 1.7: Comparison mean median mode (by Cmglee, Comparison mean median mode, CC BY-SA 3.0)

1.3.4 How to find outliers?

The statistics and assumptions discussed above are only reliable, if there are no extreme individual values considered within the data range, i. e., which have a substantial deviation from the mean value. This may occur due to errors, but may also be in fact real values with a more pronounced deviation vs. other data points. Such data can significantly affect/distort a result that is usually based on calculating an average value, it is preferable to test an experimental data set for these **outliers**.

The most frequently used tests for outliers in analytic chemistry include:

- **Dean and Dixon:** An outlier x_1^* can be distinguished for data sets with $n \leq 25$ by this method also known as *Q-test*. Thereby, the values are sorted in increasing or decreasing order (dependent on the outliers with respect to the mean value) and calculated as

$$\widehat{M} = \frac{|x_1^* - x_b|}{|x_1^* - x_k|}$$

  with $b = 2$ $(3 \leq n \leq 10)$ or $b = 3$ $(11 \leq n \leq 25)$ and $k = n$ $(3 \leq n \leq 7)$, $k = n - 1$ $(8 \leq n \leq 13)$ or $k = n - 2$ $(14 \leq n \leq 25)$.

- **Graf and Henning:** For $n \geq 25$ the outlier can be determined by calculation of the mean value and the standard deviation without consideration of the suspected outlier. If this value is within $\bar{x} \pm 4s$, the discrepancy of this value is still acceptable.

- **Grubbs:** This test can be used for an undefined number of values to investigate the lowest and/or the highest value x^* in a data series

$$\widehat{G} = \frac{|\bar{x} - x^*|}{s}$$

  with the mean value $(\bar{x})$ and the standard deviation (s).

Beratung und Kontakt

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