CONTENTS

PREFACE TO THE FIRST EDITION

The second book of the textbook "Analytical Chemistry (Analytics)" is a continuation of the first book, which includes sections "General theoretical foundations. Qualitative analysis". The content of the second book completely corresponds to the university program of analytical chemistry including a course of instrumental analysis methods for students regarding the two sections of analytical chemistry: foundations of quantitative chemical analysis and physico-chemical (instrumental) methods of analysis.

Gravimetric, chemical titrimetric methods of analysis (acid-base, oxidation-reduction, complexometric (including complexonometry), precipitation titration; titration in non-aqueous media) are considered. A number of the following physico-chemical and physical methods are characterized: optical (colorimetry, photoelectrocolorimetry, spectrophotometry, luminescent analysis), chromatographic (gas-liquid, high-performance liquid chromatography), electrochemical (conductometry, potentiometry, polarography, amperometry, coulometry). Statistical processing methods of quantitative analysis results are highlighted. Examples with solutions and exercises are presented.

Both books together completely represent the course of analytical chemistry (analytics) in a relatively significant scope specified for its studying at universities according to degree course schemes. For example, for students of pharmaceutical specialities, for these plans are currently scheduled 378 academic hours, of which 252 are classroom hours (72 hours of lectures and 180 hours of laboratory classes).

At the same time, if necessary, a concise description is given, providing the main principles of the corresponding methods. A detailed description of instruments and tools is not included since many countries produce analytical instruments of various designs.

Sufficient attention is paid to applications of the principles of probability theory (error theory) to the processing of quantitative analysis results, evaluation of the correctness and reproducibility of analytical procedures, which is illustrated by specific examples.

References include a limited number of sources; a more detailed bibliography can be found in the cited publications.

My sincere gratitude to the reviewers.

Author

I. Quantitative chemical analysis

> Only a strict quantitative experimental verification allows us to evaluate the validity and generality of the theory.
>
> *G.P. Gladyshev, President of the International Academy of Creativity ("Thermodynamic evolution theory of living beings", 1996)*

Chapter 1
INTRODUCTION TO QUANTITATIVE ANALYSIS

1.1. QUANTITATIVE ANALYSIS

In general terms, the purpose of quantitative analysis is to obtain necessary quantitative data on individual components of the system, i.e., to determine quantitatively the content of the main component, constituent parts or impurities in the analyzed sample.

The recommended definition (proposed in Journal of Analytical Chemistry back in 1975) is the following: *quantitative analysis of a substance is the experimental determination (measurement) of the concentration (quantity) of chemical elements (compounds) or their forms in an analyzed substance, which is expressed as the boundaries of a confidence interval or number indicating the standard deviation*.

Quantitative analysis is widely used in pharmaceutical analysis and is an essential part of the pharmacopoeial analysis of *any* drug product.

1.2. CLASSIFICATION OF QUANTITATIVE ANALYSIS METHODS

Quantitative analysis methods are usually classified as follows: *chemical, physico-chemical, physical, biological ones.*

Chemical methods of analysis include *gravimetric* (*weight*) and *titrimetric* (*volumetric*) *methods.*

Gravimetric methods are based on precise mass measurement of the analyzed component of a sample, separated from other components of the system, in elemental form (i.e., in the stable form of a given chemical element) or in the form of a compound with a precisely known composition. Gravimetric methods are simple to perform, highly accurate and reproducible, but quite laborious and time-consuming.

Titrimetric methods are based on measuring the volume or mass of reagent (titrant) required for reaction with the analyzed substance (analysis is based on titration). Methods are simple, highly accurate and reproducible, but in most cases *indicators* are required to determine the endpoint of the titration.

Physico-chemical and physical (*instrumental*) *methods of analysis* include *optical*, *chromatographic*, *electrochemical*, and some other methods (for example, radiometric, thermal, mass spectrometric, pycnometric, ultrasonic, etc.).

The advantages of instrumental methods of analysis include: low detection limit ($1–10^{-9}$ μg) and limit concentration (up to ~10–15 g/ml) of the analyzed substance; selectivity (it is possible to determine the constituent components of a mixture without separation and isolation); analysis rate, the possibility of automation and computerization; the objectivity of the results.

The disadvantages include a relatively large error of determination (of the order of ~5%; in some cases, up to 20%, while in chemical analysis the error of determination is usually ~0.1–0.5%), and also the complexity and high cost of necessary equipment.

Biological methods of analysis are usually not considered in the course of analytical chemistry (they are studied in courses of pharmacology, biochemistry, biology).

1.3. REQUIREMENTS FOR REACTIONS IN QUANTITATIVE ANALYSIS

Chemical analytical reactions used in the quantitative chemical analysis must meet certain requirements, the most important of which are the following.

a) Reactions must proceed quickly, to the end, and if possible, at room temperature.
b) Initial substances undergoing the reaction must react in exactly determined quantitative ratios (stoichiometrically), and without side processes.
c) Impurities must not interfere with quantitative analysis.

These general requirements are specified, complemented and defined more precisely when detailing various methods and procedures of chemical quantitative analysis.

1.4. STATISTICAL PROCESSING OF QUANTITATIVE ANALYSIS RESULTS

During quantitative analysis, the following various physical values are usually measured or determined by calculation based on the measurements: substance mass, solution concentration, liquid volume, color intensity of substance, an optical density of the medium, redox potentials, light refractive indices, and other analytical signals.

All physical values (without exception) are measured with a certain error. It is impossible to measure any physical value accurately (and the term «accurately» itself is unclear and must be specifically defined). Therefore, when performing quantitative analysis and corresponding calculations, it is necessary to take into account the errors of determination in quantitative (numerical) form.

There exists a wide variety of *error sources*.

If significant deviations from a procedure or its obvious noncompliances are allowed during the quantitative analysis, then the analysis must be repeated, excluding obviously incorrect results.

The essential rule is to repeat the incorrect analysis.

One of the founders of chemical analysis, the German scientist Karl Remigius Fresenius (1818–1897), who began his career as *a pharmacist*, wrote (in 1847):

"Each analyst always doubts the accuracy of the obtained results, and sometimes he knows in advance that they are incorrect. He may spill a few drops of solution or make any other mistake. The only thing the analyst must do in this situation is to repeat the analysis; visual loss evaluation or making any amendments is not allowed. One who does not have enough willpower for this is unfit to be an analyst, even if he is a good master in the technique of analysis and possesses sufficient knowledge. A chemist who cannot swear that the results of his work are reliable and valid, should not publish them, because if he does, he will harm not only himself but the entire science."

However, even if all the requirements of the procedure are strictly complied with, the results of individual independent tests of the same object are commonly slightly different. It is advisable to evaluate these differences quantitatively in order to understand the reliability of results. Such evaluation

usually presumes obtaining *metrological characteristics* based on the principles of the theory of probability (the error theory). In this case, it is useful to remember that *any statistical processing of experimental data is approximate and has a probabilistic meaning.*

The most important metrological terms for quantitative analysis will be considered below, such as *correctness and reproducibility* of analysis results (*metrology* is science that studies methods of measuring physical quantities).

1.4.1. Correctness and reproducibility of quantitative analysis results

In order to error detection and their numerical evaluation (especially if new analytical procedures are developed), the quantitative analysis is repeated several times, i.e., *parallel determinations are performed.* Parallel determinations are understood as achievement of several results of individual determinations for one sample under practically the same conditions.

Let μ be *a true value of determined quantity*; $x_1, x_2, ..., x_i, ..., x_n$ *are* measured (individual) values of determined quantity, which are results of *individual determinations*; *n* is total number of individual determinations.

Individual determination is meant as single performance of entire operation sequence prescribed in the analytical procedure.

Result of individual determination is a value of determined component content found during an individual determination.

Sometimes (frequently), *real value of content a* (or simply *real value of a*) is used instead of the determined quantity true value μ, meaning the experimentally obtained or calculated value of determined content, which is so close to true value that for this purpose it can be used instead of the true value.

Then, value of

$$\bar{x} = (x_1 + x_2 + ... + x_n)/n = (\sum x_i)/n \qquad (1.1)$$

is arithmetic mean (average) of individual determination results. It is considered that $\bar{x}$ is *the most probable value of determined quantity, more probable than each individual value xi.*

Correctness of analytical results is understood as analysis quality indicating that difference between the arithmetic mean and true μ (or real *a*) values of determined quantity is close to zero:

$$\bar{x} - \mu \to 0 \quad \text{or} \quad \bar{x} - a \to 0$$
$$\text{at } n \to \infty \qquad \text{at } n \to \infty.$$

In other words, correctness of analytical result indicates that obtained average value of $\bar{x}$ is close to true (or real) value of determined quantity.

Reproducibility of analytical result characterizes the degree of closeness of individual determination results *xi* to each other.

Correctness and reproducibility of quantitative analysis results depend on errors of different types.

1.4.2. Classification of quantitative analysis errors

Errors of quantitative analysis are *conventionally* divided into *systematic*, *random*, and *gross*.

Gross errors caused by non-compliance with an analytical procedure are obvious. They are eliminated by repeating analysis in compliance with all the required conditions prescribed in an analytical procedure.

A. *Systematic error:*

Systematic errors and *percent systematic errors* are distinguished*:*

Systematic error of analytical result Δ_0 is statistically significant difference between the average $\bar{x}$ and real a (or true μ) values of determined component content:

$$\Delta_0 = \bar{x} - a \quad \text{or} \quad \Delta_0 = \bar{x} - \mu. \tag{1.2}$$

Systematic error of analytical result can be more than zero, less than zero, or equal to zero.

Percent systematic error (*relative value of systematic error*) is systematic error, expressed as a percentage of real value of a (or true value of μ) of determined quantity:

$$\delta = (\bar{x} - a) \cdot 100\%/a \quad \text{or} \quad \delta = (\bar{x} - \mu) \cdot 100\%/\mu. \tag{1.3}$$

The relative value of systematic error is also denoted as Δ_0, % instead of δ symbol.

Systematic error characterizes the correctness of analytical results; therefore, the correctness of analysis can be determined in the same way as the quality of analysis, reflecting that systematic error is close to zero.

Systematic errors are caused either by permanent reasons (and they are repeated during repeating analysis), or they are changed according to the permanent law.

Thus, for example, per cent systematic error $(\Delta c/c) \cdot 100\%$ of photometric determinations (c is concentration, Δc is a systematic error of concentration determination by photometric method) is minimal within the range of optical density A changes *from* $A \approx 0.2$ to $A \approx 0.8$, and is equal to $(\Delta c/c) \cdot 100\% < 0.4\%$.

Sources of systematic errors. It is impossible to list all sources of systematic errors exhaustively. The main sources of systematic errors are the following.

Methodical errors are caused by the specifics of the analytical procedure. For example, the analytical reaction proceeds not completely; a precipitate is partially lost due to its partial solubility in solution or during its washing; impurities are co-precipitated, which leads to increase in precipitate mass, etc.

Instrumental errors are caused by the imperfections of instruments and equipment used. Thus, for example, the systematic weighing error of a laboratory analytical balance is ± 0.0002 g. Systematic error in titrimetric methods of analysis is caused by inaccurate calibrations of burettes, pipettes, volumetric flasks, volumetric cylinders, beakers, etc.

Individual errors are caused by the subjective qualities of an analyst. For example, color blindness can affect the determination of the endpoint of titration in case of visual evaluation of indicator color change.

Correctness of analytical results is determined by the presence or absence of systematic errors.

Systematic errors can be determined by the following methods.

a) *Use of reference standards.* The total composition of a reference standard must be close to the composition of analyzed sample, and content of determined component in a reference standard must be precisely known. Analysis of the reference standard is the most reliable method to recognise the presence or absence of systematic error and evaluate the correctness of the analytical result.

b) *Analysis of test object using other methods.* A test object is analyzed by a method or methods that do not cause a systematic error (metrologically certified), and analysis results are compared with data obtained during analysis of the same object by testing procedure. The comparison allows characterizing correctness testing procedure (or method) of analysis.

c) *Spike test or duplicating method* is used if reference standards and metrologically certified procedure (or method) of analysis are absent.

A sample is analyzed using a testing procedure. Then, the mass of the analyzed sample is doubled, or mass is increased (decreased) a different number of times, the content of testing component in the new sample is determined again, and the analysis results are compared.

B. *Random errors*

Random errors indicate the difference between the results of parallel determinations and characterize *reproducibility* of analysis. Causes of random errors cannot be indicated unambiguously. If an analysis is repeated many times, they either cannot be reproduced, or they have different numerical values and even different signs.

Random errors can be evaluated using methods of mathematical statistics *if systematic errors are identified and eliminated* (*or systematic errors are less than random ones*).

1.4.3. Selected terms of mathematical statistics and their use in quantitative analysis

Random quantity (in respect to quantitative analysis) is a measurable analytical signal (mass, volume, optical density, etc.) or the result of analysis.

Variant is an individual value of a random variable, i.e., individual measurement value of analytical signal or determined content.

General population is an idealized set of the results of an infinitely large number of measurements (variant) of random variables.

In most cases, a relative probability of results in the general population when performing chemical analytical determinations is described by the Gauss function (Gaussian distribution).

However, in practice it is impossible (and not necessary) to perform an infinitely large number of analytical determinations; therefore, *sampling population (sample)* is used instead of the general population.

The sample (*sampling population*) is a population of a limited number of statistically equivalent variants, considered as a random sample from the general population. In other words, the sampling population is a set of results of measurements of analytical signals or determined contents, considered as a random sample from the general population obtained under predetermined conditions.

Sample number is the number of variants n constituting the sample.

During statistical processing of quantitative analysis results, a sample described by *Student's distribution* is used. (Student was an English chemist W. Gosset, who wrote under the pseudonym "Student").

Student's distribution is preferably used with a sample size of $n < 20$.

1.4.4. Statistical processing and reporting of quantitative analysis results

Calculation of metrological parameters. In practice, the quantitative analysis does not usually include an infinitely large number of determinations, but $n = 5–6$ independent determinations, i.e., have the sample (sampling population) number of 5–6 variants. In an *optimal case* (for example, during the analysis of drug products), *it is recommended to perform 5 parallel determinations, i.e., optimal recommended sample number is* $n = 5$.

If a sample is available, the following metrological parameters are calculated according to the Student's distribution.

Average, i.e., the average value of determined value, according to (1.1), is

$$\bar{x} = (\sum x_i)/n.$$

Average of the final sample differs from the real value of a (which is usually not known) and depends on sample number n:

$$\lim \bar{x} \to a.$$

$$\text{and } n \to \infty.$$

Deviation di:

$$d_i = x_i - \bar{x} \tag{1.4}$$

— random deviation of the i-th variant from the average.

Dispersion V (sometimes denoted as s^2) shows variant spreading in relation to the average and characterizes reproducibility of the analysis. It is calculated according to formula (1.5):

$$V = (\sum d_i^2)/f = [\sum (x_{i\_} \bar{x})^2]/(n - 1), \tag{1.5}$$

where $f = n - 1$ is the so-called *number of degrees of freedom*.

If the real value of determined quantity a (or the true value of determined quantity μ) is known, for example, when working with a reference standard, then the average $\bar{x}$ is taken equal to a (or μ); then the number of degrees of freedom is $f = n$.

Dispersion of the average $V\bar{x}$ is equal to

$$V_{\bar{x}} = V/n.$$

The standard deviation (or mean root square deviation) s is a parameter of the variant dispersion in relation to the average. It is calculated as the square root of dispersion V, taken with a plus sign:

$$s = + \sqrt{V} = + [\sum (x_i - \bar{x})^2/(n - 1)]^{0.5}. \tag{1.6}$$

It is clear that $V = s^2$. Standard deviation s, as well as dispersion V, characterizes the reproducibility of quantitative analysis.

Standard deviation of the average $s_{\bar{x}}$ is determined as

$$s_{\bar{x}} = s/\sqrt{n} = \sqrt{V/n}$$

(the previous name is the mean root square error of the arithmetic mean).

Relative standard deviation sr is a ratio of the standard deviation to the mean value:

$$s_r = s/\bar{x}.$$

The lower *sr*, the higher reproducibility of the analysis.

Confidence interval (*confidence interval of the average*) is an interval, which includes the real value of determined quantity (general average) with predetermined confidence probability *P*:

$$\bar{x} \pm \Delta\bar{x}, \tag{1.7}$$

where $\Delta\bar{x}$ is the half-width of a confidence interval.

Confidence probability P is the probability of finding the real value of determined quantity *a* within the confidence interval. It changes from 0 to 1 or (which is the same) from 0% to 100%. In a pharmaceutical analysis, during quality control of drug products, the confidence probability is most often taken equal to $P = 0.95 = 95\%$ and denoted as $P_{0.95}$. In order to evaluate the correctness of procedures or methods of analysis, the confidence probability is usually considered equal to $P = 0.99 = 99\%$.

Half-width of the confidence interval $\Delta\bar{x}$ is calculated by the formula (1.8):

$$\Delta\bar{x} = t_{p,f}s/\sqrt{n}, \tag{1.8}$$

where $t_{p,f}$ *is* coefficient of normalized deviations (Student's coefficient, Student's function, Student's criteria), which depends on confidence probability *P* and number of freedom degrees $f = n - 1$, i.e., on the number of performed determinations.

The numerical values of $t_{p,f}$ are calculated for various possible values of *P* and *n* and are tabulated in reference books.

The numerical values of Student's coefficient calculated for different values of *n* and *P* are presented in table. 1.1

The more *n*, the less $t_{p,f}$. However, at $n > 5$ the decrease in $t_{p,f}$ is already relatively small, therefore, in practice, five parallel determinations ($n = 5$) are usually considered sufficient.

Relative (per cent) error of the average result:

$$\bar{\varepsilon} = (\Delta\bar{x}/\bar{x}) \cdot 100\%. \tag{1.9}$$

Table 1.1. The numerical values of Student's coefficient t for calculating the boundaries of the confidence interval with confidence probability P, sample number n, number of degrees of freedom $f = n - 1$

n	f	The value of t at confidence probability				
		0.80	**0.90**	**0.95**	**0.99**	**0.999**
2	1	3.08	6.31	12.07	63.7	636.62
3	2	1.89	2.92	4.30	9.92	31.60
4	3	1.64	2.35	3.18	5.84	12.94
5	4	1.53	2.13	2.78	4.60	8.61
6	5	1.48	2.02	2.57	4.03	6.86
7	6	1.44	1.94	2.45	3.71	5.96
8	7	1.42	1.90	2.36	3.50	5.41
9	8	1.40	1.86	2.31	3.36	5.04
10	9	1.38	1.83	2.26	3.25	4.78
11	10	1.37	1.81	2.23	3.17	4.59
12	11	1.36	1.80	2.20	3.11	4.49
13	12	1.36	1.78	2.18	3.06	4.32
14	13	1.35	1.77	2.16	3.01	4.22
15	14	1.35	1.76	2.14	2.98	4.14
16	15	1.34	1.75	2.12	2.95	4.07
17	16	1.34	1.75	2.11	2.92	4.02
18	17	1.33	1.74	2.10	2.90	3.97
19	18	1.33	1.73	2.09	2.88	3.92
20	19	1.33	1.73	2.09	2.86	3.88
21	20	1.33	1.73	2.09	2.85	3.85
22	21	1.32	1.72	2.08	2.83	3.82
23	22	1.32	1.72	2.07	2.82	3.79
24	23	1.32	1.71	2.07	2.81	3.77
25	24	1.32	1.71	2.06	2.80	3.75
26	25	1.32	1.71	2.06	2.79	3.73
27	26	1.32	1.71	2.06	2.78	3.71
28	27	1.31	1.70	2.05	2.77	3.70
29	28	1.31	1.70	2.05	2.76	3.67
30	29	1.31	1.70	2.05	2.76	3.66
31	30	1.31	1.70	2.04	2.75	3.65
41	40	1.30	1.68	2.02	2.70	3.55
61	60	1.30	1.67	2.00	2.66	3.46
121	120	1.29	1.66	1.98	2.62	3.37
∞	∞	1.28	1.64	1.96	2.58	3.29

Exclusion of gross errors. Some results of individual determinations (variants) included in the sampling population may differ significantly from the values of other variants and cause doubts about their reliability. In order for the statistical processing of the results of quantitative analysis to be reliable, the sample must be *uniform*, i.e., it should not be loaded with doubtful variants (so-called *gross errors).* These gross errors must be excluded from the total sample number, after which the final calculation of statistical parameters can be performed.

If sample number is not large $5 \leqslant n < 10$, then identification of doubtful results of the analysis (*elimination of gross errors*) is commonly performed using the so-called *Q-criterion* (control criterion *Q*), or *Q*-test. For this purpose, variants x_i are first arranged in increasing order of their numerical value from x_i to x_n, where *n* is sample number, i.e., presented as an *ordered sample.* Then, for extreme variants (minimum x_i and maximum x_n), quantity *Q* is calculated according to formulas (1.10):

$$Q_1 = (x_2 - x_1)/R; \;\; Q_n = (x_n - x_{n-1})/R, \qquad (1.10)$$

where x_2 and x_{n-1} are values of the variants closest in magnitude to the extreme variants, and

$$R = x_n - x_1$$

— *range of variability*, i.e., the difference between the maximum x_n and the minimum x_1 values of variants (between the extreme variants) constituting the sample.

Calculated values Q_1 and Q_n are compared with tabulated values at predetermined *n* and confidence probability *P.* If calculated values of Q_1 or Q_n (or both of them) are more than tabulated ones:

$$Q_1 > Q_{\text{table}} \text{ or } Q_n > Q_{\text{table}},$$

then variants x_i or x_n (or both of them) are considered as gross errors and excluded from the sample.

For the obtained sample of a smaller number, similar calculations are carried out until all gross errors are eliminated, so that the final sample is uniform and not burdened with gross errors.

The numerical values of control criterion *Q* for $P = 0.90 - 0.99$ and $n = 3 - 10$ are presented in table 1.2.

Table 1.2. The numerical values of Q-criterion at confidence probability P and sample number n

P \ n	3	4	5	6	7	8	9	10
0.90	0.94	0.76	0.64	0.56	0.51	0.47	0.44	0.41
0.95	0.98	0.85	0.73	0.64	0.59	0.54	0.51	0.48
0.99	0.99	0.93	0.82	0.74	0.68	0.63	0.60	0.57

Note. In some sources, the numerical values of Q slightly differ from the values presented in table 1.2.

During the Q-test, the confidence probability is most often taken equal to $P = 0.90 = 90\%$.

If only one of the two extreme variants x_1 and x_n is doubtful, then the Q-test can be performed only for this doubtful variant.

Example. Suppose that during five parallel analyzes, the content (%) of determined component is found equal to: 3.01, 3.03, 3.04, 3.05, and 3.11. Determine if gross errors take place or whether the considered sample is uniform.

Solution. It is obvious that only one value of 3.11 can be doubtful. Let us use Q-test. According to (1.10), the following can be written down:

$$Q_{calc} = (3.11 - 3.05)/(3.11 - 3.01) = 0.60.$$

Based on table 1.2, at $n = 5$ and $P = 0.90$, we calculate $Q_{tab} = 0.64$. Since

$$Q_{calc} = 0.60 < Q_{tab} = 0.64,$$

then variant value 3.11 is not a gross error and is not excluded.

As mentioned above, usually during quantitative analysis (for example, analysis of drug products and similar samples), the sample number (number of individual parallel determinations) equal to $n = 5$ is recommended. In these cases, gross errors are excluded using the Q-test, as described above.

If the sample number is equal to 3 or 4, i.e., n <5, then the use of Q-test is not recommended.

If the sample number is $n > 10$, then the procedure to eliminate gross errors (to check sample uniformity) is as follows.

Initially, according to the results of individual independent determinations, the average value, deviations *di* for all variants, and standard deviation *s are preliminarily calculated using formulas (1.1), (1.4), (1.6).* Then, the absolute

quantity of $|d_i|$ and the numerical value of $3s$ *are compared*. If the following ratio is correct for all variants:

$$|d_i| \leqslant 3s, \tag{1.11}$$

then gross errors are absent; the sample is uniform. If condition (1.11) is not fulfilled for all variants, then those options, for which this condition is not fulfilled, are considered gross errors at $P = 0.95 = 95\%$ and excluded from the total sample population. The less sample is obtained, for which the whole cycle of calculations is repeated again, and the relation (1.11) is used again to determine the presence or absence of gross errors. This procedure is repeated until all gross errors are excluded and the sample is uniform.

The sample number more than ten ($n > 10$) is commonly used to evaluate the reproducibility of analytical methods or procedures.

Presentation of the results of quantitative analysis. In order to present the results of quantitative analysis, the following statistical characteristics are commonly indicated and calculated: x_i *are* the results of individual determinations (variants); n is the number of independent parallel determinations (sample number); $\bar{x}$ is the average value of determined quantity; s is standard deviation; $\Delta\bar{x}$ is half-width of the confidence interval (indicating the value of confidence probability P); $\bar{x} \pm \Delta\bar{x}$ the confidence interval (the confidence interval of the average); $\bar{\varepsilon}$ is relative (per cent) error of the average result.

These characteristics constitute a necessary and sufficient minimum of values describing the results of quantitative analysis, in case if *systematic errors are eliminated or they are less than random ones*.

Sometimes, in addition, dispersion $V = s^2$, dispersion of the average $V_{\bar{x}} = V/n$, standard deviation of the average $s_{\bar{x}} = s/\sqrt{n}$, relative standard deviation $s_r = s/\bar{x}$ are also indicated. However, listing them is not necessary, since all of them are easily calculated from the above-mentioned values.

The example of statistical processing and reporting of quantitative analysis results. Let the content (%) of component determined in the analyte in five parallel unit determinations ($n = 5$) be equal to: 3.01, 3.04, 3.08, 3.16, and 3.31. It is known that systematic error is absent.

It is required to carry out statistical processing of quantitative analysis results (in order to evaluate their reproducibility) at confidence probability $P = 0.95$.

Solution. 1) Let us evaluate gross errors using the Q-criterion. The 3.31 value might be doubtful. According to formulas (1.10), we obtain:

$$Q_{\text{calc}} = (3.31 - 3.16)/(3.31 - 3.01) = 0.50.$$

The table value of Q_{table} at $n = 5$ and $P = 0.90$ is equal to (refer to table 1.2) $Q_{\text{table}} = 0.64$. Since $Q_{\text{calc}} = 0.50 < Q_{\text{table}} = 0.64$, then value 3.31 of variant is not a gross fault. The sample is uniform.

2) Calculate the average value of $\bar{x}$, deviations d_i, and the sum of squared deviations $\sum d_i^2$:

$$\bar{x} = (3.01 + 3.04 + 3.08 + 3.16 + 3.31)/5 = 3.12;$$

$$\sum d_i^2 = 0.0121 + 0.0064 + 0.0016 + 0.0016 + 0.0361 = 0.0578.$$

Table of deviations

x_i	$d_i = x_i - \bar{x}$	d_i^2
3.01	3.01 – 3.12 = –0.11	0.0121
3.04	3.04 – 3.12 = –0.08	0.0064
3.08	3.08 – 3.12 = –0.04	0.0016
3.16	3.16 – 3.12 = 0.04	0.0016
3.31	3.31 – 3.12 = 0.19	0.0361

3) Calculate standard deviation according to formula

$$s = [\sum d_i^2/(n - 1)]^{0.5} = (0.0578/4)^{0.5} = 0.12.$$

4) Calculate the half-width of the confidence interval of the average $\Delta\bar{x}$ according to formula (1.8) at $n = 5$ and P = 0.95:

$$\Delta\bar{x} = t_{P,f}s/\sqrt{n}.$$

Student's coefficient is taken from table 1.1:

$$t_{P,f} = t_{0.95;4} = 2.78.$$

Then

$$\Delta\bar{x} = 2.78 \cdot 0.12/\sqrt{5} = 0.15.$$

The confidence interval of the average:

$$\bar{x} \pm \Delta\bar{x} = 3.12 \pm 0.15.$$

5) Calculate the relative error of the average $\bar{\varepsilon}$ according to formula (1.9):

$$\bar{\varepsilon} = (\Delta\bar{x}/\bar{x}) \cdot 100\% = (0.15/3.12) \cdot 100\% = 4.8\%.$$

6) Compile a final table representing the results of the analysis.

Final table

x_i	**3.01; 3.04; 3.08; 3.16; 3.31**
n	5
$\bar{x}$	3.12
s	0.12
$\Delta\bar{x}$	0.15 ($P = 0.95$)
$\bar{x} \pm \Delta\bar{x}$	3.12 ± 0.15
ε^-	4.8%

At the final table compiling step, the presentation of statistical data processing results of quantitative analysis is completed.

1.5. EVALUATION OF ANALYTICAL METHODS BASED ON CORRECTNESS AND REPRODUCIBILITY

1.5.1. Comparison of two methods based on reproducibility (comparison of dispersions)

Let the quantitative analysis of the same object be performed using two independent methods I and II, and the following is obtained after statistical processing of the results of parallel determinations:

method I: $f_1 = n_1 - 1$; dispersion $V_1 = s_1^2$,

method II: $f_2 = n_2 - 1$; dispersion $V_2 = s_2^2$,

where *fi* and f_2; n_i and n_2 *are* numbers of degrees of freedom and sample numbers for the first and the second method, respectively. In this case, both methods (and both samples) are enumerated in such a way that the dispersion of the first sample is higher than the dispersion of the second sample: $V_1 > V_2$ *Numbers of degrees of freedom should be* $f_1 > 10$ and $f_2 > 10$.

In order to evaluate whether the difference between two dispersions V_1 and V_2 is statistically significant or not, the so-called *F*-test (*Fisher criterion*) is used according to formula (1.12)

$$F_{\text{calc}} = V_1 / V_2. \tag{1.12}$$

Since dispersions are enumerated in such a way that $V_1 > V_2$, then the quantity of $F_{\text{calc}} > 1$. Calculated value F_{calc} is compared with the tabulated

value F_{tab} of Fisher criterion, usually with a confidence coefficient $P = 0.99$. If $F_{calc} < F_{tab}$, then it means the difference between dispersions V_1 and V_2 is random, statistically insignificant; dispersions are uniform and reproducibility of the method I is worse than reproducibility of method II.

The numerical values of Fisher criterion are listed in table 1.3.

Table 1.3. The numerical values of Fisher criterion F at confidence probability $P = 0.99$ and the number of degrees of freedom of f_1 and f_2

f_2 \ f_1	1	2	3	4	5	6	8	10	12	16	20
1	4052	4999	5403	5625	5764	5859	5981	6056	5106	6169	6208
2	98.49	99.00	99.17	99.25	99.30	99.33	99.36	99.40	99.42	99.44	99.45
3	34.12	30.81	29.46	28.71	28.24	27.91	27.49	27.23	27.05	26.83	26.65
4	21.20	18.00	16.69	15.98	15.52	15.21	14.80	14.54	14.37	14.15	14.02
5	16.26	13.27	12.06	11.39	10.97	10.77	10.27	10.05	9.89	9.68	9.55
6	13.74	10.92	9.78	9.15	8.75	8.47	8.10	7.87	7.72	7.52	7.39
7	12.25	9.55	8.45	7.85	7.46	7.19	6.84	6.62	6.47	6.27	6.15
8	11.26	8.65	7.59	7.01	6.63	6.37	6.03	5.82	5.67	5.48	5.36
9	10.56	8.02	6.99	6.42	6.06	5.80	5.47	5.26	5.11	4.92	4.80
10	10.04	7.56	6.55	5.99	5.64	5.39	5.06	4.85	4.71	4.52	4.41
11	9.65	7.20	6.22	5.67	5.32	5.07	4.74	4.54	4.40	4.21	4.10
12	9.33	6.93	5.95	5.41	5.06	4.82	4.50	4.30	4.16	3.98	3.86
13	9.07	7.70	5.74	5.20	4.86	4.62	4.30	4.10	3.96	3.78	3.67
14	8.86	6.51	5.56	5.03	4.60	4.46	4.14	3.94	3.80	3.62	3.51
15	8.68	6.36	5.42	4.89	4.56	4.32	4.00	3.80	3.67	3.42	3.36
16	8.53	6.23	5.29	4.77	4.44	4.20	3.89	3.69	3.55	3.37	3.25
17	8.40	6.11	5.18	4.67	4.34	4.10	3.79	3.59	3.45	3.27	3.16
18	8.28	6.01	5.09	4.58	4.25	4.01	3.71	3.51	3.37	3.19	3.07
19	8.18	5.93	5.01	4.50	4.17	3.94	3.63	3.43	3.30	3.12	3.00
20	8.10	5.85	4.94	4.43	4.10	3.87	3.56	3.37	3.23	3.05	2.94
25	7.77	5.57	4.68	4.18	3.86	3.63	3.32	3.13	2.99	2.81	2.70
30	7.56	5.39	4.51	4.02	3.70	3.47	3.17	2.93	2.84	2.66	2.55
40	7.31	5.18	4.31	3.83	3.51	3.29	2.99	2.80	2.66	2.49	2.37
60	7.08	4.98	4.13	3.65	3.34	3.12	2.82	2.63	2.50	2.32	2.20

1.5.2. Metrological characteristics of analytical methods by correctness

Analysis of reference standard. As mentioned above, an analysis of the reference standard is performed to evaluate the correctness of this method or analytical procedure. A reference standard is a sample in which the content of determined component, i.e., the true value of determined quantity μ (or its real value *a*) is precisely defined.

Let us assume that quantitative analysis of a standard sample was carried out by the estimated method — *n* parallel determinations were made and the average value $\bar{x}$, standard deviation *s*, dispersion $V = s^2$ *were calculated*. The goal is a comparison between the average $\bar{x}$ and the true μ value and deciding whether the difference between $\bar{x}$ and μ is significant or non-significant (random or non-random), in other words, revealing the consistent error. For this purpose, one should use Student`s *t-criterion* proceeding as follows. Student's criterion (function) *t* is calculated according to formula (1.13):

$$t_{calc} = |\bar{x} - \mu| n^{0.5}/s. \quad (1.13)$$

Compare $t_{calculated}$ value with tabulated magnitude $t_{tabulated}$ of Student`s function (see table 1.1) with specified confidence probability (for example, $P = 0.95$) and given number of degrees of freedom $f = n - 1$.

If $t_{calculated} > t_{tabulated}$, then between the average $\bar{x}$ and the true value μ, there is a statistically significant difference, in other words, there is consistent error estimated by formula (1.2): $\Delta_0 = \bar{x} - \mu$.

If $t_{calculated} < t_{tabulated}$, then the difference between $\bar{x}$ and μ is statistically non-significant. The method does not contain a systematic error.

Comparison of sample quantitative analysis results by two methods (comparison of averages). Let us assume that quantitative analysis of the same sample is carried out by two independent methods I and II; it is known that one of the methods (for example Method II) provides correct results (has no consistent error), in other words, it is metrologically qualified. Then a comparison of averages $\bar{x}_1$ and $\bar{x}_2$, obtained by these two methods, allows estimating the correctness of the method I — the presence or absence of consistent error.

After statistical processing of both samples obtained by methods I and II, we have:

method I: average $\bar{x}_1$, sample number n_1, dispersion V_1; method II: average $\bar{x}_2$, sample number n_2, dispersion V_2.

Metrological comparison of methods is preferably performed at $f_1 > 10$ and $f_2 > 10$.

Further, we proceed as follows.

1) Determine whether dispersions are uniform using *F*-criterion of Fisher according to formula (1.12). Should it appear that $F_{\text{calculated}} > F_{\text{tabulated}}$ with confidence probability $P = 0.99$, then two average values shall not be compared against each other as relating to two samples of the same set (sample of large size), i.e., the difference of dispersions is statistically significant; dispersions are non-uniform.

Otherwise, if $F_{\text{calculated}} < F_{\text{tabulated}}$ with confidence probability $P = 0.99$, then since dispersions are uniform, we continue statistical treatment further.

2) Calculate *the average weighted dispersion* $\overline{V} = \bar{s}^2$ of Student $t_{\text{calculated}}$ from formulas (1.14) and (1.15):

$$\overline{V} = \bar{s}^2 [(n_1 - 1)V_1 + (n_2 - 1)V_2]/(n_1 + n_2 - 2), \quad (1.14)$$

$$t_{\text{calculated}} = (|\bar{x}_1 - \bar{x}_2|/\overline{V}^{0.5})[n_1 n_2/(n_1 + n_2)]^{0.5}. \quad (1.15)$$

3) Compare $t_{\text{calculated}}$ and $t_{\text{tabulated}}$ (table 1.1) for specified confidence probability $P = 0.99$ and degrees of freedom $f = n_1 + n_2 - 2$.

If $t_{\text{calculated}} > t_{\text{tabulated}}$, then the difference between averages is statistically significant (non-random); method II provides incorrect results.

If $t_{\text{calculated}} < t_{\text{tabulated}}$, then the difference between averages is statistically non-significant. Results obtained by both methods can be considered as one set sample.

Example of a comparison of two quantitative analysis methods by correctness and reproducibility. Suppose analysis of the same object was carried out using two methods I and II, presuming that method I provides correct results (a systematic error is absent). According to the above by comparison of two methods, it is desirable, as mentioned above, that number of degrees of freedom f_1 and f_2 for both samples was more than 10. Let us assume that in the primary statistical processing of quantitative analysis results with confidence probability $P = 0.95$ we obtained data presented in the table below (all designations in the table correspond to accepted above).

Data table of the primary statistical processing

Determined quantities	Method I	Method II
x_i	3.01; 3.06; 3.08; 3.09; 3.10; 3.12; 3.12; 3.13; 3.14; 3.15; 3.16; 3.31	3.10; 3.17; 3.18; 3.19; 3.19; 3.20; 3.20; 3.21; 3.21; 3.22; 3.24; 3.28
n	11	12

End of table

Determined quantities	Method I	Method II
$F = n - 1$	12	11
$\bar{x}$	3.12	3.20
V	0.00525	0.00183
s	0.072	0.043
$\Delta\bar{x}$	0.05	0.03
$\bar{x} \pm \Delta\bar{x}$	3.12 ± 0.05	3.20 ± 0.03
$\bar{\varepsilon}$	1.6%	0.9%

Let us compare both methods by reproducibility.

For this, we use *F*-criterion of Fisher (see above). Find out

$$F_{\text{calc}} = V_1 / V_2 = 0.00525/0.00183 = 2.87.$$

Tabulated value $F_{\text{tabulated}}$ is taken from data of table 1.3 with confidence probability $P = 0.99$ and degrees of freedom $f_1 = 11$ and $f_2 = 11$. $F_{\text{tab}} = 4.47$ is obtained. Since $F_{\text{calculated}} = 2.87 < F_{\text{tabulated}} = 4.47$, then dispersions are uniform (the difference between V_1 and V_2 is statistically non-significant). Both methods give reproducible results, reproducibility of the method II is better than reproducibility of the method I.

Let us compare both methods by correctness.

Since dispersions V_1 and V_2 are homogeneous, we use *t*-criterion after Student. Calculate the average weighted dispersion $\overline{V} = \bar{s}^2$ formula (1.14):

$$\overline{V} = \bar{s}^2 = [(n_1 - 1)V_1 + (n_2 - 1)V_2]/(n_1 + n_2 - 2) =$$

$$= [(12 - 1) \cdot 0.00525 + (12 - 1) \cdot 0.00183]/(12 + 12 - 2) = 0.003538.$$

Calculate Student`s criterion *t* calculated according to formula

$$t_{\text{calculated}} = (|\bar{x}_1 - \bar{x}_2| / \overline{V}^{0.5})[n_1 n_2/(n_1 + n_2)]^{0.5} =$$

$$= (|3.12 - 3.20|/0.003538^{0.5})[12 \cdot 12/(12 + 12)]^{0.5} = 3.29.$$

In table 1.1, we find out the tabulated value *t* tabulated with confidence probability $P = 0.99$, degrees of freedom $f = n_1 + n_2 - 2 = 12 + 12 - 2 = 22$: $t_{\text{tabulated}} = 2.83$. Since $t_{\text{calculated}} = 3.29 > t_{\text{tabulated}} = 2.83$, it can be concluded that the method II with confidence probability P = 0.99 does not provide correct results, i.e., includes a consistent error. This consistent error can be estimated given that method I in contrast to method II provides correct results, in other

words, it is possible to assume $\bar{x}_1 = a$, where a — the actual value of the determined magnitude. Then the consistent error Δ_0 of method II will be according to (1.2):

$$\Delta_0 = \bar{x}_2 - \bar{x}_1 = 3.20 - 3.12 = 0.08.$$

Percentage consistent error (relative magnitude of consistent error) δ of method II is equal according to (1.3):

$$\delta = (\bar{x}_2 - \bar{x}_1) \cdot 100\%/\bar{x}_1 = 0.08 \cdot 100\%/3.12 = 2.6\%.$$

Thus, method II produces exaggerated results.

1.5.3. Estimate of allowable divergence of parallel determination results

In the practice of quantitative analysis, a number of parallel determinations is often less than five and may be three or four. At such a small number of parallel determinations, the usual statistical processing of quantitative analysis results loses its significance. Nevertheless, the question of estimating the convergence *of parallel determination results remains. Permitted divergence of parallel determination results* $R_{\max,n,P}$ is understood as the specified upper confidence bound of the range of parallel determinations

$$R_{\max,n,P} = x_{\max} - x_{\min},$$

where $x_{\max}$ and $x_{\min}$ — maximum and minimum values of variants; n — a number of independent parallel determinations; P — confidence probability.

If the consistent error of the method is absent, then divergence of results of parallel determinations is permitted (results are converged) when the assumption (1.16) is fulfilled:

$$R_{\max,n,P} = x_{\max} - x_{\min} < L(P, n) \cdot s, \qquad (1.16)$$

where $L(P, n)$ — factor calculated according to Pearson with the confidential probability $P = 0.95$, and s — standard deviation.

Numerical values $L(P, n)$ for $n = 2, 3, 4$ and P = 0.95 are given below:

n	2	3	4
$L(0.95, n)$	2.77	3.31	3.65

If assumption (1.16) is not fulfilled, results of parallel determinations shall not be considered as convergent, in other words, their divergence is unaccep-

table. The analysis must be repeated. If during repeated independent parallel determinations the assumption (1.16) remains unfulfilled, one should use another analytical procedure.

1.6. SOME RECOMMENDATIONS ON PROCESSING OF QUANTITATIVE ANALYSIS RESULTS

Based on the above sections, it is possible to give some recommendations which are useful when results of quantitative analysis are treated.

1. The recommended number of independent parallel determinations n during the performance of quantitative analysis (especially drug products) best of all is equal to: $5 \leqslant n \leqslant 20$. With such a sample size, it is possible to perform statistical processing of analysis results (estimate of their reproducibility) using Student`s distribution.

Recommended confidence probability value is equal to P = 0.95 = 95%.

It is convenient for metrological characteristics of quantitative analysis to be finally presented in the form of the summary table described in Section 1.4.4. It is supposed that the used method of analysis has no consistent errors or consistent errors are less than random ones.

2. It is recommended to eliminate significant errors using Q-test when the sample size $5 \leqslant n < 10$ and the confidence probability $P = 0.90 = 90\%$. When the sample size is large $n > 10$, significant errors are eliminated using the formula (1.11).

3. When the sample size is small $n = 3–4$, it is possible (if necessary) to estimate allowable divergence of parallel results (convergence estimate) using factor $L(P, n)$, calculated according to Pearson, as per the formula (1.16) when the confidence probability $P = 0.95$.

4. It is desirable to estimate two methods of analysis by correctness and reproducibility with degrees of freedom $f_1 > 10$ and $f_2 > 10$ in each method and confidence probability $P = 0.99 = 99\%$.

1.7. EXAMPLES AND EXERCISES FOR CHAPTER 1

1.7.1. Examples

1. Analysis of drug product Mesatonum (for its quality control) — 1% solution for injections — by the potentiometer method has shown the following pH values of this solution: 4.50; 4.52; 4.55; 4.60; 4.70; 4.75.

Estimate the confidential interval of average pH of Mesatonum solution with confidence probability $P = 0.95$ (95%) and the relative error of average.

Solution. 1) Make clear whether gross errors exist. As may be supposed, pH value equal to 4.75 may be doubtful. Let us use the Q-criterion as the sample size $n = 6$:

$$Q_{calc} = (4.75 - 4.70) / (4.75 - 4.50) = 0.20.$$

The reference (tabulated) value Q tabulated with $n = 6$ and $P = 0.90$ is equal to 0.56. Since $Q_{calc} = 20 < Q_{tabulated} = 0.56$, then gross errors are absent. The sample is uniform.

2) Calculate the average value $\overline{pH}$, deviations ΔpH, deviation squares and sum of squared deviations:

$$\overline{pH} = (4.50 + 4.52 + 4.55 + 4.60 + 4.70 + 4.75)/6 = 4.60.$$

$$\sum \Delta pH^2 = 0.0100 + 0.0064 + 0.0025 + 0 + 0.0100 + 0.0225 = 0.0514.$$

Table of deviations

pH	ΔpH	ΔpH^2
4.50	4.50 – 4.60 = –0.10	0.0100
4.52	4.52 – 4.60 =–0.08	0.0064
4.55	4.55 – 4.60 = –0.05	0.0025
4.60	4.60 – 4.60 = 0	0
4.70	4.70 – 4.60 = 0.10	0.0100
4.75	4.75 – 4.60 = 0.15	0.0225

3) Let us calculate standard deviation s:

$$s = [(\sum \Delta pH^2)/(n - 1)]^{0.5} = [0.0514/(6 - 1)]^{0.5} = 0.10.$$

4) Find a half-width of the confidential interval $\Delta\overline{pH}$. Tabulated value of Student`s coefficient with $n = 6$ and $P = 0.95$ is equal to $t_{P,n} = t_{0.95;6} = 2.57$.

Then

$$\Delta\overline{pH} = t_{0.95;6}\, s/n^{0.5} = 2.57 \cdot 0.10/6^{0.5} = 0.10.$$

Confidential interval:

$$\overline{\text{pH}} \pm \Delta\overline{\text{pH}} = 4.60 \pm 0.10.$$

5) Calculate the relative error of average $\bar{\varepsilon}$:

$$\bar{\varepsilon} = (\Delta\overline{\text{pH}}/\overline{\text{pH}}) \cdot 100\% = (0.10/4.60) \cdot 100\% = 2.2\%.$$

6) Compile the final table.

Final table

pH	4.50; 4.52; 4.55; 4.60; 4.70; 4.75
n	6
$\overline{\text{pH}}$	4.60
s	0.10
$\Delta\overline{\text{pH}}$	0.10 ($P = 0.95$)
$\overline{\text{pH}} \pm \Delta\overline{\text{pH}}$	4.60 ± 0.10
$\bar{\varepsilon}$	2.2%

2. When determining (for quality control) foreign impurities in sample of drug product — ethyl ester of α-bromo-isovaleric acid (substance) — by gas-liquid chromatography (GLC), the total content of impurities (mass fraction W) in five parallel analyses was found to be equal, %: 1.30; 1.40; 1.50; 1.60; 1.60.

Give an account of obtained results reproducibility, calculating the confidential interval of average $W \pm \Delta W$ and the relative error $\bar{\varepsilon}$ of average result with confidence probability $P = 0.95$.

Solution. 1) Find the presence of significant errors:

$$Q_1 = (1.40 - 1.30)/(1.60 - 1.30) = 0.33;$$

$$Q_5 = (1.60 - 1.50)/(1.60 - 1.30) = 0.33.$$

Table value Q_{table} (P = 0.90; $n = 5$) = 0.64.

Calculated values Q_1 and Q_5, equal to 0.33, are less than $Q_{\text{table}} = 0.64$. Thus, significant errors are absent. The sample is uniform.

2) Calculate the average value:

$$\overline{W} = (1.30 + 1.40 + 1.50 + 1.60 + 1.60)/5 = 1.48.$$

Define deviations ΔWi, squares and sum of squared deviations, to do so compile a table of deviations:

Table of deviations

W_i	$\Delta W_i = W_i - \overline{W}$	ΔW_i^2
1.30	−0.18	0.0324
1.40	−0.08	0.0064
1.50	0.02	0.0004
1.60	0.12	0.0144
1.60	0.12	0.0144

Sum of squared deviations: $\sum \Delta W_i^2 = 0.0680$.

3) Calculate standard deviation

$$s = [\sum \Delta W_i^2/(n-1)]^{0,5} = (0,0680/4)^{0,5} = 0,1304 \approx 0,13.$$

4) Find the half-width of confidential interval:

$$\Delta\overline{W} = st_{0.95;5}/n^{0.5} = 0.13 \cdot 2.78/5^{0.5} = 0.16.$$

Confidential interval:

$$\Delta\overline{W} \pm \Delta\overline{W} = 1.48 \pm 0.13.$$

Calculate the relative error $\bar{\varepsilon}$:

$$\bar{\varepsilon} = \Delta\overline{W} \cdot 100\%/\overline{W} = 0.16 \cdot 100\%/1.48 = 10.8\%.$$

Compile the final table.

Final table

W_i	1.30; 1.40; 1.50; 1.60 1.60
n	5
$\overline{W}$	1.48
s	0.13
$\Delta\overline{W}$	0.16 ($P = 0.95$)
$\overline{W} \pm \Delta\overline{W}$	1.48 ± 0.16
$\bar{\varepsilon}$	10.8 %

3. When developing the spectrophotometric procedure for the determination of indomethacin in the drug dosage form — 5% indomethacin salve — a salve sample containing 0.0200 g of indometacin was analyzed. Mass t of indometacin determined in six parallel analyses was equal, g: 0.0196; 0.0198; 0.0199; 0.0200; 0.0202; 0.0205.

Give an account of reproducibility of analysis results calculating the confidential interval of average $\bar{m} \pm \Delta\bar{m}$ and the relative error $\bar{\varepsilon}$ of average result with confidence probability $P = 0.95$.

Estimate the correctness of analytical procedure and calculate the consistent error (if available). The true content of indometacin μ = 0.0200 g.

Solution. 1) Estimation of significant errors:

$$Q_1 = (0.0198 - 0.0196)/(0.0205 - 0.0196) = 0.22,$$

$$Q_6 = (0.0205 - 0.0202)/(0.0205 - 0.0196) = 0.33.$$

Since $Q_1 = 0.22$ and $Q_6 = 0.33$ are less than $Q_{\text{tabulated}}(P = 0.90, n = 6) = 0.56$, then significant errors are absent. The sample is uniform.

2) Calculation of average value and deviations. The average value:

$$t = (0.0196 + 0.0198 + 0.0199 + 0.0200 + 0.0202 + 0.0205) / 6 = 0.0200.$$

Table of deviations

m_i	$\Delta\bar{m}_i = \bar{m}_i - \bar{m}$	$\Delta\bar{m}_i^2$
0.0196	−0.0006	$36 \cdot 10^{-8}$
0.0198	−0.0002	$4 \cdot 10^{-8}$
0.0199	−0.0001	$1 \cdot 10^{-8}$
0.0200	0	0
0.0202	0.0002	$4 \cdot 10^{-8}$
0.0205	0.0005	$25 \cdot 10^{-8}$

Sum of squared deviations: $\sum\Delta m_i = 70 \cdot 10^{-8}$

3) Standard deviation:

$$s = [\sum\Delta m_i^2/(n - 1)]^{0.5} = [70 \cdot 10^{-8}/(6 - 1)]^{0.5} = 0.0004.$$

4) Half-width of confidential interval:

$$\Delta\bar{m} = st_{0.95;6} /n^{0.5} = 0.0004 \cdot 2.57/6^{0.5} = 0.0004.$$

Confidential interval: $\bar{m} \pm \Delta\bar{m} = 0.0200 \pm 0.0004$.

5) Average relative error:

$$\bar{\varepsilon} = (\Delta\bar{m} /\bar{m}) \cdot 100\% = 0.0004 \cdot 100\%/0.0200 = 2\%.$$

6) Final table

m_i	0.0196; 0.0198; 0.0199; 0.0200; 0.0202; 0.0205
n	6
$\bar{m}$	0.0200
s	0.0004

$\Delta\bar{m}$	0.0004 ($P = 0.95$)
$\bar{m} \pm \Delta\bar{m}$	0.0200 ± 0.0004
$\bar{\varepsilon}$	2%

7) Since the true value $\mu = 0.0200$ lies within the confidential interval, the consistent error is absent. Analytical procedure gives correct results.

4. Drug product calagel (pain-relieving and antiseptic teething gel for children) was analyzed by the high-efficiency liquid chromatography (HELC) method for the content of pharmacologically active substance — lidocaine hydrochloride. In seven parallel determinations, it was found that the content W of the specified active component is equal, as a percentage of the nominal (introduced to the drug) quantity: 100.10; 100.50; 100.70; 101.00; 101.30; 101.40; 101.40.

Give an account of analysis procedure by the reproducibility of average result and correctness with confidence probability P = 0.95 if the true value $\mu = 100\%$.

Solution. 1) An estimate of significant errors.

$$Q_1 = (100.50 - 100.10)/(101.40 - 100.10) = 0.31,$$

$$Q_7 = (101.40 - 101.30)/(101.40 - 100.10) = 0.08.$$

Values $Q_1 = 0.31$ and $Q_7 = 0.08$ are less than the tabulated (with $P = 0.90$ and $n = 7$) value $Q_{\text{tabulated}} = 0.51$ so significant errors are absent. The sample is uniform.

2) The average value:

$$\overline{W} = (100.10 + 100.50 + 100.70 + 101.00 + 101.30 + 101.40 + 101.40)/7 = 100.91.$$

Deviations and sum of squared deviations:

Table of deviations

W_i	$\Delta W_i = W_i - \overline{W}$	ΔW_i^2
100.10	−0.8	0.64
100.50	−0.4	0.16
100.70	−0.2	0.04
101.00	0.1	0.01
101.30	0.4	0.16
101.40	0.5	0.25
101.40	0.5	0.25

$$\sum \Delta W_i^2 = 1.51.$$

3) Standard deviation:

$$s = [\sum \Delta W_i^2 /(n-1)]^{0.5} = (1.51/6)^{0.5} = 0.50.$$

4) Half-width of confidential interval:

$$\Delta \overline{W} = st_{0.95;7} /n^{0.5} = 0.50 \cdot 2.45/7^{0.5} = 0.46.$$

Confidential interval:

$$\overline{W} \pm \Delta \overline{W} = 100.91 \pm 0.46.$$

5) Average relative error:

$$\bar{\varepsilon} = 0.46 \cdot 100\%/100.91 = 0.46\%.$$

Final table

W_i	100.10; 100.50; 100.70; 101.00; 101.30; 101.40; 101.40
n	7
$\overline{W}$	100.91
s	0.50
$\Delta \overline{W}$	0.46
$\overline{W} \pm \Delta \overline{W}$	100.91 ± 0.46
ε	0.46

6) The true value $\mu = 100\%$ lies beyond limits of the confidential interval. Thus, the analysis procedure is burdened with a consistent error Δ_0:

$$\Delta_0 = \overline{W} - \mu = 100.91 - 100 = 0.91.$$

The absolute value of the consistent error δ:

$$\delta = (\overline{W} - \mu) \cdot 100\%/\mu = (100.91 - 100) \cdot 100\%/100 = 0.91\%.$$

The analysis procedure produces somewhat exaggerated results.

A similar result can be obtained using t criterion according to (1.13):

$$t_{\text{calculated}} = |\bar{x} - \mu| \cdot n^{0.5}/s = |\overline{W} - \mu| \cdot n^{0.5}/s = (100.91 - 100) \cdot 7^{0.5}/0.50 = 4.82.$$

With $P = 0.95$ and $n = 7$ in table 1.1 (see above), we obtain: $t_{\text{tabulated}} = 2.45$. Since $t_{\text{calc}} = 4.82 > t_{\text{tabulated}} - 2.45$, it can therefore be concluded that analysis procedure gives the consistent error.

5. Determination of basic pharmacologically active substance in liquid drug product — ethyl ester of α-bromo-isovaleric acid (substance) by two methods — gas-liquid chromatography (GLC) and precipitation titration — gave the following results for mass fraction W of basic substance, %:

— GLC method (11 parallel determinations, $n_1 = 11$): 98.20; 98.30; 98.30; 98.40; 98.40; 98.50; 98.50; 98.60; 98.60; 98.70; 98.70;

— precipitation titration method (11 parallel determinations, $n_2 = 11$): 98.30; 98.40; 98.40; 98.50; 98.50; 98.60; 98.60; 98.70; 98.70; 98.70; 98.80.

Give an account of reproducibility of both methods with confidence probability $P = 0.95$. Perform a comparison of two methods by reproducibility and correctness with confidence probability $P = 0.99$. The true value of the drug substance content $\mu = 98.50\%$.

Solution. **A.** Let us characterise the SLC procedure by reproducibility and correctness.

1) Using the Q criterion, we find that significant errors areabsent.

2) Average value $\overline{W}_1$ and table of deviations:

$$\overline{W}_1 = (98.20 + 98.30 + 98.30 + 98.40 + 98.40 + 98.50 + 98.50 + 98.60 + + 98.60 + 98.70 + 98.70)/11 = 98.47.$$

Table of deviations

W_i	$\Delta W_i = W_i - \overline{W}_1$	$\Delta \overline{W}_i^2$
98.20	−0.47	0.2209
98.30	−0.17	0.0289
98.30	−0.17	0.0289
98.40	−0.07	0.0049
98.40	−0.07	0.0049
98.50	0.03	0.0009
98.50	0.03	0.0009
98.60	0.13	0.0169
98.60	0.13	0.0169
98.70	0.23	0.0529
98.70	0.23	0.0529

$$\sum \Delta W_i^2 = 0.4299.$$

3) Standard deviation s_1 and dispersion $V_1 = s_1^2$:

$$s_1 = (0.4299/10)^{0.5} = 0.21.$$

$$V_1 = s_1^2 = 0.21^2 = 0.0441.$$

4) Half-width of confidential interval and confidential interval:

$$\Delta\overline{W}_1 = 0.21 \cdot 2.23/11^{0.5} \approx 0.14.$$

$$\overline{W}_1 \pm \Delta\overline{W}_1 = 98.47 \pm 0.14.$$

5) Since the true value μ = 98.50 lies within limits of the confidential interval of average, then the consistent error is absent. The GLC method gives correct results.

B. Let us characterise the precipitation titration procedure by and correctness.

As seen from the presented data, gross errors are absent.

Average value $\overline{W}_2$ and table of deviations:

$$\overline{W}_2 = (98.30 + 98.40 + 98.40 + 98.50 + 98.50 + 98.60 + 98.60 + 98.70 + 98.70 + 98.70 + 98.80)/11 = 98.56.$$

Table of deviations

W_i	$\Delta\overline{W}_i = \overline{W} - \overline{W}_2$	ΔW_i^2
98.30	−0.26	0.0676
98.40	−0.16	0.0256
98.40	−0.16	0.0256
98.50	−0.06	0.0036
98.50	−0.06	0.0036
98.60	0.04	0.0016
98.60	0.04	0.0016
98.70	0.14	0.0196
98.70	0.14	0.0196
98.70	0.14	0.0196
98.80	0.24	0.0576

$$\sum \Delta W_i^2 = 0.2456.$$