**Lesson 2**

**Statistical processing of the results of quantitative determination. Validation.**

Statistical processing of the results of quantitative determination by the method of Student.

Student's criterion (t-criterion) is the most common criterion for evaluating measurement results due to its simplicity and versatility.

1. Determination of the average value of the results of the determination:



Where:

n is the number of definitions;

X1; X2; X3; Xn are the values obtained as a result of the determination.

Example: For example, determination results, X1 = 96.5%, X2 = 98.0%, X3 = 100.0%, X4 = 99.5%, X5 = 97.5%. In this case:



1. Calculating the standard deviation from the mean:



In our example:



1. Standard deviation calculation:



In our example:



1. Determination of the accuracy of determination at n-1 and α=0.95:



Where:

t α - Student's coefficient at n-1, (that is, in our case for 5-1 it is 2.776 (taken from the table)



1. Calculation regarding analysis error:



In our case:



Table of Student's coefficient values.

|  |  |  |  |
| --- | --- | --- | --- |
| **n-1** | **α=0,95** | **n-1** | **α=0,95** |
| 1 | 12,706 | 7 | 2,365 |
| 2 | 4,303 | 8 | 2,306 |
| 3 | 3,182 | 9 | 2,262 |
| 4 | 2,776 | 10 | 2,228 |
| 5 | 2,571 | 11 | 2,201 |
| 6 | 2,447 | 12 | 2,179 |

Validation of an analytical technique is the experimental proof that the technique is suitable for solving the intended problems.

This General Pharmacopoeia Monograph regulates the characteristics of analytical methods determined for the purpose of their validation, and the corresponding criteria for the suitability of validated methods intended for quality control of medicinal products: pharmaceutical substances and medicinal products.

Methods of quantitative determination, including methods for determining impurities and methods for determining the limit of content, are subject to validation. Authentication methods are validated if necessary to confirm their specificity.

During validation, the analytical method is evaluated according to the characteristics listed below, selected taking into account the typical recommendations given in the table:

 specificity;

 detection limit;

 the limit of quantitative determination (quantitation limit);

 analytical area (range);

 linearity (linearity);

 correctness (trueness);

 precision;

 stability (robustness).

Characteristics of methods determined during validation

|  |  |
| --- | --- |
| **Наименование****характеристики** | **Основные типы методик** |
| **Испытание  на подлин­ность** | **Посторонние примеси** | **Количественное определение** |
| **Количественные методики** | **Предел содержания** | **Основного действующего вещества, нормируемых компонентов** |
| Специфичность\*\*) | Да | Да | Да | Да |
| Предел обнаружения | Нет | Нет | Да | Нет |
| Предел  количественного определения | Нет | Да | Нет | Нет |
| Аналитическая область | Нет | Да | Нет | Да |
| Линейность | Нет | Да | Нет | Да |
| Правильность | Нет | Да | \* | Да |
| Прецизионность**:**– повторяемость (сходимость)– промежуточная(внутрилабораторная) прецизионность |  Нет Нет |  Да Да |  Нет Нет |  Да Да |
| Устойчивость | Нет | \* | \* | \* |

\*) can be determined if necessary;

\*\*) the lack of specificity of one analytical method can be compensated for by using another analytical method.

Revalidation (re-validation) of methods is carried out when:

 technologies for obtaining the object of analysis;

 the composition of the medicinal product (object of analysis);

 previously approved analysis methodology.

Correctness (accuracy). The correctness of the methodology is the closeness of the results obtained using this methodology to the true value. The correctness of the methodology can be determined by performing analysis of material samples prepared with quantitative accuracy. If possible, such samples should contain all components of the material, including those analyzed. Samples should also be prepared containing the analyte in an amount approximately 10% above and below the expected content. Correctness can also be determined by comparing the results with those obtained using an alternative methodology that has been previously validated.

Precision. Method accuracy is the degree of agreement between individual test results. It is measured by the deviation of individual results from the mean and is usually expressed as a standard deviation or as a coefficient of variation (relative standard deviation), provided that the full procedure is used to reanalyze individual identical samples taken from the same homogeneous batch of material.

Convergence (intralaboratory variation). This is the accuracy of a technique when performed by the same analyst under the same conditions (same reagents, equipment, setting of any parameters and laboratory) and within a short period of time. The repeatability of a method is assessed by performing complete determinations on separate, identical samples taken from the same homogeneous batch of material, and thus provides a measure of the accuracy of the method under normal operating conditions.

Reproducibility This is the accuracy of a technique when it is performed under different conditions (usually in different laboratories) on separate, presumably identical samples taken from the same homogeneous batch of material. Comparison of results obtained by different analysts using different equipment or when analyzing at different times can also provide valuable information.

Reliability (robustness or ruggedness). Reliability is the ability of a technique to produce analytical results with acceptable correctness and accuracy under changing conditions. It is a measure of the degree to which changes in operating or environmental conditions affect the results obtained from the analysis of individual, presumably identical samples from the same homogeneous batch of material.

Linearity and range (linearity and range). The linearity of an analytical procedure is its ability to produce results that are directly proportional to the concentration of the analyte in the samples. The range of a method is expressed as the highest and lowest concentrations within which the analyte is demonstrated to be detected with acceptable accuracy, accuracy, and linearity. These characteristics are determined by applying this technique to analyze a series of samples having analyte concentrations that overlap the required range.

Selectivity. The selectivity or specificity of a technique is its ability to measure an analyte without being affected by other components of the analyte. Selectivity (or lack of selectivity) can be expressed as the deviation of the results obtained when applying the method to determine the analyte in the presence of the expected amount of other components, compared with the results obtained for the same analyte without the addition of other substances. When other components are known and available, selectivity can be determined by comparing the test results of the analyte in the sample with and without the addition of potentially interfering substances.

Sensitivity. Sensitivity is the ability of a test procedure to detect small changes in concentration. Sensitivity is the slope of the calibration curve.

Limit of detection. The detection limit is the lowest level at which an analyte can be detected, but not necessarily quantified, using this technique under the required experimental conditions.

Limit of quantitation. The limit of quantitation is the lowest concentration of an analyte in a sample that can be determined with suitable accuracy and precision using the required technique. It is measured by analyzing samples containing decreasing amounts of the analyte and determining the lowest level at which an acceptable degree of accuracy and precision can be achieved. In many cases, the limit of quantitation is approximately twice the limit of detection.