**11 lesson.**

**High performance liquid chromatography.**

It is generally accepted that high-performance liquid chromatography (HPLC) was formed in the late 60s of the XX century in the process of improving traditional low-pressure liquid column chromatography: reducing the particle size of the sorbent (up to 10 μm and less) and increasing the column packing density in order to achieve greater efficiency led to a significant complication of instrumentation, which determined HPLC as an independent analytical method.

During the last decade, the most important HPLC performance for analytical applications - separation efficiency and speed, limit of detection, etc. have been improved, and HPLC can now be used as a method for working with very small samples.

High performance liquid chromatography (high pressure liquid chromatography) is a column chromatography method in which the mobile phase is a liquid moving through a chromatographic column filled with a stationary phase (sorbent). Columns for high performance liquid chromatography are characterized by high hydraulic resistance at the inlet.

Depending on the mechanism of separation of substances, the following variants of high-performance liquid chromatography are distinguished: adsorption, partition, ion-exchange, exclusion, chiral, etc., in accordance with the nature of the main manifested intermolecular interactions. In adsorption chromatography, the separation of substances occurs due to their different ability to be adsorbed and desorbed from the surface of a sorbent with a developed surface, for example, silica gel. In partition high-performance liquid chromatography, separation occurs due to the difference in the distribution coefficients of the substances to be separated between the stationary (as a rule, chemically grafted onto the surface of a stationary carrier) and the mobile phases.

Depending on the type of mobile and stationary phase, normal-phase and reversed-phase chromatography is distinguished. In normal-phase high-performance liquid chromatography, the stationary phase is polar (most often silica gel or silica gel with grafted NH2- or CN-groups, etc.), and the mobile phase is non-polar (hexane, or mixtures of hexane with more polar organic solvents - chloroform, alcohols, etc.). etc.). The retention of substances increases with increasing polarity. In normal phase chromatography, the eluting power of the mobile phase increases with increasing polarity.

In reversed-phase chromatography, the stationary phase is non-polar (hydrophobic silica gels with grafted C4, C8, C18 groups, etc.); the mobile phase is polar (mixtures of water and polar solvents: acetonitrile, methanol, tetrahydrofuran, etc.). The retention of substances increases with the increase in their hydrophobicity (non-polarity). The higher the content of the organic solvent, the higher the eluting power of the mobile phase.

In ion-exchange chromatography, the molecules of a mixture of substances, dissociated in solution into cations and anions, are separated when moving through a sorbent (cation exchanger or anion exchanger) due to different forces of interaction between the ions being determined and the ionic groups of the sorbent.

In size-exclusion (sieve, gel-penetrating, gel-filtration) chromatography, the molecules of substances are separated by size due to their different ability to penetrate into the pores of the stationary phase. In this case, the largest molecules that can penetrate into the minimum number of pores of the stationary phase are the first to leave the column, and the substances with small molecular sizes are the last to leave.

In chiral chromatography, optically active compounds are separated into individual enantiomers. The separation can be carried out on chiral stationary phases or on achiral stationary phases using chiral mobile phases.

There are other options for high performance liquid chromatography.

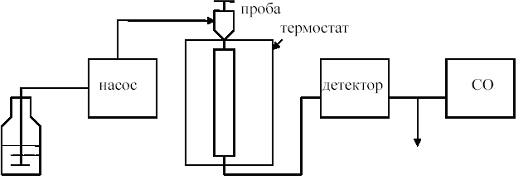
Often, the separation proceeds not by one, but by several mechanisms simultaneously, depending on the type of mobile and stationary phases, as well as the nature of the compound being determined.

Application area

High-performance liquid chromatography is successfully used for both qualitative and quantitative analysis of drugs in the tests "Identity", "Foreign impurities", "Dissolution", "Uniformity of dosing", "Quantitative determination". It should be noted that chromatography allows you to combine several tests in one sample, including "Identity" and "Quantitative determination".

Equipment

For analysis, appropriate instruments are used - liquid chromatographs.



**Liquid chromatograph.**

The composition of a liquid chromatograph usually includes the following main components:

- a mobile phase preparation unit, including a container with a mobile phase (or containers with individual solvents that are part of the mobile phase) and a system for degassing the mobile phase;

- pumping system;

- mobile phase mixer (if necessary);

- sample injection system (injector), can be manual or automatic (autosampler);

- chromatographic column (can be installed in a thermostat);

- detector (one or several with different detection methods);

- chromatograph control system, data collection and processing.

In addition, the chromatograph may include: a sample preparation system and a pre-column reactor, a column switching system, a post-column reactor, and other equipment.

Pumping system

The pumps supply the mobile phase to the column at a predetermined rate. The composition of the mobile phase and the flow rate can be constant or change during the analysis. In the case of a constant composition of the mobile phase, the process is called isocratic, and in the second - gradient. A modern liquid chromatograph pumping system consists of one or more computer-controlled pumps. This allows you to change the composition of the mobile phase according to a specific program during gradient elution. Analytical high performance liquid chromatography pumps make it possible to maintain the flow rate of the mobile phase into the column in the range from 0.1 to 10 ml/min at a column inlet pressure of up to 40 MPa. Pressure pulsations are minimized by special damper systems included in the design of the pumps. The working parts of the pumps are made of corrosion-resistant materials, which allows the use of aggressive components in the composition of the mobile phase.

Faucets

In the mixer, a single mobile phase is formed from the individual solvents supplied by the pumps, if the required mixture has not been prepared in advance. The mixing of the mobile phase components in the mixer can take place both at low pressure (before the pumps) and at high pressure (after the pumps). The mixer can be used for mobile phase preparation and isocratic elution.

The volume of the mixer can affect the retention time of the components in the gradient elution.

Injectors

Injectors can be universal, with the ability to change the volume of the injected sample, or discrete for introducing a sample of only a certain volume. Both types of injectors can be automatic ("auto-injectors" or "auto-samplers"). The sample injector (solution) is located directly in front of the chromatographic column. The design of the injector makes it possible to change the direction of the flow of the mobile phase and to preliminarily introduce a sample into a dosing loop of a certain volume (usually from 10 to 100 μL) or into a special dosing device of variable volume. The volume of the loop is indicated on its marking. The design of a discrete injector, as a rule, allows the replacement of the loop. Modern automatic injectors can have a number of additional functions, for example, they can serve as a sample preparation station: mix and dilute samples, and carry out a pre-column derivatization reaction.

Chromatography column

Chromatographic columns are usually stainless steel, glass, or plastic tubes filled with a sorbent and closed on both sides with filters with a pore diameter of 2–5 μm. The length of the analytical column can be in the range from 5 to 60 cm or more, the inner diameter is from 2 to 10 mm. Columns with an internal diameter of less than 2 mm are used in microcolumn chromatography. There are also capillary columns with an internal diameter of about 0.3–0.7 mm. Columns for preparative chromatography may have an internal diameter of 50 mm or more.

Before the analytical column, short columns (pre-columns) can be installed that perform various auxiliary functions, the main of which is the protection of the analytical column. Typically, the analysis is carried out at room temperature, however, to increase the efficiency of separation and reduce the duration of the analysis, thermostatting of the columns at temperatures up to 80 - 100 С can be used. The possibility of using an elevated temperature during separation is limited by the stability of the stationary phase, since its destruction is possible at elevated temperatures.

Stationary phase (sorbent)

Commonly used sorbents are:

 silica gel, aluminum oxide, are used in normal-phase chromatography. The retention mechanism in this case is usually adsorption;

- silica gel, resins or polymers with grafted acidic or basic groups. Scope - ion-exchange and ion chromatography;

 silica gel or polymers with a given pore size distribution (size exclusion chromatography);

 chemically modified sorbents (sorbents with bonded phases), most often prepared on the basis of silica gel. The retention mechanism is adsorption or distribution between the mobile and stationary phases. The scope depends on the type of grafted functional groups. Some types of sorbents can be used in both reversed and normal phase chromatography;

 chemically modified chiral sorbents, for example, cellulose and amylose derivatives, proteins and peptides, cyclodextrins, chitosans used to separate enantiomers (chiral chromatography).

Bonded phase sorbents can have varying degrees of chemical modification. The most commonly used bonded phases are:

– octadecyl groups [Si-(CH2)17-CH3] (sorbent octadecylsilane (ODS) or С18);

– octyl groups [Si-(CH2)7-CH3] (octylsilane or C8 sorbent);

– phenyl groups [Si-(CH2)n-(C6H5)] (phenylsilane sorbent);

– cyanopropyl groups [Si-(CH2)3-CN] (CN sorbent);

– aminopropyl groups [Si-(CH2)3-NH2] (NH2 sorbent);

– diol groups [Si-(CH2)3-OCH(OH)-CH2-OH] (sorbent diol).

Most often, the analysis is performed on non-polar bonded phases in reverse phase mode using C18 sorbent.

Bonded phase sorbents based on silica gel are chemically stable at pH values from 2.0 to 7.0, unless otherwise specified by the manufacturer. The sorbent particles may have a spherical or irregular shape and a variety of porosity. The particle size of the sorbent in analytical high performance liquid chromatography is usually 3–10 µm, in preparative high performance liquid chromatography it is 50 µm or more. There are also monolithic columns in which the sorbent is a monolith with through pores that fills the entire volume of the column.

The high separation efficiency is provided by the high surface area of the sorbent particles (which is a consequence of their microscopic size and the presence of pores), as well as the uniformity of the sorbent composition and its dense and uniform packing.

Detectors

In high performance liquid chromatography, various detection methods are used. In the general case, the mobile phase with the components dissolved in it after the chromatographic column enters the detector cell, where one or another of its properties is continuously measured (absorption in the ultraviolet or visible region of the spectrum, fluorescence, refractive index, electrical conductivity, etc.). The resulting chromatogram is a graph of the dependence of some physical or physico-chemical parameter of the mobile phase on time.

The most common detectors in high performance liquid chromatography are spectrophotometric. During the elution of substances in a specially designed microcell, the optical density of the eluate is measured at a preselected wavelength. The detector's wide linearity region makes it possible to analyze both

impurities and the main components of the mixture in one chromatogram. A spectrophotometric detector allows detection at any wavelength within its operating range (typically 190-600 nm). Multiwave detectors are also used, which allow detection at several wavelengths simultaneously, and diode array detectors, which allow recording optical density simultaneously over the entire operating wavelength range (usually 190–950 nm). This makes it possible to record the absorption spectra of the components passing through the detector cell.

The fluorimetric detector is used to detect fluorescent compounds or non-fluorescent compounds in the form of their fluorescent derivatives. The principle of operation of a fluorimetric detector is based on measuring the fluorescent emission of absorbed light. Absorption is usually carried out in the ultraviolet region of the spectrum, the wavelengths of the fluorescent radiation exceed the wavelengths of the absorbed light. Fluorometric detectors have very high sensitivity and selectivity. The sensitivity of fluorescent detectors is approximately 1000 times higher than that of spectrophotometric ones. Modern fluorescent detectors make it possible not only to obtain chromatograms, but also to record the excitation and fluorescence spectra of the analyzed compounds.

Refractometric detectors (refractometers) are used to determine compounds that weakly absorb in the ultraviolet and visible regions of the spectrum (for example, carbohydrates). The disadvantages of these detectors are their low (compared to spectrophotometric detectors) sensitivity and significant temperature dependence of the signal intensity (the detector must be thermostated), as well as the impossibility of using them in the gradient elution mode.

The principle of operation of evaporative detectors of laser light scattering is based on the difference in vapor pressures of chromatographic solvents that are part of the mobile phase and the analyzed substances. The mobile phase at the outlet of the column is introduced into the nebulizer, mixed with nitrogen or CO2, and in the form of a fine aerosol enters a heated evaporator tube with a temperature of 30–160 °C, in which the mobile phase evaporates. An aerosol of non-volatile particles of analyzed substances scatters the light flux in the dispersion chamber. By the degree of dispersion of the light flux, one can judge the amount of the determined compound. The detector is more sensitive than the refractometric one, its signal does not depend on the optical properties of the sample, on the type of functional groups in the analytes, on the composition of the mobile phase, and can be used in the gradient elution mode.

Electrochemical detectors (conductometric, amperometric, coulometric, etc.). An amperometric detector is used to detect electroactive compounds that can be oxidized or reduced on the surface of a solid electrode. The analytical signal is the magnitude of the oxidation or reduction current. The detector cell has at least two electrodes - a working electrode and a reference electrode (silver chloride or steel). An operating potential is applied to the electrodes, the value of which depends on the nature of the compounds being determined. Measurements can be carried out both at a constant potential and in a pulsed mode, when the profile of the change in the potential of the working electrode is set during one signal recording cycle. The amperometric detector uses working electrodes made of carbon materials (most often glassy carbon or graphite), and metal: platinum, gold, copper, nickel.

A conductometric detector is used to detect anions and cations in ion chromatography. Its working principle is based on the measurement of the electrical conductivity of the mobile phase in the process of elution of the substance.

Exceptionally informative is the mass spectrometric detector, which has high sensitivity and selectivity. The latest models of mass spectrometers for liquid chromatography operate in the m/z mass range from 20 to 4000 amu.

High-performance liquid chromatography also uses Fourier-IR detectors, radioactivity, and some others.

Data collection and processing system

A modern data processing system is a personal computer connected to the chromatograph with installed software that allows you to register and process the chromatogram, as well as control the operation of the chromatograph and monitor the main parameters of the chromatographic system.

**8. EVALUATION OF CHROMATOGRAPHIC CURVES**

Chromatograms can be quantified using either the height or area of the peaks. The choice of estimation method depends on the type and nature of the curves and on the technical possibilities. The height of a peak is much faster to measure than its area. The height of the peak is highly dependent on the operating conditions (temperature, speed of movement of the PF, size of the injected sample). Since changing operating conditions affect the peak area much less, they do not need to be carefully kept constant. Therefore, peak areas are currently used to evaluate separation results. Peak area measurements were previously carried out by the following methods.

1. Planimetry. The advantage of this estimation method is that the nature and shape of the curve is irrelevant in this case. Due to the low sensitivity of the planimeter and the design features, it cannot measure either too large or too small areas. The method is laborious, time consuming and less accurate than other methods (4% error).

2. Cutting and weighing peaks. This method is used relatively rarely. As with the planimetric definition, the shape of the curve does not matter. The method is time consuming but can be quite accurate, especially in the case of asymmetric curves. However, in this case, the necessary condition is the homogeneity of the paper, the constancy of its thickness and density. The disadvantage of the method is the need to cut the chromatograms. The error is about 2%.

3. Determining the peak area as the product of its height and half-width, or squaring the curve. When using this method, the height of the peak is multiplied by its width, measured at half-height of the peak.

This technique is quite simple and requires little time, but the accuracy of the determination results depends on the shape of the peak. This method cannot determine the areas of asymmetric peaks or peaks with low height and large base. The error of the method is approximately 2.5%.

4. Approximation to the area of a triangle, or trangulation. In this case, the peak area is measured as the area of a triangle.

The height is measured from the zero line to the point of intersection of the tangents to the inflection points of the curve. The technique is simple and it is easy to determine the height in this way, but sometimes there is uncertainty in determining the inflection points. In this way, it is impossible to determine the areas of narrow and high and asymmetric peaks.

5. Graphical integration. The peak is divided into vertical strips of equal width, the heights of which are then added.

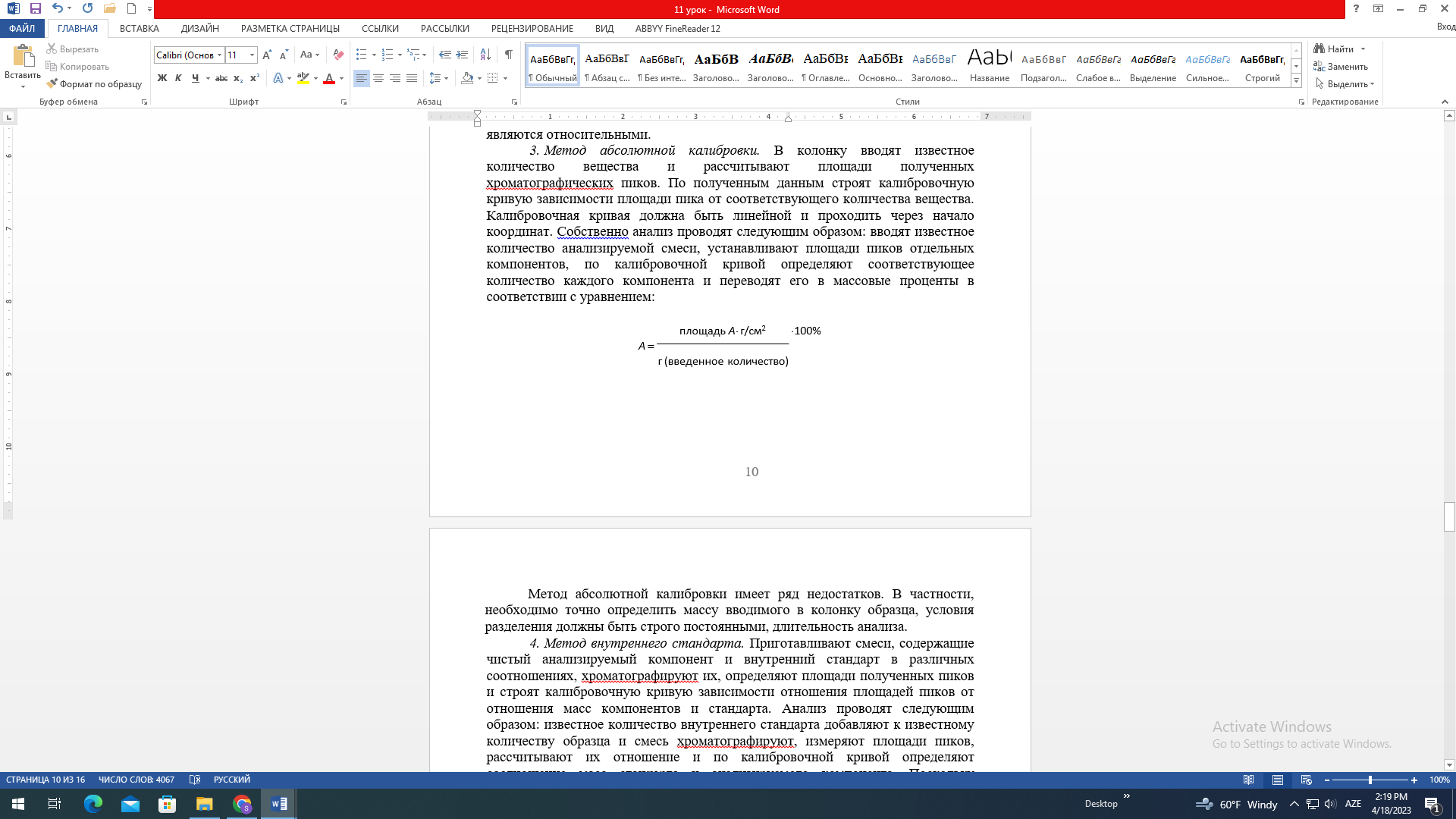
6. Electronic or mechanical integration. This method is used in modern HPLC analyzers. Initial analysis parameters and constants are entered into a computer database which can then be used. The computer program makes qualitative quantitative calculations and provides the results of the analysis.

Chromatograms can be quantified either directly or using some kind of calibration.

1. Direct method, or normalization. This method can be used when all components of the mixture are eluted from the column. The detector gives linear and reproducible data with the same sensitivity for all components.

2. Method for calculating correction factors for a flame ionization detector. A calibration mixture of compounds of known weight is prepared and after separation, the areas corresponding to the individual components are measured. Next, the ratios of areas and masses of each component are calculated, one of the ratios is taken as the standard and all correction factors lead to this value, i.e., correction factors for other compounds are obtained by dividing their ratios by the ratios for the standard. Therefore, the obtained coefficients are relative.

3. Method of absolute calibration. A known amount of the substance is introduced into the column and the areas of the resulting chromatographic peaks are calculated. Based on the data obtained, a calibration curve of the dependence of the peak area on the corresponding amount of substance is built. The calibration curve must be linear and pass through the origin. The analysis itself is carried out as follows: a known amount of the analyzed mixture is introduced, the peak areas of individual components are determined, the corresponding amount of each component is determined from the calibration curve and converted to mass percent in accordance with the equation:



The absolute calibration method has a number of disadvantages. In particular, it is necessary to accurately determine the mass of the sample introduced into the column, the separation conditions must be strictly constant, and the duration of the analysis.

4. Internal standard method. Prepare mixtures containing pure analyte component and internal standard in various ratios, chromatograph them, determine the areas of the obtained peaks and build a calibration curve for the dependence of the ratio of peak areas on the ratio of the masses of the components and the standard. The analysis is carried out as follows: a known amount of the internal standard is added to a known amount of the sample and the mixture is chromatographed, the peak areas are measured, their ratio is calculated, and the mass ratio of the standard and the analyzed component is determined from the calibration curve. Since the amount of added standard is known, the component content can be calculated from the ratio. The internal standard method has the following advantages: it is not necessary to accurately measure the amount of injected sample, it is not necessary to determine the magnitude of the detector response or keep it strictly constant, since not absolute values are determined, but their ratios. The main disadvantage of this method is the difficulty of selecting a suitable internal standard. The peak of the internal standard should be completely separated from the peaks of other compounds, but at the same time it should be located close enough to the peak of the analyte, the concentration of the standard should be approximately the same as the concentration of the analyte, and finally, the standard should be structurally similar to the analyzed compound. For complex mixtures, two or more internal standards may be added.

5. Method of standard addition. This method is the same as described above. It differs only in that the added standard is one of the components of the mixture. This method is mainly used in cases where it is impossible, for example, due to poor resolution, to pick up any other connection.

mobile phase

The mobile phase in high-performance liquid chromatography performs a dual function: it ensures the transfer of desorbed molecules along the column and regulates the equilibrium constants, and, consequently, retention as a result of interaction with the stationary phase (being sorbed on the surface) and with the molecules of the substances being separated. Thus, by changing the composition of the mobile phase in high performance liquid chromatography, one can influence the retention times of compounds, the selectivity and efficiency of their separation.

The mobile phase may consist of one solvent, often two, if necessary, three or more. The composition of the mobile phase is indicated as the volume ratio of its constituent solvents. In some cases, the mass ratio may be indicated, which should be specially stipulated. Buffer solutions with a certain pH value, various salts, acids and bases, and other modifiers can be used as components of the mobile phase.

Normal phase chromatography usually uses liquid hydrocarbons (hexane, cyclohexane, heptane) and other relatively non-polar solvents with small additions of polar organic compounds that control the eluting strength of the mobile phase.

In reverse phase chromatography, water or aqueous-organic mixtures are used as the mobile phase. Organic additives are usually polar organic solvents (acetonitrile and methanol). To optimize the separation, aqueous solutions with a certain pH value can be used, in particular buffer solutions, as well as various additives to the mobile phase: phosphoric and acetic acids in the separation of acidic compounds; ammonia and aliphatic amines in the separation of basic compounds, and other modifiers.

The purity of the mobile phase greatly affects the chromatographic analysis, so it is preferable to use solvents released specifically for liquid chromatography (including water).

When using a UV spectrophotometric detector, the mobile phase should not have a pronounced absorption at the wavelength chosen for detection. The limit of transparency or optical density at a certain wavelength of a particular manufacturer's solvent is often indicated on the packaging.

The mobile phase and analyzed solutions must be free of undissolved particles and gas bubbles. Water obtained under laboratory conditions, aqueous solutions, organic solvents previously mixed with water, as well as analyzed solutions must be subjected to fine filtration and degassing. For these purposes, vacuum filtration through a membrane filter with a pore size of 0.45 μm, which is inert with respect to a given solvent or solution, is usually used.

**MODIFIED HIGH PERFORMANCE**

**LIQUID CHROMATOGRAPHY**

Ion pair chromatography

One of the varieties of reverse-phase high-performance liquid chromatography is ion pair chromatography - which allows you to determine ionized compounds. To do this, hydrophobic organic compounds with ionogenic groups (ion-pair reagents) are added to the traditional reverse-phase high-performance liquid chromatography of the mobile phase. Sodium alkyl sulfates are usually used to separate bases; tetraalkylammonium salts (tetrabutylammonium phosphate, cetyltrimethylammonium bromide, etc.) are used to separate acids. In the ion-pair mode, the selectivity of separation of non-ionic components will be limited by the reversed-phase retention mechanism, while the retention of bases and acids increases markedly, while the shape of the chromatographic peaks improves.

Retention in the ion-pair regime is due to fairly complex equilibrium processes that compete with each other. On the one hand, due to hydrophobic interactions and the effect of displacement of the polar medium of the mobile phase, the sorption of hydrophobic ions on the surface of alkyl silica gel is possible in such a way that the charged groups face the mobile phase. In this case, the surface acquires ion-exchange properties, and retention obeys the laws of ion-exchange chromatography. On the other hand, it is possible to form an ion pair directly in the volume of the eluent, followed by its sorption on the sorbent by the reversed-phase mechanism.

Hydrophilic interaction chromatography

(HILIC chromatography)

Hydrophilic interaction chromatography is used to separate polar compounds that are poorly retained in reverse phase high performance liquid chromatography. As a mobile phase in this version of chromatography, water-acetonitrile mixtures with the addition of salts, acids, or bases are used. The stationary phases, as a rule, are silica gels modified with polar groups (amino, diol, cyanopropyl groups, etc.). More polar compounds are held stronger. The eluting power of the mobile phase increases with increasing polarity.

Ion exchange and ion high efficiency liquid chromatography

Ion-exchange chromatography is used to analyze both organic (heterocyclic bases, amino acids, proteins, etc.) and inorganic (various cations and anions) compounds. The separation of the components of the analyzed mixture in ion-exchange chromatography is based on the reversible interaction of the ions of the analyzed substances with the ion-exchange groups of the sorbent. These sorbents are mainly either polymeric ion exchange resins (usually copolymers of styrene and divinylbenzene with grafted ion exchange groups) or silica gels with grafted ion exchange groups. Sorbents with groups:

—NH3+, —R3N+, —R2HN+, —RH2N+, etc. are used to separate anions (anion exchangers), and sorbents with groups: —SO3–, —RSO3–, –COOH, —PO3–, etc. are used to separate cations (cation exchangers) .

As the mobile phase in ion-exchange chromatography, aqueous solutions of acids, bases and salts are used. Typically, buffer solutions are used to maintain certain pH values. It is also possible to use small additions of water-miscible organic solvents - acetonitrile, methanol, ethanol, tetrahydrofuran.

Ion chromatography is a variant of ion-exchange chromatography in which a conductometric detector is used to detect analytes (ions). For a highly sensitive determination of changes in the conductivity of the mobile phase passing through the detector, the background conductivity of the mobile phase must be low.

There are two main variants of ion chromatography.

The first one is two-column ion chromatography, the second variant of ion chromatography is single-column ion chromatography. Size Exclusion High Performance Liquid Chromatography

Size exclusion chromatography (gel chromatography) is a special version of high performance liquid chromatography based on the separation of molecules by their size. The distribution of molecules between the stationary and mobile phases is based on the size of the molecules and partly on their shape and polarity.

Two limiting types of interaction of molecules with a porous stationary phase are possible. Molecules larger than the maximum pore diameter are not retained at all and are eluted first, moving along with the mobile phase. Molecules with sizes smaller than the minimum pore diameter of the sorbent freely penetrate into the pores and are the last to be eluted from the column. The remaining molecules, which have intermediate sizes, are partially retained in the pores and, during elution, are separated into fractions in accordance with their sizes and, partially, the shape, penetrate into the pores of the sorbent, depending on the size and partially, depending on their shape. As a result, substances are eluted with different retention times.

Ion exclusion chromatography

The mechanism of ion-exclusion chromatography is based on the effect, as a result of which compounds in the ionized form are not retained on the ion-exchange sorbent, while compounds in the molecular form are distributed between the stationary and aqueous phases inside the pores of the ion-exchange sorbent and the mobile phase migrating in the space between the sorbent particles. Separation is based on electrostatic repulsion, polar and hydrophobic interactions between dissolved compounds and the sorbent.

The anionic groups on the surface of the sorbent act as a semi-permeable "membrane" between the stationary and mobile phases. Negatively charged components do not reach the stationary mobile phase, as they are repelled by similarly charged functional groups and eluted in the "dead" (free) volume of the column. Components in molecular form are not "rejected" by the cation-exchange sorbent and are distributed between the stationary and mobile phases. The difference in the degree of retention of the non-ionic components of the mixture is dictated by the combination of polar interactions of non-ionic components with the functional groups of the cation-exchange sorbent and hydrophobic interactions of non-ionic components with the non-polar sorbent matrix.

Chiral chromatography

The goal of chiral chromatography is to separate optical isomers. Separation is carried out on chiral stationary phases or on conventional achiral stationary phases using chiral mobile phases. As chiral stationary phases, sorbents with a modified surface, groups or substances having chiral centers (chitosan, cyclodextrins, polysaccharides, proteins, etc. (chiral selectors) are used. In this case, the same phases can be used as mobile phases as in normal-phase or reverse-phase chromatography.When using achiral stationary phases to ensure the separation of enantiomers, chiral modifiers are added to the mobile phases: chiral metal complexes, neutral chiral ligands, chiral ion-pair reagents, etc.

Ultra performance liquid chromatography

Ultra performance liquid chromatography is a variant of liquid chromatography that is more efficient than classical high performance liquid chromatography.

A feature of ultra-performance liquid chromatography is the use of sorbents with a particle size of 1.5 to 2 µm. Chromatographic columns are typically 50 to 150 mm long and 1 to 4 mm in diameter. The volume of the injected sample can be from 1 to 50 µl. The use of such chromatographic columns can significantly reduce the analysis time and improve the efficiency of chromatographic separation. However, in this case, the pressure on the column can reach 80–120 MPa, the required detector data collection frequency can increase up to 40–100 Hz, and the extracolumn volume of the chromatographic system must be minimized. Chromatography equipment and columns used in ultra high performance liquid chromatography are specially adapted to meet the requirements of this type of chromatography.

**APPLICATIONS OF HIGH PERFORMANCE LIQUID CHROMATOGRAPHY IN PHARMACEUTICAL ANALYSIS**

Over the past 20–25 years, HPLC has become the most popular method in pharmaceutical analysis, the official method included in almost all modern pharmacopoeias, including the US Pharmacopoeia, the British Pharmacopoeia, the Japanese Pharmacopoeia, the International Pharmacopoeia, and is recommended by them for determining authenticity, quantitative content, dosing uniformity, purity and stability of a variety of pharmaceutical objects.

Summing up a brief review of the current state of HPLC and the application of this method in chemical-toxicological analysis, we can say that HPLC is of great importance, and its combination with the mass spectroscopy method allows solving the most complex tasks facing the experts of the Bureau of Forensic Medical Examination.