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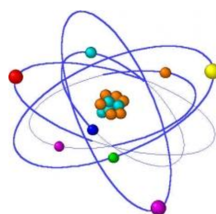
FUNDAMENTALS OF GAS AND HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Teaching and methodical manual

for foreign student



Zaporozhye, 2016

ЗАПОРІЗЬКИЙ ДЕРЖАВНИЙ МЕДИЧНИЙ УНІВЕРСИТЕТ

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ОСНОВИ ВИСОКОЕФЕКТИВНОЇ ГАЗОВОЇ ТА РІДИННОЇ ХРОМАОГРАФІЇ

Навчально-методичний посібник

для студентів англомовної форми навчання

Запоріжжя, 2016

UDC 543.544(075.8)=111
BBC 24.4Я73
F97

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Fundamentals of gas and high performance liquid chromatography: teaching and methodical manual for foreign students / A.G. Kaplaushenko, O.R. Pryakhin, B.A. Varinskiy [et al.]. − Zaporozhye, 2016. − 71 p.

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Каплаушенко А.Г., Пряхін О.Р., Варинський Б.О., Юрченко І.О.,
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Гладишев В.В., зав. кафедри технології ліків Запорізького державного медичного університету, д.фарм.н..

Основи високоефективної газової та рідинної хромаографії: навчально-методичний посібник / А.Г. Каплаушенко, О.Р. Пряхін, Б.О. Варинський [та ін.]. – Запоріжжя, 2016. – 71 с.

Навчальний посібник розглянуто та затверджено:

Цикловою методичною комісією з фармацевтичних дисциплін
(протокол №__ від ________р.),
Центральною методичною радою ЗДМУ
(протокол №__ від ________р.)

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INTRODUCTION

Gas and high performance liquid chromatography, especially in combination with mass spectrometric detection are the primary methods for analyzing complex pharmaceuticals and biological mixtures. They are pharmacopoeial methods. The study of these methods actually has a value in the preparation of pharmaceutical chemistry and pharmacognosy.

SECTION I. GAS CHROMATOGRAPHY
Plan

• Classification of methods of gas chromatography.

• Retention Indices Kovacs

• Gas-solid phase chromatography.

• Gas-Liquid Chromatography

stationary phase for gas-liquid chromatography

stationary phases

capillary columns

inactivation surface

• Gas carriers

• sample introduction system

• Selecting and temperature programming

• Detectors

• Katharometers

The flame ionization detector (FID)

electron capture detector

The thermionic detector (TTI)

The flame photometric detector (FPD)

• The use of gas chromatography

Qualitative analysis

Quantitative analysis

Classification of methods of gas chromatography

A prerequisite for the LC is a pre-transition chromatographed substances in the gas phase.

Separation is carried out in a column containing the stationary phase, solid or liquid.
It espouses the detected temperature and a constant rate of carrier gas (mobile phase).
When a mixture of substances is introduced into the chromatograph, each mobile phase component in the direction to the detector and is distributed between the stationary phase and the gas phase.

For analytical practice it is important that at a constant temperature, the amount adsorbed on the surface of Cs was is propor- tional to the concentration of the substance in the gas phase Cm:

$$C_s = kC_m,$$

ie, that the distribution is in accordance with the linear isotherm adsorption or isotherm distribution (k- constant).

In this case, each component is moved along the column at a constant rate, independent of its concentration.

Classification of methods of gas chromatography.

The gas-solid	The liquid-gas

Retention index (Kovats indices).

The system of these indices as standard substances taken paraffins (saturated hydrocarbons).

Retention Index I on any column and any conditions for the normal limit hydrocarbon shall be equal to the number of carbons multiplied by 100. So, $I_{C_6H_{14}} = 600$, $I_{C_7H_{16}} = 700$, etc.

Retention index value for any other substances of this column is determined by the formula:

$$I = 100x + 100\frac{\lg t_i - \lg t_{C_xH_y}}{\lg t_{C_{x+1}H_{y+2}} - \lg t_{C_xH_y}},$$

where x is the number of carbon atoms of paraffin exiting the column before test compound i; $t_{C_xH_y}$ - The retention time of the hydrocarbon; $t_{C_{x+1}H_{y+2}}$ - The retention time of the hydrocarbon coming immediately after the test compound.

Example:

The unknown compound has corrected retention time of 19.5 minutes. Corrected retention time of n-hexane and n-heptane, under these conditions, respectively 13.7 and 29.3 min. Using these data we calculate the Kovats index for an unknown compound:

$$I = 100 * 6 + 100\frac{\lg 19{,}5 - \lg 13{,}7}{\lg 29{,}3 - \lg 13{,}7} = 648{,}2$$

In the reference tables, we find that the calculated value closest to benzene (650).

In most homologous series of compounds retention index increased by 100 for each additional CH2 group. Retention indices of non-polar compounds are practically constant for any type NF.

Indices holding any substance at various nonpolar NF very close. The difference ΔI retention indices compounds on polar and nonpolar NF characterize the compound structure.

Gaza carriers

The choice of carrier gas depends on the type of detector.

When using a katharometer is used helium, hydrogen, nitrogen; flame ionization detector - helium, hydrogen, nitrogen; electron capture detector - nitrogen.

To measure the flow rate at the inlet of a column can be used rotameter, and the output - soap-bubble flow meter. For packed columns consumption varies between 25 and 150 ml / min and for capillary - in the range of 1-25 ml / min.

Gas-solid phase chromatography

In the gas adsorption chromatography, the stationary phase is a solid active sorbent. They may be inorganic, such as synthetic zeolite molecular sieves, carbon molecular sieves, silica gel, graphitized carbon, or they may be organic polymers.

They are typically used for separation of low molecular weight materials, such as gases and liquids.

Molecular sieves (4A, 5A, 13X) give good overall separation inorganic gases. Carbon dioxide is adsorbed irreversibly below 1600. Oxygen and nitrogen are well separated.

Carbon monoxide in the blood may be conveniently measured using 5A molecular sieves. Carbo-granular sieve is a molecular sieve for the separation of hydrocarbons from C1 to C3.

Silica gel gives a good separation of inorganic gases. Porasil - a porous silica gel with a surface of between 1.5 and 500 m2 / n and can be used as the native silica gel.

Carbon dioxide, carbon monoxide, hydrogen, and nitrogen can be separated. Oxygen and nitrogen are not separated.

Molecular sieve and silica gel column can be used for parallel analysis of exhaled gas which has value oxygen separation, nitrogen and carbon dioxide.

Chromosorb And Porapaki are polystyrene-divinylbenzene cross-linked polymers.

There are different variants with different surface area and average pore diameter. They are separated from the free dearne acids to free amines.

The alcohols from methanol to pentanol can be separated into Prapake Q or Chromosorb 102. The maximum allowable temperature of about 2500.

GC Tenax is porous polymer 2,6-diphenyl-p -phenyl oxide.

This material is used as a chromatographic phase and as a trap for the volatile substances before analysis.

Carbopak B and C is a graphitized carbon black having a surface of 12 to 100 m2 / g, respectively. They typically modified thinly coated polar stationary phase.

Complex separation of hydrocarbons from C1 to C10 can be quickly achieved. With modified Carbopak 0.2% Carbowax 1500 is a phase selection in the determination of ethanol in the blood.

Carbopak C tetragidroksietilendiamin 0.8% ethylene glycol is used to determine in the blood.

They give approximately the same order of elution of substances like Porapaki and Chromosorb, but in general with better separation.

Material support for packed columns

The behavior of the column is significantly affected by the stationary phase, but the carrier material also plays an important role, especially using a low concentration of the liquid phase.

Requirements to the support material:

It must have a very large surface area and a uniform particle size free from grinding and sufficiently strong, ie It must not be pulverized into powder during normal use.

It should be chemically inert with no effects of adsorption, and when covered with the stationary phase should be readily and uniformly packed into the column.

Usually used carriers with the surface 1-5 m2 / g, pretreated with acids, alkalis and specific reagents to decrease adsorption activity (e.g. silaniziruyuschimi).

The particle size of the carriers is about 150 to 250 microns (60-100 mesh).

The raw material for the majority of well-known carriers include diatomaceous earth (diatomaceous earth) - a sedimentary rock composed of the shells of diatomite algae.

Also, use of glass microspheres, silica, teflon and some other materials. Chromosorb P, W, G are based on diatomaceous earth materials.

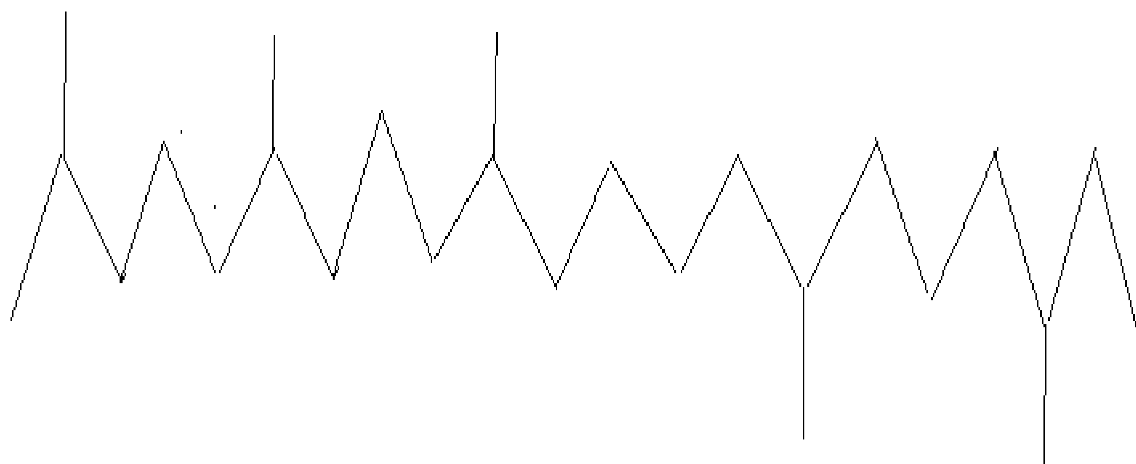
The choice of stationary phase

More than seven hundred substances are used as the stationary phase. The choice of stationary phase for a particular division as usual question of foresight, depending on experience, prejudices, intuition and desire to use the existing system if it will give the result.

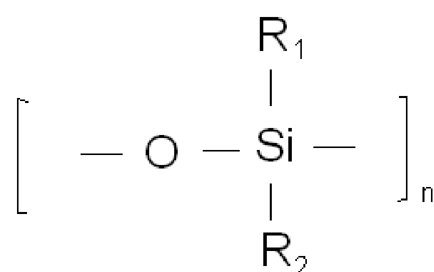
Requirements for NF:

1) providing the desired separation

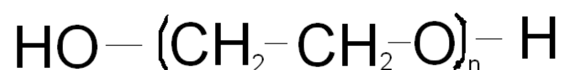
2) substances being analyzed must be dissolved in NF, otherwise retention time is very short and the separation is achieved.
Typically, the substance is chromatographed on nonpolar hydrocarbon and siloxane NF. For example, saturated hydrocarbons (squalane)



and siloxanes having the general formula



For chromatographic material capable of forming hydrogen bonds. Also, for their selective separation of non-polar compounds. For this used polar NF, for example polyethylene glycols (e.g. Carbowax 20M).



For the evaluation and selection of the NF is very convenient method of classifying them by conventional chromatographic polarity proposed Rohrschneider and improved Mack Reynolds. This method uses the so-called retention indices (Kovats indices).

Chromatographic properties of the NF (Table 1) exploring 10 standards.

Table 1 Standards for determining the constants Mack-Reynolds

Mc Reynolds constant	standard	class of materials characterized by constant Mack-Reynolds
ΔI1	Benzene	Aromatic compounds
ΔI2	1-Butanol	alcohols, nitriles, acids
ΔI3	methyl propyl	ketones, ethers, aldehydes
ΔI4	nitropropane	Nitro compounds, nitriles
ΔI5	pyridine	Nitrogen-containing heterocycles
ΔI6	2-Methyl-2-pentanol	Branching agents
ΔI7	1 Isobutane	halogenated
ΔI8	2-octene	acetylenic compounds
ΔI9	1,4-Dioxane	Ethers, polyhydric alcohols
ΔI10	cis-Gidrindan	Polycyclic Nitrogen

Retention Indices for each of the 10 standards are measured firstly on the stationary phase, which is tested and then the standard phase (squalane).

Classic Mc Reynolds constant calculated by summing the differences in retention indices between the two phases ΔI for the first 5 standard substances. They measure the polarity of the stationary phase.

However, these do not provide constant information about the shape of the peak, the temperature range, or the possibility of using in capillary columns.

The higher the value of Mc Reynolds constant, the stronger the material retained on the column. Constants Mack-Reynolds identified for the vast majority of the NF.

These data are given in the references by chromatography and very useful in selecting the NF to separate mixtures of compounds of different classes (see Table 2).

Table. 2 Constants Mc Reynolds liquid phases

The liquid phase (maximum temperature)	Mc constant Reynolds number (increase in the degree of polarity)
Squalene (1500) (standard phase)	0
Apolan-87 (2600)	71
Apiezon L (3000)	143
SE-30 (3000)	217
OV-1 (3500)	222
OV-101 (3500)	229
Apiezon L / KOH (2250)	301
OV-7 (3500)	592
OV-17 (3500)	886
Versamid 900 (2750)	986
Poly A 103 (2750)	1075
Carbowax 20M / KOH (2250)	1296
OV-225 (2500)	1813
CHDMS (cyclohexandimethyl succinate) (2500)	2017
Carbowax 20M (2250)	2308

An important characteristic of NF - temperature limits of its possible use. The lower value
corresponds to the temperature at which the liquid state retains NF. When solidification NF column efficiency decreases sharply.
The upper limit is the temperature of the beginning of this discernible to the detector NF evaporation.

The non-polar phase:

• Apiezon L

• SE-30

• O-1

• OV-101

Polar phase:

• Carbowax 20M

• OV-17

• OV-225

• SHDMS

Ramming column

Chromatography columns are steel, copper or glass tube inner diameter of 3-6 mm. Short ramming columns (1 to m) are set in the thermostat to a straightened or U-shaped form.
Columns to 3 meters in length is mounted in a spiral with a diameter of 10 to 30 cm. The column is placed for heating in a thermostat.

The ramming column stationary phase coated on granular carrier material.
First, in the GC column used exclusively ramming.

The absolute limit of the maximum length - 20 meters, and the column is obtained is very cumbersome, and the pressure drop, which is proportional to the packing density becomes very large.

Thus, for a ramming column efficiency is less than 10,000.

Capillary column

Capillary columns are made of glass, steel and quartz. Used tubing with an outer diameter of 0.5 mm, an inner diameter of 0.2 to 0.4 mm, from 10 to 50 meters long, are laid in a helix and placed in a thermostat.
The basic material - a fused silica with a low content of metal oxides. The strength of columns supported polyamide coating. They do not contain a carrier material. In this case, the liquid phase is distributed to the fixed walls of the capillaries.

Methods of application of the stationary phase:

The liquid enters the column by passing it through the stationary phase of a concentrated solution.
Evaporation (using a vacuum pump) of the solvent from the column fully filled with highly dilute solution

Methods for the immobilization of the NF in a capillary:

NF (polymer) is applied to the inner wall of the capillary how a membrane.
Polymer - a polysiloxane cross-linked and grafted to the silica surface, or polyethylene glycol. (WCOT - wall coated open tubular) (PKPS- hollow capillary walls covered).

2) the PLOT columns (Porous Layer Open Tubular) (HCCC - hollow capillary coated carrier) - include on the inner surface of a thin layer of porous adsorbent: alumina, zeolite, carbon material, or a polymer (polistiroldivinilbenzol).

3) SCOT columns (Surface coated open tubular) - have a porous surface.
Using Golay (1958) capillary columns these limitations that existed for packed columns have been overcome. Since the stationary phase is applied to the inner walls of the capillaries, the carrier gas flow is not obstructed.

Therefore, the speaker can reach lengths of 100 m, and the number of theoretical plates -100000 or more.

Furthermore, the volume of the mobile phase is significantly longer than the fixed

Sample Introduction

For the introduction of the gases used cranes dosing. Thus the sample may be introduced directly into the carrier gas stream (sample volume to 20 ul).

Liquid and solids in the injection evaporator is pre-vaporized. sample introduction system can be heated; it connects the flow of carrier gas to the column. She sealed diaphragm made of silicone rubber, called the septum.

The evaporators can be of several types (see Table 3).:

Evaporator division or splitless
Evaporator for packed columns with blowing gaskets
Evaporator cold sample introduction into the column
Evaporator with a programmed temperature vaporization
Interface for volatile compounds.
Automatic sample introduction system

The capillary column is necessary to introduce a sample of approximately 5000 times smaller than a conventional speaker. For this purpose, flow dividers used carrier gas is passed into the column is not more than 0.01% of the injected sample.

Table. 3 evaporators.

Type of evaporator	Type of column	Mode	Type of sample	Comments	The number of samples in the column
With the split / splitless	Capillary column	With the flow division	high concentrati on	Useful for large> 2 mkl input volume	Very few, most of the divider

		Splitless	low concentrati on	Useful for large> 2 l input volume	All
Cold commissioning	Capillary column		low concentrati on	Minimum sample decomposition	All
For a packed column	ramming		Any	Satisfactory if the resolution is not a problem	All

Selecting and temperature programming

Retention time generally decreases with increasing temperature. Generally, as the temperature decreases, the separation of components increases. However, at the same time increasing the duration of the analysis.

Therefore, if there is no possibility to increase the temperature, you can reduce the amount of NP. In any case, the temperature in the column should ensure the presence of analytes in the gas phase. Separation of mixtures boiling in a wide temperature range is very difficult in isothermal mode.

Components having lower affinity for the sorbent, at a high temperature quickly emerge from column unshared while components at a low temperature with a large retention time can not escape completely.

To optimize the analysis in this case, often used temperature programming. Column temperature change in analysis can be variously: stepwise, continuously, linearly or any complicated dependence.

In modern chromatographs for this purpose are programmers temperature control temperature regime in the column.

Chromatography complex mixture are often used linear programming. In this case, a constant set temperature increase rate (for example, from 100 to 2000 with a speed of 20C / min).

At low temperatures, the column exit zone of weakly sorbed substances, followed by materials area with increasing affinity for the sorbent (Table. 4).

Table. 4 Detectors

Detector	detected components	The limit of detection	Linear range
Katharometers	Do not voters	10-8 g / ml	104
PID	Compounds containing CH	10-13 g / ml	107
ECD	electronegative group	10-14 g/ml	104
TTI	P	10-15 g/ml	105
	N	10-14 g/ml	
PFD	P	10-13 g/ml	105
	S	10-11 g/ml	

Katharometers

Principle of operation

The thermal conductivity of the carrier gas is reduced in the presence of the analyte. The thermal conductivity of helium and hydrogen is about 6-10 times higher than that of organic compounds.

Design

The thermal conductivity is determined by measuring the resistance of a heated metal filament. The measured resistance and the resistance of the comparison included in the electric bridge.

Katharometers works proportional to the concentration.

Groups of compounds.

Non-selective detector, so it can be used to determine both organic and inorganic compounds.

Disadvantages

Low sensitivity and a narrow linear range. Not suitable for capillary columns

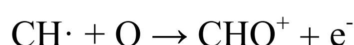
Flame Ionization

The most widely used detector.

Principle of operation

Changing the conductivity of the hydrogen flame in an electric field in contact with the flame of organic substances.

Organic compounds emerging from the column and subjected to a pyrolysis thus break up into fragments.
Upon subsequent oxidation of oxygen falling within the flame, ions are produced:



$$CH\cdot + O \rightarrow CHO^{+} + e^{-}$$

The flow of ions is recorded as a voltage drop on the collecting electrode.

Groups of compounds.

PID is sensitive to all compounds containing the C-C or C-H. That's why he became widespread. Relatively low sensitivity even to complete insensitivity can be observed with respect to functional groups such as carbonyl, alcohol, halogen or amino.

The detector is not sensitive to non-combustible gases: H2O, CO2, SO2, NO2. Because a detector responsive to the number of carbon atoms per unit time. its signal is proportional to the mass of the detectable substance.

Therefore, changes in the flow rate of mobile phase have only a negligible effect on the detector signal.

Electron capture detector

Principle of operation.

Closely related to the principle of operation of the proportional counter for measuring the X-rays. Under the action of β-emitters, such as tritium or 63Ni. The carrier gas stream is ionized and the electrons appear.

The absence of detectable compounds of the current flowing through the cell is constant.

The level of current decreases in the presence of organic compounds, especially if these compounds can capture electrons.

Groups of compounds.

Compounds that contain electrophilic (electronegative group) - halogens, peroxides, quinones, nitro or phthalates. Those. - This is a highly sensitive detector for chlorinated insektetsidov.

ECD is insensitive to amines, alcohols, and carbohydrates.

Thermionic detector

Principle of operation.

Flame detector with hydrogen-free gas mixture.

Design

Between the burner and the collecting electrode on a platinum wire is fixed glass particle containing rubidium. Plasma is formed around it, in which a compound containing N and P, to form radicals. These radicals react with rubidium steam around the glass particles, giving rubidium ion.

Rubidium cation glass charged particle is trapped, while ions containing nitrogen or moves directly to the collecting electrode or burned under the action of the electrons.

Group of compounds

Compounds containing nitrogen and phosphorus. Its sensitivity to these elements about 10,000 times greater than for carbon.

Flame Photometric Detector

Principle of operation.

Organic compounds are partially combusted in a hydrogen-air flame, while using photomultiplier measured emission at 526 nm for phosphorus and at 394 nm for sulfur.

Qualitative analysis

The relative retention time - is the ratio of the retention time of the analyte to the retention time of a substance taken as a standard.

According HFC Identification carried out as follows:

• comparing the retention time of the analyte in the test sample and reference solution.

• comparing the relative retention times of the analyte in the test sample and reference solution.

• Comparing the studied sample chromatogram with the chromatogram of the reference solution with chromatogram or given in a separate article

Often using the first method. However, in the case of non-repeatable separation conditions necessary to use the second method. For the analysis of plant and animal origin objects using a third method.

Best guaranteed identification using mass-spectrometric detection.
Relative retention data can be identified by the Kovac method, based on the fact that within a homologous series of n-alkanes a linear relationship between the logarithm of the corrected retention time and the number of carbon atoms in the compound.

Identification of the retention index produced compound by chromatography followed by chromatography under the same conditions of the two neighboring alkanes selected as standards.

Retention indices are defined for many compounds. Many available as table data for most conventional stationary phases.

They are relatively independent of temperature.

Assay

The absolute calibration - Test solution and reference solution alternately chromatography to yield at least 5 chromatograms.

For the test solution and reference solution calculated the average values of the areas or heights of the peaks of analytes. Based on these results is calculated analyte concentration.

The method of internal standard - For each chromatogram is first calculated area ratio or peak heights for the area or height of the internal standard peak. Averaged ratio obtained for the sample solution and the reference solution and the found average values calculated analyte concentration.

The HFC gas chromatography is used to identify and determine the amounts of residual solvents in the drug substances. Also confirm the quality of the substance, this information can serve as an argument in favor of a technology that has been used for the manufacture of the substance in the case of disputes about the illegal use of technology protected by international patent laws.

SECTION II. HPLC

Plan

• Historical stages of development of HPLC

• Theoretical foundations of the method

General Concepts

Chromatogram and chromatographic parameters

Classification of methods

• Types of chromatography.

Adsorption

Distribution

Ionic

gel chromatography

Ligand-binding

• Equipment

Pumps

Injectors

Columns

Detectors

• Examples of modern devices

• Examples of divisions

• Identification

• Quantitation

Creating liquid chromatography and its evolution
Four periods of history tool LC.

50 years of history tool LC can be divided into three stages. The first period began in July 1958, when it was created the first amino acid analyzer, and lasted until the early 70s. During this time, instrumental LC has gone from birth to development as a method.

Since 1967, the number of publications on experimental housing and communal services in «Analytical Chemistry» exceeds 1%, by 1972 this share exceeded 3.5%.

At the end of this period, Kirkland developed a method for separating silica particle size of 5 microns and named it HPLC.

In the second phase (mid-80s) method has become the most widely used among other analytical methods.

By 1984, the proportion of articles on instrumental LC in «Analytical Chemistry» has reached 20%. At this stage, the main merit in spreading LC belonged not so much a university scientist as instrument-making companies. Their efforts were highly efficient speakers. Pumps with no pulsation, high pressure.

HPLC has become automated, highly sensitive, fast, reliable and convenient methods for the analysis of complex mixtures of organic and inorganic substances.

By the end of the 70s there were a reverse phase C18 sorbents.

The third period can be traced back to the early 2000's. From booming HPLC method it has become a rather conservative analysis. Despite the fact that every year there are new types of sorbents, still most often used on a C18 silica gel.

Fourth step - is the last 10 years. It is characterized by the use of micro-speakers, high-speed HPLC.

Dimensions of conventional speakers comprise: 150-250 mm with an inner diameter of 3-5 mm. The diameter of the microcolumns or capillary columns is from 3 to 200 micrometers.

Rapid HPLC using a column shorter than conventional columns with a length of about 3 mm.

Theoretical foundations of the method

Basic concepts and classification of liquid chromatography methods.

Simple hardware decor paper and thin layer chromatography resulted in the widespread use of these methods in analytical practice. However, great possibilities liquid chromatography stimulated improving equipment.

The transmission of the eluent through the column under high pressure allowed to dramatically increase the speed of analysis and significantly increase the separation efficiency due to the use of fine sorbent.

HPLC method now allows you to select, quantitatively and qualitatively analyze complex mixtures of organic compounds.

Quickness, high sensitivity and accuracy determinations is an advantage compared with paper and thin layer chromatography.

Separation using a liquid mobile phase may be based on four principles:

- Adsorption (color);

- Distribution (Martin and Xingzheng);

- Ion exchange;

- Exclusion (Porat);

- The exchange of ligands (Davankov).

On the mechanism of separation are the following types of HPLC:

- Adsorption

- Distribution

- Normal-phase version

- Reversed-phase version

- Ion chromatography

- Exclusion chromatography

- Ligand-binding chromatography.

Adsorption Chromatography.

Separation by adsorption chromatography is carried out by reacting the shared material with an adsorbent such as alumina or silica gel having surface active polar centers.

The solvent (eluent) - a nonpolar liquid (normal-phase chromatography option). The mechanism of adsorption is a specific interaction between the polar surface of the sorbent and the polar (or able to be polarized) portions of the molecules of analyte (Fig. 1).

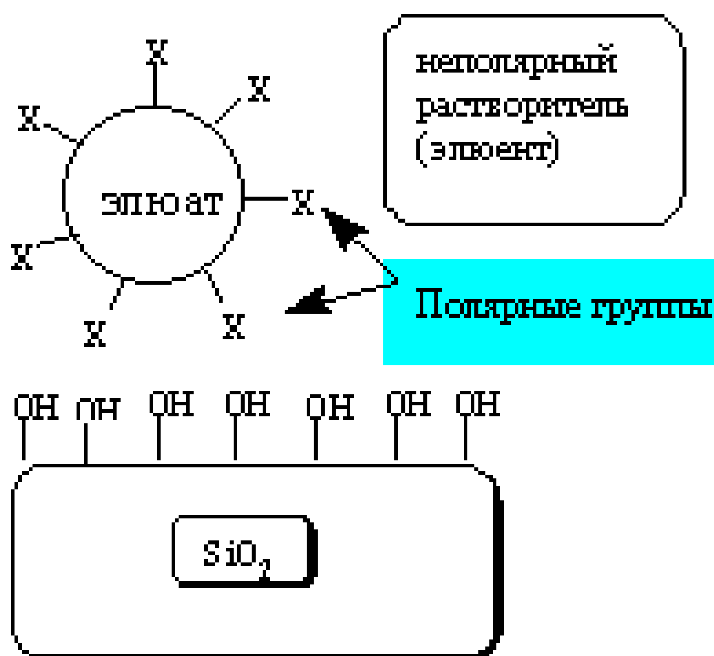


Fig. 1 adsorption liquid chromatography.

In normal phase chromatography, adsorbed on the surface of the sorbent material "pushed" (eluted) eluent actively interacting with the polar surface of the sorbent.

The less the adsorbed substance, the more it is pushed out, the smaller it is retained in the column.

Eluents polarity build in elyuotropnye series (Table. 5).

Table. 5 Elyuotropny number of solvents.

Solvent	polarity index P '	The elution force (SiO2)	UV transparency, nm
Fluoroalkane	<-2	- 0,2	200
Cyclohexane	0,04	0,03	200
n-Hexane	0,1	0,01	195
Carbon tetrachloride	1,6	0,11	265
Diisopropyl ether	2,4	0,22	220
Toluene	2,4	0,22	285
Diethyl ether	2,8	0,38	215

Dichloromethane	3,1	0,34	230
Tetrahydrofuran	4,0	0,35	210
Chloroform	4,1	0,26	235
Ethanol	4,3	0,68	205
Acetic acid	4,4	0,38	255
Dioxane	4,8	0,49	215
Methanol	5,1	0,73	205
Acetonitrile	5,8	0,50	190
Nitromethane	6,0	0,49	380
Water	10,2	Hight	170

For normal phase chromatography with increasing polarity increases "buoyancy" force. Most polar substances sorbed stronger.

Partition chromatography.

In partition chromatography, separating a mixture of substances is carried out by the difference in their distribution coefficients between two immiscible phases - eluent (mobile phase) and phase, located on the sorbent (stationary phase).

In normal-phase HPLC distribution variant using non-polar and polar groups eluent. They should be grafted to the sorbent (usually silica) surface.

As the silica gel surface modifiers (graft phase) used alkilhlorsilany substituted, containing polar groups, such as nitrile, amino and t. D. (Figure 2).

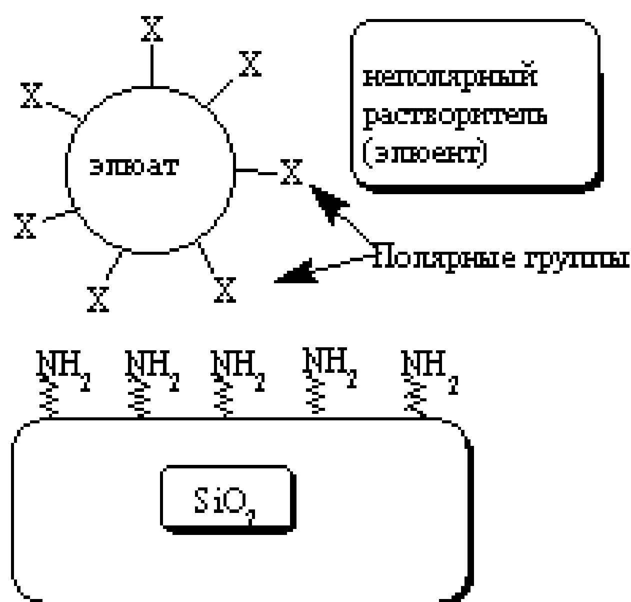


Fig. 2 Distribution grafted phase chromatography (normal phase-variant).

The use of graft phases subtle control of sorption properties of the stationary phase surface and to achieve high separation efficiency.

Reversed-phase liquid chromatography based on the distribution of the components between the polar eluent mixture and the apolar groups (long alkyl chain) grafted to the sorbent surface (Fig. 3).

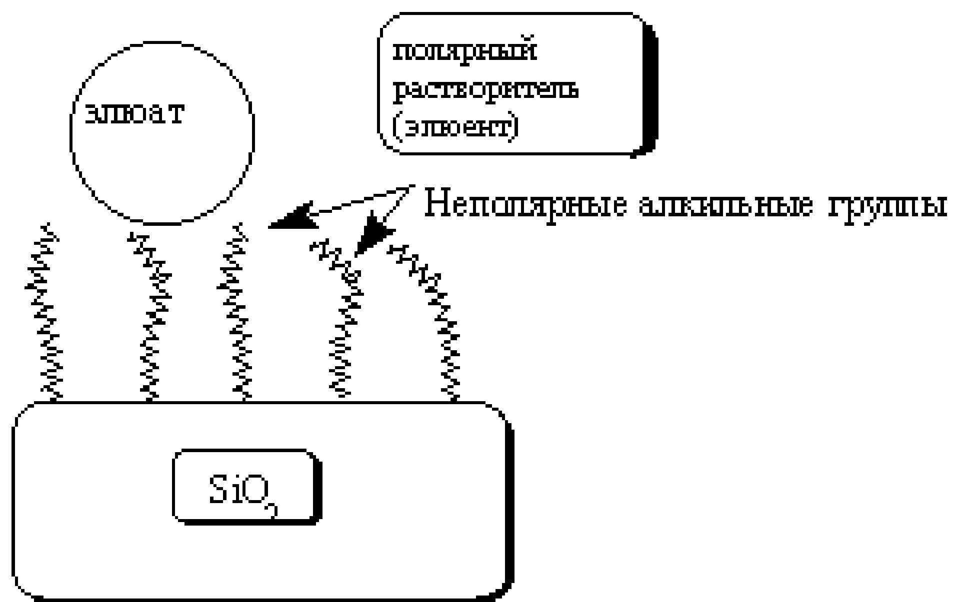
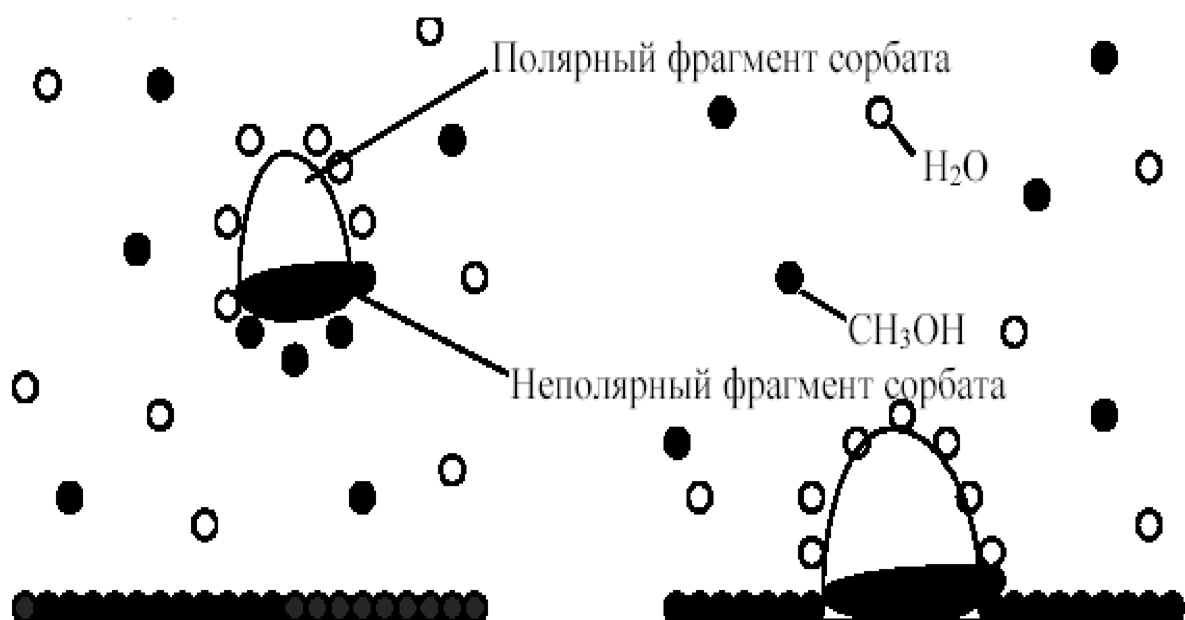


Fig. 3 Partition chromatography grafted phase (reversed-phase version).

Less commonly used variant liquid chromatography with applied phase when the liquid stationary phase is applied to the fixed carrier.



The reverse phase chromatography eluting strength increases with decreasing polarity of the solvent. The substances are retained in the column more than less of their polarity. The more of their hydrophobicity.

Exclusive (gel permeation) chromatography is a variant of liquid chromatography in which separation of the substances occurs through the distribution between the solvent molecules, located in the pores of the sorbent and the solvent, it passes between the particles.

The main parameters of chromatographic separation.

The main parameters of the chromatographic separation are the retention volume and retention time of the components of the mixture.

Retention time tR - is the elapsed time from the moment the sample introduction into the column corresponding to the peak maximum output. Multiplying the retention time on the volumetric flow rate of the eluent F, we obtain the retention volume VR:

$$VR = tR \cdot F_{\omega};$$

Corrected retention time - the time elapsed since the onset of the peak maximum is not sorbed component to the peak corresponding to the compound:

$$tR' = tR - t0;$$

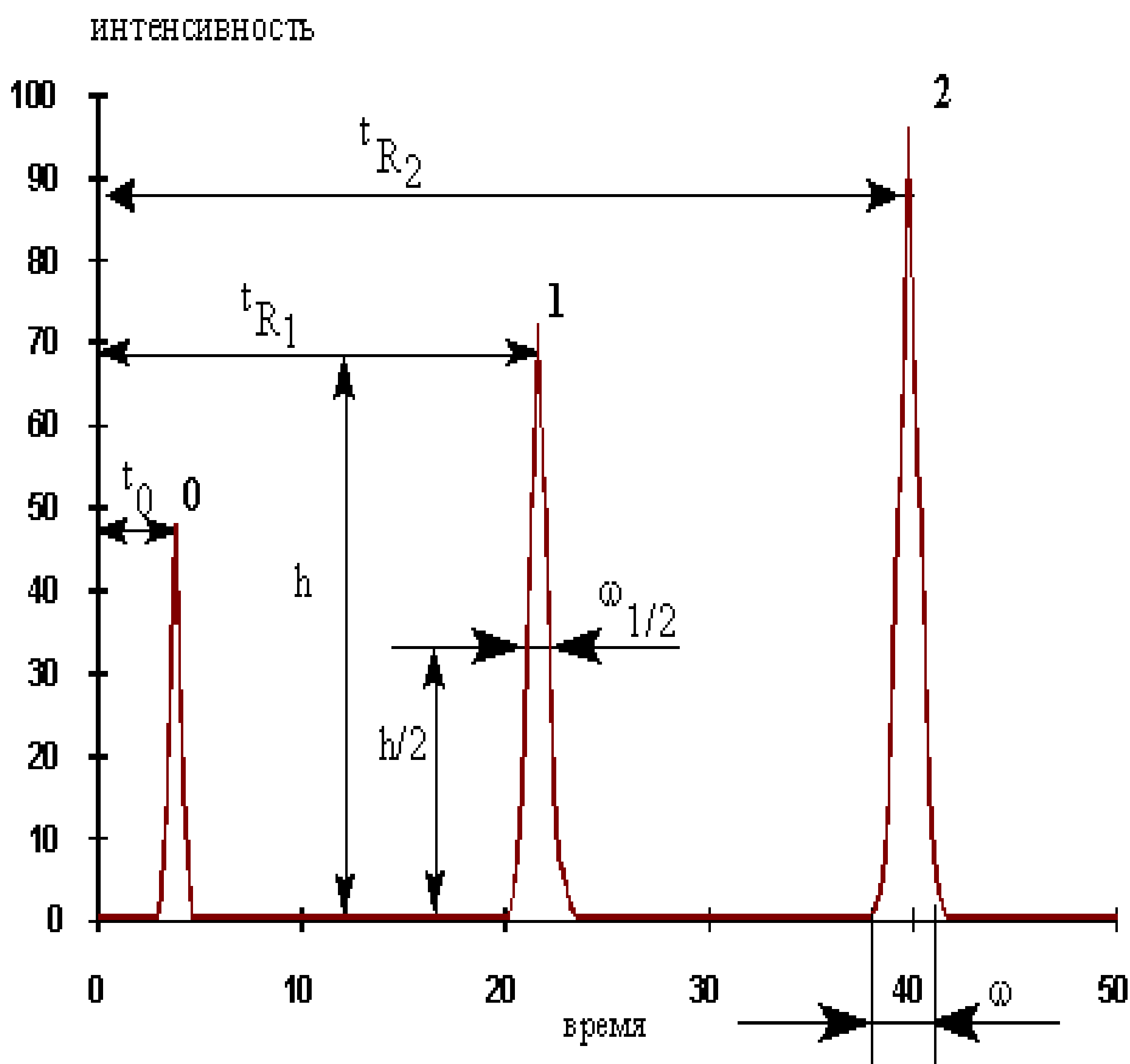
Reproduced or corrected retention volume - is the amount of the retention adjusted for dead volume V0 column, ie, on the amount of retention is not sorbed components:

$$VR' = VR - V0;$$

Retention is also a characteristic of the capacity factor k '.
It is defined as the ratio of the mass of material in the stationary phase to the weight of the substance in the mobile phase: k '= MH / mp;

The value of k 'is easily determined from the chromatogram:

$$k' = \frac{t_R - t_0}{t_0}$$



The most important parameters chromatographic separation are the efficiency and selectivity.

The effectiveness of the column, measure the height of a theoretical plate (Watts) and is inversely proportional to their number (N). That efficiency is higher than the peak thinner material exiting at the same retention time. Efficiency value can be calculated from the chromatogram of the following formula:

$$N = 5.54 \cdot (tR / \omega\, 1/2)^2,$$

where tR - retention time

w 1/2 - width of the peak in the middle of the height

Knowing the number of theoretical plates, coming to the column, the column length L, and the mean grain diameter of the sorbent dc, easily heights equivalent to a theoretical plate (HETP), and the reduced height (PHETP):

$$HETP = L/N$$

$$PHETP = HETP / d_c$$

These characteristics make it possible to compare the effectiveness of different types of columns, to evaluate the quality of the sorbent and the quality of filling column.

Selectivity dividing two substances is determined by according to the equation:

$$\alpha = \frac{t_{R1} - t_0}{t_{R2} - t_0}$$

When considering the separation of the mixture of the two components is also an important parameter is the degree of separation of the RS:

$$R_S = 2 \frac{t_{R2} - t_{R1}}{\omega_1 + \omega_2}$$

The peaks are considered authorized if the RS value is greater than or equal to 1.5. The basic chromatographic parameters connects the following equation to resolve:

$$R_S = \frac{1}{4} \frac{\alpha - 1}{\alpha} \frac{k'_2}{1 + k'_2} \sqrt{N_2}$$

The factors that determine the selectivity of separation are:

• chemical nature of sorbent;

• the composition of the solvent and its modifiers;

• The chemical structure and properties of the components of the separated mixture;

• column temperature.

Apparatus for liquid chromatography.

In modern liquid chromatography using devices of varying complexity - from the simplest systems to high-end chromatographs equipped with various additional devices.

Fig. 5 is a block diagram of a liquid chromatography comprising a minimum set of components, in one form or another are present in all the chromatographic system.

Advantages gradient elution:

- Simultaneous determination of weakly and strongly retained components.

- Reduced analysis time.

- Improved peak shape strongly retained components and lowering the detection limit.

- Removal of strongly-held impurities with column.

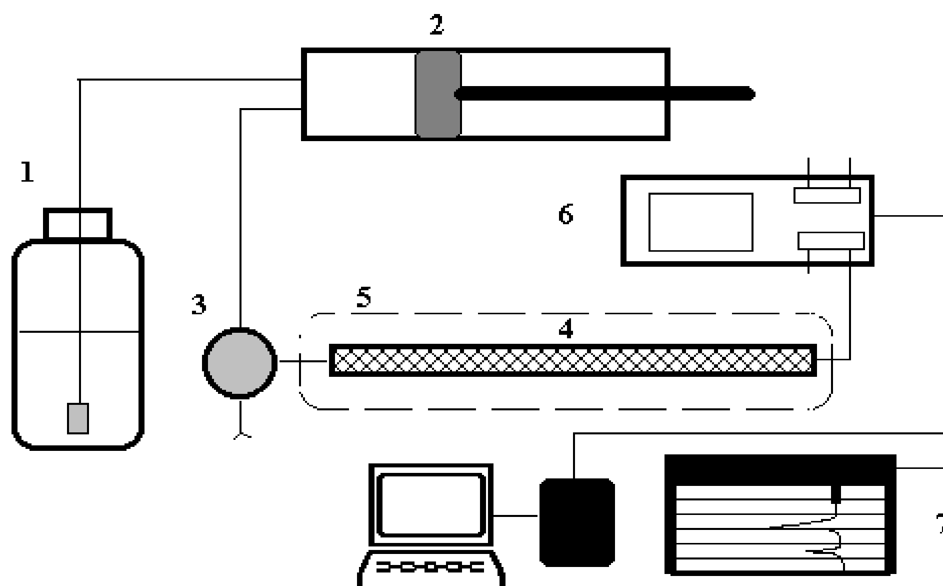


Fig. 5. A block diagram of a liquid chromatography.

Pump

It is intended to create a continuous flow of solvent. Its design is determined primarily on the system operating pressure. To operate in the range of 10-500 MPa using a plunger pump (syringe) or piston type.

The disadvantage of the first is the need for periodic stops to fill the eluent, and the second - a large complexity of the design and, as a consequence, the high price.

For simple systems with low operating pressures of 1-5 MPa successfully used inexpensive peristaltic pumps, but as this is difficult to achieve constant pressure and flow rate, their use is limited to preparative tasks.

Injector

It provides input sample mixture components to be separated on a column of sufficiently high reproducibility. (Figure 6 - Classification of devices for sample introduction). Simple sample injection system - "stop-flow" requires stopping the pump, and therefore, are less convenient than the loop dispensers designed Reodyne firm.

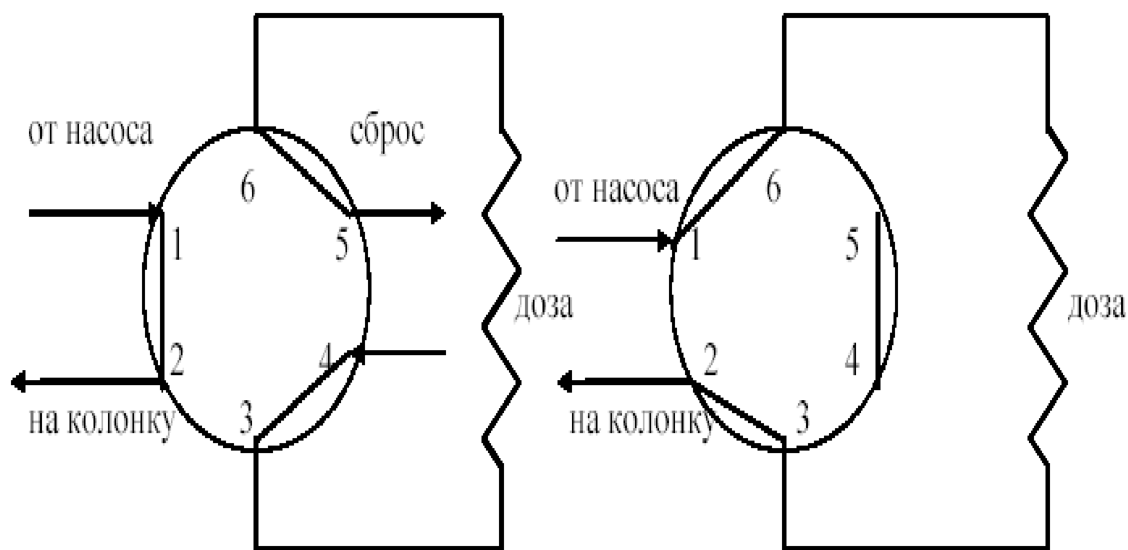


Fig. 6 Classification of devices for sample introduction

Column

HPLC are thick-walled stainless steel pipe capable of withstanding high pressure. An importantrole played density and uniformity of the sorbent in a column. For liquid chromatography, low pressure successfully use thick-walled glass columns. constant temperature by the thermostat.

Detectors

Detector - a device for detection and quantification of emerging from the column in the mobile phase flow assay mixture components.

Registration of substances carried out by converting an electric signal changes in the composition of the mobile phase.

Classification detectors

Universal (react to changes in the properties of the mobile phase) and selective (react to the presence of the analyte)

Destructive and non-destructive

Stream (sensitivity depends on the amount of material in the cell) and concentration (sensitivity depends on the concentration of the component in the eluent) to liquid chromatography have flow cell, in which the continuous measurement of any property of the flowing eluant.

The most popular types of general purpose detectors are refractometers measuring the refractive index and spectrophotometric detectors determining the optical density of the solvent at a fixed wavelength (typically in the ultraviolet region).

The advantages of refractometers (spectrophotometers and disadvantages) should include a low sensitivity to the type of defined compounds, which may or may not contain chromophore groups.

On the other hand, the application is limited refractometers isocratic system (constant eluent composition), so that the use of a solvent gradient in this case impossible.

Table. 6 Types of detectors

Detector	Measure the properties of the mobile phase	Selectivity
1. Filter photometric detector	The absorbance at a specific wavelength, which passes the filter	High
2. Spectrophotometric detector	The optical density at the selected length wave monochromator	High
3. Refractometric detector	The difference between the refractive indices of the solvent and the sample solution	Low
4. Fluorimetric detector	The emission intensity of the sample molecules in the eluent	Very high
5. Amperometric detector	Electric current of oxidation or reduction of compounds of electrochemically active	Very high
6. Conductometric detector	Conductivity ions of the sample in the eluent (water)	Low

The highest selectivity different mass spectrometry detectors.

Basic requirements to the detectors:

- Low noise and drift of the baseline.

- High sensitivity.

- Fast response.

- Wide operating range.

- Small dead volume.

- The design of the cell, preventing erosion zones and the formation of bubbles.

- Low sensitivity to changes in the background parameters (temperature, speed of the eluent, the percentage of organic modifier).

- Simplicity and reliability of the design.

- The applicability to a wide range of objects.

- Non-destructive.

The recording system in the simplest case consists of a differential amplifier and a recorder. It is also desirable presence of integrator allowing to calculate the relative areas of the peaks obtained.

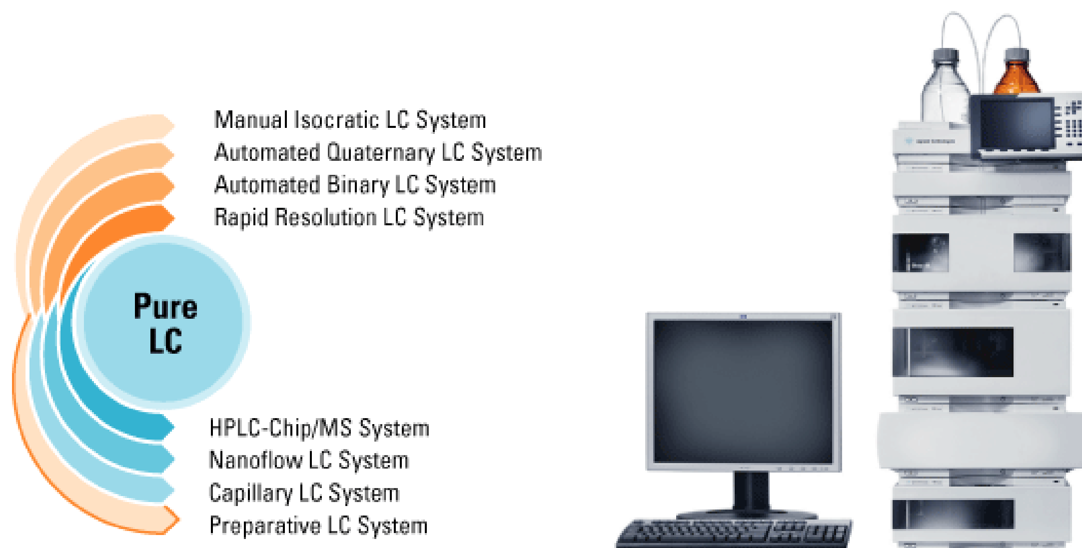
In complex systems, a chromatographic interface unit that connects to a personal computer chromatograph (8) (ADC). It provides not only the collection and processing of information, but also controls the device.

Filter the eluent water pump vacuum through a fine strainer to remove the trace Schott and degassing.

First comes the air ("zero volume")

Examples of modern devices

Modern instruments have a modular character



Agilent 1200 Series of Agilemt Technologies

Manually isocratic system:

- The flow rate to 10 mL / min with a wide selection of columns and size of applications.

- Possibility of «Upgrade» to automated 4-component gradient systems.

- Automated 4-component gradient systems
High-speed system with high-resolution liquid chromatography

- Up to 60% higher resolution and 20 times faster than conventional HPLC

- The flow rate to 5 mL / min

- Full compatibility with existing methods

- Ideally suited for use columns ZORBAX Rapid Resolution HT 1.8 µm columns

- Productivity 2000 samples per day

HPLC-Chip/MS System

— For reliable high sensitivity nanospray LC/MS

— Up to 3500 times more sensitive compared to conventional LC

— No peak dispersion for uncompromised chromatographic performance

- Sample preparation and separation columns, connection capillaries, fittings and nanospray emitter directly integrated on polymer chip

Nanoflow LC System

- Unsurpassed nanoflow performance and stability
- Up to 3500 times more sensitive compared to conventional LC
- Typical flow rates 0.1-1 μL/min, extendable up to 2.5 mL/min
- Compatibility with third-party MS platforms

Capillary LC System

Enter the world of low flow applications:

- Up to 500 times more sensitive compared to conventional LC
- Typical flow rates 1-100 μL/min, extendable up to 2.5 mL/min
- Advanced diode array detection from 190-950 nm

Another popular company is the manufacturer chromatographs Shimadzu.

Amino acids

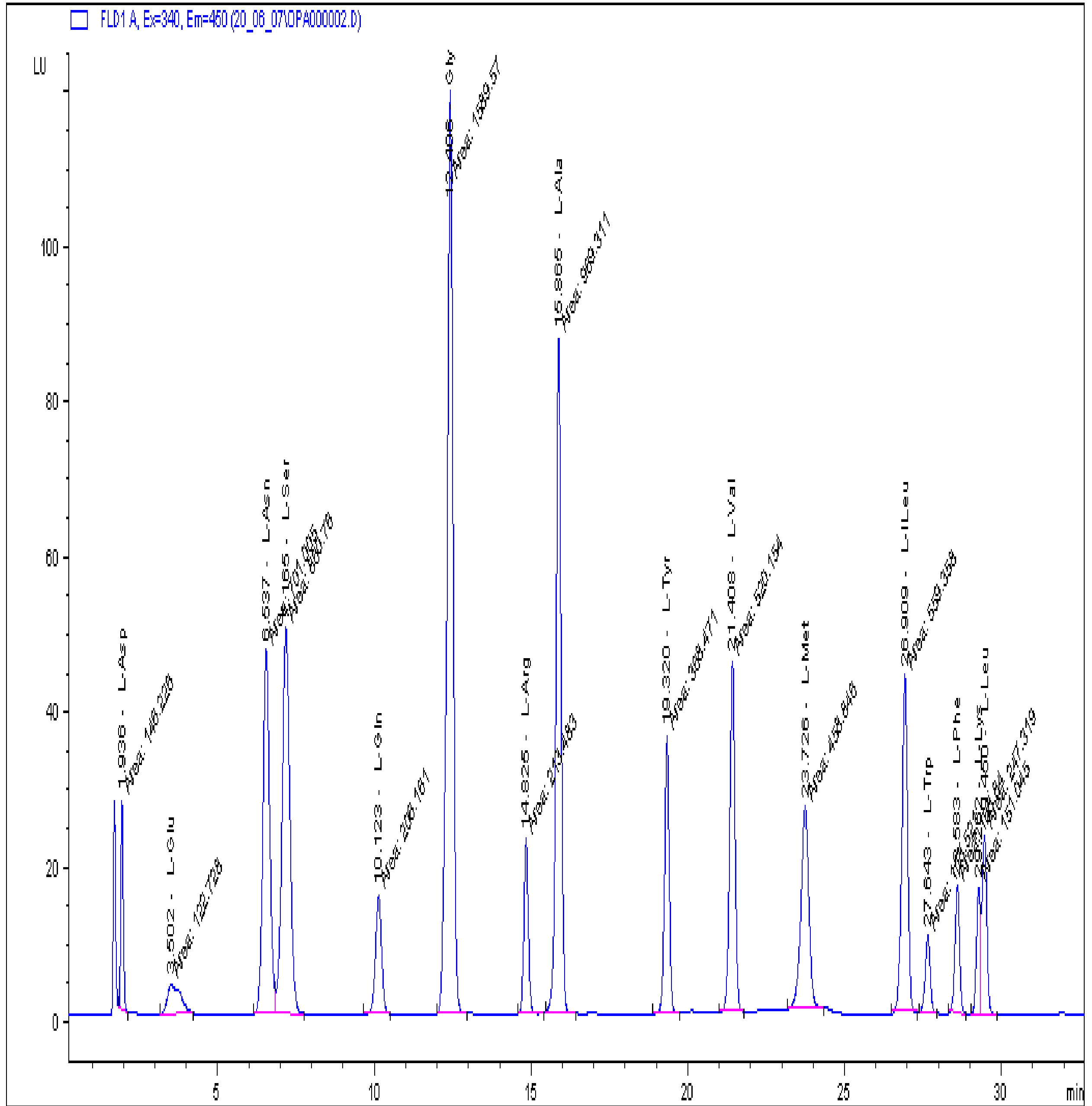


Fig. With 7 amino acids FLD chromatogram after derivatization with ortho-phthalic anhydride

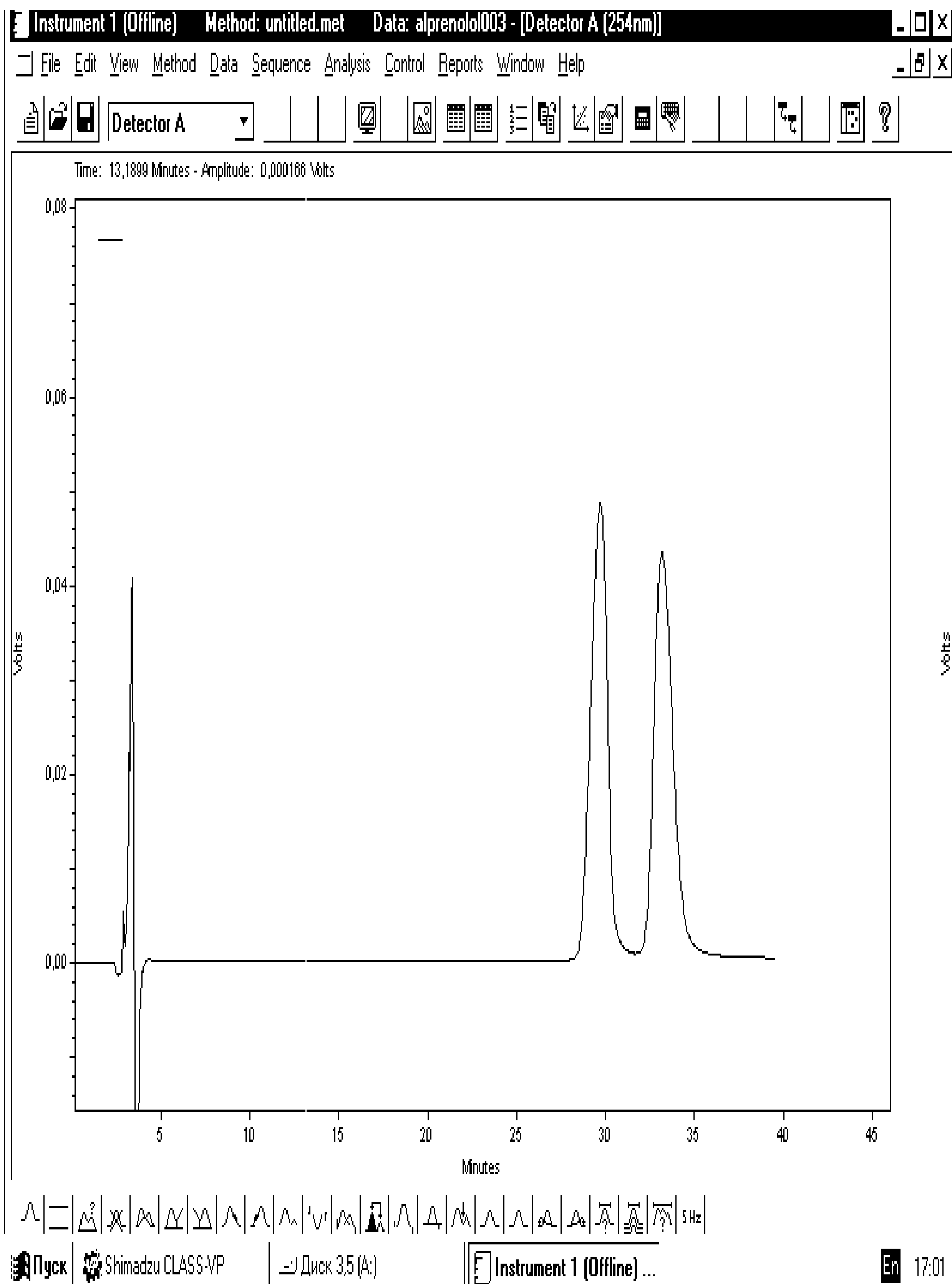


Fig. 8 Aprenolol

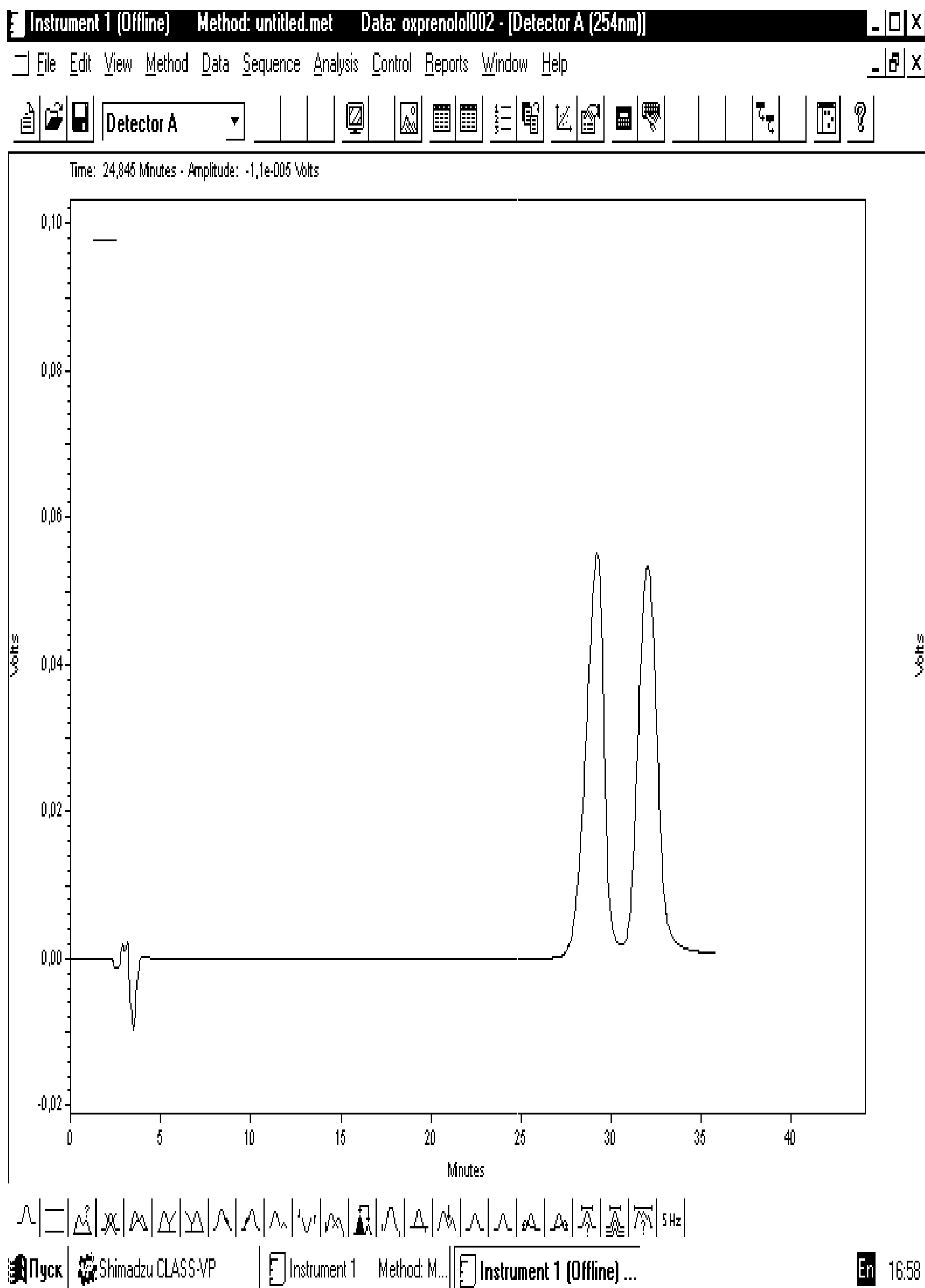


Fig. 9 Oxprenolol

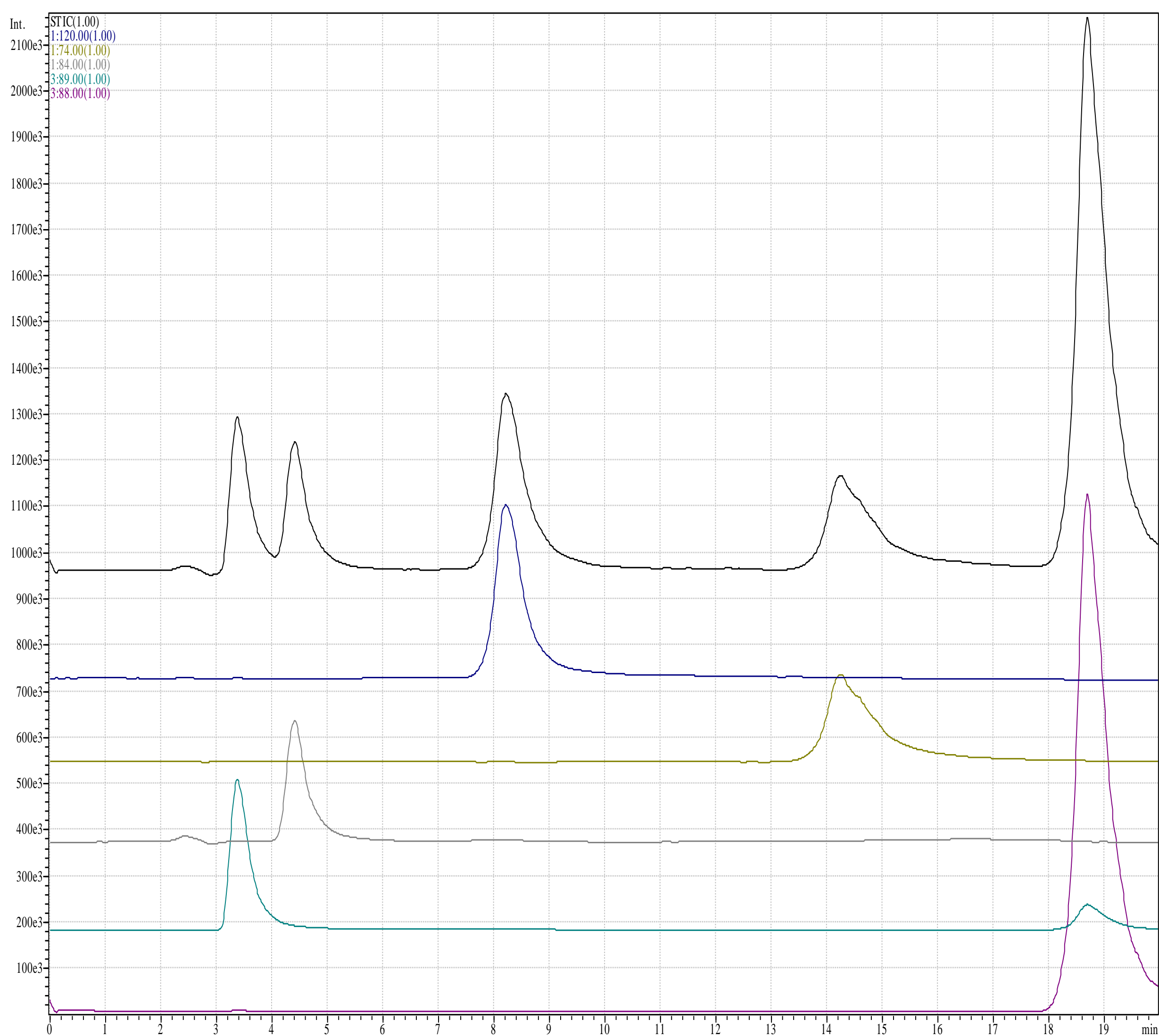


Fig. 10 HPLC-MS

- 2,2-dimethyl-1-formyl hydrazine
- 1-methyl-1,2,4-triazole
- 1,2,3-benzotriazole (internal standard)
- Diethylamine
- N, N-dimethyl guanidine

Identification

The relative retention time - is the ratio of the retention time of the analyte to the retention time of a substance taken as a standard.

According HFC Identification carried out as follows:

- Comparison of the retention time of the analyte in the test sample and reference solution.
- Comparing the relative retention times of the analyte in the test sample and reference solution.
- A comparison test sample chromatogram with the chromatogram of the reference solution with chromatogram or given in a separate article.

Quantitative determination

Absolute calibration

Test solution and reference solution alternately chromatography to yield at least 5 chromatograms. For the test solution and reference solution calculated the average values of the areas or heights of the peaks of analytes. Based on these results is calculated analyte concentration.

The method of internal standard

For each chromatogram is first calculated area ratio or peak heights for the area or height of the internal standard peak. Averaged ratio obtained for the sample solution and the reference solution and the found average values calculated analyte concentration.

SECTION III.
Gas chromatography-mass spectrometry. THEORETICAL BASIS AND THEIR USE

Plan

- Principle of the method
  - Equipment
  - Interfaces
  - Methods of ionization
- Mass analyzer
- Application to determine the composition and structure
- Application of quantitative analysis

Principle of the method

1956 - MakLaferti Golko and create the first gas chromatography-mass spectrometer

Docking gas chromatograph and mass spectrometer was completely logical, since both methods are used to analyze mixtures of organic compounds in the gas phase, and approximately equipotent sensitivity. The only problem for the unification of the two methods was working pressure.

The gas chromatograph is operated at atmospheric pressure and the mass spectrometer - under high vacuum (10-6- 10-7 mmHg). Basic principles of docking were formulated and implemented in the late 50s.
The method is primarily intended for the analysis of complex mixtures of organic compounds.

Gas chromatography mass spectrometry is to be separated by column chromatograph with serial output components from the column into the ion source of the mass spectrometer where they are ionized in the ion source, separating the resulting ions to the mass analyzer, and measuring the intensity values of m / z with a detector.

Interfaces

Initial problems associated with insufficient capacity vacuum system with a packed column and workflow carrier gas 30 mL / min, molecular separators solved by setting different types.

These devices are placed between the outlet end of column chromatograph and the ion source the mass spectrometer was designed to enrich the analyte sample by selective pumping considerably lighter carrier gas (hydrogen, helium).

The presence of the carrier gas is undesirable, as the carrier gas is the largest contributor to the creation of high pressure at the outlet of the column.

As GC interfaces mainly used:

• Membranes of silicone rubber,

• Diffusion tubes

• The molecular jet separator

Currently, molecular jet separator used for docking the quartz capillary and packed columns of large diameter (> 0.5 mm) with a mass spectrometer.

The principle of the device is based on the law of conservation of momentum. The inkjet separator helium molecules are separated from the heavier molecules assay mixture.

The outlet nozzle has a very small diameter, so gas velocity exiting the GC column, close to supersonic.

The analyte, which has a lot of movement, passes the distance between the two nozzles, and the lighter helium molecules deviate from the rectilinear motion and bilge pump.

The advent of more powerful vacuum system and capillary columns with smaller streams (0.5-2.0 ml / min) greatly facilitated the task, and the replacement of metal or glass, which is made columns in the fused silica allowed to enter the end of the column directly into the ion source. All this has made the GC-MS method is simple and effective.

Disadvantages direct entry:

- The need to provide high resistance coating stationary phase capillary column (crosslinked it is usually necessary to use stationary phases), since the interface maintains a temperature slightly above the maximum temperature thermostat columns.

- Shift of retention time compared to detectors operating at atmospheric pressure. The fact that the speaker is connected to a vacuum, but has little effect on chromatographic resolution.

Methods of ionization

Electron ionization (electron impact ionization).

In the EI sample molecules entering into the ion source from the GC column, ionized flux of thermal electrons emitted from a tungsten or rhenium filament

(cathode) and accelerated toward the anode. An electron flying near its molecule excites the electron shell.

Own the electrons of the molecule move to higher orbitals, and can leave the molecule. Chemical bonds can thus burst. The resulting ions.

Since the distribution of the internal energy directly affects the shape of the mass spectrum, and highly dependent on the energy of the electron beam, the latter is normally set at the standard level of 70 eV.

<u>Method of electron ionization gives rich fragments of the mass spectra, which provide structural information.</u>

This is due to the following factors:

• electron energy has a value at which ions are formed as much as possible.

• Change of the mass spectrum only slightly due to the electron beam energy changes. Corollary - if there is no molecular ion peak in the spectrum at an energy of ionizing electrons 70 eV, it is not his testimonies and at lower electron energy.

• Can be formed relatively large molecular ion and fragments intense peaks (depending on the molecular structure).

• In electronic form as well blow and negative ions, but the quality of the spectra are usually very poor. They maloinensivny and uninformative.

• The distribution of the internal energy of the ions is almost the same for different devices, making the range of practically independent of hardware.

Spectra were collected in a library and used for identification.

<u>Chemical ionization.</u>

Usually gives a very simple mass spectra, which provide information on molecular weight.

The substance is ionized when the gas-phase ion-molecule reactions. For this purpose the ion source at a relatively high pressure (0.01-2 mm Hg) is introduced reactant gas (methane, isobutane, or ammonia water), which as a result of ionization by electron impact generated ions.

The molecules are ionized directly by a number of reactions with the reactant gas, in which during the clashes in the analyte molecules is transferred to a small portion of energy with a narrow distribution. This explains why the CI is often called "soft" ionization method.

Soft ionization results in less fragmentation and greater molecular ion peak intensities as compared to the EI.

Advantages:

- Low level of fragmentation increases the sensitivity of CI-MS.

- The ability to adjust the selectivity by selection of the reactant gas.

- Depending on the reactant gas can be obtained robust spectra or positive or negative ions.

Disadvantages:

- Provides only limited structural information.

- The dependence of the spectrum on the conditions, especially the pressure in the ion source of various devices does not give a library of spectra.

- Soil analysis on biphenyls in registration mode, negative ions in methane CI.

- Obviously the more selectivity when using the CI compared to the EI (interfering matrix effect).

Comparison of the selectivity of EI methods (Figure 11) and HI (Fig. 12)

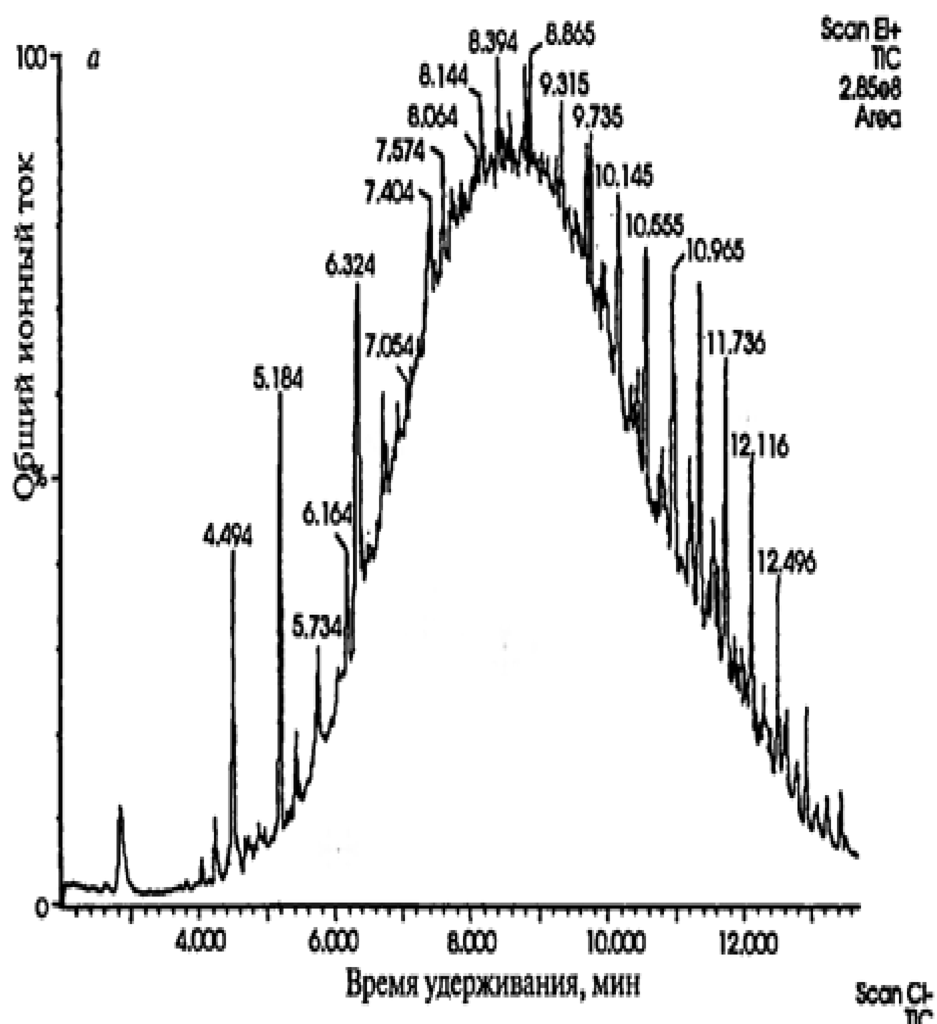


Fig. 11 The chromatogram for total ion current in the electron impact (soil analysis biphenyls) mode in EI mode (positively charged ions).

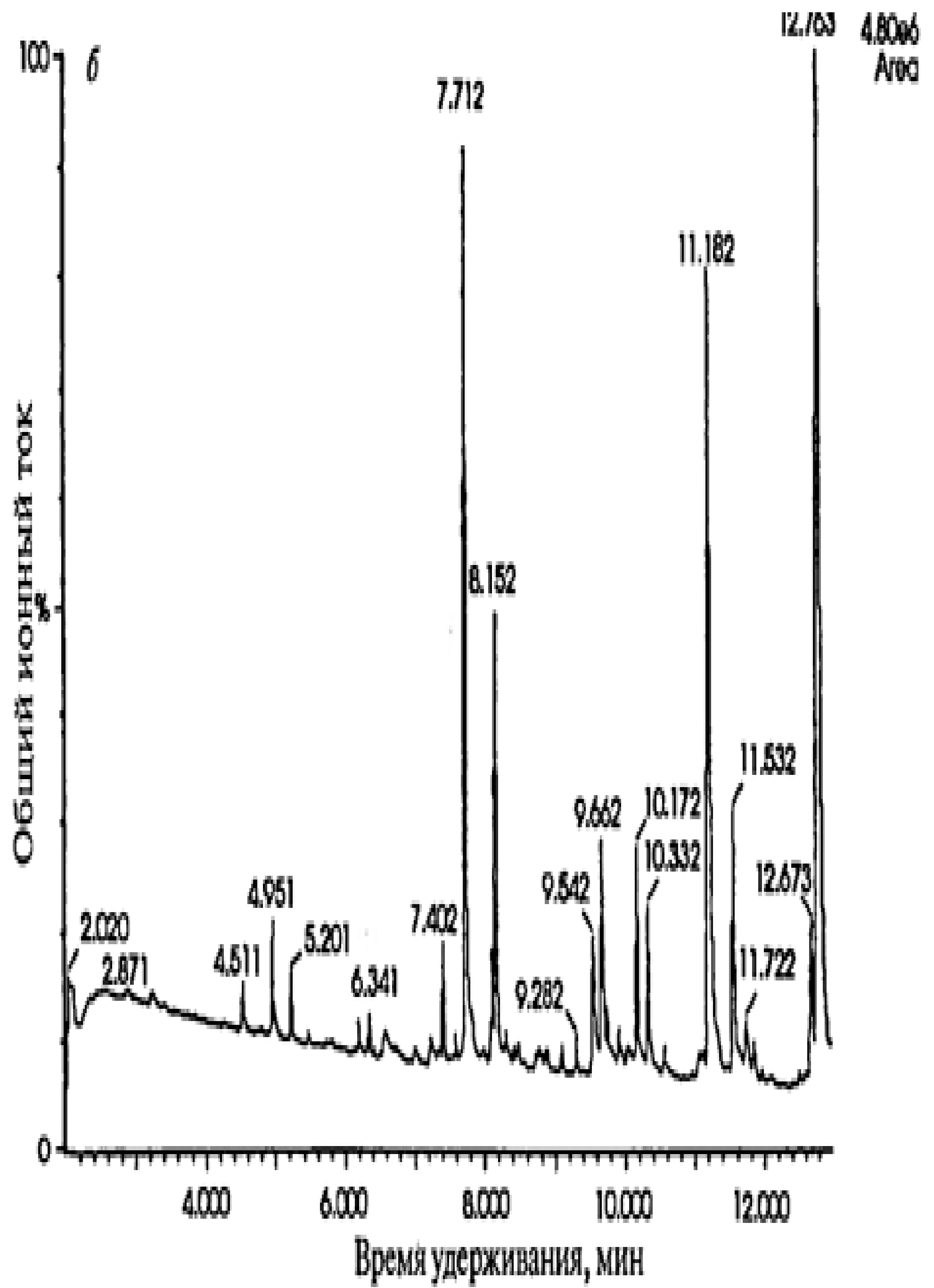


Figure 12. The chromatogram for the total ion current in the electron impact mode

Characteristics of mass spectrometers and mass spectrometry detectors

The most important technical characteristics of mass spectrometers are sensitivity, dynamic range, resolution, and scan speed.

The dynamic range (in mass spectrometry). If we analyze a mixture containing 99.99% of one compound or any element and 0.01% of any impurity, we must be sure that properly define both. To be sure of determining components in this example, must have a range of linearity in the order of 4.

Modern mass spectrometers for analysis of organic characterized by a dynamic range of 5-6 orders of magnitude, and mass spectrometers for elemental analysis 9-10 orders of magnitude. The dynamic range of 10 orders of magnitude means that the impurity in the sample will be visible even when it is 10 milligrams per 10 tons.

Resolution (resolving power) Mass Spectroscopy - quantitative measure characterizing analyzer with the ability to separate ions of adjacent mass or, in other words, to determine the precise mass ion.

For magnetic mass analyzer, for which the distance between the peaks of the mass spectrum does not depend on the mass of the ions, the resolution is a value equal to M / DM. This value is typically determined at 10% peak height.

For example, the resolution of 1000 means that the peaks with masses of 100.0 and 100.1 are separated from each other, that is, do not overlap up to 10% of the height.

For analyzers where the distance between the peaks changes in the working mass range (the larger the mass, the smaller the distance), such as quadrupole analyzers, ion traps analyzers vremyaprolëtnye, strictly speaking, the resolution has a different meaning.

The resolution, defined as M / DM, in this case the specific weight characteristic.

It makes sense to characterize these mass analyzers, the width of the peaks, the value remains constant throughout the range of masses. This width of the peaks, typically measured at 50% of their height.

For these devices the peak width at half maximum equal to 1 is a good indicator means that a mass analyzer is able to distinguish between the nominal mass, characterized by one almost all its operating range.

Nominal mass or mass number called soon to accurate mass ion integer in the scale atomic mass units. For example, the mass of the hydrogen ion H + is equal to 1.00787, and its mass number is 1.

A mass analyzer such that, basically, the nominal weight is measured is called the low-resolution analyzers. Ion traps a narrow range of masses can operate as a mass spectrometer of high resolution by providing a minimum separation of peaks spaced 1/4 a. e. m. of each other.

Mass spectrometers with double focusing (magnetic and electrostatic), ion cyclotron resonance - Instruments medium or high resolution.

Resolutions of a few thousand can also work using mass analyzers.

Resolution is closely related to another important characteristic - precision mass measurements. A simple example can illustrate the significance of this characteristic.

Molecular mass ions of nitrogen and carbon monoxide (CO) comprise 28.00615 and 27.99491, respectively (both are characterized by a mass number of 28).

These ions will be recorded by the mass spectrometer apart at a resolution of 2500, and the exact weight value will give the answer - which of the gas logs.

The most important characteristic in the analysis of organic compounds - this sensitivity. In order to achieve the greatest possible improvement in the sensitivity of the signal to noise ratio by resorting to the detection of certain selected ions.

The gain in sensitivity and selectivity while enormous, but when using a low resolution instrument has to sacrifice another important parameter - the accuracy.

You will need a lot more work to do to prove that this peak corresponds exactly to the component that you are interested in. How to solve this problem?

Use the highest resolution on the devices with a dual focus, which can achieve a high level of reliability without sacrificing sensitivity. Or using tandem mass spectrometry, where each peak corresponding to a single ion can be confirmed by the mass spectrum of the daughter ions.

Thus, an absolute record for the sensitivity of an organic gas chromatography-mass spectrometer with high resolution double focusing.

According to the characteristic of the combination of the sensitivity of reliably determining trace components are ion traps.

Classic quadrupole new generation devices have improved performance through a number of innovations Applied on them. For example, the use of curved quadrupole prefilter. It prevents ingress the neutral particles and the detector, thereby reducing noise.

The scanning speed. Mass analyzer passes ions with a certain mass and charge ratio at a certain time. In order to analyze all ions it should scan, that is, the parameters of its fields are in a given period of time to go through all the values that are required for the transmission to the detector of ions of interest.

This speed unrolling field called scanning speed and must be as much as possible (thus the scan time should be as small as possible), since the mass spectrometer should have time to measure the signal in a short time, for example during the exit of the chromatographic peaks, which can be 1 second.

The slowest mass analyzer is a magnet, its minimum scan time without much loss of sensitivity is a fraction of a second (MAT 95XP).

The quadrupole mass analyzer can expand range of tenths of a second (TSQ 7000, TRACE DSQ), and ion trap even faster (POLARIS, LCQADVANTAGE, LCQDECA).

The fastest mass analyzer is a time-of-flight (TEMPUS). It is able to record mass spectra at a rate of 40,000 per second.

Mass analyzers:

• Quadrupole mass filters
• Devices with a magnetic field
• Ion trap
• TOF
• Tandem

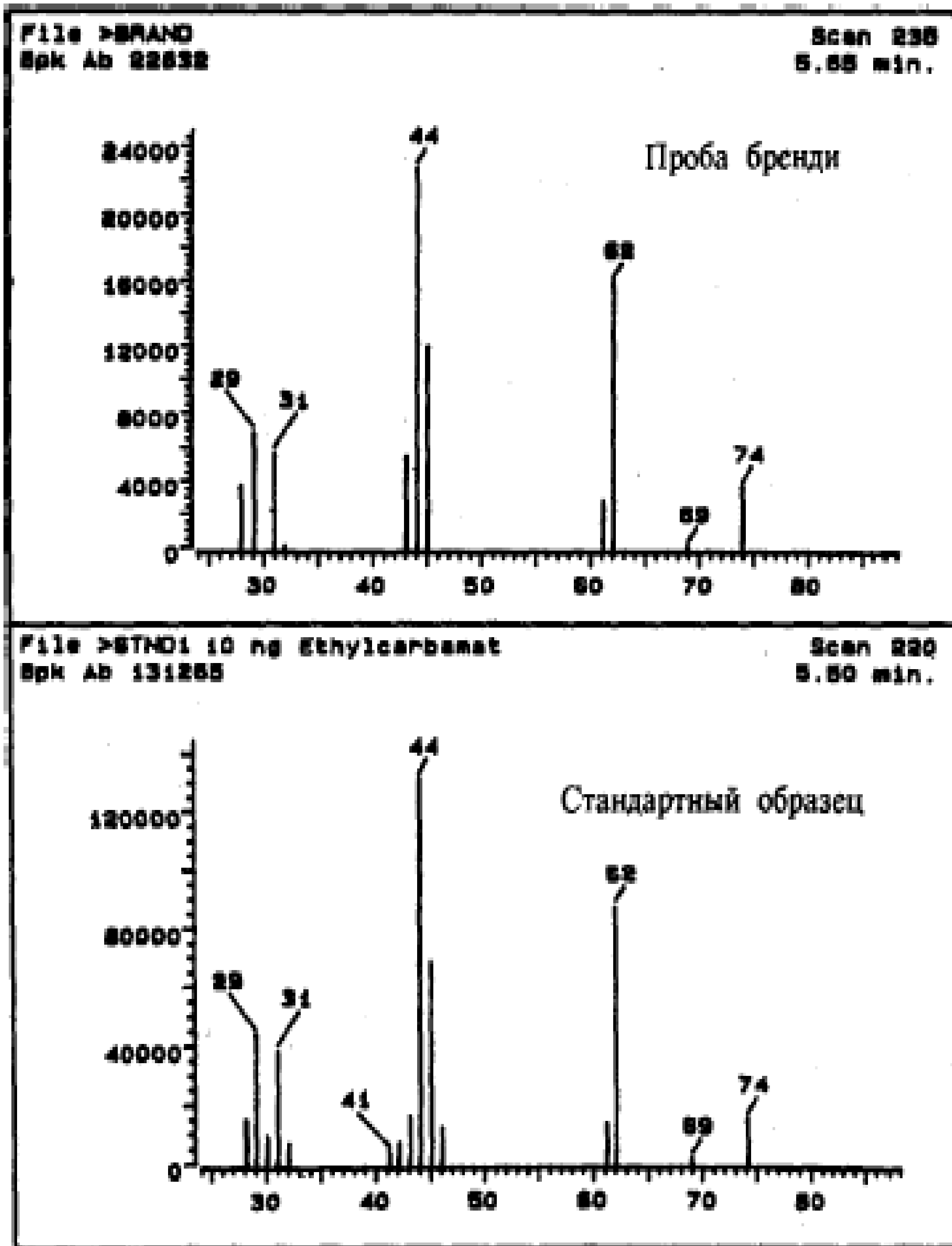


Fig. 13 Determination of ethyl carbamate in brandy

SECTION IV. Liquid chromatography-mass spectrometry.

THEORETICAL BASIS AND THEIR USE

Plan

- Principle
- Equipment
  - o Interfaces
  - o Methods of ionization
  - o Mass analyzers
- Application for establishing the composition and structure
- The use of quantitative analysis

Principle of the method

The use of high performance liquid chromatography allows to analyze heavy polar heat-labile compounds, but most of the detectors is not universal.

Recall that the main advantage of the mass selective detector is its versatility. Therefore, the search of the interface connecting the LC and MS was a very important task.

For the first time in 1974 Arpino, Baldwin and MakLafferti create the first liquid chromatography-mass spectrometer.

For a long time it was thought that it is impossible to combine liquid chromatography with mass spectrometric detection.

Large flow rate (-2 0.5 ml / min) leads to a high pressure liquid chromatography system and vacuum (10-6 mm Hg) in a mass spectrometer system has been difficult to combine.

Also necessary to introduce as much as possible in the eluate MS-detector in order to achieve maximum sensitivity, or in an interface should be held analyte enrichment.

The most often used in HPLC, methanol, acetonitrile and water.

1 ml of water, 1.24 ml of p forms. 1 ml of methanol 0.5 ml of forming a pair. Given that the vacuum of the mass spectrometer system can maintain the desired work pressure only if the inflow of vapor in the ion source does not exceed 10 ml / min, it is understood that the connector assembly (interface) LC-MS system must effectively enrich analyte approximately 100 times.

Approaches to solving the problem:

- Increase the pumping capacity of the vacuum-MS system;

- Removal of the solvent prior to introduction into the vacuum system;

- The separation of the eluate flow at the expense of sensitivity;

- Use microcolumns LC, allowing you to work efficiently with substantially lower flow rates.

Equipment

Interfaces

Band conveyer

Historically, the first interface can be considered as a conveyor belt, which appeared in 1976. Its main part is a thin ring polyimide tape.
The eluate from the column reaches the tape moving towards the ion source.

Advantages:

- Ability to use standard libraries electron impact mass spectra to identify the analytes.

- Ability to work with conventional liquid chromatography columns, because the method uses a flow rate of 1.5 ml / min.

- Inorganic salt used for the preparation do not interfere with analysis of the eluent.

- Direct input fluid

Fig. 14. Schematic diagram of the method of direct liquid injection.

To implement a direct input to set the column outlet orifice 2.5 mm. Providing in the ion source of analyte molecules undergo chemical ionization, while the solvent molecules act as the reactant gas.

Disadvantages:

- Low flow rate. Therefore it is necessary either to divide the flow by reducing the sensitivity about 20 times, or use narrow columns.

- Aperture constantly clogged.

- Particle Flow

Flow from the column is directed through the capillary into the glass diffuser, where the eluent turns into a cloud of tiny droplets, broken concentric stream of helium.

This cloud glass moves inside the vaporization chamber, the walls of which are heated and the pressure is maintained at slightly below atmospheric pressure. Thus there is a partial evaporation of the solvent, and the droplet size decreases.

Evaporating chamber ends narrow opening followed molecular beam separator with a vacuum pumping (Fig. 15).

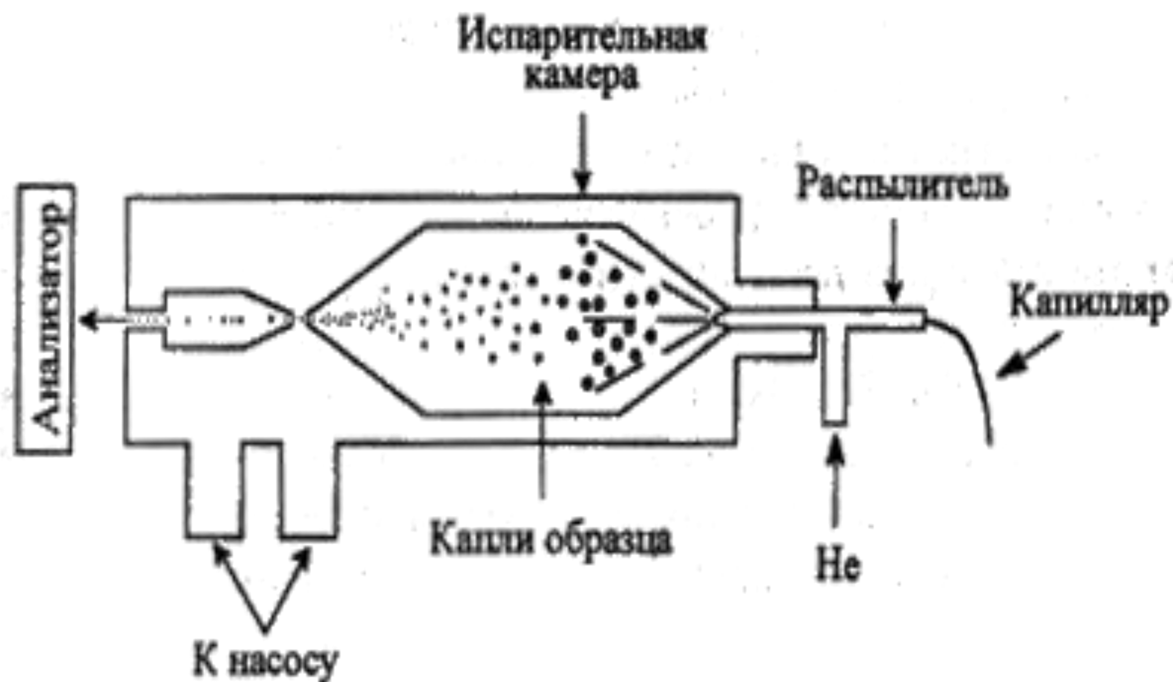


Fig. 15. The interface circuit with a stream of particles.

Departing from the evaporation chamber, particles acquire a high speed due to a narrowing of the flow. Since the heavier molecules analyte diffusion less susceptible than helium atoms and molecules of the solvent, and they are held in narrow inlet opening of the separator, and from it into the mass spectrometer.

The particles in the ion source are subjected to chemical ionization or electronic shock.

Advantages:

- Ability to use standard libraries electron impact mass spectra for identification of test substances

- Ability to work with conventional liquid chromatography columns, because the method uses a flow rate of 1.5 ml / min

- The sensitivity of the method at the level of nanograms (10-9 g).

Disadvantages:

- Inability to work with high-molecular, non-volatile and thermolabile compounds.

- Termosprey and plazmosprey.

Flow from the chromatograph column is sent to a heated capillary. The diameter of the capillary of 0.1 mm, which is significantly more than the opening for the direct liquid injection. This eliminates instances of clogging. The temperature in the capillary is maintained at the boiling point of the solvent.

As a result of capillary breaks the jet of steam that enters the ionization chamber. In the case of plasmatic spray voltage to the discharge electrode, or a beam of electrons creates chemical ionization conditions, the reagent gas play solvent vapors. If you turn off the electrode unit is in termospreya mode.

The fact that some of the compounds analyzed in the solution is in the form of positive and negative ions. Used as eluents water and methanol can be protonated or deprotonated substrate. To charge appeared also use supplements of electrolytes, such as ammonium acetate.

A jet of steam exiting the capillary, is a small spray of charged droplets. The heated ion source and running vacuum pumps make these drops when moving to decrease in size.

Ultimately field gradient on the droplets can reach critical values, which leads to the elimination of solvent shell and the appearance of free ions in the gas phase.

The push capacity directs the ions into a narrow opening leading to the analyzer, and the excess solvent is pumped pump.

Advantages:

- Ability to work with conventional liquid chromatography columns, because the method uses a flow rate of 1.5 ml / min;

- The high sensitivity of the method, and a wide range of units of nanograms to tens of micrograms;

- Ability to adjust the fragmentation of ions in the potential of changing the ejecting electrode.

Disadvantage:

- Poor reproducibility

- Do not use inorganic buffers to prepare the eluent.

Fig. 16. Schematic diagram of the thermal spray (plasma spray).

Modern methods of ionization

The most widely used interfaces that are based on the methods of ionization at atmospheric pressure.

LC-MS interfaces, ESI, APCI, APPI implement soft ionization techniques substances (without substantial fragmentation) that allows you to record the molecular ions of the test compounds.

Information about the molecular weight is one of the key parameters for the identification of substances, but no peaks of fragment ions often makes the interpretation of ambiguous results.
The possibility of fragmentation of molecular ions in order to obtain more information about their structure depends on the type of applied mass detector.

Ion fragmentation can be obtained as a result of their collisions with neutral nitrogen atoms. Collision Induced Dissosiation – CID. Inside the mass detector under low vacuum.

Since all ionization modes atmospheric composition sufficiently sensitive to the eluent, the kind of the mass spectrum depends on the chromatographic conditions.

That is why commercially available library of mass spectra (like GC-MS) is not currently established.

ESI

Electrospray interface.

I recall in 1984 - L.N. Gall, then Fenn published work electrospray method. In 2002, Fenn won the Nobel Prize.

The objects of analysis are ionized in the heat-labile molecule solution.

The principle of operation is based on the electrostatic spraying eluent. At the outlet of column liquid chromatograph eluent reported uncompensated charge by applying a voltage of several thousand volts. Further eluent enters the dispenser and is converted into fine mist droplets.

Countercurrent to the eluent stream of mist in the ionization chamber is supplied heated nitrogen. It goes along the outer channel. Droplets of fog eluent carrying uncompensated charges, evaporate quickly.

In the process of evaporation of the charge density on the surface of the droplets increases continuously and to achieve a certain limit, they are experiencing explosive fragmentation. This is repeated as long as the nitrogen in the ionization chamber are formed separate ions of analytes.

Some residual amount of the eluent is removed from the bottom of the ionization chamber to drain. The resulting ions under the influence of an electrostatic field in the inlet capillary rush mass detector.

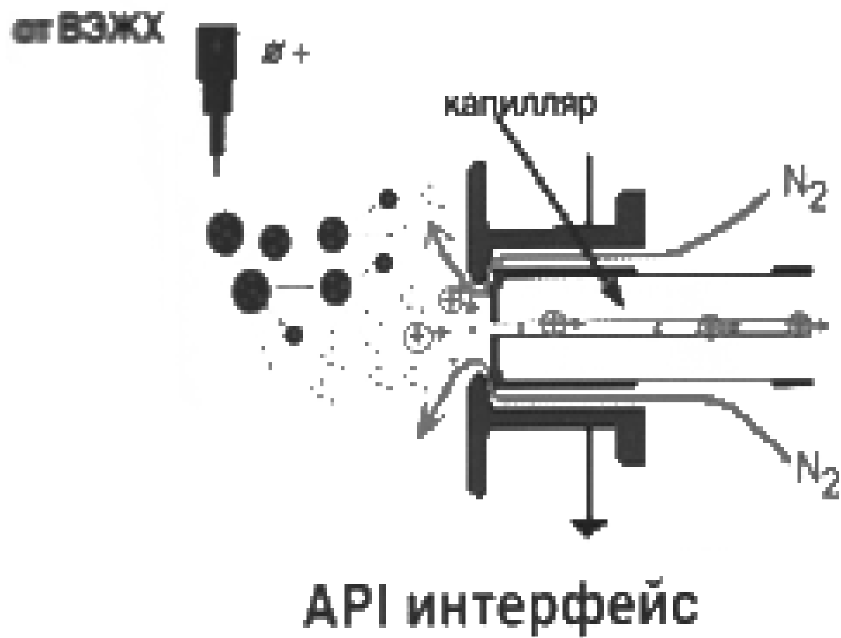


Fig. 17. The ion source (shown with permission Alsi Chrome representative Agilent Technologes).

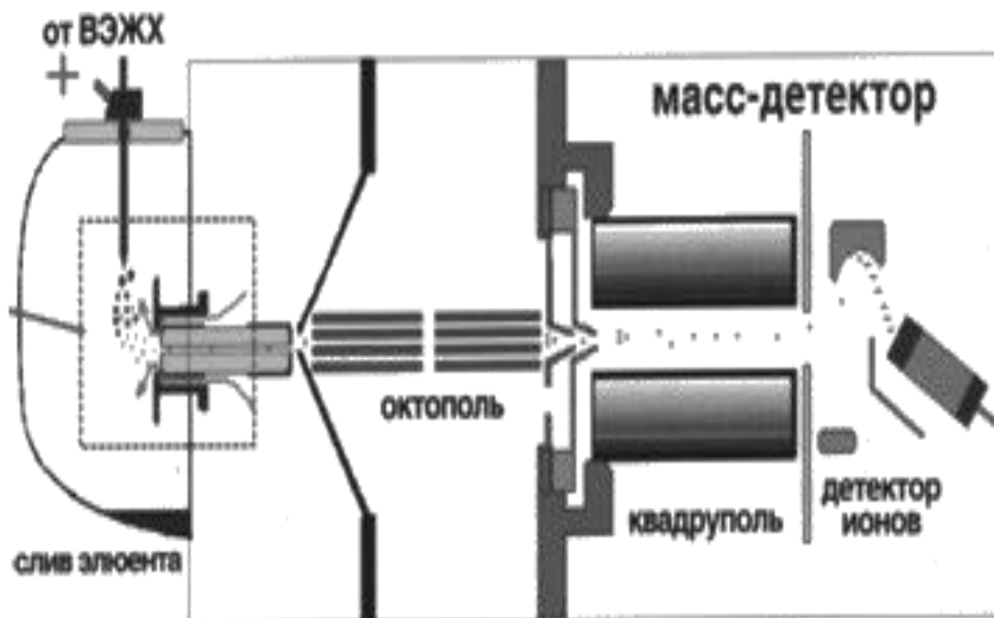


Fig. 18. The mass detector scheme with the ion source (shown with permission Alsi Chrome representative Agilent Technologes).

This interface desolvatiruet and ionizes the analyte application of a strong electric field at the same time of large molecules typically produced multiply charged molecular ions.

Having a range of masses measured by the thousands, mass detector equipped with ERI allow you to register the molecular ions of protein mass which reach tens or even hundreds of thousands. Since a mass spectrometer detects not the mass, mass to charge ratio in the operating range fall proteins carrying charges up to several thousands.

APCI

Chemical ionization at atmospheric pressure

The interface consists of a concentric air sprayer from which aerosol is fed directly into the heated tube of quartz or stainless steel.

The aerosol passes through a heated tube. During the movement of fog droplets down is their intense evaporation. Thus, in the corona discharge zone fall eluent molecules and analytes. Here in the first step preferably occurs field ionization of solvent molecules.

In the second stage the ionized solvent molecule transmit positive or negative charge of the analyte molecules, which under the action of the electrostatic field against the flow of the heated nitrogen during rush capillary inlet of the mass spectrometer.

Chemical ionization method is more stringent than electrospray, therefore in the mass spectra of analytes usually observed not only molecular ion peaks, but also some of the fragment ions carrying information about the composition of molecular analytes.

The ion source has proven itself when working with both non-polar and polar containing hetero atoms, molecules, relatively low molecular weight (2-4 kDa).

However, it turned out to be of little use for the ionization of proteins, peptides and oligonucleotides. Another disadvantage is degradation of thermolabile substances.

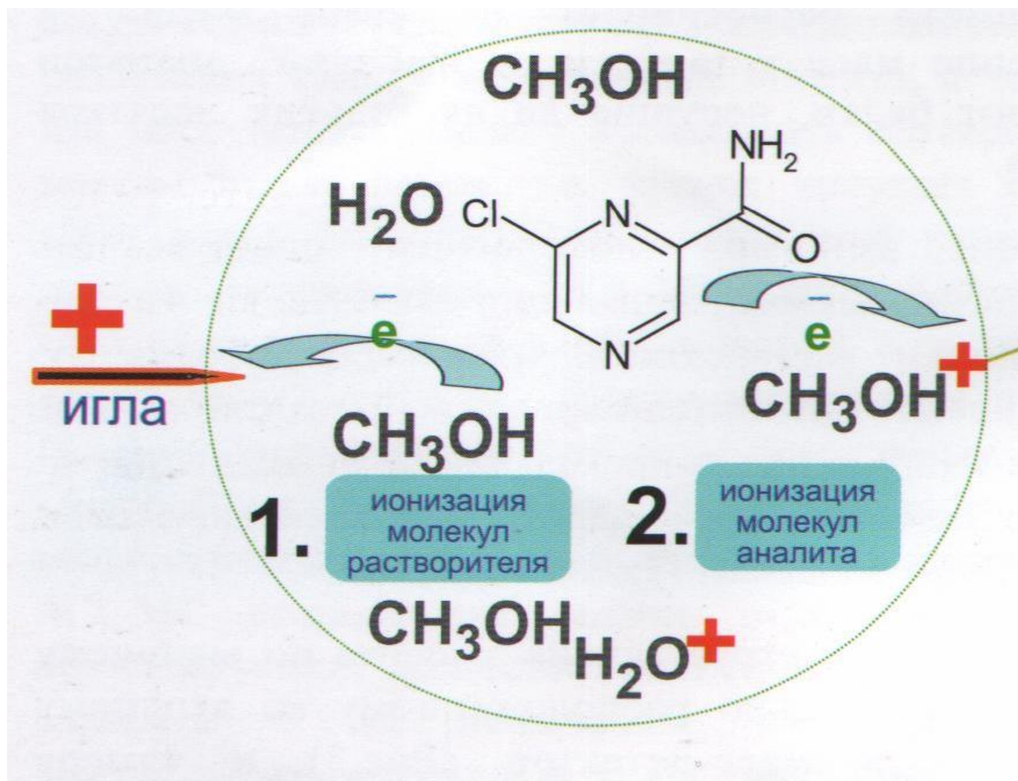


Fig. 19. Stages of chemical ionization.

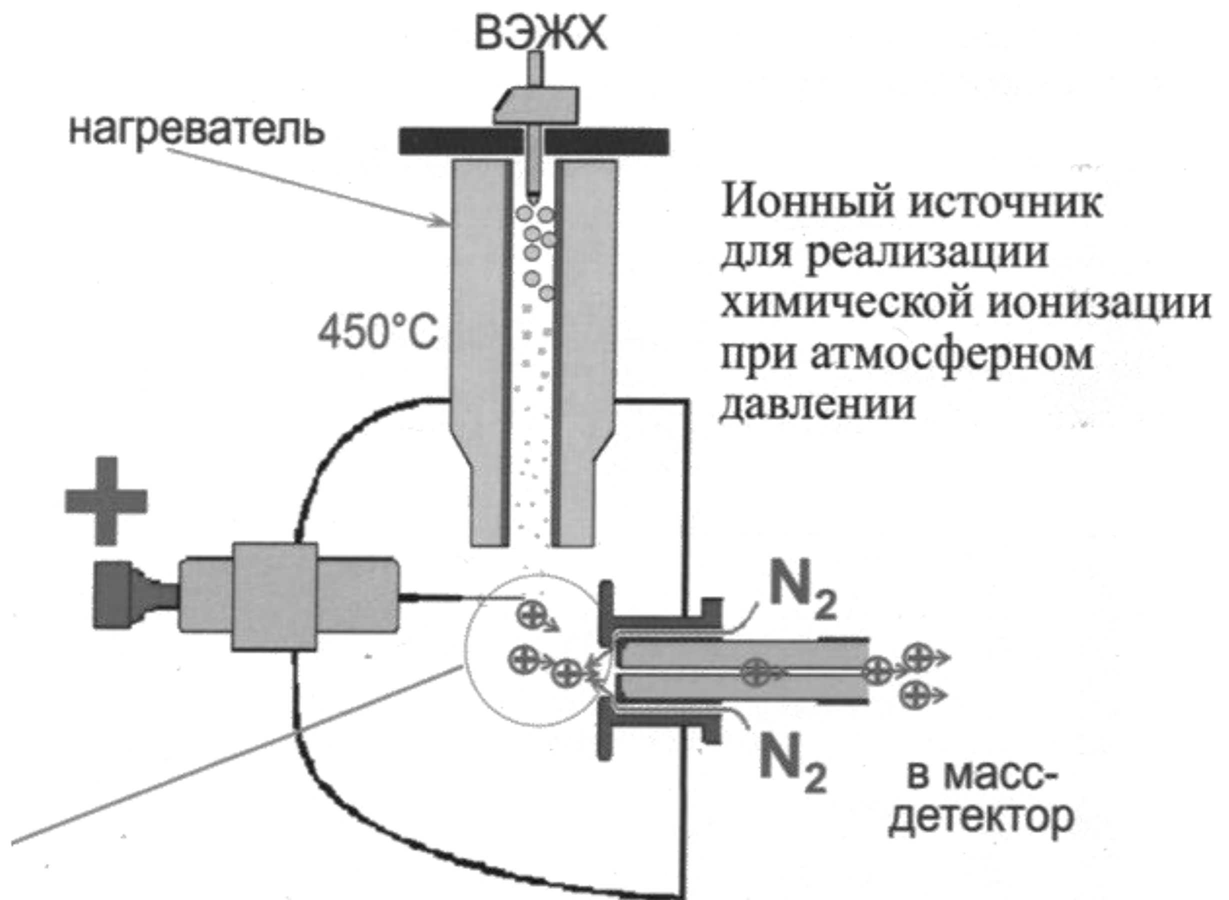


Fig. Scheme 19. Chemical Ionization ion source at atmospheric pressure.

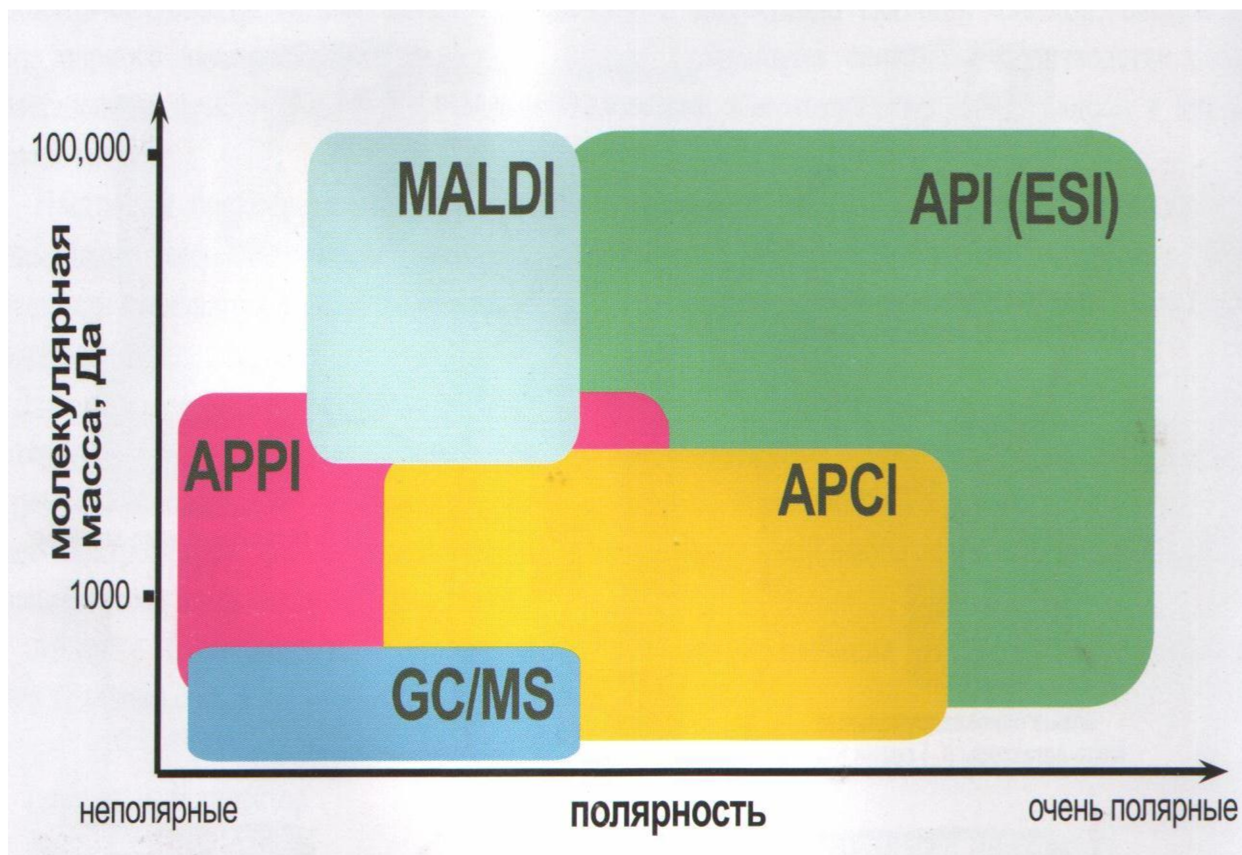


Fig. 20. Areas of application interfaces for substances of different polarity and molecular weight.

Mass analyzers:

• Q

• QQQ

• IT

• TOF

• Q-TOF

• Q-IT

простота пользования	идентификация	колич.анализ	чувствитель-ность	диапазон масс	разрешение
Q	Q-TOF	QQQ	QQQ	Q-TOF	TOF
QQQ	Ion Trap	Q	Ion Trap	TOF	Q-TOF
TOF	TOF	Q-TOF	Q	Q	QQQ
Ion Trap	QQQ	TOF	Q-TOF	Ion Trap	Q
Q-TOF	Q	Ion Trap	TOF	QQQ	Ion Trap

лучше, проще, точнее

Table. 7. Some comparative characteristics of LC-MS detectors

Application to determine the composition and structure

Qualitative determination of ingredients produced by their mass spectra and exit times. Using the HPLC-MS can confirm purity of the synthesized compounds.

Gross formula can be established by using high-resolution mass analyzers. Based fragmentation can confirm the structure of the compounds.

Application of quantitative analysis

There are masses of chromatography and mass fragmentography (SIM - registration with the specified ion m / z values).

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