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REVIEWS

Determination of Organic Impurities in Pharmaceutical Preparations

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Abstract—Data reported in the literature on methods for the determination of organic impurities in pharmaceutical preparations are systematized; the characteristics and possibilities of the methods are compared.

The intake of medicines can lead to adverse effects, which, in the majority of cases, are related to the entry of different impurities that occur in the remedy along with the pharmacologically active compound into the body. Impurities can have a strong adverse effect due to undesirable pharmacological and toxicological action, which can prevail over the positive effect of the medicine. In addition, impurities can hinder the effect of the pharmaceutical properties of the main medicinal substance. The composition of impurities allows one to draw conclusions regarding the manufacturer of the product and its adulteration, which is becoming widespread in all countries of the world. Therefore, it is necessary to strictly control the quality of pharmaceutical products and to determine the concentration of foreign impurities at all stages of production from raw materials to finished medicinal forms. According to current requirements, the concentration of detectable and identifiable organic impurities in new pharmaceutical preparations must be no higher than 0.05–0.1% depending on the preparation [1–6]. The determination of impurities at a lower level (10^{-3}% and below) is necessary only in rare cases; however, on the accumulation of the data on the detrimental effect of impurities that occur in pharmaceutical preparations even at a level of 10^{-4}–10^{-3}% [7], the decrease in the detection limits of different methods for the determination of impurities becomes more important.

TYPES AND SOURCES OF IMPURITIES

The quality of medicinal preparations is controlled by determining microbiological and chemical impurities. Chemical impurities can be of either organic or inorganic nature. Possible sources of inorganic impurities are catalysts, drying a gents, adsorbents, solvents, and equipment used in the production of the medicinal remedy, and raw materials. Inorganic impurities are commonly determined by spectrometric methods, most frequently atomic absorption spectrometry [8, 9], as well as capillary ion electrophoresis [10, 11], ion chromatography [12], and electrochemical methods [13–15]. However, the most difficult problem is the determination of organic impurities. Organic impurities are divided into two categories: impurities related to the main component in chemical nature and impurities introduced with solvents. The latter are commonly volatile organic compounds and are determined with the use of gas chromatography. The first category involves synthetic precursors of the pharmacologically active compound (raw material), intermediate products, and by-products, which are formed in the synthesis of the main component. Another possible source of impurities is the formation of products of the decomposition of the main component and its interaction with the matrix, which are accumulated in the medicinal substance with time. Light, oxygen, moisture, and heat can cause chemical reaction (cyclization, dimerization, hydrolysis, oxidation, etc.) involving the pharmacologically active compound.

Thus, the concentration of impurities in pharmaceutical preparations must be controlled at different stages of their manufacturing: in raw materials, intermediate products, unpacked medicinal substances and technical samples, and finally in ready-to-use medicinal forms (tablets, ointments, injection and infusion solutions, etc.). It is also necessary to take into account the possible formation of the products of decomposition and interaction of the main component with the matrix and to specify the proper storage and transportation conditions and the storage life of the medicinal preparation.

EXTRACTION OF ORGANIC IMPURITIES FROM PHARMACEUTICAL PREPARATIONS

For the determination of impurities in liquid samples of pharmaceutical preparations (e.g., injection and infusion solutions and technical samples), commonly the sample is previously diluted with water, and, next, extraction is performed with an organic solvent [16–19].

In the majority of cases, pharmaceutical preparations are not liquid samples but tablets, ointments, capsules, granules, syrups, etc., and solid and amorphous substances in the case of technical samples. In this case,

organic impurities are extracted using solvent extraction including accelerated solvent extraction (ASE) in a Soxhlet apparatus, microwave-assisted and ultrasonic extraction, and supercritical fluid extraction (SFE).

Solvent extraction is commonly used for the extraction of organic impurities from pharmaceutical preparations and technical samples. It is a rather inexpensive method and does not require special instrumentation. Extraction is performed with organic solvents (methanol [19–22], acetonitrile [23–25], ethanol [26, 27], etc.) or their mixtures (e.g., methanol–acetonitrile (1 : 1) [28]) in the case of the subsequent determination by high-performance liquid chromatography (HPLC) with the corresponding mobile phase [29–34]. In some cases, water is used for extraction [16, 17]. The volume of the final extract is 5–100 mL.

In the analysis of pharmaceutical preparations in the form of tablets, the required amount of tablets (from 1 to 20) is thoroughly ground into a powder. Technical samples are commonly powders, and 100–200 mg of the sample is used for analysis. For the extraction of impurities from preparations produced as creams, syrups, and capsules, 0.5–1 g, 5–10 mL, and 3–10 capsules, respectively, is taken. The extraction is performed with stirring for 5–30 min. In some cases, ultrasonic treatment for 10–30 min is used [20, 25, 33]. For the separation of the undissolved precipitate, the solution is filtered through a paper filter or centrifuged at a rate of 2500–3000 rpm for 3–10 min. The total time required for the extraction of impurities from pharmaceutical preparations by solvent extraction is from 10 min to 1 h.

The volume of a sample introduced into a chromatograph is from 1 μL (in the case of gas chromatography (GC)) to 10 μL (HPLC). Thus, for a volume of the extractant of 10–100 mL, only a small fraction (0.00001–0.001) of the extract enters the chromatograph. This approach sets the minimum value of the lower determination limits of organic impurities in pharmaceutical preparations at a level of 10^{-3}–10^{-2}%.

In spite of obvious advantages and popularity, the solvent extraction method has some disadvantages: the long time of the process (up to 1 h), the use of organic solvents, which are commonly toxic and expensive, and changes in the sample composition because of the loss of a part of determined impurities and the introduction of impurities inherent to solvents.

The use of Soxhlet extraction [34], SFE [35–39], ultrasonic solvent extraction, and ASE [40] makes it possible to decrease the amount of the solvent, to increase the extraction coefficient, and to decrease the time of extraction and the cost of sample preparation. However, currently these methods are rarely used for the extraction of impurities from pharmaceutical preparations. In some works, these methods were used for the extraction of the main component.

In ASE, the temperature is 50–200°C to increase the extraction rate and the extraction coefficient. To retain the extracting phase in the liquid state, extraction is performed at a pressure of 5–200 atm. The time of extraction is from 5 to 15 min; the volume of the final extract is 5–10 mL.

SFE with the trapping of the extracted compounds from the fluid flow in the solvent significantly decreases the time of extraction and the consumption of the solvent (up to 90%) and increases the recovery in comparison with conventional solvent extraction. This is due to the physical properties of the compounds in the critical state (densities, dielectric constants, and diffusion coefficients of dissolved compounds). In this case, the extractant is much purer than any organic solvent (99.995%), which substantially decreases the change in the composition of the extract due to solvent impurities. Supercritical carbon dioxide is most frequently used as the extracting phase in SFE. For the extraction of polar compounds, carbon dioxide is modified by the addition of a polar organic solvent (commonly methanol [35, 36, 38, 39]) with a concentration of 2–10%. For the same purpose, nitrous oxide in the supercritical state can be used as the extractant. Of particular interest is solventless SFE, which was proposed in [41, 42] and is used for the extraction of moderately volatile organic impurities from pharmaceutical preparations [43–46].

DETERMINATION OF ORGANIC IMPURITIES IN PHARMACEUTICAL PREPARATIONS

The most popular methods for the determination of organic impurities in extracts from pharmaceutical preparations are high-performance liquid chromatography, high-performance thin-layer chromatography (HPTLC), and gas chromatography. Some works deal with the determination of impurities in medicinal preparations by capillary electrophoresis (CE) and supercritical fluid chromatography (SFC) and with the use of two-column chromatographic systems. The great majority of works deal with the determination of specified (known) impurities. However, a more difficult problem is the determination of impurities when their number and composition are not known in advance. This is particularly important in the implementation of a new pharmaceutical preparation, when the reliable identification and the estimation of concentrations of the maximum possible number of impurities are required.

High-performance liquid chromatography is most frequently used for the determination of organic impurities in pharmaceutical preparations. About 80% of all works on this topic deal with the use of this method.

In the majority of cases, known impurities in pharmaceutical preparations are determined by this method using reversed-phase systems in the isocratic elution mode with ultraviolet (UV) detection, which provides high reproducibility, sensitivity, and reliability and a reasonable time of analysis. The time of analysis is

from 6 to 40 min (number of determined impurities from 1 to 20). The detection limit is within 0.05–0.1%, which meets the requirements of Pharmacopoeia. The relative standard deviation is no higher than 4–6%. Silica gel with modifying groups, most frequently C_8 or C_{18}, is commonly used as the stationary phase; adsorbents with more polar groups (–CN, –OH, and –Ph) and polymer sorbents are used less frequently. In reversed-phase HPLC, methanol–water and acetonitrile–water mixtures with different ratios of the components from 80 : 20 to 30 : 70, respectively, are commonly used for the determination of impurities in pharmaceutical preparations. The constant pH is maintained by the addition of phosphate, acetate, etc., buffer mixtures to the eluent. The flow rate of the mobile phase varies from 1.0 to 1.6 mL/min. Separation is commonly performed at room temperature; less frequently, the column is thermostatted at 40–45°C [47–49]. Gradient elution is used for the separation of impurities with different polarities [50–55]. This approach, on the one hand, improves the separation of the mixture and decreases the time of analysis and, on the other hand, complicates the analysis, impairs the reproducibility, and narrows the range of suitable detectors (only detectors unresponsive to a change in the composition of the mobile phase in the course of chromatography are suitable for gradient elution).

Normal-phase HPLC is very rarely used for the determination of organic impurities in pharmaceutical preparations [46–60], because their components and impurities are commonly polar compounds. In the majority of cases, unmodified silica gel [56, 58–60] and silica gel with modifying amino groups [57] are used as polar sorbents for the determination of impurities by normal-phase HPLC. Nonpolar organic solvents with the addition of polar modifiers, e.g., $CHCl_3$–methanol–water (4893 : 10 : 7) [58], ethanol–hexane (7 : 3) [56], hexane–ether [60], etc., are used as the mobile phase.

A UV detector is most frequently used for the determination of specified impurities in pharmaceutical preparations. It exhibits high sensitivity, stability, and reliability and allows the operation in the gradient elution mode. Fluorescence detectors [61–63], spectrophotometric detectors [64], light scattering detectors [65, 66], and electrochemical detectors [57, 67–69] are also used.

The determination of unknown impurities is a much more difficult problem. It requires the use of several independent approaches. The problem can be partially solved by HPLC using the diode-array UV detector [58, 70–75], mass spectrometer (MS) [51, 71, 76–79], and nuclear magnetic resonance (NMR) spectrometer [50, 60, 72, 80–85].

In the majority of cases, the combination of HPLC with MS is used to confirm the presence of known organic impurities. The possibilities of this combination for the identification of unknown impurities are rather limited. This is due to possible losses of information on some components of the mixture because of different ion–molecular reactions of impurities in the eluent. At soft chemical ionization, which is commonly used in the combination of HPLC with MS, only the data on the molecular mass of the unknown impurity can be obtained. Sources with spray ionization at atmospheric pressure are used in some cases: ion spray [76], electrospray [51, 71, 77], and thermospray [78, 79]. Tandem mass spectrometry (MS/MS) is used for identifying organic impurities and improving the reliability of their detection [77, 86–89].

In some cases, impurities are identified using the chromatographic extraction of fractions containing impurities, their preconcentration with solvent replacement, and the subsequent measurement of mass spectra [50, 72, 90] and NMR spectra [50, 60, 72, 80].

NMR spectrometry is finding increasing application for the identification of unknown organic impurities in pharmaceutical preparations [50, 60, 72, 80–85]. This method is highly informative and allows conclusions to be drawn on the structure of the components in a mixture of related compounds because the form of the NMR spectrum changes on even an insignificant change in the geometry of the molecule and bond orders. Commonly, NMR is used to obtain and confirm data on the structure of molecular fragments formed in MS. In some cases, only the combination of NMR and MS gives the full information on the structure of the studied component [50, 72, 82, 84, 90]. NMR spectrometry also allows the quantitative determination of impurities; however, its low sensitivity and poor reproducibility are substantial limitations of the method.

Thus, reversed-phase HPLC with UV detection is currently the most popular method for the determination of known (previously specified) organic impurities in pharmaceutical preparations, due to its high reproducibility, sensitivity, reliability, and rapidity. However, this method is unsuitable for the determination of unknown impurities. The use of the diode-array UV detector, mass spectrometer, and NMR spectrometer for this purpose involves significant technical problems, difficulties in the interpretation of the data, and insufficient informativeness. In addition, a common disadvantage of HPLC is the high cost of columns and solvents that are used as mobile phases and a decrease in the reproducibility when columns from different manufacturers are used.

Thin-layer chromatography. Classical semiquantitative thin-layer chromatography (TLC) with visual detection is rather frequently used in official Pharmacopoeia procedures. This is due to the method being simple, rapid, and inexpensive. However, its sensitivity is frequently insufficient; hence, the increasing number of works deal with the use of high-performance TLC (HPTLC) for the determination of organic impurities in medicinal preparations. HPTLC exhibits high performance and accuracy and allows the qualitative and

quantitative analysis. The method is predominantly used for the separation of rather simple mixtures containing 5–15 components, unpurified samples, and samples containing poorly elutable or nonelutable matrix components. The use of HPTLC is promising for the determination of organic impurities in pharmaceutical preparations, especially in raw materials and technical samples [91].

Unmodified Silica gel 60 F_{254} [92–96], Silica gel GF_{254} [97], and Silica gel 60 H [98] are commonly used as the sorbent. Reversed-phase plates coated with silica gel with modifying –CN [97], C_8, and C_{18} [98] groups and cellulose-based sorbents [99, 100] have been used in some works. Silufol and Sorbfil plates are widely used in Russia [101, 102]. Mixtures of two to five organic solvents are used as the mobile phases.

After the chromatography is finished, the plates are dried in a flow of air [99, 103, 104] or nitrogen [98]. Detection is commonly performed by scanning densitometry. Determination can be based on absorption in the UV and visible region (UV/Vis) [93–95, 98, 103–106] or fluorescence [97–99, 104, 106–108]. UV/Vis densitometry is used for the quantitative determination; this method is unsuitable for the identification of unknown impurities because the spectra are uncharacteristic. In routine analyses, a solution of the main component is used as the reference solution. If the UV/Vis spectra of the main component and impurities are identical (for this purpose, the molecules of the compounds must, e.g., contain a characteristic group) and their absorption maximum is known, the selection of the wavelength in scanning is unambiguous. However, in the majority of cases the absorption maxima of the main component and impurities are different; hence, the selection of the wavelength is complicated and the determination of some impurities is insufficiently sensitive. This substantially restricts the use of scanning densitometry based on the absorption in the UV/Vis region.

Fluorodensitometry is more specific and presents a basic possibility of the identification of unknown components. However, this detector was not used for the determination of unknown organic impurities in pharmaceutical preparations. Some compounds exhibit intrinsic fluorescence; however, for the detection of compounds without intrinsic fluorescence, the reaction of the analytes with a fluorescing reagent must be additionally conducted after the separation on the plate. This significantly complicates the determination procedure. With the proper selection of the wavelength, the sensitivity of the determination can be substantially increased in comparison with UV/Vis densitometry, and the detection limit can be as low as 0.5–10 ng.

MS [109, 110] and IR [92, 111] spectrometers can be also used as the detection systems in TLC. These methods commonly allow the identification of unknown organic impurities by comparing the obtained spectra with library spectra. There are works dealing with the determination of pharmacologically active compounds and their metabolites in biological matrices by TLC–MS [112, 113]; however, this method has not been used for the determination of impurities in pharmaceutical preparations.

With the use of the IR spectrometer, the detection limit is about 100 ng and the identification limit is about 1 μg [92]; this corresponds to the concentration of impurities in the analyzed preparation at a level of 0.05–0.1%.

Two-dimensional TLC makes it possible to increase the efficiency of separation. To attain a substantial increase in resolution, the conditions must be selected so that the selectivity of the mobile phase is different for the elution in different directions [97].

Although the two-dimensional TLC technique significantly improves the analytical characteristics of TLC, it is not widely used in the analysis of pharmaceutical preparations for organic impurities because of the laborious selection of the optimum set of mobile phases and a decrease in reproducibility: the relative standard deviation is commonly 2–4% in HPTLC and up to 20% in two-dimensional TLC.

HPTLC is finding increasing application in the analysis of pharmaceutical preparations for organic impurities because of the simplicity, low cost, rapidity, and high performance and efficiency of separation. A substantial limitation of the method is lower sensitivity in comparison with HPLC; however, it can successfully supplement HPLC and has significant advantages as a screening method for the analysis of initial substances and intermediate products.

Gas chromatography is the method that allows the simplest and most efficient identification and quantitative determination of unknown and specified moderately volatile organic impurities in pharmaceutical preparations. A substantial limitation in the use of GC is the requirement for thermal stability and volatility of the analytes.

Volatile and moderately volatile organic impurities in pharmaceutical preparations are formed in the course of synthesis and storage and are introduced with solvents that are used in the production. In their toxicological effect on the human body, organic solvents are divided into three groups [114]:

The first group involves strong carcinogens, such as benzene, 1,1,1-trichloroethane, 1,2-dichloroethane, 1,1-dichloroethane, and carbon tetrachloride. The use of these solvents is reasonable only in rare cases, and their concentrations in pharmaceutical products are regulated.

The second group of organic solvents involves 22 compounds that exhibit neurotoxic and teratogenic properties and have no mutagenic effect on animals: dichloromethane, acetonitrile, hexane, methanol, etc. The permissible concentrations of these solvents in ready-to-use pharmaceutical products are expressed as

the permissible daily dose, if it is known, and must be no higher than 10^{-4}%.

The third group involves 35 organic solvents that have the minimum toxicological effect on the human body and the environment: acetone, ethyl acetate, 2-propanol, ethanol, etc. The permissible concentration of these solvents in a preparation is 0.5%, and the permissible daily dose is 50 mg.

Volatile and moderately volatile organic impurities in pharmaceutical preparations are determined by equilibrium headspace analysis, purge-and-trap method, solid-phase microextraction (SPME), and direct sample introduction.

Equilibrium headspace analysis is frequently used in Pharmacopoeia in many countries [115, 116]; it is currently among the most accurate and sensitive methods. The determination of impurities in medicinal preparations by this method is commonly performed in the static automatic mode [115, 117, 118]. A sample is placed in a closed vessel, the vessel is thermostatted, and, after attaining the thermodynamic equilibrium between the sample and vapor phase, an aliquot portion of the equilibrium vapor phase is sampled and directly introduced into the separation column. To increase the distribution coefficient of the components between the liquid and vapor phases, in some cases mineral salts (commonly Na_2SO_4) are introduced into the matrix solution [116]. The volume of the analyzed sample is 10–50 mL, the thermostatting temperature is 80–110°C, the time of thermostatting is 30–90 min, and the volume of the aliquot portion of the equilibrium vapor is 0.25–2 mL. The vapor phase is introduced into the column either with [116] or without flow splitting. In the latter case, sampling can be performed with additional cryogenic or sorption preconcentration. In equilibrium headspace analysis, the detection limit of volatile organic impurities in medicinal preparations is 10^{-4}–10^{-3}% [116], and the relative standard deviation is 10–15%.

The purge-and-trap method is the dynamic version of the headspace sampler [119–121]. An inert gas is blown though a test sample, and next volatile components are separated from the carrier gas in sorption (more rarely, cryogenic [122, 123]) traps, the preconcentrated compounds are thermally desorbed from the traps with a back flow of an inert carrier gas, and further transferred into a separation column. The purge-and-trap method is more efficient and sensitive than equilibrium headspace analysis because it provides more complete separation of volatile organic impurities from the liquid matrix; however, it is more rarely used in the analysis of pharmaceutical preparations for volatile impurities. This is due to the rather low reproducibility (relative standard deviation can be up to 20%), laboriousness, and high cost of the analysis. Errors in the results of the analysis are possible because by-products of the thermal decomposition of both the analytes and the sorbent can form in the thermal desorption. The most efficient and most popular sorbents for the preconcentration of volatile organic impurities are porous polymer sorbents (Chromosorb and Porapak), among which Tenax GC (poly-2,5-diphenyl-*p*-phenylene oxide) is most widely used in the determination of impurities in pharmaceutical preparations. The purge time is 4–15 min (flow rate of the purge gas is 10–20 mL/min), the temperature of the thermal desorption from the sorption trap is 150–200°C, and the desorption time is 5–10 min. The detection limit of volatile organic impurities in pharmaceutical preparations is this case is 10^{-6}–10^{-4}%.

The SPME method is based on the preconcentration of volatile impurities by their extraction at the surface of a capillary coated with a polymer phase with the subsequent thermal desorption and simultaneous transfer into a gas chromatograph. Extraction is performed commonly from the liquid (aqueous) phase and, in some cases, from the vapor phase [124]. The length of the fused-silica capillary is 20–25 cm; its end (1 cm) is coated with a polymer stationary phase. This is commonly polydimethylsiloxane [124, 125]; polyamide, polyacrylate, carbowax, and unmodified fused silica are also used. The thickness of the sorbent layer is 10–100 μm; the time of sorption is 1–5 min (in this case, the thermodynamic equilibrium is not attained because the equilibration time can be 30–90 min). Thermal desorption is performed in the injection port of a gas chromatograph in a flow of a carrier gas at a temperature of 150–250°C. The time of thermal desorption is 2–5 min. Substantial disadvantages of SPME are the low recovery of compounds (1–3%), which leads to the use of highly sensitive detectors, and low reproducibility: the relative standard deviation can be as great as 20–25%.

Direct sample introduction. In this case, 1–2 μL of a sample is introduced directly into a chromatographic column with a special empty precolumn. Thermal focusing is used to avoid peak broadening as a result of front smearing, and to decrease sample discrimination due to the syringe (e.g., the adsorption of components on the needle walls), the sample is introduced rapidly with a hot needle. The frequent use of the direct sample introduction method is caused by such its advantages as accuracy, simplicity, rapidity, and no need in special instrumentation. A significant limitation of this sample preparation technique is low sensitivity (about 0.1%). In addition, direct sample introduction is unsuitable in the analysis of samples of unknown composition because of inevitable matrix effects, which can lead to the contamination of the precolumn and difficulties in the identification and determination of impurities. Direct sample introduction is frequently used in many Pharmacopoeia procedures for the determination of known volatile organic impurities introduced with solvents by open-tube gas chromatography at a level of 0.1% and higher [114, 116]. According to the US Pharmacopoeia procedure, open tubular columns of a large diameter (0.53 mm) with 5% phenyl–95% dimeth-

ylpolysiloxane stationary phase are used in the direct sample introduction method [116]. Columns with a special stationary phase that consisted of alternating segments coated with polydimethylsiloxane on the silicone support and polyethylene glycol on the Carbopack B support were used for this purpose in earlier works [126].

Residual solvents are determined using such types of columns as DB5, Carbowax, RTX200, etc. Their inner diameter is 0.32–0.5 mm; the thickness of the stationary phase layer is at least 1 μm. Copolymers based on polysiloxane modified with methyl, phenyl, cyanopropyl, and trifluoropropyl groups are used as stationary phases in the determination of specific volatile organic impurities. Another popular stationary phase is polyethylene glycol. The thickness of the stationary phase film is 0.4–1 μm. The inner diameter of the columns is 0.1–0.32 mm; their length is 20–30 m.

In the majority of cases, a flame-ionization detector (FID) is used in the determination of known volatile impurities in pharmaceutical preparations, because it is a highly sensitive, versatile, and inexpensive detector with a wide linear range. Such selective detectors as electron-capture and flame–photometric detectors are used extremely rarely. Unknown and specified organic impurities are commonly identified using a mass spectrometer [117, 124, 127–131]. The identification of specified impurities is particularly important when solvents of the first group are used. In addition, MS is a highly selective and highly sensitive detector, which allows the determination of trace impurities, in particular, when SPME is used as sample preparation.

A library search technique based on the comparison of the obtained mass spectrum with library spectra is used for the rapid identification of impurities. This technique allows the reliable identification of specified impurities. Internal standards with tracer isotopes are used for the quantitative determination of specified impurities.

MS analysis is most structurally informative, and the absence of basic technical difficulties in the combination of GC and MS makes the GC–MS system the most available method for the identification of organic impurities in medicinal preparations. In electron impact ionization MS, the molecular ion is fragmented yielding characteristic groups of ions. The interpretation of the resulting mass spectra allows conclusions to be drawn on the structure of the molecule. In addition, the quality of information on the structure of the molecule can be improved using tandem MS. Soft chemical ionization makes it possible to obtain information of the molecular mass of the molecule. Methodological approaches to the determination of the structure of specific impurities in medicinal preparations using GC–MS were considered in several works [127, 129, 131–133].

The combination of GC–MS with SFE without using solvents deserves special attention [43–46]. The regularities were studied for the extraction of moderately volatile organic impurities form pharmaceutical preparations as aqueous solutions and tablet forms, the active components of which belong to different classes of chemical compounds (peptides, benzodiazepines, aspirin, analgin, and dimedrol). A large number of impurities (from 16 to 56) were determined in these preparations at a level of 10^{-6}–10^{-1}% with the use of SFE–GC–MS. The number of impurities extracted by SFE increases in comparison with solvent extraction because of the transfer of the entire extract into the chromatographic–mass-spectrometric system. Based on the comparison of the results of the chromatographic–mass-spectrometric analysis of extracts obtained by solvent extraction and supercritical fluid extraction without solvents, it was demonstrated that, in the majority of characteristics (number of extracted impurities, detection limit, and time of sample preparation), SFE outperforms solvent extraction with the subsequent direct chromatographic–mass-spectrometric determination.

GC is a highly efficient, highly sensitive, and highly selective method that allows the determination of the majority of thermally stable volatile and moderately volatile organic impurities in pharmaceutical preparations. GC–MS provides the most simple and efficient identification of specified and unknown impurities mostly because of the absence of basic technical difficulties. Chromatography–mass spectrometry commonly involves less difficulties in the interpretation of spectra than HPLC–MS.

Supercritical fluid chromatography in its possibilities supplements HPLC and GC. The method exhibits higher efficiency than HPLC because diffusion and mass-transfer coefficients in supercritical fluids are significantly larger than in liquids that are used as mobile phases in HPLC. The rate of analysis can be controlled by changing the density of the mobile phase, which can be easily attained by changing the pressure and/or temperature. In addition, GC detectors, which are more sensitive than HPLC detectors, can be used in SFC. The SFC–MS system makes it possible to eliminate some significant difficulties that occur in the combination of HPLC and MS.

Supercritical carbon dioxide is commonly used as the mobile phase in the SFC determination of impurities in medicinal preparations. Carbon dioxide is a nonpolar mobile phase; therefore, a modifier (commonly methanol) is added for the elution of polar compounds. In SFC with the use of packed columns, the addition of a small amount of a modifier (<1%) leads to significant changes in retention, whereas more significant amounts of a modifier (5–30%) are required for a noticeable change in the retention for the separation on open tubular columns. In some cases, the third component (propylamine [134, 135], triethylamine [136, 137], and 1,4-dioxane [138]) is added to the mobile phase to

change specific interactions between the eluent and the stationary phase.

Open tubular columns are the most efficient; however, difficulties in the separation of polar compounds, low performance, and the absence of good instruments for SFC on open tubular columns are responsible for the fact that open tubular columns are rarely used for the SFC determination of impurities in pharmaceutical preparations [139, 140]. In this case, the stationary phase is silica gel modified with diol [137, 141–143], aminopropyl [136, 137], cyanopropyl [140], or cyano [141] groups.

UV detectors are most frequently used; light scattering, fluorimetric, and electron-capture detectors are used more rarely. The first of these exhibits the nonlinear response in the region of low concentrations, and the last two detectors are selective, which precludes the determination of all impurities including unknown. The selection of the sorbent in the column, the mobile phase modifier, and its amount are most important parameters in the selection of conditions of the separation and determination of impurities by SFC on packed columns. The gradient elution with the variation of the concentration of the modifier allows the separation of impurities largely different in polarity. The selectivity can be improved by changing the temperature of the column.

SFC in combination with MS commonly employs chemical ionization at atmospheric pressure [144, 145], which allows the determination of only the molecular mass of an unknown impurity rather than its structure. NMR [146] and IR [147] spectrometers can be used for the identification of unknown components by SFC; however, they are not used for the identification of unknown organic impurities in pharmaceutical preparations.

The widest area of application of SFC in the determination of impurities in medicinal preparations is the separation and determination of enantiomers. In routine analyses for the determination of enantiomer impurities, SFC is more popular than HPLC because of the higher efficiency of SFC, shorter time of analysis, and lower cost. Both packed [134, 135, 138, 148–150] and open tubular [139] columns are used in this method. Sorbents with chiral stationary phases based on cellulose [134, 148, 150] and silica gel [135, 138, 139] are used in packed columns.

SFC is a promising method for the analysis of medicinal preparations for organic impurities. The reason is that the method is highly sensitive, does not require expensive solvents, and allows changes in the conditions of analysis, which makes it possible to easily control such parameters as the selectivity, resolution, and time of analysis. The limitation of the method is its low reproducibility (in comparison with HPLC).

Capillary electrophoresis. In recent years, this method has received increasing acceptance for the determination of organic impurities (primarily specified) in medicinal preparations being alternative to HPLC. Capillary zone electrophoresis (CZE) is used for the separation and determination of basic [151–158] and acidic [159–162] organic impurities. Separation is performed in fused-silica capillaries with a length of 25–60 cm, an inner diameter of 50–100 μm, and an outer diameter of 300–400 μm. At a voltage of 2–45 mV (current strength 15–100 μA) and a temperature of 15–30°C, the components are separated within 10–20 min. The UV detectors that are used in this method allow the determination of only specified impurities. Basic impurities are determined using electrolytes with low pH, commonly phosphate and citrate buffer solutions with pH 2–4. A significant advantage of CZE over HPLC in the determination of basic compounds is the solution of the problem of asymmetric peaks with broad tailing. In addition, a substantial advantage is the fact that the electrolytes that are used in this method exhibit low absorbance in the UV region and, hence, detection is possible at short wavelengths of 190–210 nm. Many pharmacologically active compounds and their specific impurities have an absorption maximum in this region [151]. To improve such analytical characteristics as the migration time, selectivity, and resolution, complexing agents (cyclodextrin [152–154] and EDTA [155]), ion-pair reagents (tetrabutylammonium and tetrapropylammonium [154]), and organic solvents (isopropanol and methanol [152]) are added to the electrolyte.

Acidic impurities are determined using electrolytes with high pH, e.g., phosphate (pH 7) and borate (pH 9.5) buffer solutions. The selectivity and resolution can be also improved by the addition of cyclodextrin, ion-pair reagents, and organic solvents. Organic solvents are added to decrease the electroosmotic current and to decrease the ionization of acids. The addition of polymer compounds [152] such as polyethylene glycol or cellulose leads to a decrease in electroosmotic current because of the increase in the viscosity of the electrolyte solution.

Micellar electrokinetic chromatography (MEKC) is used for the determination of the neutral components of a sample [163–165]. Micelles are formed on the addition of surfactants to a buffer solution at concentrations exceeding the critical micelle concentration. These micelles are hydrophobic inside and charged outside, which leads to electrophoretic mobility in electric field. Sodium dodecyl sulfate is most frequently used as the surfactant. Selectivity and resolution can be improved by the addition of cyclodextrin, ion-pair reagents, and organic solvents (commonly acetonitrile) to the electrolyte. Selectivity can be additionally improved by varying the type and concentration of the surfactant [166] or by combining surfactants.

In CZE and MEKC, specified impurities are identified by the comparison of the relative migration time of the compound in the sample and in a reference solution. Single works deal with the identification of known

impurities with the use of a diode-array detector [167] and the determination of the molecular mass of the impurity by the combination of CZE and MS [168].

Capillary electrochromatography (CEC) combines the high selectivity of HPLC and the high efficiency of CE. CEC employs the same principles of separation and stationary phases as HPLC; however, the transport of eluents (aqueous–organic buffer solutions) and components of the sample is due to migration under the action of electric field. Fused-silica capillary columns with a length of 8–50 cm and an inner diameter of 50–100 μm and sorbents based on silica gels Hypersil and Spherisorb ODSI with a particle size of 3 μm are used. In [169], norgestimate and its specific impurities (decomposition products) were determined at a level of 0.1%. A three-component mixture of organic solvents was used as the mobile phase. Successful separation was performed within 15 min, whereas the HPLC separation with gradient elution requires more than 30 min. Only few works have dealt with the determination of organic impurities in pharmaceutical preparations by this method [169–171].

CEC exhibits the high selectivity and high efficiency of separation (number of theoretical plates per meter can be up to 10^4–10^6). In the determination of polar and ionogenic organic impurities in medicinal preparations, CEC is highly competitive with HPLC. CEC allows the successful separation of impurities with different polarity. In addition, a significant advantage of CEC is the short time of separation. However, a significant disadvantage of this method is low sensitivity (about 0.1%); therefore, CEC is currently uncompetitive with HPLC, and even more with GC, in the determination of organic impurities in medicinal preparations.

Two-column systems are chromatographic systems consisting of two columns arranged in series. The use of this system makes it possible to eliminate some difficulties that occur, e.g., in the identification of impurities in the HPLC–MS system, to determine impurities eluted simultaneously with the main component, and to improve such analytical characteristics as sensitivity, efficiency, resolution, and selectivity. The possibilities of the two-column system for the determination of organic impurities in pharmaceutical preparations were demonstrated in single works with examples of model mixtures [172–174]. The separation and fractionation of analyzed mixtures are performed using systems of two types: HPLC–HPLC [174] or HPLC–GC [172, 173]. The HPLC–GC system is actually a more "rapid" analogue of the system for the separation of impurities from the main component by preparative HPLC and their subsequent identification by GC–MS. Unknown organic impurities were identified at a level of 0.01–0.1%.

One method proposed for the determination of unknown moderately volatile organic impurities in liquid extracts of pharmaceutical preparations improves the selectivity of the determination and decreases the detection limit of impurities because of the use of a two-step open-tube chromatography in the off-line mode and preconcentration [175–177]. The method is applicable to the determination of impurities more and less volatile than the main component. The possibilities of the method were demonstrated with an analysis of diazepam, in which 28 impurities were determined at a level of 10^{-4}–10^{-2}%, and phenazepam, in which 26 impurities were determined at a level of 10^{-4}–10^{-1}%.

Thus, a consideration of the modern methods for the extraction and determination of organic impurities in ready-to-use pharmaceutical preparations and technical samples has demonstrated that existing approaches fail utterly to provide the reliable determination of the number of unknown organic impurities in pharmaceutical preparations. The vast majority of publications deal with the determination of specified impurities and the main component by HPLC, HPTLC, CE, and, more rarely, HPLC–MS. Only minimal attention has been given to the identification of unknown impurities in pharmaceutical preparations. However, the use of chromatography–mass spectrometry for the analysis of extracts obtained by SFE without organic solvent and impurity concentrates extracted from liquid extracts by two-step off-line chromatography makes it possible to decrease the detection limit by several orders of magnitude, to increase the number of detected impurities, and to improve the reliability of their identification.

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