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REVIEWS

Analytical Methods for Studying the Stability of Pharmaceutical Compositions and the Compatibility of Their Components

N. O. El'tsova[a], G. B. Golubitskii[b, *], and E. V. Budko[a]

[a] *Chair of General and Bioorganic Chemistry, Kursk State Medical University, ul. K. Marksa 3, Kursk, 305004 Russia*
[b] *Pharmstandart-Leksredstva JSC, ul. 2-ya Agregatnaya 1a/18, Kursk, 305039 Russia*
**e-mail: iirogirg@narod.ru*

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Abstract—A review of publications on studying the stability of pharmaceutical preparations and interactions of their components is presented. Volumetric and physical factors activating decomposition processes are considered. Specific features of mixture analysis by optical, chromatographic, and thermoanalytical methods are discussed together with the advantages and disadvantages of methods and possibilities of their combination.

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Analytical chemistry is an essential constituent and indispensable approach to the quality control of pharmaceutical preparations. Analytical methods are widely used at all stages of the production and storage of pharmaceutical preparations to reveal their identity and quantify active substances, study the kinetics of drug release, and assess the concentrations of foreign impurities and other quality characteristics [1, 2]. In developing new pharmaceutical preparations, of importance is not only the acknowledgment of the fact that the pharmaceutical preparation satisfies the imposed requirements [3] but also the understanding of reasons for undesirable deviations from these. therefore, a novel trend in the study of interactions between the components of a dosage form in the production and storage of pharmaceutical preparations has been recently developed at the intersection of analytical and pharmaceutical chemistry. Analysts revealed that different chemical (acids, bases, and their salts) and physical (polymorphism, particle size, state of aggregation) states of active pharmaceutical ingredients (APIs) are technologically not equivalent. In particular, the polymorphism phenomenon is widespread among salicylates: acetylsalicylic acid can be met in six crystal forms, capable of changing to one another on abrasion, pressing, granulation, and pelleting; moistening exerts a promoting effect. Polymorphic modifications differ by their solubility, melting temperatures, and stability to oxidation and other treatments. Being carriers of active ingredients, pharmaceutical excipients of natural and synthetic origin can interact with one another, with AFIs, and with the environment. Substances can form bonds of different types: van der Waals, hydrogen, or covalent and also form inclusion compounds, complexes, etc. All this can affect the stability of pharmaceutical preparations [4].

The diversity of new pharmaceutical preparations and the complexity of their chemical composition call for the further development of analytical basics for detecting changes possible in the preparation and storage of drugs. Many works on component interactions and the detection of products of drug decomposition have been published. However, review publications in this urgent and scientifically interest field of chemical analysis are scanty. This paper provides an attempt to at least partially fill the gap and reveal the main trends and problems in this direction.

METHODS FOR STUDYING STABILITY AND DETERMINING DECOMPOSITION PRODUCTS IN PHARMACEUTICAL MIXTURES

Methodological approaches to sample preparation. Studies of the stability of a compound under the action of external factors form the first step in the study of the stability of pharmaceutical compositions as constituents of dosage forms. Analysts use different stress conditions to model processes, such as acid and basic hydrolysis, oxidation, photolysis, and thermal decomposition. The reagents are often 0.1 M NaOH, 0.1 M HCl, and 3% H_2O_2 [5]. Oxidation processes were observed at room temperatures using 3% (24 h) and 30% (48 h) H_2O_2. Studies were conducted at both room and, to accelerate the processes, elevated temperatures. Thus, ornidazole solutions were heated to 80°C for 72 h using 0.1 M HCl and for 12 h (1 M HCl

and 5 M HCl). Stability in 0.1 M NaOH was studied by heating to 80°C for 8 h. Stability in neutral aqueous solutions was investigated at 80°C for 120 h and, in a phosphate buffer solution with pH 8, at 40°C for 24 h. Ornidazole samples were stored for 60 h under UV irradiation and for 6 days at 75°C [6]. Other effects of temperature and time on AFI solutions were described: 10 min at 100°C, 60 min at 60°C, 60 min at room temperature, and 60 min at 4°C for enalapril and maleate [7], and 91 days at 60°C for piracetam [8].

In the work, samples of water-insoluble AFI, for example containing ritonavirs, were dissolved in methanol and then mixed with acid solutions (0.5 M HCl at 75°C) or alkalies (0.1 M NaOH at room temperature), or diluted with water (75°C for 12 h) [9]. Samples of enalapril powder were stored in ampoules at 40, 50 and 80°C and also at 40°C and a relative humidity of 75% within 1, 2, 3, and 4 weeks. After that, solutions of the concentration 1 mg/mL were prepared from the samples [7]. The photolysis of ornidazole was studied for solid samples and using dissolution in a phosphate buffer solution, in 0.1 M HCl, and in distilled water at different time intervals (up to 30 days) [6].

When studying the stability of AFIs in multicomponent pharmaceutical preparations, substances were heated to 100°C for 2 h [10]. In the study of analgin, aqueous–alcoholic solutions and solutions in 0.1 M HCl were prepared. The decomposition products were detected by HPLC, TLC, and UV spectrophotometry. Processes in TLC were accelerated by heating the chromatographic plate [11, 12].

Spectrometric and mass-spectrometric techniques. Spectral techniques in UV and IR spectral ranges are widely used for the identification and quantitative determination of pharmaceutical preparations and products of their decomposition [2]. These techniques ensure rapid nondestructive analysis and provide a simple method for the control of processes accompanied by spectral changes.

Spectrometry is often used to study decomposition processes; however, in the case of the similarity of spectral properties of an impurity and the main substance, the detection of decomposition products may be difficult. The advantages of the method can be realized using derivative spectra. For example, the determination of doxazosin mesylate (I) and celecoxib (II) in the presence of products of their decomposition was based on recording the first derivatives at 256 and 269 nm for I and II, respectively. The method ensures the determination of substances and products of their decomposition in concentration ranges of 0.8–12 and 1–20 μg/mL (I and II, respectively); the recovery was higher than 99%. This approach was used to control the stability of doxazosin mesylate and celecoxib in powders, laboratory mixtures, and pharmaceutical dosage forms [13]. The use of derivative spectrophotometry for determining clozapine in the presence of the main product of its decomposition was also described. Absorption spectra of clozapine and of the product of its decomposition in methanol substantially overlap, hindering their direct determination. Accurate, well separated peaks at 305 and 315 nm were recorded for the second and third derivatives, respectively. Solutions were prepared in 0.1 M NaOH and in 0.1 M HCl. To construct a calibration curve, changes in the absorbance of mixtures containing the AFI and the product of its decomposition in different ratios were recorded; concentrations were calculated using the regression equation [14].

Method of IR spectrometry was used to study the structure of a yellow compound, being an undesirable impurity and a product of analgin decomposition. It was found that the decomposition product is a secondary amine (3334 cm^{-1}) bearing no sulfoxide group. In the wavenumber range 3500–3200 cm^{-1}, the spectra of analgin and possible products of its decomposition differed substantially: analgin (basic nitrile) exhibited a complicated broad band (3600–3400 cm^{-1}); 4-methylaminoantipyrine (4-MAAP), a narrower band at 3334 cm^{-1}; and aminoantipyrine, two intense narrow bands (3432 and 3322 cm^{-1}). A broad band in the region 1230–1150 cm^{-1} and well-defined bands at 610 and 1055 cm^{-1} ($R–SO_2–OH$ sulfonic acid) were clearly seen only for analgin. Based on these facts, analysts concluded that the main product of analgin decomposition is 4-MAAP [15].

Mass spectrometry (MS) is also used. The sensitivity and resolution of this method are 10 times higher, but it is much more expensive. Electrospray ionization mass spectrometry was used to study the widely used phenylephrine compound [16]. The stability of the substance in hydrochloride and hydrogen tartrate salts was studied by analyzing aqueous solutions of a pharmaceutical preparation before and after its UV irradiation. The main decomposition product, phenylephrine derivative with an unsaturated side chain, was detected. It was shown that phenylephrine hydrogen tartrate is less stable than its hydrochloride because of the higher lability of the tartaric acid anion.

Nuclear magnetic resonance (NMR) spectrometry is widely used for the qualitative and, sometimes, quantitative analysis. Previously the development of routine procedures of NMR spectrometry was limited by the high cost of the equipment, its low sensitivity, and problems with the reproducibility of the results compared to other analytical methods. Nowadays proton NMR spectrometry has become quite suitable for the routine analysis of complex mixtures. An analysis is short, usually does not require complex sample preparation or using additional reagents, and is nondestructive. ^{1}H and ^{13}C NMR spectrometry was used for the detection of ampicillin and similar penicillanic acid and 6-aminopenicillanic acid derivatives in the concentration range $(0.5–4.5) \times 10^{-4}$%. Maleic acid was used as an internal standard for the quantitative determination. The results obtained by NMR spec-

trometry were compared with the data of HPLC using the British Pharmacopoeia procedure. It was shown that, at a confidence level of 95%, the results obtained by the two methods were identical. Analysis by ^{13}C NMR took more time. The direct determination of ampicillin in blood serum was performed by recording certain pulse sequences after sample preparation by solid-phase extraction [17].

Chromatography methods. Present-day chromatography methods, HPLC and GLC with different detectors (UV, MS, etc.), are widely used in pharmaceutical analysis. No other method can be compared to chromatography in the efficiency of analysis of complex mixtures [1, 2, 18–21]. This is due to achievements in the development of new highly selective adsorbents and the enhancement of the sensitivity and resolution of spectrophotometric, fluorimetric, electrochemical, and mass-spectrometric detectors. Different versions and procedures are used to study stability; these are quite often named methods (stability-indicating methods) [22]. A series of publications demonstrate wide possibilities of HPLC for studying the stability of pharmaceutical preparations. Most of procedures were intended for the quality control of pharmaceutical preparations and ensured the revelation of the products of AFI decomposition in complex compositions.

The detection of the decomposition products of zonisamide [23], valdecoxib in tablets [24], lactone and *cis*-aminoindanole in an indinavir substance [25]; syn-1-cefuroxime axetil, syn-2-cefuroxime axetil, anti-1-cefuroxime axetil, anti-2-cefuroxime axetil in a cefuroxime axetil substance [26]; and clozapine in the presence of the main product of its decomposition [14] were described. Lovastatin, methylsimvastatin, anhydrosimvastatin, and other impurities in a simvastatin substance were detected by microemulsion chromatography [27]. The stability of pemetrexed disodium was studied on an EXPERT 3 C18 column (3.0) in the gradient mode using spectrophotometric (230 nm) detection [28]. Entacapone and products of its decomposition in tablets were separated on an EXPERT RP-18 (5.0) column and detected by a diode-array detector at 305 nm [29].

One of the main components of the validol substance, menthyl isovalerate, is synthesized in the presence of conc. H_2SO_4, catalyzing the esterification reaction. According to studies by the developers of its production technology, a number of accessory substances, including mixtures of isomeric products of menthol decomposition, menthenes, form under these conditions. To determine foreign impurities and products of decomposition of Validol tablets, analysts used a fused-silica capillary column with an OV-101 liquid stationary phase [30].

The specificity of an HPLC procedure developed to control the purity and stability a cardiocyclide in the substance and solid dosage form was assessed in [31]. Analysts used a chromatograph operated in the linear gradient mode with two mobile phases and a spectrophotometric detector operated at 210 nm. The procedure allowed the separation of the cardiocyclide from its most probable impurities. Among these were intermediates of the last two synthesis stages (some of which were hydrolysis products) and a number of unidentified impurities in the substance and the solid dosage form. The procedure also allows the detection of the products cardiocyclide decomposition formed under the influence of environmental factors and to gain information on the stability of the substance and the dosage form in their production and storage.

The selectivity and high sensitivity of the method are equally important in the studies of decomposition processes. The last property is of particular importance for toxic substances. A procedure for the determination of 4-aminophenol in concentrations to 1.0 ng/mL in solutions of paracetamol substance and to 4.0 ng/mL in solutions of multicomponent anesthetic pharmaceutical preparations was described in [32]. The amperometric detection of 4-aminophenol was optimized and the best potential (+325 mV) was found. A stable symmetric peak of a decomposition product and a rather small peak of paracetamol were recorded. The mobile phase was a mixture of CH_3OH with a 0.05 M LiCl solution (18 : 82, with an additive of H_3PO_4).

To assess the concentration and study the mechanism of the formation of a product of analgin decomposition in Pentalgin N and Pentalgin FS tablets, analysts used a kinetic plot chromatography method [15]. They succeeded to exclude the systematic error due to growth of the decomposition product the concentration in the test solution. The sample was dissolved in a polar organic solvent and chromatographed under the optimized conditions; not less than two chromatograms were obtained for each solution. The peak area of 4-methylaminoantipyrine was calculated in the initial instant of time S_0 using the equation $S_0 = S_n - t(S_n - S_1)/\Delta t$, where S_n is the peak area of 4-MAAP in the last chromatogram; S_1 is the peak area of 4-MAAP in the first chromatogram; t is the time from the moment of sample dissolution to the elution of the peak of 4-MAAP in the last chromatogram, min.; Δt is time interval between the moments of elution of the peak of 4-MAAP in the first and last chromatograms, min. Then the concentration of 4-MAAP in the sample was calculated using the response factor of analgin relative to 4-MAAP. The response factor K_{dev} was calculated by the equation $K_{dev} = (S_{an}c_{4\text{-MAAP}})/(S_{4\text{-MAAP}}c_{an})$, where S_{an} and c_{an} are the peak area and concentration of analgin and $S_{4\text{-MAAP}}$ and $c_{4\text{-MAAP}}$ are the peak area and concentration of 4-MAAP, respectively.

Kinetic curves for the decomposition of ascorbic acid at different pH of solution were obtained by HPLC in [33]. The behavior of kinetic curves demonstrates that, at pH 1.0–4.4, the rate of ascorbic acid

Determination of impurities in pharmaceutical preparations by chromatographic methods

Substance, dosage form, c_{lim} (c_{min})	Column, mm	Stationary phase (particle size, μm)	Mobile phase (flow rate, mL/min)	*t*, °C	Detector (conditions)	References
4-Aminophenol in a paracetamol substance (1.0 ng/mL), tablets and capsules (4.0 ng/mL)	100 × 4.6	Luna C18 (5.0)	18 : 82 CH_3OH–0.05 M LiCl pH 4.0 (H_3PO_4)		A(0.325 V)	[32]
4-Methylaminoantipyrine in analgin substance and preparations	3.9 × 150, precolumn 3.9 × 20	Nova-Pak C18 (4.0)	10:25:65 acetonitrile–0.025 M potassium phosphate solution–water		SF (244 nm)	[15]
Valdecoxib and its impurity SC77852 in tablets	150 × 4.6	XTerra RP18 (5.0)	52 : 48 CH_3OH–1% $(C_2H_5)_3N$ pH 7.35 (H_3PO_4) (1.0)		SF (220 nm)	[24]
Zonisamide (antiepileptic agent) and products of its decomposition	150 × 4.6	XTerra RP18 (5.0)	18 : 82 CH_3CN–0.1 MNaH_2PO_4 pH 3.0 (H_3PO_4) (1.5)		SF (240 nm)	[23]
Clozapine in the presence of its main decomposition product			40 : 60 CH_3CN–H_2O		SF (230 nm)	[14]
Lactone and *cis*-aminoindanole in indinavir substance (antiviral agent)	150 × 4.6	XTerra RP18 (5.0)	35 : 65 CH_3CN–2.5% NaDDS, 0.5% $(C_2H_5)_3N$ pH 7.0 (H_3PO_4) (1.25)	20	SF (214 nm)	[25]
Lovastatin, methyl simvastatin, anhydro simvastatin, and other impurities in simvastatin substance (hypolipidemic drugs); 10–200 ng/mL	50 × 4.6	XTerra TM (3.5)	0.9 : 1.7 : 7 : 90.4 diisopropyl ether–NaDDS–C_4H_9OH–0.025 M Na_2HPO_4 pH 7.0 (0.3)	30	SF (238 nm)	[27]
Isovaleric acid menthyl ester and isomeric products of menthol decomposition	50 m × 0.3 mm	Fused-silica capillary column with an OV-101 liquid stationary phase	Carrier gas N_2 at a flow rate of 20 mL/min and flow splitting of 1 : 20. Flow rates of H_2 and air are 20 and 200 mL/min, respectively. Sample volume 1.0 μL	100 → 240 °C (5 K/min)	Injector and detector temperatures 250°C	[30]
Pemetrexed disodium (antitumor drug) and products of its decomposition	150 × 4.6	ACE3 C18 (3.0)	85 : 15 0.1% H_3PO_4–CH_3CN	25	SF (230 nm)	[28]
Syn-1-cefuroxime axetil, syn-2-cefuroxime axetil, anti-1-cefuroxime axetil, anti-2- cefuroxime axetil in a cefuroxime axetil substance (antibiotic); 1.5 μg/mL	150 × 4.6	XTerra C18 (5.0)	8 : 92 CH_3CN–0.02 M NaDDS pH 2.5	50	SF (280 nm)	[26]
Enalapril maleate and products of its decomposition (0.5–10 μg/mL)	250 × 4.6	PLRP -S	A 0.002 M phosphate buffer solution (pH 6.8)–CH_3CN; 95 : 5 and 34 : 66.	70	SF (215 nm)	[7]
Entacapone (antiparcinsonian drug) and products of its decomposition in tablets	250 × 4.6	Ace RP18 (5.0)	65 : 35 H_2O pH 3.0 (H_3PO_4)–CH_3CN (2.0)	25	SF (305 nm)	[29]

decomposition increases, at pH 5.4–7.2 it decreases, and with the further growth of pH to 8.4 it virtually does not change. The peak area at pH 4.4–5.4 decreased by approximately 25% in 50 min. The stability of the substance in solutions increased on the addition of acetonitrile, methanol, and ethanol. It was shown that the optimum solvent for the determination of ascorbic acid was CH_3CN–0.1% H_3PO_4 (1 : 19).

In recent decades, the HPLC–MS method, which ensures a substantial improvement of selectivity and sensitivity, has become of particular importance for the analysis of pharmaceutical preparations. This method was used, for example, to study mixtures containing enalapril maleate [7]. Samples were subjected to stress conditions and then analyzed. The results confirmed the formation of three main decomposition products irrespectively of the conditions; total impurities comprised about 0.5%, which well agrees with the data from other sources. New decomposition products were found after the action of high temperatures; their structures were determined and a mechanism of enalapril decomposition was proposed.

The short list of works presented above on the use of chromatography in studies of the stability of pharmaceutical preparations demonstrates wide possibilities and good prospects of this method in pharmaceutical studies. Characteristic features of some chromatographic procedures for determining decomposition products in pharmaceutical preparations are summarized in the table.

Thermoanalytical methods are widely used to determine decomposition temperatures of polymers, the moisture content of materials, and the concentrations of organic and inorganic components in the composition of a studied substance. Thermogravimetry (**TG**) was used to assess changes in sample mass (measured on a thermobalance) with temperature on its programmable variation or with time for a given change in weight at a constant temperature. To interpret the results, analysts used differential curves, allowing the detection of the instant of time or temperature at which the most pronounced change in weight was observed. Differential scanning calorimetry (**DSC**) allows one to compare changes in enthalpies with the reference data for isobaric processes. The limiting stage in solid-phase process can be determined by (a) particle diffusion in the reaction zone or from it; (b) chemical reaction itself with product redistribution in the reaction medium [34].

Both TG and DSC were used to study the heat stability of calcium and disodium salts of the fosfomicin antibiotic [34]. Antibiotic salts are often use as pure substances form or in combinations with several fillers in dosage forms. Both TG and DSC curves were recorded in an air flow in the temperature range 298–703 K with a step of 5 K/min. For the sodium salt, researchers observed weight loss in the range 298–423 K with a corresponding endothermic peak in the DSC curve due to water evaporation (initial water concentration 17.7%) and then an about 20% loss of weight with a strong exothermal effect (503–603 K) due to the complete oxidative decomposition of the substance. The kinetic parameters of the processes were used to determine the expiration dates of pure components and their dosage forms.

Changes in the concentration of piracetam substance in its storage were studied in [35]. The obtained DSC data demonstrated the possibility of developing a procedure for predicting the expiration date of a pharmaceutical preparation. The sample studied was polymorphic and included three forms. Note that only one of these forms exhibited biological activity, whereas the other ones could be considered impurities. The researchers used the method of accelerated ageing; thermograms were obtained on a DSM-10MA microcalorimeter manufactured at the Institute for Biological Instrumentation of the Russian Academy of Sciences [8].

Thus, thermoanalytical methods offer valuable information on physical properties due to specific features of crystal structures of substances and their mixtures and on processes occurring on heating. These methods provide powerful instruments for the study of interactions between the components.

A combination of different methods allows an analyst to obtain complex information on substances and their mixtures and to more precisely assess the state of substances when they are brought into contact with each other. It was found that, whereas the IR spectra of the samples to be compared fully coincided, distinctions were observed in the DSC curves of verapamil substances from different producers [36]. The temperatures and melting heat of the substance were also different, and one verapamil substance did not crystallize on subsequent cooling and changed into an amorphous form. DSC curves were also used also to control of the purity of the verapamil substance.

Thermogravimetric analysis and mass spectrometry under nonisothermal heating were used to study the kinetics of thermal decomposition and to predict the expiration date of ascorbic acid [37]. A weighed portion of about 3 mg was heated from 50 to 500°C at a rate of 4 K/min in nitrogen or air at a gas flow rate of 80 mL/min. Decomposition proceeded in three stages; their profiles were similar; however, the process was accelerated at the initial stage in the presence of air. The results allowed the calculation of the expected shelf-life of the substance in nitrogen atmosphere at 25°C and a relative humidity of 90% (two years).

To characterize and determine the main products of risperidone decomposition, Tomar et al. used a complex of methods, including IR, NMR, MS, and HPLC–MS [38]. The study was performed before and after the action of stress factors (acid, bases, and hydrogen peroxide) within 8 h. Treatment with an acid and a base led to the formation of hydroxyrisperidone; the reaction with peroxide gave risperidone-N-oxide.

The same decomposition products were detected for dosage forms.

A combination of HPLC with MS and NMR, which allows not only the separation but also the identification of unknown products, is of special promise for the control of stability and identification of the decomposition products of pharmaceutical preparations. The approach described in [39] combined product separation by HPLC–MS, semipreparative HPLC for purification and structure refinement, and NMR spectrometry for the identification of unknown substances. The procedure allowed not only the determination of the decomposition mechanism, but also the extraction of a necessary amount of unknown products for the confirmation of their structures by NMR spectrometry. The decomposition product was also detected in an HPLC–MS study of a dosage form. Using MS and NMR spectrometry, analysts proved that 5-hydroxymethylfurfural was the decomposition product of lactose, a filler of this dosage form [40].

Two previously unknown impurities were detected in citalopram by HPLC. Their structures were determined based on MS data and confirmed by NMR and IR spectrometry [41].

A complex of methods was used to assess the stability of promising cytotoxic drugs 1-(4-metoxyphenylethyl)-11H-benzo[e]-1,2-dihydropyrido-[3,2-c][1,2,5]-oxathiazepine-5,5-dioxide after the action of stress factors [42]. Mixtures were preseparated on an LC 1100 chromatograph equipped with a degasser, an automatic sampler, and a Waters XTerra C18 column. A mobile phase (a solution of ammonium formate and acetonitrile) gradient and detection at 240 nm were used. Then a MS analysis was carried out on an LCQ-Advantage mass spectrometer with an ion trap at 250°C in a nitrogen flow. Samples for NMR study were dissolved in $CDCl_3$. The decomposition of AFIs treated with acids and (or) oxidants was studied; the formation of ten decomposition products was demonstrated; their structures were elucidated; decomposition kinetics was determined; and a rapid procedure of HPLC analysis with UV detection was developed.

A combined use of optical, chromatographic, and thermoanalytic procedures along with the influence stress factors and (or) the conditions accelerated ageing allowed a more efficient investigation of the occurring processes and the characterization of the decomposition products formed compared to the separate use of each procedure. A comparison of a number of methods (UV spectrophotometry, TLC, and HPLC) has indicated their quite satisfactory performance characteristics in the presence of decomposition products and good applicability to the analysis of dosage forms [14].

METHODS FOR STUDYING SUBSTANCE INTERACTIONS IN PHARMACEUTICAL COMPOSITIONS

In pharmaceutical technology, pharmaceutical excipients (PEs) form an abundant group of substances of natural and synthetic origin, acting as solvents, thickeners, stabilizers, emulsifiers, preservatives, dyes, disintegrants, etc. in the production of dosage forms. The search for a technologically acceptable composition of a dosage form implies changes in the qualitative and quantitative composition of PEs based on their industrial characteristics. An example of optimizing the structure of a PE can be provided by the mathematical evaluation of the contribution of each component to the resulting physical properties of a mixture made in [43]. However, pharmaceutical excipients can interact with other components of a formulation and such processes can affect not only the physical and chemical properties of pharmaceutical preparations, but also their efficiency. For example, it is known that Twin-80 accelerates the absorption of vitamins A, D, and E and improves the bioavailability of acetylsalicylic acid, while poly(ethylene oxide) decelerates the absorption of phenobarbital but accelerates the absorption of laevomycetin. Pharmaceutical excipients directly affect the stability of AFIs. Lactose favors the inactivation of isoniazid and magnesium stearate enhances the decomposition of acetylsalicylic acid. Therefore, the PE set cannot be compiled only on the basis of their technological properties [4].

Studies of compatibility are very important for the creation of stable pharmaceutical preparations; they take a lot of time and resources of pharmaceutical laboratories [44]. The researchers seldom use only one method and usually conduct complex studies to gain exhaustive information on intermolecular processes.

Specific features of sample preparation. Interactions between the components of pharmaceutical compositions may be desirable and planned or undesirable; in the latter case they must be prevented.

When studying interactions, analysts often directionally prepare possible interaction products. For example, polyvinylpyrrolidone complexes with ascorbic acid and pyridoxine were obtained in ethanol on heating followed by the solvent removal [45]; then powders were dissolved in water and ethanol for the study of structures by IR and UV spectrometry. Rutin–β-cyclodextrin inclusion complexes were obtained by suspending powders in 40 mL of purified water [46]. Stirring was carried out for 116 h under controlled temperature (25 ± 0.01°C) and light protection for preventing decomposition. Then solutions were filtered through a 0.45-μm Whatman® PTFE filter, water was evaporated under vacuum at 30°C, and a solid complex was obtained as a pale yellow powder; the powder was washed with a small amount of water and dried to a constant weight. Model mixtures of a gemfibrozil substance with γ-cyclodextrin were pre-

pared by grinding, evaporation, and coprecipitation [47]. Solid mixtures of triclosan with β-cyclodextrin and its water-soluble copolymer with epichlorohydrin were obtained by simultaneous grinding in a high-speed mill [48]. The systems studied were prepared by grinding the components in a mortar under an acetone layer followed by drying at 100°C to a constant weight [49]. Samples of indometacin and saccharin cocrystals were obtained by mechanical grinding or simultaneous thermal treatment [50].

In the study of undesirable and (or) hypothetical interactions, the test substances were subjected to treatments most similar to those under industrial conditions: wetting, simultaneous grinding, and drying at elevated temperatures [51, 52]. The compatibility of AFIs and PEs was studied under isothermal conditions: binary mixtures were stored at 50°C and a humidity of 95% for 12 days which, according to the authors, most adequately reproduced the actual storage conditions of pharmaceutical preparations [44].

Thermoanalytical methods and complex studies on their basis. Studies of compatibility by DSC offer considerable advantages in preliminary technological research [44, 53].

The thermal stability of sodium and calcium salts of an antibiotic in the presence of PEs was studied in [34]. Both TG and DSC curves in the temperature range 298–703 K were recorded at a rate of temperature variation 5 K/min. A comparison of TG and DSC curves and kinetic data suggests that the processes revealed by TG and DSC for pure AFIs are also observed in ready dosage forms. It was shown that changes in DSC curves on the addition sodium succinate to succinic acid were more pronounced than those for the calcium salt. Similar differences were typical for TG curves. The temperature of the beginning of oxidative decomposition for the dosage form containing succinic acid and its sodium salt was about 9 K lower than that for the pure sodium salt. A decrease in the enthalpy of decomposition for dosage forms in comparison to AFIs was also noted.

The DSC method was used to assess the compatibility of ibuproxam with some PEs [52]. Based on the results obtained, a conclusion was made that ibuproxam is compatible with corn starch, avicel, and sodium carboxymethyl cellulose. Interactions were observed with polyethyleneglycol 4000, palmitic acid, stearic acid, and calcium and magnesium stearates. It was shown that ibuproxam interactions with polyvinylpyrrolidone and polyvinylpyrrolidone K30 can be due to mechanical impact. Scanning electron microscopy was additionally used to interpret the results.

Both TG and DSC were used to assess the compatibility of an acetaminophen with fillers (polyvinylpyrrolidone, magnesium stearate, citric acid, aspartame, mannitol, cellulose, and starch) in some widespread pharmaceuticals and solid binary mixtures [54]. The additivity of the calorimetric peaks of pure components was revealed for the majority of dosage forms and solid binary mixtures; this confirmed the compatibility of acetaminophen with all fillers except for mannitol.

To assess the compatibility of ketoprofen with a number of PEs (corn starch, microcrystalline cellulose, colloid silica, lactose, polyvinylpyrrolidone K30, magnesium stearate, and talc), Tit et al. prepared their mixtures in the ratio 1 : 1 by weight [53]. The DSC curve for each mixture was compared to curves for working reference samples. Weight losses in the temperature range 235–400°C were revealed. Infrared spectrometry and X-ray diffraction spectrometry were used as additional methods for interpreting the results of DSC. Changes in the profiles of thermoanalytical curves for some binary mixtures were indicative of interactions proceeding on heating. The results of analysis of DSCs curve have shown that all studied fillers are compatible with ketoprofen and that the physical interaction observed does not mean pharmaceutical incompatibility.

As was noted above, interactions in some cases can lead to positive changes in a pharmaceutical system. Thus, an analysis of the spectral properties of tautomeric forms of S- and R-omeprasole sodium showed a difference in their interactions with mannitol. Because the solubility of S-omeprasole weaker depends on the pH of the medium compared to the solubility of R-omeprasole, by adding mannitol one can affect the bioavailability of the pharmaceutical preparation. The study was performed by DSC, IR spectrometry, ATR IR spectrometry, and local thermal analysis. A decrease in melting temperature and peak broadening was detected in the DSC curve of S-omeprasole sodium; these changes were not observed for the R-isomer. The conclusions were confirmed by IR spectrometry [55].

Thermoanalytical studies confirmed that diclofenac sodium can behave as a plasticizer because of the reduction of the glass transition temperature and that its properties depend on the particle size of the matrix of the ammonium methacrylate copolymer [56]. DSC and TG were chosen as methods for the assessment of the thermal stability and properties of polymer films containing diclofenac sodium. The possibility of interactions between AFI and PEs was confirmed by Raman spectrometry; its results were indicative of a strong ion interaction between the studied substances.

The formation of indometacin and saccharin cocrystals was studied by DSC and Fourier-transform infrared microspectroscopy [50]. All samples were obtained by mechanical grinding or simultaneous thermal treatment. Measurements were performed at certain time intervals in the continuous process of cocrystal growth. The results of IR spectrometry suggested that OH and NH groups in both structures form hydrogen bonds (shifts from 3371 to 3347 cm^{-1} and from 1693 to 1682 cm^{-1} for indometacin, and also

from 3094 to 3136 cm^{-1} and from 1718 to 1735 cm^{-1} for saccharin, respectively). The melting temperatures of the ground mixture and of the cocrystal virtually coincided. Storage at 25°C and humidity of 40% within more than seven months favored cocrystallization.

Studies of the influence of component ratio on the properties of caffeine–paracetamol mixtures were conducted on a DTA installation, brand DTAP-500 [57]. Binary mixtures with different component ratios with a step of 5% in molar mass were prepared by grinding in a mortar with the addition of a small amount of ethanol followed by drying at 105°C. The phase diagram was typical for simple eutectics. For the detailed study of the properties of the system, researchers determined the rates of paracetamol and caffeine transfer into the solution using a "rotating basket" setup from FARMTEST (Germany). The solvent was water; concentrations in the samples were determined by UV spectrophotomery. The eutectic composition of the studied system was characterized by an abnormal increase in solubility and by the rate of component dissolution. A paracetamol mixture with urea was studied by DTA. Phase diagrams of the system were obtained on a DTAP-500 setup and confirmed by X-ray powder diffraction [49]. The solubility of the samples in water at 25°C was determined simultaneously; the concentration of paracetamol was found by UV spectrophotometry. The rate of paracetamol release from the eutectic mixture was much higher.

A microcalorimetric method based on DSC was used in [58] to assess interactions in binary mixtures containing naproxen in combination with amorphous hydroxypropyl-β-cyclodextrin (**HP-β-CD**), sodium salt of β-cyclodextrin sulfobutyl ether, acetyl-β-cyclodextrin, and acetyl-γ-cyclodextrin. Changes in the DSC curves of model mixtures were indicative of the interaction of naproxen with HP-β-CD, acetyl-β-cyclodextrin, and acetyl-γ-cyclodextrin.

In thermoanalytical methods, interactions were studied at high temperatures. Real processes at standard storage temperatures may differ from each other. Thus, in the study of dosage forms containing polyethyleneglycol or other matrices by DSC, one should take into account the possibility of substance amorphization on heating [35]. From this point of view, methods in which studies are performed at room temperature, HPLC, MS, and IR spectrometry, seem preferable [54].

Optical methods and complex studies on their basis. Ultraviolet spectrophotometry is one of methods most widely used to study inclusion complexes. Complexes of ascorbic acid with glycine, alanine, γ-aminobutyric acid, lysine, methionine, and acetylmethionine were studied. The IR spectrum of the lysine complex exhibits a broad and intense band at 3550–3100 cm^{-1}, which was not presence in the spectra of individual substances; it is due to intermolecular hydrogen bonds involving OH–groups. In IR spectra of complexes, the bands of –C=O– and –C=O– groups of the ascorbic acid ring are shifted towards lower frequencies compared to those in the spectrum of ascorbic acid, which suggests the formation of ascorbate (ionized form). Complexes of glycine and (or) its ethyl ester with ascorbic acid due to a hydrogen bond can form because of the favorable arrangement of interacting groups in the substance molecules. In IR spectra of ascorbic acid complexes with methionine and acetylmethionine, the frequency of the –C=O– group does not change. It was supposed that methionine and acetylmethionine are also attached to the OH– group at the C3 atom of ascorbic acid via a hydrogen bond [45]. The IR spectra of ascorbic acid mixtures with chlorpheniramine exhibit changes typical for the transition of the ionized acid into the molecular form. Interactions in this case are also due to the formation of hydrogen bonds, which is attested by changes in the region absorption of nitrogen-containing groups of chlorpheniramine. Diethylamine is a stronger base compared to chlorpheniramine. The spectrum of a mixture with diethylamine demonstrates acid characteristics of ascorbic acid and its ability to form hydrogen bonds, also with the amino group [10, 59]. Nicotinamide is a weak base; therefore, the formation of an ionic $-N^+O^-$ bond in its interaction with ascorbic acid is low-probable. Hydrogen bonds are formed by OH– groups at C3 and C5 atoms of ascorbic acid with the pyridine nitrogen atom. Binding of different compounds to ascorbic acid can give complexes of different structures via different mechanisms [60].

To obtain magnetically controlled particles forming a basis for a new class of pharmaceutical preparations, information on active functional groups in AFIs and the mechanisms of their interactions with magnetite, the carrier of pharmaceutical substances, is necessary. Compounds capable of interacting with iron ions were chosen among pharmaceutical substances, such as isoniazid, acetylsalicylic acid, sodium mefenamate acid, sodium salycylate, quiinosol, novocaine, vikasol, and *p*-aminobenzoic acid. The interaction products in the magnetite–AFI system were studied by correlating characteristic IR bands of the analyzed samples to those of mother substances. A necessary condition for interaction is the availability of an amino group or a pyridine nitrogen atom in the AFI structure; the interaction is due to the donor–acceptor mechanism [61].

The property of polyvinylpyrrolidone to form complexes with AFIs is used in medical practice for the synthesis of pharmaceutical preparations with prolonged action; complex formation with cyclodextrin improves the availability and efficiency of AFIs by increasing their solubility. UV spectrophotometry, including versions utilizing the second derivative of the absorption spectrum, was used to study inclusion complexes of bromazepam with β-cyclodextrin

and β-hydroxypropylcyclodextrin [62]. Both UV and IR spectrometry were used in the study of 9-fluorenone-2-carboxylic acid ester complexes with 2-hydroxypropyl-β-cyclodextrin [63]. A combination of optical method with the method of phase solubility confirmed an increase in the solubility and efficiency of the studied AFIs because of the formation of their compounds with cyclodextrin.

The interaction of gemfibrozil with γ- and hydroxypropyl-γ-cyclodextrin in aqueous solution and in the solid state was studied in [47] by fluorescence spectrometry, NMR spectrometry, X-ray diffraction, IR spectrometry, thermal analysis, and solubility measurements. The fluorescence of gemfibrozil was enhanced in the presence of hydroxypropyl-γ-cyclodextrin. The solubility of a gemfibrozil in the presence of hydroxypropyl-γ-cyclodextrin increased linearly, and the constants of complex formation at pH 2.8 found by different methods virtually coincided.

Complexes of praziquantel with β-cyclodextrin were analyzed by 2D NMR spectrometry (^{1}H-^{2}D-NMR) [64]. The ratio of components in the complex was found to be 1 : 1; information for the substantiation of the mechanism of intermolecular interaction was obtained.

The UV spectrum of rutin substantially differs from the spectrum of its complex with β-cyclodextrin: both absorption bands are shifted to longer wavelengths (256 → 260 nm; 351 → 355 nm) and the intensity ratio in the maxima decreases (1.44 for rutin; 1.24 for the complex). Based on these studies, the complex stoichiometry was successfully determined. A conclusion about the mutual arrangement of molecules was made using data of circular dichroism spectrometry. Information on intermolecular hydrogen bonds was obtained by NMR spectrometry. It is clear that the success of the experiment depends on the method chosen [46].

A study of triclosan interactions with β-cyclodextrin and its water-soluble copolymer with epichlorohydrin (**EPI–β-CD**) in solution and in the solid phase was performed by Sohajda et al. using a complex of analytical methods [65]. Two-dimensional NMR spectrometry confirmed the assumption about different types of interaction in AFI–cyclodextrin and AFI–polimeric cyclodextrin systems. The results of studies by DSC, X-ray powder diffraction, and Fourier-transform IR spectrometry were indicative of the high affinity EPI–β-CD to triclosan: a reaction in the solid phase gave water-soluble amorphous products [48]. Inclusion complexes of the host-guest type formed by aspartame with different cyclodextrins were studied by NMR titration and capillary electrophoresis. The stability of the complexes formed by aspartame and twenty-one different cyclodextrins was determined at pH 2.5, 5.2, and 9.0 (aspartame occurs mainly as monocation, zwitter-ion, and monoanion, respectively). The derivatives of cyclodextrin differed by the structures of side chains, substituents, and the ionic form.

The compatibility of two new AFI ({4-[(4-pyridinyl-2-thiazolyl-2-amino)methyl]cyclohexyl}amide 2-propanesulfonic acid and mono-{2-[(*Z*)-4-(3,3-dimethylbutyrylamino)-3,5-difluorobenzoylimino]thiazolyl-3-methyl}ester of phosphoric acid) with PEs (talc, starch, lactose, crospovidone, polyvinylpyrrolidone, magnesium stearate, microcrystalline cellulose and hydroxypropylmethylcellulose, primojel, etc.) used in solid compositions was investigated by Fourier-transform IR spectrometry and HPLC after storage under isothermal conditions [46]. It was shown that potential problems of the compatibility of AFI and PEs can be revealed by IR spectrometry after three days of storage. Changes in IR spectra were detected for mixtures with magnesium stearate and primojel. Studies conducted by HPLC demonstrated that, after twelve days, the maximum decrease in the concentration of AFIs was observed in mixtures with crospovidone, polyvinylpyrrolidone, hydroxypropylmethylcellulose and primojel.

Different interactions between components were revealed for systems microcrystalline cellulose–benzoimidazolylmethyl carbamate after mechanical activation. These were the formation of hydrogen bonds, van-der-Waals interactions, and, probably, chemical interactions with the formation of free radicals, which depended on time, process conditions, method of stirring, etc. [66]. Model mixtures with the ratio AFI : PEs = 1 : 1 were studied before and after mechanical activation. The formation of AFI and PE agglomerates of different types was detected. IR spectra were recorded on a Specord 75 IR spectrophotometer in the wavenumber region 4000–400 cm^{-1}. Samples were prepared by pressing a sample portion with KBr. For the system microcrystalline cellulose–trichlorophene, Fazilova et al. studied mother substances, tablets, and model mixtures in the ratio 1 : 1 [67]. IR spectra were recorded on a Specord 75 IR spectrometer in the wavenumber region 4000–400 cm^{-1}; samples were prepared by pressing sample portions with KBr. X-Ray diffraction studies on a DRON-3M diffractometer and microscopic studies on an MBI-6 optical microscope, a REM-200 electron microscope, and a PEM-100 transmission electron microscope were conducted simultaneously. The results obtained confirmed the absence of chemical interactions between cellulose with trichlorophene with a possibility of changes in the crystal structure of components.

Chromatographic methods and complex studies on their basis. Benzamycin, which is a mixture of benzoyl peroxide and erythromycin, possesses high reactivity and may contain decay products. Gradient HPLC using volatile mobile phases and mass-spectrometric detection revealed that erythromycin during storage oxidized and then was benzoylated [68]. Both freshly prepared samples of benzamycin and pharmaceutical preparations after storage for 2 and 18 months were

investigated. Using isocratic ion-pair HPLC on a C18 column, Dousa et al. found new, previously unknown impurities in anti-cold pharmaceutical preparations containing phenylephrine and sucrose [51]. As was shown by HPLC–MS and NMR spectrometry, phenylephrine was unstable in the presence of sugars and undergoes condensation with aldehydes. The following decomposition products were identified: 1-[5-(hydroxymethyl)-2-furyl]-2-methyl-1,2,3,4-tetrahydroisoquinolinediol-4,8 and 1-[5-(hydroxymethyl)-2-furyl]-2-methyl-1,2,3,4-tetrahydroisoquinoline-4,6-diol. The same impurity was then found in other anti-cold pharmaceutical preparations containing phenylephrine and sucrose. It is clear that particular industrial methods must be developed to exclude undesirable processes and that the use of sucrose must be excluded.

In developing a dosage form of a pharmaceutical preparation containing ephedrine hydrochloride, a problem of the underestimation of the results of analysis by 15–20% arose [69]. It was supposed that this was due to the interaction of ephedrine with croscarmelose sodium. The hypothesis was confirmed by the data of HPLC and IR spectrometry. A possible mechanism of the process was selected and methods for the elimination of the interfering effect were proposed. For combined antitubercular pharmaceutical preparations containing rifampicin and isoniazid, a decrease in the activity of rifampicin because of its interaction was AFIs was proved [70]. A new HPLC procedure was attracted to solve this problem. It was shown that the mechanism of interaction of the above AFIs in the solid phase was similar to that in acid media. The results obtained clearly demonstrate that the composition and technology of this pharmaceutical preparation must exclude the possibility of interactions between AFIs in the presence of gastric juice acid.

PROSPECTS OF THE STUDY OF THE STABILITY OF PHARMACEUTICAL COMPOSITIONS AND THE COMPATIBILITY OF THEIR COMPONENTS

Using the data on the mechanisms of component interaction and decomposition, one can gain positive results in the quality control of pharmaceutical preparations. For example, to prevent decomposition, one can reduce the time of stirring and drying temperature, prevent the interaction of components by varying industrial parameters or the composition of components. The knowledge of changes in the structure components in a mixture allows one to vary the rate of AFI dissolution in the media of an organism and to potentially affect bioavailability.

A comparison of methods for studying the stability and interaction of components showed that IR spectrometry, HPLC, DSC and TG, both separately and in combination, are used most often.

Interactions are most often studied by spectrometric techniques. Infrared spectrometry is a routine method of the quality control of pharmaceutical substances; it is characterized by high information content and specificity. This method is preferable at the initial steps of work and, in some cases, can reveal changes unobservable in, for example, HPLC studies. Using IR spectrometry, one can gain an idea of reaction mechanisms and also specific information on the functional groups in a substance structure; this method allows the detection of the compatibility (or incompatibility) of components in the cases when chromatographic methods are low-informative.

Thermal analysis is an efficient and reliable method for studying the stability and compatibility of substances; is provides an instrument for screening interactions in preliminary technological studies. Studies of heat stability allow calculations of the expiration times of AFIs and pharmaceutical preparations and the regulation of storage conditions.

The use of chromatography in studies of the stability of pharmaceutical preparations demonstrates its high possibilities and good prospects. In combination with mass spectrometry detection it provides the most powerful present-day method of structure determination of the products of AFI and PE decomposition.

Other analytical methods are used less often. Note that discrepancies between the results obtained by different methods are often observed in the studies of interactions and compatibility. This is, possibly, associated with different behaviors of the studied processes under different conditions (temperature, medium) because of the specific features of the methods used.

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