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Stability of Glucosamine Dosage Forms

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Abstract—Stability of medical products (tablets, granules, sup-positories, and ointments), containing glucosamine salts, has been studied by various methods: spectrophotometry, HPLC, capillary electro-phoresis, and thin-layer chromatography. It is shown that glucosamine salts gradually destruct in solutions to 5-hydroxymethylfurfural.

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It is known that the components of drug dosage forms undergo destruction on storage. The regulatory documents determine the contents of admixtures both in the substance and in the dosage form which includes excipients along with the active substances. This is just changes in the appearance and detection of destruction products are taken into account in making decisions on further utility of drugs.

In studying stability of glucosamine dosage forms, which was the subject of the present work, we had first of all focus on destruction products of glucosamine and find out what admixtures may be present in its dosage forms. It might be suggested that glucosamine, like glucose, in the normal state is present as an inactive cyclic form, and, therefore, its destruction begins with ring opening to form a reactive acyclic molecule which may undergo dehydration, oxidation, epimerization, or polymerization under the action of temperature or other external factors [1–3]. Based on the data on glucose destruction, we can suggest that the thermal destruction of glucosamine occurs by a dehydration mechanism and forms 5-hydroxymethylfurfural (HMF) via a number of intermediate stages [2, 4].

5-Hydroxymethylfurfural, in terms of the median lethal dose, is a low-toxic compound (LD_{50} 875 mg kg^{-1}). However, research on its effect on the central nervous system showed that it is by no means harmless, and, therefore, the HMF content in drug dosage forms should be regulated [5].

In exploring the possibility of identification and quantitation of destruction products in glucosamine dosage forms we relied on the practice of determination of HMF in food products.

According to State Standard 29032-91, all food products produced by thermal treatment of fruits and vegetables should be controlled for HMF. The allowed content of this substance is no more than 10–20 mg kg^{-1}. The quantitative determination of HMF is based on measuring the optical density of its reaction product with *p*-toluidine and barbituric acid. Using thin-layer chromatography, HMF is extracted from the analyzed product with an organic solvent (ethyl acetate) and detected by a yellow spot appearing after treatment of the plate with a benzidine solution. This procedure is applied with citrus fruit processing products. A photometric procedure of HMF determination in furyl alcohol is described [6].

Zenkevich et al. [7] suggested standardization of sugar dye for coloring confectionery products, beverages, and elixirs in food and pharmaceutical industries by the HMF content determined by reversed phase high-performance liquid chromatography (HPLC), using benzoic acid, coumarin, nitrobenzene, and anethole as internal standards [7]. The same instrumental technique was also suggested for determination of HMF in electrical insulating oils [8].

According to the Manual on Quality and Safety Control of Biological Food Additives (R 4.1.1672-03), the HMF contents are regulated in juices and honey. This substance is determined by HPLC with a methanol−water−acetic acid (15:84:1) mobile phase. Gas-liquid chromatography is recommended in the regulatory documents (State Standard R 53419-2009)

for the determination of the HMF admixture in ethanol.

Along with the above-mentioned methods, a practice of HMF determination by capillary electrophoresis is known (instruction to the Kapel' capillary electrophoresis system, St. Petersburg, 2001).

EXPERIMENTAL

In the present work we studied stability of novel glucosamine dosage forms for medical and veterinarian applications. These dosage forms contain, along with glucosamine, other biologically active substances which improve the efficiency of the active substance (these preparations were developed at the Pyatigorsk State Pharmaceutical Academy). These are the following dosage forms.

Flexiprofene pellets (glucosamine sulfate, Ketoprofene); Ketoprofene is included in this formulation as an analgesic agent with a minimum ulcerogenic side effect [9].

Flexiactive pellets (glucosamine sulfate, willow bark extract, calcium ascorbate, manganese citrate) are developed for veterinary as a potent antiarthritic drug [10].

Dichlofenac suppositories with glucosamine hydrochloride can also be used as an effective antiinflammatory remedy with a much attenuated ulcerogenic action of Dichlofenac [11].

Glucosamine granules containing burdock or birch tree extracts, as well as pectin, aspartame, and citric acid act as an efficient antiarthritic and anti-inflammatory remedy and are intended for patients with intestinal tract diseases [12].

Sportactive granules (glucosamine sulfate, *Rhaponticum carthamoides* extract, potassium orotate, calcium gluconate) act to enhance the physical efficiency and stimulate recovery processes after physical load [13].

Dental gel (glucosamine hydrochloride, nettle and kalanchoe juices) exhibits an expressed reparative activity in the therapy of periodontal diseases [14].

Furthermore, we analyzed Aminoarthrine pellets (glucosamine hydrochloride, 0.3 g, producer the "Moscow Pharmaceutical Factory" OOO) for HMF.

In developing a regulatory document (FSP 42-0314-1478-01) for the glucosamine hydrochloride substance we based on the spectophotometric procedure used to determine HMF and its related substances in Dona Glucosamine (glucosamine sulfate, Italy). The procedure allows detection of a 0.001% admixture in the preparation. In view of the fact that HMF and related substances are regulated admixtures ($\leq$ 0.05%) in the domestic drug "Glucose, 10% and 20% Solution for Injections," we chose conditions for the determination of this admixture at the required level (optical density of a 0.4% solution should not be higher than 0.275).

Aminoarthrine pellets (glucosamine hydrochloride) were analyzed using our modified spectrophotometric procedure involving extraction to separate glucosamine destruction products from the pellet excipients [15, 16]. The extractant was ethanol (95%).

Since the other analyzed drug dosage forms (Sportactive granules, granules with dry extracts, Flexiprofene and Flexiactive pellets) are multicomponent compositions, it is necessary to study the mutual effect of ingredients in these preparations on their stability [17].

In this research we used 0.4% solutions of glucosamine salts and solutions of other ingredients (as well as their mixtures) in ethanol (50%) or water in concentrations proportional to their contents specified in the corresponding drug formulations. Stability of solutions of substances was studied with the aim to determine the fastest destroyed substance.

Part of the solutions in sealed ampules was kept in a drying oven at 100°C, whereas the others (control) were kept without heating. The mutual effect of ingredients was assessed by a change in the color after keeping for a day. Before heating only solutions of dry and liquid extracts and juices of nettle and kalanchoe were colored. After 2-h heating, glucosamine solutions acquired color, and, therewith, the color of glucosamine sulfate solutions got stronger in the presence of liquid and dry extracts, calcium ascorbate, and nettle and kalanchoe juices and got weaker in the presence of manganese citrate and citric acid. All other ingredients revealed no mutual effect and no effect on glucosamine sulfate (hydrochloride) over the entire observation period. Color enhancement after heating was also observed in solutions of dry and liquid extracts and juices.

The absorption spectra showed that the colorless solutions of Ketoprofen, Dichlofenac, and potassium orotate remained stable throughout the entire experiment. In the spectra of heated glucosamine

sulfate (hydrochloride) solutions we observed appearance of maxima at 230 and 280 nm, depending on heating time, whereas the control solution showed no bands in this spectral region. After 40-min heating, the optical density of the solutions at 280 nm was higher than the reference value (0.275) specified in the FSP 42-0314-1478-01 for the "Glucosamine Hydrochloride" substance.

The resulting data, while preliminary, give us grounds to state that if the first destroyed substance in solutions is glucosamine sulfate (hydrochloride), then in solid forms (granules, pellets, suppositories) we will deal with an analogous process. Thus, stability of glucosamine dosage forms can be judged about by the appearance of glucosamine destruction products.

Ketoprofen, Dichlofenac, potassium orotate, dry and liquid extracts, as well as juices entering the composition of the suggested drug forms absorb in the UV spectral region, which makes direct spectrophotometric analysis an unsuitable technique for determination of admixtures in glucosamine sulfate (hydrochloride) in these preparations.

Ketoprofen, Dichlofenac, and other light-absorbing components and admixtures in glucosamine sulfate (hydrochloride) can be determined using a modified spectrophotometric procedure (based on the difference in the optical densities of the solutions), as well as HPLC with UV detection.

The modified spectrophotometric procedure was tested on the determination of the shelf life of Flexactive pellets. It was found that the optical density of a dry extract of willow bark at 280 nm remains unchanged during two years of storage, i.e. we can suggest that dry components of the extract undergo no destruction within two years [17]. Therefore, we considered it possible to determine admixtures in glucosamine sulfate in Flexactive pellets in the presence of their ingredients by the difference in the optical densities of solutions of the pellets and a solution of an unheated willow bark extract.

The results of the determination of admixtures in Flexactive pellet series stored in ambient conditions showed that the difference in the optical densities was no larger than 0.275 throughout the entire observation period (2.5 years). These data allowed the the shelf life of Flexactive pellets to be established at 2 years.

However, our developed procedure allows no more than preliminarily assessing the minimum allowable storage time for glucosamine dosage forms containing light-absorbing components. This is explained by the fact that for this analysis one has to have the same series of light-absorbing components as those used to produce the analyzed drugs. This especially relates to drugs containing extracts of natural medicinal herbs, the contents of biologically active substances in which is not subject to stringent regulation.

The feasibility of HPLC with UV detection was explored on an example of Dichlofenac suppositories with glucosamine hydrochloride (for separation of HMF and Dichlofenac).

The Manual on Quality and Safety Control of Biological Food Additives (R 4.1.1672-03) recommends for HMF determination by this method the highly toxic methanol as a mobile phase. We used a safer eluent, acetonitrile: phosphate buffer (55:45), pH = 3, as a mobile phase for separations on a Separon-C_{18} sorbent [18].

Since the absorption maximum of HMF is at 280 nm and Dichlofenac absorbs at 276 nm, we used for measurements the common wavelength, specifically 280 nm.

We studied the applicability of this procedure to furfural, the closest analog of HMF in structure and properties. The retention time of furfural in ethanol solution under the above-mentioned conditions was 3.8 min and remained unchanged in six successive runs. The total content of HMF and related compounds was calculated using the scaling coefficient K (ratio of the specific absorption of furfural to the specific absorption of HMF), equaling 1.09. As seen from the chromatogram in Fig. 1, the destruction product of glucosamine is well separated both from furfural and Dichlofenac Sodium.

The applicability of HPLC with UV detection with HMF as internal standard was demonstrated by the example of separation of Ketoprofen and admixtures in glucosamine sulfate in Flexiprofen pellets. The mobile phase was initially water, then acetonitrile was added, and its concentration was increased to 20% over the course of 10 min; in doing so, we observed elution of all destruction products of glucosamine sulfate. To elute Ketoprofen and its possible destruction products, the concentration of acetonitrile was increased from 20 to 60% over the course of 5 min.

As seen from the chromatograms in Fig. 2, the peaks of destruction products of glucosamine sulfate and Ketoprofen are well separated; consequently,

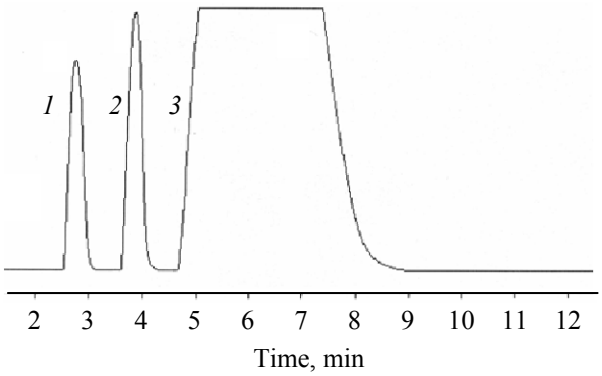

Fig. 1. Chromatogram of compositions of Diclofenac with glucosamine hydrochloride (suppositories): (*1*) destruction product of glucosamine hydrochloride; (*2*) furfural (int. reference); and (*3*) Diclofenac.

admixtures in glucosamine can be identified and quantified in the presence of Ketoprofen.

The developed procedure allows establishing the expiration date of Flexiprofen pellets and was included into the project of technical specifications for this dosage form for veterinary [19].

Let us consider one more example of the use of HPLC: establishment of the shelf life of glucosamine granules with dry extracts of burdock roots and birch leaves. Thermostated (90°C, 120 min) 0.4% solutions of glucosamine salts and solutions of other ingredients of the granules in concentrations proportional to their concentrations in the dosage forms were analyzed by reversed-phase HPLC (Aquilon Stayer HPLC with UV detector) at the following conditions: column Luna C_{18} (150×4.6 mm), eluent acetonitrile–water (acetonitrile gradient from to 20%), detection at 280 nm.

It was found that dry extracts of birch leaves (burdock roots), pectin, aspartame, and citric acid undergo no destruction under the above-mentioned conditions: The retention times and peak shapes and heights were almost the same before and after heating. The chromatogram of glucosamine hydrochloride before heating showed neither peak of glucosamine hydrochloride nor other peaks. The chromatogram of a 4% glucosamine hydrochloride solution (until ~0.05% of admixtures appeared) showed a number of peaks (total area 202 mV s) assignable to glucosamine destruction products (Fig. 3).

Comparison of the chromatograms of glucosamine hydrochloride destruction products and HMF shows that the former chromatograms contains a peak of HMF with the retention time 8.64 min. However, in the elution range of all glucosamine destruction products, there are peaks of biologically active components of dry extracts of birch and burdock. This circumstance makes it impossible to calculate the total content of HMF and its related compounds.

In terms of the total peak area of glucosamine hydrochloride destruction products (> 202 mV s), the substance fails to fit the requirements of the regulatory document by the parameter "foreign admixtures." However, taking into account the total peak area of granule components and stability of dry birch and burdock extracts, citric acid, and aspartame, we can estimate the total peak area at which the contents of HMF and its related compounds will fit the suggested standard (≤ 0.05%). Such determination is only

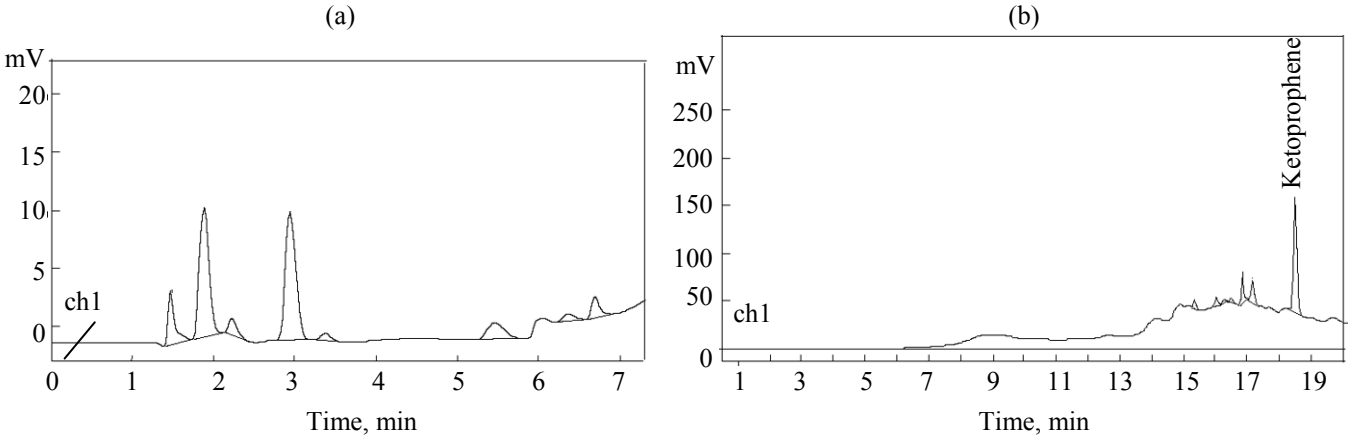

Fig. 2. Chromatograms of (a) 0.4% solution of glucosamine sulfate and (b) 0.02% solution of Ketoprofene after thermal treatment.

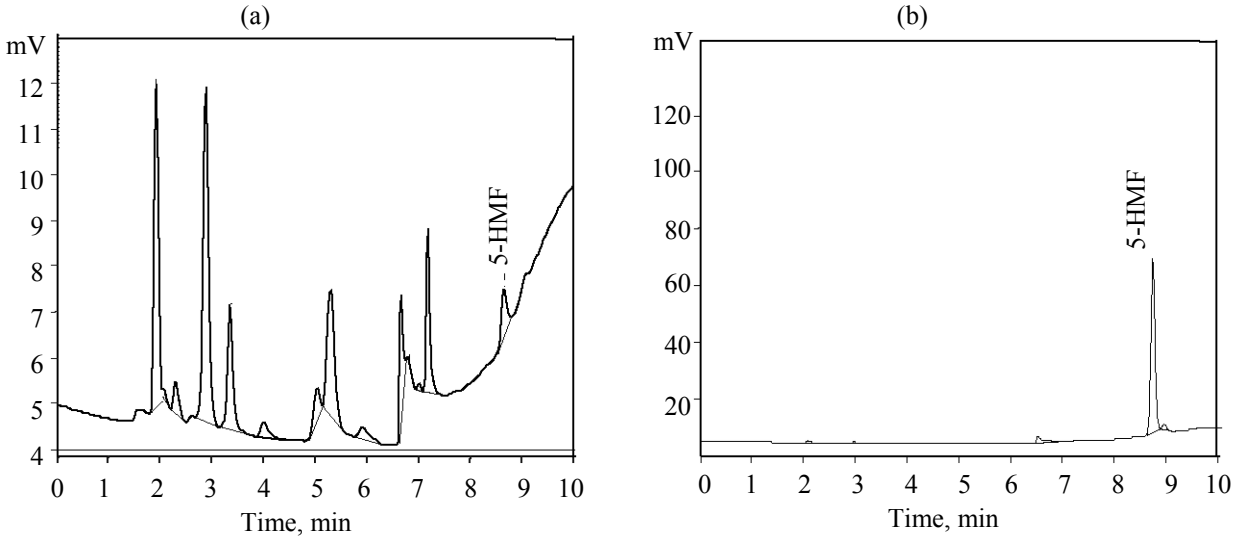

Fig. 3. Chromatograms of (a) glucosamine hydrochloride after its destruction and (b) reference sample of HMF.

suitable for preliminary assessment of the expiration date of such dosage forms, since for analysis one should have only those dry extracts which were used to prepare each specific batch [20].

Along with HPLC, an effective technique for HMF analysis is capillary electrophoresis. To determine this admixture in glucosamine sulfate in Sportactive granules, we made use of micellar electrokinetic capillary chromatography, one of the widely used techniques of capillary electrophoresis. As the basis we took the procedure for the determination of HMF, described in the manual to a Lumex Kapel' instrument. Analysis was performed under the following conditions: capillary diameter 50 μm, working length 65 cm; voltage 20 kV; sample injection at 50 mbar s^{-1}; electrolyte 50 mM phosphate buffer (pH 7.5). The retention time of HMF was 4.12 min.

In the electrophoregram of a 0.4% solution of glucosamine sulfate (Fig. 4) after thermal treatment, which contained up to 0.05% of HMF, the total peak area in the range 4.0–5.0 min was 0.154 mV s. Consequently, in terms of the total peak area of glucosamine sulfate destruction products (> 0.150 mV s) the glucosamine sulfate substance does not fit the standard by the parameter "foreign admixtures" (~0.05% of HMF).

The possibility of determination of HMF and related compounds in drug dosage forms was studied using model mixtures of granules. The model mixtures containing no glucosamine sulfate (placebo) and the model mixture of granules were analyzed in the above-described conditions.

The electrophoregram showed unidentified peaks in the range 2.9-3.8 min. On addition of HMF to a solution of the placebo mixture, a peak at 4.12 min appeared. On addition of glucosamine sulfate to the placebo solution, peaks of glucosamine destruction products appear, separated from peaks of the host solution, which allows the found conditions to be used to determine glucosamine destruction products in Sportactive granules during their storage [21].

In conclusion let us dwell on a procedure we developed to determine HMF in a dental ointment containing glucosamine hydrochloride, with the aim to establish its expiration date [22]**.** The procedure is based on planar chromatography. Here both HPLC and GLC are unsuitable, since the polymer film from the

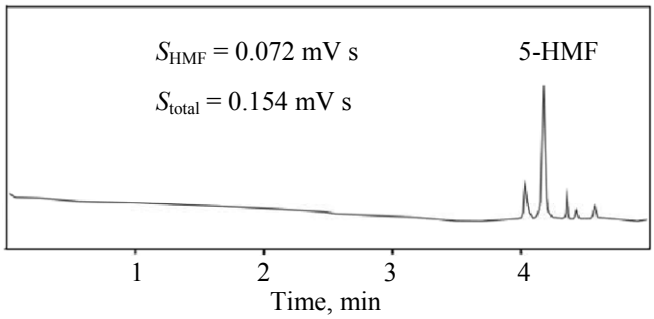

Fig. 4. Electrophoregram of a 0.4% solution of glucosamine sulfate after thermal treatment.

ointment can be extracted with HMF and contaminate chromatographic columns. Analysis conditions: Silufol plates; solvent ethyl acetate, extractant diethyl ether, solvent diethyl ether; extractant sample size 20.0 μl; volume of a 0.2% solution of the HMF reference in ethyl acetate 2 μl; developing agent a freshly prepared 5% solution of benzidine in ethyl acetate. The chromatogram after development contains a yellowish brown spot at the same level as that of the reference sample (delay factor 0.75). Computer processing of the cheromatogram (Videodensimeter Sorbfil software) by two characteristics: spot area and spatial "volume," using color intensity as the third coordinate. A mass–spot area calibration plot is constructed. The calibration plot is linear over the concentration range in focus (0.01 to 0.05 mg ml^{-1}), correlation coefficient 0.996; relative error of the determination is no more than ±5.67%, selectivity 100%. The highest recovery of HMF (to 41%) is reached by extraction with 5 portions of ether (by 5 ml).

The described work contributed, to a certain degree, to the practice of development of new drugs. The procedures of spectrophotometric determination of glucosamine destruction products were included into the regulatory documentation for glucosamine hydrochloride and Aminoarthrine drugs. The procedures based on HPLC, capillary electrophoresis, and planar chromatography were used in the development of projects of pharmacopeial descriptions for Flexiprofen pellts, glucosamine suppositories with Dichlofenac, Sportactive granules, and dental ointment, respectively.

The developed procedure were used to establish shelf lives under ambient conditions for Flexac-tive pellets, Sportactive granules, Dichlofenac suppositories with glucosamine hydrochloride, and dental ointment (2 years), glucosamine hydrochloride (sulfate) granules with dry birch and burdock extracts (1.5 years), and Flexiprofen pellets (1.5 and 3 years).

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