THE STATE OF WATER IN ALPIZARIN

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In continuation of systematic investigations into the hydration characteristics of phytopreparations and standard samples produced at the Institute of Medical and Aromatic Plants, we have studied a parent compound of the antiviral drug alpizarin [1] and the corresponding State standard Sample (SSS).

The base active compound in alpizarin is the xanthone glycoside mangiferin (2-C-β-D-glucopyranosyl-1,3,6,7-tetrahydroxyxanthone) with the formula $C_{19}H_{18}O_{11}$, which is contained in the drug in amounts not less than 96%.

The purpose of this work was to study the hygroscopic properties of this substance and the character of its interaction with water. From the standpoint of practical analysis, the most important task consists in characterization of the hygroscopicity of alpizarin SSS, since any uncertainty in the content of water in this sample would affect the accuracy of parent compound determination in various medicinal forms of the drug.

Since the solubility behavior of a substance is closely related to its crystal structure, in particular, to polymorphism, we have attempted to evaluate the instability of this factor in alpizarin by methods of thermal analysis. Previously, this approach was successfully used to study polymorphism in flacoside [2] and arbutin preparations.

MATERIALS AND METHODS

We have studied the State Standard Sample (SSS) of alpizarin (series 10698), a sample of the parent compound (series 30896) fully conforming to requirements of the normative documentation on alpizarin, and two batches of the drug (series 60298 and 90298) whose solubility in an acetone – water mixture deviated from these requirements to decrease in the following order: 30896 > 60298 > 90298.

The thermoanalytical procedure included DSC measurements on a Perkin-Elmer DSC-2 differential scanning calorimeter (sample heating rate, 20 K/min; sensitivity, $R = 20$ mcal/sec; nitrogen flow rate, 20 ml/min) and gravimetric measurements on a Perkin-Elmer AD-2 ultramicrobalance (accuracy, 0.0001 mg). The results of the water content determination were compared to the control data obtained by the classical microtitration method of K. Fischer. The total bound water content was also determined by measuring the total weight loss on drying (TWLD) for samples kept in a thermal box at 105°C.

The conditions of preset humidity were created in a desiccator containing phosphorus pentoxide together with special acid and salt solutions, which provided a controlled relative humidity in the range from 0.025 to 0.98.

Data obtained by titration with the Fischer reagent showed that both SSS and the parent compound of alpizarin differ from the flavonoid-containing preparations studied

TABLE 1. Water Content in Various Alpizarin Samples

Sample	Water content, %	
	K. Fischer	TWLD, %
Initial samples:		
State Standard 10698	0.68	–
Parent substance 30896	0.55	0.52
60298	0.73	0.72
90298	0.92	0.95
Samples dried for 3 h at 105°C		
State Standard 10698	0	0
Parent substance 30896	0	0
60298	0	0
90298	0	0
Samples dried for 3 h at 105°C and exposed for 100 h at $P/P_s = 0.98$:		
State Standard 10698	1.23	1.29
Parent substance 30896	1.55	1.52
60298	1.80	1.78
90298	1.38	1.40

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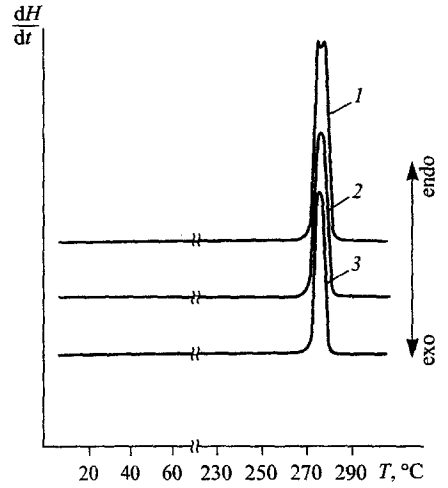

Fig. 1. DSC thermograms (*1*) for alpizarin SSS in the initial state (sample weight, 4.54 mg) and for the same sample (*2*) upon drying for 3 h at 105°C (4.45 mg) and (*3*) after moisturized by exposure for 48 h at $P/P_s = 0.98$ (4.35 mg).

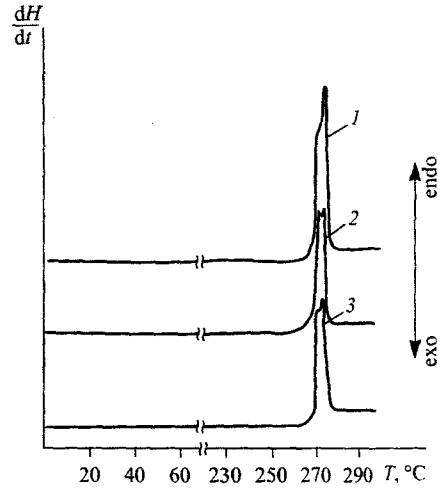

Fig. 2. DSC thermograms of (*1*) the alpizarin parent substance (series 30896) in the initial state (sample weight, 5.24 mg) and for the same sample (*2*) upon drying for 3 h at 105°C (5.48 mg) and (*3*) after moisturized by exposure for 48 h at $P/P_s = 0.98$ (5.34 mg).

previously [2 – 5] by having a comparatively low water content (0.5 – 0.9%).

As is known, titration of the carbonyl-containing compounds by the Fischer reagent may encounter some problems related to the formation of ketals and acetals as a result of methanol attachment or the side reaction of bisulfite addiction. This factor may affect the result of water determination, making it different from the true value [6]. However, coincidence of the results of alpizarin titration according to Fischer and the TWLD data shows that alpizarin does not react with components on the Fischer reagent (Table 1).

In order to evaluate the accuracy of bound water determination provided by the Fischer method, we have performed 13 independent measurements for alpizarin samples of the same commercial batch. The resulting metrological characteristics determined for $f = 12$ were as follows: $\bar{x} = 0.966\%$; $S^2 = 0.0081$; $S = 0.0899$; $P = 95\%$; $t(p,f) = 2.18$; $\Delta X = 0.1960$; $E_1 = \pm 20.3\%$; $E_3 = \pm 11.7\%$.

Thus, the relative error of three parallel determinations, usually performed during the titration according to Fischer, is $E_3 = \pm 11.7\%$.

Taking into account rather small weights of the samples and their low water content (varying from 0.87 to 1.11%), the reproducibility of analytical data can be considered as satisfactory.

It was found that DSC thermograms exhibited virtually the same character for all the alpizarin samples studied, showing a single phase transition manifested by an endothermal peak in the temperature interval from 263 to 289°C. Sometimes, the peak top acquired a two-hump shape (Table 2, Fig. 1). As is known, resolution of a DSC thermogram depends on the sample weight. We have studied the influence of this factor on the thermograms of alpizarin and established that the greater the sample weight, the more pronounced the peak top splitting. For this reason, all subsequent analyses were performed for alpizarin samples having comparable weights.

Taking into account that the endothermal peak falls within a rather narrow temperature interval and is characterized by a high enthalpy value, this feature can be interpreted as a first-order phase transition corresponding to the melting of alpizarin accompanied by decomposition of the compound. This interpretation was confirmed by the value of the

TABLE 2. Temperature Intervals and Enthalpies of Phase Transitions in Alpizarin SSS and Parent Substances

Sample	Peak I			
	ΔT, °C	T_{max1}, ° C	T_{max2}, ° C	ΔH, J/g
Initial samples:				
State Standard 10698	267 – 285	270	273	169
Parent substance 30896	268 – 285	269	275	152
60298	265 – 288	265	–	170
90298	263 – 285	265	–	163
Samples dried for 3 h at 105°C				
State Standard 10698	265 – 287	272	–	174
Parent substance 30296	263 – 280	265	270	199
60298	264 – 289	266	275	191
90298	263 – 285	264	–	178
Samples dried for 3 h at 105°C and exposed at $P/P_s = 0.98$:				
State Standard 10698	265 – 289	271	–	170
Parent substance 30296	263 – 280	263	269	183
60298	264 – 285	265	269	192
90298	265 – 285	263	266	189

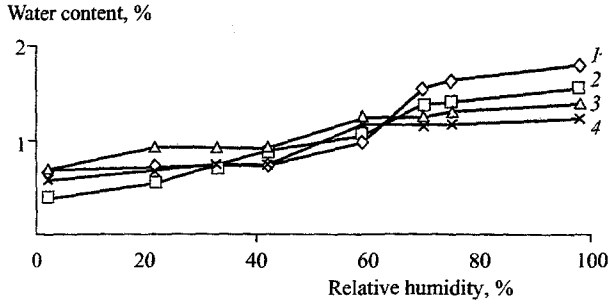

Fig. 3. Water sorption isotherms measured at 20°C for (*4*) alpizarin SSS (10698) and three parent substances (*1*) 60298, (*2*) 30896, and (*3*) 90298.

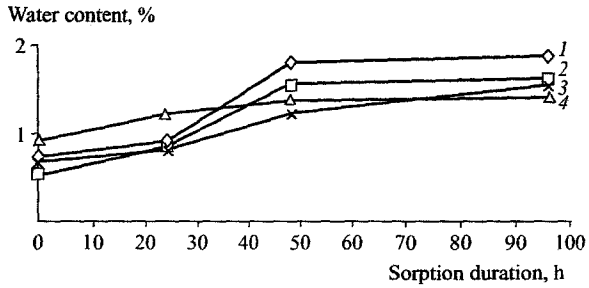

Fig. 4. Dynamics of water vapor sorption by (*4*) alpizarin SSS (10698) and three parent substances (*1*) 60298, (*2*) 30896, and (*3*) 90298.

melting temperature determined for alpizarin SSS in a Koefler block (269 – 270°C, with decomp.) and by the published data [7].

Drying of the alpizarin samples at 105 and 135°C for 3 h had virtually no effect on the phase transition enthalpy, sometimes only slightly changing the shape of the enthalpy peak top (Figs. 1 and 2). Explanation of this fact would require additional experiments.

The fact that the thermograms of alpizarin display no phase transition due to the evaporation of water can be explained by the extremely low hygroscopicity of the parent compound, which was confirmed by experiments of the sorption capacity of alpizarin (Figs. 3 and 4).

As seen from the water sorption isotherms and the data presented in Table 1, the samples practically did not absorbed water (the water content remained equal to approximately 1%) during a 48-h exposure at a relative humidity of $P/P_s = 0.98$. It was only after a very long (100 h) exposure at this humidity that the water content somewhat increased (to 1.2 – 1.8%). On the DSC thermograms, the presence of absorbed water was manifested as a phase transition corresponding to the evaporation of this water, which could be observed only upon a 3 – 4-fold increase in the sample weight and the instrument sensitivity. Note that the purest sample (alpizarin SSS) exhibited minimum water sorption capacity.

The pronounced hydrophobicity of alpizarin, in contrast to the behavior observed for other flavonoids [2 – 5], is probably explained by the coplanar configuration of the dibenzo-γ-pyrone nucleus favoring close crystal packing and hindering water penetration into the crystal even under the conditions of high relative humidity.

Thus, the results of thermal analysis of the alpizarin SSS and various samples of the parent compound exposed to the atmosphere in a broad range of relative humidity showed that alpizarin possesses very low hygroscopicity. This eliminates the requirement for storage in a dry place as stated in the normative documentation for alpizarin.

It was also established that differences in the solubility of various series of alpizarin samples are not related to polymorphism of the substance studied.

REFERENCES

1. L. P. Smirnova, V. I. Glyzin, T. B. Shemeryankina, and E. F. Ob'edkova, RF Patent No. 2,032,413 (06.09.91); *Byull. Izobret.*, No. 10 (1995).
2. M. Yu. Vyaznikova, S. S. Nikolaeva, L. P. Smirnova, et al., *Khim.-Farm. Zh.*, **31**(1), 39 – 41 (1997).
3. M. Yu. Vyaznikova, S. S. Nikolaeva, V. A. Bykov, et al., *Khim.-Farm. Zh.*, **31**(2), 42 – 45 (1997).
4. M. Yu. Vyaznikova, S. S. Nikolaeva, L. P. Smirnova, et al., *Khim.-Farm. Zh.*, **31**(2), 39 – 41 (1997).
5. M. Yu. Vyaznikova, S. S. Nikolaeva, L. P. Smirnova, et al., *Khim.-Farm. Zh.*, **31**(1), 36 – 38 (1997).
6. *Eugen Scholz Reagents for Karl Fischer titration*, Hydranal-Manual (1995), p. 84 – 86.
7. V. I. Glyzin, G. G. Nikolaeva, and T. D. Dargaeva, *Natural Xanthones* [in Russian], Nauka, Novosibirsk (1986), p. 87.