CHROMATO-SPECTROPHOTOMETRIC DETERMINATION OF PSOBERAN AND PSORALEN IN BLOOD PLASMA

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A procedure has been developed for the quantitative determination of the natural photosensitizers psoberan and psoralen in blood plasma. The furocoumarins are extracted from the plasma with chloroform and are separated from accompanying substances by the TLC method. The quantitative determination is made spectrophotometrically. The sensitivity threshold of the method is 1 μg/ml.

Psoralen and psoberan are used as photosensitizing agents for the treatment of chronic dermatoses [1-6]. These substances are taken internally in the form of tablets but the fact that they are sparingly soluble may limit their absorbability and therefore it is a matter of practical interest to determine the concentrations of psoralen and psoberan in the blood plasma. There is no such information in the literature.

EXPERIMENTAL

The conditions for isolating furocoumarins from plasma and determining them spectrophotometrically were selected with the use of psoberan- and psoralen-blood as models. These substances are readily soluble in chloroform and, on the basis of the results of a study of their distribution between aqueous (plasma) and organic (chloroform) phases, this solvent was selected as the extractant. To determine the completeness of the extraction of psoberan or psoralen from the plasma, solutions of these substances with concentrations of 0.1, 0.2, 0.4, 0.6, 0.8, and 1.0 mg/ml were prepared and were added to the control plasma. Preliminary experiments had shown a linear relationship between the optical density and the concentration (1-10 μg/mg) of furocoumarins.

Procedure. Heparinized blood in a volume of 4 ml was centrifuged at 3000 rpm for 15 min. To 2 ml of the supernatant liquid were added 2 ml of a 0.2 M solution of HCl (for breaking down the complex with albumin [7]) and 2 ml of a solution of the furocoumarin in a tenfold volume of chloroform. The substance was extracted by stirring with a magnetic stirrer for 15 min. The organic phase was separated from the aqueous phase in a separatory funnel, and the extraction with chloroform was repeated twice.

The combined chloroform extracts were evaporated to dryness in a rotary evaporator at 40°C. The dry residue was dissolved in 2 ml of chloroform and, depending on the amount of the substance in the plasma, from 0.2 to 0.5 ml of the resulting solution was deposited on a Silufol UV-254 plate. Beside it was deposited 0.1 ml of a 0.025% chloroform solution of standard psoralen and a pure band of the background was left as a blank in spectrophometry.

The plate with the deposited samples was dried in the air for 10 min and was placed in a chromatographic chamber in the petroleum ether—ethyl acetate (2:1) system. When the solvent front had reached the end of the plate (20-30 min), the latter was removed, dried in the air until the smell of the solvents had disappeared, and examined in UV light at 254 nm. The zone of violet fluorescence of the substance at the level of the standard was marked and so was a band of the pure solvent of equal area for control. Then the marked zones were transferred into 100-ml flasks with ground stoppers, covered with 10 ml of 95% ethanol, and eluted by heating in a thermostated bath at 50-55°C for 3 h with periodic shaking. After cooling, the eluate was filtered through a folded paper filter and its optical density was determined at 298 nm (psoberan) or 246 nm (psoralen) in a cell with a layer thickness of 10 mm in comparison with the eluate from the control zone.

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The amount of furocoumarins (X, μg/ml) in the plasma was determined from the formula

$$X = \frac{D_1 \cdot C_0 \cdot V_e \cdot 1000}{D_0 \cdot V},$$

where D_1 and D_0 are the optical densities of the solution under investigation and of the solution of standard psoralen, respectively; C_0 is the concentration of the standard psoralen solution, mg/ml; V is the volume of the extract deposited on the chromatogram, ml; and V_e is the volume of eluate, ml.

The percentage extraction of the fluorocoumarins added to the control plasma in the concentrations shown above amounted to 86.9 ± 5.48 (n = 6, P = 0.95). The sensitivity threshold of the method is 1 μg/ml.

In experiments on animals, the procedure for extracting the furocoumarins from the blood plasma was the same as described above for model mixtures.

The blood for analysis was taken from random-bred white rats weighing 230-250 g. Tablets of the furocoumarins were given to the fasting animals at the rate of 50 mg per 1 kg of body weight. Rats were sacrificed by decapitation 0.5, 1, 2, 4, 6, and 8 h after the administration of the preparation, and the concentrations of furocoumarins in the blood plasma were determined. It was established that the maximum concentration of psoberan and psoralen in the plasma was observed 2 h after the administration of the tablets, and for three series of psoberan tablets it amounted to 7.4, 7.8, and 8.5 μg/ml, and for the psoralen tablets to 5.1, 5.8, and 6.4 μg/ml. After 8 h, furocoumarins were no longer detected in the blood plasma.

SUMMARY

A procedure for determining psoberan and psoralen in blood plasma is proposed.

LITERATURE CITED

1. U. Rakhmankulov, L. N. Shubina, V. Z. Mazgutov, T. T. Shakirov, and N. K. Abubakirov, Drupe Scurfpea (*Psoralea drupacea*) — a Medicinal Plant [in Russian], Tashkent (1979).
2. A. V. Khamaganova, V. N. Glozman, and S. F. Frantseva, Pediatriya, No. 4, 59 (1979).
3. A. V. Braitsev, Vestn. Dermatol., No. 9, 53 (1975).
4. G. I. Marzeeva, L. Ya. Trofimova, and M. M. Kirsanova, Vest. Dermatol., No. 5, 8 (1982).
5. M. B. Sultanov, Khim.-Pharm. Zh., No. 12, 108 (1981).
6. R. A. Kapkaev, B. L. Lyuban, and V. N. Akovbyan, Vestn. Dermatol., 12, 48 (1983).
7. I. Schmid and F. W. Koss, J. Chromatogr., 146 (Biomedical Application, 3), 498 (1978).