Silene nutans L. It has been shown that it is 2β,3β,14α,20R,25-pentahydro-5β-cholest-7-en-6-one. Characteristic features of the mass spectrum of 22-deoxyecdysterone due to the absence of an oxygen function at C-22 have been discussed.

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CHROMATO-PHOTOCOLORIMETRIC DETERMINATION OF DIGOXIN

D. M. Popov and M. B. Mavlyanova

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Procedures have been developed for the quantitative determination of digoxin as such and in solutions for injection and tablets by a chromato-photocolorimetric method with the aid of which it is possible to obtain reliable results with adequate accuracy. The relative error of the determination does not exceed ±4.0%.

The quantitative determination of digoxin is carried out by a biological method [1]. Photocolorimetric, spectrophotometric, and chromatographic methods of analyzing digoxin have been described [5, 6, 10, 11]. It is reported in [2-4] that direct photocalorimetric and spectrophotometric methods are unsuitable for the investigation of the stability of preparations containing cardiac glycosides, and chromatographic methods have been developed mainly for the qualitative estimation of digoxin.

In view of this, the tasks of the present work were: 1) to study the chromatographic separation of digoxin from the foxglove and the breakdown products of digoxin; 2) to develop a procedure for the chromato-photocolorimetric determination of digoxin as such, in tablets, and in solutions; and 3) in order to establish the reliability of the results obtained, to compare them with those found by high-pressure liquid chromatography (HPLC) and by biological analysis.

EXPERIMENTAL

Standard digoxin, the ordinary substance, a solution for injection, and digoxin tablets were investigated. For the separation of digoxin, foxglove glycosides, and the products of its degradation we used ascending chromatography on Silufol UV-254 plates (Czechoslovakia) as the most readily available of the standard sorbents. The biological activity was determined on common frogs by the method of subcutaneous injection [1]. Analysis of digoxin by the HPLC method was performed by a procedure that we had developed previously [7].

We studied more than 20 solvent systems for the separation of foxglove glycosides. In the chloroform–acetone (1:1) solvent system, digoxin was separated from digitoxin and lantosides A, B, and C (Fig. 1).

Factors that have an unfavorable influence on the stability of drugs both in their production and during their storage are high temperatures, UV light, acidity or alkalinity of the glassware, and acidic or alkaline tablet fillers. In the choice of a system of solvents and reagents for revealing the zones of adsorption, digozin subjected to treatment with UV rays and to the action of alkalis and acids and also to high temperatures was

All-Union Scientific-Research Institute of Pharmacy, Moscow. Tashkent Pharmaceutical Institute.

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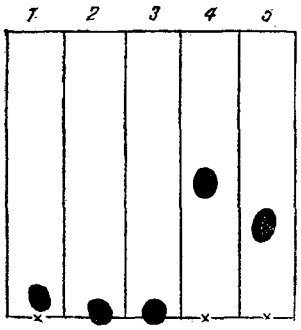

Fig. 1

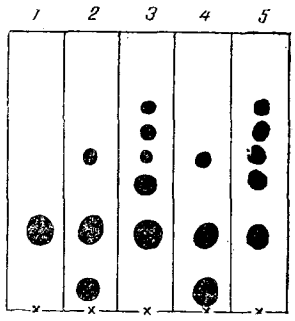

Fig. 2

Fig. 1. Chromatograms of solutions of glycosides: 1) lanatoside A; 2) lanatoside B; 3) lanatoside C; 4) digitoxin; 5) digoxin.

Fig. 2. Chromatograms of solutions: 1) standard digoxin; 2) digoxin that had been stored at 60°C; 3) digoxin irradiated with UV rays; 4) digoxin decomposed with alkali; 5) digoxin decomposed with acid.

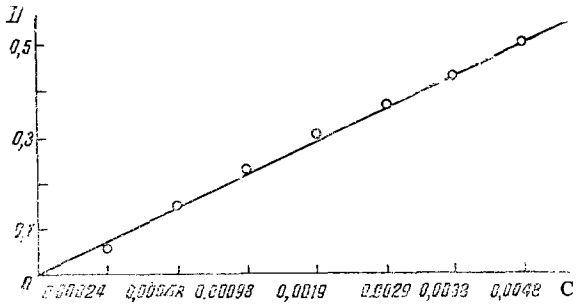

Fig. 3. Calibration graph for the determination of digoxin.

chromatographed. In the chloroform—acetone (1:1) solvent system the products of the degradation of digoxin formed under the action on it of various factors (high temperature, UV light, alkalis, acids) were well separated from digoxin (Fig. 2). A 1% solution of vanillin in 10% perchloric acid proved to be the most suitable agent for revealing the foxglove glycosides, since it well showed the glycosides and their degradation products.

It did not appear possible to use a direct UV-spectrophotometric method for analyzing the eluates, since the eluates from the sorbents (Silufol, silica gel, etc.) had strong absorption at a wavelength of 220 nm [8]. For the quantitative determination of digoxin we used a known photocolorimetric method based on the reaction with sodium picrate [9].

The absorption maximum of the products of the reaction of digoxin with sodium picrate is observed at a wavelength of 490 nm.

To select the working concentrations of digoxin we plotted a calibration graph.

Construction of a Calibration Graph. A 0.1000-g sample (accurately weighed) of standard digoxin was dissolved in 90 ml of chloroform—95% ethanol (1:1) in a 100-ml measuring flask and, after the material had dissolved, the volume of the solution was made up to the mark with the same mixture (solution A).

Seven Silufol plates with dimensions of 15 × 15 cm were used. Five spots were applied: on plate 1, 0.005 ml; on plate 2, 0.01 ml; on plate 3, 0.02 ml; on plate 4, 0.04 ml; on plate 5, 0.06 ml; on plate 6, 0.08 ml; and on plate 7, 0.1 ml of solution A at each point, and chromatography was carried out by the ascending method in the chloroform—acetone (1:1) system. After the solvent system had traveled 12-13 cm, the band from the first spot was cut out, dried in the air, sprayed with a 1% solution of vanillin in 10% perchloric acid solution, and

TABLE 1. Results of the Quantitative Determination of Digoxin in Substance by the Chromato-Photocolorimetric Method (n = 6)

Sample no.	Digoxin taken, g	Metrological characteristics				
		digoxin found		$S_{\bar{X}}$	ε_α	A, %
		r, $\bar{X}$	%, $\bar{X}$			
1	0,0500	0,0497	99,29	0,90	2 32	±2.34
2	0,0500	0,0503	100,61	0,41	1,10	±1,·0
3	0,0500	0,0497	99,41	1,50	3,85	±3,87
4	0,0500	0,0488	95,68	0,83	2,14	±2,24
5	0,0500	0,0495	98,22	0,82	1,95	±1,99

TABLE 2. Results of the Quantitative Determination of Digoxin in Model Samples of Tableting Mixtures by the Chromato-Photocolorimetric Method (n = 6)

Sample no.	Weight of preparation, g	Digoxin taken in one tablet, mg	Metrological characteristics			
			digoxin found in one tablet, mg $\bar{X}$	$S_{\bar{X}}$	ε_α	A, %
1	1,0002	0,243	0,235	0,0029	0,0075	±3,17
2	0 9826	0,248	0,242	0,0030	0,0078	±3,21
3	1,0056	0,251	0,244	0,0031	0,0079	±3,23

placed in the drying cabinet at 70-80°C for 10 min. The developed band was used to detect the zones of adsorption of the digoxin. The zones corresponding to digoxin were scraped off and eluted with 4 ml of 70% ethanol for 30 min, and each extract was then filtered through a paper filter into a 5-ml measuring flask. The deposit on the filter was washed with 1 ml of 70% ethanol, and the volume of the solution was brought up to the mark with the same ethanol.

To 3 ml of each eluate was added 1.5 ml of a neutral solution of picric acid and 0.5 ml of a 2% solution of caustic soda, and the mixture was carefully stirred. After 10 min, the optical density of each solution was measured with the aid of a photoelectric colorimeter at 490 nm in a cell with a layer thickness of 10 mm using as comparison solution a mixture of 3 ml of 70% ethanol, 1.5 ml of a neutral solution of picric acid, and 0.5 ml of a 2% solution of caustic soda.

Observance of the Bouguer—Lambert—Beer law was found within the concentration limits from 0.00048 to 0.0048%. The sensitivity of the determination was 0.0048 mg/ml of digoxin (Fig. 3).

Procedure for the Determination of Digoxin as Such. About 0.05 g (accurately weighed) of digoxin was dissolved in 90 ml of chloroform—95% ethanol (1:1) in a 100-ml measuring flask and after it had dissolved the volume of the solution was brought up to the mark with the same mixture.

At each of four points on a Silufol plate with dimensions of 15 × 15 cm was deposited 0.12 ml of the solution obtained. At a fifth spot was deposited 0.12 ml of standard solution B and chromatography was performed in the chloroform—acetone (1:1) solvent system. The strip with the standard digoxin was cut off and was sprayed with a 1% solution of vanillin in 10% perchloric acid, and the subsequent procedure was as described in the plotting of the calibration graph. The percentage of digoxin the preparation (X) was calculated from the formula

$$X = \frac{D \cdot 0{,}0005 \cdot 100 \cdot 100}{D_0 \cdot a},$$

where D and D_0 are the optical densities of the solution under investigation and the solution of standard digoxin, respectively; and a is the weight of the preparation, g.

The amount of digoxin in the preparation should be not less than 97.0%.

The procedure was checked on five batches of digoxin powder. The results obtained are shown in Table 1, from which it follows that the chromato-photocolorimetric method can be used to analyze small amounts of digoxin (0.29 mg) with adequate accuracy. The relative error of the determination at a confidence level of 0.95 does not exceed ±4%.

TABLE 3. Results of the Quantitative Determination of Digoxin in Model Samples of Solutions by the Chromatophotocolorimetric Method (n = 6)

Sample no.	Digoxin taken, mg in 1 ml	Metrological characteristics			
		digoxin found, mg in 1 ml, $\bar{X}$	$S_{\bar{X}}$	ε_α	A, %
1	0,252	0,257	0,0028	0,0079	±3,06
2	0,250	0,248	0,0021	0,0058	±2,35
3	0,296	0,292	0,0021	0,0058	±2,00

TABLE 4. Results of the Quantitative Determination of Digoxin in Tablets and in Solutions by Various Methods

Batch number of the preparation	Amount of digoxin found in 1 tablet or in 1 ml		Found by the biological method, FAU
	chromato-photo-colorimetric method	HPLC method	
Tablets containing 0.00025 g of digoxin in each			
00200380	0,000236	0,000241	3,30
0570781	0,000247	0,000245	3,50
0150580	0,000247	0,000246	3,50
Digoxin solution, 0.025%, for injection			
0400180	0,000241	0,000238	3,50
080372	0,000230	0,000228	3,30
010468	0,000235	0,000230	3,30

Procedure for Determining Digoxin in Tablets. About 1 g (accurately weighed) of a powder of the ground tablets was placed in a bottle, 3 ml of chloroform–95% ethanol (1:1) was added, and extraction was carried out on a vibration apparatus for 30 min. The bottle and the solid on the filter were washed with 2 ml of the same mixture and chromatography was carried out as described in the determination of digoxin as such. The amount of digoxin in one tablet (X) was calculated from formula

$$X = \frac{D \cdot 0{,}0005 \cdot 5 \cdot b}{D_0 \cdot a},$$

where b is the mean weight of a tablet, g.

The amount of digoxin in one tablet should be 0.000225–0.000275 g.

The procedure was checked in three model samples of tablet mixtures (Table 2). The relative error of the determination does not exceed +4%.

Procedure for the Determination of Digoxin in Solutions. A separatory funnel was charged with 4 ml of a 0.025% solution of digoxin and after the addition of 6 ml of water and 30 ml of chloroform 95% ethanol (5:1) the mixture was shaken for 5 min. The chloroform layer was filtered into an evaporating dish through a filter with 4 g of anhydrous sodium sulfate that had been wetted with chloroform. The digoxin-extracting operation was repeated two times more with 30 ml of chloroform–95% ethanol (5:1) each time, the chloroform extracts being collected in the same evaporating dish. The filter with the anhydrous sodium sulfate was washed with 10 ml of the same mixture and the filtrate was evaporated on the water bath to about 2 ml. The last traces of solvent were driven off with a stream of air. The residue in the dish was dissolved in 2 ml of chloroform–95% ethanol (1:1) and chromatography was carried out as in the determination of digoxin as such. The amount of digoxin in 1 ml of the preparation in grams (X) was calculated from the formula

$$X = \frac{D \cdot 0{,}0005 \cdot 2}{D_0 \cdot 4} = \frac{D \cdot 0{,}0005}{D_0 \cdot 2}.$$

The amount of digoxin in 1 ml should be 0.000225-0.000275 g.

A trial of model samples of solutions of digoxin with the aid of the procedure developed showed that sufficiently accurate results were obtained. The relative error of the determination does not exceed ±3.5%.

Preparation of a Solution of Standard Digoxin. A 50-ml measuring flask was charged with 25 ml of solution A prepared for the plotting of the calibration graph, and the volume of the solution was brought up to the mark with chloroform–95% ethanol (1:1) (solution B), and chromatography of the resulting solution, which contained 0.0005 g of standard digoxin in 1 ml, was carried out as in the construction of the calibration graph.

The results of the analysis of industrial samples of tablets and solutions of digoxin by various methods are given in Table 4, from which it can be seen that the chromato-photocolorimetric method gave reliable results correlating with those of the HPLC method. Consequently, the procedures developed can be used for the analysis of digoxin during storage, since the separation of the degradation products with the aid of thin-layer chromatography permits the analysis of the remaining glycoside by the photocolorimetric method.

SUMMARY

Procedures have been developed for the quantitative determination of digoxin as such in solutions for injection, and in tablets by a chromato-photocolorimetric method, with the aid of which it is possible to obtain reliable results with adequate accuracy. The relative error of the determination does not exceed ±4.0%.

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