CHROMATO-PHOTOCOLORIMETRIC DETERMINATION OF TSELANID (LANATOSIDE C)

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Methods have been developed for the chromato-colorimetric determination of tselanid (lanatoside C) as such and in tablets and solutions. The chloroform–methanol–water (80:19:1) solvent system was used. Chromatography was performed by the ascending method on Silufol UV-254 plates. The revealing agent was a 1% solution of vanillin in 10% perchloric acid. Quantitative determination was performed photocolorimetrically by the reaction with sodium picrate. The methods developed have given accurate results correlating with those of high-performance liquid chromatography and the biological method. The relative error of this determination does not exceed ±4% [1].

It is recommended to perform the quantitative determination of tselanid (lanatoside C) in parallel with the biological method by a photocolorimetric method with xanthydrol after paper chromatography [1]. The method is laborious and requires large amounts of time. High-performance liquid chromatography [2] cannot be widely used in the practice of laboratories for analytical control because of the lack of instruments. At the present time, the photocolorimetric method in combination with thin-layer chromatography is recommended for the analysis of cardiac glycosides [3, 4].

The aims of the present work were: 1) to choose a system of solvents for the separation of glycosides and the products of their degradation, a reagent revealing both the glycosides and the products of their decomposition, and a solvent for the elution of the tselanid from the sorbents; 2) the development of a procedure for the chromato-photocolorimetric determination of tselanid as such and in tablets and in solutions; and 3) the comparison of the results obtained with those of biological analysis and of high-performance liquid chromatography.

EXPERIMENTAL

The standards used were standard lanatoside C (WHO) and tselanid-standart (USSR). The tselanid was studied as such and in the form of tablets and solutions produced by the domestic industry.

Tselanid and the products of its decomposition were separated by ascending chromatography on Silufol UV-254 plates (Czechoslovakia) as the most suitable of the standard sorbents. A solvent mixture — chloroform–95% ethanol (1:1) — in which tselanid dissolves well and can be extracted from the fillers of tablets was selected experimentally. Biological activity was determined on common frogs by subcutaneous injection and was expressed in frog activity units (FAUs) [1]. The high-performance liquid chromatography of tselanid was carried out by procedures that we have developed [2].

The problem posed was solved in several stages. In the choice of systems of solvents and reagents for revealing the zones of adsorption, tselanid subjected to the action of high temperature, UV radiation, acid, and alkali was analyzed. The results of the investigation are shown in Fig. 1.

In the solvent system chloroform–methanol–water (80:19:1), the degradation products formed under the action of various factors are well separated from tselanid. This system of solvents was then used for the quantitative determination of tselanid by the chromato-photocolorimetric method. A 1% solution of vanillin in 10% perchloric acid has proved to be the most suitable reagent for revealing digitalis glycosides and both the glycosides and the products of their degradation are shown up well by it. For the elution of the glycosides from the chromatograms we used 70% ethanol.

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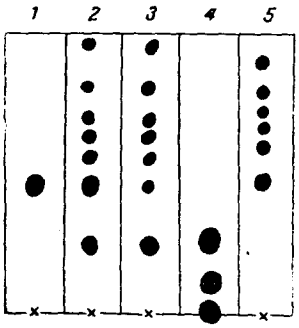

Fig. 1. Chromatograms of solutions of tselanid: 1) tselanid-standart; 2) tselanid stored at 60°C; 3) tselanid subjected to UV irradiation; 4) tselanid decomposed by alkali; 5) tselanid decomposed by acid.

The direct UV-spectrophotometric method could not be used for analyzing the eluates, since the eluates from the Silufol sorbents had strong absorption at a wavelength of 220 nm [3]. For the quantitative determination of tselanid we used a known photocolorimetric method based on the reaction with sodium picrate. The optical density of the products of the reaction of tselanid with sodium picrate was measured at a wavelength of 490 nm [5].

Plotting the Calibration Curve. With accurate weighing, 0.1000 g of tselanid-standart was dissolved in 90 ml of chloroform–95% ethanol (1:1) in a 100-ml measuring flask. After complete dissolution, the volume of the solution was made up to the mark with the same mixture.

Six Silufol UV plates with dimensions of 15 × 15 cm were used. At each of five spots on each plate, 0.01 ml of the solution obtained was deposited on the first plate, 0.02 ml on the second, 0.04 ml on the third, 0.06 ml on the fourth, 0.08 ml on the fifth, and 0.1 ml on the sixth, and ascending chromatography was performed in the chloroform–methanol–water (80:19:1) system.

After the solvent system had traveled a distance of 12-13 cm, the band from the first spot was cut off, dried in the air, sprayed with a 1% solution of vanillin in 10% perchloric acid, and placed in the drying chest at 70-80°C for 10 min. The strip so obtained was used for detecting the zones of absorption of tselanid. The zones corresponding to the tselanid were scraped off and eluted with 4 ml of 70% ethanol for 30 min. Then the extract was filtered into a 5-ml measuring flask. The residue on the filter was washed with 1 ml of 70% ethanol and the volume of the solution in the flask was made up with the same ethanol to the mark.

To each of 3-ml portions 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 mixtures were carefully stirred. After 15 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 the comparison solution a mixture of 3 ml of 70% ethanol, 1.5 ml of neutral picric acid solution, and 0.5 ml of 2% caustic soda solution.

Conformance to the Bouguer–Lambert-Beer law was observed in the range of concentrations from 0.00024 to 0.00384%, the sensitivity of the determination being 0.0024 mg/ml of tselanid.

The results obtained were used for drawing up a procedure for the chromato-photocolorimetric determination of tselanid as such and in tablets and solutions.

Procedure for the Determination of Tselanid as Such. About 0.05 g (accurately weighed) of tselanid powder was dissolved in 90 ml of chloroform–95% ethanol (1:1) in a 100-ml measuring flask and, after complete dissolution, the volume of the solution was made up to the mark with the same mixture.

At each of four points on a Silufol UV-254 plate with dimensions of 15 × 15 cm was deposited 0.12 ml of the solution so obtained. At a fifth spot, 0.12 ml of the solution of tselanid-standart was deposited and chromatography was performed by the ascending method in chloroform–methanol–water (80:19:1). The strip with the tselanid-standart was cut out and sprayed with the revealing agent. The subsequent procedure was the same as in the plotting of the calibration curve.

TABLE 1. Results of the Quantitative Determination of Tselanid by the Chromato-Photocolorimetric Method

Preparation No.	Tselanid taken, g	Metrological characteristics				
		tselanid found		$S_{\bar{x}}$	ε_α	A
		g $\bar{x}$	% $\bar{x}$			
120779	0,0256	0,0250	97,66	0,449	1,063	±1,10%
10181	0,0250	0,0241	96,22	0,399	1,109	±1,15%
20181	0,0250	0,0245	98,22	0,824	1,953	±1,99%
30181	0,0250	0,0238	95,63	0,831	2,136	±2,23%
150581	0,0250	0,0240	96,13	0,539	1,322	±1,38%

TABLE 2. Results of the Quantitative Determination of Tselanid in Model Samples of Tableting Masses by the Chromato-Photocolorimetric Method

Sample No.	Weight of preparation, g	Tselanid taken calculated to one tablet, mg	Metrological characteristics			
			tselanid found in one tablet, mg, $\bar{x}$	$S_{\bar{x}}$	ε_α	A
1	1,0020	0,248	0,242	0,0013	0,0032	±1,31%
2	0,9858	0,250	0,251	0,0012	0,0031	±1,24%
3	0,9787	0,251	0,243	0,0034	0,0088	±3,61%
4	1,0102	0,245	0,237	0,0018	0,0045	±1,91%
5	1,0098	0,252	0,245	0,0038	0,0098	±4,00%

TABLE 3. Results of the Quantitative Determination of Tselanid in Model Samples of Solutions by the Chromato-Photocolorimetric Method

Sample No.	Tselanid taken, calculated to 1 ml, mg	Metrological characteristics			
		tselanid found in 1 ml, mg $\bar{x}$	$S_{\bar{x}}$	ε_α	A
1	0,500	0,497	0,0045	0,0121	±2,49%
2	0,503	0,514	0,0045	0,0125	±2,43%
3	0,591	0,583	0,0041	0,0114	±1,96%
4	0,202	0,203	0,0018	0,0050	±2,44%
5	0,205	0,200	0,0017	0,0044	±2,20%

The percentage of tselanid in the preparation (X) was calculated from the formula

$$X = \frac{D \cdot 0.0005 \cdot 100 \cdot 100}{D_0 \cdot a} = \frac{D \cdot 5}{D_0 \cdot a},$$

where D and D_0 are the optical densities of the solution under test and the solution of tselanid-standart, respectively; a is the weight of the preparation, g; and 0.0005 is the amount of tselanid in 1 ml of standard solution, g.

The amount of tselanid in the sample should be from 95.0 to 105.0%.

The method developed was tested on five samples of tselanid. The results obtained are presented in Table 1.

Thus, it is possible to analyze small amounts of tselanid (0.12 mg) by the chromato-photocolorimetric method with adequate accuracy. The relative error of the determination at a confidence level of 0.95 does not exceed ±3%

Procedure for Determining Tselanid in Tablets. About 1 g (accurately weighed) of a powder of ground tablets was placed in a bottle to which 4 ml of chloroform–95% ethanol (1:1) was added, and the whole was shaken on a vibration apparatus for 30 min. Then it was filtered into a 5-ml measuring flask. The bottle and the residue on the filter were washed with 1 ml of chloroform–95% ethanol (1:1) and the volume of the solution was made up to the mark with the same mixture.

TABLE 4. Results of the Determination of Tselanid in Industrial Samples of Tablets and Solutions for Injection

Serial No. of the preparation	Tselanid found by the method given				
	chromato-photo-colorimetric		high-performance liquid chromatography		biological
	mg	recalculated to FAUs	mg	recalculated to FAUs	FAUs
0,250 mg tselanid tablets					
230780	0,245	3,52	0,241	3,62	3,75
111282	0,252	3,78	0,250	3,75	3,75
121282	0,254	3,81	0,255	3,83	3,88
0,02% solution of tselanid for injection					
011079	0,144	2,16	0,143	2,14	2,20
021079	0,106	1,58	0,109	1,64	1,60
031079	0,151	2,27	0,147	2,21	2,30

At each of four points on a 15 × 15 cm plate was deposited 0.012 ml of the resulting solution. At a fifth point, 0.12 ml of a solution of tselanid-standart was deposited, and the subsequent procedure was as in the determination of powdered tselanid.

The amount of tselanid in a tablet in grams (X) was calculated from the formula

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

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

The amount of tselanid in one tablet should be between 0.000225 and 0.000275 g.

The procedure was checked on specially prepared mixtures of tselanid with the fillers used in the tablets (Table 2). The relative error of the determination does not exceed ±4%.

Procedure for Determining Tselanid in Solutions. In a separatory funnel, 2 ml of a 0.05% solution or 5 ml of a 0.02% solution of tselanid was placed and to this was added 8 ml of water in the first case and 5 ml of water in the second case together with 30 ml of chloroform–95% ethanol (5:1) and the funnel was shaken for 5 min. After layer separation, the chloroform layer was filtered into an evaporating dish through a paper filter with 4 g of anhydrous sodium sulfate that had been moistened with the chloroform–95% ethanol (5:1) mixture. The extraction operation was repeated twice more with 30 ml of solvent each time, the chloroform extracts being collected in the same evaporating dish. The filter with the sodium sulfate was washed with 10 ml of the same mixture. The filtrate was evaporated on the water bath to approximately 2 ml, and the remainder of the solvent was driven off by blowing with air. The residue in the dish was dissolved in 2 ml of chloroform–95% ethanol (1:1).

At each of four points on a 15 × 15 cm plate was deposited 0.12 ml of the resulting solution. At a fifth point was deposited 0.12 ml of a solution of tselanid-standart, and the subsequent procedure was the same as in the determination of tselanid in a powder.

The amount of tselanid in 1 ml of preparation in grams was calculated from the formula

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

where a is the number of milliliters of solution taken for analysis.

The amount of tselanid in 1 ml of a 0.02% solution should be between 0.00018 and 0.00022 g and in 1 ml of a 0.05% solution between 0.00045 and 0.00055 g.

Preparation of the Solution of Tselanid-standart. In a 100-ml measuring flask, 0.0500 g (accurately weighed) of tselanid-standart (FS 42-990-75) was dissolved in 90 ml of chloroform–95% ethanol (1:1) and, after dissolution, the volume of the solution was made up to the mark with the same mixture.

At each of the five points on a 15 × 15 cm plate was deposited 0.12 ml of the solution obtained, and the subsequent procedure was the same as in the construction of the calibration curve. The standard solution contains 0.0005 g of tselanid-standart in 1 ml.

The method was checked on five model samples of tselanid solutions. The results obtained are given in Table 3, from which it can be seen that the relative error of the determination in the analysis of tselanid solutions was less than ±3%.

The results of an analysis of industrial samples of tablets and solutions of tselanid are given in Table 4, from which it follows that the results of the chromato-photocolorimetric method correlate with those obtained by the use of high-performance liquid chromatography and the biological method [1]. On the basis of the investigations performed, procedures have been developed for the quantitative determination of small amounts of tselanid (0.12 mg) that are 4-5 times faster than the procedures included in pharmacopoeias. Reliable results can be obtained by the procedures developed, since the separation of the degradation products by thin-layer chromatography makes it possible to analyze the remaining glycoside.

SUMMARY

1. Conditions for the chromatographic separation of glycosides and the products of their degradation have been investigated and solvents have been selected for extracting glycosides from tablets and sorbents. Procedures have been developed for the quantitative chromato-colorimetric determinations of tselanid as such and in tablets and solutions. The relative error of the determination does not exceed ±4%.

2. The results of the chromato-photocolorimetric method correlate with those obtained by the biological method and by high-performance liquid chromatography. The procedure developed can be used for the analysis of glycosides during storage.

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MASS SPECTROMETRY OF PENNOGENIN GLYCOSIDES

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The electron-impact mass spectra of five unesterified pennogenin glycosides, which contain the M^+ or $(M - H_2O)^+$ peaks, have been obtained. The characteristic features of the fragmentation of these compounds have been studied. In addition to ions characterizing the successive elimination of carbohydrate units, fragments have been detected which show the breakdown of the terminal pyranose ring. Five new directions of the fragmentation of the spirostanol skeleton due to the presence of an OH group at C-17 have been found.

Pennogenin (PG) and its glycosides are discussed in a number of publications [1-10] the mass-spectral information in which relates almost entirely to features of the fragmentation of the aglycon due to the presence of an OH group at C-17. A report by Japanese authors recently appeared on the acquisition of the electron-impact (EI) mass spectra of glycosides of PG and of diosgenin and of the corresponding furostanols where the possibility was shown of

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