QUANTITATIVE DETERMINATION OF CELANIDE BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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UDC 615.22:547.918:582.951.64/074:543.544

Methods have been developed for the quantitative determination of celanide [lanatoside C] in substances, in solutions for injection, and in tablets by high-performance liquid chromatography, with the aid of which it is possible to determine small amounts of celanide (0.025 mg/ml) with adequate accuracy. The relative error of the determination does not exceed ±4.0%.

It is recommended that the quantitative determination of celanide [lanatoside C] should be carried out, in parallel with the biological method, by a photocolorimetric method using the reaction with xanthydrol after paper chromatography [1]. The method is laborious and requires a long time and the biological method is therefore the main one for the analysis of celanide and preparations containing it. In recent years, high-performance liquid chromatography has come into use for the analysis of cardiac glycosides and other drugs, and it possesses a number of advantages (objectivity, high degree of separation of the components, adequate accuracy) as compared with other methods [2, 3].

The aim of the present investigation was to select the conditions and develop a procedure for the analysis of celanide in the preparation, in the solution for injection, and in tablets by high-performance liquid chromatography.

EXPERIMENTAL

For the investigation we used standard celanide, the solution for injection, and celanide tablets. Chromatography was performed on a Hewlett-Packard model 1084B liquid chromatograph (USA) with a UV detector having a variable wavelength. The conditions of chromatographic analysis have been given elsewhere [2-4]. In the analysis we used a wavelength of 217 nm and a column 25 cm long with an internal diameter of 2.5 mm filled with the sorbent Lichrosorb RP-8 with a particle diameter of 10 μm, a dosing device adjusted for a volume of 10 μl, and a column temperature of 50°C, the eluent being 40% ethanol at a rate of flow of 1 ml/min. The areas of the peaks on the chromatograms were measured with the aid of a Terminal LC 79850B integrator.

The concentration of celanide was calculated by the internal-standard method and also by the method of absolute calibration. As the internal standard we used nitrobenzene, which is easily separated from celanide, absorbs at a wavelength of 217 nm, and issues from the chromatogram after a residence time close to that of celanide (Fig. 1).

Preparation of the Internal Standard (for the internal-standard method). A 100-ml measuring flask was charged with 0.2000 g (accurate weight) of nitrobenzene (density, g/cm^3, 1.2030-1.2040; refractive index 1.5524-1.5530; boiling point 209.5-211.5°C), and it was dissolved in 90 ml of 95% ethanol. After complete dissolution, the volume of the solution was made up to the mark with the same alcohol (solution A).

From solution A, 5 ml was transferred into a 100-ml measuring flask, where it was diluted with 35 ml of 95% ethanol, and the volume of the solution was made up to the mark with water (solution B).

Construction of a Calibration Curve (absolute-calibration method). In a 100-ml measuring flask, 0.1000 g (accurate weight) of standard celanide was dissolved in 90 ml of 95% ethanol, and after complete dissolution the volume was made up to the mark with the same ethanol.

All-Union Scientific-Research Institute of Physical Culture, Moscow. Translated from Khimiya Prirodnykh Soedinenii, No. 4, pp. 476-479, July-August, 1983. Original article submitted October 22, 1982.

0009-3130/83/1904-0447$07.50

Into seven 50-ml measuring flasks were transferred 1.25, 2.5, 5.0, 10.0, 15.0, 20.0, and 25.0 ml, respectively, of the solution obtained, sufficient 95% ethanol was added to each to give the same final concentration as in the eluent, and the volumes of solutions were made up to the marks with water.

The solutions were chromatographed. From the results obtained, a calibration curve was plotted in the coordinates S versus C, where S is the area of the celanide peak and C is the concentration of celanide in the corresponding solution.

The calibration curve of the relationship between the area of the peak of the celanide and its concentration has a linear nature within the range of concentrations from 0.025 to 0.05%. The sensitivity of the determination is 0.025 mg/ml.

Determination of the Proportionality Coefficient (internal-standard method). To 2 ml of each solution prepared for the plotting of the calibration curve was added 2 ml of solution B of nitrobenzene, and after careful mixing the resulting solutions were chromatographed.

To determine the proportionality coefficient a graph was plotted in the coordinates S_C/S_{NB} versus C_C/C_{NB}, where S_C/S_{NB} is the ratio of the areas of the celanide and nitrobenzene peaks on the chromatograms and C_C/C_{NB} is the ratio of the percentages of celanide and nitrobenzene in the solutions studied. The proportionality factor K was determined from the graph; for various celanide series it proved to be 0.275 ± 5%.

Method of Determining Celanide in Substance. About 0.02 g (accurately weighed) of celanide was placed in a 100-ml measuring flask and dissolved in 40 ml of 95% ethanol. After complete dissolution, the volume of the solution was made up to the mark with water. To 2 ml of the resulting solution was added 2 ml of nitrobenzene solution B and after careful mixing the chromatographic analysis was performed.

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

$$X = \frac{S_C \cdot 0.01 \cdot 100}{S_{NB} \cdot a \cdot K},$$

where S_C is the area of the celanide peak; S_{NB} is the area of the nitrobenzene peak; 0.01 is the concentration of nitrobenzene, %; a is the weight of the sample, g; and K is the proportionality coefficient, 0.275.

The amount of celanide in the preparation must be between 95.0 and 105.0%.

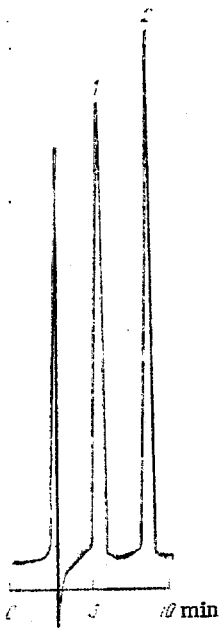

Fig. 1. Chromatograms of standard solutions:
1) celanide; 2) nitrobenzene.

TABLE 1. Results of the Quantitative Determination of Celanide in a Powder by High-Performance Liquid Chromatography

Sample No.	Celanide taken, g	Celanide found						
		internal-standard method			absolute-calibration method			
		g	%	metrological characteristics	g	%	metrological characteristics	as FAU in sample
1		0.0186	95,98	$\bar{X}$ = 97.01	0,0190	97,94	$\bar{X}$ = 98,36	317
		0.0193	99,67	$S_{\bar{x}}$ = 0.61	0,0199	102 58	$S_{\bar{x}}$ = 1.98	
	0,0194	0,0189	97,55	E_z = 1.56	0,0194	100.00	E_z = 2,78	
		0.0188	97.15	A = ±1.62%	0,0188	96,91	A = ±2.83%	
		0,0186	95,67		0 0190	97,97		
		0,0186	96.06		0,0184	94,85		
2		0,0207	103.38	$\bar{X}$ = 102,68	0,0209	104,50	$\bar{X}$ = 100 83	335
		0,0205	102,74	$S_{\bar{x}}$ = 0,73	0,0199	99,50	$S_{\bar{x}}$ = 0 98	
	0,0200	0,0210	105,40	E_z = 1,87	0,0206	103 00	E_z = 2.52	
		0,0206	103,04	A = ±1.82%	0,0201	100,50	A = ±2.50%	
		0,0200	100,33		0,0197	98 50		
		0,0202	101.17		0,0198	99,00		
3		0,0196	98,00	$\bar{X}$ = 100,75	0,0199	99,50	$\bar{X}$ = 100.17	333
		0,0201	100,50	$S_{\bar{x}}$ = 0,83	0,0200	100,00	$S_{\bar{x}}$ = 0,51	
	0,0200	0,0200	100.00	E_z = 2,14	0,0199	99,50	E_z = 1,32	
		0,0204	102,00	A = ±2.13%	0,0198	99,00	A = ±1,31%	
		0,0200	100,00		0,0201	100.50		
		0,0208	104,00		0,0205	102,50		

The method developed was checked on three samples of celanide material. The results obtained are presented in Table 1.

As can be seen from Table 1, with the aid of this method it is possible to determine small amounts of celanide (2.5 mg) with adequate accuracy. The relative error of the determination at a confidence level of 0.95 does not exceed ±3.0%.

Celanide is marketed in the form of a 0.02% solution of celanide for injections and as tablets each containing 0.00025 g of celanide.

Procedure for Determining Celanide in the Solution for Injection. To 2 ml of the preparation was added 2 ml of nitrobenzene solution B and after careful mixing the chromatographic analysis was performed.

The amount of celanide in grams in 1 ml of preparation (X) was calculated from the formula

$$X = \frac{S_C \cdot 0.01}{S_{NB} 100 \cdot K} .$$

The amount of celanide in one milliliter of preparation should be 0.00018-0.00022 g.

The method developed was checked on six model samples of celanide solution. Satisfactory results were obtained falling within the standard permissible deviations for physicochemical methods. The relative error of the determination for a confidence level of 0.95 does not exceed ±4.0%.

On studying the conditions for the extraction of celanide from tablets, it was established that celanide is extracted almost completely in 30 min by a single treatment with 6 ml of methanol when its amount in a tablet is 0.002 g.

Procedure for Determining Celanide in Tablets. About 0.8 g (accurately weighed) of a powder of ground tablets was placed in a bottle, 6 ml of methanol was added, and the celanide was extracted by shaking in a vibration apparatus for 30 min. Then the extract was filtered into a 10-ml measuring flask. The bottle and the residue on the filter were washed with 3 ml of methanol, and the volume of the methanolic solution was made up to the mark.

To 2 ml of the resulting solution was added 2 ml of nitrobenzene solution B, and the resulting solution was chromatographed. The amount of celanide in one tablet in grams was calculated from the formula

TABLE 2. Results of the Quantitative Determination of Celanide in Solutions and Tablets Obtained by High-Performance Liquid Chromatography and by a Biological Method

Batch number of the preparation	Amount of celanide in 1 ml 0.00018-0.00022 g and in one tablet 0.000225-0.000275 g	Found by the biological method: in 1 ml – 2.5-3.5 FAU; in one tablet – 3.5-4.5 FAU
	0.02% solutions for injection	
011079	0.000143	2,20
021079	0,000109	1,60
031079	0,000147	2,30
041079	0'000117	1,60
051079	0,000157	2,30
	0.000250 g tablets	
230780	0 000241	3,75
170374	0,000245	3,88
070173	0,000241	3,75

$$X = \frac{S_C \cdot 0{,}01 \cdot b}{S_{NB} \cdot a \cdot 10 \cdot K} \cdot$$

where a is the weight of the preparation, g; and b is the mean mass of one tablet.

The amount of celanide in one tablet should be 0.000225-0.000275 g.

The method for the quantitative determination of celanide in tablets was checked on six model samples. The method possesses good reproducibility and adequate accuracy. The relative error of the determination ranges from ±2.0 to ±3.5%.

In addition to the HPLC method, the celanide samples were studied by a biological method. The analytical results obtained by the two methods are presented in Table 2, from which it can be seen that both methods show a fall in the concentration of celanide in solutions.

SUMMARY

Methods have been developed for the quantitative determination of **celanide in substances,** in solutions for injection, and in tablets by high-performance liquid chromatography, with the aid of which it is possible to determine small amounts of celanide (0.025 mg/ml) with adequate accuracy. The relative error of the determination does not exceed ±4.0%.

LITERATURE CITED

1. State Pharmacopoeia of the USSR [in Russian], 10th edn., Moscow (1968), p. 165.
2. V. Ya. Davydov, **A. V. Kiselev,** D. M. Popov, and Yu. M. Sapozhnikov, Khim-farm Zh., No. 4, 102 (1978).
3. V. Ya. Davydov, A. V. Kiselev, and D. M. Popov, Khim-farm. Zh., No. 3, 111 (1980).
4. V. Ya. Davydov, A. V. Kiselev, et al., J. Chromatogr., 11, No. 10, 591 (1978).