DEVELOPMENT OF A METHOD FOR QUANTITATIVE DETERMINATION OF DEXPANTHENOL IN 5% GEL DOSAGE FORM AND ITS VALIDATION.

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Abstract. This article presents the results of a study on the development and validation of a high performance liquid chromatography technique for the quantitative determination of dexpanthenol in 5% anti-inflammatory gels. On the basis of the validation, the criteria for the acceptability of the method were established and it was proved that the proposed method for the quantitative determination of dexpanthenol is specific, linear in the range of its application, correct and precise.

The developed validated method for the quantitative determination of dexpanthenol was proposed for inclusion in the draft pharmacopoeial monograph of the manufacturer.

Keywords: Dexpanthenol, Pharmacopoeial,

Pharmacological properties

Dexpanthenol (Dexpanthenolum, R-2,4-Dihydroxy-N- (3-hydroxy-propyl) -3,3-dimethylbutanamide) is a pantothenic acid derivative. Replenishes the deficiency of pantothenic acid, has anti-inflammatory and dermatoprotective effects, stimulates regeneration processes.

Available in the form of ointments, creams, sprays, aerosols. Combined preparations based on it, such as Combisept ointment (dexpanthenol; chloramphenicol; benzalkonium chloride (BC)), etc.

There are a number of modern physicochemical methods of analysis used to determine dexpanthenol in drugs, among which the method of reversed-phase liquid chromatography is the most commonly used and is described in modern scientific literature [1-4].

In order to increase the range of drugs produced by domestic pharmaceutical manufacturers, increase their bioavailability and reduce the negative effects of existing dosage forms, we have developed a 5% gel based on dexpanthenol.

Research Objective - development and validation of a unified method for the quantitative determination of dexpanthenol in a new gel dosage form using the HPLC method, which will make it possible to objectively evaluate it in a dosage form (DF).

Materials and research methods. As an object of research, we used prototypes of gels using APIs that meet the requirements of the European Pharmacopoeia: dexpanthenol (ND RB 0103 S-2010), the components of which were: carbomer, 400

(GF RB, volume 2, p. 192); water, purification of the sorbent disodium editate, almond oil - 1500 (GF RB, volume 2, p. 192).

A liquid chromatograph "Agilent 1260 infinity" (Angilent Technologies Germany) was used for research, which consists of: a four-channel plunger pump from "Agilent" (up to 400 bar) with a flow rate of 0.001-10 ml / min with a compartment for solvents and a feed adjustment step solvent 0.001 ml / min "Agilent"; a vacuum degasser for 4 channels with a capacity of up to 10 ml / min "Agilent"; block for automatic injection of samples with injection volume from 0.1 µl to 100 µl with a step of 0.03 µl "Agilent".

Dexpanthenol was identified using an Agilent diode-array spectrophotometric detector.

A system consisting of a buffer solution (monobasic sodium phosphate with pH up to 2.2 ± 0.1 for chromatography) and acetonitrile for chromatography in the ratio (75:25) was used as a mobile phase.

A test solution of 5% gel and a solution of a working standard sample (WSS) of dexpanthenol were prepared at a concentration of 0.2 mg / ml.

The methodology was validated in accordance with the guidelines of the International Conference on Harmonization using parameters, specificity, linearity, accuracy (repeatability and within laboratory accuracy), limit of detection (LOD) and limit of quantification (LOQ), as well as reliability [5-7] ... Validation characteristics were established on experimental gel samples obtained under laboratory conditions.

The specificity of the technique was confirmed by analyzing solutions of the finished Lf (test solution) and the "base" containing all the components of the Lf except for dexpanthenol (in 6 replicates). The criterion of acceptability was the absence on the chromatogram of the "base" solution of a peak with a retention time corresponding to the release time of the dexpanthenol peak.

Linearity was assessed by analyzing at least five concentration levels in the gel in triplicate, covering values in the range 80% ÷ 120% of the nominal dexpanthenol content (30-70 µg / ml). Linearity calculations were carried out using the least squares method according to the experimentally measured values of "y" for the given values of the argument "x". The criterion of acceptability was the correlation coefficient of the linear relationship (r), which should be at least 0.99.

To prove the convergence of the test method, six samples of the same LF series were analyzed sequentially by one chemist in one day on the same equipment.

The intra-laboratory precision was established by two analysts on different days on different equipment by sequential analysis of 6 samples of the same LF series. The acceptance criterion was: the relative standard deviation (RSD,%), calculated for the quantitative content of dexpanthenol, obtained under repeatability conditions, should not exceed 2.0%; The difference in the values of the variances S12 and S22 of the mean results of two samples obtained under conditions of intra-laboratory reproducibility when determining the content of dexpanthenol in identical Lf samples should be statistically significant.

The accuracy of the method for the quantitative determination of dexpanthenol was determined in three laboratory samples at concentrations that are within the

range of application of the method: 45.00 mg / g, 50.00 mg / g, 55.00 mg / g (90%; 100% and 110% of nominal content) using 6 reproductions for each concentration. The model samples contained an exactly known amount of dexpanthenol.

The test results were checked for sample homogeneity; data burdened with gross errors were excluded from the sample. Confirmation of the correctness of the data obtained was carried out by calculating the bias | xcp - μ | and checking the significance of the difference between the random variable X cf and the constant μ (the accepted reference value).

As a result of the studies carried out, the method for the quantitative determination of dexpanthenol in LF was substantiated. For this, 2,000 g (accurately weighed) of the test gel is placed in a 100 ml volumetric flask, 60.0 ml of water is added, stirring vigorously for 20 minutes in a hot ultrasonic bath (about 700 C) until the gel dissolves. The resulting mixture, constantly shaking, is cooled to room temperature, the volume of the solution is brought to the mark with the same solvent. 5.0 ml of the resulting solution is placed in a volumetric flask with a capacity of 25.0 ml and the volume is adjusted with water, stirred. The resulting solution is filtered through a membrane filter with a pore diameter of 0.45 μm.

To prepare a CO solution, an exact weighed portion (0.100 g) of dexpanthenol is dissolved in 50 ml of purified water in a 100 ml volumetric flask by shaking until it is completely dissolved, the volume of the solution is brought to the mark with the same solvent and mixed. 5.0 ml of the resulting solution is placed in a 25.0 ml volumetric flask and diluted to the mark with a mixture of water for chromatography. The resulting solution is not filtered.

Based on the analysis of literature data and the results of the studies carried out, the following optimal conditions for the chromatography of dexapanthenol were determined: chromatographic column Eclipse XDB C18 (150cm x 4.6mm) filled with cyanosilyl silica gel for chromatography P with a particle size of 5μm (for example, the column “Waters Spherisorb CNRP "Or similar, column temperature - 40 ˚C; mobile phase A: solution of sodium phosphate monobasic with a concentration of 1.38 g / l, brought to pH (2.2 ± 0.1) with phosphoric acid; mobile phase B: acetonitrile for chromatography; isocratic elution with the ratio of mobile phase A and mobile phase B (75:25) v / v; mobile phase speed - 1.0 ml / min; spectrophotometric detector: recording wavelength - 210 nm; volume of injected sample - 20.0 μl The results are shown in Figures 1 and 2.

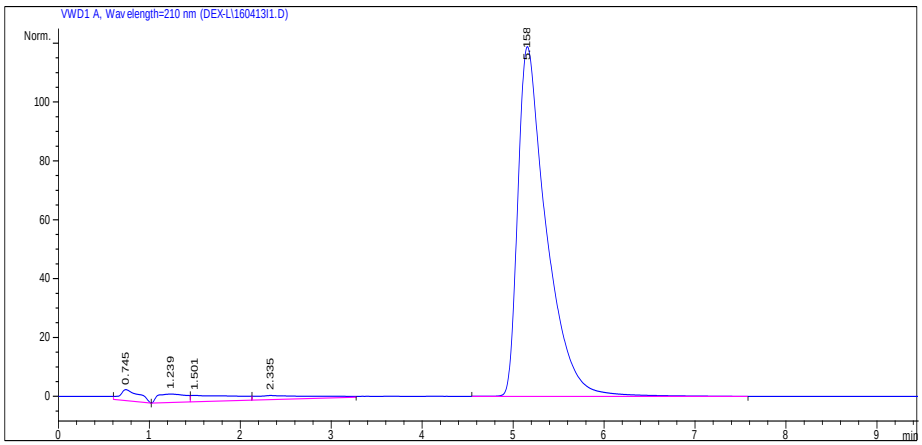

Fig. 1. Typical chromatogram of dexpanthenol PCO solution under selected conditions

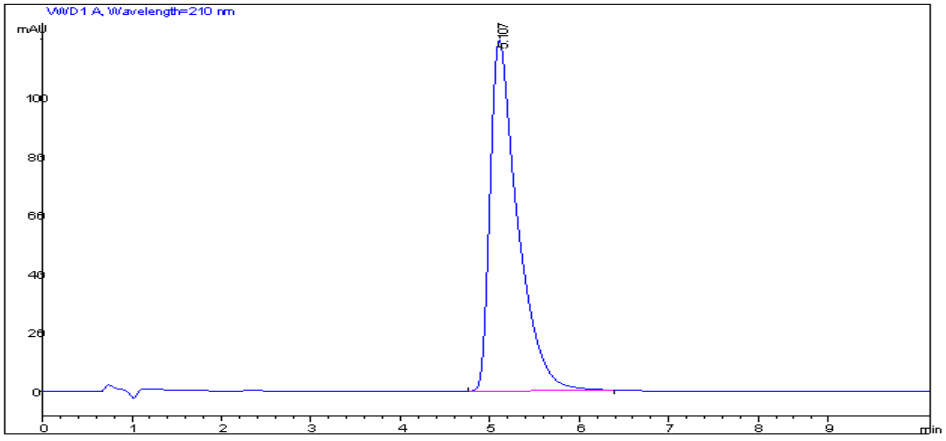

Fig. 2. Typical chromatogram of the test gel solution under the selected conditions

From the chromatograms of the CO and the test solution presented in Figures 1 and 2, it can be seen that the peaks with a retention time of 5.1 min correspond to dexpanthenol. The resolution between the peaks was 3.42, which is more than the regulated value of 1.5. The relative standard deviation, calculated from the peak release times from the results of six repetitions of the analysis, was 0.939%.

The efficiency of the chromatographic column was evaluated by the number of theoretical plates and the asymmetry factor of the peaks.

The quantitative determination of dexpanthenol was carried out by comparing the areas of dexpanthenol peaks in the chromatogram of dexapanthenol RS (Figure 1) with the corresponding peak in the chromatogram of the test solution (Figure 2).

When calculating the quantitative content of dexpanthenol in the gel, we also took into account the weight of the weighed samples of the test sample, the mass of the PCO taken for analysis, and the percentage of dexpanthenol in the PCO, in terms of the anhydrous substance.

The quantitative content of dexpanthenol (X, mg) was calculated using the following formula:

$$X = \frac{S_1 \cdot m_0 \cdot 5 \cdot 100 \cdot 25 \cdot P \cdot b}{}$$

$$S_0 \cdot 100 \cdot 50 \cdot m_1 \cdot 5 \cdot 100$$

where: S is the average value of the dexpanthenol peak area in the chromatogram of the test solution;
S - is the average value of the peak area of a standard dexpanthenol solution;
m - is the weight of the sample of dexpanthenol RM, mg;
m - is the weight of the test sample, mg;
P - is the actual content of dexpanthenol in CO, in%
B - is the average weight of the gel, mg.

The results of the quantitative determination of dexpanthenol and the metrological characteristics of the developed method are shown in Table 1.

Table 1.

Metrological characteristics of methods for the quantitative determination of dexpanthenol at F = 4, t (P, f) = 2.78, P = 95%

X_{av}, %	S^2	S	Δ x	ε , %
5,1				

As can be seen from table 1, the content of dexpanthenol in the gel is 5.1 ± 0.04%. The relative error of a single determination of dexpanthenol in Lf is 2.66%. According to the developed technique, a quantitative analysis of dexpanthenol was carried out in 5 series of gels. The analysis results are presented in Table 2.

The reliability of the HPLC method was assessed by making changes in the buffer pH (± 0.1), composition of the least mobile phase (± 10%), flow rate (± 0.1 ml / min), oven temperature (± 2 °), wavelength (± 2 nm) and a bar of another batch of stationary phase.

The results of the study of the specificity of the method showed that under the chosen test conditions, neither the solvent used, nor the mobile phase, nor the placebo components distort the results of the quantitative determination of dexpanthenol.

The specificity of the method was determined by comparing the area and retention time of the peaks of an aqueous solution of 5% dexpanthenol gel, obtained according to the proposed method of quantitative determination, and a solution of dexpanthenol CO (Figs. 1 and 2).

The linearity of the method was carried out at five levels of dexpanthenol CO concentration from 80 to 120% of the theoretical dexpanthenol content in the gels. The experimental data were processed by the least squares method, and a regression equation was drawn up. The criterion for the acceptability of linearity is the correlation coefficient (r), which must be at least 0.99. Provided that its value is close to one, then the set of analyzed data can be described by a straight line.

The analytical area of the technique was determined by the interval of experimental data obtained and satisfying the linear model. The results of the tests performed are presented in table 1.

Table 2.

Results of studying the linear dependence of the technique

Content RM of dexpan-tenol in% of standardized	Concentration RM of dexpanthenol, mg / ml (in measured solution)	Analytical signal (peak area)	Coefficient correlations
80	40,6	2238,478	0,9999
90	45,6	2514,153	
100	50,2	2754,727	
110	55,3	3034,589	
120	60,5	3319,939	
b=054,217; a=-37,649			

The precision of the method is characterized by repeatability, intralaboratory and interlaboratory reproducibility and is the value of the standard deviation (RSD), which should be no more than 15%.

To determine the convergence, the test was carried out on different days with the same specialist on the same gel sample in 6 replicates (table 3) under the same conditions.

Table 3.

Results of determining the convergence of the method

The true value of the determined value is 5%					
sample	results Definitions	Standard deviation SD	Relative standard deviation RSD%	Coefficient of variation sb	Student criterion tab.
1 day					
1	0,0513	**0,00096**	**1,92**	**3,92**	**2,57**
2	0,0510	**0,00096**	**1,92**	**3,92**	**2,57**
3	0,0498	**0,00096**	**1,92**	**3,92**	**2,57**
4	0,0489	**0,00096**	**1,92**	**3,92**	**2,57**
5	0,0500	**0,00096**	**1,92**	**3,92**	**2,57**
6	0,0513	**0,00096**	**1,92**	**3,92**	**2,57**

2 day					
1	0,0499	**0,00063**	**1,42**	**2,57**	**2,57**
2	0,0500	**0,00063**	**1,42**	**2,57**	**2,57**
3	0,0520	**0,00063**	**1,42**	**2,57**	**2,57**
4	0,0509	**0,00063**	**1,42**	**2,57**	**2,57**
5	0,0500	**0,00063**	**1,42**	**2,57**	**2,57**
6	0,0514	**0,00063**	**1,42**	**2,57**	**2,57**

The convergence of the test method for the quantitative determination of dexpanthenol was established. The relative standard deviation (RSD,%) calculated for the dexpanthenol content and obtained under repeatability conditions did not exceed 2.0% and amounted to 0.348%.

The in-laboratory convergence of the test procedure for the quantitative determination of dexpanthenol was confirmed. Fisher's coefficient F (P, f1, f2) does not exceed the table value Ftabl (0.95; 5; 5). The results are shown in table 4.

Table 4.

Intralaboratory and interlaboratory reproducibility of the method for quantitative determination of dexpanthenol

Sample №	1-laboratory		2-laboratory
	1-analyst	2-analyst	
	Dexpanthenol content, g		
1	0,0513	0,0499	0,0513
2	0,0510	0,0500	0,0510
3	0,0498	0,0520	0,0498
4	0,0489	0,0509	0,0489
5	0,0500	0,0500	0,0500
6	0,0513	0,0514	0,0513
Statistical characteristics			
In-laboratory precision			interlaboratory precision
$\bar{x}$	0,050	0,0507	0,0504
S	0,00096	0,00063	0,00097
S^2	0,0000009	0,0000004	0,00000095
RSD,%	1,92	1,42	1,92
Variation interval $\Delta\bar{x}$	0,001	0,00066	0,00102
Relative standard deviation,%	0,78	0,507	0,79

The correctness of the method for determining the quantitative content of dexpanthenol was confirmed by appropriate tests on model samples for three concentrations. The correctness of the method was established by measuring the quantitative content of dexpanthenol in gels with the addition of CO dexpanthenol at the rate of 80, 100, 120% of the content in Lf. The relative error in experiments with additives does not exceed the relative error of the average result, which indicates the correctness of the method and the absence of a systematic error. The obtained results of the determination are not burdened with a systematic error. The average recovery rate for 9 measurements was 99.37% for dexpanthenol. The results of the study to determine the correctness are presented in table 5.

Table 5.

Accuracy of quantitative determination of dexpanthenol in 5% gels

№ definitions	Dexpanthenol content, mg / g	Added CRM of dexpanthenol, mg	Expected value, mg / g	Experimentally found value	
				Received content, mg / g	Percent recovery, %
1	50	25	74	73,0	98,64
2				73,5	99,32
3				73,7	99,59
1	51	50	101	100,0	99,0
2				101,0	100,0
3				100,0	99,0
1	52	75	127	126,0	99,21
2				127,0	100,0
3				126,5	99,61

An indicator for the quantitative determination of dexpanthenol has been established, which is included in the regulatory documents for the gel - not less than 5.0%.

Thus, the proposed spectrophotometric method for the determination of dexpanthenol in gels complies with the following validation indicators: correctness, precision, specificity, and linearity, and is included.

Findings

Based on the research results, the most acceptable conditions for sample preparation of dexapatenol gel and the conditions for its analysis were selected.

A unified HPLC method has been developed that allows the identification and quantitative determination of dexpanthenol in its Lf.

The optimal conditions for chromatography were selected, which made it possible to elute the analyte in a short period of time and ensure the expressiveness of the analysis (the time of chromatography is ~ 5 min).

The developed method of quantitative determination was validated in accordance with the established requirements and its specificity, linearity in the range

of application, correctness and precision were proved. The results obtained confirm the guarantee of obtaining the expected and reproducible results, validation was carried out.

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