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Development and validation of reversed phase high performance liquid chromatography method for determination of dexpanthenol in pharmaceutical formulations

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Abstract

This paper describes the validation of an isocratic HPLC method for the assay of dexpanthenol in aerosol and gel. The method employs the Vydac Proteins C4 column with a mobile phase of aqueous solution of trifluoroacetic acid and UV detection at 206 nm. A linear response ($r > 0.9999$) was observed in the range of 13.0–130 μg mL^{-1}. The method shows good recoveries and intra and inter-day relative standard deviations were less than 1.0%. Validation parameters as specificity, accuracy and robustness were also determined. The method can be used for dexpanthenol assay of panthenol aerosol and gel with dexpanthenol as the method separates dexpanthenol from aerosol or gel excipients.

Keywords: Dexpanthenol; Column liquid chromatography; Validation; Quality control

1. Introduction

Dexpanthenol (butanamide, provitamin B_5) has the formula $C_9H_{19}NO_4$ and IUPAC name (2R)-2,4-dihydroxy-*N*-(3-hydroxypropyl)-3,3-dimethylbutanamide, also called pantothenyl alcohol (Fig. 1). Dexpanthenol is one of the components of multi-vitamin preparations, parenterals and some of local cosmetic preparations. It has as much biological activity as pantothenic acid and is also more stable, especially in aqueous solution. Pantothenic acid is a constituent of coenzyme A, which is known to have an important role in the metabolism of carbohydrates, fats and nitrogen compounds. It is used externally to increase epithelialization of burn and scratch wounds as well as ulcers.

Owing to its chemical structure, dexpanthenol has the low molar absorptivity at high wavelength due to the lack of UV chromophores. That is why the USP [1] proposed the non-aqueous titrimetric method for its assay both in the raw material and in its pharmaceutical preparations.

Bukowska et al. [2] proposed for determination of panthenol in bulk and in pharmaceutical forms the colorimetric titration method. Wang et al. [3] used differential pulse voltammetry for its determination in cosmetics and pharmaceutical formulations.

Classical method for the dexpanthenol determination is microbiological assay with *Acetobacter suboxydans* [4]; microbiological method with *Pideococcus acidilactici* has been used in [1] for dexpanthenol determination as an ingredient of multivitamin preparations.

Several methods have been reported on the analysis of dexpanthenol depending on hydrolysis of dexpanthenol into the primary amine which then formed colored products with some reagents such as iodine [5], 1,2-napthahynone-4-sulphonate [6], hydroxylamine [7], ninhydrine [8], vanillin [9].

Dexpanthenol as alcohol may be determined by using gas–liquid chromatographic methods [10,11]. Liquid chromatographic methods either directly or after hydrolysis and derivatization with fluorescamine were also reported [12,13].

TLC has been used to separate dexpanthenol in multi-vitamin preparations and detected after derivatization with ninhydrine [14].

The purpose of the present work is to develop and validate a new and simple liquid chromatography method with UV detection for quantitative analysis of dexpanthenol in pharmaceutical

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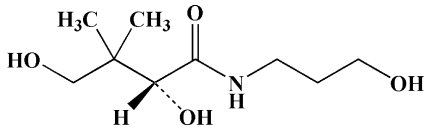

Fig. 1. Chemical structure of dexpanthenol.

preparations such as gel and aerosol. All pharmaceuticals contain about 50 mg g^{-1} of dexpanthenol in a gel or aerosol base.

2. Experimental

2.1. Chemicals and reagents

Dexpanthenol substance was obtained from "BASF AG" Chemical Company (Germany) and has been analyzed by the EP non-aqueous titremetic method [15] and was found to contain 99.9% of dexpanthenol calculated with reference to the anhydrous substance.

Trifluoroacetic acid was of analytical reagent grade and was purchased from Merck (Darmstadt, Germany). Double distilled water was used in all experiments.

2.2. Equipment

The development and validation of the assay was performed on a Hewlett Packard HPLC system (Agilent Technologies, Walbronn, Germany) provided with a Series 1050 pump, a Series 1050 spectrophotometric detector with the variable wavelength and a Series 3395 integrator. The pH value was determined with a Beckman Φ-200 pH meter (Beckman Instruments, Fullerton, CA, USA). For the determination of peak purity the Waters 2695 Separation Module (Waters, Milford, MA, USA) with Waters 996 Photodiode Array Detector (Waters) was used.

2.3. Preparation of solutions and chromatographic conditions

2.3.1. Chromatographic conditions

The separation was achieved using a Vydac Proteins C4, 250 mm × 4.6 mm, 5 μm, column (Grace Vydac, CA, USA). The mobile phase was prepared by adding 0.60 mL of trifluoroacetic acid to 1.0 L of double distilled water (approximately 0.1%, w/v). Mobile phase was degassed by sonication and vacuum prior to use. The mobile phase flow rate was 1.0 mL min^{-1} and the injection volume was 20 μL. The chromatographic runs were carried out at 30.0 ± 0.1 °C. UV detection was performed at 206 nm.

2.3.2. Stock and working standard solutions

Dexpanthenol stock solution was prepared by adding 65.60 mg of dexpanthenol to a 100-mL volumetric flask, dissolving this quantity in double distilled water and filling to the mark with the same solvent. Working standard solutions were prepared by the dilution of the dexpanthenol stock solution with mobile phase to obtain five different concentrations within the range of interest, in this case, 13.11, 39.32, 65.53, 91.75 and 131.1 μg mL^{-1}.

2.3.3. Reference standard solutions

Reference standard solutions of dexpanthenol were prepared by weighting 51.2 mg of dexpanthenol and diluting to obtain the dexpanthenol concentrations 40.96, 46.08, 51.20, 56.32 and 61.44 μg mL^{-1} for the evaluation of the precision and accuracy study of the proposed method.

2.3.4. Preparation of sample solution

An accurately weighed portion of gel or aerosol contents equivalent to 50 mg of dexpanthenol (sample weight about 1.0 g) was transferred to a 100-mL volumetric flask. After it, about 60 mL of the mobile phase was added to the flask, shacked vigorously during 10 min, sonicated for 5 min, brought to volume with the same solvent and filtered through 0.45-μm membrane filter (Westboro, MA, USA). The first 5 mL of the filtrate was rejected.

3. Results and discussion

According to the low log P value for dexpanthenol (−0.83 ± 0.34, calculated using ACD/Labs Software V. 4.01/11, Advanced Chemistry Development Inc., Toronto ON, Canada) it can be expected that dexpanthenol will not retain when conventional HPLC C18 columns are used. That is the case, because our preliminary experiments showed that even small amount of organic modifier presented in the mobile phase resulted in dexpanthenol small retention. Practically it was eluted in the column dead volume. When the organic modifier was excluded from the mobile phase, we could not obtain reproducible chromatograms; the well known "ligand folding" or "collapse" effect was observed. The effect was in progressively decreasing or instability of the retention times and deterioration of peak shape that is directly related to the length of exposure of C18 absorbent to 100% aqueous eluent. The decreasing of the bonded group length results in retention time increasing. But the simplest bonded group length decreasing does not lead to the good chromatographic results: dexpanthenol peak symmetry using Nucleosil 100-7 C2 (250 mm × 4.0 mm i.d.) column is about 4 when water or acidified water is used as mobile phase. Experiments showed that it needs to change not only bonded group length, but sorbent porosity. Optimal sorbent for dexpanthenol analysis should have small bonded group length (C1–C4), large pore diameter and will be able to work with 100% aqueous mobile phase without "collapse" effect.

Vydac proteins C4 reversed-phase column consists of butyl aliphatic groups bonded to the surface 300 Å pore diameter silica. Surface chemistry of Vydac Proteins C4 sorbent allows avoiding "collapse" effect. It proves to be an ideal medium for high-performance reversed-phase chromatography of proteins and other molecules (including small molecules), which had previously been difficult separated by RP HPLC procedure. That is why Vydac Proteins C4 was chosen as optimal sorbent for dexpanthenol analysis.

For Vydac Proteins C4 sorbent, it is common practice to include a trifluoroacetic acid (TFA) in the mobile phase as acidic modifier [16] that allows to increase the efficiency of the chromatographic peaks, to decrease and control the retention and selectivity for different analysis. The modifier masks basic entities, reducing mixed-mode retention and improving peak symmetry.

Trifluoroacetic acid is typically present in a mobile phase at concentrations of 0.05–0.1% [16]. Varying concentration of trifluoroacetic acid has a suitable affect on selectivity and peak shapes. Moreover, it has little UV adsorption at low wavelength (200–220 nm).

Choosing TFA concentration in mobile phase was based on peak parameters (symmetry, theoretical plates and retention factor), run time and pH value of mobile phase. The concentration ranges studied for the acidifier was from 0.01% to 0.20% (v/v) (step 0.05%). It is obvious that the addition of high amount of TFA would not benefit the dexpanthenol, which could elute near the dead volume. Furthermore, mobile phases with TFA concentration near 0.2% (v/v) show pH values about 1.0–1.5 that is unacceptable for use with bonded silica columns. On the other hand, it was observed that when the concentration of TFA was decreased the dexpanthenol retention time increased; high retention time and bad peak symmetry was obtained.

An acceptable separation with a retention time of 10 min (k is about 5) for dexpanthenol and good peak symmetry was obtained using mobile phase with aqueous trifluoroacetic acid solution with TFA concentration about 0.06% (v/v) and 0.09% (w/v).

3.1. Validation of the test procedure

The objective of validation of an analytical procedure is to demonstrate that it is adequate for its intended purpose. To meet current pharmaceutical regulatory guidelines (i.e., ICH [17,18], USP [1], EP [15]) a number of parameters must be investigated in order to validate analytical methods such as precision, accuracy, specificity, linearity and robustness study.

3.1.1. Specificity (selectivity)

The selectivity of assay was determined by placebo analysis. Placebos of gel or aerosol formulations containing all the normal ingredients except dexpanthenol were prepared for this study. They were treated in the same manner as the normal samples, and chromatograms were injected for study of other ingredient interference on the selectivity of the dexpanthenol separation. The chromatograms obtained from placebo, samples and dexpanthenol standard solution are shown in Fig. 2 (for gel formulation) and Fig. 3 (for aerosol formulation).

Fig. 2 and Fig. 3 show that despite the presence of the peaks of pharmaceutical formulation base components on the chromatogram were obtained, the peak of the dexpanthenol was satisfactory separated from those ones. The peak purity was always more than 98.0%.

3.1.2. Linearity of response

A linearity relationship was evaluated across the range of analytical procedure. Since the nominal working concentration for assay test is about 50–60 μg mL^{-1} (about 1 μg of dexpanthenol injected into column), the linearity was performed over the range of 13.0–130 μg mL^{-1} (approximately from 20 to 200% of nominal range of analyte [19]).

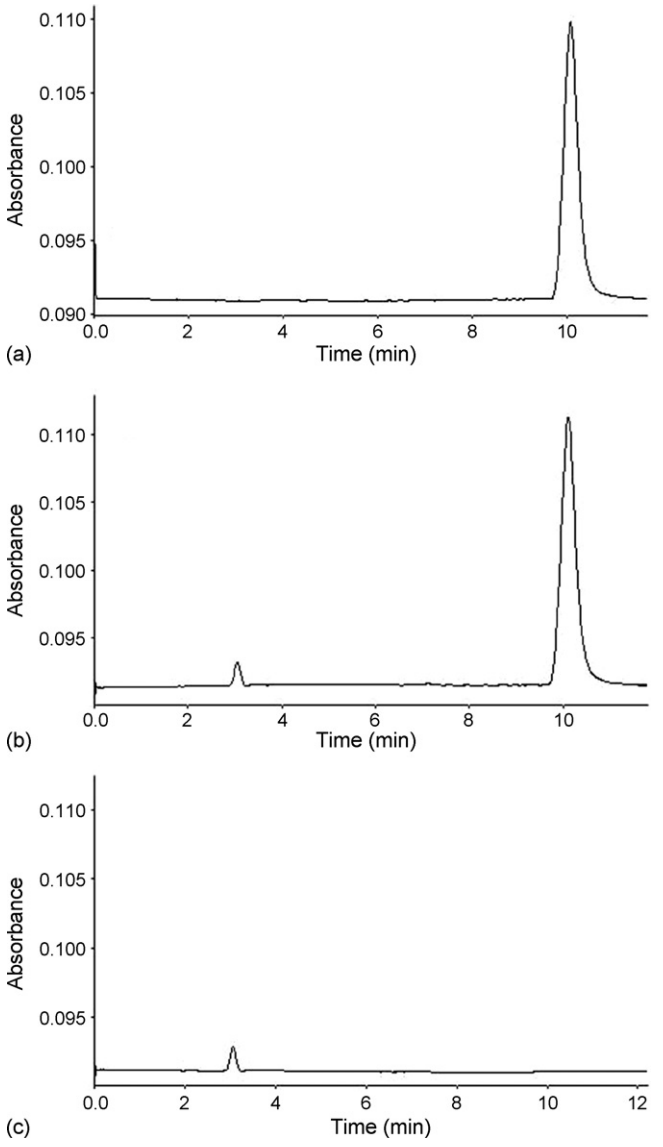

Fig. 2. Chromatogram of dexpanthenol reference substance 51.2 μg mL^{-1} (a), panthenol gel sample about 50 μg mL^{-1} of dexpanthenol (b) and placebo sample (C).

The regression line was calculated as $Y = A + BX$, where X was the dexpanthenol concentration (μg mL^{-1}) and Y was the response (peak area expressed as AU). The calibration curve was obtained using the linear least squares regression procedure. The representative linear equation was $Y = (-2.2 \pm 3.1) \times 10^4 + (47.79 \pm 0.15) \times 10^3 X$. The correlation coefficient (r) value is 0.9999.

Since the coefficient of correlation is not suitable as a general acceptance criterion to the linearity performance of an analytical procedure [20], the relative standard error of slope was used as a parameter with respect to precision of the regression. This parameter should be comparable to the relative standard devi-

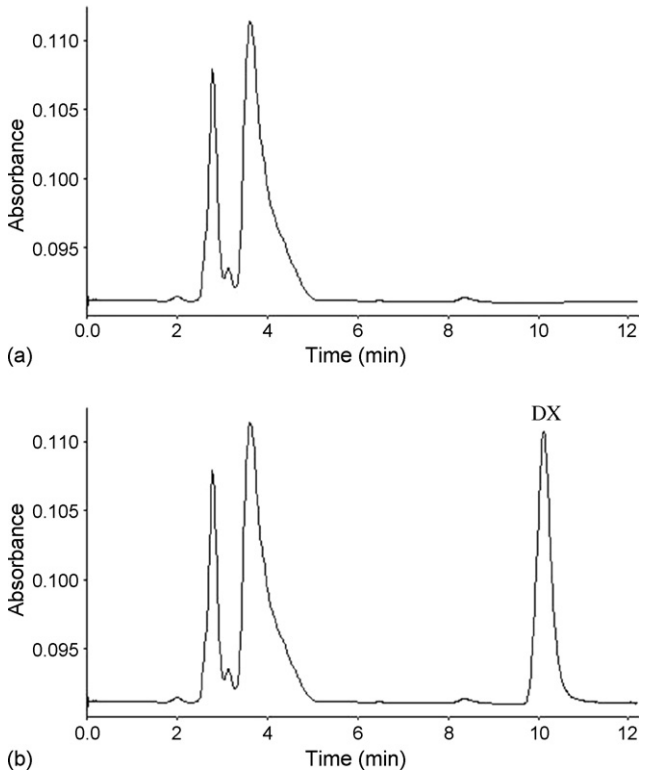

Fig. 3. Chromatogram of dexpanthenol aerosol sample about 50 μg mL^{-1} of dexpanthenol (a) and placebo sample (b).

ation obtained in precision studies in the given concentration range [20]. Obtained relative standard error of slope is about 0.2–0.3%, that is less than R.S.D. of precision (mean value is about 0.7%).

3.1.3. Limits of detection and quantitation

The limits of detection (LOD) and quantitation (LOQ) were calculated in accordance with the 3.3*s/m* and 10*s/m* criteria, respectively, where *s* is the standard deviation of the peak area (for five replicates) for the sample and *m* is the slope of the calibration curve, determined from linearity investigation [17,18]. The LOD and LOQ were calculated and were found to be 2.1 μg mL^{-1} and 6.2 μg mL^{-1}, respectively.

3.1.4. Accuracy

Accuracy was determined by analyzing a sample of known concentration (reference standard solutions) and comparing the measured value with the true value, and using the method of standard additions.

Tables 1 and 2 summarizes the accuracy results, expressed as percent recovery and relative standard deviation (R.S.D.) for both approaches. The method showed good recovery.

3.1.5. Stability of the dexpanthenol solutions

The stability of the dexpanthenol solutions during time was investigated. The dexpanthenol working standard solution with its concentration 51.20 μg mL^{-1} onto the HPLC system at 0, 1, 3, 5, 7, 9 and 24 h after preparation was injected. The data indicate that after 9 h the chromatographic peak area of dexpanthenol decreased insignificantly. It can be concluded that dexpanthenol solution was stable at least for 9 h after its preparations. After 24 h when sample at assay concentration was stored at ambient temperature under laboratory light conditions the significant rise in the assay levels was observed. Correlation of freshly prepared standard solutions and those been stored after 24 h was about 92–97%. It would be preferable that standard and sample solutions to be freshly prepared prior to injection.

Table 1
Results of accuracy determination by analyzing the samples of known concentrations

Level (μg mL^{-1})	Amount recovered (μg mL^{-1})	%Recovery	Mean (%) (n = 5)	%R.S.D.
80% (40.96)	40.66	99.27	99.28	0.05
	40.67	99.30		
	40.65	99.25		
	40.64	99.22		
	40.69	99.35		
90% (46.08)	46.13	100.10	100.12	0.07
	46.15	100.15		
	46.13	100.11		
	46.17	100.20		
	46.10	100.05		
100% (51.20)	50.97	99.55	99.46	0.08
	50.90	99.41		
	50.91	99.43		
	50.89	99.40		
	50.95	99.51		
110% (56.32)	56.04	99.50	99.48	0.10
	55.99	99.42		
	56.09	99.59		
	55.97	99.38		
	56.05	99.52		
120% (61.44)	61.83	100.64	100.68	0.07
	61.88	100.71		
	61.86	100.68		
	61.81	100.61		
	61.90	100.75		

3.1.6. Precision

Precision may be measured as repeatability, intermediate precision and reproducibility. Reproducibility refers to the use of analytical procedure in different laboratories.

The repeatability (intra-day) and intermediate (inter-day) precision of the method were demonstrated by analyzing dexpanthenol reference standard solutions of concentration 40.96, 51.20 and 61.44 μg mL^{-1} during 1 day and each of 3 days under the same conditions. The results obtained from these analyses are listed in Table 3 as mean recovery (%). Table 3 shows there was no significant differences between assay results either within-day or between days, implying that the precision of the proposed method was good (R.S.D. less than 1.0%).

Repeatability and especially reproducibility of the proposed method was investigated by analysis of drug synthetic mixture in different laboratories. The results of repeatability and

Table 2
Results of accuracy determination by method of standard additions

Theoretical concentration ($\mu g\,mL^{-1}$)	Dexpanthenol concentration after addition ($\mu g\,mL^{-1}$)	Dexpanthenol concentratoin[a] found ($\mu g\,mL^{-1}$)	Recovery		
			$\mu g\,mL^{-1}$	%	Δ (%)
40.96	53.97	54.11	41.10	100.34	0.34
	66.98	67.02	41.00	100.10	0.10
	79.99	79.89	40.86	99.76	−0.24
51.20	64.21	64.12	51.11	99.82	−0.18
	77.22	77.28	51.26	100.12	0.12
	90.23	90.33	51.30	100.20	0.20
61.44	74.45	74.59	61.58	100.23	0.23
	87.46	87.35	61.33	99.82	−0.18
	100.47	100.58	61.55	100.18	0.18
Mean value				100.06	
R.S.D. (%)				0.16	

[a] Mean value of the three determinations.

Table 3
Summary of repeatability (intra-day) and reproducibility (inter-day) precision data for dexpanthenol

Spike level (%)	Intra-day[a] mean ± R.S.D. (%)	Inter-day[a], recovery amount ± R.S.D. (%)			Inter-day mean ± R.S.D. (%)
		Day 1	Day 2	Day 3	
80	99.3 ± 0.4	99.6 ± 0.5	99.9 ± 0.8	100.5 ± 0.8	100.1 ± 0.9
100	101.1 ± 0.7	99.6 ± 0.9	100.1 ± 0.3	99.5 ± 0.7	99.8 ± 0.8
120	100.4 ± 0.8	100.8 ± 0.2	100.9 ± 0.4	100.5 ± 0.6	100.7 ± 0.5

[a] Mean value of the five determinations.

Table 4
Assay precision: repeatability and reproducibility study

Sample (dexpanthenol theoretical concentration = 50 mg g^{-1})	Laboratory 1 (Kharkov, Ukraine)	Laboratory 2 (Kiev, Ukraine)		Laboratory 3 (Moscow, Russia)	
	Found (%)	Found (%)	Δ (%)	Found (%)	Δ (%)
Gel, sample 1	100.5 ± 0.8	101.2 ± 0.4	0.7	100.9 ± 0.5	0.4
Gel, sample 2	103.4 ± 0.2	104.6 ± 0.7	1.2	104.2 ± 0.2	0.8
Aerosol, sample 1	99.2 ± 0.5	100.0 ± 0.4	0.8	98.7 ± 0.9	−0.5
Aerosol, sample 2	107.3 ± 0.8	105.9 ± 0.8	−1.4	106.4 ± 0.5	−0.9
Calculated *F*-criteria*		5.88		4.21	
Repeatability (R.S.D. of standard solution for assay; five injections)	1.22	0.95		0.47	

*Theoretical *F* value for *P* = 95% is 6.39.

reproducibility study were listed in Table 4. When results from assay of the same drugs by different laboratories were compared (Table 4), statistical analysis using the variance ratio *F*-test showed there were no significant difference between the results. The calculated *F* values were less than the theoretical values at 95% confidence level. These results show that the method is rugged and precise.

3.1.7. Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate, variations in method parameters, and provides an indication of its reliability during normal usage. In order to the robustness study of the proposed method deliberate modifications in pH values of the

Table 5
Results for robustness test study

Parameter	Variations	*k*	$N\,(\times 10^3)$[a]	*T*
Mobile phase flow-rate	0.8 mL min^{-1}	6.52	18.5	1.38
	1.0 mL min^{-1}	5.18	20.9	1.24
	1.2 mL min^{-1}	4.25	22.6	1.12
Column temperature	25 °C	5.22	20.5	1.26
	30 °C	5.18	20.9	1.24
	35 °C	5.14	21.1	1.24
Mobile phase pH	2.10	4.82	22.0	1.15
	2.30	5.18	20.9	1.24
	2.50	5.86	19.1	1.47

[a] Theoretical plates per meter.

Table 6
Application of the procedure to the pharmaceutical formulation

Pharmaceutical formulation (Company)	Declared composition	Found (mg)	Recovery (%)
Panthenol aerosol, 130 g (Ankerpharm GmbH, Germany)	463 mg of dexpanthenol in 10 g of aerosol	482.2 ± 0.5	104.1
Aerosol Panthenol 116 g (Micropharm, Ukraine)	44 mg of dexpanthenol in 1 g of aerosol	44.3 ± 0.4	100.7
Panthenol-sprey (Chauvin Ankerpharm GmbH, Germany)	463 mg of dexpanthenol in 10 g of aerosol	467.2 ± 0.8	100.9
Cornergel, eye-gel 5% (Dr. Mann Pharma GmbH, Germany)	50 mg of dexpanthenol in 1 g of gel	52.1 ± 0.3	104.2
Pantestin-Darnitsa, gel 15 g (Darnitsa, Ukraine)	50 mg of dexpanthenol in 1 g of gel	49.8 ± 0.7	99.6
Dolobene, gel (Ratiopharm GmbH, Germany)	25 mg of dexpanthenol in 1 g of gel	26.1 ± 0.5	104.4

mobile phase were made. The results are shown in Table 5. It can be seen that every employed condition, the chromatographic parameters are in accordance with established value [21]. A change of +0.2 unit of pH around 2.30 (pH of mobile phase) had no impact on chromatographic performance.

Tailing (symmetry) factor (T) and number of theoretical plates (N) was calculated by formulas: $T = W/(2\mathrm{Wa})$ and $N = 5.545(t_R/W_{1/2})^2$, where W is the peak width at 5% height from baseline Wa the peak front edge width at the same height and $W_{1/2}$ is the peak width at half height.

According to the data of robustness test study we proposed criteria for system suitability test (tailing factors, theoretical plates number and repeatability (R.S.D.) of retention time and peak area for replicate analysis). It is used to verify that the resolution and repeatability of the system are adequate for the analysis intended. The criteria for system suitability test proposed for the determination of dexpanthenol in drugs should be:

- The symmetry factor for the dexpanthenol peak, determined by analysis of the standard solution, should be not more than 1.5;
- The column efficiency on dexpanthenol peak should be not more than 4500 theoretical plates per column (18,000 theoretical plates per meter);
- The relative standard deviation of the dexpanthenol peak area for five replicate injection of the standard solution should be not more than 1.5%.

All obtained results were within the acceptable range.

3.2. Application of the method to pharmaceutical analysis

The six drugs studied are all currently administrated in our country. Pharmaceutical formulation was presented as aerosol (Panthenol aerosol 130 g, Aerosol Panthenol 116 g and Panthenol-sprey) and as gel (Cornergel, Pantestin-Darnitsa and Dolobene). Table 6 shows the composition declared by the manufacturers, and those ones found according to the recommended procedure. The recoveries obtained agreed with the declared compositions.

4. Conclusions

A new reversed-phase liquid chromatographic method for the determination of dexpanthenol contents in pharmaceutical formulations was developed. The developed method is very simple and results were obtained confirm suitable accuracy, specificity and precision. Therefore, the developed RP HPLC method was proved to be suitable for the dexpanthenol determination in aerosol and gel pharmaceuticals.

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