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Journal of Pharmaceutical and Biomedical Analysis
27 (2002) 729–735

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

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Colorimetric and fluorimetric methods for determination of panthenol in cosmetic and pharmaceutical formulation

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Received 11 April 2001; received in revised form 16 May 2001; accepted 29 May 2001

Abstract

Two methods are suggested for determination of panthenol. The first is colorimetric method where panthenol is subjected to alkaline hydrolysis; the resulting β-alanol is allowed to react with vanillin (Duquenois reagent) in presence of McIlvain buffer pH 7.5. The color developed is measured at 406 nm. The linearity range was found to be 50–500 μg/ml while the lower limit of detection was about 10 μg/ml. The second is a sensitive and reliable modified fluorimetric method is also suggested, whereas panthenol, after alkaline hydrolysis is treated with ninhydrin. The fluorescent product was found to have excitation λ_{max} at 385 nm and emission λ_{max} at 465 nm. The method showed high sensitivity with linearity range from 0.01 to 3 μg/ml. The lower limit of detection (LOQ) reached 0.005 μg/ml. Validation of both methods was carried out and the two methods were applied for determination of panthenol in some cosmetic and pharmaceutical formulations.

Keywords: Panthenol; β-alanol; Duquenois reagent; Colorimetry; Ninhydrin; Fluorimetry

1. Introduction

Panthenol, butanamide or vitamin B_3 has the formula $C_9H_{19}NO_4$ and IUPAC name (±)-2,4-dihydroxy-*N*-(3-hydroxypropyl)-3,3-dimethyl-butyramide, it is also called (±)-pantothenyl alcohol. Panthenol is one of the components of multivitamin preparations, parenterals and some of the local cosmetic preparations. The USP XXIV stated a non-aqueous titrimetric method for its assay [1]. Despite the low molar absorptivity of panthenol at (280 or 254 nm) due to the lack of UV chromophores, yet direct spectrophotometric assay has been reported [2]. Several methods have been reported on the analysis of panthenol depending on hydrolysis of panthenol into the primary amine, β-alanol, that forms colored products with reagents such as iodine, ninhydrin or even ferric hydroxamate [3–5]. As an alcohol, it has been determined by gas–liquid chromatographic methods [6,7]. Liquid chromatographic methods either directly or after hydrolysis

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0731-7085/02/$ - see front matter
PII: S0731-7085(01)00510-6

and derivatization with fluorescamine were also reported [8–10]. Fluorimetric method for panthenol after its hydrolysis to the β-alanol and reaction with ninhydrin was reported [11]. The use of ^{1}H NMR spectroscopy to analyze panthenol in mixtures has been reported [12]. Determination of panthenol in cosmetic and pharmaceutical formulations by differential pulse voltammetry has also been reported [13].

In the present study, panthenol after its alkaline hydrolysis to β-alanol was subjected to react with vanillin using Duquenois reagent [14], the reagent forms colored product with primary amines that can be measured at 406 nm. This simple reaction has never been used for quantitation of panthenol. The latter is also one of the components of most cosmetic products and some pharmaceutical. This gave the necessity of finding a simple, sensitive and reliable method beside the compendial one. The second fluorimetric assay is achieved by reacting the β-alanol with ninhydrin in presence of acetaldehyde instead of butyraldehyde in the method reported by Panier and Close [11]. The proposed methods were applied for the analysis of panthenol in some pharmaceutical and cosmetic products.

2. Experimental

2.1. Reagents and chemicals

All reagents and chemicals are of analytical grade.

1. Panthenol standard: was kindly supplied by EVA laboratories, Cairo, Egypt. The standard has been analyzed by the USP XXIV non-aqueous titrimetric method and was found to contain 99.8% of panthenol calculated on the dried basis.
2. Standard panthenol stock solution (1 mg/ml) for colorimetry: dissolve 500 mg of panthenol in 5 ml of distilled water. Add 2 ml of 0.5 N sodium hydroxide and 30 ml of distilled water. Place the solution on a boiling water bath for 1 h then neutralize to pH 7 with 2 ml of 0.5 N sulphuric acid and complete to 50 ml with distilled water. Dilute 10 ml of the solution with distilled water to 100 ml.
3. Standard panthenol stock solution (10 μg/ml) for fluorimetry: dissolve 5 mg of panthenol in few ml of distilled water. Add 2 ml of 0.5 N sodium hydroxide and 30 ml of distilled water and complete as under 1.2. Starting from (add 2 ml of 0.5 N sodium hydroxide…).
4. McIlvaine buffer: mix 35.5 ml of 0.2 M disodium hydrogen phosphate with 64.5 ml of 0.1 M citric acid and adjust the pH to 7.5 with 0.1 N sodium hydroxide.
5. Duquenois reagent: mix 2 g of vanillin with 0.3 ml of acetaldehyde and the complete to 100 ml with ethyl alcohol. The reagent is stored in dark.
6. Acetate buffer (1 M) pH 4.7.
7. Ninhydrin reagent: about 14 mM of ninhydrin solution in sodium carbonate/bicarbonate buffer (1 M) pH 9.1 and should be freshly prepared.
8. Acetaldehyde solution (0.2 M) in water; should be freshly prepared.

2.2. Apparatus

A dual beam ultra-violet/visible spectrophotometer model UV-1601 PC, Shimadzu, Japan.
A spectrofluorimeter Model RF-540, Shimadzu, Japan.

2.3. Samples

2.3.1. Pharmaceutical formulations

The following products are purchased from the local market:

- Panthenol cream (consists of panthenol 5% in a cream base);
- Panthenol ampoule 250 mg/ml (Nile Co for pharmaceutical);
- Panthenol shampoo (consists of panthenol 5% in a shampoo base);
- Sunscreen gel (contain 5% panthenol in a gel base).

No more information about the above mentioned cosmetic products could be obtained from manufacturers.

2.3.2. Sample treatment

Mix 1 g of cream, gel of shampoo with 1 g of anhydrous sodium sulphate and dissolve and add 5 ml of distilled water, mix well, filter and wash if necessary. Add 2 ml of 0.5 N sodium hydroxide. Add 30 ml of distilled water and mix well. Allow the solution to stand on a boiling water bath for 1 h then neutralize to pH 7 with 2 ml of 0.5 N sulphuric acid, complete to 50 ml with distilled water. For colorimetric determination, dilute 10 ml of the solution with distilled water to 100 ml in a volumetric flask. For fluorimetric determination, dilute 1 ml of the solution used for colorimetric determination to 100 ml in a volumetric flask.

2.4. Procedure

2.4.1. Colorimetric procedure and calibration

Into 10 ml volumetric flask transfer different aliquots of the hydrolyzed standard or sample solution prepared for colorimetry (0.5–5) ml to provide final concentration range 50–500 μg/ml. To each flask add 1 ml of Duquenois reagent, 1 ml of McIlvaine buffer pH 7.5 and complete to volume with distilled water. Allow standing at room temperature for 35–40 min then measure the absorbance of the color at 406 nm. Carry out a blank experiment using 1 ml of buffer pH 7.5 instead of sample. Calculate the concentration from calibration graph representing the absorbance versus concentration or from the regression equation.

2.4.2. Fluorimetric procedure

Into 100 ml volumetric flask transfer 50 ml of acetate buffer pH 4.7, 1 ml of the standard or sample hydrolyzate (for fluorimetry) and dilute the solution to 100 ml with distilled water. Transfer 1, 2 and 10 ml of the resulting solution equivalent to final concentrations 0.005, 0.01 and 0.05 μg/ml into 20 ml glass stoppered test tubes. Futhermore, transfer 0.2, 2, 4 and 5 ml of the original hydrolyzate to provide final concentrations 0.1, 1, 2 and 3 μg/ml into glass stoppered test tubes. Furthermore, transfer 0.2, 2, 4 and 6 ml of the original hydrolyzate to provide final concentrations 0.1, 1, 2, and 3 μg/ml into glass stoppered test tubes. To each tube add 0.5 ml of acetate buffer pH 4.7, followed by 0.5 ml of acetaldehyde solution and finally 0.5 ml of ninhydrin reagent solution. Carry out a blank experiment using 1 ml of acetate buffer instead of sample. In all cases, the solution is mixed well and tubes are loosely capped and allowed to stand for 45 min in a water bath at 60 °C. Cool and dilute the solutions quantitatively to 20 ml with distilled water. Record the fluorescence intensity at 465 nm setting excitation wavelength at 385 nm using a suitable spectrofluorimeter. Calculate the concentration from the calibration curve representing concentration versus the fluorescence intensity or from the regression equation.

3. Results and discussion

3.1. Panthenol hydrolysis

The UV absorption spectrum of panthenol shows poor ultra-violet absorption [9]. The question of hydrolysis has arisen long years ago and many researchers used either acid or alkaline hydrolysis to produce the reactive amino alcohol that is capable of undergoing condensation reactions with many reagents [11]. It should be noted here that the proposed methods depend on the reaction with the hydrolyzate. The methods can be applied to analyze a sample once before hydrolysis and another after hydrolysis. The difference will show the extent of degradation. Therefore, they are considered as stability indicating methods.

3.2. Colorimetric method

Duquenois reagent is used for the determination of amino-group containing compounds. The reagent consists mainly of vanillin, which contains an aldehyde group. On the other hand, panthenol after hydrolysis produces an aminopropyl alcohol or β-alanol, which is on turn a primary amino-alcohol that reacts with the aldehyde group of vanillin via a condensation mechanism to give colored product [14].

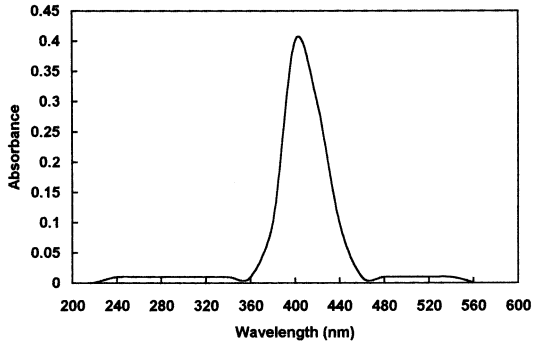

Fig. 1. Absorption spectrum of the color produced by reacting hydrolyzed panthenol with Duquenois reagent.

The McIlvaine buffer pH 7.5 was necessary to achieve the reaction and to obtain the color. Trials are carried out to use buffers with different pH values. However, the pH value 7.5 was found to be the best to obtain the color in a specified time 35–40 min at room temperature and maximum wavelength at 406 nm. Fig. 1 shows the absorption spectrum of the color produced by the reaction. The change in buffer concentration and pH led to change in λ_{max}. Study of the reaction time to obtain maximum absorption under the assay conditions is presented in Fig. 2.

3.2.1. Method validation

Standard panthenol after hydrolysis to β-alanol was analyzed by the proposed method to check the limit of detection (LOD), that was down to 10 μg/ml. Calibration curve relating the absorbance to concentration of panthenol is constructed to prove linearity. The regression equation was found as follows:

$$\text{Panthenol concentration} = \frac{\text{Absorbance} + 0.0059}{0.0019}$$

with regression coefficient = 0.9997. The limit of quantitation (LOQ) was in range 50–500 μg/ml. The data for accuracy and precision of both standard and dosage forms spiked with the panthenol are presented in Tables 1 and 2. The degree of reproducibility of results obtained under a variety of conditions is indicating by the small standard deviation (S.D.), which is less than 2% or the confidence limit, which is also less than 2. The method was also tried by using different instrument and another operator in the same laboratory and data compared together revealed insignificant difference, which may indicate the method robustness.

The data presented in Table 1 for the analyses of standard panthenol were compared with the data

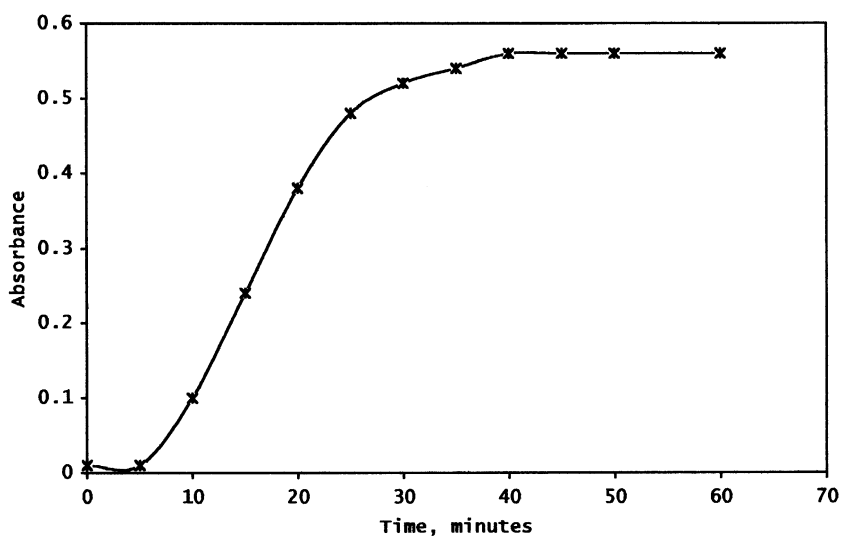

Fig. 2. Reaction time of 300 μg/ml of hydrolyzed panthenol with Duquenois reagent.

Table 1
Determination of panthenol by the proposed colorimetric method using the Duquenois reagent at 406 nm

Taken (μg/ml)	Absorbance	Found (μg/ml)	Recovery (%)
50	0.09	50.26	100.53
60	0.11	60.79	101.34
70	0.13	71.34	101.88
80	0.15	79.21	99.01
90	0.168	91.34	101.46
100	0.19	101.84	101.84
200	0.38	200.26	100.13
300	0.56	297.63	99.21
400	0.75	397.63	99.41
500	0.96	508.16	101.63
Mean			100.64
S.D.			1.134
Confidence limits[a]			0.741

[a] Confidence interval at a significance level $\alpha = 5\%$.

obtained for analyses of standard panthenol by using the USP XXIV method. It was clear that no significant difference between methods. As for the specificity of the method, it has been explained by increasing the specificity by choice of the suitable buffer and reaction conditions that may be suitable for any amino-group containing compound.

3.3. Fluorimetric method

This method has been reported in 1964 by Panier and Close [11], however, they used butyraldehyde to achieve the fluorescence reaction with ninhydrin, yet the use of acetaldehyde in this study did not alter the reaction results. Moreover, the reaction time under the specified conditions has been found shorter, that is about 30 min rather than 45 min stated by Panier and Close. Study of the optimum reaction time for fluorescence formation is presented in Fig. 3.

3.3.1. Method validation

Certainly the fluorimetric method is so sensitive than the colorimetric one that the detection limits (LOD) were about 0.005 μg/ml of panthenol. A calibration graph representing the concentration of panthenol versus the fluorescence intensity is constructed to check linearity. The regression equation was found as follows:

$$\text{Panthenol concentration} = \frac{\text{Fluorescence intensity} - 0.0269}{29.966}$$

with regression coefficient = 1. The limit of quantitation (LOQ) of the method has range 0.01–3 μg/ml.

Table 2
Accuracy and precision data obtained for the proposed colorimetric method using the Duquenois reagent for determination of panthenol in different products

Product	Taken (mg)	Mean absorbance	Mean found (μg/ml)	Mean found (g%)	Mean recovery (%)[a]
Shampoo	8	0.116	63.95	4.99	99.80
	12	0.178	96.40	5.04	100.80
	20	0.303	162.54	5.08	101.56
Mean					100.40
S.D.					0.880
Sunscreen gel	11	0.164	89.39	5.08	101.56
	13	0.189	102.41	4.93	98.60
	16	0.236	126.76	4.96	99.16
Mean					99.77
S.D.					1.570
Ampoules	1.0	0.35	188.86	251.89	100.76
	1.5	0.53	283.60	252.09	100.84
	2.0	0.71	376.58	251.05	100.42
Mean					100.67
S.D.					0.223

[a] Average of three separate experiments.

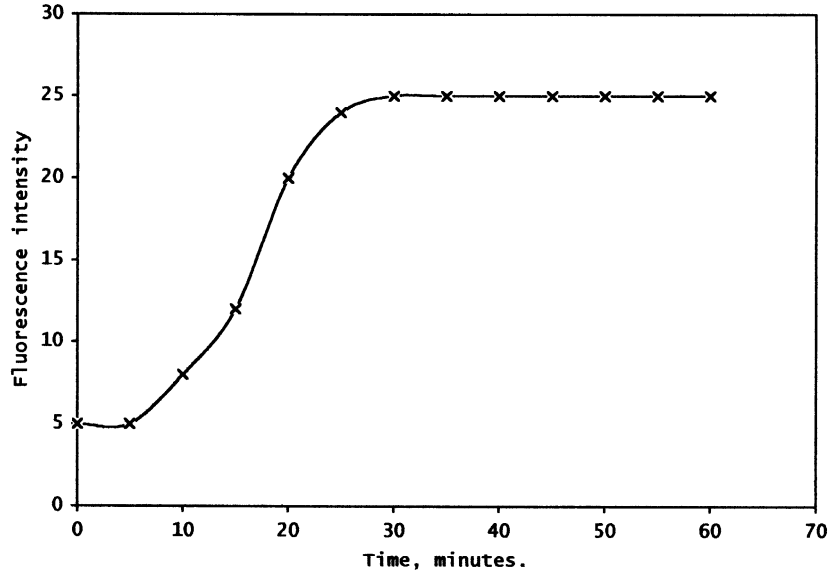

Fig. 3. Reaction time of 1 μg/ml of hydrolyzed panthenol with ninhydrin.

Table 3
Fluorimetric determination of panthenol by the ninhydrin method

Taken (μg/ml)	Fluorescence intensity	Found (μg/ml)	Recovery (%)
0.1	3.0	0.099	99.22
0.4	12.0	0.400	99.89
0.8	24.0	0.800	100.00
1.2	36.0	1.200	100.04
1.6	47.9	1.598	99.85
2.0	60.0	2.002	100.10
2.4	72.1	2.405	100.22
2.8	84.0	2.803	100.12
3.0	89.7	2.992	99.75
Mean			99.91
S.D.			0.298
Confidence limits[a]			0.206

[a] Confidence interval at a significance level $\alpha = 5\%$.

It was obvious that higher concentration exhibit quenching. In order to ascertain the accuracy and precision of the method, spiking of commercial pharmaceutical and cosmetic products with standard panthenol was done and the results presented in Tables 3 and 4 showed a small S.D. less than 1% and confidence interval (C.I.) less than 1.

4. Conclusion

The colorimetric method for determination of panthenol existed lower sensitivity than the studied fluorimetric method but it was found more sensitive and accurate than the official USP 2000 non-aqueous titrimetric method. It also offered a wide range of determination and good applicabil-

Table 4
Accuracy and precision data obtained for fluorimetric determination of panthenol in different products

Product	Taken (mg)	Fluorescence intensity	Found (μg/ml)	Found (g%)	Recovery (%)[b]
Shampoo	0.125	30.1	1.00	5.02	100.36
	0.250	61.1	2.04	5.10	101.90
	0.500	120.5	4.02	5.03	100.51
Mean	100.92				
S.D.	0.853				
Confidence limits[a]					0.591
Cream	0.125	30.5	1.02	5.08	101.69
	0.250	60.4	2.01	5.04	100.74
	0.500	120.6	4.02	5.03	100.59
Mean					101.01
S.D.					0.598
Confidence limits[a]					0.414
Sunscreen gel	0.125	29.9	1.00	4.98	99.69
	0.250	60.7	2.02	5.06	101.24
	0.500	119.2	3.98	4.97	99.42
Mean					100.12
S.D.					0.979
Confidence limits[a]					0.678

[a] Confidence interval at a significance level $\alpha = 5\%$.
[b] Average of three separate experiments.

ity to the analysis of pharmaceutical dosage forms. The use of Duquenois reagent gave a stable color and accurate results.

The modified fluorimetric method was suited to be simpler. The high sensitivity and accuracy were sustained. Getting fluorescence in a shorter reaction time reduced the analysis time. The method also provided a wider analytical range than that reported in 1964 by Panier and Close [11].

The two methods were found suitable for the analysis of panthenol at different levels in different cosmetic and pharmaceutical formulations.

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