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Development of an HPLC-UV method for the simultaneous determination of allantoin and D-panthenol in cosmetic products containing *Aloe vera* extracts

Burhan Ceylan[1*] and S. Evrim Kepekci Tekkeli[2]

[1] Department of Pharmacognosy, Faculty of Pharmacy, Harran University, Sanlıurfa, Turkey

[2] Department of Analytical Chemistry, Faculty of Pharmacy, Istanbul Health and Technology University, Istanbul, Turkey

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ABSTRACT

A simple, fast and selective analytical method has been developed for the simultaneous determination of allantoin and D-panthenol in cosmetic products containing *Aloe vera* extracts. The proposed method depends on reversed-phase liquid chromatography with isocratic flow profile of the mobile phase composed of acetonitrile–10 mM phosphoric acid (pH 2.5) (85:15, v/v), with a C18 column at 30 °C. The analytes were detected with UV–vis. detector at 210 nm. The injection volume was 20 µL. The linearity ranges were found to be 0.2–20 and 0.1–10 µg mL^{-1} for allantoin and D-panthenol, respectively. LOD values were found to be 0.07 µg mL^{-1} and 0.03 µg mL^{-1}, LOQ values were found to be 0.2 and 0.1 µg mL^{-1} for allantoin and D-panthenol, respectively. No interference was observed from concomitants. The developed method was applied to the analysis of 10 different type cosmetic products. It is foreseen that the method will be able to be used in order to carry out routine analysis, quality control and standardization in cosmetic products containing allantoin and D-panthenol.

KEYWORDS

allantoin, D-panthenol, high performance liquid chromatography, *Aloe vera*, cosmetic products, UV detection

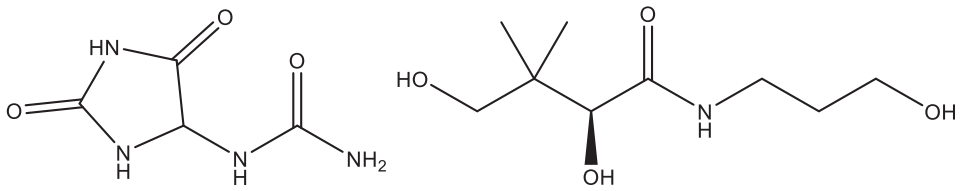
1. INTRODUCTION

Aloe vera (*Aloe barbadensis* Miller) is a plant belonging to the Liliaceae family, most important species of the *A. vera* genus. This plant, whose homeland is South Africa, grows naturally in North Africa, the Nile region of Sudan and the southwest of Turkey [1]. *A. vera* is a perennial plant with succulent and spiny leaves. The root is light brown, strong and fibrous tissue. *A. vera* products have long been used in health food products, medicine and cosmetics for various purposes. It is also used in many formulations as gel, beverage, powder, capsule and cream [2].

A. vera contains over 200 nutrients including water-soluble and fat-soluble vitamins, minerals, enzymes, polysaccharides, amino acids, flavonoids, phenolic compounds and organic acids. It is the parts of the *A. vera* plant that are used externally and internally as a therapeutic in various ailments, the whole plant leaf, the juice secreted from the vascular bundles and the gel found in the fleshy part of the leaves. The laxative, wound healing, antioxidant, anti-inflammatory, immunostimulant, antimicrobial, antitumor, analgesic and neuroprotective effects of *A. vera* plant have been reported [3–7].

Allantoin is a chemical compound with formula $C_4H_6N_4O_3$ (Fig. 1). It is also called 5-ureidohydantoin or glyoxyldiureide. Allantoin is a major metabolic intermediate in most organisms including animals, plants and bacteria. The occurs as a natural mineral compound.

*Corresponding author. Department of Pharmacognosy, Faculty of Pharmacy, Harran University, 63050 Haliliye-Sanlıurfa, Turkey.
Tel.: +904143183000.
E-mail: burhanceylan@harran.edu.tr, b.ceylan022@gmail.com



(a) (b)

Fig. 1. Chemical structures of allantoin (a) and D-panthenol (b)

Allantoin is a commonly used ingredient found in many popular skin care products. It comes from herbal extracts, usually found in the roots of the comfrey plant (*Symphytym caucasicum* L.), *Aloe vera*, *Matricarica chamomilla* L. and other herbs. Allantoin is a versatile, safe (non-toxic and suitable for all skin types) and effective skin care ingredient with many different benefits, including softening and moisturizing the skin [8].

Panthenol, also known as pantothenol (well-known B-complex component), serves as the alcohol analogue of pantothenic acid and, as such, functions as a provitamin of B5 (Fig. 1). Within living organisms, it undergoes rapid oxidation to become pantothenic acid. Panthenol finds applications in pharmaceuticals, cosmetics, and personal care products, where it serves as a moisturizer and aids in wound healing. It is incorporated into formulations such as ointments, lotions, shampoos, nasal sprays, eye drops, lozenges, and contact lens cleaning solutions. In ointments, panthenol is employed to treat conditions like sunburns, mild burns, minor skin injuries, and related disorders. Its benefits encompass enhanced skin hydration, itch and inflammation reduction, improved skin elasticity, and acceleration of the healing rate for epidermal wounds. On occasion, it is combined with allantoin for these purposes. Panthenol exists in two enantiomeric forms, D and L, with only D-panthenol (dexpanthenol) exhibiting biological activity. In the realm of cosmetics, panthenol is available in either the D form or as a racemic mixture of D and L (DL-panthenol) [9].

These two cosmetic raw materials (allantoin and D-panthenol) have skin moisturizing, wound healing and many pharmacological effects. In addition, these two substances are found in combination in cosmetic products containing *A. vera*. Because of the role of the combination in therapy, simultaneous determination of the cosmetic substances is very important and there is a lack of such an analytical method in the literature. When we look at the literature, there are many methods for the determination of allantoin and D-panthenol. Biological fluids (human plasma and urine), cosmetic and pharmaceutical products were used in these analysis methods. In these methods different techniques were used such as hydrophilic liquid chromatography [10], HPLC-UV [11], HPLC combined with MS/MS [12], ion-pair HPLC [13], hydrophilic interaction chromatography [14], supercritical fluid chromatography [15], different pulse voltammetry [16]. None of these methods provide simultaneous determination of allantoin and D-panthenol, so they are not appropriate to use them for the analysis of cosmetics, including the components as combinations.

The aim of this study is to use body oil, face creams, moisturizers, shampoo, body creams, toothpaste, hair conditioner, etc.in cosmetic products containing *A. vera*, it is aimed to develop and validate a HPLC method that will allow analysis of allantoin and D-panthenol together without interfering with other components. According to the developed method, the analysis of cosmetic products claimed to contain allantoin, *A. vera* and D-panthenol will be carried out easily and quickly in routine laboratories.

2. MATERIALS AND METHODS

2.1. Instrumentation and reagents

HPLC analyses were conducted using a Shimadzu (Japan) LC 20 liquid chromatograph, comprising of an LC-20AT pump, SIL AT-HT autosampler component, and a SPD-20A HT UV detector set at 210 nm. Additionally, a CTO 10 AC column oven was employed for temperature control. Chromatographic separation was achieved isocratically at 30 °C, utilizing a GL Sciences (Japan) C18 (ODS) column with dimensions of 4.6 mm I.D., 150 mm length, and a particle size of 5 µm. The mobile phase consisted of acetonitrile-10 mM orthophosphoric acid (pH 2.5), supplemented with 1 mL L^{-1} triethylamine, and was delivered at a flow rate of 0.9 mL min^{-1}.

Allantoin and D-panthenol were procured from Sigma Aldrich, St. Louis, Missouri, USA. Acetonitrile, orthophosphoric acid, and triethylamine were all of HPLC grade and sourced from Merck, Darmstadt, Germany. Water was purified using the Human from Japan ultrawater purification system.

2.2. Chemicals and solution (preparations of stock solutions)

The stock standard solutions of allantoin and D-panthenol were dissolved in 100.0 µg mL^{-1} acetonitrile. Before conducting measurements, stock solutions were appropriately diluted with acetonitrile to create working standard solutions at different concentrations. HPLC analyses were performed using 20 µL aliquots of these working standard solutions. The peak areas versus concentration of the cosmetic raw components were used to analyse the chromatograms.

2.3. Preparation of the calibration curves

To prepare the calibration curves, we analyzed working standard solutions of allantoin and D-panthenol at different concentrations. Linear least-squares regression analysis was employed to establish the linear concentration ranges for both compounds. Each concentration was studied in five replicates, and the equations for the calibration curves were calculated in the form of $y = ax + b$, where y represents the peak areas, and x corresponds to the concentrations of the cosmetic raw materials in μg mL^{-1}.

2.4. Extraction process for application the method to cosmetic products

Various extraction techniques were explored to extract allantoin and D-panthenol from cosmetic preparations, including liquid-liquid extraction (LLE) with different extraction solvents and solid phase extraction (SPE) with various cartridge types and sizes (C18-N, C8, NH2, C18 Resprep cartridges; 6 mL, 1,000 mg). Once the new method was developed and validated, it was applied to 10 different types of cosmetic products. Initially, the LLE technique was tested with different extraction solvents (acetonitrile, methanol, chloroform, dimethyl sulfoxide and dimethylformamide), solvent mixtures (1:1 and 1:2) and varying volumes of extraction solvents to optimize the efficient extraction of allantoin and D-panthenol from the cosmetics. Following the LLE phase, SPE techniques were experimented with, using different elution solvents and cartridge types. It was observed that LLE exhibited higher efficacy with better recovery values. For sample preparation, cosmetic samples (0.1 mL or 0.1 g) were briefly mixed with 0.5 mL of ethanol and 0.5 mL of acetonitrile. These mixed solutions were then centrifuged at 4,000 rpm for 10 min at room temperature. The resulting supernatant was subsequently filtered through 0.45 μm polyethersulfone filters (Dainippon Seiki, Kyoto, Japan). Finally, the samples were analyzed using HPLC. All measurements were repeated six times to ensure the accuracy of each compound's analysis. The nominal contents of cosmetic raw materials were calculated using the regression equations derived from the calibration graphs.

3. RESULTS AND DISCUSSION

3.1. Method development

The HPLC method (Preliminary experiments were conducted to determine the most suitable chromatographic conditions. Various types of columns (C2, C8 and C18) were evaluated at different temperatures (25, 30 and 35 °C). The highest resolution, characterized by sharper and more symmetrical peaks, was achieved when using a C18 column with dimensions of 4.6 mm I.D, 250 mm length, and a 5 μm particle size, maintained at a temperature of 30 °C. Different mobile phases (80:20 and 90:10) including both acidic and aqueous solutions, were tested at various flow rates (0.8 and 1.0). It was observed that the acidic mobile phase yielded favourable results. Consequently, an o-phosphoric acid solution (10 mM) containing 1 mL L^{-1} triethylamine was selected as the acidic component. Acetonitrile was chosen as the organic modifier. The optimal mobile phase composition was determined to be acetonitrile–phosphoric acid (pH: 2.5) in a ratio of 85:15 (v/v), with a flow rate of 0.9 mL min^{-1}, resulting in a high-resolution value. For quantification purposes, the wavelength was set at 210 nm. Under these conditions, the retention times for the cosmetic raw materials were found to be 3.30 ± 0.02 and 4.50 ± 0.01 min for allantoin and D-panthenol, respectively. Peak areas and resolution values were measured to identify the optimal conditions for analysis. Representative chromatograms are shown in Fig. 2a–c.

3.2. Method validation

The method's validation was conducted in accordance with the guidelines provided by the International Conference on Harmonization (ICH) [17].

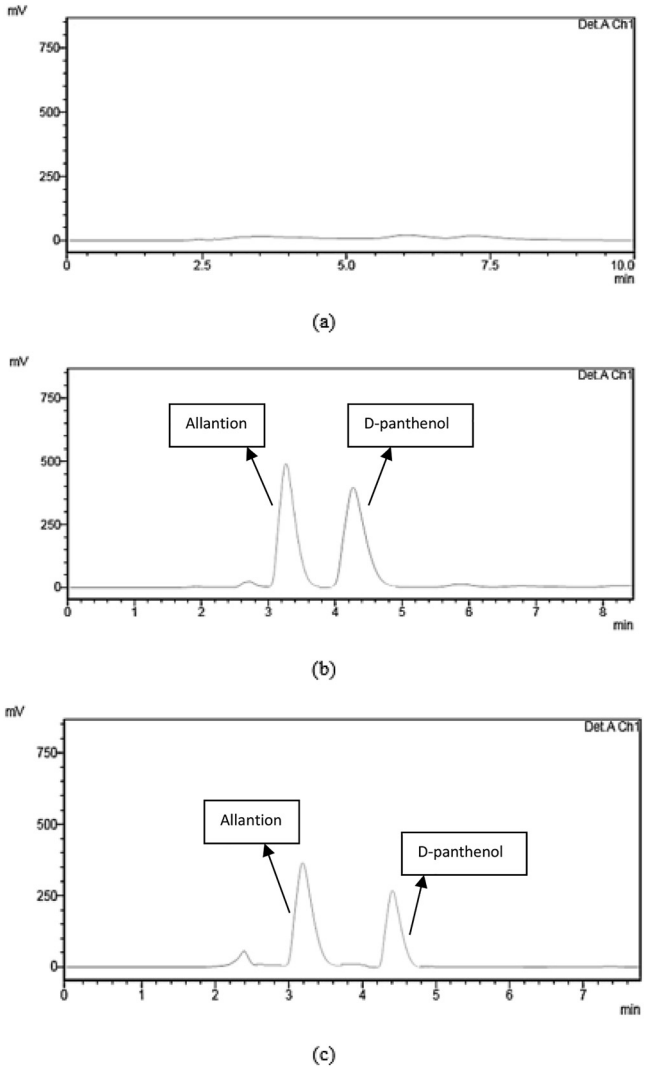


Fig. 2. (a): blank (aqueous medium), (b): standard solution (10 μg mL^{-1} standard allantoin and D-panthenol solutions), (c): real sample (hand cream)

3.2.1. Linearity range and calibration curve. The linearity ranges for the analysis were established as 0.2–20 µg mL^{-1} for allantoin and 0.1–10 µg mL^{-1} for D-panthenol. The regression equations for the calibration curves were determined as follows:

- For allantoin: y = 1558.1x + 119.2 (with an r^2 value of 0.9993)
- For D-panthenol: y = 1518.2x + 240.2 (with an r^2 value of 0.9956)

The limits of detection (LOD) and quantification (LOQ) values were determined using the formulas LOD or LOQ = k * SDa/b, where k = 3 for LOD and 10 for LOQ, SDa represents the standard deviation of the intercept, and b is the slope. In this context, the LOD values were identified as 0.07 µg mL^{-1} for allantoin and 0.03 µg mL^{-1} for D-panthenol. Likewise, the LOQ values were determined to be 0.2 µg mL^{-1} for allantoin and 0.1 µg mL^{-1} for D-panthenol. You can find additional analytical parameters in Table 1.

3.2.2. Precision. The precision of the method was evaluated in terms of inter-day and intra-day precision at three concentration levels (low, medium, high). These analyses were conducted for both allantoin and D-panthenol on the same day and across seven different days, with each test performed five times. The relative standard deviation (RSD) values ranged from 0.22% to 1.84% for intra-day precision and from 1.66% to 2.43% for inter-day precision. These results demonstrate the method's excellent repeatability.

Table 1. Results of analytical parameters for the proposed method

Parameter	Allantoin	D-panthenol
Linearity range* (µg mL^{-1})	0.2–20	0.1–10
Regression equation	y = 1558.1x + 119.2	y = 1518.2x + 240.2
Slope±SD	1558.2 ± 8.20	1518.2 ± 5.8
Intercept±SD	119.2 ± 15.76	240.2 ± 12.4
Correlation coefficient, r^2	0.9993	0.9956
LOD (µg mL^{-1})	0.07	0.03
LOQ (µg mL^{-1})	0.2	0.1

*n = 5 correspond to replicate analysis for each level.

3.2.3. Recovery. Recovery experiments were carried out to determine the accuracy of the method for the quantification of allantoin and D-panthenol. Recovery of the method was checked at three different concentration by spiking of the standards to the cosmetic formulations which are given in Table 2 and the following equation was used for the recovery experiments.

$$Recovery\% = ((Ct - Cu))/Ca)$$

Where C_a represents the proportion of the purified analyte introduced to the formulation, C_u represents the proportion of the analyte contained within the formula, and C_t is the overall concentration of the analyte discovered. Table 2 displays the findings from the recovery research and the examination of the industrial cosmetic formulations specimen types. The average percent recoveries obtained from the method were found to be quantitative, ranging from 99.06% to 103.60%. These results indicate a high level of accuracy in the method, as the recoveries are very close to 100%, suggesting that the method is reliable for determining the analyte concentrations in the cosmetic formulations.

3.2.4. Stability. The stability of the working standard solutions of the cosmetic raw materials was assessed under various storage conditions, including room temperature in the dark, in autosampler conditions for 48 h, and refrigerated at 4 °C for 1 month. The results of the stability studies indicated that the samples remained stable under these conditions. Specifically, the samples were found to be stable when stored at room temperature for 48 h, as well as when kept in autosampler conditions for the same duration. Additionally, refrigeration at 4 °C for 1 month did not compromise the stability of the samples. In all tested storage conditions, both allantoin and D-panthenol were determined to be stable, suggesting that the method can reliably analyze these cosmetic raw materials without concerns about sample degradation during storage.

3.2.5. Robustness. The robustness of the method was evaluated by introducing variations in key parameters, including the flow rate, column oven temperature, and the proportions of acetonitrile and the acidic solution in the mobile phase. Specifically, the mobile phase composition was modified from the initial 85:15 (acetonitrile–acidic

Table 2. Results of recovery studies by standard addition method

	Existing concentration (µg mL^{-1})	Added concentration (µg mL^{-1})	Found concentration (mg mL^{-1}) (Mean ± S.D)	Recovery (%)	RDS of intraday variation	RDS of interday variation
Allantoin	10	0.2	10.11 ± 0.05	99.11	0.55	2.10
		5	14.88 ± 0.07	99.20	1.21	1.75
		10	19.70 ± 0.03	98.50	1.84	2.43
D-panthenol	5	0.1	5.8 ± 0.02	103.60	0.95	1.66
		2.5	7.43 ± 0.09	99.06	0.51	1.87
		5	10.08 ± 0.01	100.80	0.22	1.39

*n = 5 correspond to replicate analysis for each level.

Table 3. Results from robustness experiments

Condition	Value	Recovery (%)		RSD (%)	
		Allantoin	D-panthenol	Allantoin	D-panthenol
Flow rate mL min^{-1}	0.8	99.73	100.88	0.34	1.45
	1.0	99.32	100.19	1.15	0.95
Mobile phase composition (ACN:aqueous phase)	80:20	97.56	98.89	1.05	1.10
	90:10	98.35	99.78	0.58	1.01
Column temperature	25	98.96	99.36	0.74	1.45
	35	99.44	98.95	1.31	1.22

For each concentration $n = 5$.

Table 4. Cosmetic raw material concentrations and method reproducibility

Cosmetic product	Recovery	% RSD	Allantoin concentration (μg mL^{-1})	D-panthenol concentration (μg mL^{-1})
Toothpaste	% 91.3	1.44	6.48	0.68
Hair shampoo	% 88.6	2.73	6.77	7.51
Body lotion	% 90.4	1.27	7.02	9.64
Liquid hand soap	% 96.5	1.35	1.53	3.72
Hand cream	% 95.6	2.14	15.08	9.88
Face cream	% 97.5	0.97	11.33	8.64
Hair cream	% 89.4	2.11	8.84	7.41
Eye cream	% 88.6	1.24	4.96	9.27
Body shampoo	% 83.4	2.62	10.23	9.11
Body gel	%90.6	1.70	17.44	9.92

solution) to two different ratios: 80:20 and 90:10. The column temperature was adjusted from the initial 30 °C to 35 °C and 25 °C, while the flow rate was altered from 0.9 to 0.8 and 1.0 mL min^{-1}. It's noteworthy that these changes in the method's parameters did not have any significant impact on the peak areas. Low relative standard deviation (RSD) values, as indicated in Table 3, demonstrate the robustness of the method. This suggests that the method is capable of providing consistent and reliable results even when slight variations in these parameters are introduced, which is an essential characteristic for its practical applicability.

3.3. Analysis of real samples

After the method and validation studies, the concentrations of allantoin and D-panthenol in cosmetic products containing *Aloe vera* were determined and listed in Table 4. %RDS values were quite low compared to our previous study [18].

4. CONCLUSIONS

In this study we developed a method in order to determine a widely used allantoin and D-panthenol cosmetic formulations. Due to the fact that allantoin has many cosmetic effects (as mentioned with the references in the text) it is used in so many cosmetic preparations. Allantoin is mostly found in *Aloe vera* sp. Generally cosmetic products that include allantoin are launched as a preparation consisting of *Aloe vera* extract, coexisting with D-panthenol due to its similar effects. In this context we aimed to developed a new HPLC method that provides the determination the allantoin and D-panthenol concentrations in 10 different kinds of cosmetic such as body lotion, shampoo, creams etc. with. Additionally, our method is very fast, simple and cost reduced. According to the validation studies, it was shown that this method is fairly sensitive, selective, precise and accurate. There are some methods for the determination of allantoin in the literature, but none of them provides an assay in cosmetics. They provide quantification in biological samples, plant extracts or human urine. In addition, there is no method in the literature that analyzes allantoin and D-panthenol simultaneously cosmetic products. It is foreseen that the method will be able to be used in order to carry out routine analysis, quality control and standardization of cosmetic products including allantoin and D-panthenol.

Conflict of interest: The authors declare no conflict of interest.

Informed consent: Informed consent was obtained from all individual participants included in the study.

Author contribution: All the authors have accepted responsibility for the entire content of this submitted manuscript and approved submission.

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