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DETERMINATION OF ALLANTOIN AND CHLOROHYDROXYALUMINIUM ALLANTOINATE IN COSMETIC AND PHARMACEUTICAL PRODUCTS BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

JIRO KAWASE*, HIDEKO UENO and KAZURO TSUJI
Tochigi Research Laboratories, Kao Soap Co. Ltd., 2606, Akabane, Ichikai-machi, Tochigi (Japan)
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SUMMARY

A high-performance liquid chromatographic method is described for convenient and direct determination of allantoin derivatives, by which as little as 0.1 μg of the compounds can be determined without any conventional sample preparation required to eliminate interferences. Chromatographic separation on a resin-based strong cation exchanger (H^+) using water as eluent is combined with sensitive and selective post-column reaction of the allantoin derivatives. After dissolution of the sample in hot water, the determinations take 12 min per sample. The relative standard deviation is less than 1.8% and quantitative recoveries are obtained. Several commercial cosmetic and pharmaceutical products were analysed successfully. The method can be applied to test the stability of the compounds in formulations.

INTRODUCTION

Several reports have been published on the determination of allantoin and the chlorohydroxy aluminium allantoinate (ALCA) in various cosmetic and pharmaceutical products[1–6]. Most of these determinations are based on the detection of hydrolysed products, are indirect and need cumbersome sample preparation.

We are interested in the stability of allantoin and/or ALCA in several cosmetic and pharmaceutical products. Here we report a new high-performance liquid chromatographic (HPLC) method for convenient and direct determination of allantoin derivatives in commercial products by a novel post-column reaction technique. The selective colour reaction of allantoin derivatives is combined with an efficient separation of the compounds on a strong cation exchanger.

EXPERIMENTAL

Apparatus

A schematic diagram of the liquid chromatograph is shown in Fig. 1. Sample solutions were injected via a variable sample injector. An Hitachi 635 pump was used to force eluent through the analytical column at a flow-rate of 1.0 ml/min. Acid-

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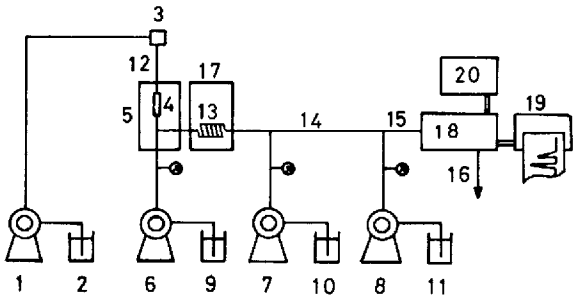


Fig. 1. Schematic diagram of the proposed method. 1 = Pump; 2 = eluent; 3 = sample injector; 4 = analytical column; 5 = water-bath; 6–8 = acid-resistant pumps; 9 = hypochlorite reagent; 10 = nitrite reagent; 11 = iodide reagent; 12 = PTFE tubing (0.25 mm I.D. × 0.5 m); 13 = PTFE reaction coil (0.5 mm I.D. × 5 m); 14,15 = PTFE tubing (0.5 m × 0.5 mm I.D.); 16 = PTFE suppressor tubing (5 m × 0.5 mm I.D.); 17 = water-bath; 18 = detector; 19 = recorder; 20 = data processor.

resistant pumps (Nihon Seimitsu NMP-2u, Tokyo, Japan) were used for post-column reagents at a flow-rate of 0.4 ml/min. The jacketed glass column (250 × 8 mm I.D.) was packed with TSK-Gel LS-211 (H^+, 12 μm, Toyo Soda, Japan) by a slurry packing procedure. The post-column reaction coils were made of PTFE tubing (0.5 mm I.D.). The effluent from the post-column reaction was monitored at 370 nm with UV detector (UVIDEC III) in combination with a multirange recorder and a data processor (Shimadzu Chromatopac ElA).

Reagents

Allantoin of analytical grade was obtained from Wako (Osaka, Japan). Chlorohydroxyaluminium allantoinate (supplier's specifications: allantoin–aluminium–chloride–nitrogen (40.0 ± 4:14.8 ± 1.5:9.5 ± 1.5:14.2 ± 5 w/w) was obtained from Kawaken (Japan).

The eluent was deionized water. The post-column reagents were as follows: hypochlorite reagent, 0.5 *M* phosphate buffer (pH 5.0) containing 1% of sodium hypochlorite; nitrite reagent, 0.5% sodium nitrite aqueous solution; iodide reagent, 0.5% potassium iodide aqueous solution.

Sample preparation

Stock solutions of allantoin and ALCA were prepared by dissolving 1.00 g of each compound in 80–100 ml of hot water and diluting to 200 ml in a volumetric flask. By further dilution, working standard solutions each containing 50 μg/ml were prepared.

The samples (tooth-pastes, creams, suppositories, shampoos, lotions, lipsticks, powders etc.) were extracted with hot water. In some cases such as soaps, samples were extracted with hot 5% *n*-propanol aqueous solution. The extracts were diluted in deionized water to give final allantoin concentrations in the range of 10–120 μg/ml. The supernatant solutions obtained by centrifugation were analysed.

RESULTS AND DISCUSSION

Chromatographic separation of allantoin and ALCA

Variation of the counter-ion attached to a resin-based cation exchanger alters

the chromatographic behaviour towards allantoin and low-molecular-weight urea derivatives[7]; a resin-based cation exchanger alters the chromatographic behaviour towards allantoin and low-molecular-weight urea derivatives[7]; a resin in the lithium form is superior for both allantoin and urea derivatives using water as the mobile phase, giving well-shaped peaks and good resolutions.

We intended to separate allantoin and ALCA by cation exchange of the aluminium ion of the latter. In this study, resin in the proton form was chosen for the following reasons: the column life is longer than that of the lithium form; amine compounds and urea derived from commercial products and/or the degradation of allantoin derivatives do not elute and the determination takes less time with no interferences.

In the chromatographic separation, ALCA can be determined as allantoin as described below. The effect of the column temperature is large: raising the column temperature reduced the capacity factor of allantoin and decreased peak tailing. A temperature of 70°C was therefore selected.

Post-column detection of allantoin derivatives

We find that allantoin derivatives can be detected by the following post-column reactions:

$$\text{Allantoin derivatives} + \text{NaOCl} \rightarrow \text{N-chloramine}$$
$$\text{NaOCl} + \text{NaNO}_2 \rightarrow \text{NaCl} + \text{NaNO}_3$$
$$\text{N-chloramine} + \text{I}^- \rightarrow \text{I}_3^-$$

Allantoin derivatives from the analytical column are converted into the corresponding N-chloramines with hypochlorite. The excess of hypochlorite is then selectively destroyed with nitrite. The N-chloramines finally react with iodide to form triiodide which is monitored at 370 nm.

The effects of the pH of the reaction system and the reaction temperature on the sensitivities of both allantoin and ALCA were studied as described previously[7].

Varying the pH of the hypochlorite reagent in the range pH 4.0–8.0, it was found that allantoin and ALCA together had two maximum sensitivities at pH 5.0 and 7.5 when the reaction temperature was fixed at 40°C. The sensitivity at pH 5.0 is higher than that at pH 7.5, and a pH of 5.0 was therefore selected.

On varying the reaction temperature, the sensitivity of both allantoin and ALCA decreased dramatically with increasing reaction temperature in the range 40–80°C when the pH of the hypochlorite reagent was fixed at 5.0. A reaction temperature of 40°C was selected.

Determination of allantoin and ALCA

With the proposed method, calibration curves for both allantoin and ALCA were linear between the peak areas and the concentrations in the range of 0.2–3 μg, passing through the origin. The detection limits were less than 0.1 μg.

Table I shows the formulae of allantoin and ALCA. The purity of commercially available ALCA is not as high as that of allantoin. The content of allantoin is important and it should be measured first. From the results of the elemental analyses shown in Table II, the calculated molar ratio of the elements agrees well with the theoretical one, except for aluminium. This shows that commercially available ALCA

TABLE I

THEORETICAL FORMULAE OF ALLANTOIN AND ALCA

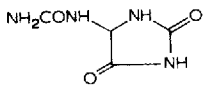
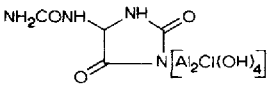
	Formula	*MW*
Allantoin	$C_4H_6N_4O_3$ 	158.12
ALCA	$C_4H_5N_4O_3 \cdot Al_2Cl(OH)_4$ 	314.57

TABLE II

ELEMENTAL ANALYSIS OF COMMERCIALLY AVAILABLE ALCA*

		C	*N*	*Al*	*Cl*
Observed (%, w/w)		12.0	14.3	14.8	9.5
Molar ratio,	Calculated**	4.00	4.09	2.19	1.07
	Theoretical***	4.00	4.00	2.00	1.00

* Obtained from Kawaken. It contains water as an impurity, and oxygen in ALCA is consumed as Al_2O_3 in the elemental analysis, so that the H and O contents were useless in the calculation of allantoin content.

** Calculated from the elemental analysis (standardized by the carbon atom).

*** Calculated from the empirical formula of ALCA (Table I).

TABLE III

ALLANTOIN CONTENT IN THE ALCA

Allantoin (%, w/w)	
*Calculated**	*Observed***
39.5	39.4

* Calculated from the equation obtained from the results of Tables I and II: $\frac{158.12}{12.0 \times 4} \times 0.12 \times 100\%$.

** Obtained from the proposed HPLC method using allantoin as the standard.

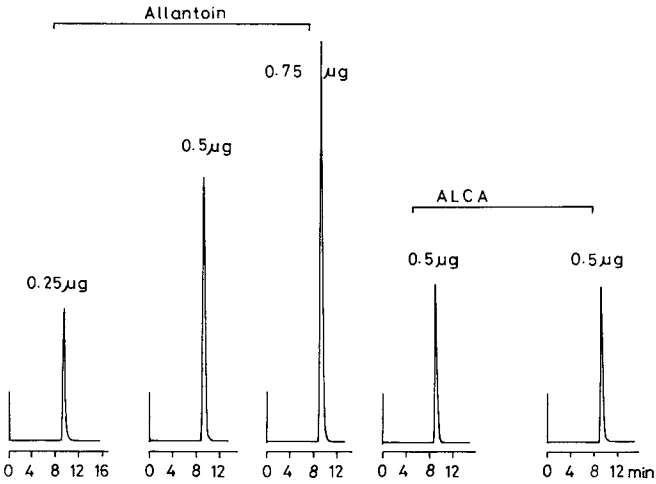


Fig. 2. Representative chromatograms obtained with the proposed method. Values on the peaks represent the amounts injected into the column.

contains some inorganic compounds as impurity, such as aluminium compounds. The results permit the calculation of the allantoin content from the content of the corresponding organic compounds in the ALCA. Then, using the weight per cent of carbon in the ALCA, the content of allantoin can be calculated as shown in Table III.

In the proposed method, the ALCA elutes as allantoin from an analytical column by cation exchange of the aluminium ion, and can be determined as allantoin. Table III shows that the HPLC results are in good agreement with the calculated ones. These data permit the calculation of the ALCA content, by multiplying the observed allantoin content by the factor calculated from Table III:

$$(\%)\text{ALCA commercially available} = (\%)\text{allantoin} \times 100/39.5$$

Representative chromatograms obtained with the proposed method are shown in Fig. 2.

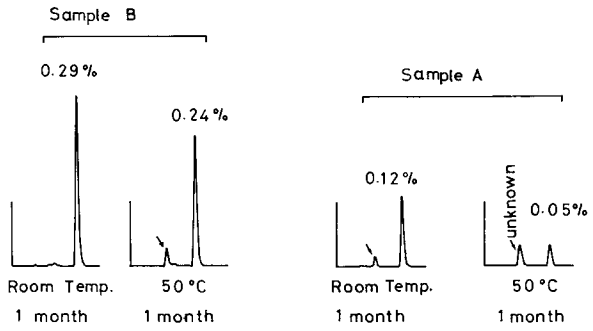


Fig. 3. Stability tests of ALCA in formulations. Sample A is a common synthetic preparation of any cosmetic product. Sample B is an improvement of the formulation on the preparation. Each sample was stored under normal (room temperature) and accelerated conditions (50°C) as indicated under the peaks.

TABLE IV

DETERMINATION OF ALLANTOIN AND ALCA IN SYNTHETIC PREPARATIONS

n = 4 in each case. C.V. = Coefficient of variation.

Sample	*Allantoin derivative*			*C.V.* (%)	*Recovery* (%)
		Added (%)	*Found* (%)		
1 Tooth-paste	ALCA	0.250	0.245	1.42	98.1
2 Tooth-paste	ALCA	0.250	0.249	1.26	99.6
3 Tooth-paste	Allantoin	0.150	0.147	1.54	98.0
4 Soap	Allantoin	0.100	0.098	0.98	98.0
5 Soap	Allantoin	0.100	0.100	1.04	100.0
6 Skin cream	Allantoin	0.100	0.099	1.74	99.0
7 Shampoo	ALCA	0.200	0.197	1.43	99.0
8 Ointment	Allantoin	0.300	0.295	1.76	98.2
9 Tincture	Allantoin	0.300	0.294	1.69	98.0

* Main base of formulations: 1, calcium carbonate as an abrasive; 2, calcium hydrogenphosphate as an abrasive; 3, aluminium hydroxide as an abrasive; 4, fatty acid sodium salts; 5, sodium lauryl sulphate; 6, sodium monolauryl phosphate.

Commercial products analysis

The precision and accuracy of the method were tested by adding known amounts of allantoin or ALCA to commercial products which do not contain allantoin derivatives. Each sample was analysed immediately after preparation, because of the instability of allantoin derivatives. Table IV shows that the precision and recovery were good.

Stability tests of allantoin derivatives

It is well known that the stability of both allantoin and ALCA are very sensitive to some synthetic formulations. In our method, the main factors affecting the stability have been screened out. Representative stability tests are shown in Fig. 3. Sample A is a common synthetic preparation of any cosmetic product. The initial ALCA content is 0.3%. Sample B shows the dramatic improvement in stability. In this way, the method can be effectively applied to improve the stability of allantoin derivatives in formulations.

REFERENCES

1 T. Kaito, K. Sagara, Y. Ito, K. Nakamura and T. Anmo, *Yakugaku Zasshi*, 97 (2) (1977) 165–170.
2 J. Lutomski and B. Jernas, *Pharmazie*, 31 (2) (1976) 131–132.
3 Z. Dudzik, *Farmacja Pol.*, 25 (4) (1969) 255–258.
4 S. A. Katz, R. Turse and S. B. Mecca, *J. Soc. Cosmet. Chem.*, 15 (6) (1964) 303–310.
5 D. J. Weber and J. W. Higgins, *J. Pharm. Sci.*, 59 (12) (1970) 1819–1821.
6 I. Bonadeo and G. Bottazzi, *Riv. Ital. Essenze Profumi*, 50 (2) (1968) 78–80.
7 J. Kawase, H. Ueno, A. Nakae and K. Tsuji, *J. Chromatogr.*, 252 (1982) 209–216.