Stability Assay of Allantoin in Lotions and Creams by High-Pressure Liquid Chromatography

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Abstract □ A high-pressure liquid chromatographic method for indicating stability is described for the rapid quantitative analysis of allantoin in lotions and creams. Allantoin was extracted from the preparations using distilled water containing 70% (v/v) methanol and separated from interferences by reversed-phase chromatography. The separation was carried out using an amino column (250 × 4.5-mm i.d.) and a mobile phase of distilled water containing 70% (v/v) acetonitrile. Quantitation was accomplished using a UV detector at 220 nm. The assay has a relative standard deviation of ~1.7% (n = 10) and the average recovery from laboratory prepared samples was 100%.

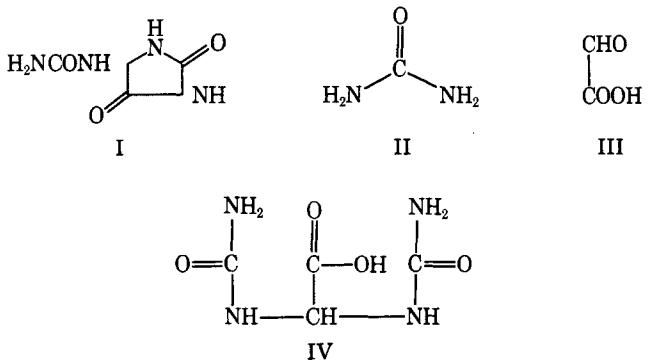
Keyphrases □ Allantoin—stability assay in lotions and creams by high-pressure liquid chromatography, degradation □ Stability—assay of allantoin in lotions and creams, high-pressure liquid chromatography, degradation □ Degradation—allantoin in lotions and creams, stability assay, high-pressure liquid chromatography

Allantoin and its derivatives have been used in various cosmetic preparations including skin creams, lotions, shampoos, lipsticks, and shaving preparations. A suitable analytical method is required for routine quality control as well as stability studies in the pharmaceutical/cosmetic industry. Allantoin (I) can be unstable in alkaline conditions and is known to hydrolyze to urea (II) and glyoxylic acid (III), perhaps *via* allantoic acid (IV):

Several methods (1–3) have been reported that are based on this hydrolysis route and subsequent colorimetric determination of III with phenylhydrazine. A fluorometric determination of sodium glyoxylate using phenylephrine hydrochloride described previously has been applied to the determination of allantoin in pharmaceutical preparations (4). However, for stability tracking, these procedures are deficient, because they measure urea or glyoxylic acid which are the products of normal allantoin degradation, rather than the residual intact allantoin.

Other techniques reported include titration (5, 6), classical chromatography (7), and a TLC separation with UV assay of allantoin at 220 nm (8, 9). In addition, a chromatographic procedure had been followed in these laboratories based on the separation of allantoin *via* TLC and determination by densitometry after chromophore formation.

All of these procedures are either time consuming or nonspecific. To overcome these problems, an assay procedure for allantoin in topical cream and lotion products by high-pressure reversed-phase liquid chromatography was investigated. The method described provides rapid, specific determination of intact allantoin with minimal sample preparation.



EXPERIMENTAL

Chemicals and Reagents—All reagents and chemicals were either HPLC or American Chemical Society grade and were used without further purification.

Apparatus—The liquid chromatograph[1] was fitted with a 50-μl septumless injector[2] and a variable wavelength UV detector[3] (set at 220 nm).

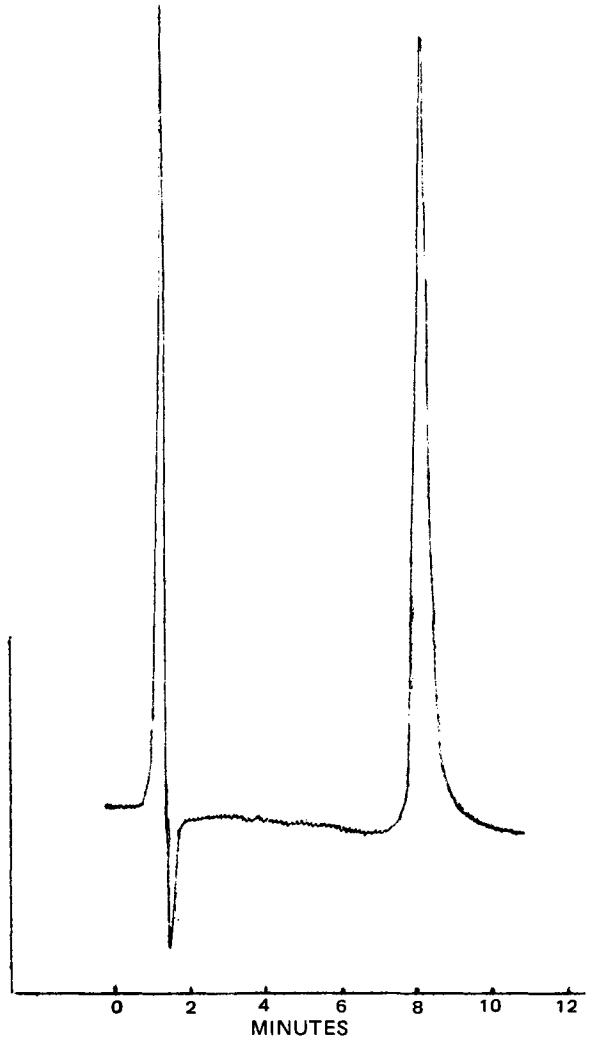


Figure 1—*HPLC of allantoin standard.*

[1] SP8000 Liquid Chromatograph equipped with a Printer/Plotter, Spectra-Physics, Santa Clara, Calif.
[2] Auto Injector model 725, Micromeritics Instruments Corp., Norcross, Ga.
[3] Spectroflow Monitor SF 770, Schoeffel Instruments Corp., Westwood, N.J.

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Table I—Recovery Data for Spiked Samples

	Topical Lotion	Amount Added, mg	Amount Found, mg	Amount Recovered, %
	1	23.0	22.32	97.05
	2	26.0	26.35	101.35
	3	39.1	39.3	100.50
	4	45.0	44.82	99.6
	5	51.0	50.98	99.96
Average				99.69
RSD, %				1.62
	Topical Cream			
	1	26.2	26.25	100.2
	2	33.5	33.3	99.3
	3	37.5	38.2	101.8
	4	42.4	41.8	98.6
	5	46.4	46.4	100.0
Average				100.0
RSD, %				1.20

Column—A 250 mm × 4.5-mm i.d. column containing 5 μm amino (NH_2) packing[4] was used.

Chromatographic Conditions—The chromatographic solvent was 70% (v/v) acetonitrile in distilled water. The solvent was vacuum filtered through a 0.50-μm (47 mm) filter[5] and vacuum degassed for 2 min with stirring before use. The temperature was ambient, the solvent flow rate was 2.5 ml/min, and the inlet pressure was ~2000 psi. Detector sensitivity was 0.04 aufs and the chart speed was 5.08 mm/min.

Standard Solutions—Standard solutions containing 30, 40, and 50 mg of allantoin/100 ml were prepared in distilled water containing 70% (v/v) methanol. Sonication was used to complete dissolution and the solutions were filtered using a 0.5-μm syringe filter[5] (25 mm) prior to injection.

Table II—Assay Results for Topical Cream Containing 2.0% Allantoin

Topical Cream	Allantoin, %	Percent of Label
1	2.03	101.5
2	1.95	97.5
3	1.98	99.0
4	1.98	99.0
5	2.01	100.5
6	2.05	102.5
7	2.05	102.5
8	2.03	101.5
9	2.02	101.0
10	1.98	99.0
Average	2.01	100.4
RSD, %	1.7	

Assay for Commercial Products—Samples of creams and lotions were transferred from their commercial container to glass jars and mixed thoroughly. An accurately weighed 2.0-g sample was transferred to a 150-ml beaker, 30 ml of distilled water added, and the contents stirred for ~20 min. The contents of the beaker were then quantitatively

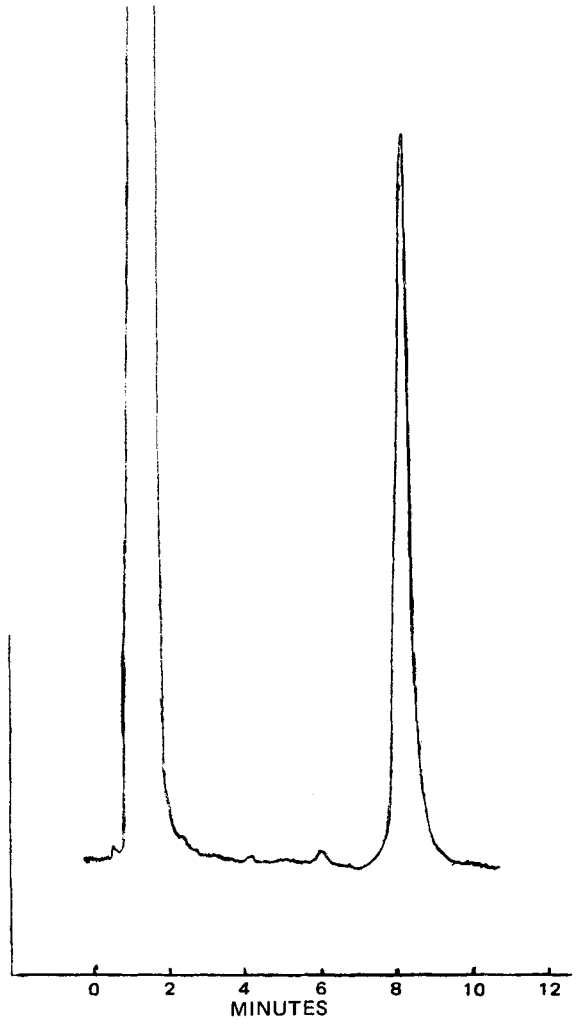

Figure 2—*HPLC of commercial cream sample.*

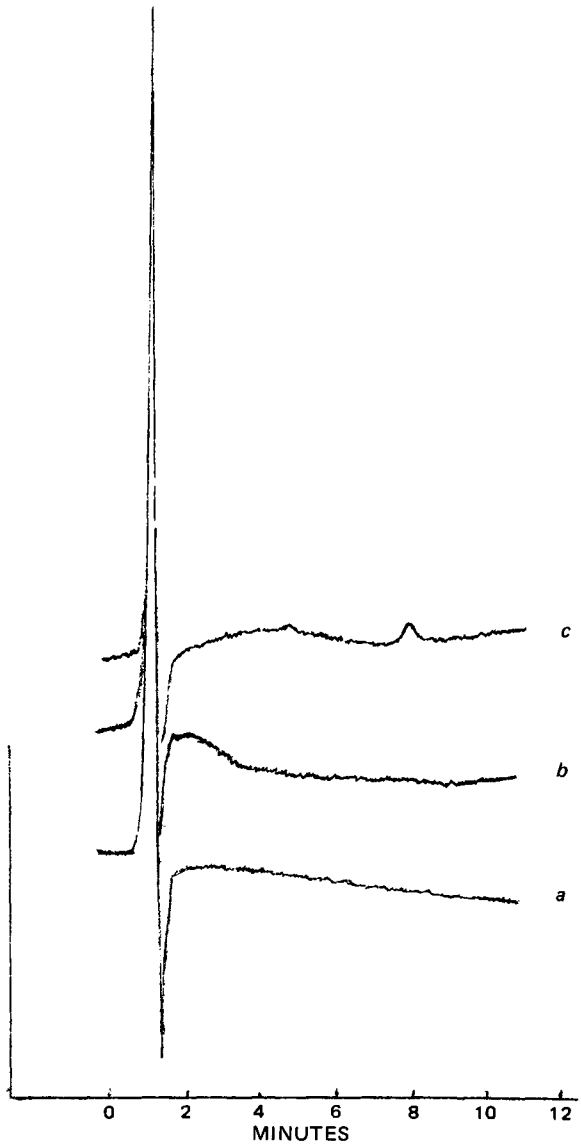

Figure 3—*HPLC of pure materials (a) blank, (b) urea, (c) allantoic acid.*

[4] Amino (NH_2) column, IBM Instruments Inc., Danberry, Conn.

[5] Type FH 0.5 μm, Fluoropore (PTFE), Millipore Corp., Bedford, Mass.

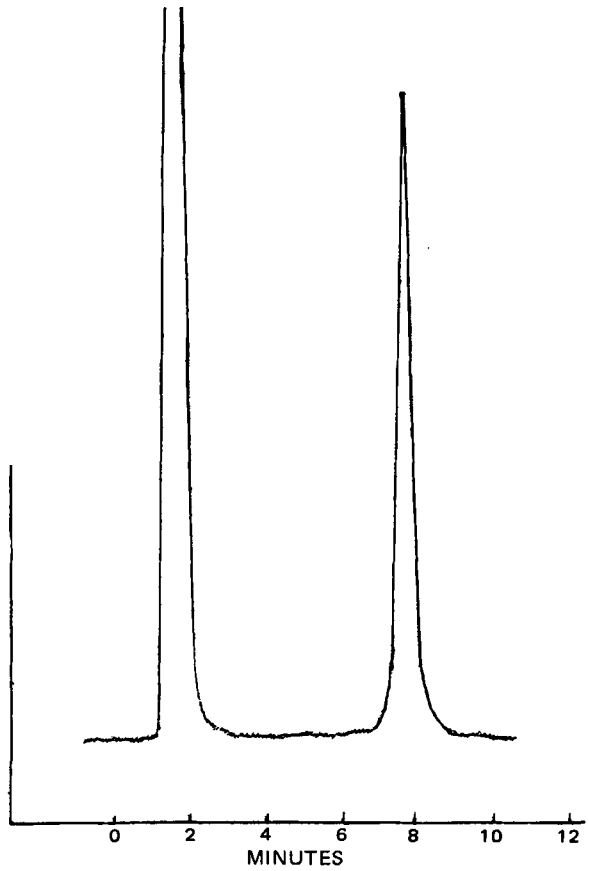

Figure 4—*HPLC of commercial lotion sample.*

transferred to a 100-ml volumetric flask, diluted to volume with methanol, mixed well, and filtered using a 0.5-μm syringe filter[5] (25 mm) prior to injection.

Spiked Samples—Accurately weighed quantities of allantoin were admixed with placebo portions of the topical creams and lotions. These samples were formulated to contain 1–2.5% (w/w) allantoin, and were assayed as described previously for commercial products.

Quantitation—Since peak heights of allantoin were directly proportional to concentration, all results were calculated by interpolation from a standard curve.

RESULTS AND DISCUSSION

Although satisfactory separations were achieved for samples of lotions and creams using distilled water containing 70% acetonitrile, for economic reasons, attempts were made to substitute methanol. However, no acceptable separations were obtained with various proportions of methanol water, therefore, acetonitrile was used for all further work.

The recovery data for allantoin from spiked samples of creams and lotions, presented in Table I, illustrate the validity of the method for these formulations. Average recoveries for cream and lotion were 100 and 99.7%, respectively. Linearity between peak height and concentration was excellent over the range of 0.3–0.5 mg of allantoin/ml with a correlation coefficient of 0.999. This method has been used routinely in a stability program and found to be accurate and precise with a *RSD* of 1.7% (n = 10). These data are summarized in Table II, and chromatograms obtained for allantoin standard and commercial cream sample are presented in Figs. 1 and 2, respectively. Since no sample clean up is required, assay time was ~15 min.

As noted earlier, allantoin is reported to degrade to urea and glyoxylic acid in alkaline conditions, perhaps through allantoic acid. To demonstrate the selectivity of the method for intact allantoin, standard and sample preparations containing allantoin were artificially degraded with alkali and analyzed according to the proposed conditions. No significant peaks were observed due to the degradation products at the retention time of allantoin peak, which was reduced in height.

To further demonstrate the stability-indicating nature of the method, several additional experiments were completed. Commercially available

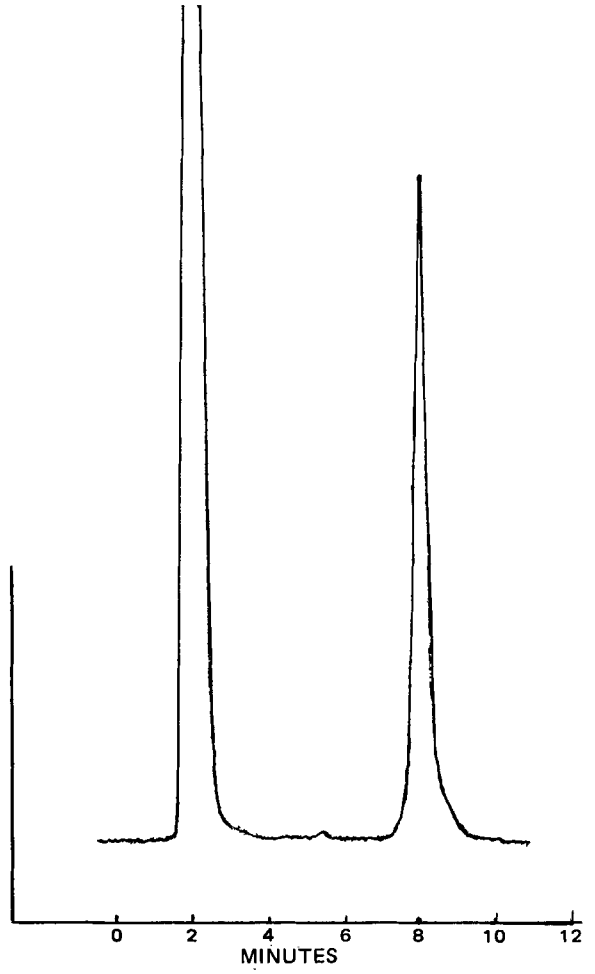

Figure 5—*HPLC of commercial lotion sample spiked with pure allantoic acid.*

urea and glyoxylic acid were chromatographed, and no peak was observed at the retention time of allantoin (Fig. 3). This was further confirmed by introducing 40–50 mg of these compounds to standard and sample preparations containing allantoin. In addition, commercially available allantoic acid was chromatographed as described (Fig. 3). It eluted at the same time as allantoin; however, when 0.4 mg/ml of allantoic acid (corresponding to 100% conversion from allantoin) was introduced to a standard allantoin solution (0.4 mg/ml) and chromatographed, an increase in allantoin peak height of <2% resulted.

A commercial lotion sample containing 40 mg of allantoin was analyzed in duplicate (Fig. 4). The samples were then spiked with 35–40 mg of allantoic acid and reanalyzed for allantoin (Fig. 5). Evaluation of these results indicated that within experimental error allantoic acid (if present) does not interfere in the analysis of allantoin.

It is concluded that the proposed method is selective for intact allantoin in the presence of probable degradation products.

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