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ANALYSIS OF ANTHRALIN IN DERMATOLOGICAL PRODUCTS BY REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

A reversed-phase liquid chromatographic method is described for the determination of anthralin in topical products. The factors affecting the degradation of anthralin solid and solutions are discussed. Ultraviolet spectra of degradation products are presented. The assay method has been thoroughly optimized and is shown to be specific, precise, and accurate.

INTRODUCTION

Anthralin is a very effective agent against psoriasis. Commercial anthralin products for topical usage are available in different strengths. Until recently, the assay method for analyzing these products for anthralin was spectrophotometry[1]. The interference by a known degradation product, danthron, was corrected for in this method by the use of absorbance measurements at two wavelengths and the use of known absorptivities for the two compounds at these wavelengths. This method assumes that danthron is the only degradation product which interferes in this assay.

The anthralin degradation has long been known, mostly observed as the darkening of anthralin raw material and products on exposure to air, light, or heat. By using thin-layer chromatography (TLC) followed by spectrophotometry, Elsabbagh *et al.* were able to separate, identify, and quantitate the two degradation products of anthralin: danthron and dianthrone[2]. Dianthrone was also shown to have absorbance maximum (368 nm) close to that of anthralin (356 nm), hence the spectrophotometric method[1] is only partially stability-indicating. Two other methods using TLC for separation prior to colorimetric or fluorometric determination of anthralin and degradation products were reported but no analytical data was presented[3,4].

Caron and Shroot reported the first high-performance liquid chromatography (HPLC) method for the assay of anthralin, danthron (quinone), and dianthrone (dimer)[5]. Both normal-phase (silica) and reversed-phase (C_{18}) HPLC methods were reported. Although these methods have been demonstrated to be superior to the spectrophotometric method, the accuracy of the methods was not fully established

with "spiked" samples. Also, the sample preparation includes an evaporation step, requiring long analysis times.

In this paper, we report a simple and accurate HPLC method for the determination of anthralin and its degradation products in topical products.

EXPERIMENTAL

Apparatus

A Waters Assoc. (Milford, MA, U.S.A.) Model 204 liquid chromatograph equipped with a Model 6000A low-volume deplacement pump, a Model U6K universal injector, a Model 440 absorbance detector (365 nm), and a μBondapak C_{18} column (30 cm × 3.9 mm I.D.) was used. The chromatograms were recorded using a Houston Instruments (Austin, TX, U.S.A.) Model OmiScribe B5117-1 strip chart recorder. The absorption spectra of the component chromatographic peaks were recorded using Model 1040A photodiode array HPLC detector (Hewlett-Packard, Palo Alto, CA, U.S.A.) coupled with an HP-85 computer and 7470A graphics plotter. An intelligent sample processor, Waters Model WISP 710A, was used in making multiple sample injections.

Unless otherwise mentioned, the eluent was acetonitrile–water–acetic acid (60:39.5:0.5, v/v/v) containing 0.05% (w/v) sodium hexanesulfonate. The flow-rate was 2.5 ml/min.

A Hewlett-Packard Model 3352D laboratory data system was used to determine the peak areas.

Reagents and chemicals

USP-grade anthralin was obtained from City Chemicals (New York, NY, U.S.A.). Danthron was purchased from Pfaltz and Bauer, (Stamford, CO, U.S.A.), and anthracene from Aldrich (Milwaukee, WI, U.S.A.). HPLC grade acetonitrile and sodium hexanesulfonate and also ACS-grade chloroform were obtained from Fisher Scientific (Rochester, NY, U.S.A.). The water from a Milli-Q water purification system (Millipore, Bedford, MA, U.S.A.) was used in preparing the HPLC eluent. Dianthrone was provided by our Medicinal Chemistry Department.

Unless otherwise stated, all solvents and eluent were degassed with argon before use. The head-space over all solutions was flushed with argon. Exposure of anthralin solutions to UV light was minimized by the use of low-actinic glassware.

The product and placebo used in method validation were experimental formulations provided by our Product Development Department. Applicability of the method was tested on several commercial products.

Standard preparation

A stock solution of anthralin standard (250 μg/ml) was prepared in chloroform. A 10-ml aliquot of this solution was mixed with 10 ml of anthracene solution (1 mg/ml in acetonitrile), 30 ml of acetonitrile and 0.25 ml of acetic acid.

Sample preparation

Separate procedures were used for hydrous products (creams, gels, lotions) and anhydrous products (sticks, ointments).

Hydrous products. The sample was dispersed and diluted in acetonitrile to an anthralin concentration of *ca.* 0.25 mg/ml. A 10-ml aliquot of this solution was mixed with 10 ml of anthracene solution, 10 ml of chloroform, 20 ml of acetonitrile, and 0.25 ml of acetic acid.

Anhydrous products. The sample was dispersed and diluted in chloroform to an anthralin concentration of *ca.* 0.25 mg/ml. A 10-ml aliquot of this solution was mixed with 10 ml of anthracene solution, 30 ml of acetonitrile, and 0.25 ml of acetic acid.

All samples were filtered through a Whatman No. 42 filter prior to HPLC analysis.

RESULTS AND DISCUSSION

Chromatography

Preliminary chromatographic studies of an anthralin–danthron mixture using octadecylsilane (C_{18}) and phenyl columns revealed that the two compounds are chromatographically very similar and that complete resolution of the two is difficult to achieve with columns normally used in analysis (less than 5000 theoretical plates). However, by proper choice of eluent to achieve the best separation and by choosing a detector wavelength (365 nm) more sensitive to anthralin than danthron, it appeared possible to develop an accurate assay method for anthralin. The best resolution between anthralin and danthron was obtained using an acetonitrile–water–acetic acid (60:39.5:0.5) eluent with 0.05% sodium hexanesulfonate (Fig. 1). Anthracene, which is not a degradation product of anthralin, was selected as the internal standard because of its good peak shape and resolution from anthralin and its degradation products.

The solvent used for sample preparation was found to be very important since it affects the peak shape. The effect of chloroform on the peak shape is demonstrated

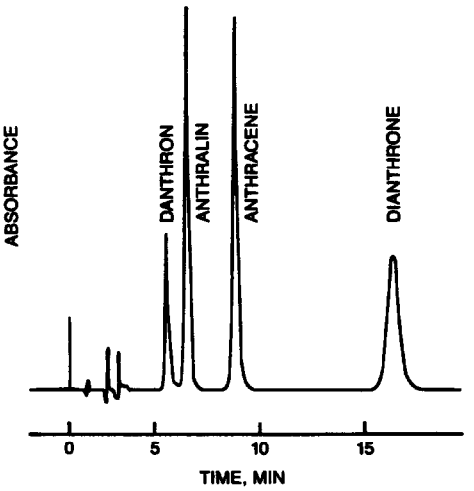

Fig. 1. Chromatographic separation of a mixture of danthron, anthralin, dianthrone, and anthracene (internal standard). Column, μBondapak C_{18}; eluent, acetonitrile–water–acetic acid (60:39.5:0.5) with 0.05% sodium hexanesulfonate; flow-rate, 2.5 ml/min; detector, 365 nm; injection volume, 10 μl.

TABLE I

EFFECT OF CHLOROFORM ON ANTHRALIN PEAK

Concentration of anthralin, 49.6 μg/ml; HPLC injection volume, 10 μl.

Solvent composition (%, v/v)*		*Anthralin (normalized response)*	
Acetonitrile	*Chloroform*	*Peak height*	*Peak area*
100	0	100.0	100.0
75	25	93.2	100.9
50	50	69.0	101.3

* All solutions contained 0.25% acetic acid.

by the data in Table I. Only a slight increase in the peak area, 1.3%, was observed with an increase in chloroform content from 0 to 50%. However, a marked decrease, 31% was observed in the peak height. This trend is not linear with the increase in chloroform content; a relatively smaller effect was observed at a lower concentration of chloroform. In the assay method reported here, we have kept the chloroform concentration the same in both sample and standard solutions prepared for HPLC analysis. The chloroform concentration in these solutions was selected in such a way to permit preparation of stock standard solutions in chloroform and also permit analysis of both hydrous and anhydrous products using the same standard solution.

Degradation of anthralin

The degradation of anthralin in solutions and in products is well known. It is

TABLE II

EFFECT OF SOLVENT ON THE STABILITY OF ANTHRALIN

All solutions protected from light and stored at ambient temperature (25 ± 3°C). Anthralin concentration, 50 μg/ml.

Solvent modifications	*Storage time (h)*	*Anthralin concentration (% of initial)*			
		Deaerated methanol	*Deaerated acetonitrile*	*Non-deaerated methanol*	*Non-deaerated acetonitrile*
Pure solvent	1	88.4	100.5		
	4	81.9	100.4		
	8	68.8	101.2		
Solvent with 0.25% acetic acid	1	99.7	99.8	100.5	
	4	100.9	100.3	100.3	
	8	100.0	99.7	98.6	
	24	100.4	100.1	99.0	
Solvent with 20% chloroform and 0.25% acetic acid	1	99.7	99.8	100.5	100.3
	4	100.9	100.6	100.3	100.2
	8	100.0	100.2	98.6	99.2
	24	100.4	98.4	99.0	93.1

generally observed as a darkening in color and also as changes in UV spectra. The rate of degradation is enhanced by UV light, heat, and oxygen[6]. The solvent and also the pH were reported to have a significant effect on the degradation[6–8]. Accurate determination of anthralin is thus possible only if we minimize the degradation during analysis by proper choice of solvent for sample preparation and eluent for HPLC analysis.

We studied the degradation of anthralin (0.5 mg/ml) in chloroform, methanol, and acetonitrile under controlled conditions and the results are summarized in Table II. Anthralin is most stable in chloroform, with or without dearation, and light has no noticeable effect. Significantly more degradation was observed in methanol than in acetonitrile. These results are in agreement with earlier reports[6–8]. Regular white, fluorescent light in the laboratory significantly increasded the degradation in non-deaerated acetonitrile. No noticeable degradation was observed for at least 24 h, both in methanol and acetonitrile containing 0.25% acetic acid. Similar stability was observed in methanol with 10^{-4} *M* HCl. In the eluent, which also contained 0.25% acetic acid, anthralin was found to be stable for at least 24 h. To eliminate essentially all degradation during sample analysis, all the solvents used were deaerated with argon, and all anthralin solutions were protected from light by low-actinic glassware.

Solid anthralin showed 2.5% degradation when exposed to white fluorescent light in a light cabinet (*ca.* 1000 foot candles) for 2 weeks. Heating the solid at 60°C for 2 weeks led to 3% degradation. To minimize degradation, anthralin standard was stored in a refrigerator and protected from light and air (head-space flushed with argon).

It is also preferable to store small amounts in small containers rather than to have a large amount in a single container. Otherwise, frequent handling of anthralin will expose it to light, air, and moisture, leading to faster degradation.

UV spectra of degradation products

The efficiency of HPLC for the separation of the anthralin degradation prod-

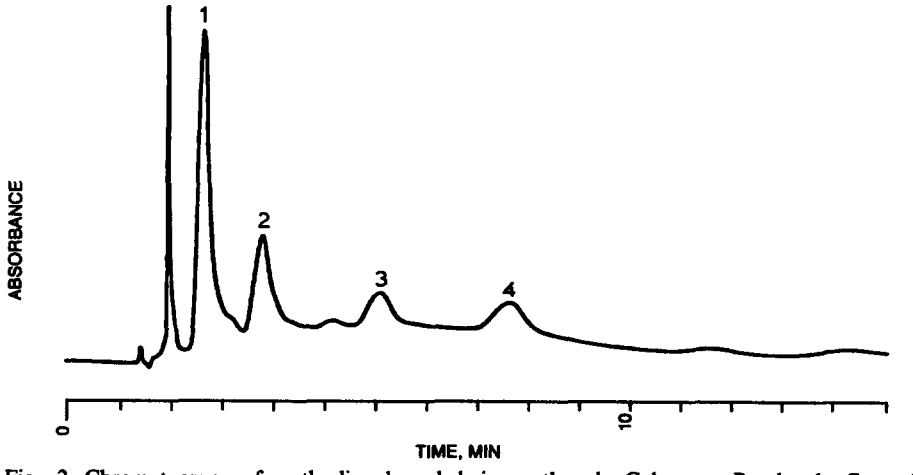

Fig. 2. Chromatogram of anthralin degraded in methanol. Column, μBondapak C_{18}; eluent, acetonitrile–water–acetic acid (75:24.5:0.5) with 0.05% sodium hexanesulfonate; flow-rate, 2 ml/min; detector, 254 nm. Peaks: 1 = danthron; 2 = dianthrone; 3 and 4 = unknowns.

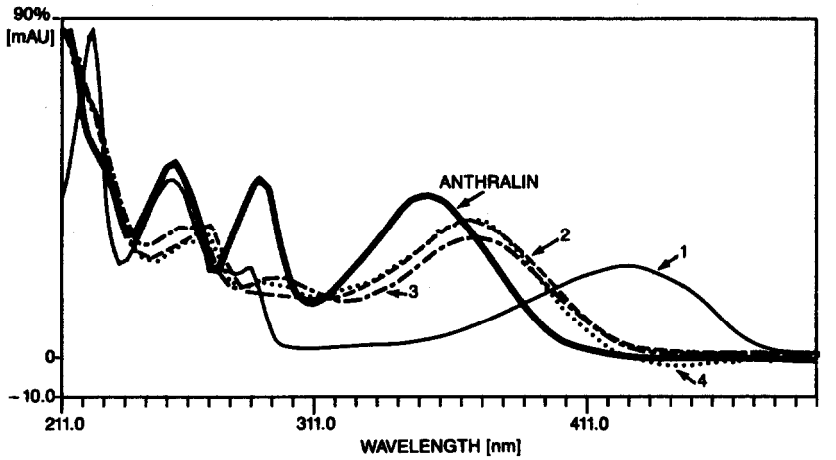

Fig. 3. Absorption spectra of degradation products of anthralin corresponding to the peaks in Fig. 2. Spectra 1, 2, 3, and 4 correspond to Peaks 1, 2, 3, and 4 of Fig. 2, respectively. The spectrum for anthralin, is included for comparison.

ucts and the ability of the photodiode array spectrophotometric HPLC detectors to record the full spectra simultaneously with the chromatogram permit us to obtain the spectra of the purest degradation products. Fig. 2 shows the chromatogram of the solid residue obtained by degrading anthralin in methanol at 35°C for 6 months. The residue was washed with methanol prior to dissolution in chloroform and HPLC analysis. Four major peaks and three minor peaks (other than anthralin) were observed. (The peak at 2 min is due to chloroform, the sample solvent.) Two major peaks were identified to be due to danthron and dianthrone by their retention times and UV spectra (Fig. 3). The two unidentified peaks have spectra similar to that of dianthrone. Because of this similarity in the UV spectra of the degradation products, interpretation of anthralin degradation solely on asbsorbance data[8] could be misleading. The UV spectra of anthralin and its degradation products in Fig. 3 are probably the best UV spectra attainable since the separation and spectra acquisition were done simultaneously. Since the separation and the recording of spectra in all other techniques are sequential operations, the observed spectra may not be due to a truly single component.

Method validation

Linearity of detector response for anthralin was studied by using standard solutions containing different concentrations of anthralin while keeping the anthracene (internal standard) concentration constant. The results are summarized in Table III. The data show that the response is linear over the concentration range studied. Both peak height and peak area data gave a correlation coefficient of 0.999 and a y-intercept equivalent to less than 1% of the normal response, corresponding to 0.5 μg of anthralin.

Analysis of the control blanks for anthralin gel and anthralin stick formulations by the proposed HPLC method showed that the placebo interference is negligible (less than 0.1% contribution to the response of formulations with 1% anthralin).

TABLE III

LINEARITY OF DETECTOR

Amount injected (μg)		*Response ratio, anthralin/anthracene*	
Anthralin	*Anthracene*	*Peak area*	*Peak height*
0.388	4.04	0.281	0.346
0.766	4.04	0.566	0.697
0.970	4.04	0.703	0.878
1.164	4.04	0.850	1.038
1.549	4.04	1.135	–
	Correlation coefficient	0.999	0.999
	Slope	0.74	0.90
	y-intercept/*y*-normal	0.8	0.02

TABLE IV

ACCURACY DATA

Spiked sample		*Absolute recovery (%)*	
Control blank (mg)	*Anthralin (mg)*	*Peak area*	*Peak height*
350	4.95	101.0	101.4
346	4.95	101.8	101.8
353	4.95	101.8	101.8
498	4.95	100.6	101.2
512	4.95	100.0	101.4
493	4.95	102.0	102.0
610	4.95	101.8	101.6
605	4.95	101.6	101.4
657	4.95	101.8	101.8
	Mean	101.4	101.6

TABLE V

PRECISION DATA

Anthralin gel (mg)	*Anthralin assay (%)*	
	Peak area	*Peak height*
345.1	1.116	1.123
355.2	1.142	1.105
341.8	1.094	1.095
492.8	1.112	1.102
501.5	1.098	1.086
494.4	1.127	1.104
598.9	1.139	1.107
591.8	1.073	1.073
612.0	1.122	1.080
Mean	1.114	1.097
R.S.D. (%)	2.0	1.4

Nine control blank gel formulations spiked with anthralin standard solution were prepared and each sample as a whole was analyzed. The results are shown in Table IV. The average absolute recovery observed was 101.4% using the peak area data and 101.6% using the peak height data. The data in Table IV also shows that the amount of control blank in the sample has no noticeable effect on the assay results.

Nine analyses of anthralin gel formulation gave results with a relative standard deviation (R.S.D.) of 2.1% using peak area and 1.4% using peak height data (Table V). Variation in sample size used for analysis showed no significant effect on the assay results.

Application of the method to an anthralin stick formulation and also to marketed anthralin products showed that the method is suitable with no noticeable interference in analysis. Although both peak areas and peak heights can be used for quantitation, peak height data generally appear to be more accurate and precise.

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