High-Pressure Liquid Chromatographic Determination of Anthralin in Ointments

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Abstract □ Anthralin concentration was determined in different ointments by high-pressure liquid chromatography, and results were compared with those of the USP assay technique. Significant differences existed between the claimed and the actual levels of anthralin in some preparations. Varying quantities of anthralin breakdown products were found, and their concentrations appear to be related to the nature of the formulation.

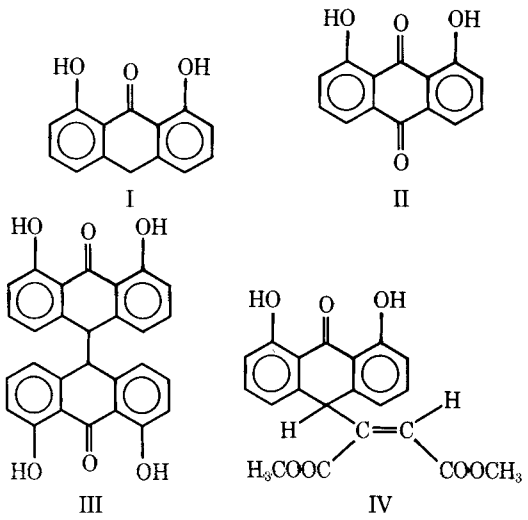
Keyphrases □ Anthralin—high-pressure liquid chromatographic determination in ointments □ High-pressure liquid chromatography—determination of anthralin in ointments □ Ointments—high-pressure liquid chromatographic determination of anthralin

Anthralin, applied percutaneously in various formulations, has been used in the treatment of psoriasis for over 100 years (1). This reactive chemical entity can undergo predictable chemical changes in the vehicle, depending on the presence of basic ingredients and/or oxygen. The end-products are claimed to be inactive in the chemotherapy of psoriasis (2). Ponec-Waelsch and Hulsebosch (3) postulated that anthralin and zinc hydroxide interact in zinc oxide pastes (Lassars' paste). These workers, however, used UV spectrophotometry for the assay and, as discussed recently (4), quantification of the breakdown products can only be estimated following prior separation of the compounds by TLC.

High-pressure liquid chromatography (HPLC), a selective, sensitive, reproducible, and rapid assay procedure, seemed a better choice for this assay and is compared in the present study to the standard USP method (5).

EXPERIMENTAL

Materials—Acetic acid[1] was analytical reagent grade; isooctane, isopropyl ether, methanol, chloroform, and *n*-hexane were HPLC grade[2] and were used as received after filtration and degassing.



[1] Prolabo Normapur.
[2] Merck.

1,8-Dihydroxy-9-anthrone (anthralin, I) was purified by column chromatography. Its chemical derivatives, 1,8-dihydroxy-9,10-anthraquinone (quinone, II) and 1,8,1′,8′-tetrahydroxy-10,10′-dianthrone (dimer, III) (6), and the internal standard, 1,8-dihydroxy-9-anthron-10-yl maleic acid dimethyl ester (IV) (7), were synthesized[3] following published procedures.

Instrumentation—Two high-pressure liquid chromatographs were used. One was equipped with a septumless injector[4], a reciprocating pump[5], a normal-phase 25-cm column[6], a UV-visible detector[7] set at 254 nm, and a strip-chart recorder[8]. The other chromatograph[9] was equipped with a reversed-phase 25-cm column[10] also set at 254 nm (referenced at 500 nm). The mobile phases compositions and conditions used are listed in Table I. Under these conditions, the response of each compound was linear in the working range (1–50 μg/ml).

Products Investigated—Commercially available samples were used, except in one case where the formulation was obtained directly from the

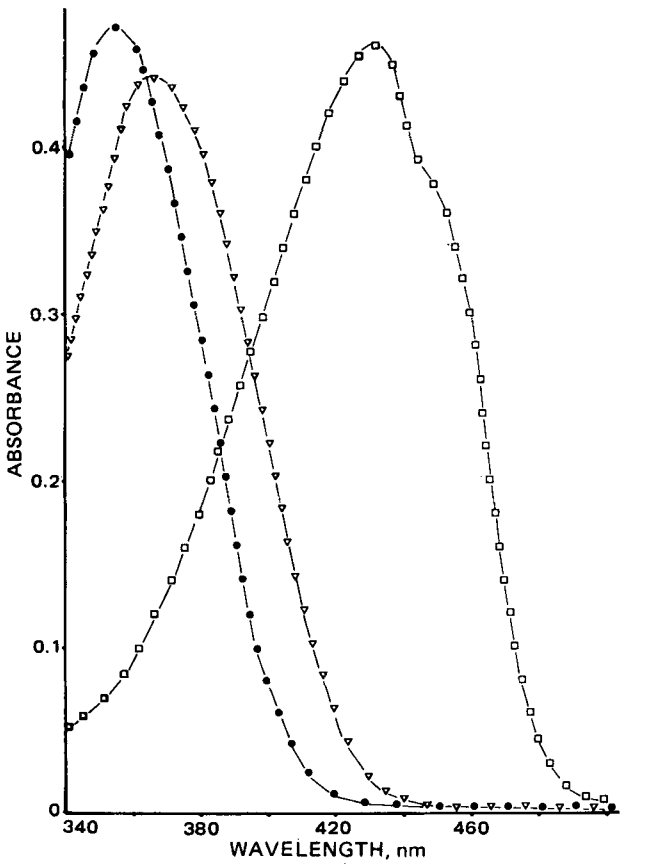


Figure 1—*Visible spectra at 10 μg/ml in chloroform of anthralin (●), the dimer (▽), and the quinone (□). Molar absorptivities (ε) at 345 and 432 nm are, respectively, 10,650 and 100 for anthralin, 16,970 and 220 for the dimer, and 1680 and 10,890 for the quinone.*

[3] Laboratoirès de Recherches Fondamentales, L'Oréal, Aulnay-sous-Bois, France.
[4] Waters Associates model U6K.
[5] Waters Associates model 6000A.
[6] Merck Lichrosorb RT 250-4-5 μm-Si60.
[7] Waters Associates model 440.
[8] Omniscribe.
[9] Hewlett-Packard model 1084 B.
[10] Merck Lichrosorb RT 250-4-5 μm-RP18.

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Table I—Analytical Conditions and Chromatographic Properties Observed in HPLC Separation of Anthralin, the Quinone, and the Dimer

Packing	Analytical Conditions	Compound	Retention Time, min
Normal phase (SiO_2)	Mobile phase (percent volume); isooctane (44.5), isopropyl ether (49.9), methanol (5.4), acetic acid (0.2); flow: 1 ml/min; pressure: 81 bars; temperature: 30°	Anthralin	3.70
		Quinone	3.35
		Dimer	4.52
		Internal standard	5.25
Reversed phase (C_{18})	Mobile phase (percent volume): water (24.9), methanol (74.9), acetic acid (0.2); flow: 2 ml/min; pressure: 254 bars; temperature: 60°	Anthralin	4.90
		Quinone	4.52
		Dimer	8.42
		Internal standard	3.68

pharmacy of a local hospital. The ointment bases and theoretical concentrations of anthralin are given in Table II.

Analytical Procedure—Ointment theoretically corresponding to ~200 μg of anthralin was weighed and spiked with exactly 200 μg of internal standard in the appropriate solvent (chloroform or *n*-hexane) from a freshly prepared (1 mg/ml) stock solution.

The extraction was performed by shaking the mixture for 15 min on a vortex mixer in the presence of 5 ml of chloroform or *n*-hexane and filtering the resulting solution through a solvent-resistant filter[11]. An aliquot (1 ml) of this solution was then evaporated to dryness under nitrogen at room temperature, and the residue was redissolved in the same amount of the elution solvent and subjected to HPLC analysis.

RESULTS AND DISCUSSION

The USP describes an assay procedure for the quantification of anthralin as the starting material for the preparation of ointments or in the ointment itself. This method is based on the absorbances at 354 and 432 nm but suffers from overlapping and interference between the absorption maxima of anthralin, dimer, and quinone (Fig. 1). In contrast, the described method allows the quantification of anthralin and its major oxidation products in the presence of each other (Fig. 2).

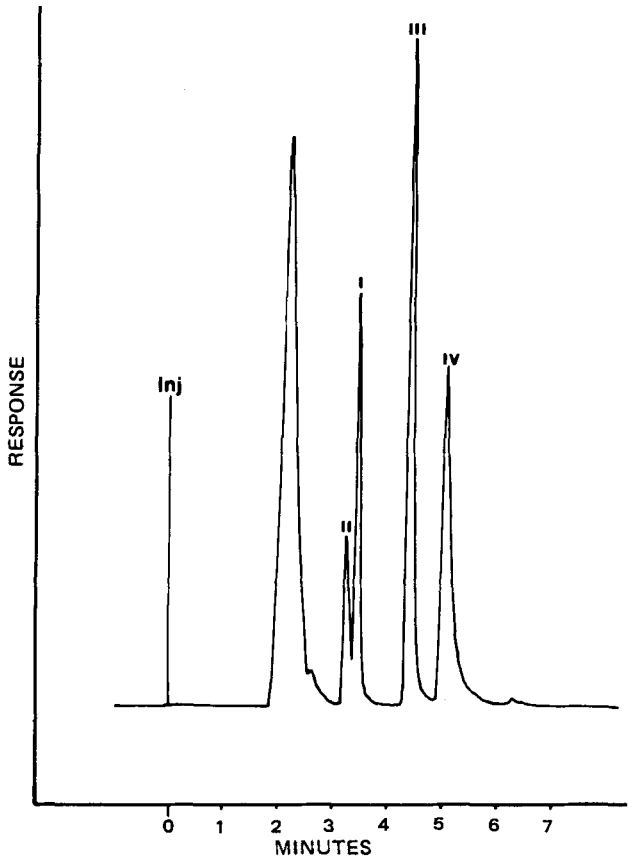

Figure 2—*Example of the chromatogram of a formulation extract (Sample 11) obtained on a silica gel phase.*

[11] Millipore FHLP 013, 0.5 μm.

Table III lists the results obtained by HPLC and USP techniques for the percentages of anthralin in the formulations studied.

Table IV lists the results obtained for anthralin, the quinone, and the dimer, with chloroform as the extraction solvent, on the two chromatographic systems; for solubility reasons, *n*-hexane is unsuitable as an extraction solvent for the dimer.

In nine out of the 12 formulations studied, 100 ± 5% of the material could be accounted for. Since the possible variation in the original anthralin used by the manufacturers could not be estimated, in cases where significantly less products was found, the extraction procedure was verified by adding increasing amounts of pure anthralin standard to the ointment before extraction.

The possibility of anthralin decomposition during extraction can be rejected since the standard deviation in the results obtained by the two extraction procedures was the same as the error in the analytical procedures itself. Decomposition during chromatography on either system can also be rejected since the normal and reversed-phase results are in good agreement.

Consequently, the difference between the theoretical and actual concentrations of anthralin in the ointment is related to the vehicle itself, in particular the zinc oxide pastes (Base D, Table III). Three out of the four such preparations (Samples 9, 11, and 12) contained <40% of the theoretical amount of anthralin; in two cases, a marked tendency towards the formation of the corresponding dimer (81 and 91%) was noted.

For Base C (Sample 10), low overall recovery was obtained with the highest amount of quinone. This low recovery can be explained by the

Table II—Composition of Vehicles and Theoretical Anthralin Concentration of Ointments

Cream Base	Anthralin Concentration, %	Code
Powder-in-cream urea	0.1	A
Oil-in-water cream	0.1	B
Anhydrous water washable	0.2	C
Zinc oxide–petrolatum–salicylic acid–lanolin–starch	0.2	D
Zinc oxide–petrolatum–salicylic acid–starch	0.22	D
Oil-in-water cream	0.25	B
Zinc oxide–petrolatum–salicylic acid starch	0.44	D
Salicylic acid–cetyl alcohol–sodium lauryl sulfate–paraffin–petrolatum	0.44	E
Oil-in-water cream	0.5	B

Table III—Percentage of Anthralin [a] Related to the Theoretical Amount Compared to USP Assay Results

Sample	Base Code	Anthralin, % HPLC	Anthralin, % USP
1	A	90.8 ± 2.5	113
2	B	90.1 ± 1.2	105
3	B	79.8 ± 2.5	94
4	B	71.5 ± 1.9	103
5	D	70.4 ± 2.4	85
6	E	70.2 ± 2.3	109
7	E	68.4 ± 1.8	112
8	B	66.0 ± 2.5	106
9	D	37.1 ± 2.1	53
10	C	26.9 ± 1.2	82
11	D	18.8 ± 1.3	79
12	D	3.3 ± 1.6	74

[a] Average of results obtained with chloroform and *n*-hexane extractions with the two chromatographic systems.

Table IV—Percentage of Recovery of Anthralin and Its Decomposition Products [a]

Sample	Anthralin, %	Dimer, %	Quinone, %	Total Recovery, %
1	88.5	7.2	1	96.7
2	92	9	2	103.0
3	80.9	15.5	2.2	98.6
4	72.6	25	1.3	98.9
5	71.2	4.7	1.1	77.0
6	70.5	25.1	1.8	97.4
7	69.0	22.4	1.7	93.1
8	67.4	29.5	1	97.9
9	39.0	10.4	1.2	50.6
10	29.8	2.5	12.9	45.2
11	17.3	81.1	5.9	104.3
12	4.4	90.9	5.1	100.4

[a] Chloroform extraction; average of data obtained using both chromatographic systems.

formation of high molecular weight materials, and an HPLC method is currently being developed for the analysis of these products.

The surprising disparity between the USP and HPLC assay results can be rationalized when the data in Table IV are considered. Although both methods rely on UV absorption, the USP technique cannot quantify the dimer and merely includes it with the anthralin level. However, a discrepancy between the results is still evident after correcting the USP data for the presence of dimer.

The majority of these ointments contained ~70% (range 44–92%) of the theoretical amount of anthralin, the remaining 30% being various quantities of the dimer, quinone, and, perhaps, high molecular weight materials.

The need to check anthralin and its preparations must be emphasized, and the danger of further decomposition of samples in use cannot be ignored.

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High-Performance Liquid Chromatographic Determination of Stereoselective Disposition of Carprofen in Humans

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Received January 26, 1981, from the **Department of Biochemistry and Drug Metabolism and the* ‡*Department of Pharmacokinetics and Biopharmaceutics, Hoffmann-La Roche Inc., Nutley, NJ 07110.* Accepted for publication March 25, 1981.

Abstract □ A high-performance liquid chromatographic (HPLC) assay was developed for the determination of the ratios of the (*S*)-(+) and (*R*)-(−) enantiomers of the anti-inflammatory drug carprofen in blood, urine, and feces. The procedure relies on: (*a*) extraction and purification of carprofen from biological fluids, (*b*) reaction of carprofen with (*S*)-(−)-α-methylbenzylamine to form the two diastereomeric (S)-(−)-α-methylbenzylamides *via* the 1,1′-carbonyldiimidazole intermediate, (*c*) purification of the reaction mixture by extraction of the diastereomeric derivatives into hexane at pH 11, and (*d*) analysis of the diastereomeric derivatives by HPLC with UV detection. The (*S*)-(+):(*R*)-(−) ratios in the blood of three subjects receiving single 100-mg oral doses of carprofen were greater than unity up to 16 hr after dosing. The mean ± *SD* of the ratios in the early blood samples (0.5, 1, and 2 hr) was 1.21 ± 0.09, while the mean of the ratios in the later blood samples (4, 6, 8, 12, and 16 hr) was slightly higher (1.48 ± 0.17). The blood level fall-off curves for the (*S*)-(+) and (*R*)-(−) enantiomers were similar in each of the three subjects for the 4–16-hr period. The carprofen enantiomers were excreted stereoselectively by humans. An excess of the (*S*)-(+) enantiomer relative to the (*R*)-(−) enantiomer was excreted in the urine as the ester glucuronide, while unchanged (*R*)-(−) enantiomer predominated in the feces. The total urinary plus fecal excretion of the enantiomers (0–96 hr) revealed only a slight excess of the (*S*)-(+) enantiomer over the (*R*)-(−) enantiomer, which amounted to 2.1–4.9% of the dose. Since the amount of carprofen (free and glucuronide) excreted in 96 hr by the three subjects only accounted for 62–72% of the dose, no definitive statement could be made relative to the possible inversion of the carprofen chiral center.

Keyphrases □ Carprofen—stereoselective disposition determination in humans using high-performance liquid chromatography □ Anti-inflammatory agents—carprofen, metabolism in humans, and rats, high-performance liquid chromatography □ High-performance liquid chromatography—determination of stereoselective disposition of carprofen in humans

Carprofen [(D,L)-6-chloro-α-methylcarbazole-2-acetic acid] is presently undergoing extensive clinical evaluation as a nonsteroidal anti-inflammatory agent. The possible stereoselective disposition of carprofen is of interest since it is a racemic compound with a chiral center at the α-carbon position. Other α-methylarylacetic acids have been shown to undergo stereoselective disposition and inversion at the α-carbon (1–5).

A TLC procedure was previously developed (6) for the quantitation of the enantiomers of [^{14}C]carprofen as their diastereomeric (*S*)-(−)-α-methylbenzylamides. This TLC procedure has been used to study stereoselective elimi-