Liquid Chromatographic Analysis of Dichlorophen and Its Major Impurity

KIRIT A. SHAH ˣ, SUSAN A. SAMUELS, MICHAEL K. MICHAELS, and JAMES W. WARREN

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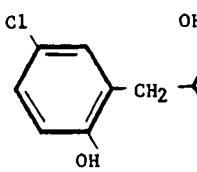
Abstract □ A rapid and simple high-performance liquid chromatographic (HPLC) method for the analysis of dichlorophen in raw material and in dichlorophen-toluene soft gelatin capsules for veterinary use was developed using a reverse-phase technique. This HPLC system was shown to isolate dichlorophen from its major impurity (the trimer). Three formulations were assayed and were found to contain 7.14, 7.90, and 8.4% of the trimer. A C-18 column was used with a mobile phase of methanol–water (75:25). The flow rate was 1.5 mL/min, and the effluent was monitored at 290 nm for both dichlorophen and the trimer. Dichlorophen and the trimer had retention times of 6.5 and 9.0 min, respectively.

Keyphrases □ Dichlorophen—HPLC, major impurity □ HPLC—analysis of dichlorophen and its major impurity

Dichlorophen (I), 2,2′-methylenebis(4-chlorophenol), is an anthelmintic which is used mainly for the removal of ascarids and hookworms and as an aid in the removal of tapeworms from dogs and cats. Dichlorophen is used in combination with toluene and is available in a soft gelatin capsule formulation for veterinary use. The USP XX (1) does not contain an official monograph for dichlorophen or dichlorophen capsule formulations; however, the British Pharmacopoeia (2) does for dichlorophen tablets. Dichlorophen is often contaminated with a trimer (II), 4-chloro-2,6-bis(5-chloro-2-hydroxybenzyl)-phenol, which is a result of the synthesis process. The trimer constitutes 5–10% by weight of dichlorophen.

The BP (2) method for dichlorophen assay involves a nonaqueous titration. Various analytical methods have appeared in the literature (3–7) for the determination of dichlorophen. Unfortunately, these methods cannot detect the presence of the trimer impurity. The present method offers a rapid and simple high-performance liquid chromatographic (HPLC) procedure for the simultaneous determination of dichlorophen and the trimer in raw material and capsule formulations.



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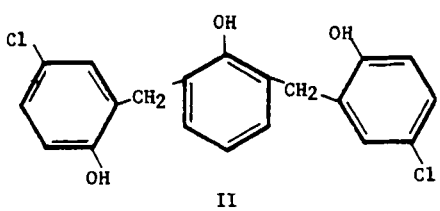

II

Table I—Analysis of Raw Material Samples of Dichlorophen [a]

Sample	HPLC Assay		Methoxide Titration Method
	Dichlorophen	Trimer	Dichlorophen
1	93.6	7.5	97.0
2	93.7	9.9	96.8
3	95.2	7.6	96.9
4	94.9	3.3	98.4
5	93.9	7.5	97.7
6	95.1	6.1	97.3
Mean	94.40	6.98	97.35
SD [b]	0.74	2.18	0.61

[a] Reported as a percentage. [b] Standard deviation.

EXPERIMENTAL

Materials—Dichlorophen[1] and the trimer[1], were used as received. Methanol and water were HPLC quality[2]. A gradient liquid chromatograph[3] equipped with two single-piston pumps[4] was operated in the isocratic mode. A 20-μL loop injector[5], variable-wavelength detector[6], electronic data printer-plotter[7], and nonpolar column (3.9 mm × 30 cm[8]) were used throughout. The mobile phase consisted of methanol–water (75:25) and was pumped at 1.5 mL/min. The detector was set at 290 nm and sensitivity was 0.018 AUFS.

Sample Preparation—Stock solutions of dichlorophen (60 mg/dL) and the trimer (30 mg/dL) were prepared in methanol. Solutions of dichlorophen containing 5, 9, 12, 15, and 18 μg/mL were prepared in methanol. Solutions of the trimer containing 6, 9, 12, 18, and 24 μg/mL were also prepared in methanol.

One capsule was cut, the fill material was squeezed into a 500-mL graduated cylinder, and the volume was adjusted with methanol. This was allowed to stand for 4 h, and then 8 mL of solution was diluted to 100 mL with methanol. Five milliliters of this diluted solution was further diluted to 50 mL with methanol to produce a solution having approximate concentration of 16 μg/mL. Twenty microliters of the resulting solution was used for analysis.

Calculations—Since linearity experiments indicated that the peak areas (and also peak heights) were related directly to the concentrations of dichlorophen (range 6–18 μg/mL) as well as the trimer (range 6–24 μg/mL), the results were calculated using:

$$\frac{Ph_a}{Ph_s} \times \frac{C}{L} \times D \times 100 = \text{Percent of label claim}$$

Where Ph_a is the peak area for the unknown solution, pH_s is the peak area for standard solution, C is the concentration of standard solution (g/mL), L is label claim (g), and D is the dilution factor (mL)

RESULTS AND DISCUSSION

Dichlorophen contains two phenolic groups. Use of the phenolic properties is made in several methods for the assay of dichlorophen (8); the results ob-

[1] Generously supplied by Givaudan, Clifton, N.J.
[2] Fisher Scientific Co., Fair Lawn, N.J.
[3] Model 344; Beckman Instruments, Berkeley, Calif.
[4] Model 112; Beckman Instruments, Berkeley, Calif.
[5] Model 210; Beckman Instruments, Berkeley, Calif.
[6] Model 165; Beckman Instruments, Berkeley, Calif.
[7] Model 3353; Hewlett-Packard, Palo Alto, Calif.
[8] μ-Bondapak C_{18}; Waters Associates, Milford, Mass.

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Table II—Analysis of Dichlorophen-Toluene Capsule Formulations by the HPLC Method[a]

Number	Formulation A		Formulation B		Formulation C	
	Dichlorophen	Trimer	Dichlorophen	Trimer	Dichlorophen	Trimer
1	93.2	7.8	99.8	7.5	97.8	9.1
2	92.8	7.4	102.1	7.2	96.4	8.8
3	95.1	7.8	98.6	6.7	97.2	8.6
4	89.9	8.4	100.7	7.3	96.3	7.9
5	93.9	8.1	99.3	7.0	95.4	7.6
Mean	92.98	7.90	100.10	7.14	96.62	8.4
SD^b	1.93	0.37	1.35	0.30	0.92	0.63

[a] Reported as a percentage of the theoretical label claim of 1 g. [b] Standard deviation.

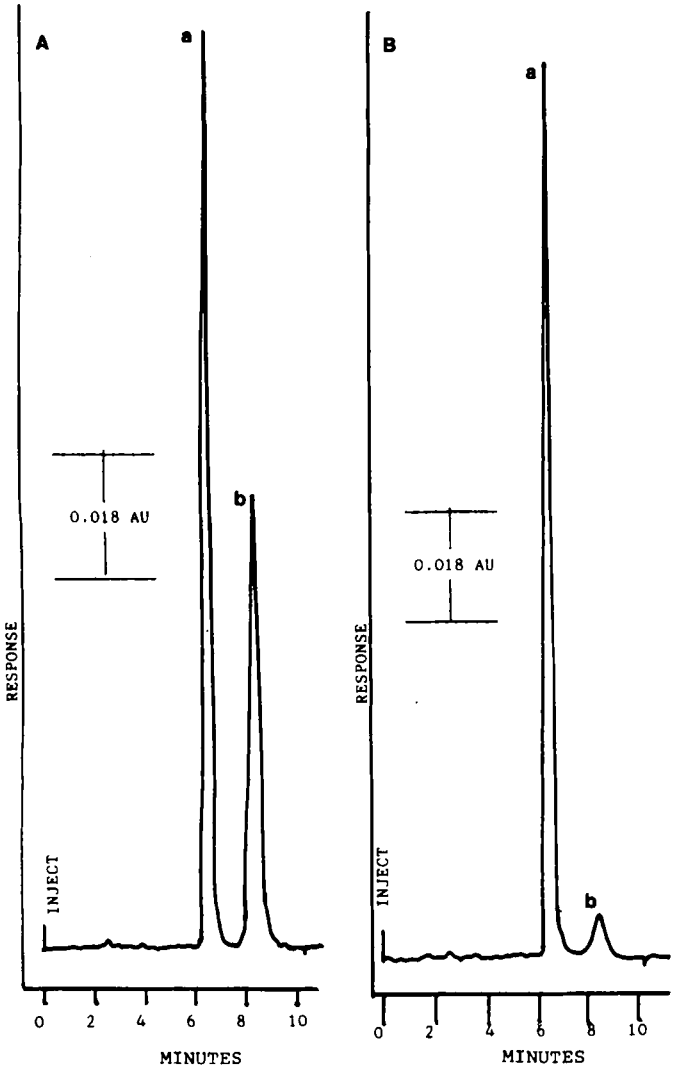

Figure 1—*Typical chromatograms of a standard solution of dichlorophen (19 μg/mL) and trimer (19 μg/mL) (A) and dichlorophen-toluene soft gelatin capsules containing 1 g of dichlorophen. Key: (a) dichlorophen; and (b) trimer.*

tained using these methods included the presence of the trimer. The chromatogram shown in Fig. 1A was obtained for a standard mixture of dichlorophen and the trimer. Figure 1B represents a chromatogram from a dichlorophen-toluene soft gelatin veterinary capsule formulation.

The calibration curve for dichlorophen was linear (r^2 = 0.99) over the concentration range of 6–18 μg/mL. The trimer calibration curve was also linear (r^2 = 0.99) over a concentration range of 6–24 μg/mL. The precision of this method was determined using 20 μL of each of a series of 10 standard solutions containing 12 μg/mL of dichlorophen and 12 μg/mL of the trimer. The coefficient of variation averaged 1.76% for dichlorophen and 2.02% for the trimer.

The recovery data for dichlorophen were determined by adding different amounts of the reference standard equivalent to one-third of the amount of dichlorophen contained in the dichlorophen-toluene veterinary capsules. The analysis of five such samples resulted in recovery values of 99.3, 100.0, 99.7, 101.0, and 99.8% of the total quantity expected. The mean and standard deviation were 99.96 and 0.64, respectively. The recovery data for the trimer were also determined by adding different amounts of the trimer equivalent to one-third of the amount of the trimer contained in the dichlorophen-toluene capsules. The analysis of five such samples resulted in recovery values of 98.2, 98.9, 98.9, 96.5, and 100.5% of the total quantity expected. The mean and standard deviation were 98.60 and 1.46, respectively. Therefore, it was determined from the above data that the assay provides satisfactory accuracy and precision.

Application of the HPLC assay to commercial source dichlorophen raw material provided the results given in Table I. The methoxide titration produced a higher percentage of dichlorophen because this value represents a summation of dichlorophen and the trimer. However, the HPLC procedure differentiated dichlorophen from the trimer, producing lower percentages.

Application of the HPLC procedure to three commercial dichlorophen-toluene soft gelatin veterinary capsules produced the results given in Table II. All formulations were labeled as having 1 g of dichlorophen. Mean dichlorophen potencies were 92.98, 100.10, and 96.62%, with respective standard deviations of 1.93, 1.35, and 0.92. The trimer means were 7.90, 7.14, and 8.40, with respective standard deviations of 0.37, 0.30, and 0.63.

The dichlorophen content for formulations A, B, and C were also determined using the methoxide titration method. This method provided mean dichlorophen potencies of 99.9, 105.0, and 103.0% for formulations A, B, and C, respectively. Again, the methoxide titration provided higher percentages of dichlorophen. The method presented here is rapid, precise, accurate, and simpler than the methoxide titration method. The trimer content in dichlorophen raw material can be easily determined.

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