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Note

High-performance liquid chromatographic analysis of technical Ruelene insecticide

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Ruelene insecticide which contains the active ingredient, 4-*tert.*-butyl-2-chlorophenyl methyl methylphosphoramidate (common name, crufomate) has found use as an anthelmintic and insecticide[1,2]. Besides the active ingredient, this product contains two significant impurities, 4-*tert.*-butyl-2-chlorophenyl dimethyl phosphate (triester) and 4-*tert.*-butyl-2-chlorophenyl N,N[1]-dimethylphosphorodiamidate (diamidate).

Technical Ruelene insecticide has been analyzed internally by an infrared (IR) method using the $POCH_3$ vibration. Although giving good results, it requires two scans, a differential scan for impurities and a scan for assay. Based on the concentrations for those impurities having $POCH_3$ absorption, a correction of the assay value is made.

Gas chromatography[3–5] has been used for trace crufomate determination. It has also been used internally for assay; however, extensive column conditioning and frequent standardization are necessary to obtain satisfactory results.

This product has been analyzed gravimetrically[6] based on phosphorus content but this approach is non-specific.

High-performance liquid chromatography (HPLC) seemed like a viable approach for analysis of the technical product.

EXPERIMENTAL

Reagents

(a) 4-Chlorophenol, 99%+, Cat. No. 18578-7 (Aldrich, Milwaukee, WI, U.S.A.).

(b) Crufomate, 99% (Dow Chem.). Purity and identification were made by appropriate tests.

(c) Triester (see b).

(d) Diamidate (see b).

(e) 4-*tert.*-Butyl-2-chlorophenol (see b).

(f) Acetonitrile, distilled-in-glass grade (Burdick & Jackson Labs., Muskegon, MI, U.S.A.).

(g) Eluent, 50% v/v acetonitrile–water.

(h) Diamidate–triester stock solution. Weigh 25 mg of the diamidate and triester standard to the nearest 0.01 mg into the same 25-ml volumetric flask. Dissolve and dilute to volume with acetonitrile.

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(i) Calibration solution. Weight 20–30 mg 4-chlorophenol to the nearest 0.01 mg into a 4-dram vial. Into the same vial weigh 40–60 mg of crufomate standard. Pipet in 2 ml of diamidate–triester standard (h) and follow with 8 ml of acetonitrile.

Apparatus

Liquid chromatograph-modular: a unit composed of a model M-6000 pump (Waters Assoc., Milford, MA, U.S A.), a Model 70-10 injection valve with 20-μl loop (Rheodyne, Berkeley, CA, U.S.A.), a variable wavelength detector, Model LC 55 (Perkin-Elmer, Norwalk, CT, U.S.A.), a computing integrator, Model 3380A, Hewlett-Packard, Avondale, PA, U.S.A.), a μBondapak C_{18} reversed-phase column (30 cm $\times$ 3.9 mm), Cat. No. 27324 (Waters Assoc.) protected with a guard column (50 $\times$ 2.1 mm) packed with pellicular Corasil C_{18} (Waters Assoc.).

Preparation of sample

Weigh 20–30 mg 4-chlorophenol internal standard to the nearest 0.01 mg into a 4-dram vial. Into the same vial weigh 40–60 mg of Ruelene insecticide sample. Add 10 ml of acetonitrile$_1$

Determination

Inject 20-μl aliquots of the calibration solution (i) until the response factor varies $<1\%$ for consecutive injections. Inject 20-μl duplicate aliquots of the sample solution. Record the peak areas on a computing integrator and average the results. Calculation

$$R_F = P_{ir} W_r / W_{ir} P_r$$

where R_F = response factor, P_{ir} = internal standard peak area in reference standard solution, P_r = crufomate standard peak area in reference standard solution, W_{ir} = weight in grams of internal standard and W_r = weight in grams of crufomate standard.

$$\% \text{ crufomate} = P_c W_{is} R_F P / P_{is} W_s$$

where R_F = response factor, P_c = peak area of crufomate peak in sample solution, P_{is} = peak area of internal standard peak in sample solution, W_s = weight in grams of sample; W_{is} = weight in grams of internal standard and P = purity of standard.

RESULTS AND DISCUSSION

High-performance liquid chromatograms for a Ruelene insecticide sample and standard are presented in Fig. 1.

Coefficient of variation data for the analysis of technical Ruelene insecticide was obtained by analyzing one sample five times on two successive days. Results are given in Table I. The values for the diamidate and triester were obtained in the same run as the assay value therefore their coefficients of variation would be expected to greater than crufomate.

Recovery data was obtained by preparing synthetic samples and analyzing these by the method. Results are presented in Table II.

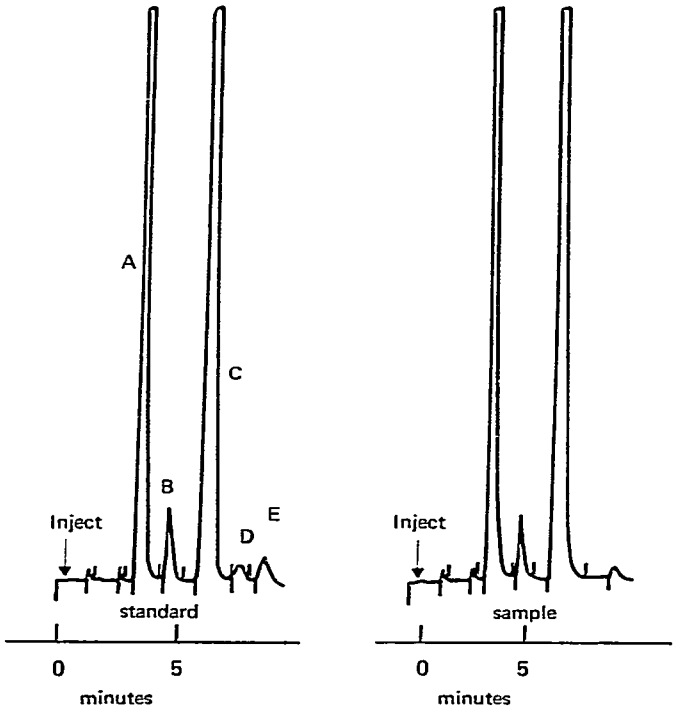

Fig. 1. Liquid chromatogram for 4-chlorophenol (internal standard) (A), diamidate (B), crufomate (C), 4-*tert.*-butyl-2-chlorophenol (D) and triester (E).

TABLE I

PRECISION STUDY FOR THE ANALYSIS OF RUELENE INSECTICIDE

	Diamidate (%)	*Crufomate* (%)	*Triester* (%)
	2.64	94.08	1.75
	2.61	93.91	1.75
	2.67	94.17	1.86
	2.61	93.69	1.79
	2.63	93.21	1.69
	2.43	93.78	1.73
	2.42	93.72	1.84
	2.44	93.85	1.89
	2.45	93.63	1.81
	2.44	93.79	1.83
Mean (%)	2.53	93.78	1.79
S. D. (%)	0.10	0.26	0.063
Coeff. of Var. (%)	4.0	0.28	3.5

The retention times for a number of potential impurities were determined. None of these was found to interfere with the internal standard or assay peak. They are shown in Table III.

TABLE II

ANALYSIS OF KNOWN MIXTURES OF RUELENE INSECTICIDE

Mixture	*Diamidate (%)*			*Crufomate (%)*			*4-tert.-Butyl-2-chlorophenol (%)*		*Triester (%)*		
	By wt.	*By LC*	*Recovery*	*By wt.*	*By LC*	*Recovery*	*By wt.*	*By LC*	*By wt.*	*By LC*	*Recovery*
1	3.86	3.48	90	92.40	92.49	100.1	0.60	0.62	3.09	3.13	101
2	6.12	5.35	87	88.92	88.56	99.6			5.31	4.96	93
3	2.51	2.42	96	95.38	95.72	100.4	0.59	0.61	1.36	1.52	112
4	1.00	1.17	117	94.15	94.25	100.1			5.00	4.85	97
5	1.06	1.21	114	97.15	97.13	100.0	0.50	0.50	1.18	1.29	109
6	3.54	3.25	92	93.04	93.25	100.2	0.55	0.59	2.64	2.87	109
7	5.14	4.55	89	92.10	92.12	100.0	1.20	1.38	1.48	1.56	105
8	2.52	2.35	93	92.88	93.03	100.2			4.96	4.60	92
9	1.04	1.14	110	92.15	92.50	100.4	0.49	0.55	6.56	6.32	96
10	3.14	2.88	92	95.59	95.56	100.0			1.17	1.27	109
Mean recovery (%)			98			100.1					102
Coeff. of var. (%)			11			0.23					7

TABLE III

HPLC RETENTION TIMES FOR RUELENE INSECTICIDE AND POTENTIAL IMPURITIES

Component	*Time (min)*
Solvent front	1.04
4-*tert.*-Butyl-2-chlorophenyl methyl phosphate dimethylamine salt	1.18
Methyl phenyl N-methylphosphoramidate	2.13
2-Chlorophenyl methyl N-methylphosphoramidate	2.56
4-Chlorophenyl methyl N-methylphosphoramidate	2.71
4-Chlorophenol (internal standard)	3.26
2,4-Dichlorophenyl methyl N-methylphosphoramidate	3.56
4-*tert.*-Butyl-2-chlorophenyl N,N¹-dimethylphosphorodiamidate (diamidate)	4.50
4-*tert.*-Butyl methyl N-methylphosphoramidate	4.58
4-*tert.*-Butyl-2-chlorophenyl methyl N-methylphosphoramidate (crufomate)	6.32
4-*tert.*-Butyl-2,6-dichlorophenyl methyl N-methylphosphoramidate	7.49
4-*tert.*-Butyl-2-chlorophenyl dimethyl phosphate (triester)	8.97
Bis(4-*tert.*-butyl-2-chlorophenyl) methyl phosphate	>30
Bis(4-*tert.*-butyl-2-chlorophenyl) methylphosphoramidate	>30
Tris(4-*tert.*-butyl-2-chlorophenyl) phosphate	>30

A wavelength of 270 nm was chosen for monitoring the separation because it is very near the wavelength of maximum absorption for crufomate and related impurities.

A methanol–water eluent was also tried for this separation; however, 4-*tert.*-butyl-2-chlorophenol was not separated from the other components.

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