Determination of *N*-Hydroxynaphthalimide Diethyl Phosphate (Maretin) in Milk and Eggs by in Situ Fluorometry

Jean-Guy Zakrevsky and Victorin N. Mallet*

A procedure is described for the analysis of Maretin at the 0.01-ppm level in eggs and milk by in situ fluorometry. Following extraction, the oils and fats are removed by solvent partitioning and Maretin is hydrolyzed to naphthostyril. Egg extracts require further cleanup by column chromatography. Naphthostyril is then separated on silica gel by two-dimensional thin-layer chromatography and its fluorescence is measured by direct scanning of the spot.

Maretin (*N*-hydroxynaphthalimide diethyl phosphate), also named naphthalaphos, is an anthelmintic used for the control of various endoparasites of sheep, cattle, and poultry.

Anderson et al. (1966) proposed a method for the determination of Maretin in animal tissues in which the compound, after initial extraction and purification by liquid–liquid partition and column chromatography, was hydrolyzed and the fluorescence measured in benzene. Cleanup was incomplete and because of this, background interferences were high.

The method was greatly improved by Thornton and Schumann (1972) who modified the cleanup procedure and analyzed for residues in milk. Maretin was first hydrolyzed after extraction and its hydrolysis product, naphthostyril, was separated on a Florisil column. Interfering substances were successfully removed and a better limit of detection was obtained.

Mallet et al. (1974) proposed a method for the determination of Maretin in milk in which the natural fluorescence of the compound was measured directly on a silica gel thin-layer chromatogram. The method was both rapid and simple but since the fluorescence of Maretin is unstable on silica gel layers, the instrumental measurements had to be done quickly and chromatograms could not be kept for further reference.

In this study, a modification of this method is proposed (see Mallet et al., 1974) whereby the pesticide is hydrolyzed prior to chromatography. The technique is then applied to the determination of Maretin in milk for comparison purposes. The method is extended to the analysis of Maretin in eggs.

EXPERIMENTAL SECTION

Materials and Apparatus. Technical Maretin (98%) was obtained from Chemagro Corporation (Kansas City, Mo.). Stock solutions were prepared (1 μg/μl) in methylene chloride. Florisil (60–100 mesh, Fisher Scientific Co.) was heated in an oven at 130° for at least 48 hr and then deactivated by adding 3% water.

A Farrand visible–ultraviolet Chromatogram Analyzer equipped with a 1P28 photomultiplier tube was used for fluorometric analysis. Excitation filter no. 7-54 (230–420 nm) and emission filter no. 3-73 (greater than 405 nm), both available from Corning Glass Work (Corning, N.Y.), were used.

Extraction of Milk. The procedure utilized was similar to that of Thornton and Schumann (1972). However, the chloroform extract should be dried for 5 min with 30 g of anhydrous magnesium sulfate following partition with water. The mixture was then filtered through a 32-cm Whatman No. 2-V filter paper.

Department of Chemistry, Université de Moncton, Moncton, New Brunswick, Canada.

Extraction of Eggs. This procedure is similar to that developed by Thornton (1968). An egg was weighed and then broken into a Waring Blendor jar. The weight of the shell was subtracted. The egg was blended for 5 min with 200 ml of acetone and the mixture was filtered with vacuum through a Whatman No. 42 filter paper in a Büchner funnel. The filter cake was reblended with 200 ml of chloroform and the mixture was again filtered. The filter cake was rinsed with 100 ml of chloroform. The combined extracts were transferred to a 1000-ml separatory funnel and shaken for 30 sec. The phases were allowed to separate and the lower layer was drained through a 32-cm Whatman No. 2-V fluted filter paper into a 1000-ml round-bottomed flask. The extract was evaporated to dryness on a rotary vacuum evaporator at 35°.

The residue was transferred to a 50-ml volumetric flask using small portions of Skellysolve-B and the volume was completed to the mark. A 25-ml aliquot was pipetted into a 250-ml separatory funnel and 75 ml of Skellysolve-B was added. Acetonitrile (100 ml) was added and the funnel was shaken for 30 sec. The two phases were allowed to separate and the lower acetonitrile layer was drained into a 500-ml round-bottomed flask. The extraction was repeated twice using fresh 100-ml portions of acetonitrile. The combined extracts were evaporated under vacuum on a rotary evaporator at 35°.

Hydrolysis. The procedure of Thornton and Schumann (1972) was followed.

Florisil Column Cleanup (Egg Samples Only). Refer to the procedure of Thornton and Schumann (1972). The column was prepared by first placing a plug of glass wool at the bottom of a chromatographic tube (20 mm i.d.) and adding 3 g of anhydrous sodium sulfate followed by 10 g of Florisil. The adsorbent was topped with 3 g of sodium sulfate. The column was washed with 20 ml of benzene and the residue from the previous step was transferred to the column using small portions of benzene. A volume of 165 ml of 4% acetonitrile in benzene (v/v) was added and the first 65 ml was collected in a graduated cylinder and discarded. The next 100 ml was collected in a 125-ml round-bottomed flask and the eluate was evaporated under vacuum at 35°.

Thin-Layer Chromatography. The residue was transferred to a 15-ml graduated centrifuge tube using small portions of *n*-hexane. The solvent was evaporated to dryness on the rotary evaporator (35°). The residue was redissolved in methylene chloride. For milk analysis, the volume was completed to the 0.2-ml mark. Egg extracts were diluted to 0.1 ml. A 10-μl (milk extract) or a 20-μl (egg extract) aliquot was spotted with a 25-μl microsyringe at the bottom of a chromatographic plate (silica gel H, 250 μ). The first development was made to the 10-cm line in *n*-hexane–acetone (7:3) and the second development was done in carbon tetrachloride–methanol (100:7) to a distance of 10 cm in a direction perpendicular to the first migration. Standards

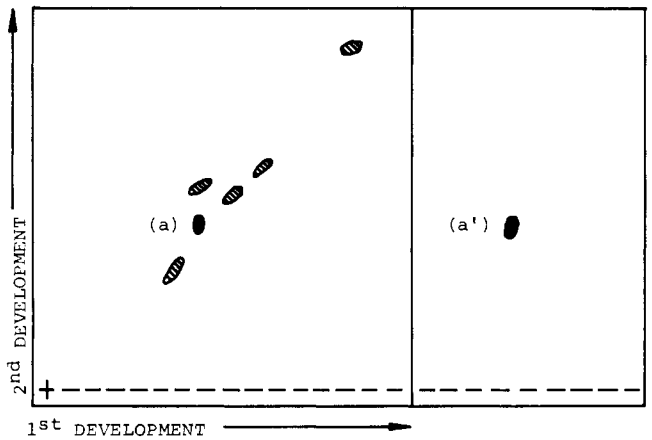

Figure 1. Thin-layer chromatogram of naphthostyril in a milk extract after hydrolysis: (a) naphthostyril; (a') naphthostyril standard.

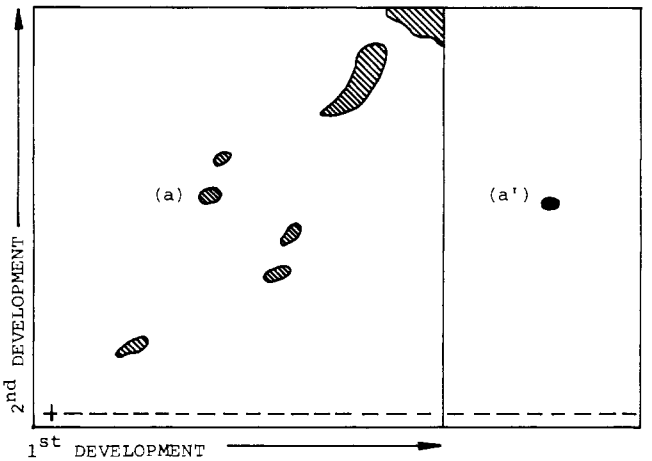

Figure 2. Thin-layer chromatogram of naphthostyril in egg extract after hydrolysis: (a) naphthostyril; (a') naphthostyril standard.

Table I. Recovery of Maretin from Eggs and Milk

Sample	Quantity added, μg	Concn, ppm	Quantity recovered, μg	Recovery, %
Milk	20.0	0.10	19.6	98
			19.9	99
				Av 99
	2.0	0.01	2.1	105
			2.1	105
			2.0	100
				Av 103
Eggs	5.0	0.10	4.5	90
			4.3	86
				Av 88
	0.50	0.01	0.48	96
			0.48	96
				Av 96

were developed simultaneously with the sample during the second development.

After locating the naphthostyril spot under the uv lamp, the fluorescence was measured at an emission wavelength of 495 nm while excitation was set at 365 nm.

RESULTS AND DISCUSSION

Thin-layer chromatography is used as the final cleanup step before fluorometric determination of residues of Maretin in milk or egg samples. Two-dimensional chromatography is very efficient in removing co-extractives that would otherwise interfere with the fluorometric analysis. Typical thin-layer chromatograms are shown in Figure 1 for milk and Figure 2 for eggs. In both cases, sufficient separation for subsequent quantitative evaluation of the chromatogram could not be obtained with a one-dimensional system. Moreover, two-dimensional chromatography using different eluting solvents provides more certainty in identifying the pesticide.

Naphthostyril is very stable on silica gel under uv or visible light. After several hours of exposure to uv light, no change in the fluorescence intensity is observed, whereas the fluorescence of Maretin disappears completely after only a few hours (Mallet et al., 1974). The precision of the technique is therefore better and the analysis need not be performed immediately following the development of the chromatogram. Furthermore, a chromatogram can be stored indefinitely for future reference. Although the procedure is lengthier due to the hydrolysis step, it is more reliable.

Column chromatography, which was used by Thornton and Schumann (1972) for the cleanup of milk extract, is not needed. However, egg samples require a more rigorous preliminary cleanup and consequently column chromatography is necessary.

Recovery experiments were conducted from milk and egg samples at the 0.10- and 0.01-ppm levels (Table I). Recoveries from milk samples were in the 98–105% range with little variation between the results obtained at two different concentrations. The precision is better than that attained with the previous in situ method (Mallet et al., 1974). Lower recoveries are observed for the analysis of eggs but this may be attributed to a loss resulting from the use of the supplementary Florisil column cleanup. Interference from the extract also causes variations in the results. As low as 5 ng of Maretin in the form of naphthostyril can be detected instrumentally on silica gel and the response is linear up to a concentration of 10 μg per spot. Thus, it should be possible to work comfortably at concentrations much lower than those reported here.

LITERATURE CITED

Anderson, R. J., Anderson, C. A., Yagelowich, M. L., *J. Agric. Food Chem.* **14,** 43 (1966).
Mallet, V. N., Zakrevsky, J.-G., Brun, G. L., *Bull. Soc. Chim. Fr.* **9–10,** 1755 (1974).
Thornton, J. S., Chemagro Corporation Research Department, Report No. 22052, 1968.
Thornton, J. S., Schumann, S. A., *J. Agric. Food Chem.* **20,** 635 (1972).

Received for review December 6, 1974. Accepted February 19, 1975. This work was supported by Agriculture Canada.