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Note

Methods for the determination of maleic hydrazide, ethoxyquin and thiabendazole in wastewaters*

DILNA M. VICTOR*, REX E. HALL, JEFF D. SHAMIS and STUART A. WHITLOCK
Environmental Science and Engineering, Inc., P.O. Box ESE Gainesville, FL 32602 (U.S.A.)
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Pesticides have been extensively manufactured and used in the past 10 years. The EPA has been entrusted with the task of monitoring industrial effluents and any wastestreams affected by these effluents. To achieve this end, methods for the analysis of the pesticides must be developed or modifications made to existing methods. This paper discusses simple methods developed for the analysis of maleic hydrazide, ethoxyquin and thiabendazole.

Earlier methods for the determination of these fungicides and plant growth regulators have been relatively involved. Maleic hydrazide has been analyzed in fruits and vegetables by distillation–spectrophotometry[1] or by high-performance liquid chromatography (HPLC) with ultraviolet (UV) absorption at 313 nm[2].

Ethoxyquin has been determined by thin-layer chromatography[3] and by gas chromatography (GC) with electron-capture detection of the derivative formed by mixing with heptafluorobutyric anhydride[4]. More recently, ethoxyquin was determined by extraction from apples and analysis on HPLC with fluorometric detection[5]. HPLC determinations of thiabendazole have been used often although derivatization and analysis by GC with electron-capture[6] and with flame ionization detection[7] have been successful. HPLC methods generally have been less complicated. HPLC with UV detection has been used by Austin *et al.*[8] and Farrow *et al.*[9]. Hydrolysis and subsequent HPLC analysis using a fluorometer was used by Maeda and Tsuji[10].

In earlier methods development, ethoxyquin and thiabendazole were placed initially into the same group. However, their polarities were found to be quite different. Ethoxyquin could be extracted from basic water with hexane, while salt and methylene chloride were required for extraction of thiabendazole from basic water. Florisil Sep-Paks (Waters) were used for sample cleanup. Ethoxyquin elutes with hexane; however, thiabendazole was mostly retained with methylene chloride. These incompatible polarities led to the development of different methods for ethoxyquin and thiabendazole.

Preliminary investigations using maleic hydrazide showed that it had a UV shoulder at 220 nm and a secondary maximum at 305 nm. A strong response to the electrochemical detector was seen at an optimum applied potential of 1.05 V. Reversed-phase

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HPLC conditions were developed, and a detection limit of 1.0 μg/l was achieved by direct aqueous injection.

This paper reports on methods developed at ESE for the analysis of these pesticides in wastewater. These methods involve sample filtration through a 0.45-μm filter and analysis by HPLC using direct aqueous injection and fluorometric detection for ethoxyquin and thiabendazole and electrochemical detection for maleic hydrazide.

EXPERIMENTAL

Apparatus

A liquid chromatograph equipped with a reversed-phase 250 × I.D. 4.6 mm Ultrasphere octadecylsilane (ODS) column (particle size 10 μm) (Altex Scientific) and an Altex 110A pumping system were used. The system was interfaced to a fluorescence spectrophotometer (Perkin-Elmer 650-105) for ethoxyquin and thiabendazole, or an electrochemical detector with glassy carbon electrode (Bioanalytical Systems LC-2A) for maleic hydrazide. Samples were introduced through an injector valve (Rheodyne) with either 100-μl or 250-μl loops.

The mobile phases used were: methanol–0.01 *M* phosphate buffer, pH 2 (5:95) for maleic hydrazide analysis, methanol–0.043 *M* phosphate buffer, pH 2(80:20) for ethoxyquin and methanol–triethanolamine–acetic acid buffer, pH 8.2 (70:30) for thiabendazole. A 5 cm × 2.1 mm I.D. silica precolumn is recommended owing to the pH of the buffer in the thiabendazole analysis.

Reagents and chemicals

All solvents used were HPLC grade. Chemicals used were reagent grade (Baker Analyzed). Standards of maleic hydrazide, ethoxyquin, and thiabendazole were obtained from the United States Environmental Protection Agency (EPA), Research Triangle Park, NC, U.S.A.

Buffer solutions: pH 2, O.1 *M* phosphate buffer: 5.83 g of KH_2PO_4 and 3.9 ml of 85% phosphoric acid were added to 1 l of HPLC water; pH 7.0, 0.043 *M* phosphate buffer: dissolve 2.58 g of KH_2PO_4 and 4.18 g of K_2HPO_4 in 1 l of HPLC water; pH 8.2, triethanolamine–acetic acid buffer: add 8 ml of triethanolamine (Eastman 1599) and 1 ml of glacial acetic acid (ACS) to 1 l of HPLC water.

Method

Maleic hydrazide, ethoxyquin, and thiabendazole standards were dissolved in methanol and were spiked into publicly-owned treatment works (POTW) water for samples. The pH of each solution was adjusted to 2 for maleic hydrazide, 7–9 for thiabendazole, and 5–9 for ethoxyquin with dilute sodium hydroxide or sulfuric acid.

The sample solutions consisting of maleic hydrazide, ethoxyquin, or thiabendazole in POTW water were filtered through a 0.45 μm Nylon 66 filter using filter holder (stainless steel with Leur connection – Rainin 38-101). The syringe and filter holder were rinsed with acetone or methanol and HPLC water between samples. Low-level samples of maleic hydrazide may require rinsing of glassware, syringe, and injector loop with dilute sodium hydroxide between samples. Samples were then injected directly into the liquid chromatograph.

RESULTS AND DISCUSSION

The HPLC conditions above proved to be acceptable for the analyses of maleic hydrazide, ethoxyquin and thiabendazole. Chromatograms obtained under these conditions are shown in Figs. 1–3. The retention times and estimated detection limits are presented in Table I. This detection limit was calculated from the minimum detectable response of the electrochemical detector at 1.05 V (or the fluorescence detector) being equal to five times the background noise, using a 100-μl or 250-μl injection. Recoveries

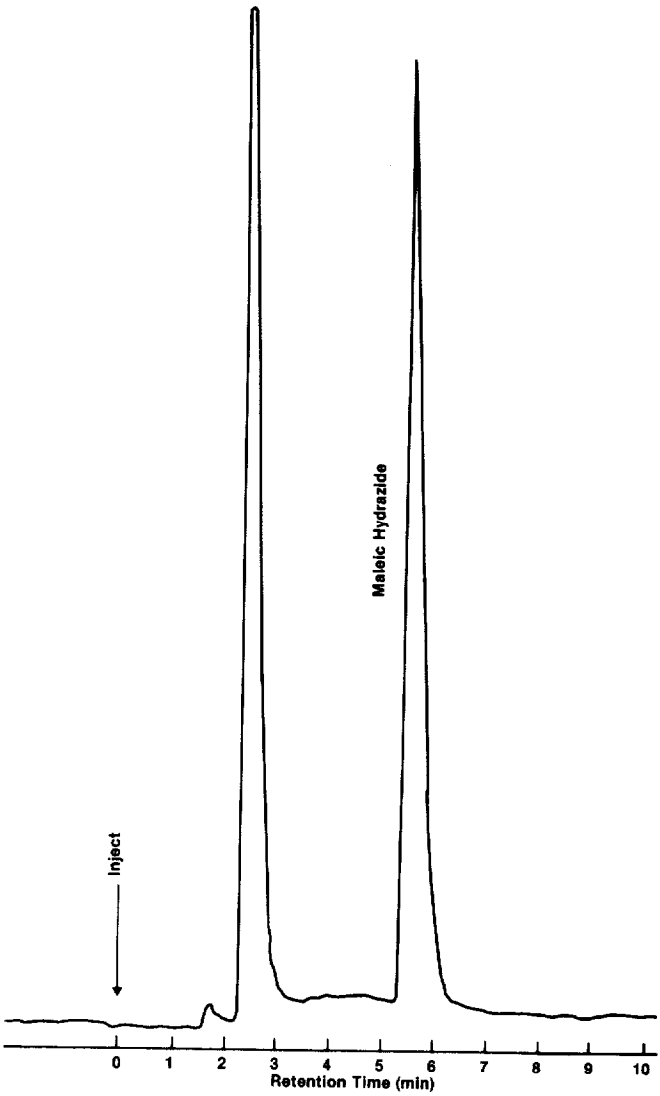
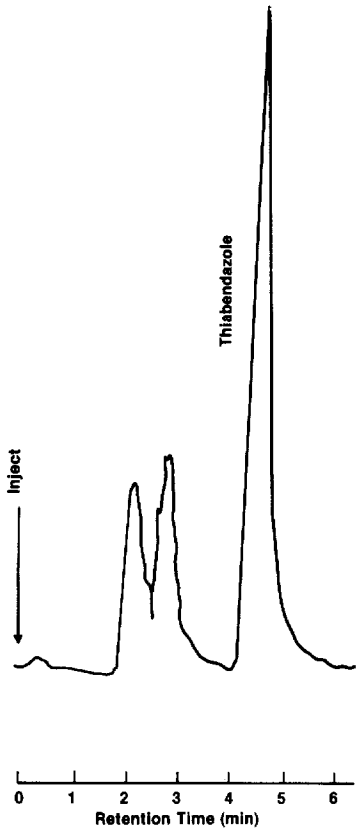


Fig. 1. HPLC chromatogram of maleic hydrazide.



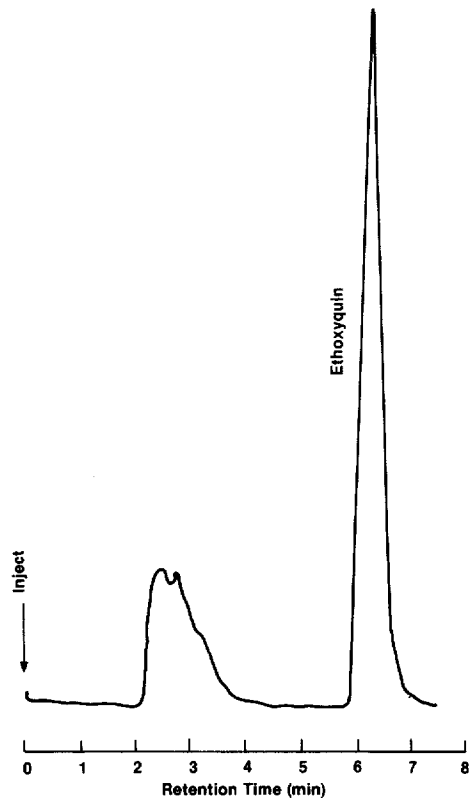


Fig. 2. Chromatogram of thiabendazole in wastewater sample.

Fig. 3. HPLC chromatogram of ethoxyquin in wastewater sample.

TABLE I

RETENTION TIMES AND ESTIMATED DETECTION LIMITS OF MALEIC HYDRAZIDE, THIABENDAZOLE, AND ETHOXYQUIN

Conditions specified in the text.

Analyte	*Retenion time (min.)*	*Estimated detection limit (μg/l)*
Maleic hydrazide	5.5	1
Thiabendazole	4.5	1
Ethoxyquin	6.4	1

for the validation of the above methods for these pesticides are excellent as can be seen in Table II. These recoveries were calculated as:

$$\text{Percentage recovery} = \frac{\text{fortified levels} - \text{sample level}}{\text{fortification}} \times 100$$

and the relative standard deviations as:

$$\text{R.S.D.} = \frac{\text{standard deviation}}{\text{percentage recovery}} \times 100$$

No interferences were observed owing to the specificity of the detectors used. Blanks were run with each set of samples. Chromatograms of POTW water blanks analyzed by HPLC using an electrochemical detector and a fluorescence detector are shown in Figs. 4 and 5, respectively.

Residual chlorine was found to degrade ethoxyquin, and to some extent, thiabendazole, as can be seen in Table III. Sodium thiosulfate also appeared to have some degradative effect on both compounds, but could prevent them from further degration

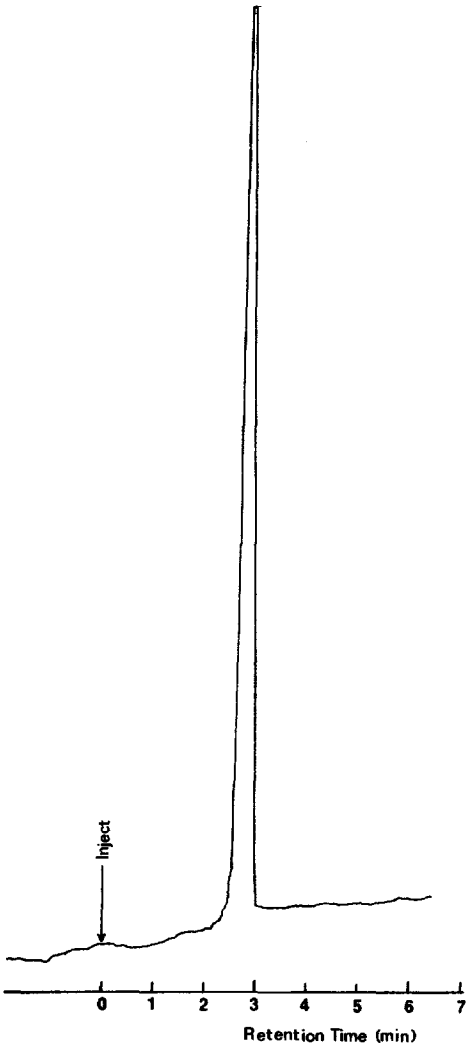


Fig. 4. HPLC chromatogram of POTW blank using electrochemical detection.

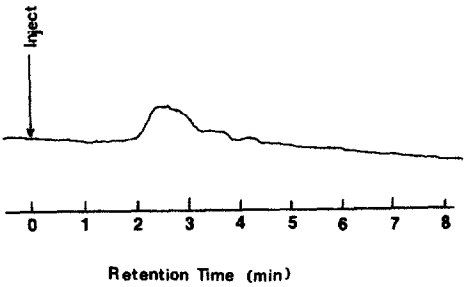


Fig. 5. HPLC chromatogram of water blank using fluorescence detection.

TABLE II

RECOVERIES FOR MALEIC HYDRAZIDE, ETHOXYQUIN AND THIABENDAZOLE FROM WASTEWATERS

	Level	*Range (μg/l)*	*Replicates*	*Average recovery (%)*	*Standard Dev. (%)*
Maleic hydrazide	Low	20	7	96.9	38.8
	High	500	7	120.0	12.0
Ethoxyquin	Low	10	7	94.7	4.9
	High	500	7	106.4	6.5
Thiabendazole	Low	12.5	7	100.2	9.5
	High	615	7	92.8	4.5

TABLE III

THIABENDAZOLE/ETHOXYQUIN METHODS DEVELOPMENT TESTS

Test/sample	*Percentage recovery*	
	Ethoxyquin	*Thiabendazole*
3 ppm hypochlorite added to 2 ppm standard,	0.0	69.4
then 20 ppm sodium thiosulfate added	0.0	78.8
20 ppm sodium thiosulfate added to 2 ppm analyte standard, then 3 ppm sodium hypochlorite added	78.7	80.5
	80.9	85.7

by residual chlorine. Adsorption of analyte on any particulate matter in the water matrix and subsequent removal of this particulate matter with some analyte on filtration are recognized. However, no such problem was encountered in the POTW matrix used.

Results for stability studies of a 7-day storage period are shown in Table IV. Two replicates each at 4°C and room temperature were analyzed. Maleic hydrazide and thiabendazole were relatively stable at both 4°C and room temperature, while complete decomposition of ethoxyquin was observed.

The simplicity of these methods and their excellent recoveries gives them an obvious advantage in routine analyses. Cleanup is generally not necessary because of

TABLE IV

STABILITY STUDIES FOR A 7-DAY STORAGE PERIOD

	Percentage recovery	
	4°C, dark	*Room temp., dark*
Maleic hydrazide*	109.5	108.3
	109.5	109.0
Ethoxyquin**	21.0	0
	21.5	0
Thiabendazole***	91.2	103.2
	109.6	93.2

* 100% POTW water acidified to pH 2 for 0.5 h before spiking, stored at pH 2.
** 100% POTW water adjusted to pH 2 for 0.5 h and adjusted back to pH 7.
*** 100% POTW.

the specificity of the detectors used, a fluorometric spectrophotometer for ethoxyquin and thiabendazole, and an electrochemical detector with glassy carbon electrode for maleic hydrazide.

These methods may be applicable to industrial wastewaters, except excessively contaminated samples, and to affected waterways with little or no cleanup.

ACKNOWLEDGEMENT

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