Use of HPTLC for Simultaneous Determination of Three Fungicides in Tomatoes

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Key Words

HPTLC
Residue analysis
Fungicides
Tomato

Summary

A simple, rapid, and sensitive high-performance thin layer chromatographic method for analysis of the residues of tricyclazole, thiram, and folpet in tomatoes has been established and validated. The sample was extracted by mechanical vibration at room temperature with acetone–dichloromethane 1:1 (*v*/*v*) and the extract was directly applied as bands to glass-backed silica gel 60F_{254} HPTLC plates. The plates were developed with hexane–acetone 6+4 (*v*/*v*) in an unsaturated glass twin-trough chamber. Evaluation of the developed HPTLC plates was performed densitometrically. The results indicated that the detection limits of tricyclazole (R_F = 0.26.), thiram (R_F = 0.65), and folpet (R_F = 0.77) were 1.2 × 10^{-8}, 3.0 × 10^{-8}, 4.0 × 10^{-8} g, respectively. Recoveries of the pesticides from tomatoes with this analytical method were 67.66–98.02%, and RSD were 0.13–22.06%. The precision and accuracy of this method were generally fit for analysis of pesticide residues in tomatoes.

1 Introduction

Many pesticides used in agriculture have polluted the environment and living organisms. Gas chromatography (GC) and high-performance liquid chromatography (HPLC) are the main modern analytical techniques widely used to monitor the presence of these compounds in different matrixes [1]. Contemporary thin layer chromatography (TLC) has developed from simple, inexpensive, qualitative screening TLC into highly efficient, instrumental, quantitative HPTLC with clearly improved detection and quantification [2], and has become a complement to GC and HPLC in the pesticide residue analysis [3]. For example, thin-layer chromatography in combination with fiber optical (diode-array) scanning densitometry has been used for identification of fenitrothion in apples and fresh apple juice [4]. HPTLC has advantages, including:

– simplicity of operation;

– the availability of many sensitive and selective reagents for detection and confirmation without interference of the mobile phase;

– the ability to repeat detection and quantification at any time with changed conditions, because fraction representing the entire sample are stored on the plate;

– in-system calibration for quantitative analysis; and

– cost effectiveness because many samples can be analyzed on a single plate with low solvent usage [5].

Tomatoes are among the most popular vegetables in the world. The monitoring of multiresidues of pesticides in vegetables is very important because of its public health, environmental monitoring, and foreign trade [1]. Several recent papers have reported the presence of pesticide residue in tomatoes. Reversed-phase high-performance liquid chromatography (RP-HPLC) with UV detection [1], liquid chromatography–electrospray ionization–tandem mass spectrometry [6], or gas chromatography (GC) with different detectors [7, 8] have usually been used for analysis of pesticides residues in tomatoes. In this work, an HPTLC method has been established for multiresidue determination of three fungicides in tomatoes. The fungicides were extracted and concentrated, using the cleaning ability of thin-layer chromatography and no additional purification procedure.

2 Experimental

2.1 Materials and Chemicals

Thiram (98%) was from Germany. Tricyclazole (99.4%) and folpet (99.7%) were from the National Center for Pesticide Quality Inspection, Beijing, China. Acetone, hexane, dichloromethane, and methanol were analysis-grade. Anhydrous sodium sulfate was heated for 8 h at 450°C and stored in a tightly capped bottle until used.

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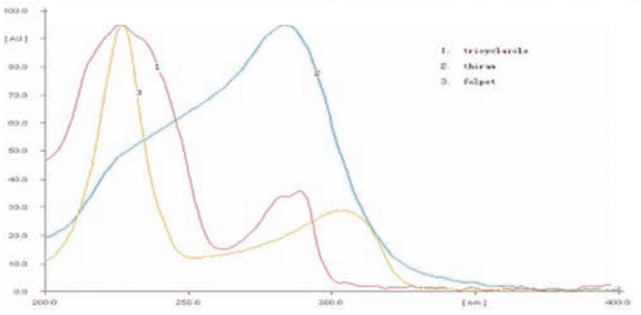

Figure 1

Overlaid UV absorption spectra of tricyclazole, thiram, and folpet standards, acquired with the Camag TLC Scanner 3.

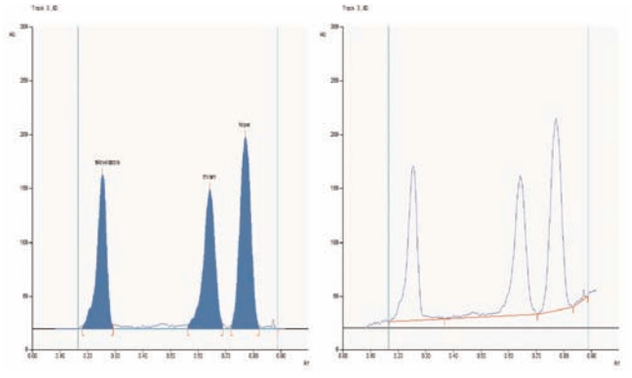

Figure 2

Chromatogram obtained from tricyclazole, thiram, and folpet standards.

2.2 Standard Solutions

Stock calibration solutions (2000 mg L^{-1} folpet, 1000 mg L^{-1} thiram, and 600 mg L^{-1} tricyclazole) were prepared in methanol and stored in glass-stoppered bottles at 4°C. The stock solutions (1 mL) were mixed and diluted to 10 mL with methanol to furnish a mixed standard solution of the three pesticides. Working solutions of appropriate concentration were prepared daily by diluting this stock standard solution.

2.3 Sample Preparation

Tomatoes were sampled in our kitchen garden where the fungicides of interest were never used. Sample (10 g) was weighed into a 150-mL conical flask with stopper and a known amount of spiking solution was added. After equilibration, the fortified samples were mixed with 50 mL acetone–dichloromethane 1:1 (*v*/*v*) and 3 g anhydrous sodium sulfate and extracted by mechanical vibration at room temperature for 30 min. The extract was filtered through a glass funnel containing 5 g anhydrous sodium sulfate into a 150-mL round-bottomed flask. Extraction of the sample was repeated with another 50 mL extraction solution and the filtrate was collected in the same flask. The combined extracts were evaporated to dryness at 35–40°C. The residue was transferred to a glass tube, by use of three 1-mL portions of methanol, and concentrated to 2 mL at 35°C by use of an N-Evap112 nitrogen evaporator (Organomation Associates, USA). Control experiments without fortification were performed in the same way but without addition of the pesticides. Three replicate analyses were performed for both sets of experiments.

Table 1

Regression equations and detection limits of selected fungicides.

Fungicide	R_F	Regression equation	Correlation coefficient [%]	Detection limit [ng]
Tricyclazole	0.26	$Y = 0.3209X + 22.31$	0.9909	12
Thiram	0.65	$Y = 0.3534X + 10.42$	0.9931	30
Folpet	0.77	$Y = 0.2462X + 22.32$	0.9980	40

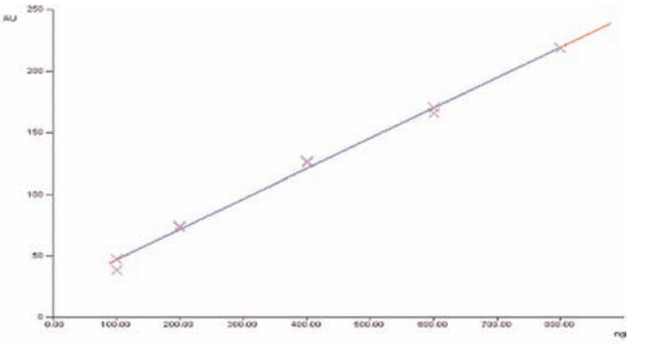

Figure 3

Calibration plot for determination of folpet.

2.4 Chromatography

Chromatography was performed on 10 cm × 20 cm glass-backed silica gel $60F_{254}$ HPTLC plates (E. Merck Germany) previously prewashed by development with methanol and activated at 110°C for 30 min. Solutions were applied as 6.0-mm bands by means of a Camag (Muttenz, Switzerland) Linomat V applicator equipped with a 100-µL syringe. The plates were developed with hexane–acetone 6:4 (*v*/*v*) as mobile phase by the linear ascending technique in an unsaturated Camag glass twin-trough chamber. The development distance was 70 mm. After development the plates were freed from mobile phase in a stream of air and evaluation was performed densitometrically with a Camag TLC Scanner 3 controlled by an external PC running Wincats software (Version m1.4.2). Absorbance was measured at 235 nm (**Figure 1**) using the deuterium lamp. Peak heights were recorded for all the tracks.

3 Results and Discussion

3.1 Optimization of the Chromatography

Initial trial experiments were conducted to select a suitable mobile phase for accurate estimation of the fungicides. Finally, hexane–acetone 6:4 (*v*/*v*) was selected as the optimum for development of the chromatogram. This mobile phase gave dense and compact bands on the plate and well resolved peaks on the densitograms. The R_F values of tricyclazole, thiram, and folpet were 0.26, 0.65, and 0.77, respectively. Typical chromatograms obtained from tricyclazole, thiram, and folpet are shown in **Figure 2**.

Table 2

Recovery of the fungicides from tomatoes.

Fungicide	Fortification level [mg kg^{-1}] (n = 3)	Volume applied [µL]	Recovery [%]	RSD [%]
Tricyclazole	0.12	50	92.62	7.23
	0.6	25	83.50	10.54
	3.0	10	81.98	0.31
Thiram	0.2	50	72.82	22.06
	1	25	67.66	12.09
	5	10	73.92	4.63
Folpet	0.4	50	98.02	0.13
	2	25	85.99	4.33
	10	10	87.08	2.84

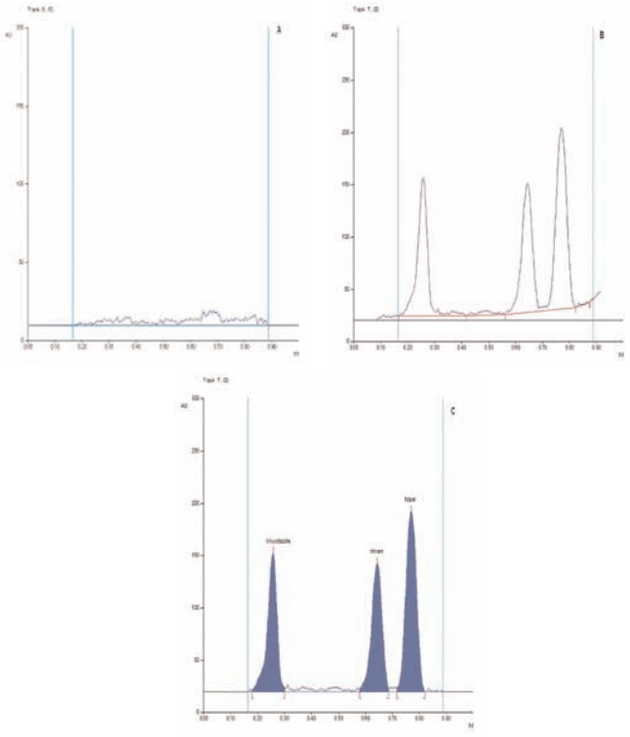

Figure 4

Chromatograms obtained from tricyclazole, thiram, and folpet in an extract from tomato: A, unspiked sample; B and C, spiked samples.

3.2 Calibration Plot

A series of standard solutions was prepared from the stock solution of tricyclazole, thiram, and folpet such that amounts in the range 10–300 ng were applied to the plate. Calibration plots were constructed for each fungicide by plotting the peak height (Y-axis) against the amount of each fungicide (X-axis). The linear regression equations for the fungicides are given in **Table 1**. The calibration plot for folpet is shown in **Figure 3**.

Limits of detection for tricyclazole, thiram, and folpet were measured by reducing the amounts of the fungicides applied to the plates; they were 12, 30, and 40 ng, respectively (Table 1). The sensitivity of UV detection of the fungicides is quite good.

3.3 Recovery Studies

Recoveries of tricyclazole, thiram, and folpet from spiked tomatoes (fortification levels 0.12–3.0, 0.2–5.0, and 0.4–10.0 mg kg^{-1}, respectively) by this method were 67.66–73.92, 81.98–92.62, and 85.99–98.02%, respectively, and the relative standard deviations (RSD) were 4.63–22.06, 0.31– 7.23, and 0.13–4.33%, respectively. Each measurement was performed in triplicate. The precision and accuracy of this HPTLC method were generally fit for analysis of residues of the fungicides in tomatoes (**Table 2**).

Because there was no interfering substance on the plates from samples of tomatoes, the method is specific for determination of selected fungicides residues in this fruit. HPTLC chromatograms obtained from an extract of spiked tomatoes are shown in **Figure 4**.

4 Conclusion

In the work discussed in this paper successfully developed a HTPLC method with densitometric scanning for simultaneous determination of tricyclazole, thiram, and folpet residues in tomatoes. Because no sample purification is needed, this HPTLC method enables simpler, faster, and less expensive analysis. Determinations of pesticide residues in food and environmental matrixes by HPTLC will be of high importance in the foreseeable future [9].

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