Liquid Chromatographic Determination of Novel Aminithiazolecarboxamide Fungicide Residues in Soil and Crops Using Online Solid-Phase Extraction

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Abstract

A simple and convenient liquid chromatographic method has been developed and applied to the analysis of the novel aminothiazolecarboxamide fungicide, ethaboxam, in soil and crops. After the isolation and concentration of analyte from soil and crops, clean-up and separation of sample solutions are performed by high-performance liquid chromatography with online solid-phase extraction. Good linearity ($r^2 > 0.9995$), recovery [for soil, 95.3–98.4%, and crops (grape, red pepper), 92.9–95.9%], and repeatability are achieved in the calibration range of 0.1–10.3 µg/mL. The limit of detection is the 2.5 parts per billion (ppb) (40 g of soil) and the 20 ppb (25 g of crops), respectively. This assay method shows the suitability for the residual analysis of ethaboxam in soil and crops.

Introduction

Many compounds containing the 2-aminothiazole components have been found to show a wide range of biological activities. Also, this aminothiazole groups have found high fungicidal activity in agricultural application (1). High efficacy against *Oomycete* fungi such as *Plasmopara viticola* and *Phytophthora infestans* is very considerable because the *Oomycete* family has been found to be highly resistant to the phenyl amide fungicide (e.g., metalaxyl). Recently, the 2-aminothiazole group introduced novel aminothiazolecarboxamide derived fungicide, ethaboxam (2–3). Its efficacy has been evaluated, showing a high fungicidal activity against *Plasmopara viticola* and *Phytophthora infestans* (4).

Currently, most sample pretreatment relating chromatography apply labor and time consuming procedure such as a liquid–liquid extraction (LLE) or offline solid-phase extraction (SPE) (or both). First, a conventional off-line SPE method and applied sample clean-up for analytical method development of residual analysis in soil and crops was selected. However, this offline SPE method showed interference in high-performance liquid chromatography (HPLC) with UV detection. Previously published were LC methods for the determination of herbicide residues in soil using online SPE with column switching (5). Compared with offline SPE, the online SPE method is able to perform sequential and automated analysis including sample extraction, clean-up, and separation. Its merits are free of time and labor consumption in routine analysis. These analytical techniques have already been put into widespread use and are applicable in drug analysis in biological fluids (6–10).

In this study, the development of an HPLC method for the residual analysis of novel aminothiazolecarboxamide fungicide in soil and crops using automated online column switching techniques is described.

Experimental

Chemicals and reagents

Ethaboxam was supplied by LG Life Science Ltd. (Figure 1). All solvents used in this study were HPLC grade and purchased from J.T. Baker (Phillipsburg, NJ). Trifluoroacetic acid (TFA) was purchased from Aldrich (Milwaukee, WI). Distilled and deionized water from a Milli-Q water purification system was used (Millipore, Bedford, MA).

Chromatographic conditions

Chromatography was carried out on Waters Alliance 2690 HPLC system, gradient programmable Waters 510 pump, six port

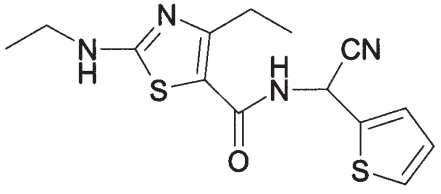

Figure 1. The structure of ethaboxam.

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column switching valve (Rheodyne Lab pro) and Waters 484 tunable wavelength detector (Waters, Milford, MA). Data process was performed on a model 746 integrator (Waters). Sample clean-up was accomplished with a Microsorb CN (4.6- × 100-mm i.d., 5 µm) (Varian, Palo Alto, CA). The columns used for the analysis and the trapping analyte were a Capcellpak C_{18} (4.6- × 250-mm i.d., 5 µm) (Shiseido, Tokyo, Japan) and a Spheri-5 polyfunctional C_{18} (4.6- × 30-mm i.d., 5 µm) (Applied Biosystems, Foster City, CA), respectively.

The mobile phase of the sample clean-up process consisted of a mixture of solvents A [0.1 % TFA in acetonitrile (ACN)] and B (0.1% TFA in water) as follows: 20% of A for 0–10 min, 80% of A for 10–20 min, and 10% of A for 20–37 min. Sample analysis was performed as follows: 25% of A for 0–22 min, 75% of A for 22–35 min, 25% of A for 35–37 min. The system set-up of the online column switching HPLC is given in Figure 2 and the timetable of the analysis in Table I. A 50-µL of sample solution was injected on online column switching HPLC. The sample was loaded on the clean-up column, in which sample clean-up took place. Then, after valve switching at 6 min, the clean-up column was connected to the trap column into which the sample was transferred. The clean-up column was disconnected at 10 min by valve switching while the sample was back-flushed from the trap column to the analytical column and then progressed to the washing and re-equilibrating step. All mobile phase used was filtered and degassed through a 0.45-µm membrane filter. Sample clean-up and analysis processes were performed at 1.0 and 1.2 mL/min of flow rate, respectively. The temperatures of clean-up, trap, and analytical column used were controlled at a constant 3°C. The analyte was monitored by UV detection at a wavelength of 240 nm.

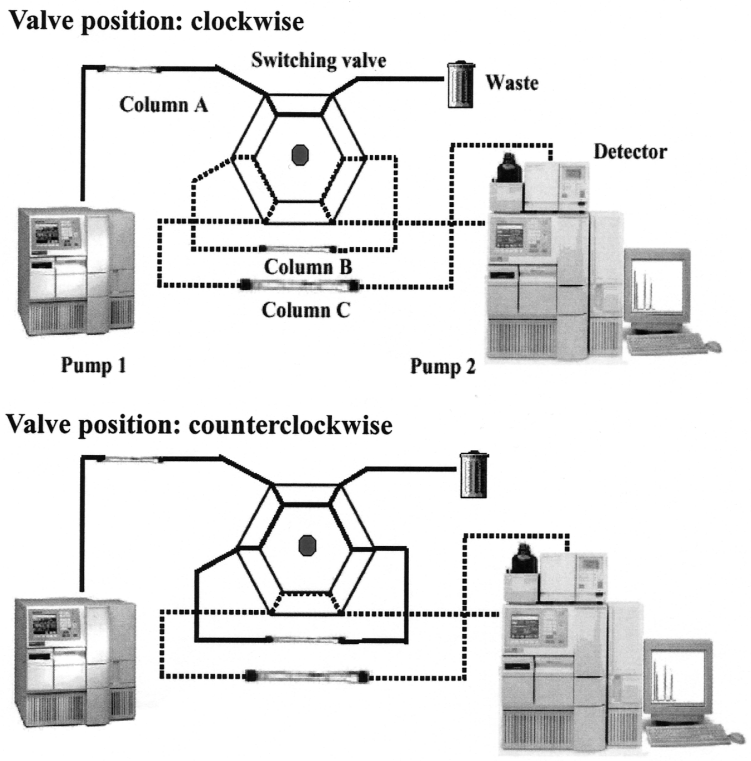

Figure 2. System set-up of online column switching HPLC.

Table I. The Timetable of Online Column Switching HPLC*

Time (min)	Switching valve position	Flow stream	Comment
0–6	+	Pump 1–col. A–waste, Pump 2–col. B–col. C–UV detector	Sample injection & clean-up
6–10	–	Pump 1–col. A–col. B–waste, Pump 2–col. A–UV detector	Sample trap
10–End	+	Pump 1–col. A–waste, Pump 2–col. B–col. C–UV detector	Sample analysis & re-equilibrium step of clean-up column

* Clockwise (+) and counterclockwise (–). Col. A, clean-up column; col. B, trap column; and col. C, analysis column.

Sample preparation

A primary stock standard was prepared at the concentration of 1.0 mg/mL by accurately weighing 100.0 mg of ethaboxam into a 100-mL volumetric flask and filled to volume with water. Aliquots of the primary stock standard solution were transferred to screw-capped glass vials. Further dilutions were performed at the concentration of 10, 5, 2, 1, 0.2, and 0.1 µg/mL. An amount of 40 g of soil (25 g of crops) was weighed into 200-mL bottles equipped with Teflon-lined caps. Recovery samples were prepared by spiking the appropriate standard solution into the soil or crop to obtain the concentration of 0.25 and 1.00 µg/g for soil and 1.00 and 4.00 µg/g for crops, respectively. Each sample was suspended in 100 mL of ethyl acetate–acetone (3:2) solvent and shaken for 2 h. The extracted samples were filtered. The filtrate was completely dried under nitrogen at room temperature, then 1 (soil) or 5 mL (crops) of 40% aqueous acetonitrile solution was added to each residue. The resulting solutions were filtered with 0.45-µm membrane syringe filter and transferred to insert of autosampler vials. An amount of 50 µL was analyzed by online column switching HPLC.

Results and Discussion

In order to develop the sample clean-up method (the first step) retention time of ethaboxam was determined on the clean-up column. The sample solution of standard and blank (soil and crops) were then examined on the online column switching HPLC (Figure 3). The optimal condi-

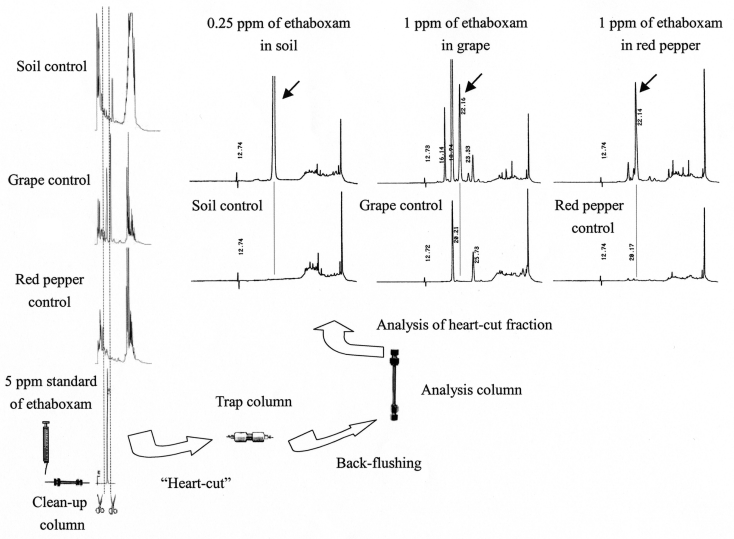

Figure 3. Flow diagram and result chromatograms of residual analysis in soil and crops.

Table II. Recovery of the Online SPE Analysis in Soil and Crops

	Concentration spiked (ppm)	Recovery (%) Rep. 1	Rep. 2	Rep. 3	Average (± SD*)	LOD (ppm)	LOD quantity (ng)
Soil 1	0.25	95.3	92.1	98.4	95.3 (± 3.2)	0.0025	5
	1.00	96.3	97.2	95.6	96.4 (± 0.8)		
Soil 2	0.25	94.0	100.4	100.8	98.4 (± 3.8)		
	1.00	91.3	99.6	100.4	97.1 (± 5.0)		
Grape	1.00	93.5	94.0	92.4	93.3 (± 0.8)	0.020	
	4.00	93.4	95.0	90.3	92.9 (± 2.4)		
Red pepper	1.00	98.1	93.7	96.0	95.9 (± 2.2)		
	4.00	95.0	92.3	95.6	94.3 (± 1.8)		

* SD, standard deviation.

tion of mobile phase in the sample clean-up process was found to be 20% ACN containing 0.1% TFA. The analyte was eluted from 6 to 10 min on the clean-up column. A switching interval of 4.0 min was selected for elution of analyte from the clean-up column to trap column. The analyte for this switching time was quantitatively transferred to the trap column. As a final step, 10 min after sample injection the switching valve was returned to its initial position. The analyte was eluted to the analytical column from the trap column in the back-flush mode. Because of the strong eluting strength of the mobile phase consisting of 25% aqueous ACN containing 0.1% TFA, the analyte was desorbed from the trap column. Column washing and re-equilibrating steps were performed on the clean-up column and new sample application was carried out 10 min later. Ethaboxam was eluted at 22.1 min without interference; good peak shape was achieved as shown in Figure 3.

For the test of specificity, different sources of blank soil and crops were checked for the presence of interfering peaks in their chromatogram. In addition, the chromatographic system was checked for injection carry-over. There were no interfering peaks present in chromatograms corresponding to the retention time of ethaboxam as shown in Figure 3. If there is an interference of excess 50% of the limit of quantitation (LOQ) in one or more blank samples, it is unlikely that the method will be sufficiently specific to be applied to residual analysis. Therefore, the method would need to undergo further method development to obtain the required specificity or the LOQ would be recalcu-

Table III. Results of the Online SPE Analysis in Laboratory Soil

Soil	Treatment quantity of ethaboxam	Number of treatment	Days after treatment	Residues of ethaboxam (ppm) Rep. 1	Rep. 2	Rep. 3	Average (± SD)	Half-life (days)
Lab soil 1	Blank	–	–	< 0.0025	< 0.0025	< 0.0025	< 0.0025	Residues (ppm) = $0.762 \times e^{-0.0415t}$ $r^2 = 0.968$ $t_{1/2}$ = 17 days
	1 ppm	1	0	0.760	0.774	0.751	0.762 (± 0.012)	
			1	0.789	0.823	0.823	0.812 (± 0.020)	
			3	0.465	0.505	0.473	0.481 (± 0.021)	
			7	0.414	0.416	0.420	0.417 (± 0.003)	
			14	0.382	0.386	0.392	0.387 (± 0.005)	
			30	0.164	0.184	0.175	0.174 (± 0.010)	
			60	0.056	0.062	0.058	0.059 (± 0.003)	
Lab soil 2	Blank	–	–	< 0.0025	< 0.0025	< 0.0025	< 0.0025	Residues (ppm) = $0.767 \times e^{-0.0461t}$ $r^2 = 0.992$ $t_{1/2}$ = 15 days
	1 ppm	1	0	0.786	0.748	0.768	0.767 (± 0.019)	
			1	0.704	0.741	0.753	0.733 (± 0.026)	
			3	0.619	0.612	0.613	0.615 (± 0.004)	
			7	0.449	0.447	0.448	0.448 (± 0.001)	
			14	0.312	0.342	0.320	0.325 (± 0.016)	
			30	0.191	0.171	0.185	0.182 (± 0.010)	
			60	0.044	0.047	0.045	0.045 (± 0.002)	

Table V. Results of the Online SPE Analysis in Crops

Crops	Number of treatment	Treatment days before harvest	Residues of ethaboxam (ppm) Rep. 1	Rep. 2	Rep. 3	Average (± SD)
	Blank	–	< 0.020	< 0.020	< 0.020	< 0.020
	4	50, 40, 30, 21	0.318	0.316	0.300	0.311 (± 0.010)
	4	30, 21, 14, 7	0.618	0.711	0.611	0.645 (± 0.056)
	4	21, 14, 7, 3	0.848	0.918	0.823	0.862 (± 0.049)
Grape	5	50, 40, 30, 21, 14	0.270	0.368	0.365	0.334 (± 0.056)
	5	40, 30, 21, 14, 7	1.055	1.041	0.965	1.02 (± 0.048)
	5	30, 21, 14, 7, 3	1.807	1.595	1.658	1.687 (± 0.109)
	6	50, 40, 30, 21, 14, 7	0.851	1.215	1.067	1.044 (± 0.183)
	7	50, 40, 30, 21, 14, 7, 3	2.431	2.749	2.664	2.615 (± 0.165)
	Blank	–	< 0.020	< 0.020	< 0.020	< 0.020
	3	30, 21, 14	0.723	0.725	0.731	0.726 (± 0.004)
	3	21, 14, 7	1.045	0.918	1.004	0.989 (± 0.065)
	4	30, 21, 14, 7	1.044	1.009	1.108	1.054 (± 0.050)
	4	21, 14, 7, 3	1.122	1.097	1.024	1.081 (± 0.051)
Red pepper	5	45, 30, 21, 14, 7	1.352	1.329	1.577	1.1419 (± 0.137)
	5	30, 21, 14, 7, 3	1.559	1.636	1.602	1.599 (± 0.039)
	6	45, 30, 21, 14, 7, 3	1.643	1.684	1.669	1.665 (± 0.021)
	6	30, 21, 14, 7, 3, 1	2.422	2.398	2.103	2.308 (± 0.178)
	7	45, 30, 21, 14, 7, 3, 1	4.122	4.188	4.127	4.146 (± 0.037)

lated. Six calibration standards were employed over the range 0.1 to 10 µg/mL and determined for five separate days. All correlation coefficients (r^2) were better than or equal to 0.9995. The precision and accuracy showed no significant deviation and were measured with acceptable value, as shown in Table II. Intrabatch (within-a-day assay, n = 3) precision (standard deviation) and accuracy (%) were 0.8–5.0 and 95.3–98.4% for soil and 0.8–2.4 and 92.9–95.9% for crops. The limit of detection (LOD) of ethaboxam in soil and crops was 2.5 ppb (40 g of soil) and 20 ppb (25 g of crops). The results of residual analysis of ethaboxam in soil are shown in Table III and IV. The half-life was 15–17 days for laboratory soil and 9 days (the number of times for fungicide treatment, 1) and 11–12 days (the number of times for fungicide treatment, 2) for field soil. Table V shows results of ethaboxam residues in crops. The number of treatment and treatment days were varied before harvest. In the case of seventh treatment, ethaboxam residues were 2.615 and 4.146 ppm, respectively, in grape and red pepper.

Table IV. Results of the Online SPE Analysis in Field Soil

Soil	Treatment quantity of ethaboxam	Number of treatment	Days after treatment	Residues of ethaboxam (ppm) Rep. 1	Rep. 2	Rep. 3	Average (± SD)	Half-life (days)
	Blank	–	–	< 0.0025	< 0.0025	< 0.0025	< 0.0025	
			0	3.850	4.007	3.731	3.863 (± 0.138)	
			1	2.498	2.662	2.694	2.618 (± 0.105)	Residues (ppm) = $3.863 \times e^{-0.0816t}$
			3	2.243	2.381	2.092	2.239 (± 0.145)	$r^2 = 0.989$
960 g/a		1	7	1.731	1.933	1.711	1.792 (± 0.123)	$t_{1/2}$ = 9 days
			14	1.570	1.560	1.536	1.555 (± 0.017)	
			32	0.211	0.254	0.212	0.226 (± 0.025)	
Field soil 1			62	0.020	0.023	0.021	0.021 (± 0.002)	
			0	7.855	8.341	8.662	8.286 (± 0.406)	
			1	4.054	3.939	3.843	3.945 (± 0.106)	
			3	3.239	3.170	3.360	3.256 (± 0.096)	Residues (ppm) = $8.286 \times e^{-0.0648t}$
	960 g/a	2	7	3.590	3.726	3.250	3.522 (± 0.245)	$r^2 = 0.929$
			14	0.952	1.038	0.973	0.988 (± 0.045)	$t_{1/2}$ = 11 days
			32	0.900	0.883	0.952	0.912 (± 0.036)	
			62	0.080	0.071	0.093	0.082 (± 0.011)	
	Blank	–	–	< 0.0025	< 0.0025	< 0.0025	< 0.0025	
			0	5.859	6.399	5.519	5.926 (± 0.444)	
			1	3.528	3.960	3.783	3.757 (± 0.217)	Residues (ppm) = $5.926 \times e^{-0.0792t}$
	960 g/a	1	7	1.265	1.267	1.270	1.267 (± 0.003)	$r^2 = 0.956$
			14	0.682	0.730	0.757	0.723 (± 0.038)	$t_{1/2}$ = 9 days
			32	0.292	0.324	0.368	0.328 (± 0.038)	
Field soil 2			62	0.028	0.024	0.020	0.024 (± 0.004)	
			0	6.080	6.361	5.163	5.868 (± 0.627)	
			1	3.267	3.543	3.811	3.54 (± 0.272)	
			3	3.343	3.406	2.763	3.17 (± 0.354)	Residues (ppm) = $5.868 \times e^{-0.0595t}$
	960 g/a	2	7	2.515	3.401	3.074	2.997 (± 0.448)	$r^2 = 0.966$
			14	2.758	3.110	2.547	2.805 (± 0.284)	$t_{1/2}$ = 12 days
			32	0.470	0.446	0.528	0.481 (± 0.042)	
			62	0.128	0.121	0.131	0.127 (± 0.005)	

Conclusion

Ethaboxam in soil and crops was successfully separated by automated online column switching HPLC without interference. The quantitative analysis of ethaboxam residues could be accomplished within 35 min including sample clean-up and analysis. The results of recovery, precision, and sensitivity were acceptable in residual analysis.

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