Journal of Health Science, **49**(6) 492–496 (2003)

Determination of Fenoxaprop-Ethyl in Agricultural Products by HPLC with Photometric Detection and Mass Spectrometry

Susumu Ishimitsu,*, [1] Kimihiko Yoshii, Yukari Tsumura, and Yasuhide Tonogai

National Institute of Health Sciences, Osaka Branch, Division of Food Chemistry, 1–1–43 Hoenzaka, Chuo-ku, Osaka 540–0006, Japan

(Received March 26, 2003; Accepted July 31, 2003)

A method was developed for the determination of 6-chloro-2,3-dihydrobenzoxazol-2-one (CDHB) generated by the acid decomposition of fenoxaprop-ethyl and fenoxaprop in agricultural products. Fenoxaprop-ethyl and fenoxaprop were extracted from agricultural products using acetonitrile, and the extract was acidified by 0.5 mol/l hydrochloric acid to make CDHB. The CDHB was extracted again into ethyl acetate and cleaned up using Sep-Pak® Plus Diol and Bond Elut® AccuCAT cartridge columns. The recoveries from brown rice, wheat, cotton seed, onion, carrot, sweet potato and cabbage exceeded 70% by HPLC (UV). However, soybean, green soybeans and kidney beans showed many interference peaks in the UV spectra, and Florisil column chromatography was necessary for additional purification. The fortified peaks were confirmed by liquid chromatograph/mass spectrometry (LC/MS) with electrospray ionization (ESI), and the CDHB peak was quantitatively determined. Almost the same result was obtained by HPLC (UV) and LC/MS selected ion monitoring (SIM). Consequently, for agricultural products which included many interfering peaks during UV detection, using LC/MS (SIM) significantly improved the quantitative and qualitative analyses and the number of interfering peaks was fewer than by UV detection.

[1]Present address: National Institute of Health Sciences, Division of Safety Information on Drug, Food and Chemicals, 1–18–1, Kamiyoga, Setagayaku, Tokyo 158–8501, Japan
*To whom correspondence should be addressed: National Institute of Health Sciences, Division of Safety Information on Drug, Food and Chemicals, 1–18–1, Kamiyoga, Setagayaku, Tokyo 158–8501, Japan. Tel.: +81-3-3700-1141; Fax: +81-3-3700-1483; E-mail: ishimits@nihs.go.jp

Key words —— fenoxaprop-ethyl, pesticide, HPLC, liquid chromatograph/mass spectrometry

INTRODUCTION

The Ministry of Health, Labour and Welfare (MHLW) in Japan has set maximum residue limits (MRL) in agricultural products for 229 pesticides under the Food Sanitation Law.[1] Among them, we proposed HPLC determination as the official analytical method for emamectin,[2] clethodim,[3] azimsulfuron, flazasulfuron and halosulfuron-methyl[4] in these products. The MHLW intends to set the MRL and the official analytical method for fenoxaprop-ethyl in agricultural products in the near future.

Fenoxaprop-ethyl is widely used in Japan[5] as an herbicide to control annual and perennial grasses such as grains, seeds and vegetables. Fenoxaprop-ethyl is gradually decomposed from fenoxaprop to 6-chloro-2,3-dihydrobenzoxazol-2-one (CDHB) in the field.[6] The Ministry of Agriculture, Forestry and Fisheries earlier declared the official analytical method only for the determination of fenoxaprop-ethyl in agricultural products to be by gas chromatography with nitrogen phosphorus detector (GC-NPD).[7] Hirahara et al. reported the determination of fenoxaprop-ethyl in polished rice and brown rice using HPLC instead of GC.[8] However, there are some objections to these analytical methods, and therefore the determination of fenoxaprop and CDHB in agricultural products has not yet been measured.

In the present study, we examined the method of determining CDHB generated by the acid decomposition of fenoxaprop-ethyl and fenoxaprop. We propose that our established method, presented here, be adopted as the official Japanese analytical method for the determination of fenoxaprop-ethyl in agricultural products.

MATERIALS AND METHODS

Samples —— Brown rice, wheat, soybean, onion, carrot, sweet potato, green soybeans, kidney beans and cabbage were purchased from local markets in Osaka. The cotton seeds were provided by MHLW.
Reagents —— Acetone, acetonitrile, *n*-hexane and ethyl acetate were of pesticide residue analytical

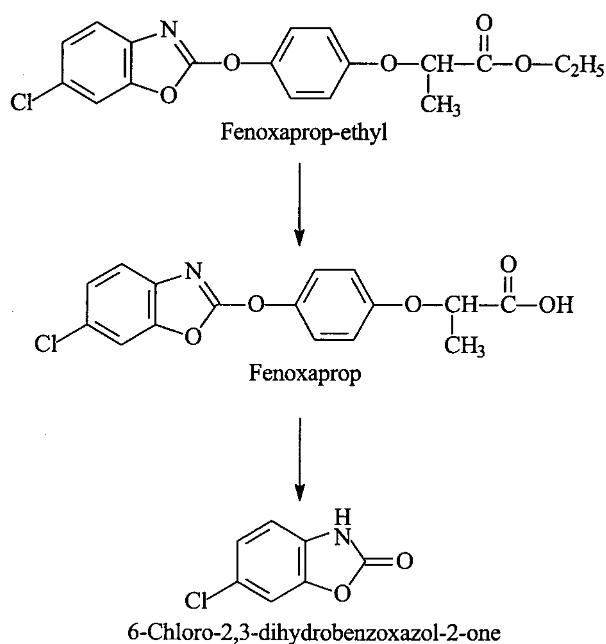

Fig. 1. Chemical Structures of Fenoxaprop-Ethyl and Its Metabolites

grade (Wako Pure Chemical Industries, Ltd., Osaka, Japan).

Hydrochloric acid, sodium chloride, trichloroacetic acid, diatomaceous earth Celite 545, sodium sulfate and Florisil PR: Special grade (Wako Pure Chemical Industries).

Sodium Sulfate and Florisil PR: Activated at 120°C for 12 hr.

Standard Materials: Fenoxaprop-ethyl and fenoxaprop were obtained from Hayashi Pure Chemical Industries, Ltd., Osaka. CDHB was provided by MHLW. The chemical structures of these compounds are shown in Fig. 1.

Pesticide Standard Solution: Standard solutions of fenoxaprop-ethyl, fenoxaprop and CDHB (1000 μg/ml) were prepared by dissolving each pesticide in acetonitrile. For the recovery experiments, the standard solution was diluted with acetonitrile (10 μg/ml).

HPLC: Mobile phase A, 0.01% trichloroacetic acid in distilled water; mobile phase B, acetonitrile.

Liquid Chromatograph/Mass Spectrometry (LC/MS): Mobile phase A, 0.2% acetic acid in distilled water, mobile phase B, 0.2% acetic acid in acetonitrile.

Cartridge Columns: Bond Elut® AccuCAT cartridge column (Varian, Harbor City, CA, U.S.A.). Sep-Pak® Plus Diol cartridge column (Waters Corp., Milford, MA, U.S.A.).

Florisil Column: Add 5 g of a slurry of Florisil PR (60–100 mesh) in *n*-hexane to a glass column (1.5 cm i.d. × 30 cm), followed by 5 g anhydrous sodium sulfate. Electric homogenizer (Nihonseiki, Tokyo Japan). Rotary evaporator (Shibata Scientific Technology, Tokyo).

HPLC and LC/MS Analysis —— *Apparatus*: The HPLC and LC used in our previous study.[4] The MS used a Shimadzu QP-2010.

Operating Parameters of HPLC: The mobile phase flow rate was adjusted to 1.0 ml/min during the analysis. The system was equilibrated at 30% mobile phase B in mobile phase A; a 30-min linear gradient to 100% mobile phase B was begun and held for 5 min. The other conditions were as follows: temperature for column separation, 40°C, and ultraviolet detection wavelength, 235 nm.

Operating Parameters of LC/MS: The stainless steel column (2.0 mm i.d. × 150 mm) was packed with Shim-pack VP-ODS. The mobile phase flow rate was adjusted to 0.2 ml/min during the analysis. The system was equilibrated at 3% mobile phase B in mobile phase A; a 10-min linear gradient to 97% mobile phase B was begun and held for 7 min. When the gradient was completed, the mobile phase was returned to 97% A, 3% B and held for 5 min to re-equilibrate the column. The other chromatographic conditions were as described for the operating parameters of the HPLC.

MS Conditions: Analytical mode, electrospray ionization (ESI negative); drying gas (N_2) flow, 4.5 l/min; probe voltage, 4.5 kV. The selected ion for monitoring was *m/z* 168 for CDHB.

Extraction —— Ten g of the shredded sample (brown rice, wheat, soybean, cotton seed) was placed in a stainless steel cup to which 20 ml of water was added and allowed to stand for 2 hr, and then 100 ml of acetonitrile was added. Twenty g of chopped onion, carrot, sweet potato, green soybeans, kidney beans or cabbage was placed in a stainless steel cup to which 100 ml of acetonitrile was added. The mixture was homogenized for 3 min and then filtered through filter paper with 7 g of Celite 545 (10 mm thickness) into a 300-ml round-bottom flask. The extract was rinsed, filtered with 50 ml of acetonitrile, and evaporated to dryness with a rotary evaporator. The extract was transferred to a 300-ml separatory funnel to which was added 20 g of sodium chloride, 100 ml of 0.5 mol/l hydrochloric acid solution and 100 ml of 30% ethyl acetate in *n*-hexane, and vigorously shaken for 5 min. Another 50 ml of 30% ethyl acetate in *n*-hexane was added, and

the solution was shaken again for 5 min. The organic layers were collected in a 300-ml round-bottom flask and evaporated to dryness with a rotary evaporator.

In the case of brown rice, wheat, soybean, and cotton seed, the organic layers were collected in a 200-ml Erlenmeyer flask, dehydrated with *ca*. 20 g of anhydrous Na_2SO_4, and allowed to stand for 15 min. They were then filterd through filter paper to separate the anhydrous Na_2SO_4. The flask was then rinsed with an additional 20 ml of 30% ethyl acetate in *n*-hexane and evaporated to dryness with a rotary evaporator. The extract was transferred to a 100-ml separatory funnel, to which was added 30 ml of *n*-hexane and 30 ml of *n*-hexane saturated with acetonitrile, and vigorously shaken for 5 min. Another 30 ml of *n*-hexane saturated with acetonitrile was added, and the solution was shaken again for 5 min.

The organic layers were collected in a 300-ml round-bottom flask and evaporated to dryness with a rotary evaporator.

Acid Decomposition —— The extract was added to 10 ml of 0.5 mol/l hydrochloric acid solution and warmed at 50°C for 30 min. The solution was transferred to a 300-ml separatory funnel, to which was added 100 ml of 10% sodium chloride solution and 100 ml of 30% ethyl acetate in *n*-hexane, and vigorously shaken for 5 min. Another 50 ml of 30% ethyl acetate in *n*-hexane was added, and the solution was shaken again for 5 min. The organic layers were collected in a 200-ml Erlenmeyer flask, dehydrated with *ca*. 20 g of anhydrous Na_2SO_4, and allowed to stand for 15 min, then filtered through filter paper to separate the anhydrous Na_2SO_4. The flask was rinsed with an additional 20 ml of 30% ethyl acetate in *n*-hexane and evaporated to dryness with a rotary evaporator.

Cleanup ——

Procedure A: A Sep-Pak® Plus Diol connected to Bond Elut® AccuCAT cartridge columns were conditioned with 10 ml of *n*-hexane before use. The residue was dissolved in 5 ml of *n*-hexane and charged onto the columns. The columns were rinsed with 30 ml of 5% acetone in *n*-hexane. A Sep-Pak® Plus Diol cartridge column was then detached from the Bond Elut® AccuCAT cartridge column and eluted with 30 ml of 50% acetone in *n*-hexane. The eluate was evaporated to dryness with a rotary evaporator and dissolved in 1 ml of acetonitrile (test solution A: brown rice, wheat, cotton seed, onion, carrot, sweet potato, cabbage).

Procedure B: A Florisil column was conditioned with 10 ml of *n*-hexane before use. Test solution A (soybean, green soybeans, kidney beans) was evaporated to dryness with a rotary evaporator. The residue was dissolved in 5 ml of *n*-hexane and charged onto the column. The column was rinsed with 150 ml of 5% acetone in *n*-hexane, followed by elution with 150 ml of 30% acetone in *n*-hexane. The eluate was evaporated to dryness with a rotary evaporator and dissolved in 1 ml of acetonitrile (test solution B: soybean, green soybeans, kidney beans).

Quantification —— The sample solution was automatically injected into the HPLC and the LC/MS systems for residue analysis. The concentration of CDHB was calculated based on a peak area calibration curve. This curve was constructed with CDHB generated from fenoxaprop-ethyl by acid decomposition with hydrochloric acid. The injection was performed three times for each sample to test the reproducibility.

Recovery Test —— Chopped samples were fortified with 0.1 or 0.2 μg/g of fenoxaprop-ethyl. The recovery data represent three replications.

RESULTS AND DISCUSSION

HPLC Retention Time, Linearity and Limit of Detection

The retention times of CDHB, fenoxaprop and fenoxaprop-ethyl are 9.5, 17.5 and 23.8 min, respectively. In the present study, trichloroacetic acid was used as the mobile phase; acetic acid could not be used because CDHB was detected as a broad peak.

The linear dynamic range of the detector response at 235 nm for CDHB was examined and appeared to be from 0.05 to 2 μg/ml injected on-column. The detection limits of the analytes in agricultural products were 0.02 μg/ml for CDHB (S/N > 3).

Acid Decomposition

In the first step of our study, we attempted the determination of fenoxaprop generated by the acid decomposition of fenoxaprop-ethyl. However, fenoxaprop was not detected by HPLC; hence, this compound is not resistant to acids, and it is necessary to determine CDHB. We examined the percent reaction from fenoxaprop-ethyl and fenoxaprop to CDHB by acid decomposition with hydrochloric acid. This result is shown in Table 1. The recovery of CDHB generated from fenoxaprop-ethyl was calculated based on the corresponding CDHB standard, and the optimum decomposition conditions were found to be around 30 min at 50°C. The same result

Table 1. Recovery of CDHB from Fenoxaprop-Ethyl and Fenoxaprop by Acid Decomposition with Hydrochloric Acid

Temperature (°C)	Time (min)	Recovery of CDHB(%) Fenoxaprop-ethyl	Fenoxaprop
40	30	89.7	88.4
	60	96.0	95.1
50	30	95.5	95.5
	60	95.6	94.2
60	30	95.7	94.8
	60	95.0	95.1

Solution containing 2 μg of fenoxaprop-ethyl and fenoxaprop in 10 ml of 0.5 mol/l hydrochloric acid was incubated at several temperatures for different periods. After incubation, the preparation of the test solution was as described in the Extraction section. The calculated recoveries of CDHB generated from fenoxaprop-ethyl and fenoxaprop are based on the corresponding CDHB standard.

was obtained from fenoxaprop to CDHB.

Cleanup

The extracts of the samples before cleanup were directly injected into the HPLC. There were too many interfering peaks to measure on the chromatogram, therefore, a cleanup procedure was necessary before the HPLC analysis. In this study, several cartridge columns (Sep-Pak® Plus Diol and Bond Elut® AccuCAT, Certify II, SAX, PRS, NH2, SCX and PSA) were tested, and the recoveries and cleanup of the extract were compared for each column. The Sep-Pak® Plus Diol (organic solvent type) and Bond Elut® AccuCAT (ion exchange type) cartridge columns gave the best recovery. The obtained fortified samples of brown rice, wheat, soybean, cotton seed, onion, carrot, sweet potato, green soybeans, kidney beans and cabbage were injected into the HPLC. Except for the soybean, green soybeans and kidney beans, the recovery and cleanup was satisfactory using these 2 columns. Therefore, a second cleanup with another column was necessary for the three beans. We evaluated Florisil and Silicagel columns for sample purification. Using a Silicagel column, CDHB from agricultural products could not be measured because too many interfering peaks appeared in the UV spectra; using a Florisil column, the HPLC chromatograms had fewer interfering peaks.

Typical chromatograms of a kidney bean-fortified 0.1 μg/g (A) sample of the pesticides and a kidney bean blank (B) are shown in Fig. 2. The HPLC chromatogram of this sample shows an interfering peak close to that of CDHB.

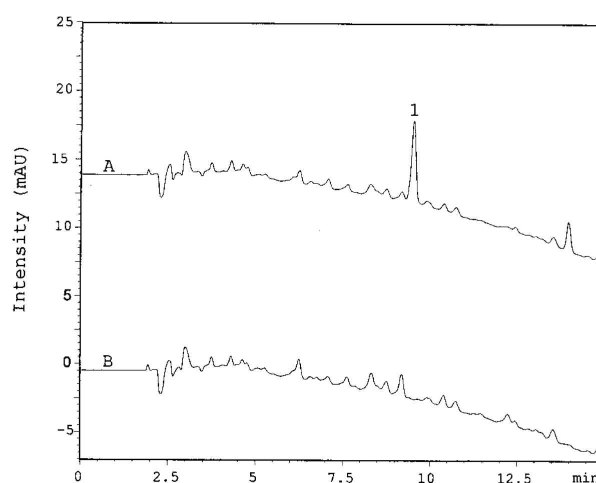

Fig. 2. Typical HPLC Chromatograms of Agricultural Product Samples

Peaks: 1 = 6-Chloro-2,3-dihydrobenzoxazol-2-one. Chromatograms are kidney bean-fortified to 0.1 μg/g (A) and the kidney bean blank (B). Twenty μl of a sample was injected.

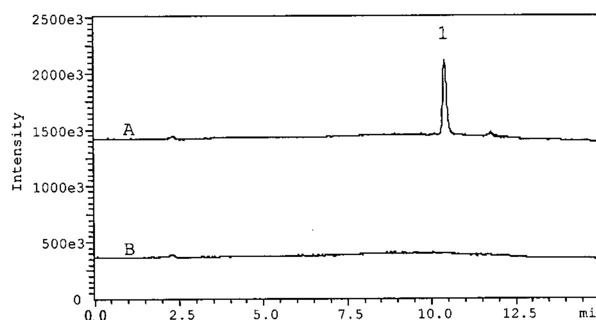

Fig. 3. Selected Ion Monitoring (SIM) Chromatograms of Agricultural Product Samples by LC/MS (ESI)

Peaks: 1 = 6-Chloro-2,3-dihydrobenzoxazol-2-one. SIM chromatograms are kidney bean-fortified to 0.1 μg/g (A) and the kidney bean blank (B). Ten μl of a sample was injected.

The detected peaks in the samples were then measured by LC/MS (ESI). It is well known that LC/MS detection has fewer interfering peaks than UV detection, because LC/MS can detect the specific m/z of a target compound. The selected ion mode for monitoring was m/z 168 for CDHB. Figure 3 shows a kidney bean-fortified 0.1 μg/g (A) sample of the pesticides and a kidney bean blank (B) by LC/MS selected ion monitoring (SIM). The peak of CDHB in the kidney bean blank was not detected. In the LC/MS measurement, trichloroacetic acid is known to interfere with the ionization of an analyte in the mass spectrometer. Using a semi-micro column, we did not use trichloroacetic acid but acetic acid, because the addition of acetic acid

Table 2. Recovery of Fenoxaprop-Ethyl Added to Agricultural Products by HPLC and LC/MS

Sample	Fortified level (μg/g)	HPLC	LC/MS
		Recovery (%, mean ± S.D.)[a]	
Brown rice	0.2[b]	81.3 ± 5.0	84.1 ± 3.3
Wheat	0.2	80.6 ± 3.8	80.0 ± 2.8
Soybean	0.2	76.7 ± 3.5	71.9 ± 2.5
Cotton seeds	0.2	83.1 ± 4.4	78.9 ± 1.9
Onion	0.1[c]	89.9 ± 5.1	87.6 ± 2.8
Carrot	0.1	82.7 ± 2.8	85.6 ± 3.0
Sweet potato	0.1	88.1 ± 2.7	94.3 ± 3.7
Green soybeans	0.1	73.2 ± 2.9	71.6 ± 1.8
Kidney beans	0.1	77.9 ± 3.4	77.5 ± 1.7
Cabbage	0.1	75.2 ± 3.3	73.2 ± 2.5

a) Average ± standard deviation of 3 determinations. *b*) Fortified by fenoxaprop-ethyl (2 μg/10g). *c*) Fortified by fenoxaprop-ethyl (2 μg/20g).

in the mobile phase did not broaden the peak of CDHB. The linear dynamic range for CDHB was examined and appeared to be from 0.01 to 2 μg/ml injected on-column. The detection limits of the analytes in agricultural products were 0.004 μg/ml for CDHB (S/N > 3).

Recovery Test

The recoveries of fenoxaprop-ethyl in 10 agricultural products fortified at 0.1 or 0.2 μg/g are shown in Table 2. The recoveries were greater than 70% by HPLC; the coefficient of variation of the recovery was within 10%. The calculated amount of CDHB generated from fenoxaprop-ethyl, based on the corresponding CDHB standard, and the calculation from CDHB to fenoxaprop-ethyl was found to have a molecular ratio of 2.13. In Japan, the commonly accepted range of recovery is 70–120%. Almost the same result was obtained by LC/MS (SIM). This indicated that when measuring pesticides in agricultural products including many interfering peaks during UV detection, using the SIM of LC/MS significantly improved the quantitative and qualitative analyses. However, use of LC/MS for the quantitative analysis was not adopted as the official Japanese analytical method.

REFERENCES

1) Ministry of Health, Labour and Welfare, Japan (2002) Announcement No. 94.
2) Yoshii, K., Kaihara, A., Tsumura, Y., Ishimitsu, S. and Tonogai, Y. (2001) Simultaneous determination of residue of emamectin and its metabolites, and milbemectin, ivermectin, and abamectin in crops by liquid chromatography with fluorescence detection. *J. AOAC Int.*, **84**, 910–917.
3) Ishimitsu, S., Kaihara, A., Yoshii, K., Tsumura, Y., Nakamura, Y. and Tonogai, Y. (2001) Determination of clethodim and its oxidation metabolites in crops by liquid chromatography with confirmation by LC/MS. *J. AOAC Int.*, **84**, 1172–1178.
4) Ishimitsu, S., Kaihara, A., Yoshii, K., Tsumura, Y., Nakamura, Y. and Tonogai, Y. (2002) Simultaneous determination of azimsulfuron, flazasulfuron and halosulfuron-methyl in grains, seeds, vegetables and fruits by HPLC. *J. Health Sci.*, **48**, 335–340.
5) *Nouyaku Handbook*, Association of Plant Quarantine, Tokyo, pp. 326–327 (1994).
6) *Code of Federal Regulations, No. 40, Part 180.430*, the Office of the Federal Register National Archives and Records Administration, Washington, p. 445 (1995).
7) *Nouyaku Touroku Horyukijun Handbook*, Kagaku Kougyou Nippousha, Tokyo, pp. 668–669 (1998).
8) Hirahara, Y., Ohta, M., Kimura, M., Narita, M., Sekiguchi, Y., Miyodhi, T., Itoyama, T., Miyata, M., Hasegawa, M., Koiguchi, S., Kamakura, K., Maeda, K., Yamana, T. and Tonogai, Y. (1995) Determination of fenoxaprop-ethyl in polished rice and brown rice by HPLC. *J. Food Hyg. Soc. Japan*, **36**, 289–292.