Journal of Food and Drug Analysis
1998. 6(3) : 559-572

藥物食品分析
第六卷 第三期

Analytical Methods for the Determination of Acifluorfen and Bentazone Residues in Crops

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ABSTRACT

Methods using high performance liquid chromatography (HPLC) were developed to determine acifluorfen and bentazone in crops. Twenty samples of dry bean crops and rice were purchased from traditional markets and analyzed. Acifluorfen and bentazone being weak acids, were selectively extracted as ion pair compounds formed with tetrabutylammonium ion into dichloromethane under basic conditions. This technique was used for cleanup prior to determination of acifluorfen and bentazone by reverse phase liquid chromatography with UV detector. A residue of acifluorfen was extracted from crops with acetonitrile and acetonitrile-H_2O (9:1, v/v) and a residue of bentazone was extracted from crops with acetone. The extracts were evaporated to dryness, dissolved in pH 8.0 phosphate buffer, and washed with n-hexane and dichloromethane. Ion pair compounds were formed by adding tetrabutylammonium ion into an aqueous phase, and then extracted into dichloromethane. The dichloromethane phase was evaporated and the residues were dissolved in organic solvent and determined by HPLC. Recovery studies were carried out by spiking the standards of acifluorfen and bentazone at the levels of 0.05~0.15 and 0.25~0.75 ppm, respectively. The average recoveries were determined to be 91.6~97.6% and 88.6~91.7%, respectively. The detection limits in crops were 0.02 and 0.05 ppm for acifluorfen and bentazone, respectively. No residues of acifluorfen or bentazone were detected in dry bean crop samples, nor was bentazone residue found in rice samples.

Key words: **pesticide residues, acifluorfen, bentazone, high performance liquid chromatography (HPLC), ion-pair extraction, crop.**

INTRODUCTION

Acifluorfen and bentazone which belong to the selective contact herbicides are widely used on crops. The chemical structures, physical-chemical properties, reaction mechanisms, and applications of these two herbicides are shown in Table 1 and 2. Acifluorfen is allowed to be applied to dry bean crops and bentazone is permitted to be used in dry bean crops as well as rice in Taiwan according to the "Tolerances for Residues of Pesticides" [1] announced by the Department of Health. The tolerances for residue levels of acifluorfen and bentazone are 0.1 and 0.5 ppm, respectively. Gas

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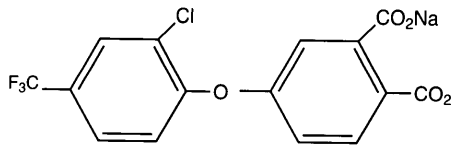
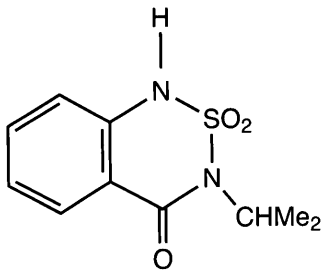
Accepted for Publication: May 13, 1998

Table 1. Chemical structure, physical-chemical properties, mechanism of reaction and applications of sodium acifluorfen [1,2]

Chemical structure	
Chemical names	sodium 5-(2-chloro-α,α,α-trifluoro-*p*-tolyloxy)-2-nitrobenzoate (IUPAC); sodium 5-[2-chloro-4-(trifluoromethyl)phenoxy]-2-nitrobenzoate (CA)
Trade names	Blazer (Rohm&Haas); Tackle 2S (Rhone-Poulenc); RH-6201 (Rohm&Haas)
Physical-chemical properties	MW: 383.7 Physical form: White powder Melting point: 124~125 ℃ Solubility: In water at 25 ℃, > 250 g/l. In methanol, ethanl, ethyl acetate, dimethyl sulphoxide, and dimethylformamide > 500 g/kg at 25 ℃; in chloroform, carbon tetrachloride, dichloromethane, benzene, xylene, and hexane < 10 g/kg at 25 ℃. Stability: Decomposed by UV light, with a half-life of ca.110 hours.
Mode of action	Selective contact herbicide, absorbed by the foliage and roots, with negligible translocation. Activity is enhanced by sunlight.
Applications (Tolerance level)	Dry bean crops (0.1 ppm)
Toxicity to mammals	Acute oral LD_{50} for rats 1300 mg/kg
Degradation and metabolism	In soil, readily photodecomposes to non-herbicidal products. Half-life in soil is ca. 30-60 days. In plants, rapidly metabolized.

chromatography (GC) and high performance liquid chromatography (HPLC) methods are routinely used to determine the residues of both these two herbicides in agricultural products. The GC method [3~5] however has not been adopted by some laboratories because this method requires the derivatization with diazomethane, which is a highly toxic and easily explosive reagent. Because of these factors, we decided to substitute GC method with an HPLC method. It is very important to select the extraction solvents, cleanup conditions, and detectors to reduce the interference from co-extractives when the HPLC method is applied. Ion-pair reverse phase liquid chromatography has been used to analyze the acifluorfen in feeds [6]. HPLC method is also available for determining acifluorfen in soil and water [7]. Takeda et al [8] developed an analytical method which was capable of analyzing five different pesticides (including bentazone) simultaneously. However, this method requires an HPLC equipped with a postcolumn photoreactor system prior to fluorometric detection and the extraction procedure for this method is complex. Hogendoorn and Goewie [9] developed an on-line clean-up column-switching procedure for sample cleanup operation. Both these two methods developed by Takeda et al [8] and Hogendoorn and Goewie [9] are nevertheless unsuitable for most laboratories because they require specialized equipment which is not routinely available in most of the laboratories. In this study, a method of extraction and ion-pair cleanup operation was adopted from the report of Alerblom and Alex [10] with slight modifications

Table 2. Chemical structure, physical-chemical properties, mechanism of reaction and applications of bentazone [1,2]

Chemical structure	
Chemical names	3-isopropyl-(1H)-benzo-2,1,3-thiadiazin-4-one2,2-dioxide (IUPAC); 3-(1-methylethyl)-(1H)-2,1,3-benzothiadiazin-4(3H)-one 2,2-dioxide (CA)
Trade names	Basagran (BASF); BAS 351H (BASF)
Physical-chemical properties	MW: 240.28 Physical form: Colourless crystals. Melting point: 137~139℃ Solubility: In water at 20℃, 500 mg/l. In acetone 1507, ethanol 861, ethyl acetate 650, diethyl ether 616, chloroform 180, benzene 33, cyclohexane 0.2 (all in g/kg at 20℃). Stability: Very resistant to hydrolysis in both in acidic and alkaline media. Decomposed by UV light.
Mode of action	Selective contact herbicide, absorbed mainly by the foliage, with very little translocation, but also absorbed by the roots, with translocation acropetally in the xylem. Inhibits photosynthesis.
Applications (Tolerance level)	Rice and dry bean crops (0.5 ppm)
Toxicity to mammals	Acute oral LD_{50} for rats 1100, mice 400, rabbits 750, cats 500 mg/kg.
Degradation and metabolism	Persistence in soil is < 6 weeks. Rapidly metabolized in tolerant plants, forming extractable conjugates which are incorporated into plant components.

and was applied for the analyses of acifluorfen in dry bean crops as well as bentazone in rice and dry bean crops. The fortification recovery was carried out in triplicate. The purpose of this study was to develop an analytical method with high recovery and good reproducibility. The detection limit of this method was expected to be lower than the tolerance levels announced by Department of Health.

MATERIALS AND METHODS

I. *Materials*

The samples of rice (*Oryza sativa* L.), red bean (*Phaseolus radiatus* L. *var. aurea* Prain), soybean (*Glycine hispida*), mung bean (*Phaseolus aureus*) and peanut (*Arachis hypogaea*) were purchased from traditional markets and retailers.

II. *Chemicals*

A residue grade acetone, hexane, and dichloromethane, LC grade acetonitrile and methanol, and reagent grade anhydrous sodium

sulfate, phosphoric acid, sodium hydrogen phosphate, sodium dihydrogen phosphate, acetic acid, and tetra-n-butyl-ammonium phosphate (TBA) were used in this study. The standards of sodium acifluorfen and bentazone were obtained from Riedel-de Haen AG (Germany). The purity of standards was 99% as labeled.

III. *Methods*

(I) *Preparation of Standard Solutions*

One hundred mg of sodium acifluorfen or bentazone was accurately weighed into a 100 ml volumetric flask and dissolved to volume in acetonitrile (for acifluorfen) or methanol (for bentazone) as a stock solution. This solution was then diluted to a final concentration of 10 μg/ml with acetonitrile (for acifluorfen) or methanol (for bentazone) as a standard solution.

(II) *Sample Preparation*

1. *Analysis of Acifluorfen*

One hundred ml of acetonitrile was added to 40 g milled dry bean crops and shaken thoroughly for 5 min. After filtration, the pellets and container were washed with 100 ml of acetonitrile/water (9/1, v/v), which was then filtered. The combined filtrates were evaporated to dryness at 35~40℃ under reduced pressure. The residue was then dissolved in 50 ml of 0.05M pH 8.0 phosphate buffer solution, then transferred into a separation funnel in which hexane (50 ml) was added and shaken vigorously for 1 min. The hexane phase was discarded. Dichloromethane (50 ml) was then added to separation funnel and shaken for 1 min. After disposal of the dichloromethane layer, the TBA solution (0.02M in pH 8.0 phosphate buffer; 4 ml) was added and shaken for 2 min. This solution was allowed to stand for 10 min and then extracted twice with dichloromethane (70 ml) for 2 min. The combined dichloromethane layers were dehydrated over an anhydrous sodium sulfate and evaporated to dryness at 35~40℃ using a rotary evaporator. The residue was dissolved in 2 ml of acetonitrile and filtered through a 0.45 μm membrane prior to HPLC analysis.

2. *Analysis of Bentazone*

Each test sample was ground with a homogenizer and 20 g was weighed into a container. Eighty ml of acetone was then added and shaken for 5 min. After filtration, the pellets and container were washed with 80 ml of acetone. The combined filtrates were evaporated to dryness at 35~40℃ under reduced pressure. The residue was dissolved in 50 ml of 0.05M pH 8.0 phosphate buffer solution, then transferred into a separation funnel in which hexane (50 ml) was added and shaken vigorously for 1 min. The hexane phase was discarded. The dichloromethane (50 ml) was then added to a separation funnel and shaken for 1 min. After the dichloromethane layer was discarded, the TBA solution (0.02M in pH 8.0 phosphate buffer; 2 ml) was added and shaken for 2 min. This solution was allowed to stand for 10 min and then extracted twice with dichloromethane (70 ml) for 2 min. The combined dichloromethane layers were dehydrated over an anhydrous sodium sulfate and evaporated to dryness at 35~40℃ using a rotary evaporator. The residue was dissolved in 5 ml of methanol and filtered through a 0.45 μm membrane prior to HPLC analysis.

(III) *High Performance Liquid Chromatography (HPLC) Analysis*

1. *Acifluorfen*

HPLC analysis was carried out by using a Shimadzu HPLC system (Japan) equipped with a Model L6200 pump system, a CBM-10A communication module and a SIL-10A auto-injector. The separation was performed on a Merck Lichrospher 60 RP-Select B column (5 μm, 250 x 4.0 mm i.d.). Detection was carried out on a SPD-10A UV-VIS detector set at 283 nm and chromatography data was controlled by a CLASS data management system. A mobile phase of acetonitrile/water with pH 3.0 adjusted by 0.05 M phosphate buffer solution (50/50, v/v) pumped at a flow rate of 1.0 ml/min was used. The injection volume was 20 μl.

2. *Bentazone*

The same HPLC system used for the analysis

of acifluorfen was used to analyze the bentazone. An HP ODS Hypersil HPLC column (5 μm, 250 x 4.0 mm i.d.) was used as a separation column. Detection was carried out on a SPD-10A UV-VIS detector set at 254 nm and chromatography data was managed by a C-R4A integrator. A mobile phase of methanol/0.1 M acetic acid solution (45/55, v/v) pumped at a flow rate of 0.8 ml/min was used. The injection volume was 20 μl.

(IV) *Gas Chromatography / Electron Impact-mass Spectrometry (GC / EI-MS) Analysis*

GC/MS analysis was performed by using an HP-5890 series II GC equipped with an HP 5970B quadrupole mass selective detector (MSD) and an HP 340C ChemStation data management system. A J&W Scientific DB-17 column (30 m x 0.25 mm i.d.) was used. The column oven temperature was held at 50℃ for 2 min and then programmed to 250℃ at 10℃/min. Both temperatures of injection port and interface to MSD were 250℃. The injection volume was 1 μl. The carrier gas was helium and the head pressure was adjusted to 8 psi.

(V) *Identification and Quantification Analysis*

The standard solutions of acifluorfen and bentazone were diluted with acetonitrile and methanol, respectively, to make up the solutions with a series of concentrations ranging from 0.5 to 5.0 μg/ml. Twenty μl of standard solutions with different concentrations was then injected into HPLC and the standard curves were made by plotting the peak areas versus concentrations. The identification and quantification of acifluorfen or bentazone in tested samples were carried out by comparing the retention times and peak area counts of the peaks of standards to the peaks from samples. The injection volume of both tested samples and standard solution was 20 μl. The content of acifluorfen or bentazone in ppm was calculated as (C x V) / M, where C is the concentration

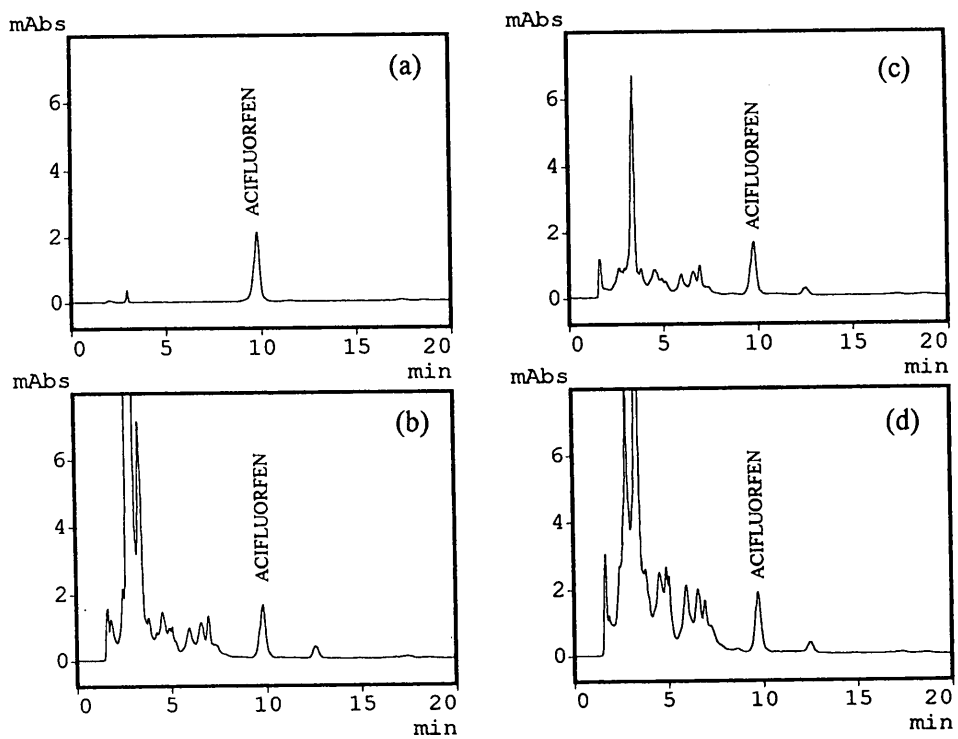


Figure 1. HPLC chromatograms of red bean spiked with 0.1 ppm acifluorfen. (a) acifluorfen standard (b) extracted with acetone (c) extracted with acetonitrile (d) extracted with acetonitrile and acetonitrile: H_2O (9:1, v/v) HPLC Column: Lichrospher 60 RP-Select B; mobile phase: CH_3CN: pH 3.0 H_2O (50:50, v/v); Flow rate: 1.0 ml/min; UV detector: 283nm.

(μg/ml) of acifluorfen or bentazone in sample solution; V is the volume (ml) of solvent to dissolve the residue before HPLC analysis; M is the sample weight (g).

(VI) *Recovery Test*

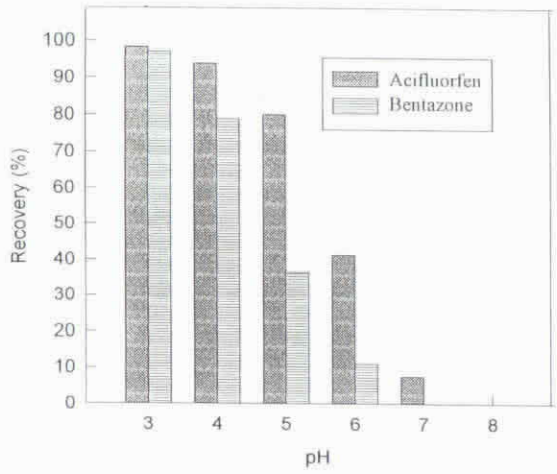


Figure 2. Recovery of acifluorfen and bentazone using phosphate buffers ranged from pH 3 to 7.

Recovery tests were carried out in triplicate by spiking the acifluorfen or bentazone at three concentration levels (0.05, 0.10, and 0.15 ppm for acifluorfen or 0.25, 0.5, and 0.75 ppm for bentazone) into 40 g (for acifluorfen) or 20g (for bentazone) of test samples. Blank tests without spiking standards were also performed. The samples were prepared as described above, and recoveries of these two herbicides from tested samples were obtained by HPLC analysis.

(VII) *Detection Limit Test*

The detection limit was evaluated by spiking the different levels of standards (0.01~0.10 ppm) to 40 g (for acifluorfen) or 20 g (for bentazone) of test samples followed by HPLC analysis. The preparation of samples was as described above.

RESULTS AND DISCUSSION

I. *Solvent Effect on the Extraction of Acifluorfen*

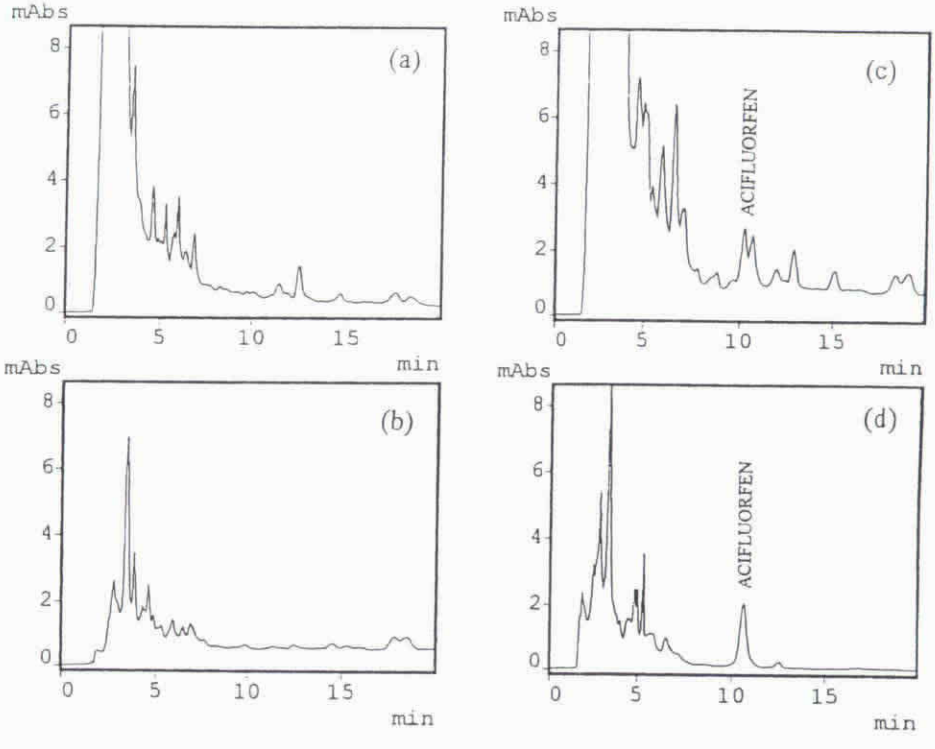


Figure 3. Clean-up effect of ion-pair extraction in determination of acifluorfen residues in red bean. HPLC chromatgrams of (a) impurities of n-hexane layer (b) impurities of dichloromethane layer (c) before cleanup (d) after cleanup. HPLC conditions are shown in Figure 1.

and Bentazone

(I) *Acifluorfen*

The solvent effect on the extraction of acifluorfen in crops has been well documented. Roy et al [6] assayed acifluorfen in feeds using HCl-ethyl acetate as an extraction solvent system which was capable of giving a high recovery and good reproducibility results. Alder et al [4] reported a GC-ECD (electron capture detector) method to analyze the acifluorfen in soybean. This method was able to give more than 70% recovery by using acetonitrile-1N HCl (70/30, v/v) as an extraction solvent system followed by derivatization with diazomethane. Gennari et al [7] assayed acifluorfen in soil using numerous solvent combinations, methanol-water, methanol-0.1N HCl, methanol-1N HCl, or methanol-0.1N NaOH. A solvent system of methanol-0.1N NaOH (80/20, v/v) was found to be capable of giving a superior recovery and reproducibility. Recovery was poor (<50%) for the other solvent combinations.

In this study, numerous solvent systems including acetonitrile-1N HCl (70/30, v/v), methanol-0.1N NaOH (80/20, v/v), ethyl acetate, acetone, acetone-1N HCl (70/30, v/v), acetonitrile, and acetonitrile-water (9/1, v/v) were compared for maximizing recovery of acifluorfen from red bean samples. Preliminary results showed that the extraction solvents containing alkaline solution were not suitable for application on the dry bean crops since the gelatinization of starch caused by the alkaline effect resulted in a difficulty for filtration and larger amount of co-extractives. The extraction solvents containing acid solution also led to larger amount of co-extractives which were difficult to remove by cleanup operation. Of extraction solvents evaluated, the acetone and acetonitrile were considered as superior solvents which were capable of giving more than 80% recovery of acifluorfen. Acetonitrile was especially chosen to be an extraction solvent because it gave less co-extractives and increased the rate of separation between organic layer and water layer during the partition process. Because of the higher polarity of acifluorfen, the polar extraction solvents are recommended for use in maximizing the recovery. We found that a recovery of higher 90% was obtainable (Table 3) and much less interference was observed from co-extractives (Figure 1) when red bean sample containing fortified acifluorfen was extracted with acetonitrile and the container and residues subsequently washed with acetonitrile-water (9/1, v/v).

(II) *Bentazone*

Methanol was widely used as an extraction solvent for bentazone analysis [3, 10]. However, when methanol was applied on the extraction of bentazone from rice and dry bean crops, the partition of methanol extracts yielded an emulsion between two phases and resulted in low recovery. The results of this study showed that a higher than 90% recovery could be achieved by using acetone as an extraction solvent.

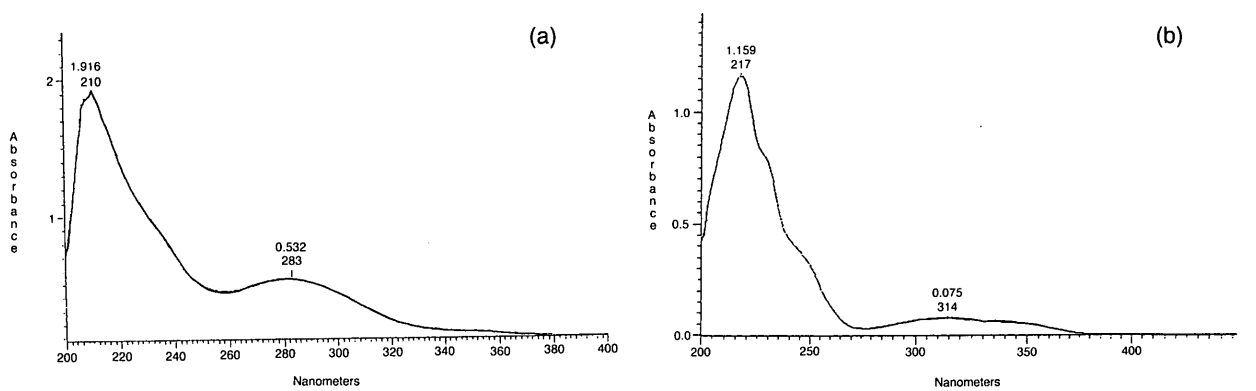


Figure 4. UV scanning spectrum of (a) acifluorfen 20 μg/ml and (b) bentazone 20 μg/ml (in methanol).

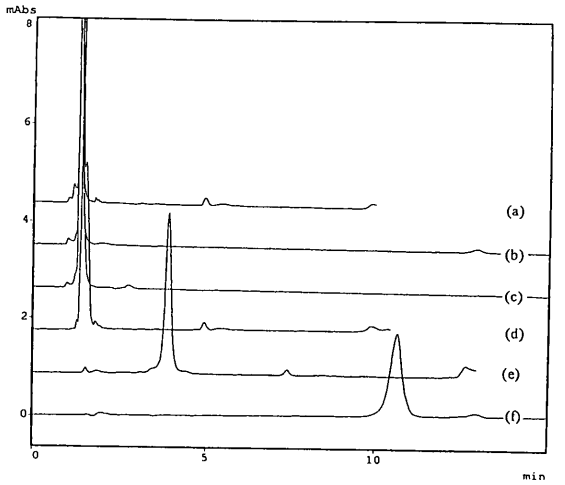


Figure 5. Retention time of acifluorfen in various mobile phases (a) CH_3CN: H_2O= 70:30 (v/v), (b) CH_3CN:H_2O=50:50 (v/v), (c) CH_3CN:H_2O= 70:30 (v/v), (d) CH_3CN: pH 3.0 H_2O=70:30 (v/v), (e) CH_3CN: pH 3.0 H_2O= 60:40 (v/v) and (f) CH_3CN: pH 3.0 H_2O= 50:50 (v/v).

Table 3. Recovery of acifluorfen spiked into red bean

Sample (Crop type)	Spiked level (ppm)	Recovery [a] (%)
Red bean (Dry bean crop)	0.05	97.6(8.9) [b]
	0.10	97.1(4.1)
	0.15	91.6(4.8)

[a]: average of triplicate.

[b]: value in the parenthesis is coefficient of variation (CV, %)

Table 4. Recovery of bentazone spiked into rice and red bean

Sample (Crop type)	Spiked level (ppm)	Recovery [a] (%)
Rice (Rice)	0.25	91.2(1.9)[b]
	0.50	89.8(2.6)
	0.75	88.6(1.7)
Red bean (Dry bean crop)	0.25	91.7(2.8)
	0.50	91.4(1.9)
	0.75	89.8(2.1)

[a]: average of triplicate.

[b]: value in the parenthesis is coefficient of variation (CV, %)

II. *Cleanup Operation*

Cleanup operation was used to prevent the UV interference from co-extractives when a method of reverse phase HPLC was carried out. An ion-pair cleanup procedure was adopted from Akerblom and Alex [10] with slight modifications. Acifluorfen and bentazone, which are usually used in the treatment of weeds, are weakly acidic with a pKa of 3.5 [6] and 3.2 [10], respectively. The ionization and polarity of these two compounds differ in various pHs and thus the solubility in dichloromethane are varies. Hence, the pH effect on the recovery was tested by adding 2 μg/ml of the standards for both acifluorfen and bentazone to a separation funnel containing equal amounts of dichloromethane and phosphate buffer solution with different pH. The separation funnel was then shaken for 10 min. The results showed the recoveries of both these compounds to be close to 100% when the pH 3.0 buffer was used as shown in Figure 2. The recoveries were decreased with increasing pH. Recoveries of both acifluorfen and bentazone decreased to 0% when the buffer solutions with pH above 8.0 and 7.0, respectively, were used. This indicated both of these herbicides were not dissolved in dichloromethane within this pH range.

To take advantage of this solubility property of acifluorfen and bentazone, sample extracts were dissolved in a buffer solution of pH 8.0 and washed with hexane and dichloromethane for removal of impurities. The water phase was then fortified with ion-pair reagent (TBA) which was able to change the polar molecules of acifluorfen and bentazone into the less polar ones, which then could be selectively extracted by dichloromethane. Figure 3 demonstrates the cleanup effect of ion-pair extraction for HPLC determination of acifluorfen in red bean. The reaction time for the interaction between herbicides and ion-pair reagent (TBA) to form a non-polar complex was based on the properties of herbicides and test samples. In order to completely react herbicides with ion-pair reagent, the sample mixtures was shaken and allowed to stand briefly after adding the ion-

pair reagent. Our study showed the recovery of acifluorfen, compared to the direct extraction without standing process, was increased by 15% after adding TBA, shaking for 2 min, and standing for 10 min.

III. *HPLC Conditions*

(I) *Wavelength Determination*

Figure 4 shows the UV-VIS scanning spectra of acifluorfen and bentazone at the concentrations of 20 μg/ml in methanol. Two absorbance maxima at 210 and 283 nm were present in UV-VIS spectrum of acifluorfen as shown in figure 4 (a). Because of too much interference from co-extractives when 210 nm was used as a detection wavelength, the wavelength at 283 nm was selected instead. The spectrum of bentazone as demonstrated in Figure 4(b) showed a strong absorbance maximum at 217 nm, a shoulder peak at 254 nm, and a weak peak at 314 nm. The wavelength of 217 nm was not suitable for HPLC detection because of a higher effect of interfering substances in the extracts. The absorbance at 314 nm was not strong enough to serve as a detection wavelength. Therefore, a absorbance at 254 nm, which was adopted by Akerblom and Alex [10], was selected to detect the bentazone in this study.

(II) *Mobile Phase Determination*

Because acifluorfen and bentazone are weak acidic compounds, control of mobile phase under acid conditions to prevent the dissociation of these two compounds is necessary when reverse phase HPLC is used. These two herbicides can be well retained in C_{18} column so as to resolve from

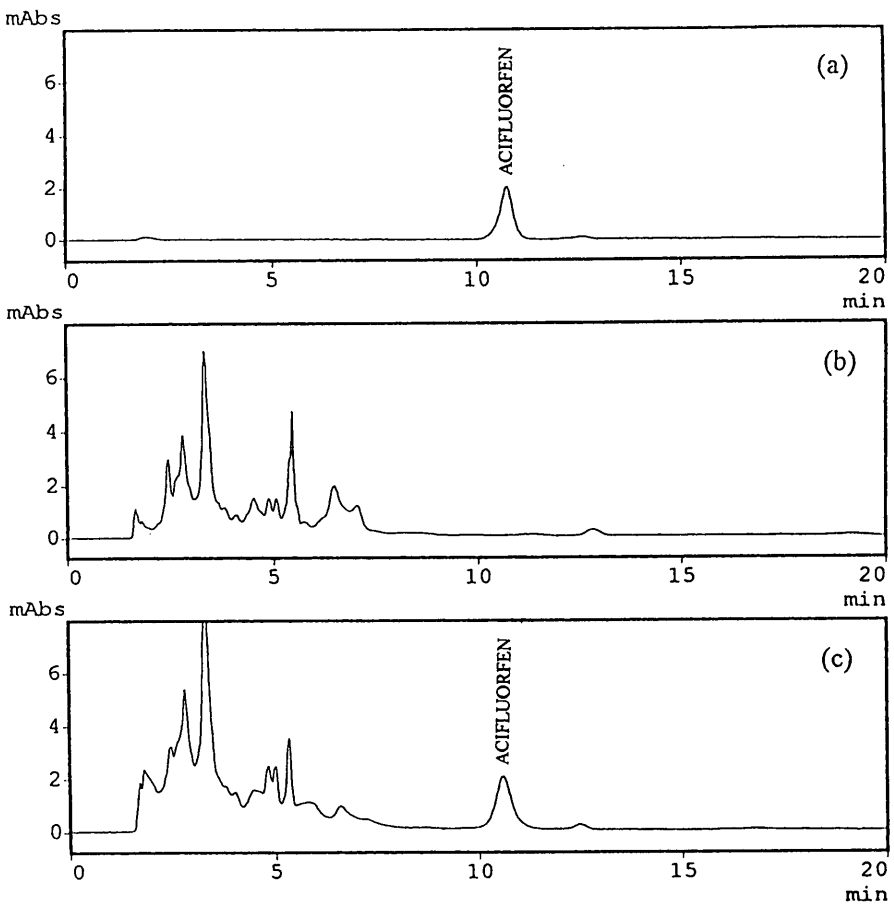


Figure 6. HPLC chromatograms of (a) acifluorfen standard (b) red bean blank (c) red bean spiked with 0.1 ppm acifluorfen. HPLC conditions are shown in Figure 1.

impurities by adding an alkaline ion pair reagent to form a complex with target compounds. Figure 5 demonstrates the retention times of acifluorfen in various mobile phases. Of the mobile phases used, a mobile phase of acetonitrile-pH 3.0 water (50:50, v/v) was preferred because it gave a longer retention time (10.9 min) enough to separate the acifluorfen from impurities. The mobile phase for bentazone analysis was adopted from that reported by Akerblom and Alex [10], but was modified to methanol-0.1M acetic acid (45:55, v/v), which was able to yield a shorter retention time at 7.4 min (Figure 7) so as to accelerate the bentazone analysis.

IV. *Standard and Recovery Curves*

The regression coefficients of acifluorfen for standard and recovery curves were 0.9957 and 0.9823, respectively, and the regressioin coefficients of bentazone for standard and recovery curves were determined to be 0.9980 and 0.9983~0.9989. They showed the satisfactory linearity.

V. *Fortification Recovery Test*

Table 3 gives the recovery of fortified acifluorfen at the levels of 0.05~0.15 ppm from red bean. The average recoveries ranged from 91.6 to 97.6% and the coefficients of variation were between 4.1~8.9%. The recovery of fortified bentazone at the levels of 0.25~0.75 ppm from rice and red bean is shown in Table 4. The average recoveries from rice and red bean ranged from 88.6 to 91.2% and 89.8~91.7%, respectively, and the coefficients of variation for both were located at 1.7~2.8%. The results showed a satisfactory

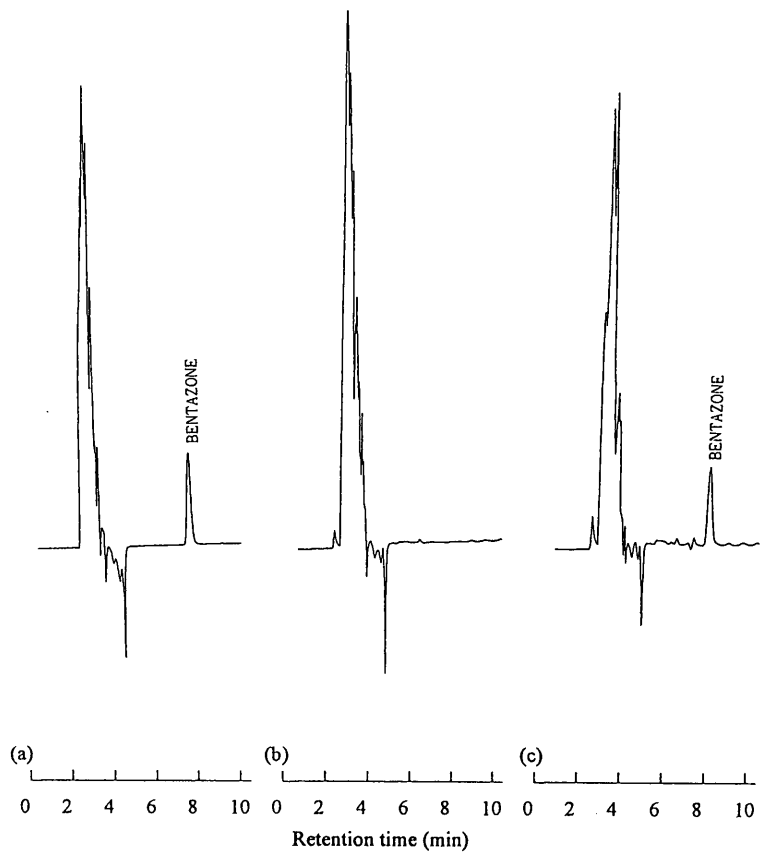


Figure 7. HPLC chromatograms of (a) bentazone standard (b) rice blank (c) rice spiked with 0.5 ppm bentazone standard. HPLC Column: ODS Hypersil; Mobile phase: MeOH: 0.1 M acetic acid (45:55, v/v); Flow rate: 0.8 ml/min; UV detector: 254nm.

recovery and reproducibility. Figure 6 and Figure 7 shows the HPLC chromatograms of acifluorfen in red bean and bentazone in rice samples, respectively. The compounds of interest were well resolved from other co-extractives as compared to the sample blanks without fortification of acifluorfen or bentazone (Figures 6 and 7). These results indicate that the method used in this study provides a satisfactory cleanup.

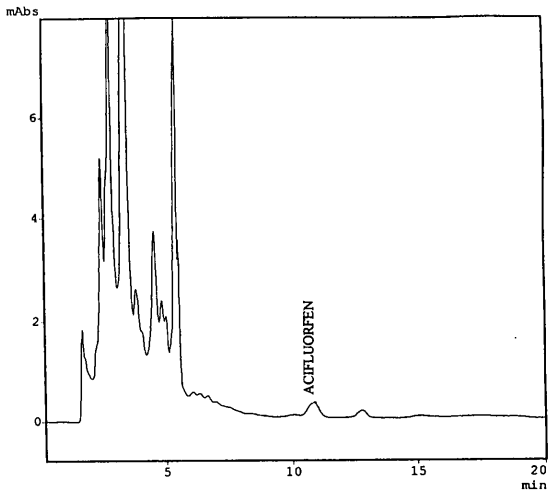


Figure 8. HPLC chromatogram of the detection limit of acifluorfen in red bean. HPLC conditions are shown in Figure 1.

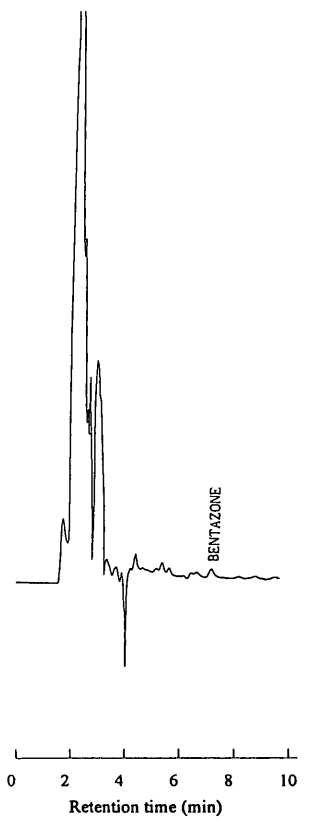


Figure 9. HPLC chromatogram of the detection limit of bentazone in rice. HPLC conditions are shown in Figure 7.

VI. *Detection Limit Test*

By using the methods described above, the detection limits of acifluorfen and bentazone for tested samples were taken to be 0.02 and 0.05 ppm, respectively (Figure 8 and 9), which were lower than the tolerance levels announced by Department of Health. This indicates these methods developed in our laboratory were sensitive and could be used as standard methods to determine acifluorfen and bentazone in rice and dry bean crops.

VII. *Gas Chromatography-Mass Spectrometry (GC-MS) Analysis*

The GC-MS is not suitable for the analysis of acifluorfen because acifluorfen is easily decomposed during GC analysis. Bentazone can be analyzed by GC-MS and its mass spectrum is shown in Figure 10. The m/z 240 is molecular ion. The fragments of m/z 240, 198, 161, and 119 are suggested to be the ions for the selected ion monitoring (SIM) detection. This GC-MS is used for the further identification purpose.

VIII. *Investigation of Commercial Products*

Twenty test samples including rice, red bean, mung bean, soybean, and peanut samples were collected and analyzed for the determination of acifluorfen and bentazone. No acifluorfen and bentazone were found in dry bean crops. Likewise no bentazone was found in rice samples. Although soybean and peanut extracts had higher oil content and more impurities than the others, the peak of bentazone was well resolved from those impurities. This reveals the method used in this study is qualified for the analysis of those herbicides in rice and dry bean crops.

CONCLUSIONS

Analysis of acifluorfen and bentazone residues in agricultural products was established in this study. Acifluorfen recoveries from red bean was in the range of 91.6~97.6% and its coefficients of variation were 4.1~8.9%. Bentazone recoveries from rice and red bean were between 88.6~91.7% and its coefficients of variation were 1.7~2.8%. The detection limits for acifluorfen and bentazone were 0.02 and 0.05 ppm, respectively.

This study presents an analytical method which is simple, reproducible, and able to provide high recoveries for the analysis of acifluorfen and bentazone. This is the first report to apply the ion-pair extraction for cleanup operation on acifluorfen analysis.

ACKNOWLEDGEMENTS

We thank Dr. C. W. Chen for his translation work.

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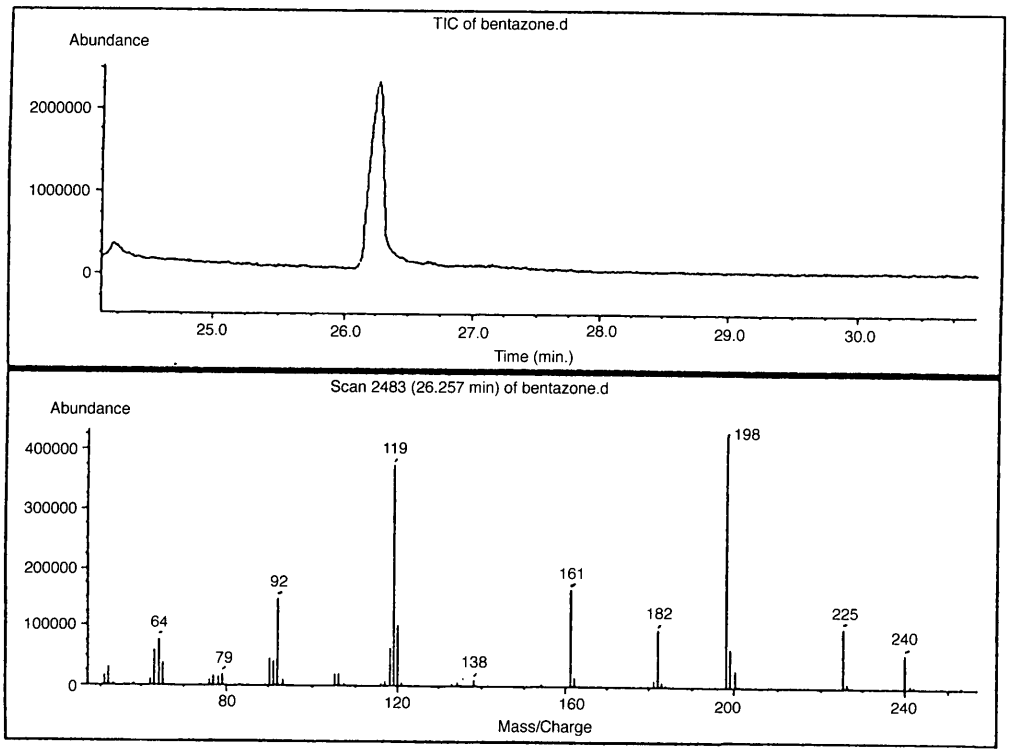

GC-MSD conditions:
Column: DB-17; Initial temp.: 50 ℃; Initial time: 2 Min;
Rate: 10 ℃ /min; Final temp.: 250 ℃; Final time: 20 min;
MSD interface temp.: 250 ℃ ;
Injector temp.: 250 ℃.

Figure 10. GC-MS spectrum of bentazone standard.

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Journal of Food and Drug Analysis. 1998. 6(3)

農產品中亞喜芬及本達隆殘留量之檢驗

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摘　要

本研究發展出以高效液相層析法檢驗作物中亞喜芬（acifluorfen）及本達隆（bentazone）殘留量之方法，並進行市售乾豆類及米類計二十件農產品之抽樣調查。亞喜芬及本達隆屬弱酸性殺草劑，在鹼性下會解離而溶於水中，添加鹼性對偶離子試劑後形成複合物而溶於二氯甲烷中，本方法即利用此一選擇性對偶離子萃取技術來淨化檢體。亞喜芬以乙腈及乙腈：水（9:1, v/v）自作物中抽出，本達隆以丙酮抽出，減壓濃縮至無溶媒後以pH 8.0磷酸緩衝液溶出，以正己烷及二氯甲烷去雜質，添加鹼性對偶離子試劑後以二氯甲烷萃取，所得檢液以高效液相層析儀配合C_{18}層析管及UV檢測器進行分析，亞喜芬於283 nm波長，本達隆於254 nm波長偵測。添加亞喜芬於紅豆中，檢體濃度爲0.05～0.15 ppm，所得平均回收率爲91.6～97.6%，最低檢出限量爲0.02 ppm；添加本達隆於白米及紅豆中，檢體濃度爲0.25～0.75 ppm，所得平均回收率爲88.6～91.7%，最低檢出限量爲0.05 ppm。以建立之檢驗方法分析市售檢體，乾豆類十件並未檢出亞喜芬，而乾豆類及米類計十件亦未檢出本達隆。

關鍵詞：農藥殘留量，亞喜芬，本達隆，高效液相層析，對偶離子萃取，農產品。