

Residue Analysis of Quinclorac in Soil by Supercritical Fluid Extraction and Fluorogenic Derivatization Coupled with High Performance Liquid Chromatography

Yong-Whan Kim

R&D Team, Agro Div., Oriental Chemical Industries, 50 Sokong-Dong, Seoul 100-718, Korea

Abstract : A new analytical method was developed by HPLC after supercritical fluid extraction and fluorogenic derivatization for the determination of quinclorac (3,7-dichloro-8-quinoline carboxylic acid) in soil. The graminicide quinclorac was extracted from soil by supercritical fluid extraction. Supercritical carbon dioxide at 7000 psi (80°C) modified with 30% of methanol extracted quinclorac from soil samples at the level of 0.1 ng g⁻¹ with 96% recovery. Extracted quinclorac was determined by HPLC as a fluorescent derivative. Derivatization was made with 4-bromomethyl-7-methoxycoumarin (4-Br-Mmc) using 18-crown-6-ether as a catalyst. The conversion was completed within 30 min and the limit of detection was 0.5 ppb to prove that the procedure could be used in the residue analysis of the pesticides containing carboxylic acid group. (Received October 6, 1997; accepted October 21, 1997)

Introduction

Quinclorac¹⁾ (3,7-dichloro-8-quinolinecarboxylic acid) is a quinoline carboxylic acid herbicide developed by BASF AG in 1985 and has been widely used for rice and turf-grass to control barnyardgrass, crabgrass, and some broadleaf weeds. In Korea, quinclorac has been used since 1989 for transplanted rice for the first time in the world. Although quinclorac showed extremely high safety to rice, carry-over residue resulted in the phytotoxic effect against following crops such as tomato at the level of 10 ppb or higher in soil.²⁾ Very sensitive detection methods at least 1 ppb detection are thus essential because the conventional base extraction methods detect only 5 ppb level owing to organic acid interference originated from soil. In general, supercritical fluid extraction(SFE)³⁻⁵⁾ is known to be very efficient and selective method which could be suitable for residue analysis. This paper discussed the supercritical fluid extraction of quinclorac from soil, and the fluorogenic labeling method⁶⁾ using 4-bromomethyl-7-methoxycoumarin for the ppb level detection of quinclorac.

Materials and Methods

Materials

Quinclorac standard was prepared by recrystallization of technical grade compound (BASF, Germany). Soil sample, which was obtained from Agrochemical Experimental Station of Oriental Chemical Industries (Jincheon, Korea), was a clay soil (texture, light clay; pH 7.3; moisture content 3.5%; organic matter content 7.3%; C.E.C. 18.1 meq/100 g). Supercritical carbon dioxide was purchased from Korea Special Gases (Osan, Korea). HPLC grade solvents were obtained from Tedia (USA) or Junsei (Japan). Other chemical reagents were purchased from Aldrich (USA).

Instrumental Analysis

The purity of quinclorac standard was analysed by differential scanning calorimetry (DSC, Perkin Elmer model 7 series). The kinetic study and residue analysis of quinclorac-Mmc ester were performed by high performance liquid chromatograph equipped with Waters 600E pump system, Waters 717 autosampler, Waters 996 photo diode array detector and Waters 470 scanning fluorescence detector. The sample was chromatographed on a 15 cm×4.6 mm×3 μm column (Spelcosil LC-18, Spelco, USA). The mobile phase was 30% water in methanol. The sample was run isocratically at 0.9 ml/min. The detection wavelength was 324 nm and the injection volume was 20 μl. The mobile phase in this study was 30% water in methanol at a flow rate of 1 ml/min.

찾는말 : quinclorac, SFE, fluorescence detection, 4-bromomethyl-7-methoxycoumarin, 18-crown-6-ether

*연락처

A Nuclear magnetic resonance spectrometer (Bruker 300 AM, Germany) was used to elucidate the structure of quinclorac-Mmc. A Hitachi fluorescence spectrophotometer (Model F-4010, Japan) was used to get the fluorescence spectra. The excitation wavelength was set at 324 nm and the emission wavelength was set at 400 nm. Extraction was carried out using a model SFX 3560 supercritical fluid extractor (Isco Inc, USA). Methanol was used as modifier and mixed via T-junction at 0-30% (v/v) concentration. Extraction parameters were as follows; 40-80°C and at 3000-8000 psi for 2 min with static mode followed by 15 min with dynamic extraction mode. The average flow rate was 1.5 ml/min.

Preparation of quinclorac-Mmc ester standard

For the reaction kinetics and fluorescence studies, quinclorac 4-methyl-7-methoxycoumarin ester (quinclorac-Mmc ester) was synthesized on a semipreparative scale. Quinclorac (0.242 g) was neutralized with 10% methanolic potassium hydroxide solution. After the solvent had been evaporated, 0.5 g of potassium carbonate, 3 ml of crown ether solution and 0.8 g of Br-Mmc in 50 ml of acetone were added and the mixture was refluxed for 1 h (Fig. 1). During the reaction, the reaction mixtures were sampled at given times, and the conversion yields were monitored with HPLC by the peak height of quinclorac-Mmc ester using external standard method. The quinclorac-Mmc ester was recrystallized from ethanol to yield an authentic reference compound for the fluorescence spectral measurements and the detection limit studies. The purity was checked by HPLC at 254 nm and differential scanning calorimetry. The derivative structure was elucidated by proton NMR.

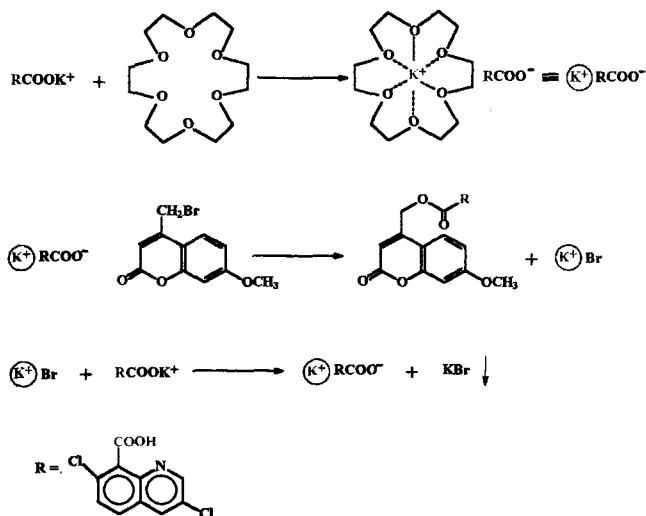


Fig. 1. Derivatization of quinclorac with 4-bromomethyl-7-methoxycoumarin with crown ether catalyst.

Fluorescence studies

Stock solutions of 2.5 ppm of quinclorac-Mmc ester were prepared in five different solvents: methanol, and 10%, 20%, 30% and 40% water in methanol (v/v). Spectrum was measured with 1 ml of each stock solution to obtain optimum analysis condition.

Recovery experiment

Four gram of soil samples were fortified with three different levels (10, 25 and 100 ppb) of quinclorac and extracted after 24 hours. Soil samples were air dried for 30 min before extraction. Soil samples (4 g) were transferred to 10 ml extraction cartridge topped with 0.5 g of anhydrous magnesium sulfate and extracted with supercritical fluid extractor. The elute was collected in pressurized (25 psi) 25 ml teflon capped glass tube filled with 10 ml of acetone cooled with carbon dioxide. The collection solution was transferred to 20 ml round-bottomed flask, and then evaporated to dryness with rotary evaporator. The residue was transferred to 100 ml separatory funnel with 50 ml of 0.1 N sodium hydroxide solution. The aqueous solution was washed twice with 25 ml of methylene chloride and then acidified with 1 N hydrochloric acid to $\text{pH} < 2$. The acidified solution was extracted twice with 25 ml of methylene chloride. The organic layer was dried on anhydrous sodium sulfate and then evaporated to dryness. The residue was transferred with 20 ml of acetone to 50 ml 1-neck round bottomed flask equipped with reflux condenser. To the solution, 20-30 mg of anhydrous potassium carbonate, 2 ml of crown ether solution (0.07 g of 18-crown-6-ether in 100 ml of acetonitrile) and 2 ml of 4-Br-Mmc solution (0.11 g of 4-Br-Mmc in 100 ml of acetone) were added. The reaction mixture was refluxed for 30 min, and then was evaporated under vacuum. The residue was partitioned twice with 25 ml of methylene chloride and water. The organic layer was dried on anhydrous sodium sulfate and evaporated to dryness. The residue was dissolved in hexane/ethyl acetate (9:1) and transferred to 500 mg Florisil Sep-Pak (Alltech, USA). Twenty milliliters of hexane/ethyl acetate (9:1) was eluted to discard and 4 ml of hexane/ethyl acetate (7:3) fraction was then collected. The residue was dissolved in 4 ml of methanol after evaporation and analyzed by HPLC.

Results and Discussion

Extraction of quinclorac

Supercritical fluid extraction, which has replaced

many conventional extraction methods, is free from toxic or flammable organic solvents. Preliminary supercritical extraction work done in my laboratory with supercritical carbon dioxide at 3000-8000 psi and 40-80°C (2 min static mode followed by 15 min dynamic mode) failed to recover quinclorac from soil samples fortified to 100 ppb level (recovery < 10%).⁷ When the experiments were performed with different ratio of methanol as modifier as reported by Hills,⁵ the recovery of quinclorac increased as percentage of methanol increased (Table 1). Extraction fluid carbon dioxide containing 30% methanol gave 96-103% recovery at 10-100 ppb spiking level when extracted at 7000 psi and 80°C for 2 min in the static mode and 15 min in the dynamic mode.

Derivatization of quinclorac

The use of crown ether to solvate the metal cations of organic salts is well known.⁸ The conversion of quinclorac to quinclorac-Mmc ester to get a high sensitive derivative in fluorescence detection was completed within 30 min (Fig. 2) and the conversion yield, determined by external standard method with HPLC, was quantitative. The chemical structure of quinclorac-Mmc ester was elucidated by proton NMR. (DMSO-*d*₆, TMS; 3.90, -OCH₃, 3H, s; 5.71, -OCH₂-, 2H, s; 6.73, -OC(O)CH=C-, 1H, s; 6.85, CH₃OC=CH-, 1H, s; 6.90, -C=CH-C(OCH₃)-, 1H, s; 7.50-7.85, Cl-CH=C-CH=C-, 2H, m; 8.25, -N=CH-C(Cl)=C-, 1H, s; 9.92, Cl-C=CH-C-, 1H, s).

Fluorescence properties

For the optimum analysis condition of HPLC, fluorescence properties of quinclorac-Mmc was studied. The fluorescence spectra of quinclorac-Mmc ester were

Table 1. Recoveries of quinclorac from soil samples by SFE. The flow rate was 1.5 ml/min. The soil samples were extracted at 80°C for 2 min in the dynamic mode.

Spiking Level (ppb)	Extraction Pressure (psi)	% of Modifier (MeOH, v/v)	Recovery (%)
100	3000	0	<10
100	4000	0	<10
100	5000	0	<10
100	5000	15	62
100	6000	15	70
100	7000	15	71
100	8000	15	73
100	5000	30	75
100	6000	30	91
100	7000	30	96
100	8000	30	96
25	7000	30	103
10	7000	30	98

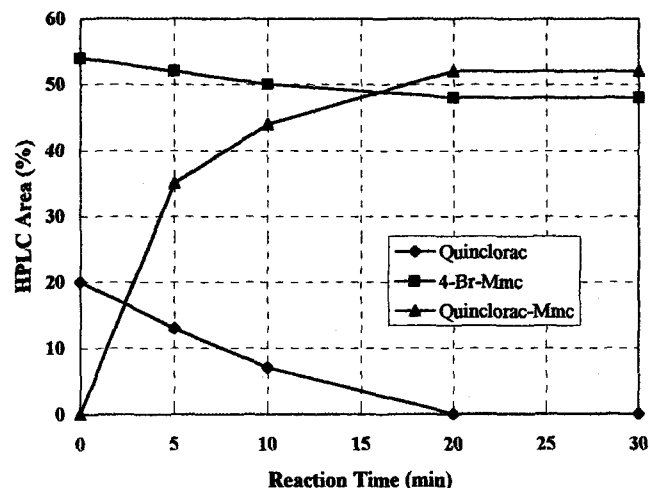


Fig. 2. Kinetics of quinclorac-Mmc ester formation. Reaction scale: 0.01 mM quinclorac, 3 eq. 4-Br-Mmc, 3.7 eq. potassium carbonate, and 0.4 eq. crown ether under reflux condition.

obtained in solvents used in reverse phase HPLC. For the five solvent systems examined (methanol, and 10%, 20%, 30%, and 40% water in methanol), the excitation maximum appeared at 324 nm and the emission maximum at 400 nm (Fig. 3). Being consistent with other work,⁹ the fluorescence intensity increased with the increase of water content in the solvent.

Limit of detection

When 30% water in methanol was used as HPLC eluent, the limit of detection (LOD) was 20 pg (1 ppb) as quinclorac-Mmc ester (11.2 pg (0.55 ppb) as quinclorac by using conversion factor 0.56 based on molecular weight), and the peak height of quinclorac-Mmc

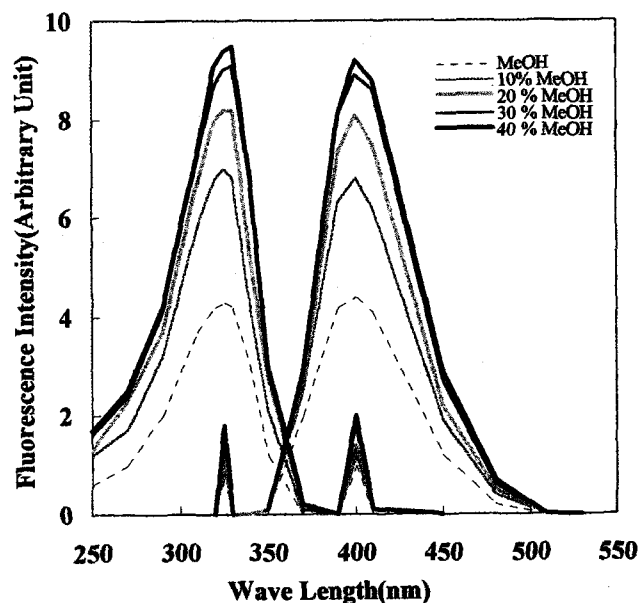


Fig. 3. Fluorescence spectra of quinclorac-Mmc ester obtained in aq. methanol systems.

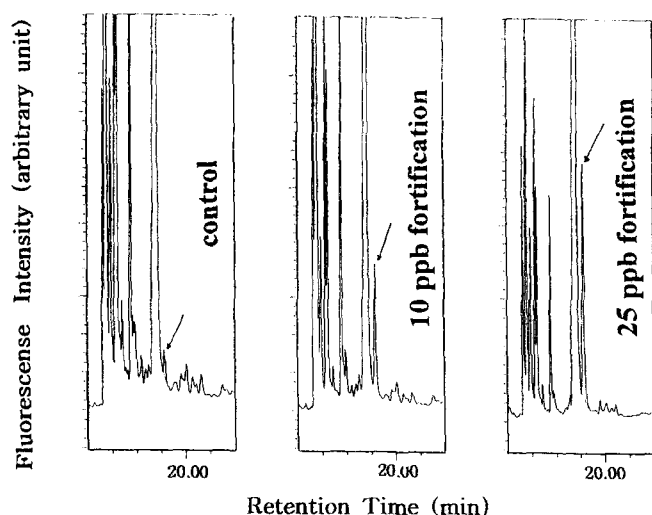


Fig. 4. HPLC-fluorescence chromatograms of quinclorac-Mmc from fortified soil.

shows linearity in the range of 20 pg-1 ng. This result indicates the usefulness of the fluorogenic technique in quantitative residue analysis. The impurity peaks did not interfere the quantitative analysis of quinclorac-Mmc ester (Fig 4). The quinclorac-Mmc peak was confirmed by comparison of HPLC retention time and UV-spectrum using photodiode array detector with those of authentic sample, which shows that this method can be used for the residue analysis of quinclorac in soil.

Conclusions

The SFE procedure coupled with 4-bromomethyl-7-methoxycoumarin derivatization of quinclorac was applicable to the quantitative determination of its residue in soil samples at ppb levels. Because the extraction was carried out with relatively non-toxic carbon dioxide instead of toxic or flammable organic solvents and can be run fast (typically thirty min for a 4 g soil sample), this method can be a powerful alternative to the conventional soxhlet extraction or aqueous base extraction method.¹⁰⁾ Moreover, fluorogenic labeling with 4-Br-Mmc using crown ether as a catalyst is highly selective, quantitative and sensitive method. Although this method is more expensive, it showed many advantages

over the conventional method in the analytical results, can therefore be used in the residue analysis of the pesticides containing carboxylic acid group(s).

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초임계추출과 형광유도체를 이용한 HPLC 에서의 Quinclorac 의 토양중 잔류분석

김용환(동양화학공업)

초록 : 감도와 재현성이 높은 quinclorac 의 토양중 잔류분석방법 개발을 위해 CO₂를 이용한 초임계추출후 4-bromomethyl-7-methoxycoumarin 로 형광유도체화하여 HPLC 상에서 분석하는 방법을 개발하였다. 추출변수로 추출압력과 modifier인 methanol 의 함량을 변화시켜, 100 ppb 처리토양에서 7000 psi (80°C), 30% (methanol/CO₂, v/v) 조건을 이용하여 96% 의 회수율을 얻었다. 18-crown-6-ether 를 촉매로 한 형광유도체 반응은 상온에서 30분이내에 정량적으로 완결되었으며 HPLC-형광분석검출기를 이용하여 토양중 quinclorac 의 최소검출 수준을 0.5 ppb 까지 낮출 수 있었다. 이 방법은 기존의 방법에 비해 간편하고 감도가 높아 carboxylic acid 를 함유한 농약의 잔류분석에 이용될 수 있다고 판단된다.

찾는말 : quinclorac, SFE, fluorescence detection, 4-bromomethyl-7-methoxycoumarin, 18-crown-6-ether

* 연락저자