Analysis of Phthalide, Fenobucarb and Inabenfide in Unpolished Rice Using Selective Gel Permeation Chromatography

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We studied a simple cleanup method using selective gel permeation chromatography (GPC) for the analysis of phthalide, fenobucarb and inabenfide in unpolished rice. These pesticides in ground unpolished rice were extracted with acetonitrile. Acetonitrile layer was separated by salting-out with NaCl. The residue was loaded to GPC (acetone-cyclohexane, 20:80, v/v). After fenobucarb, inabenfide and phthalide were fractionated by GPC, each eluant was determined by GC-NPD, HPLC and GC-ECD, respectively, without further cleanup. Average recoveries (n = 5) of fenobucarb, inabenfide and phthalide from fortified rice were 98, 90 and 81% at the 0.2 μg/g level and the limit of detection was 0.01 ppm, 0.01 ppm and 0.005 ppm, respectively.

Keywords: gel permeation chromatography, unpolished rice, phthalide, fenobucarb, inabenfide, fractionation.

INTRODUCTION

Gel permeation chromatography (GPC) has become a powerful and useful cleanup technique for the analysis of organochlorine pesticides and polychlorinated biphenyls (PCBs) in fatty foods for removing lipids and other high molecular weight compounds. Furthermore, GPC is effective in removing co-extractive material from non-fatty foods such as spinach. Specht et al. have reported the application of GPC and silica gel cleanup to more than 400 pesticides and their metabolites in vegetables and fruits. There have also been reports of the application of a gel permeation column using Bio-Beads S-X3 for cleanup of pesticide residues in non-fatty foods. Recently, GPC columns of hard gel type such as the polystyrene divinylbenzene copolymer CLN pak EV2000 have been used instead of S-X3 resin. GPC has already been employed as a cleanup tool in the multi-residue method as listed in the Pesticide Analytical Manual (Vol. 1) of the U.S. FDA, The Netherlands, Japan, Sweden, and other countries.

GPC removes co-extractives such as chlorophyll and lipids from samples and successfully separates pesticides. Currently, however, the separated pesticides recombine as one fraction upon determination of multi-residue analysis. For a precise analysis of multi pesticide residues, it is necessary to conduct another cleanup using a SPE cartridge of silica or Florisil. When analyzing specific pesticide residues in foods, GPC should be employed as a cleanup tool in the fractionation of each pesticide.

Phthalide (a fungicide) and fenobucarb (an insecticide) were frequently detected in unpolished rice using routine analyses. Therefore, we examined a simpler and more selective method of determining levels of phthalide, fenobucarb, and inabenfide (a plant growth regulator) in unpolished rice using acetonitrile extraction, GPC fractionation without further cleanup, and Gas Chromatography with ECD for phthalide, with NPD for fenobucarb, and with high performance liquid chromatography (HPLC) for inabenfide.

MATERIALS AND METHODS

1. Apparatus

1.1. Gel permeation chromatograph

The apparatus used included an HPLC pump, Hitachi L-6000 (Hitachi, Japan); a Reodyne manual injector with a sample loop of 5 ml and injection volume of 2 ml; a GPC column, Shodex CLN pak EV2000 (polystyrene divinylbenzene copolymer gel, Showa Denko, Japan), 2 cm i.d. × 30 cm and an EV-Guard column, 2 cm i.d. × 10 cm; an elution mixture, acetone/cyclohexane (2:8), flow rate of 4 ml/min, detection at 230 nm; and a fraction collector, Advantec SF2120 (Advantec, Japan). Fenobucarb, inabenfide, and phthalide were collected from 18 to 22 min, from 22 to 26 min, and from 29.5 min to 34.5 min, respectively.

1.2. Gas chromatograph for fenobucarb and phthalide

1) fenobucarb: A Model HP5890 series II equipped with a nitrogen phosphorus detector (NPD, Agilent Technologies Inc., USA), a DB-5 and DB-200 capillary column 30 m × 0.32 mm × 0.5 μm (J & W Scientific, USA); a helium carrier gas flow, 5 ml/min, injection temperature, 240°C, detector temperature, 280°C; column temperature program: held at 60°C for 1 min, a ramp of 10°C/min to 200°C, then a ramp of 5°C/min to 280°C, splitless injection at a volume of 1 μl by HP7796 autoinjector.

2) phthalide: GC-17A equipped with an electron capture detector (ECD, Shimadzu, Japan), a Rtx-CLPesticide capillary column 30 m × 0.32 mm × 0.5 μm (Restek Corp., USA); a helium carrier gas flow, 5 ml/min, injection temperature, 240°C, detector temperature, 280°C; a column temperature program: held at 60°C for 1 min, a ramp of 10°C/min to 200°C, then a ramp of 5°C/min to 280°C, splitless injection at a volume of 2 μl by a Shimadzu AOC-20A autoinjector.

1.3. HPLC for inabenfide

A Shimadzu 10A Series (Shimadzu, Japan), Shimadzu ODS-II column 150 mm × 4.6 mm, 5 μm, mobile phase, flow rate 1 ml/min, solvent A, 40% acetonitrile in water, solvent B, 90% acetonitrile in water; gradient systems, a linear gradient from 0%
of solvent B (20 min) to 100% of solvent B (25 min) and held for 5 min, 20 \( \mu l \) injection volume, a Reodyne injector and detection at 270 nm.

2. Chemicals

Acetonitrile, acetone, cyclohexane, ethyl acetate, and \( n \)-hexane for determining pesticide-residue grade were purchased from Wako Pure Chemical Ind. (Japan). Anhyd. Na\( \text{SO}_4 \) and NaCl—pesticide-residue grade, were also obtained from Wako. Fenobucarb, inabenfide, and phthalide were purchased from Hayashi Pure Chemical Ind. (Japan); working standard mixtures in acetone, containing 10 ppm for each pesticide, were used for spiking samples and preparing calibration standards.

3. Sample Extraction and Cleanup

A 20-g sample of ground unpolished rice was added to 20 ml of water and left to stand for 1 hr. Then, 100 ml of acetonitrile was added and the mixture was homogenized for 1 min and centrifuged at 3000 rpm for 5 min. Fifty milliliters of acetonitrile was added to the precipitated residue, which was again homogenized and centrifuged as mentioned above. Ten grams of NaCl was added to the combined acetonitrile extract and shaken vigorously for salting out. The acetonitrile layer was separated and evaporated \textit{in vacuo} until dry. The residue was dissolved in 30 ml of ethyl acetate and dehydrated with anhyd. Na\( \text{SO}_4 \). After evaporation of the filtrate, the residue was dissolved in 4 ml of acetone/cyclohexane (2 : 8). The sample solution was transferred to a vial and centrifuged for 5 min at 3000 rpm. Two milliliters of supernatant was loaded to GPC. The fractions of fenobucarb, inabenfide, and phthalide were collected at 18–22 min, 22–26 min, and 29.5–34 min, respectively. After the evaporation of each fraction \textit{in vacuo}, the residue was dissolved with 2 ml of acetone, and determined by NPD-GC in the case of fenobucarb, 2 ml of 50% acetonitrile in water and by HPLC in the case of inabenfide, and 2 ml \( n \)-hexane and by ECD-GC in the case of phthalide.

RESULTS AND DISCUSSION

1. Elution Profiles of Fenobucarb, Inabenfide and Phthalide

Figure 1 showed the elution profiles of fenobucarb, inabenfide and phthalide from the GPC column. Mean elution times were 20.3±0.1 min (RSD 0.5%), 23.0±0.2 min (0.7%), and 31.6±0.2 min (0.5%), respectively. CLNpak EV2000, a hard type gel, restrains swelling during the change of solvents, provides a good reproducible elution time for injected compounds, and has a relative standard deviation (RSD) within 1%. The reproducibility of the elution times of the three pesticides for GPC supports the suitability of using CLN pak EV 2000 and the good reproducibility of fractionation for each pesticide.

The peak of phthalide was recorded from 29.5 to 34 min. Although the peaks of fenobucarb and inabenfide did not separate completely, we collected two fractions (18–22 min and 22–26 min), by vertically dividing them into two peaks.

2. Chromatograms after Fractionation by GPC

A comparison of the chromatograms of the combined pesticide fraction (A) with all the pesticide eluents, and the phthalide fraction (B) is given in Fig. 2. Chromatogram B showed a marked reduction in background and was clearer than chromatogram A. The phthalide peak found in a rice extract was able

![Fig. 1. Fractionation of fenobucarb, inabenfide, and phthalide by GPC.](image)

![Fig. 2. Comparison of gas chromatograms of the combined pesticide fraction and phthalide fraction in rice.](image)

A: combined pesticide fraction of blank rice extract; B: phthalide fraction of blank rice extract; C: phthalide found in phthalide fraction of rice extract; D: 0.1 ppm phthalide standard solution. The signal for each pesticide is indicated by an arrow.
to be determined without interfering peaks (C). Generally, it is necessary to conduct a cleanup process using a Florisil cartridge before determining organochlorine pesticides by ECD-GC after GPC. However, the eluate of the selective fractionation of phthalide in this study could be analyzed for targeted pesticides without further cleanup.

Figure 3 provides a comparison of the chromatograms of the combined pesticide fraction and fenobucarb fraction. The latter chromatogram has an obviously reduced background (B) compared with the former (A). The fenobucarb found in the rice extract was able to be determined quite easily (D).

HPLC-UV chromatograms after the fractionation of inabenfide by GPC are shown in Fig. 4. The blank rice extract showed no peak at the same retention time (B). The inabenfide in the fortified rice extract was determined without interfering peaks (C).

The pesticide fractions collected by GPC have often required cleanup with a silicagel cartridge. For analyzing specific pesticides in this with the GPC procedure, we found it is useful to collect individual pesticide fractions for GC or HPLC without further cleanup.

3. Recovery from Fortified Unpolished Rice

Table 1 lists the recovery rates for the three pesticides in fortified unpolished rice. Three pesticides showed good rates of recovery (81.2–97.8%), and the RSD was within 10%.

The limit of detection was 0.01 ppm for fenobucarb, 0.01 ppm for inabenfide and 0.005 ppm for phthalide. With this method, three pesticide residues in unpolished rice could be measured at 1/5 to 1/20 of the concentration set by the Maximum Residue Limits for Agricultural Chemicals in foods or Standards for Withholding Registration in Japan.

In conclusion, the present study showed that fractionation by gel permeation chromatography (GPC) with a CLN pak EV 2000 column was a valuable tool for the cleanup of targeted or selected pesticide residues in unpolished rice without further cleanup.

REFERENCES


