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Short communication

Simplified determination of combined residues of prochloraz and its metabolites in vegetable, fruit and wheat samples by gas chromatography

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Abstract

This paper describes a gas chromatographic method for the determination of combined residues of prochloraz and its metabolites in various plant materials, including vegetables, fruits, seeds, grains and roots. The method is much simpler than previous ones as it does not require steam distillation; it consists of preliminary extraction of the sample, hydrolysis with pyridine hydrochloride in a test tube, extraction of the 2,4,6-trichlorophenol without the aid of steam distillation/continuous extraction, derivatization with diazomethane and gas chromatographic determination using electron-capture detection. The method is very rapid and can be done in a test tube; detection limit is from 0.01 to 0.50 mg/kg; recoveries are dependent on concentration of analyte and sample matrix; the determined values were found in the region of 72–102%.

Keywords: Vegetables; Wheat grain; Fruits; Food analysis; Pesticides; Prochloraz; Trichlorophenol

1. Introduction

Prochloraz (IUPAC name N-propyl-N-[2-(2,4,6-trichlorophenoxy)ethyl]imidazole-1-carboxamide) is a fungicide belonging to the class of imidazoles. It is used in treating cereal crops against eyespot fungus, on oilseed rape, citrus and tropical fruit, field legumes and beet. Prochloraz undergoes different transformations. In plants there is firstly a breaking of the imidazole ring with the formation of N - formyl - N - propyl - N - [2 - (2,4,6 - trichlorophenoxy)ethyl]ethylurea and N - propyl - N - [2 - (2,4,6 - trichlorophenoxy)ethyl]ethylurea, which are then degraded to 2,4,6-trichlorophenol (2,4,6-TCP), present in the plants in both the free and conjugated forms, together with traces of 2,4,6-trichlorophenoxyacetic acid.

Since European regulations require prochloraz residues to be expressed as 2,4,6-TCP, a method is needed whereby all final metabolites can be determined.

Analytical methods are available for the analysis of free and conjugated prochloraz and its metabolites. There are various GLC methods for the analysis of free prochloraz in cereals (grains and straw) [1], in citrus fruits [2] and in fruit and vegetables [3]. HPLC methods exist for citrus fruits [4,5] and water [6,7].

Some of these methods involve Soxhlet extraction and all use steam distillation to recover 2,4,6-TCP [8]. Not all laboratories are equipped for this type of procedure, and these techniques are also extremely long and thus not suitable for routine analyses. The method we present here does not involve Soxhlet extraction, hydrolysis is done in a test tube, and the hydrolysis products are extracted, after acid–base

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partition, with a solvent. The final determination, after methylation with diazomethane, is by gas chromatography with electron-capture detection (ECD).

2. Experimental

2.1. Reagents and solvents

Acetone, dichloromethane, hexane and methyl *tert.*-butyl ether (MTBE) were all of pesticide grade; they were obtained from Fluka (Switzerland) as was pyridine hydrochloride.

Prochloraz, 2,4,6-TCP and 2,4,5-TCP, used as analytical reference (99.9%), were from Dr. Ehrenstorfer Company (Germany).

Diazomethane was obtained as Diazald (Aldrich).

2.2. Extraction and hydrolysis

2.2.1. Apples, peaches, sugar-beet and vegetables

25 g of sample, taken from a representative number of chopped fruits or vegetables, were extracted in a blender with exactly 50 ml of dichloromethane for 2 min at top speed. The extract was centrifuged and exactly 3 ml (or 1 ml in the case of sugar-beet leaves) of the dichloromethane phase transferred into a 200 mm×20 mm I.D. (Pyrex) test tube fitted with a screw stopper. The organic solution was slightly basified with sodium hydroxide in methanol (20 μl, 1 *M*) to prevent loss of any 2,4,6-TCP that might be present, and evaporated to dryness under nitrogen.

1 g of dry pyridine hydrochloride was added to the residue and the test tube hermetically sealed with the stopper and heated to 220°C for 90 min in the dry-heater.

The test tube was then cooled and 10 ml of water was added.

The aqueous solution was extracted three times with 2 ml of hexane–MTBE (70:30) and the organic phase is transferred into a test tube. 3 ml of 0.25 *M* sodium hydroxide followed by 1 ml of saturated sodium sulphate were added to this. The test tube was shaken for 1 min and the upper phase discarded; 2.5 ml of 0.5 *M* HCl were added to the aqueous phase and extracted with exactly 1.5 ml of a solution of the internal standard (2,4,5-TCP; 0.25 ppm) in hexane–MTBE (70:30). The extract was collected in a test tube, methylated with diazomethane in diethyl ether (five drops) and after 20 min was injected into the gas chromatograph.

2.2.2. Wheat, grains and seeds

About 100 g of sample were ground in a Ultra Centrifugal mill. 3 g of the resulting flour (0.5 g in the case of straw) were placed with about 0.5 g of dry sodium sulphate in a 100 mm×20 mm I.D. test tube fitted with a screw stopper and extracted in a stirrer for 30 min with 6 ml of acetone (5 ml for the straw). The extract was centrifuged, and exactly 2.5 ml (1.5 ml for the straw) of the acetone phase were transferred into a 200 mm×20 mm I.D. screw stopper test tube. The organic solution was slightly basified with sodium hydroxide in methanol (20 μl, 1 *M*), to prevent loss of any 2,4,6-TCP that might be present and evaporated to dryness under nitrogen.

1 g of dry pyridine hydrochloride was added to the residue, and the test tube hermetically sealed and heated to 220°C for 90 min in the dry-heater.

The procedure then continued as for the fruit and vegetables.

2.3. Standard curve

A series of solutions was prepared by successive dilution of a 1000 mg/l solution of 2,4,6-TCP in hexane: 0.05, 0.1, 0.2 and 0.4 mg/l. A concentrated solution of internal standard (2,4,5-TCP) was added to each of these solutions, to produce a concentration of 0.25 mg/l in each.

2.4. Gas–liquid chromatography

Gas chromatograph: Mega HRGC Model 5300-HT (Carlo Erba Instruments, Milan, Italy).

Column: Capillary SE-54 fused-silica column, 20 m×0.32 mm I.D.; thickness, 0.25 μm (Mega-Legnano/Italy).

Temperature: 100°C; for 7 min, 1°C/min to 103°C then at the maximum rates to 240°C for 5 min. Injector: Split type (split ratio 1:5). Detection: ECD, 320°C; make-up gas: N_2 at 1.5 kg/cm^2; pulse width: 1 μs; ref. current: 1.2 nA; pulse voltage: 3.5 V.

Table 1
Mean recoveries (%) ±S.D. ($n=3$)

mg/kg added	Apples	Sugar beet root	Sugar beet leaves	Wheat	Wheat straw	Tomato
0.05	82.3±2.1	79.6±3.3	88.6±2.8	89.6±3.1	72.6±4.2	99.6±4.3
0.10	93.0±4.8	93.0±4.8	92.7±3.9	101.0±3.7	82.3±5.4	102.2±5.1

2.5. Calculation

The conversion factor between prochloraz (molecular mass=376.7) and 2,4,6-TCP, (M_r=197.5) must be taken into account in all calculations. Considering the stoichiometry of the hydrolysis reaction and noting that one mole of prochloraz produces one mole of 2,4,6-TCP, then:

(mg/kg) prochloraz = 1.906 (mg/kg) 2,4,6-TCP

(mg/kg) 2,4,6-TCP = 0.525 (mg/kg) prochloraz

2,4,6-TCP concentration in the final extract as read from calibration curve should be multiplied by volume of the final extract, divided by mass of the sample present in the final extract and then easily converted into prochloraz residues.

2.6. Recoveries

Three 0.05 and three 0.10 mg/l fortified samples were prepared from untreated samples.

3. Results and discussion

The method described offers marked advantages over traditional methods using Soxhlet extraction and steam distillation. Approximately 20 samples may be prepared at the same time, making the method quick; it does not require large quantities of glassware and thus particularly suited for routine analysis. Recoveries, as shown in Table 1, are very satisfactory. As the gas chromatograms show (Figs. 1 and 2), the extracts analysed are very clean and thus do not require further clean-up operations. Hydrolysis with

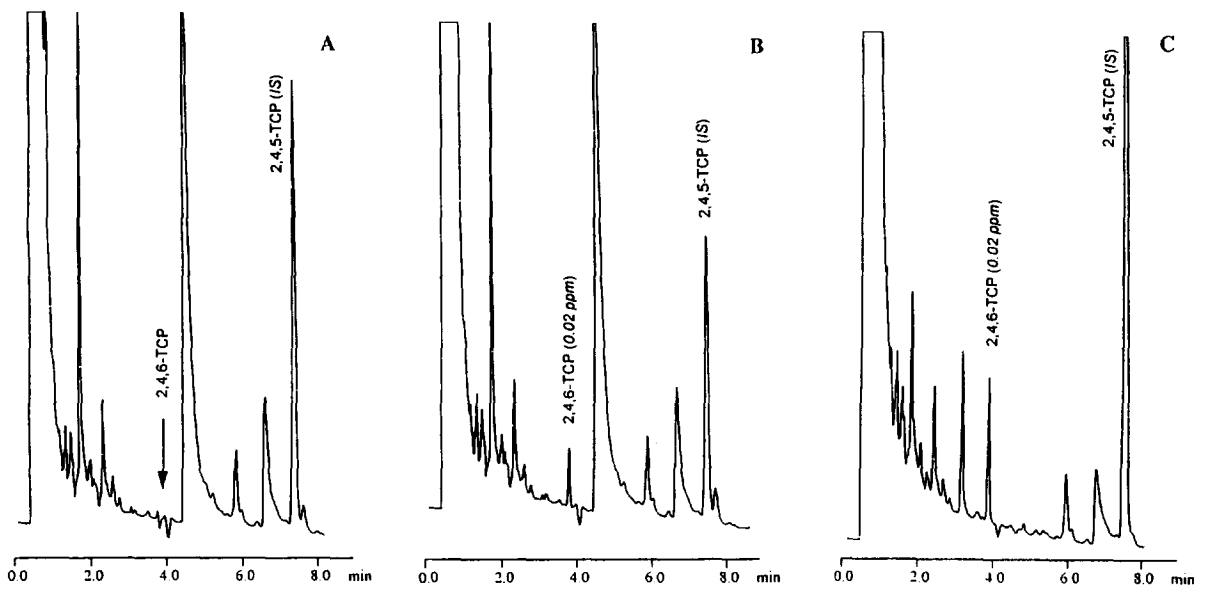

Fig. 1. Gas chromatograms of sugar-beet sample: (A) root untreated, (B) root and (C) sugar-beet leaves.

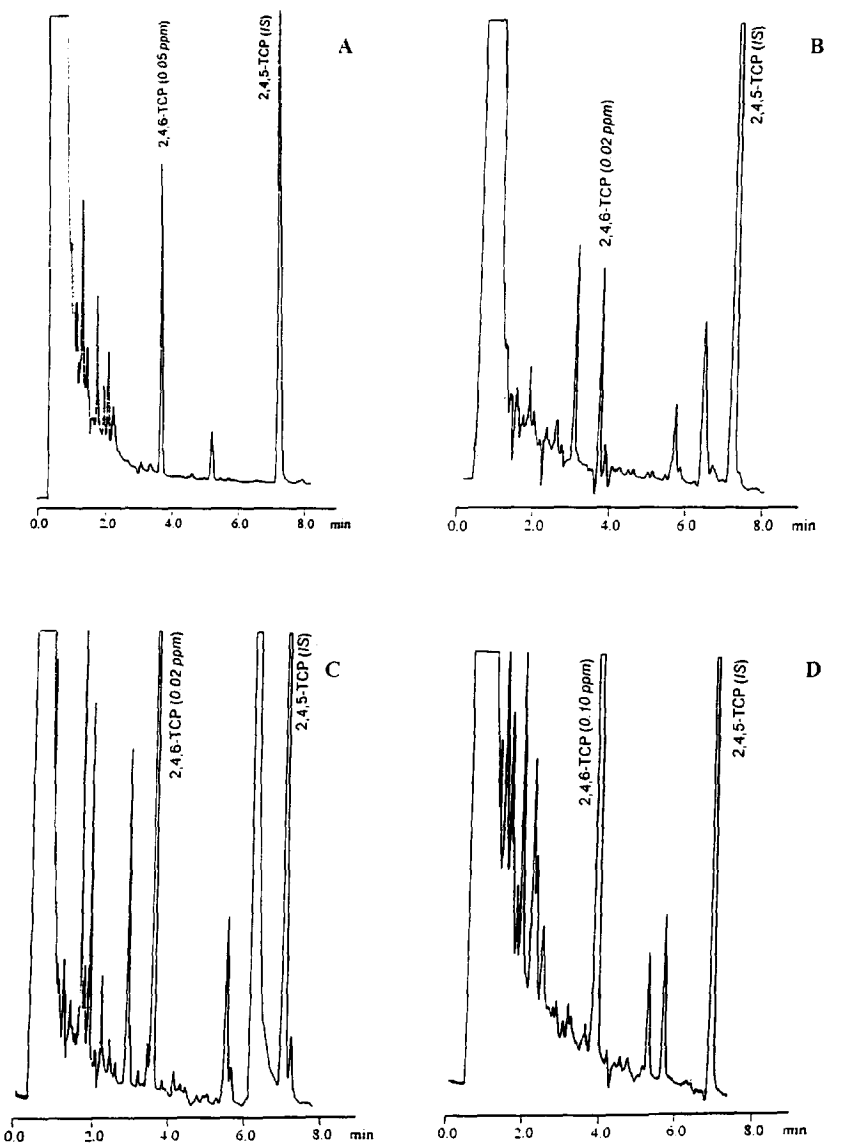

Fig. 2. Gas chromatograms. (A) Test solution of prochloraz after hydrolysis to 2,4,6-TCP and derivatisation with diazomethane and 2,4,5-TCP as internal standard; (B) samples of wheat; (C) wheat-straw; (D) apple.

pyridine hydrochloride was done in a dry-heater with an aluminium clock, holding up to 20 test tubes. The advantage of using test tubes is that only small quantities of pyridine hydrochloride are required, and that several samples can be hydrolysed at the same time. Before doing the analyses the purity of the pyridine hydrochloride should be verified since some of these lots were polluted with 2,4,6-TCP. The method we used gave recoveries and standard deviations that were very acceptable, without using steam distillation, a time-consuming technique that does not allow the analysis of many samples at the same time.

The sensitivity of ECD to methylated 2,4,6-TCP is

very high, giving sensitivity limits that are well below those set by the regulations for pesticide residues.

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