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Article

An Alternative LC-UV Procedure for the Determination of Prochloraz Residues in Fruits

***Sandro Navickiene*[*,a] *and Maria Lúcia Ribeiro*[b]**

[a] *Departamento de Química, Universidade Federal de Sergipe, 49100-000 São Cristóvão-SE, Brazil*

[b] *Instituto de Química, Universidade Estadual Paulista, 14810-970 Araraquara-SP, Brazil*

Um método alternativo, utilizando cromatografia líquida com detecção espectrofotométrica, para análise de procloraz como o produto de degradação 2,4,6-triclorofenol em manga, mamão e laranja é descrito. Acetato de etila, acetona e diclorometano foram testados para a extração de procloraz das frutas. Após a extração, procloraz foi submetido a uma reação com cloridrato de piridina para gerar o derivado 2,4,6-triclorofenol. A análise foi realizada por cromatografia líquida com detector espectrofotométrico e por cromatografia gasosa com detecção por captura de elétrons. Recuperações médias de frutas fortificadas (0,1 e 0,2 mg kg^{-1}) variaram de 80 a 94% com coeficiente de variação entre 5,6% e 12,6% (n=8). Os limites de detecção e quantificação foram 0,05 e 0,1 mg kg^{-1}, respectivamente. O método alternativo foi aplicado a amostras de manga e mamão, as quais foram tratadas por imersão em solução da formulação de procloraz sob condições de laboratório. Além disso, amostras de frutas de mercados locais foram analisadas.

An alternative method using liquid chromatography with UV detection for the determination of prochloraz as 2,4,6-trichlorophenol in mango, papaya and orange is described. Ethyl acetate, acetone and dichloromethane were tested for extraction of prochloraz from the fruits. After extraction the residue of prochloraz was derivatized with pyridine hydrochloride. The analysis was carried out using liquid chromatography with UV detection and gas chromatography with electron-capture detection. Average recoveries of prochloraz from spiked fruits (0.1 and 0.2 mg kg^{-1}) ranged from 80% to 94% with relative standard deviations between 5.6% and 12.6% (n=8). Detection and quantification limits were 0.05 and 0.1 mg kg^{-1}, respectively. The LC-UV method was applied to mango and papaya samples submitted to dip treatment with a prochloraz formulation under laboratory conditions. In addition, fruit samples obtained from local markets were analysed.

Keywords: fruit, food analysis, prochloraz, fungicides, pesticides, derivatization

Introduction

The fungicide prochloraz, *N*-propyl-*N*-[2-(2,4,6-trichlorophenoxy)-ethyl]imidazole-1-carboxamide, Figure 1, has been employed to control leaf scab and grey mold on fruits, vegetables and ornamentals.[1, 2] In Brazil, prochloraz has been released by the legislation for field application on apple, orange, tomato, wheat, rice and ornamentals and also as a postharvest fungicide on mango and papaya.[3] However, a potential problem affecting the quality of the fruit is the appearance of fungicide residues. So, regular monitoring of fruit for fungicide content is required.

Prochloraz undergoes different transformations. In plants, the primary metabolic step is a breaking of the imidazole ring with the formation of *N'*-formyl-*N*-propyl-*N*-[2-(2,4,6-trichlorophenoxy)ethyl]urea and *N*-propyl-*N*-[(2-(2,4,6-trichlorophenoxy)ethyl)]urea, which are then degraded to 2,4,6-trichlorophenol, present as free and conjugated metabolites, together with traces of 2-(2,4,6-trichlorophenoxy)-acetic acid.[1,2]

Some methods for determining prochloraz in fruits such as orange, lemon, apricot, apple and banana have been described in the literature. These methods generally include liquid-liquid or matrix solid-phase dispersion extraction. Gel permeation chromatography has been used as a clean-up technique. Analyses have been carried out by liquid chromatography (LC) with mass selective detection and gas chromatography (GC) with electron-capture or mass selective detection.[4-9] To date, no LC-UV method after a derivatization reaction for analysis of prochloraz residues in fruit has been reported in the literature.

* e-mail: sandnavi@ufs.br

The paper reports an alternative LC-UV method for the determination of prochloraz as its 2,4,6-trichlorophenol derivative in mango, papaya and orange samples. Additional objectives in this work were to compare this proposed method with the GC-ECD procedure and to apply it to analyse prochloraz in mango and papaya submitted to dip treatment with a prochloraz formulation under laboratory conditions, and in fruit samples taken from local markets.

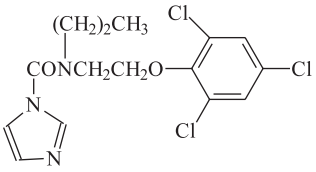

Figure 1. Molecular structure of the prochloraz.

Experimental

Reagents

Methanol, acetone, dichloromethane, toluene, diethyl ether, ethyl acetate and n-hexane (Mallinckrodt Baker Inc., Paris, KY, USA) were nanograde. Analytical-grade pyridine hydrochloride was purchased from Sigma (St. Louis, MO, USA).

LC-grade water was obtained by filtering deionized water through a 0.45 μm filter with a Waters Millipore (Milford, MA, USA) system. Methanol and water were degassed using a Branson 5200 (Branson Ultrasonic Corporation, Danbury, CT, USA) ultrasonic bath.

Certified standards of prochloraz (98.4% pure) and 2,4,6-trichlorophenol (99.5% pure) were purchased from Dr. Ehrenstorfer (Augsburg, Germany). The individual stock solutions of the analytes were prepared by diluting 1.0 mg of the standards in 10.0 mL of methanol to obtain a concentration of 100 μg mL^{-1}. The working standard solutions were prepared by diluting the stock solutions as required.

Apparatus

Liquid chromatographic analyses were carried out using a Waters liquid chromatograph (Waters Assoc., Milford, MA, USA) equipped with two solvent delivery pumps (Model 501), injector (Model U6K), UV-Vis absorbance detector (Model 486) and an integrator (Model 746). A stainless steel analytical column, LiChrospher 100 RP-18 (250 x 4.6 mm i.d., 5 μm; Merck) connected to a LiChrospher 100 RP-18 guard column (20 x 4.6 mm i.d., 5 μm; Merck) was used. The compound was analysed in the isocratic mode using methanol-water: 70:30 (v/v) at a flow rate of 0.8 mL min^{-1} with UV absorption at 220 nm.

Gas chromatographic analyses were carried out using a Varian 3300 gas chromatograph equipped with an electron-capture detector (ECD), an on-column injector, and a connected Varian 4290 reporting integrator. The megabore column was a ZB-1701 fused-silica column (30 m x 0.53 mm i.d., 1.25 μm; Zebron-Phenomenex, Torrance, CA). The injector and detector were operated at 240 °C and 300 °C, respectively. The oven temperature was programmed as follows: 140 °C for 1 min, increasing to 265 °C at 10 °C min^{-1} and holding for 10 min. Nitrogen was the carrier (2 mL min^{-1}) and makeup (28 mL min^{-1}) gas.

Sample preparation

Fruit samples (mango, orange and papaya) were purchased from local markets. The fruits were triturated separately using a household blender, homogenized and stored in individual jars at –18 °C until analysis.

Extraction and derivatization procedures

For hydrolysis of prochloraz residues in 2,4,6-trichlorophenol, a derivatization reaction proposed by De Paoli *et al*. has been used:[4] (*i*) *extraction procedure*: a 5 g portion of the fruit sample was weighed into a glass-stoppered flask. 10.0 mL of acetone were added and the flask was shaken for 20 min on a mechanical shaker (Thermolyne, Dubuque, Iowa, USA); (*ii*) *derivatization procedure*: a fraction of the extract (1 mL) was transferred to a test tube. A 1 g portion of dry pyridine hydrochloride was added and the test tube sealed with a stopper and heated to 220 °C for 90 min in a glycerine bath. The test tube was cooled and 10 mL of water was added. The aqueous solution was extracted two times with 5 mL of diethyl ether:n-hexane (1:4, v/v), and the organic phase was transferred into another test tube. 5 mL of 0.1 mol L^{-1} of KOH was added. The test tube was shaken for 1 min and the upper phase discarded; 5 mL of 1 mol L^{-1} HCl were added to the aqueous phase and this was extracted two times with 5 mL of toluene. An aliquot (1 μL) was injected into the GC-ECD system. For LC-UV analysis, an aliquot of 2 mL of the toluene phase was taken to dryness under a gentle stream of nitrogen. Residues were redissolved in 2 mL of methanol and an aliquot (20 μL) was injected into the LC-UV system.

Recovery studies

Recovery studies were carried out with untreated

mango, orange and papaya samples. Samples of 20 g of each fruit were spiked with appropriate volumes of prochloraz standard solution. The fortified fruits were left to stand for a few minutes before extraction to allow the spike solution to penetrate the fruits. Recovery assays were performed at 0.1 and 0.2 mg kg^{-1}. At each fortification level eight replicates were analysed. The extraction and derivatization procedures described above were followed.

Treatment conditions

The experiments were performed on mature mango (*Mangifera indica* L.) and papaya (*Carica papaya* L.) under laboratory conditions. To carry out this study, mango and papaya were sorted to eliminate those with defects and selected for uniform size. Mango and papaya samples were placed separately in plastic boxes (20 fruits per box). The treatments were carried out in duplicate during 3 min, by dipping the samples in aqueous suspensions of SPORTAK 450 CE® (450 g L^{-1}) at the dose: 110 mL per 100 L of water.[13] The fruits were left to dry at room temperature and stored for 29 days at 10 °C and 85-90% relative humidity (RH). Samples were taken before the SPORTAK 450 CE® application and also at 0, 7, 14, 29 days after application.

Results and Discussion

Chromatographic conditions

In preliminary experiments, taking into account the unsatisfactory peak shape when prochloraz was injected directly into the LC-UV system a derivatization procedure, already established for the determination of prochloraz in apple, sugar beet root and leaves, wheat, wheat straw and tomato using GC-ECD, was tested.[4] Therefore, LC-UV analyses of prochloraz as its 2,4,6-trichlorophenol derivative were conducted on a conventional LiChrospher 100 RP-18 reversed-phase column. To evaluate the mobile phase, different ratios of methanol-water were tested with respect to optimal peak form, separation efficiency and short elution time. Methanol (70%) in water using the isocratic mode with a flow rate of 0.8 mL min^{-1} shows the best conditions with respect to the analysis of the prochloraz derivative. The UV-Vis detector was operated at 220 nm. The identification of prochloraz as 2,4,6-trichlorophenol was carried out by comparison of the retention time obtained with the corresponding 2,4,6-trichlorophenol certified standard. Figure 2A shows the chromatogram of prochloraz submitted to the derivatization reaction with pyridine hydrochloride and Figure 2B shows prochloraz injected directly into the LC-UV system. Figure 3 shows the chromatograms of the fruit control samples and fortified papaya sample. The total running time of the LC-UV analysis was 10 min.

Since, prochloraz degrades when it is injected directly without derivatization into the gas chromatograph, the

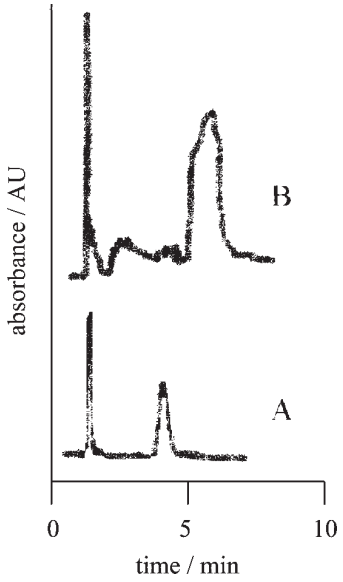

Figure 2. LC-UV chromatograms of: (A) prochloraz converted to 2,4,6-trichlorophenol, 0.1 mg kg^{-1}, (B) prochloraz standard solution without derivatization reaction, 0.1 mg kg^{-1}. For chromatographic conditions, see Experimental.

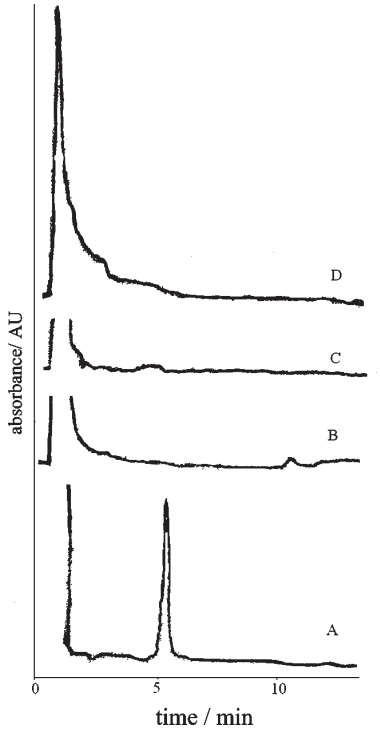

Figure 3. LC-UV chromatograms of (A) papaya sample fortified with prochloraz (as 2,4,6-trichlorophenol), 0.1 mg kg^{-1}; (B) untreated orange sample; (C) untreated papaya sample; (D) untreated mango sample. For chromatographic conditions, see Experimental.

procedure based on the one proposed by De Paoli *et al.*[4] was used for its derivatization, Figure 4. With relation to the GC-ECD analysis, during the optimization of the chromatographic conditions, different initial temperatures (90, 110, 120 and 140 °C) were tested. From this, an initial temperature of 140 °C proved to be the most suitable with respect to peak form. Figure 5 shows the chromatograms of an orange control sample, fortified orange sample and standard solution of the pesticide studied. The total running time of GC-ECD analysis was 10 min.

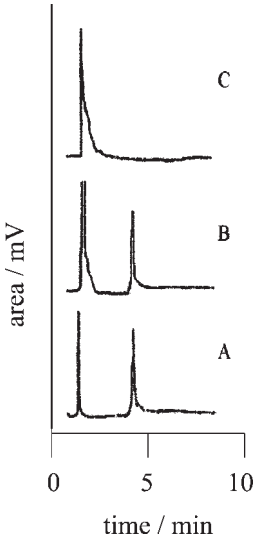

Figure 4. GC-ECD chromatograms of (A) 2,4,6-trichlorophenol certified standard, 0.1 mg kg^{-1}, (B) prochloraz converted to 2,4,6-trichlorophenol, 0.1 mg kg^{-1}, (C) blank of hydrolysis reaction with pyridine hydrochloride. For chromatographic conditions, see Experimental.

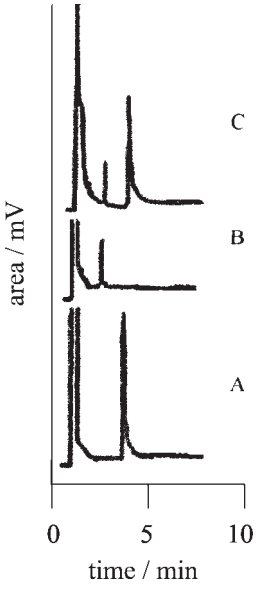

Figure 5. GC-ECD chromatograms of (A) prochloraz standard (as 2,4,6-trichlorophenol), 0.1 mg kg^{-1}, (B) untreated orange sample; (C) orange sample fortified with prochloraz, 0.1 mg kg^{-1}. For chromatographic conditions, see Experimental.

Extraction procedure

Preliminary investigations were performed for choosing the extraction solvent. Dichloromethane, acetone and ethyl acetate were tested. Acetone was selected, since it presented the highest recoveries (80-100%) for extraction of the compound from mango, orange and papaya. Despite the suitable recoveries (79-89%), the use of dichloromethane in the method is not favorable, because of environmental concerns. The recovery tests using ethyl acetate were in the range of 70-79%. For the recovery experiments untreated fruit samples were used. Recoveries were calculated by comparison with the appropriate working standard solutions. A 20 g portion of untreated fruit was fortified at two different concentrations (0.1 and 0.2 mg kg^{-1}) and quantified using the external standard method. The results of the average recoveries ranged from 80% to 94%, with relative standard deviation (RSD) values of 5.6% to 12.6%, as can be seen in Table 1. Each recovery analysis was repeated 8 times. The precision and accuracy were considered adequate for the validation of the method.[11] Standard solutions were injected after every ten samples to monitor changes in the chromatographic conditions. The chromatograms of the fruit extracts were satisfactory, without any interference in the retention time of the fungicide for both techniques. The amounts of 2,4,6-trichlorophenol obtained were corrected with the following factor to convert to the amounts of prochloraz [mol. wt. of prochloraz (376.7) / mol wt. of 2,4,6-triclhorophenol (197.5) = 1.91]. The Brazilian legislation[3] establishes maximum residue limits (MRLs) for prochloraz for papaya, mango and orange matrices. The MRL values are 1.0, 0.2 and 0.5 mg kg^{-1}, respectively.

Table 2 shows some differences between the method studied and the De Paoli *et al.*[4] and Lafuente and Tadeo[5] methods. The comparison emphasizes the recovery values,

Table 1. Recovery of prochloraz from fortified fruits employing LC-UV and GC-ECD

Matrix	Spiked level (mg kg^{-1})	%Range of recovery (%mean[a]; %RSD) LC-UV	GC-ECD
Papaya	0.1	89-98 (94; 10.7)	78-88 (82; 8.9)
	0.2	78-83 (81; 8.6)	75-82 (80; 7.9)
Mango	0.1	85-101 (82; 9.2)	80-87 (82; 7.5)
	0.2	77-104 (89;12.6)	80-92
Orange	0.1	85-92 (82; 5.6)	70-104 (83; 11.0)
	0.2	88-96 (83; 8.3)	80-96 (88; 7.5)

[a] n=8 analyses.

Table 2. Comparison of several methods for the determination of prochloraz in fruit

Prochloraz	Spiked	Analytical procedure		Average*(%RSD)	LOD (mg kg^{-1})
Matrix[g]	Level (mg kg^{-1}) [solvent, mL]	Extraction [column, technique]	Clean up		
orange[a] [5]	0.1 [acetone, 10]	10 mL of acetone, shake for 20 min, 2 g of NaCl Hydrolysis with pyridine hydrochloride, 1 g [LiChrospher 100 RP-18, LC-UV]	partition	82 (5.6)	0.05
apple[b] [25]	0.1 [DCM, 50]	50 mL of DCM Hydrolysis with pyridine hydrochloride, 1 g [SE-54, GC-ECD]	partition	93 (9.8)	0.01-0.5
orange[c] [20]	3.0 [ethyl acetate, 180]	60 mL of ethyl acetate, 10 mL of 0.5 mol L^{-1} NaOH, 2.5 g Na_2SO_4, 2.5 g NaCl repeat with 2 x 60 mL of ethyl acetate, 20 mL 0.5 mol L^{-1} HCl [RP-18 reverse phase 10 μm, LC-UV]	partition hexane at acidic pH	73 (4.0)	0.04

DCM: dichloromethane; [a]present LC-UV method (*n=8); [b]ref. 4 (*n=3); [c]ref. 5 (*n=4).

coefficients of variation, extraction and clean-up procedures. Also these methods require large volumes of solvent and large amounts of sample. The present method has comparable results at the same level of concentration (De Paoli *et al*.[4]) and emphasizes the reduced number of steps involved in the analytical procedure (Lafuente and Tadeo[5]). On the other hand, the chromatographic peak for the unhydrolysed prochloraz obtained by Lafuente and Tadeo was symmetric and relatively broad.

Linearity

Under the chromatographic conditions described, good linearities and correlation coefficients were achieved for the compound studied. Replicates (n=3) of six standard pesticide solutions of different concentrations were found to be linear in the range from 0.4 to 5.0 μg mL^{-1} for both chromatographic techniques. The equations for the calibration curves were y=11942.35x+239.66 for LC-UV and y=24974.94x-40.73 for GC-ECD. The determination coefficients obtained for the prochloraz were 0.9997 (LC-UV) and 0.9991 (GC-ECD).

Limits of Detection (LOD) and Quantification (LOQ)

The criteria established by Thier and Zeumer[11] to find LOD and LOQ were used in this study for both chromatographic techniques. The LOD for prochloraz was 0.05 mg kg^{-1}. The LOQ was determined as the lowest concentration of the compound that gives a response that could be quantified with RSD of the less than 20% and a recovery at least 70%. Thus, the LOQ value for this compound was 0.1 mg kg^{-1}.

Prochloraz degradation

In this study, prochloraz residues were detected in all mango and papaya samples submitted to the dip treatment with an aqueous suspension of SPORTAK 450 CE® (prochloraz as active ingredient) using the LC-UV procedure. For mango, the initial concentration of 9.5 mg kg^{-1} (0 day) decays to 7.2 mg kg^{-1} (7 days), 3.4 mg kg^{-1} (14 days), and dropped to 0.1 mg kg^{-1} in 29 days. For papaya, the initial concentration of 12.5 mg kg^{-1} (0 day) decays to 7.5 mg kg^{-1} (7 days), 2.1 mg kg^{-1} (14 days), and dropped to 0.4 mg kg^{-1} in 29 days. On the basis of the MRLs established by the Brazilian legislation for these matrices (1.0 mg kg^{-1} for papaya and 0.2 mg kg^{-1} for mango), these fruits can be considerable acceptable for human consumption 29 days after treatment, considering this experiment was done under laboratory conditions.

Real samples

The LC-UV method was applied to the analysis of the 126 real fruit samples (mango, orange and papaya) obtained

from commercial markets located in Araraquara, São Carlos, Jabotical and Ribeirão Preto, São Paulo State, Brazil. Prochloraz residues were not found in any of these samples under the experimental conditions described.

Conclusions

An alternative LC-UV method for determining prochloraz as the 2,4,6-trichlorophenol derivative in mango, orange and papaya is described. The comparison between LC-UV and GC-ECD methods shows similar recoveries at the same levels of concentration, LOQ and linearity, demonstrating the good performance of the LC-UV method to analyse the prochloraz residues in fruit samples. To corroborate these features, results were presented from fruits submitted to dip treatment with a prochloraz formulation. The LOQ achieved by the method were lower than the Brazilian MRL values, making the method suitable for routine analysis. A decay study of prochloraz in mango and papaya was carried out under laboratory conditions, in which it was observed that the remaining prochloraz residues were lower than the Brazilian MRL for this fungicide in mango and papaya matrices, 0.2 and 1.0 mg kg^{-1}, respectively, after an interval of 29 days.

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