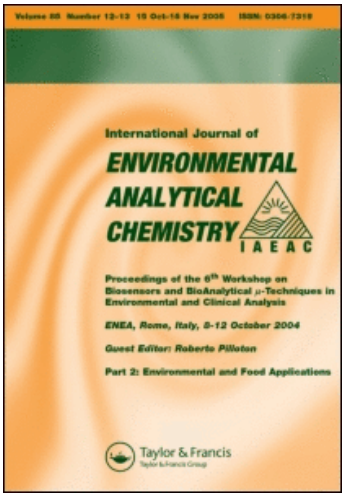

International Journal of Environmental Analytical Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713640455

Residues Analysis of Post-Harvest Imidazole Fungicides in Citrus Fruit by H PLC and GLC

M. T. Lafuente[a]; J. L. Tadeo[a]
[a] Laboratorio de Anαlisis, Departamento Nacional de Citricultura, I.V.I.A., Moncada, Valencia, Spain

To cite this Article Lafuente, M. T. and Tadeo, J. L.(1985) 'Residues Analysis of Post-Harvest Imidazole Fungicides in Citrus Fruit by H PLC and GLC', International Journal of Environmental Analytical Chemistry, 22: 1, 99 — 108
To link to this Article: DOI: 10.1080/03067318508076412
URL: http://dx.doi.org/10.1080/03067318508076412

Intern. J. Environ. Anal. Chem., 1985, Vol. 22, pp. 99–108
0306-7319/85/2202-0099 $18.50/0

Printed in Great Britain

Residues Analysis of Post-Harvest Imidazole Fungicides in Citrus Fruit by HPLC and GLC†

M. T. LAFUENTE and J. L. TADEO

Laboratorio de Análisis, Departamento Nacional de Citricultura, I.V.I.A., Moncada, Valencia, Spain

(*Received December 18, 1984; in final form April 4, 1985*)

The determination of imazalil and prochloraz fungicide residues has been carried out by HPLC with an UV detector at 204 nm and by GLC with an electron capture detector (ECD).

In both cases fungicide residues were extracted with hexane/acetone (90:10, v/v) after pH adjustment and purified by a liquid–liquid partitioning process. When HPLC was used for prochloraz and imazalil analysis, it was necessary to eliminate the interfering substances with a further clean-up process. This was also required when samples with low residue levels were analyzed by GLC.

Recovery was always higher than 70%. The detection limit was 0.04 ppm for the HPLC method and 0.02 for the GLC method.

Imazalil and prochloraz residues in "Washington Navel" oranges and "Hernandina" clementine fruits, dipped in a 1000 ppm fungicide solution, are reported.

KEY WORDS: Fungicide residues, HPLC, GLC, imazalil, prochloraz, citrus fruit.

†Presented at the 14th Annual Symposium on the Analytical Chemistry of Pollutants. Barcelona, November 22–24, 1984.

1. INTRODUCTION

Post-harvest diseases of citrus fruits are mainly produced by fungal pathogens. They can cause important economic losses. Thus fungicides treatments are necessary in order to overcome this problem.[1] The fungicides employed are generally toxic and they can present some hazards to public health. Therefore, legal requirements of many countries are increasing, making it necessary to determine fungicide residues at very low levels.

Imazalil (1-[2-(2,4-dichlorophenyl)-2-(2-propenyloxy)ethyl]-1H-imidazole), is widely used in citrus fruits, due to its high activity against *Penicillium* spp.[1-4] the main fungi that cause citrus decay in Spain, and its effectivity to control benzimidazole-resistant strains.[1,3,5] Prochloraz (1-[N-propyl-N-2-(2,4,6-trichlorophenoxy)ethylcarbamoyl]imidazole), another imidazole derivative with similar fungitoxic activity[1,3] is under research as a post-harvest fungicide in citrus.

Imazalil residues in citrus fruits have been determined by gas–liquid chromatography (GLC) with electron capture detector (ECD)[6-8] and by reverse-phase liquid chromatography (HPLC) with UV detector.[9] Prochloraz residues have been mainly determined by GLC (ECD) in cereals,[10] vegetables, apples and pears.[11] Recently, an HPLC method has been proposed for prochloraz residue analysis in citrus fruit.[12]

In this study we report two alternative procedures for determining imazalil and prochloraz residues in whole fruit, peel, albedo and pulp of two citrus varieties, using a GLC method with electron capture detector and an HPLC method with an UV detector.

2. EXPERIMENTAL

2.1 Apparatus and equipment

The liquid chromatograph used was a Hewlett–Packard model 1084 B equipped with a 20-μl loop injector, a 4.6×200 mm stainless steel column (RP 18 reverse-phase 10 μm), a 79875 A variable wavelength UV detector, and a 79850 B recorder. A 80:20 mixture of methanol and 0.25% ammonia was used as eluent; the solvent flow rate was 1.3 ml/min, the temperature ambient, the injection volume

20 μl and the detection wavelength 204 nm. The gas chromatograph used was a Perkin–Elmer Sigma 3 B equipped with an ECD ^{63}Ni and a 1 m $\times \frac{1}{8}''$ i.d. stainless steel column packed with 3% of OV-17 on gas-chrom Q (60–80 mesh). The detector and injector temperature were 350°C and 300°C respectively. The column-oven temperature was 240°C to determine imazalil and 265°C to determine prochloraz. The carrier gas was nitrogen at a flow rate of 30 ml/min and 30 ml/min by-pass. Homogenization was made in a Du Pont Sorvall Omni-mixer and spectrophotometric readings in a Varian Cary 210.

2.2. Reagents and samples

The reagents employed in this study were sodium sulfate (anhydr.), sodium hydrogen carbonate, sodium carbonate, sodium chloride (anhydr.) and ammonia, all AR grade (PANREAC). Solvents used were acetone, hexane, and methanol, all reagent grade (PANREAC). The analytical prochloraz standard (97.4% purity) was obtained from FBC Ltd. (Hauxton, Cambridge, U.K.) and imazalil R23979 (95% purity) from Janssen Pharmaceutica (2340 Beerse, Belgium).

Two citrus varieties were used: "Washington Navel" orange (*Citrus sinensis* (L.) Osbeck) and "Hernandina" clementine (*Citrus clementina* Hort. ex. Tan.). The fruits were hand-dipped for 2 min in aqueous solutions containing 1000 ppm imazalil or 1000 ppm prochloraz and analyzed after storage at 18°C for 2 or 7 days.

2.3. Procedure

Sample preparation and extraction Whole citrus fruit, peel, albedo, or pulp (10 oranges "Washington Navel" or 20 clementines "Hernandina") were quartered and ground in a food chopper. A representative 20 g sample was weighed into the 250 ml glass flask of a Sorvall homogenizer. To the sample, 75 ml hexane-acetone (90:10, v/v), 10 ml 0.5 N NaOH, 2.5 g anhydrous Na_2SO_4 and 2.5 g NaCl were added and the mixture was homogenized for 2 min at high speed. The mixture was then filtered under vacuum through Whatman No. 1 filter paper, using a Buchner funnel. The extraction was repeated twice using 75 ml hexane-acetone (90:10, v/v) each time. The extract was transferred to a 250 ml round-bottom flask, the organic solvent

was removed on a rotary evaporator until the aqueous solution was left.

Clean-up In this study we report two clean-up procedures:

A) The aqueous solution was made slightly basic with a $CO_3^{=}$—HCO_3^{-} buffer (pH = 9.2), the solution transferred to a 100 ml separating funnel and the fungicide extracted by shaking for 2 min with three 30 ml portions of hexane-acetone (90:10, v/v). The combined hexane-acetone extract was dried by filtering through anhydrous Na_2SO_4 into a 100 ml round-bottom flask, and the solvent removed on a rotary evaporator under vacuum at 40°C. Acetone (10–15 ml) was added, and the solution transferred to a 15 ml tube and stored in a freezer until ready for GLC determination.

B) The aqueous solution was acidified with 20 ml 0.5 N HCl, transferred to a 100 ml separating funnel, washed twice by shaking with 25 ml hexane for 2 min and the hexane discarded. The aqueous layer was neutralized with sodium hydrogen carbonate (2 g) and extracted with three 30 ml portions of hexane-acetone (90:10, v/v), shaking vigorously for 2 min each time. The aqueous phase was discarded and the hexane-acetone removed under vacuum after passing through anhydrous Na_2SO_4. The residue was dissolved with acetone (10–15 ml), and transferred to a 15 ml tube which was stored until ready for GLC or HPLC determination.

Detection and determination The determination of imazalil and prochloraz fungicide residues has been carried out by HPLC with an UV detector at 204 nm and by GLC with an ECD. The acetone solution was evaporated to dryness in a gentle stream of air. The residue was dissolved in an adequate volume of solvent, hexane when it was analyzed by GLC or methanol for HPLC analysis. In this case, samples were previously filtered by pressing through a 1-μm millipore filter. Fungicide concentrations were calculated by comparing integration counts or peak heights obtained for samples with those obtained for the standards.

3. RESULTS AND DISCUSSION

Imazalil and prochloraz were extracted from citrus fruit with hexane-acetone (90:10, v/v). Because of the weakly basic property of

imidazoles due to the "lone pair" electrons on the nitrogen atom, it was necessary to make the fruit homogenate slightly basic (10 ml 0.5 N NaOH), in order to achieve a high recovery. Ethyl acetate can also be used for extraction, fungicide recovery and the amount of interfering substances being similar to those of hexane-acetone (data not presented).

According to the sensitivity and specificity of the chosen technique for residue determination, an adequate removal of impurities will be needed. In this study two clean-up procedures (A or B) are reported. In method B, the aqueous solution from the extraction step was acidified with diluted HCl (H_2SO_4 can also be used), and partitioned with hexane. Ethyl acetate instead of hexane was also assayed, but no improvement in removing interfering substances was obtained and the fungicide recovery was lower. After hexane partition, the fungicide is re-extracted into hexane-acetone (90:10, v/v), upon neutralizing the acid-aqueous solution back with $NaHCO_3$.

In method A, the fungicide is re-extracted directly from the initial aqueous solution made slightly basic. Samples purified by method A can only be analyzed by GLC, and a more complete clean-up (method B) is needed to determine imazalil and prochloraz by HPLC, because the sensitivity and selectivity of the UV detector for these compounds is lower than that of the ECD. When residue levels are very low, as it occurs in the pulp, samples can be scarcely diluted and clean-up by method B is necessary before the HPLC or GLC determination.

After the clean-up (method B), imazalil and prochloraz residues were analyzed by reverse-phase HPLC with an UV detector and aqueous methanol as eluant. The use of an alkaline eluant was necessary to avoid the peak tailing shown by these fungicides in a neutral mobile solvent, which is probably caused by their basic property as secondary amines. Therefore, residue determination was improved by adding 0.25% ammonia solution to methanol. A methanol proportion in the mobile phase between 77% and 80% was found adequate to achieve a good separation of these compounds from impurities at a reasonable retention time (5 to 6 min). With a methanol proportion higher than 80% the separation from interfering substances is difficult, and when lower than 77% the fungicides t_r quickly increases (t_r about 9 min with 75% methanol), without a clear improvement in resolution.

Other mobile phases like acetonitrile, methanol-0.002 M Na Cl-phosphate buffer[9] and methanol-acetonitrile (50:50, v/v)-0.01 M K_2HPO_4, were also assayed with the same RP-18 reverse-phase column, but no improvement was obtained over the methanol-ammonia eluant. A Lichrosorb Si-60 column with hexane-isopropanol as mobile phase was also used, in this case it was impossible to separate the fungicides from the interfering compounds.

The analysis of imazalil and prochloraz residues in whole fruit, peel and pulp can be properly done by the reported HPLC method after clean-up by method B. Nevertheless, the residues determination in albedo was not possible, even with method B, due to interfering substances. Thus, an additional clean-up process or the use of another HPLC detector should be necessary.

Imazalil and prochloraz residues were also analyzed by GLC with an ECD. This technique allows the residue determination in whole fruit, peel and even albedo using the simple and short clean-up of method A. Pulp can also be analyzed by GLC but in this case, as pointed before, purification through method B is necessary.

Taking into account that these compounds have several nitrogen atoms in their molecules, it would be also possible to analyze them by GLC using the specific nitrogen-phosphorus detector (NPD). In our case, residues determination with NPD gave preliminary good results, being an alternative detector to be used with these fungicides.

The required time to analyze samples by GLC is, in our conditions, lower than that of HPLC, since the clean-up process of method A is shorter than that of method B and the retention time of prochloraz and imazalil is shorter in GLC (about 2 min) than in HPLC (about 6 min) analysis. In Figure 1, representative chromatograms of imazalil and prochloraz standards as well as treated and untreated samples analyzed by HPLC or GLC are shown.

The linearity range for these compounds was wider with the HPLC–UV method than with the GLC–ECD one. In our conditions residues were determined by using standards between 1 to 10 ppm in HPLC and 0.3 to 3 ppm in GLC.

The detection limit was 0.01 ppm of imazalil and 0.02 ppm of prochloraz by GLC analysis and 0.04 ppm of each one by HPLC determination.

Fungicides recovery through methods A and B, determined by

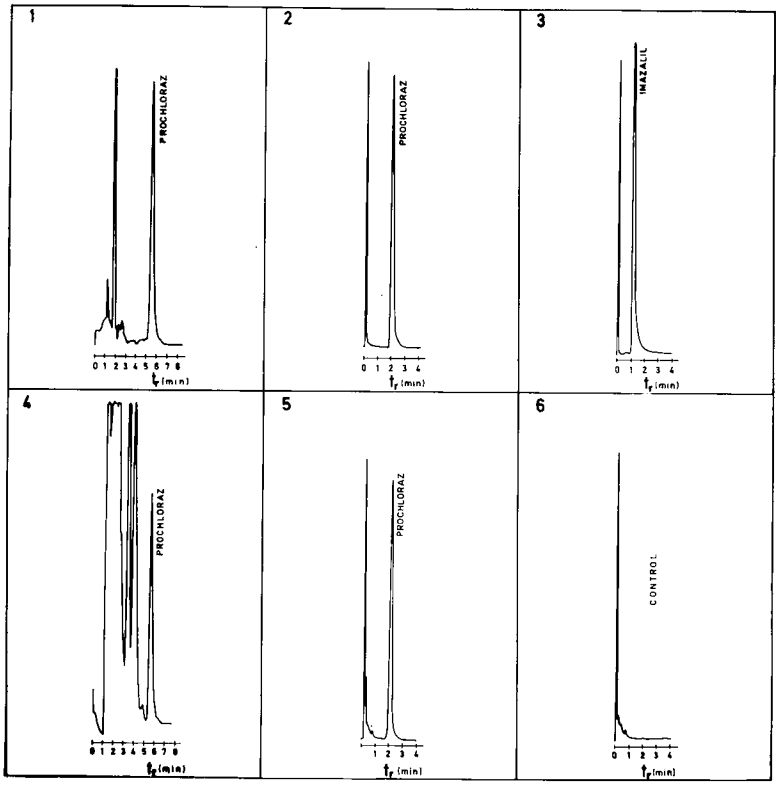

FIGURE 1 GLC and HPLC chromatograms of fungicides standards and sample extracts: 1 Standard (100 ng, HPLC); 2 and 3 Standard (20 ng, GLC); 4 and 5 Whole fruit of "Washington Navel" treated with 1000 ppm prochloraz (HPLC and GLC respectively); 6 Whole fruit of untreated "Washington Navel" oranges (GLC).

HPLC or GLC in different parts of the fruit, is shown in Table I. The average recovery obtained through method B was 89% for imazalil and somewhat lower, 77% for prochloraz. With method A, the values obtained were higher, the average recovery of imazalil being 93% and that of prochloraz 91%. Recovery through any method was always higher than 70%.

Imazalil and prochloraz residues found in "Washington Navel" oranges and "Hernandina" clementines are shown in Table II. The fruits were hand-dipped in water solutions containing 1000 ppm

TABLE I

Recoveries (%) of imazalil and prochloraz from samples analyzed by GLC or HPLC after clean-up through method A or B. Values obtained are the mean of, at least, four replicate experiments.

	Method B		Method A		
Fungicide	Pulp[a]	Whole fruit[b]	Whole fruit[b]	Peel[a]	Albedo[a]
Imazalil	88±4	90±6	98±2	86±4	95±3
Prochloraz	81±4	72±6	85±3	88±4	99±2

[a]Samples analyzed by GLC.
[b]Samples analyzed by HPLC.

TABLE II

Imazalil and prochloraz residues (ppm) found in "Washington Navel" oranges and "Hernandina" clementines hand-dipped in aqueous solution containing 1000 ppm fungicide and analyzed through method A by GLC (ECD) after storage at 18°C for 2 days. Values obtained are the mean of two replicate experiments.

	Imazalil		Prochloraz	
Sample	Washington Navel	Hernandina	Washington Navel	Hernandina
Pulp	0.08	0.14	0.08	0.11
Albedo	0.90	2.01	0.49	0.62
Whole fruit	4.4	5.9	4.6	4.5
Peel	8.7	16.7	13.7	14.3

fungicide, concentration that is normally used in packing houses, and analyzed by GLC (ECD) after storage at 18°C for 2 days. Residues of these fungicides were analyzed in whole fruit, peel, pulp and albedo, to determine their penetration in the fruit. The values obtained for both products in pulp are nearly the same, but prochloraz residues in albedo are approximately the half or one-third of those of imazalil. In whole fruit and peel results are also similar, except in the case of imazalil residues in "Washington Navel" peel, which are lower than those of prochloraz. Residue levels of both fungicides were considerably higher in the peel than in the whole fruit; the pulp had the lowest fungicide content, less than 3% of that of whole fruit.

TABLE III

Prochloraz residues (ppm) found in "Washington Navel" oranges and "Hernandina" clementines hand-dipped in aqueous solution containing 1000 ppm fungicide, and analyzed by HPLC (method B) and by GLC (method A) after storage at 18°C for 7 days. Values obtained are the mean of two replicate experiments.

	Washington Navel		Hernandina	
Sample	HPLC	GLC	HPLC	GLC
Pulp	0.09	0.08	0.12	0.10
Albedo	—	0.52	—	0.63
Whole fruit	3.9	4.0	4.2	4.4
Peel	11.1	10.4	14.2	13.8

Table III shows the HPLC or GLC values of prochloraz residues found in "Washington Navel" oranges and "Hernandina" clementines analyzed after storage at 18°C for 7 days. In all cases, results obtained in samples analyzed by HPLC through method B are similar to those obtained by GLC through method A. Thus, either of the chromatographic methods described above can be used to determine imazalil and prochloraz residues in citrus fruit.

4. CONCLUSIONS

The proposed HPLC and GLC methods can be used for the determination of imazalil and prochloraz residues in "Washington Navel" oranges and "Hernandina" clementines.

The required time to analyze samples was shorter with the GLC than with the HPLC method. This was mainly due to the faster clean-up procedure used in GLC for all types of samples except pulp. Moreover, the retention time of fungicides was shorter in GLC.

On the other hand, the linearity range for these compounds was wider with the HPLC than with the GLC method, although the detection limit was somewhat lower with the former.

Residue levels of both fungicides in whole fruit were about 5 ppm. The concentrations found in peel were considerably higher than those in whole fruit, while in pulp they were less than 3%. In albedo

imazalil residues were about 10% of those of peel and prochloraz levels were less than 5%. The obtained values indicate the low penetration of these imidazole fungicides in the fruit.

References

1. J. J. Tuset, J. Piquer, J. García, J. M. Martínez and M. Roca, *Proc. Int. Soc. Citriculture* **2,** 784 (1981).
2. E. Laville, *Fruits* **28,** 545 (1973).
3. J. W. Eckert, B. F. Bretschneider and M. Ratnayake, *Proc. Int. Soc. Citriculture* **2,** 804 (1981).
4. G. E. Brown, *Proc. Int. Soc. Citriculture* **2,** 815 (1981).
5. L. G. Houck, *Proc. Int. Soc. Citriculture* **1,** 263 (1977).
6. J. Wynants, *Proc. Int. Soc. Citriculture* **3,** 1107 (1977).
7. R. Greenberg and Ch. Resnick, *Pestic. Sci.* **8,** 59 (1977).
8. M. T. Lafuente, J. J. Tuset, J. Piquer, A. Estellés and J. L. Tadeo, *Anales I.N.I.A. Ser Agríc.* **26,** 83 (1984).
9. S. Norman and D. Fouse, *J. Assoc. Off. Anal. Chem.* **61,** 1469 (1978).
10. L. Somerville, *Med. Fac. Landbouww. Rijksuniv. Gent.* **45,** 841 (1980).
11. M. A. Maclaine Pont, H. P. Vogelezang and C. Siegmann-Knoester, *Med. Fac. Landbouww. Rijksuniv. Gent.* **45,** 835 (1980).
12. M. T. Lafuente and J. L. Tadeo, *J. High Resolut. Chromatogr. & Chromatogr. Commun.* **7,** 268 (1984).