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A New Green Analytical Procedure for Monitoring Sub-nanogram Amounts of Chlorpyrifos on Fruits Using Flow Injection Chemiluminescence with Immobilized Reagents

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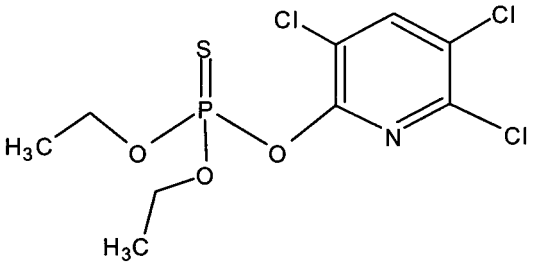
A novel green method using flow injection chemiluminescence with controlled-reagent-release technology has been investigated for the rapid and sensitive monitoring of sub-nanogram amounts of chlorpyrifos. The analytical reagents involved in chemiluminescence (CL) reaction, luminol and periodate, were both immobilized on an anion-exchange column. The CL signals produced by the reaction between luminol and periodate, which were eluted from the column through water injection, were decreased in the presence of chlorpyrifos. The decrease of CL intensity was linear over the logarithm of concentration of chlorpyrifos ranging from 0.48 to 484.0 ng·mL^{-1} ($r^2 = 0.9969$), and the limit of detection was 0.18 ng·mL^{-1} (3σ). At a flow rate of 2.0 mL·min^{-1}, the determination of chlorpyrifos, including sampling and washing, could be performed in 0.5 min with a relative standard deviation of less than <3.0%. The proposed method was applied successfully in an assay of remnant chlorpyrifos on fruits such as orange and shaddock with the recovery of 94.4−107.4%. The change of the concentration of chlorpyrifos in a water sample was also investigated, and the variation rate was 99.96% during 35 h in the open air.

KEYWORDS: Chlorpyrifos; chemiluminescence; flow injection; immobilized reagents; fruits

INTRODUCTION

Chlorpyrifos [*O*,*O*-diethyl *O*-(3,5,6-trichloro-2-pyridinyl) phosphorothioate] is a broad-spectrum organophosphorus pesticide,



which was widely used in agriculture and even in and around homes and in nonresidential settings as a termiticide. Chlorpyrifos is a cholinesterase inhibitor, which can cause cholinesterase inhibition in humans; that is, it can overstimulate the nervous system causing nausea, dizziness, confusion, and, at high exposures, respiratory paralysis and death.

Recently, considerable attention has been focused on the threat to human health coming from the dietary food, drinking water, and residential risks caused by the residues of chlorpyrifos (*1*, *2*). Restricted residue tolerance limits have been established by the WHO Expert Group on Pesticide Residues early in 1982 (*3*). To minimize the health risk of chlorpyrifos, accordingly, precise and accurate methods are needed for the assay of chlorpyrifos and its residue in drinking water, food, and terrestrial, aquatic, and atmospheric systems (*4*).

A variety of methods have been developed for detecting, measuring, and/or monitoring chlorpyrifos in environmental samples, biological samples, foodstuff, etc. The classical analytical procedures, widely applied, consisted essentially of pretreatments such as extractions with a solvent, water partition, or cleanup on a chromatographic column, followed by GC or HPLC (*5*−*8*). Several immunoassays and enzymatic methods, owing to the biological nature of chlorpyrifos, including enzyme-linked immunosorbent assay (ELISA) (*9*−*14*) and kinetic enzymatic method (*15*), have been developed. In addition, spectrometry (*16*−*19*) and electrochemistry methods (*20*, *21*) have been investigated for the determination of chlorpyrifos as useful approaches.

Chemiluminescence (CL) combined with a flow injection (FI) system is an attractive and promising analytical method offering sensitive and rapid detection, with simple handling for on-line or real-time monitoring. A few CL methods have been proposed for the determination of pesticides, including the biological methods based on the enzymatic reactions for paraoxon and dimethoate parathion (*22*, *23*) as well as the chemical method involving an H_2O_2−luminol CL system for dichlorvos pesticide (*24*). We have reported on the luminol−$Fe(CN)_6^{3-}$ CL system for the determination of environmental species resorcinol and hydrazine (*25*−*27*) using FI technology. However, there has

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been no report dealing with a CL method for monitoring chlorpyrifos so far.

It is well-known that the fast oxidation reaction between luminol and periodate in alkaline medium produces a strong CL signal (*28*). We found that the CL intensity from the oxidation between luminol and periodate would be inhibited in the presence of chlorpyrifos. To achieve a homogeneous mixing of CL reagents, which will result in a more stable background and reproducible results, both CL reagents were immobilized on anion-exchange resin. Through injection of 100 μL of water, the CL reagents on the resin were eluted and the CL intensity was decreased in the presence of chlorpyrifos, by which chlorpyrifos could be detected. The decrement of CL was linear over the logarithm of the chlorpyrifos concentration range of 0.48−484.0 ng·mL^{-1} with a relative standard deviation of <3.0%. The proposed procedure was applied successfully in an assay of chlorpyrifos residue on orange and shaddock; the variation of chlorpyrifos concentration in solution was monitored during 35 h.

EXPERIMENTAL PROCEDURES

Reagents. All chemicals used were of analytical reagent grade. Double-distilled water was used throughout. Chlorpyrifos was obtained from Xi'an Modern Chemistry Research Institute (Xi'an, China). Luminol (Fluka, Biochemika) was obtained from Xi'an Medicine Purchasing and Supply Station, China. Potassium periodate was purchased from Xi'an Chemical Reagent Plant.

A standard solution of chlorpyrifos (100 mg·mL^{-1}) was prepared by dissolving 0.101 g of chlorpyrifos with 6 mL of methanol (45 g/100 g in methanol) (*29*) and double-distilled water to 100 mL in a brown calibrated flask. Working strength solutions were prepared freshly before analysis as described above for fear that hydrolysis might occur. Luminol was used as supplied to prepare a 0.25 mol stock standard solution in 0.5 mol·L^{-1} sodium hydroxide in a 1000 mL calibrated flask. A 0.04 mol·L^{-1} stock standard solution of KIO_4 was made by dissolving the solid in distilled water and diluting to 250 mL in a calibrated flask.

Preparation of Immobilized Reagents Column. Amberlyst A-27 (from Rohm and Haas Co.) (2.0 g) was shaken with 50 mL of 0.25 mol·L^{-1} luminol or 0.04 mol·L^{-1} potassium periodate for 12 h, and then the resin was filtered, washed with doubel-distilled water, and dry-stored. The most convenient method to determine the amounts of luminol and potassium periodate immobilized was to measure the losses of these reagents from the immobilization solutions. The concentration was detected at 360 nm for luminol and at 225 nm for potassium periodate by UV−vis. In the proposed method, the amounts of luminol and potassium periodate immobilized were 1.99 ± 0.01 mmol·g^{-1} (n = 3) and 1.01 ± 0.02 mmol·g^{-1} (n = 3) resin, respectively.

To prepare an immobilized reagents column, resins containing immobilized luminol (0.05 g) and potassium periodate (0.1 g) were well mixed together and packed into a glass column (i.d. = 3.0 mm and total volume of ~0.5 mL), and the column was plugged with glass wool at both ends to prevent the resin from leaking.

Apparatus. The FI system employed for this work is shown in **Figure 1**. A peristaltic pump (Shanghai meter electromotor plant, model ND-15, 15 rpm) was used to generate the flows. PTFE tubing (1.0 mm i.d.) was used in the flow system. The immobilized reagents column was placed in front of a six-way valve, by which 100 μL of CL reagents eluted by water quantitatively was injected into the carrier. Before reaching the flow cell, the streams of luminol, potassium periodate, sodium hydroxide, and analyte were merged in a mixing tube (50.0 mm in length). The CL emission cell is a spiral glass tube (1.0 mm i.d., 15.0 cm length), producing a large surface area exposed to the adjacent photomultiplier tube (PMT) (Hamamatsu, model IP28). The sample compartment and PMT were light-tight. The CL signal produced in the CL emission cell was detected without wavelength discrimination, and the PMT output was amplified and quantified by a luminosity meter (Xi'an Keri Electron Device Ltd., model GD-1) connected to a recorder (Shanghai Dahua Instrument and Meter Plant, model XWT-206).

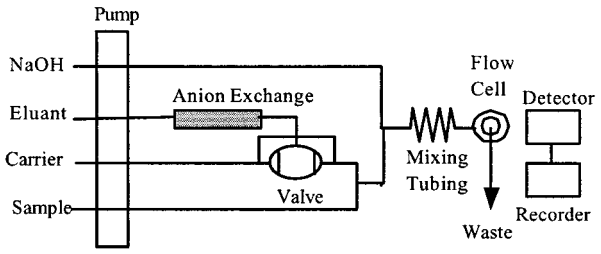

Figure 1. Schematic diagram of the flow injection system for chlorpyrifos determination.

Table 1. Preparation, Washing Steps, and Determination of Fruit Samples

step	events
1	clean up the orange (or shaddock) samples
2	dip the three oranges (or shaddocks) separately into three beakers containing 500 mL of a solution of chlorpyrifos (100 μg·mL^{-1}), respectively, for 2 min
3	dip the dried oranges (or shaddocks) into double-distilled water for 10 min and then analyze the eluted solutions
4	repeat step 3
5	repeat step 3 once again (for orange samples)

Procedures. The carrier water and the solutions (sodium hydroxide, sample, and eluant) were propelled at a constant flow rate on each flow line. Until a stable baseline was recorded, 100 μL of eluant solution, flowing through the reagents-immobilized column and containing luminol and periodate quantitatively eluted, was injected into the carrier stream, which was then mixed with the sample stream. The mixed solution was then delivered to the CL cell in the alkaline medium, and the peak height of the CL signal was detected with the PMT and the luminometer. The concentration of sample was quantified by measuring the decreased CL intensity, $\Delta I = I_0 - I_s$, where I_0 and I_s are CL signals in the absence and in the presence of chlorpyrifos, respectively.

Sample Preparation. The oranges and shaddocks were purchased from the local market. The samples were divided into two groups for oranges and shaddocks (also known as pomelo), respectively. Six oranges (weighing 114, 121, 106, 180, 165, and 191 g) and six shaddocks (weighing 311, 320, 302, 346, 377, and 365 g) were prepared and determined for the remnant chlorpyrifos. In each group, three samples were determined to be a control group, which were prepared following the same procedure but without the cleanup step. The procedures involving preparation, washing, and determination were carried out as the steps showed in **Table 1**.

RESULTS AND DISCUSSION

CL Intensity—Time Profile. Before the FI method was carried out, the batch method for the CL profiles was used. Without any special eluant, the mixture of luminol and periodate rinsed by water gave an evident CL signal in alkaline medium. As **Figure 2** shows, the CL intensity reached a maximum 10 s after injection and then died within 25 s. When the sample was added into the above mixing solution, a decreased CL signal was recorded. The decrease of the CL emission was proportional to the logarithm of chlorpyrifos concentration.

Designation for the FI-CL System. The assay could be carried out by a continuous flow mode in two different manifolds. To evaluate the different designations for the FI-CL system, Na_3PO_4 (5.0×10^{-5} mol·L^{-1}) was proposed as eluant instead of water, which was proved to be more suitable in the

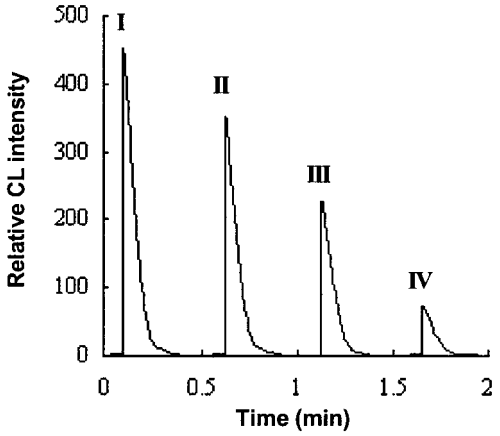

Figure 2. Chemiluminescence time profile in the batch system: I, CL intensity in the absence of chlorpyrifos; II, CL intensity in the presence of 2.5 ng·mL^{-1} chlorpyrifos; III, CL intensity in the presence of 25.0 ng·mL^{-1} chlorpyrifos; IV, CL intensity in the presence of 250.0 ng·mL^{-1}chlorpyrifos.

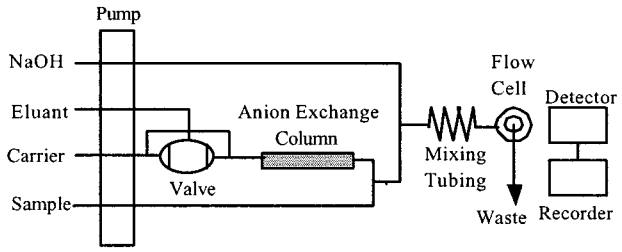

Figure 3. Schematic diagram of the alternate flow injection system for chlorpyrifos determination.

Table 2. Character of Eluants for Chlorpyrifos Determination

type of CL intensity	relative CL intensity[a]				
	H_2O	NaCl	Na_2CO_3	Na_2SO_4	Na_3PO_4
I	255	386	151	519	431
II	190	294	121	377	313
III	65	92	30	142	118

[a] The concentration of each eluant was 1.0×10^{-4} mol·L^{-1}. I, CL intensity in the absence of chlorpyrifos; II, CL intensity in the presence of 1.5 ng·mL^{-1} chlorpyrifos; III, decrease of CL intensity.

following section, because there would be no obvious CL intensity versus background when water was used as eluant in the manifold illustrated in **Figure 3**. Through injection of 100 μL of eluant, the reagents on the anion-exchange resin column were eluted, and in the presence of chlorpyrifos, the CL intensity decreased and the decrease of CL intensity was recorded. It was found that when the column with immobilized reagents was put in front of or behind the valve, two significantly different results were observed. The whole analysis process, including sampling and washing, could be accomplished in 0.5 min if the column was put in front of the valve manifold, that is, as shown in **Figure 1**, whereas the process took >2.0 min if the column was put behind the valve manifold (**Figure 3**). **Figure 1** gave the better precision; therefore, the manifold depicted in Figure 1 was chosen for subsequent work.

Selection of Eluant. One hundred microliters of different kinds of eluant was injected through the resin column, releasing different amounts of luminol and periodate, thus producing the CL emission. The results are shown in **Table 2**. It was found that sodium sulfate gives a maximum CL emission, whereas sodium carbonate shows some inhibitory effects on the CL reaction. Nevertheless, it was observed that a continuous flow

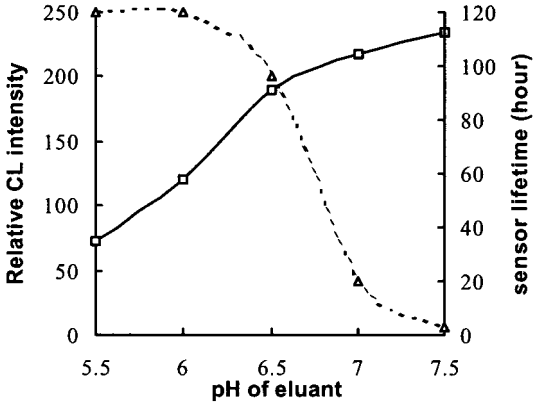

Figure 4. Effect of eluant pH on (△) column lifetime and (□) CL intensity.

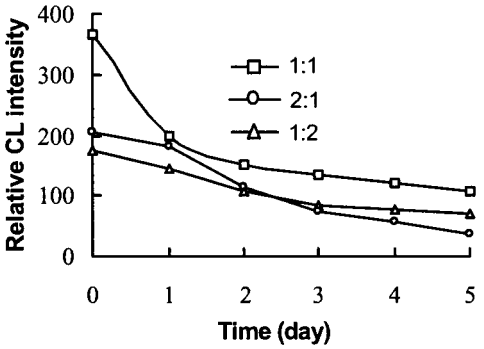

Figure 5. Effect of molar ratio of luminol and periodate on CL intensity and sensor lifetime.

of salt solution through the column results in a rather short lifetime of the immobilized reagents cartridge, down to only 5−10 h. It was shown that the immobilized luminol and periodate anions on the anion-exchange resin undergo dissociation with water, thus releasing trace amounts of luminol and periodate from the column, and the decrease of the CL signal in the presence of chlorpyrifos could be easily observed. In this case, the column could be reused for >80 h. Water was then selected as eluant in subsequent work, as a compromise between higher CL intensity and longer lifetime of the column (discussed under Operational Stability of the Flow System).

Effect of pH on CL and the Lifetime of the Column with Immobilized Reagents. The best pH of eluant (water) on the analytical performance was evaluated. It was found that the increase of pH in eluant leads to the increase in CL intensity but a considerable decrease in the lifetime of the immobilized reagents column (**Figure 4**). This phenomenon is probably due to the increasing quantities of hydroxide ions in the eluant. A pH of 6.5 was then chosen as a compromise between column lifetime and sufficient CL intensity. Therefore, double-distilled water had a compatible pH and was used as eluant directly throughout. In this case, the column with immobilized CL reagents could be used for >80 h in a continuous-flow system.

Effect of Molar Ratio of Immobilized Luminol and Periodate. To examine the influence of the mixing ratio, resins (0.15 g) with different mixing ratios were packed into a column with the same internal diameter and volume. By the injection of water at a fixed volume of 100 μL, different amounts of luminol and periodate were eluted from the resins and emitted CL signals with different intensities. As **Figure 5** shows, the CL intensity dropped drastically from the beginning to the next day, and then it went down slowly. The most stable CL signal was found with a molar ratio of 1:2 (luminol to periodate), and

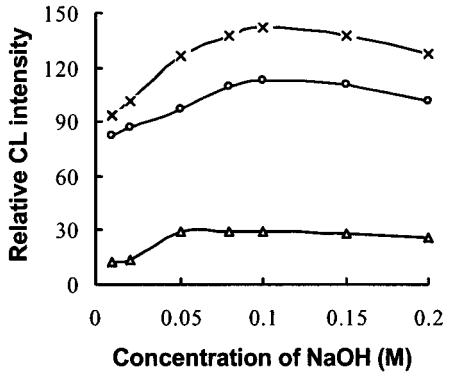

Figure 6. Effect of concentration of sodium hydroxide on CL intensity: (○) CL intensity in the presence of chlorpyrifos (I_s): (×) CL intensity in the absence of chlorpyrifos (I_0); (△) decrease of CL intensity (ΔI).

a middling CL intensity is for measuring an inhibitive effect of chlorpyrifos on CL reaction.

Effect of Sodium Hydroxide Concentration. It was found that luminol reacts with periodate and emits a CL signal readily in an alkaline medium. As **Figure 6** shows, a sodium hydroxide concentration of <0.05 mol·L^{-1} leads to an apparent decrease in ΔI. The maximum intensity was found with 0.1 mol·L^{-1} sodium hydroxide. When the concentration of sodium hydroxide is >0.2 mol·L^{-1}, there is a scattering effect in the flow cell due to the discrepancy between the refractive indices of various components. Thus, 0.1 mol·L^{-1} sodium hydroxide was selected as an optimal condition.

Effect of Flow Rate and Length of Mixing Tubing. The CL signal was related to the flow rate of carrier and eluant. The signal-to-noise ratio decreased at a higher flow rate because the higher flow rate would influence the rate of contact of eluant molecules with the ion-exchange resin. Nevertheless, the high flow rate could lead to an unstable baseline and shortening of the immobilized reagents column lifetime. The lower flow rate caused broadening of the peak and slowing of the sampling efficiency. A rate of 2.0 mL·min^{-1} was then chosen as a suitable condition with good precision and lower reagent consumption. The length of the mixing tubing was also adjusted to yield maximum light emission in the cell. It was found that 5.0 cm of mixing tubing afforded the best results with regard to sensitivity and reproducibility.

Performance of Proposed Method for Chlorpyrifos Measurements. A series of standard solutions were injected into the manifold depicted in **Figure 1** under the optimized conditions to test the linearity for the determination of chlorpyrifos. The decrement of CL intensity was found to be proportional with the logarithm of chlorpyrifos concentration. As **Figure 7** shows, the linear range is from 0.48 to 484.0 ng·mL^{-1}, and the regression equation is

$$\Delta I = 136.4 \log C_{\text{chlorpyrifos}} - 42.21 \qquad r^2 = 0.9969$$

The relative standard deviations of five determinations were 2.58, 2.25, and 1.64% with chlorpyrifos concentrations of 1.5, 15.0, and 150.0 ng·mL^{-1}, respectively. The limit of detection was 0.18 ng·mL^{-1}. At a flow rate of 2.0 mL·min^{-1}, the determination of analyte could be performed in 0.5 min, including sampling and washing, giving a throughput of ~120 times per hour with a relative standard deviation of $<3.0\%$.

Interference Studies. The interference of foreign substances was investigated by analyzing a standard solution of chlorpyrifos (2.5 ng·mL^{-1}) to which increasing amounts of interfering analyte were added. The tolerable concentration ratios with respect to

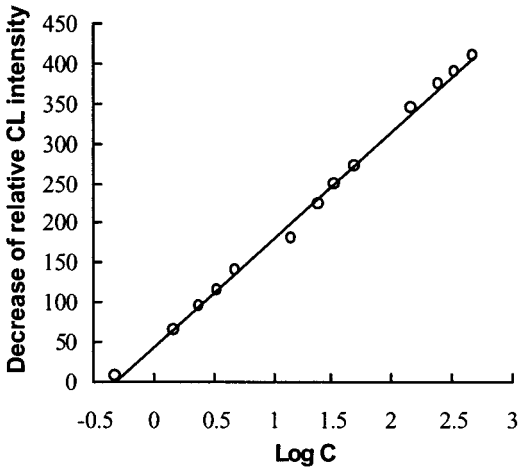

Figure 7. ΔI vs logarithm of chlorpyrifos concentration. The concentration range of chlorpyrifos is from 0.48 to 484.0 ng·mL^{-1}.

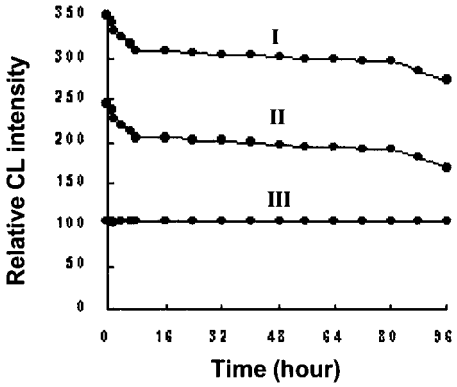

Figure 8. Stability of the flow sensor: I, CL intensity in the absence of chlorpyrifos (I_0); II, CL intensity in the presence of 2.5 ng·mL^{-1} chlorpyrifos (I_s); III, decrease of CL intensity (ΔI).

2.5 ng·mL^{-1} chlorpyrifos for interference at 5% level were over 2000 for Cl^-, NO_3^-, Ac^-, I^-, SO_4^{2-}, PO_4^{3-}, $Cr_2O_7^{2-}$, borate, oxalate, tartrate, salicylic acid, and malic acid, 800 for NH_4^+, Mg^{2+}, Ca^{2+}, Ba^{2+}, Zn^{2+}, Ni^{2+}, Mn^{2+}, and Cr^{3+}, 600 for methanol, ethanol, urea, Tween 80, CTMAB, and poly(vinyl alcohol), 200 for mannitol and dichlorvos pesticide, and 20 for Cu^{2+} and Fe^{2+}/Fe^{3+}.

Operational Stability of the Flow System. One hundred microliters of water was flow-injected through the system in the presence of 2.5 ng·mL^{-1} chlorpyrifos solution, and the ΔI was recorded to test the operational stability of the immobilized reagents column. The experiment lasted for 10 days, and the flow system was regularly used for >8 h per day. **Figure 8** shows the stability of the immobilized reagents column, and the average ΔI was calculated in 10 spot check determinations with RSDs of $<3.0\%$. The flow system showed remarkable stability and could be easily reused for >80 h.

Assay of Chlorpyrifos Residue on Orange and Shaddock. Following the method described under Experimental Procedures, the concentration of remnant chlorpyrifos on orange and shaddock was assayed directly without any pretreatment by determination of the eluted solutions of the prepared samples after appropriate dilution (with factors of 2.5−10 for orange sample and 10−100 for shaddock sample). The results of clean and control samples are listed in **Tables 3** and **4**, with recovery varying from 94.4 to 107.4% and RSDs of $<3\%$.

The concentration of remnant chlorpyrifos on orange and shaddock was also determined by HPLC (Waters column

Table 3. Results of Remnant Chlorpyrifos on Orange[a] ($n = 5$)

orange/cm^2 [b]		added, ng·mL^{-1}	found, ng·mL^{-1}	RSD %	recovery %	content in eluant, μg·mL^{-1} by proposed method	content in eluant, μg·mL^{-1} by HPLC	total content, μg·mL^{-1}	total chlorpyrifos on fruits, μg·cm^{-2}
1/115	1st[c]	0	24.4	2.54	99.4	0.244	0.230	61.1/250	0.530
		40.0	64.2	1.61					
	2nd	0	17.9	2.60	96.5	0.179	0.178	26.9/150	0.234
		22.0	39.2	1.71					
	3rd	0	24.4	2.25	99.2	0.061	0.059	9.2/150	0.080
		20.0	44.3	1.67					
2/132	1st	0	26.3	2.41	95.9	0.263	0.269	65.8/250	0.497
		40.0	64.7	1.58					
	2nd	0	17.0	2.73	97.6	0.170	0.175	25.5/150	0.192
		22.0	38.4	1.82					
	3rd	0	26.3	2.20	98.2	0.066	0.071	9.9/150	0.074
		20.0	46.0	1.63					
3/101	1st	0	21.9	2.22	94.4	0.219	0.224	54.6/250	0.543
		40.0	59.6	1.62					
	2nd	0	20.8	2.24	97.1	0.208	0.214	31.2/150	0.310
		22.0	42.2	1.73					
	3rd	0	30.2	1.94	103.0	0.075	0.081	11.3/150	0.112
		20.0	50.8	1.69					
4/156	1st	0	32.0	2.68	96.7	0.320	0.326	80.1/250	0.513
		40.0	70.7	1.70					
	2nd	0	14.8	1.90	96.8	0.148	0.145	22.2/150	0.142
		22.0	36.1	1.52					
	3rd	0	25.3	1.23	104.8	0.063	0.068	9.47/150	0.061
		20.0	46.2	0.63					
5/143	1st	0	33.8	1.98	99.7	0.338	0.333	84.5/250	0.591
		40.0	73.7	1.40					
	2nd	0	11.8	2.14	99.6	0.118	0.123	17.6/150	0.123
		22.0	33.7	1.46					
	3rd	0	28.5	1.76	102.7	0.071	0.066	10.7/150	0.075
		20.0	49.1	1.34					
6/168	1st	0	35.1	1.65	105.9	0.351	0.357	87.7/250	0.522
		40.0	77.4	1.11					
	2nd	0	10.6	1.86	96.6	0.106	0.110	15.9/150	0.095
		22.0	31.9	1.52					
	3rd	0	23.8	1.44	100.3	0.058	0.060	8.94/150	0.053
		20.0	43.9	1.04					

[a] Oranges 1–3 were prepared with cleanup procedure, and oranges 4–6 were prepared without cleanup procedure. [b] Area of surface of the orange. [c] 1st, 2nd, 3rd: time the prepared sample was dipped in 250 or 150 mL of water for 10 min.

μBondapak C_{18}, 4.6 × 150 mm, mobile phase of MeOH/H_2O), and the eluant that samples were immersed in was determined directly without dilution. The comparison of the assay of residue chlorpyrifos between the proposed method and HPLC has been investigated through the calibration graph of results obtained by the proposed method versus that by HPLC, and the results for the remnant chlorpyrifos on orange and shaddock were validated to be well in agreement with those obtained by HPLC. The regression equation is

$$Y_{CL} = 1.0022X_{HPLC} + 0.0003 \qquad r^2 = 0.9999$$

Monitoring of the Variation of the Concentration of Chlorpyrifos during 35 h. Following the method described under Experimental Procedures, the proposed method has been applied in monitoring of the variation of the concentration of chlorpyrifos in the solution (methanol/H_2O = 1:20) of 200 μg·mL^{-1} exposed to the sunlight in the open air. The standard addition method was utilized on the samples for quantification, to which a known quantity of chlorpyrifos was added in chlorpyrifos samples. The experimental results are listed in **Table 5**, and the results indicated that ~89% chlorpyrifos has been varied in the first 10 h and up to 99.96% during 35 h in the open air. The *t* test and recoveries are presented with average recoveries ranging from 93.9 to 107.0% and RSDs of <3%.

Possible CL Mechanism of the Reaction. A possible CL mechanism of luminol−periodate−chlorpyrifos was proposed. To support the possible reaction mechanism of chlorpyrifos, four different solutions at the same concentration in the flow system were studied. The absorbance of different solutions was measured by UV at 254 nm, and the results are listed in **Table 6**. It was found that the rate of the reaction of periodate with chlorpyrifos in solution was very fast. It was obvious that the absorption intensity of chlorpyrifos decreased quickly in the presence of periodate. It was also found that the product of reaction between periodate and chlorpyrifos could not oxidize luminol chemiluminescently. Hence, the mechanism of the inhibition effect of chlorpyrifos on the luminol−periodate CL system could be presented as

$$\text{chlorpyrifos (red. state)} \xrightarrow{IO_4^-} \text{chlorpyrifos (ox state)}$$

$$\text{periodate} + \text{luminol} \xrightarrow{OH^-} \text{aminophthalate} + h\nu\ (\lambda_{max}\,425\text{nm})$$

Conclusions. The presented CL method is simpler, quicker, and more sensitive than the existing methods. A newly designed

Table 4. Results of Remnant Chlorpyrifos on Shaddock[a] ($n = 5$)

shaddock/cm^2 [b]	c	added, ng·mL^{-1}	found, ng·mL^{-1}	RSD %	recovery %	content in eluant, μg·mL^{-1} by proposed method	content in eluant, μg·mL^{-1} by HPLC	total content, μg/100 mL	total chlorpyrifos on fruits, μg·cm^{-2}
1/220	1st	0	17.3	2.20	107.4	1.73	1.72	172.9	0.783
		50.0	71.0	1.40					
	2nd	0	37.9	2.21	99.7	0.379	0.373	37.9	0.172
		50.0	87.7	0.90					
2/253	1st	0	14.4	2.31	103.1	1.43	1.44	143.6	0.574
		50.0	65.9	1.51					
	2nd	0	40.7	2.07	102.8	0.407	0.413	40.7	0.162
		50.0	92.1	1.23					
3/181	1st	0	11.5	2.49	99.4	1.15	1.16	114.9	0.638
		50.0	61.2	1.62					
	2nd	0	33.8	2.16	103.4	0.338	0.343	33.8	0.188
		50.0	85.5	1.34					
4/228	1st	0	27.9	1.18	102.4	2.79	2.77	278.6	1.22
		50.0	79.1	0.82					
	2nd	0	43.2	1.83	99.8	0.432	0.438	43.2	0.189
		50.0	93.1	0.83					
5/246	1st	0	31.5	1.75	96.4	3.15	3.16	315.0	1.28
		50.0	79.7	1.15					
	2nd	0	39.7	2.43	104.4	0.397	0.390	39.7	0.160
		50.0	91.9	1.10					
6/259	1st	0	23.2	1.97	103.6	2.32	2.30	232.1	0.896
		50.0	75.0	1.75					
	2nd	0	45.6	2.31	97.2	0.456	0.462	45.6	0.176
		50.0	94.2	0.91					

[a] Shaddocks 1–3 were prepared with cleanup procedure, shaddocks 4–6 were prepared without cleanup procedure. [b] Area of surface of the shaddock. [c] 1st, 2nd: time for which the prepared sample was dipped in 100 mL of water for 10 min.

Table 5. Results of Variation of Chlorpyrifos Concentration during 35 h[a]

interval hour	added, ng·mL^{-1}	found, ng·mL^{-1}	RSD %	recovery %	t, $t_{0.05,5} = 2.57$	content in solution, μg·mL^{-1}	variation ratio %
0.1	0.0	40.0	1.39	100.9	0.73	1.99×10^2	0.11
	30.0	70.2	0.83				
1.0	0.0	27.6	1.85	96.8	2.06	1.38×10^2	31.05
	30.0	56.6	1.05				
2.0	0.0	32.7	1.66	102.3	1.72	1.02×10^2	48.90
	30.0	63.4	0.88				
4.0	0.0	25.2	1.82	97.5	1.85	78.7	60.67
	30.0	54.4	0.92				
6.0	0.0	19.0	1.91	99.2	0.47	59.5	70.24
	30.0	48.4	1.14				
10.0	0.0	13.1	2.18	102.6	1.46	40.9	79.57
	30.0	43.8	1.18				
23.0	0.0	13.0	2.39	98.5	0.78	16.3	91.86
	30.0	42.6	1.33				
31.0	0.0	6.2	2.42	93.9	2.48	7.69	96.15
	30.0	34.3	1.64				
35.0	0.0	0.14	2.89	107.0	2.77	8.72×10^{-2}	99.96
	30.0	32.2	1.69				

[a] Average of five determinations

manifold offers a reagentless process and reduced background noise. Advantages including low reagent consumption and the use of water as eluant contributed to the "green" analytical procedure due to the minimization of potential pollution to the environment. With good selectivity, an application to the residue of chlorpyrifos on fruits is one unique feature of the present method. The green analytical procedure offers the promise for routine assay of chlorpyrifos residues in environmental samples, agricultural products, and foodstuffs.

Table 6. Results of Absorbance at Different Solutions by UV at 254 nm

species[a]	A[b]	species[a]	A[b]
chlorpyrifos[c]	0.548	luminol + chlorpyrifos[c]	0.636
periodate	0.074	periodate + chlorpyrifos[c]	0.270
luminol	0.093		

[a] Same concentration and injection volume (2.0×10^{-4} mol·L^{-1}, 25 μL). [b] Average of five determinations. [c] Solutions were prepared freshly.

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