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Article

Determination of Picloram in Waters by Sequential Injection Chromatography with UV Detection

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Este trabalho descreve um procedimento de cromatografia por injeção seqüencial para a determinação de picloram em águas explorando a baixa pressão de uma coluna monolítica C_{18} de 2,5 cm de comprimento. A separação do analito da matriz foi obtida em menos de 60 s usando como fase móvel uma mistura de acetonitrila e H_3PO_4 5,0 mmol L^{-1} na proporção 20:80 (v v^{-1}) e vazão de 30 µL s^{-1}. Detecção foi feita a 223 nm com uma cela de 40 mm de caminho óptico. O limite de detecção do método é adequado para monitorar o nível de concentração máximo permitido para picloram em água potável (500 µg L^{-1}). A frequência de amostragem é de 60 análises por hora, consumindo 300 µL de acetonitrila por análise. A metodologia foi aplicada a águas de rio fortificadas, não sendo observadas diferenças estatisticamente significativas em comparação com a metodologia convencional de HPLC-UV.

This paper describes a sequential injection chromatography procedure for determination of picloram in waters exploring the low backpressure of a 2.5 cm long monolithic C_{18} column. Separation of the analyte from the matrix was achieved in less than 60 s using a mobile phase composed by 20:80 (v v^{-1}) acetonitrile:5.0 mmol L^{-1} H_3PO_4 and flow rate of 30 µL s^{-1}. Detection was made at 223 nm with a 40 mm optical path length cell. The limits of detection and quantification were 33 and 137 µg L^{-1}, respectively. The proposed method is sensitive enough to monitor the maximum concentration level for picloram in drinking water (500 µg L^{-1}). The sampling frequency is 60 analyses per hour, consuming only 300 µL of acetonitrile per analysis. The proposed methodology was applied to spiked river water samples and no statistically significant differences were observed in comparison to a conventional HPLC-UV method.

Keywords: sequential injection chromatography, monolithic column, picloram, waters

Introduction

The presence of pesticides in surface and ground waters is a consequence of the extensive use of these chemicals in agriculture and their runoff down through the soil profile.[1] Picloram (4-amino-3,5,6-trichloro-2-pyridinecarboxilic acid) is a herbicide widely used to control weeds in crops of sugar cane (pre-emergency), rice, pasture and wheat (pos-emergency).[2] This herbicide can stay active in soil for long time, depending on the type of soil, soil moisture and temperature. It may exist at toxic levels to plants for more than one year after application at normal rates.[2,3] It is chemically adsorbed onto clay particles and natural organic matter occurring in soils. If the soil is poor in clay or organic matter contents, the herbicide may be easily leached to surface and ground waters.[3-6]

Determination of picloram is usually made by gas-liquid chromatography with electron capture detector or mass spectrometry detectors,[7] although several high performance liquid chromatography methods have already been proposed using either UV absorption or mass spectrometry detection modes.[8-11] These methods are very sensitive, but require the use of large sample volumes, besides to extensive extraction steps, derivatization reactions and expensive instrumentation, so that new sensitive methods that reduce the time of analysis and the use of organic solvents are needed. Electroanalytical methods are known to attend the demand for minimal sample treatment and low consumption of organic solvents,[12,13] but these techniques are liable to matrix effects such as passivation

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of the electrode surface by naturally occurring organic matter, besides to interference from any other electroactive substances with $E_{1/2}$ close to that one of the analyte.

Sequential injection chromatography (SIC) is a relatively new liquid chromatography technique[14] that explores the potentialities of sequential injection analysis for flow programming[15] and the low backpressures provided by C_{18} monolithic stationary phases. This technique uses low cost instrumentation and has been applied especially in the determination of components of pharmaceutical products,[16] although determinations of herbicides and pesticides[17,18] and amino acids[19] have been described. The present paper shows that the SIC capabilities for separation of simple mixtures can be explored for monitoring the maximum concentration levels of picloram in natural waters.[20] To achieve this goal a SIC method was developed and applied for determination of the herbicide in spiked natural waters collected in the São Paulo State, Brazil.

Experimental

Apparatus and reagents

A SIChrom™ - accelerated liquid chromatography system was provided by FIAlab® Instruments (Bellevue, WA, USA) and schematized in Figure 1, where PP is a piston pump model S17 PDP from Sapphire Engineering™ (Pocasset, MA, USA) with capacity of 4.0 mL, built in ULTEM®, having a ceramic piston (P) for solution propelling and aspiration. The frontal port (FP) of PP is connected to the central port of a rotary selection valve (SV) by the holding coil (HC), which is made of 2 m of 0.8 mm i.d. Teflon tubing (capacity of 1.0 mL). The rear port (RP_1) of PP is connected to the main solvent reservoir (MP_1) through ports 9 and 10 of SV (Figure 1). An additional port in the pump body (RP_2) is connected through a 4-way valve to a relief valve (RV) from Up-Church Scientific (Oak Harbor, WA, USA) that opens to waste at pressure > 500 psi. A Cheminert® Valco10-port multi-position valve (SV) model C25 stream selector C25-3180 EMH (Valco Instruments, Houston, TX, USA) was used to select and drive sample and mobile phase solutions though the system. Port 2 of SV is connected to a 5 mm long guard column coupled to a 25 mm reverse phase C_{18} Chromolith Flash monolithic column, both from Merck KGaA (Darmstadt, Germany). Detection was made by molecular absorbance spectrophotometry using an USB 4000 spectrometer (Ocean Optics, Dunedin, FL, USA) coupled to an SMA-Z-40 µvol PEEK flow cell (FIAlab Instruments, Bellevue, WA, USA) with 40 mm of optical path length and 10 µL of internal volume.

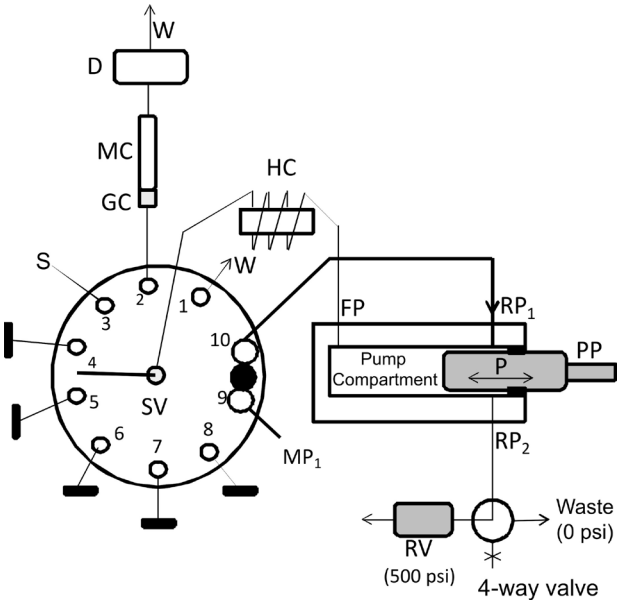

Figure 1. SIC manifold to perform determination of picloram in river waters. PP = piston pump, P = piston; RP_1 = rear port 1, RP_2 = rear port 2, RV = relief valve, HC = holding coil (2 m of 0.8 mm i.d. PTFE tubing), W = waste, SV = 10 port selection valve, GC = 5 mm C_{18} monolithic guard column, MC = 25 mm C_{18} monolithic column, D = UV-Vis detector (223 nm), S = sample/standard solution, MP_1 = mobile phase for isocratic elution composed by 20:80 ACN: 5.0 mmol L^{-1} H_3PO_4. The selection valve is shown in the fill-position, in which a circular groove connects ports 9 and 10 allowing the pump to fill. In any other rotor position, the circular groove does not connect adjacent ports.

A DH 2000 Deuterium Tungsten Halogen lamp (Mikropack GmbH, Germany) was used as light source. Two 600 µm diameter optical fibers (20 inches long) were used to transmit radiation from the source to the flow cell and to the spectrometer. Connections of port 2 of SV to the pre-column and from the column outlet to flow cell are made, respectively, with 40 and 18 cm long 0.25 mm i.d. PEEK (polyetheretherketone polymer) tubing. Connection of the MP_1 reservoir to SV (port 9) is made with 1/8 o.d. Teflon tubing. Connection of SV (port 10) to RP of PP is made with 1.0 mm i.d. PEEK tubing. Port 3 of SV is connected to the sample reservoir (S), and, to minimize the sample consumption, this connection is made with 15 cm of 0.25 mm i.d. PEEK tubing (7.4 µL). Ports 4 to 8 were not used in the proposed methodology and were kept blocked with solid Teflon tubing. The system is controlled by the FIAlab for Windows software.

An LC 9A Shimadzu high performance liquid chromatograph (HPLC), equipped with a SPD 6 AV UV detector and the LC Workstation Class-LC 10 software was used in comparison studies. An SBC18 Zorbax-HP column (3.5 µm, 150 mm × 4.6 mm) connected to a C18 Phenomenex guard column was used. Sample injection was made with a rotary Rheodyne valve using a 20 µL sample loop.

Acetonitrile (ACN) of HPLC grade was supplied by J.T. Baker (Phillipsburg, NJ, USA). Water used in all experiments was distilled and deionized using the Simplicity 185 system from Millipore (Billerica, MA, USA) coupled to an UV lamp. Mobile phases were filtered through 0.45 μm LCR-PTFE membranes prior to use. In all experiments a helium stream was used to remove dissolved air from the mobile phases. All other reagents used in this work were of analytical grade from Merck, Sigma or Aldrich. A stock 1000 μg L^{-1} solution of picloram was prepared by dissolving the solid standard (Riedel-de Haën, purity > 97.4%, molar mass 241.46 g mol^{-1}) in ethanol. Working solutions were prepared by diluting these stock solutions in distilled deionized water.

Analysis of picloram

First, the pump compartment of PP, as well as HC, and the flow cell are filled with the mobile phase MP_1 composed by (20:80) ACN: 5.0 mmol L^{-1} phosphoric acid. Column conditioning was made by performing three cycles of aspiration of 4.0 mL of MP_1 inside the pump compartment at a flow rate of 100 μL s^{-1}, followed by emptying the pump through port 2 of SV, which is connected to GC, MC and D (Figure 1), at a flow rate of 30 μL s^{-1}. Cleansing of the sampling line is made by aspirating 50 μL of sample or standard to the holding coil, followed by discarding 300 μL (sample plus mobile phase) through port 1 of the selection valve. For calibration and analysis 1500 μL of MP_1 are aspirated inside the pump compartment through ports 9 and 10 of SV (Figure 1), followed by aspiration of 100 μL of sample/standard solution inside HC through port 3 of SV (100 μL s^{-1}). Next, the pump is emptied through port 2 at a flow rate of 30 μL s^{-1}, performing the sample injection and analyte elution simultaneously to the acquisition data from the UV-Vis detector. Ports 4 to 8 of SV are blocked.

HPLC analyses of picloram were made by isocratic elution with a mobile phase constituted by (50:50) acetonitrile : 0.10% (m/v) phosphoric acid. The analyses were made at a flow rate of 1.0 mL min^{-1} and injecting a sample volume of 20 μL. The UV detector monitored the absorbance at 223 nm.

Samples

Water samples were collected in two reservoirs located in the metropolitan area of São Paulo (Brazil) used as water supplies for São Paulo City. Other samples were collected in reservoirs of Atibaia River, near to the municipality of Americana (São Paulo State), in an agricultural area dominated by sugar cane cultivation, where herbicides are extensively used. A tap water sample collected at the laboratory was also analyzed. Water samples were filtered through a 0.45 μm cellulose acetate membrane and stored in glass bottles at 4 °C. Quantification was performed by external calibration, preparing the standards in deionized water. Blank in all experiments was deionized water. Recovery experiments were performed by spiking the samples with 0.50 mg L^{-1} of picloram, adopting a delay time of 24 h between the spike and the analysis.

Results and Discussion

Method development

The composition of the mobile phase was studied by varying the content of ACN from 10 to 30% (v/v) in relation to the 5.0 mmol L^{-1} H_3PO_4 aqueous phase. The 30:90 ACN: 5.0 mmol L^{-1} H_3PO_4 did not provide suitable separation between picloram and the unretained peak. Mobile phase constituted by 20:80 ACN:5.0 mmol L^{-1} H_3PO_4 provided the best compromise between separation of picloram from the matrix and the length of the chromatographic runs, which were unnecessarily increased at lower ACN concentrations (10:90 ACN:5.0 mmol L^{-1} H_3PO_4).

The influence of sample volume was studied by injecting 25 to 200 μL of a 5.0 mg L^{-1} picloram solution. Peak areas increased linearly with the sample volume (S_v) up to 100 μL, obeying the equation Area = (0.0223 ± 0.002) S_v + (0.01 ± 0.02), r = 0.9998, but for larger volumes the signals leveled off and carryover between subsequent injections was observed, so that 100 μL was used in the application of the method. Flow rates between 10 and 60 μL s^{-1} were studied. Although at 60 μL s^{-1} the analysis could be made in about 30 s, implying in a sampling throughput of 120 analyses *per* hour, the flow rate of 30 μL s^{-1} was used in the next studies to avoid leaking problems through the relief valve (RV, Figure 1), reaching a sampling throughput of 60 analyses *per* hour. Flow rate of 30 μL s^{-1} is not applicable with conventional syringe pumps conventionally used in SIA systems because of phase mobile leaking through the two way syringe valve .[16]

Figures of merit

The repeatability of the method was evaluated at two concentration levels, 0.25 and 5.0 mg L^{-1}, resulting in variation coefficients of 4.4 and 1.0%, respectively. Figure 2 shows the chromatograms of a typical calibration curve for picloram concentrations between 0.25 and 5.0 mg L^{-1}, obtained by injecting a sample volume of 100 μL and eluting the column at a flow rate of 30 μL s^{-1}. Peak area (Y) versus picloram concentrations (C) fitted to the linear equation

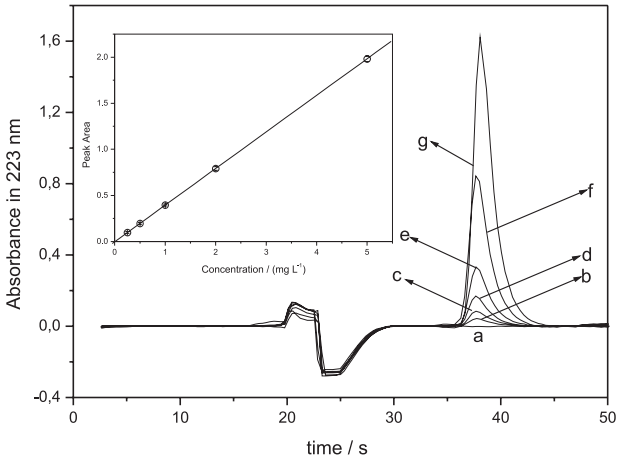

Figure 2. Sequential Injection Chromatograms for calibration of the SIC system obtained by injecting 100 µL of standard at flow rate of 30 µL s^{-1} in a mobile phase composed by 20:80 ACN: 5.0 mmol L^{-1} H_3PO_4 Picloram concentrations of the calibration solutions were: (a) blank, (b) 0.25, (c) 0.50, (d) 1.0, (e) 2.0, (f) 5.0 e (g) 10 mg L^{-1}. The inset shows the linear correlation between peak area and picloram concentration.

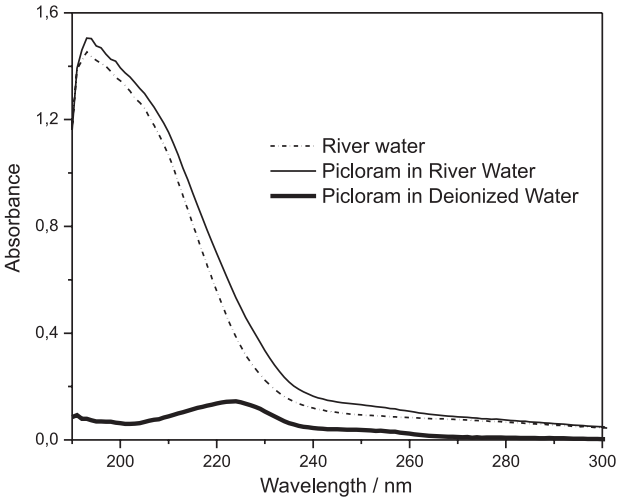

Figure 3. Absorption spectrum of a 0.50 mg L^{-1} picloram solution in deionized water superposed to the spectra of a river water sample and river water spiked with 0.50 mg L^{-1} of picloram.

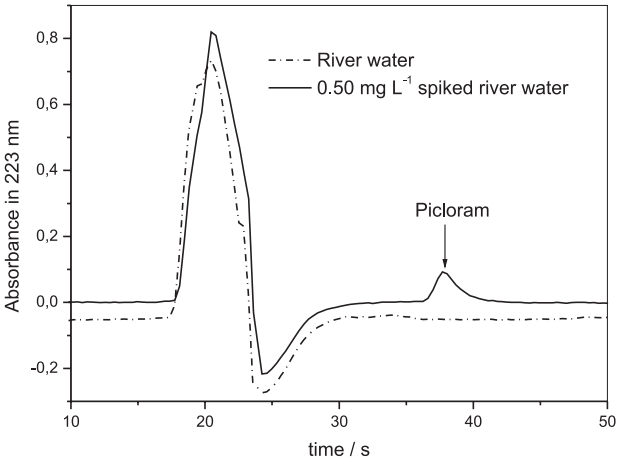

Figure 4. Sequential injection chromatograms of a picloram free river water superposed to a chromatogram of the same sample spiked with 0.50 mg L^{-1} of picloram, exhibiting the absence of interference peaks in the retention time of the analyte. The large and high peak at 20.5 s is assigned to unretained substances with strong absorption of UV radiation.

$Y = (0.397 \pm 0.001)C - (0.002 \pm 0.001)$ with r = 0.9998. The limits of detection (LOD) and quantification (LOQ) were 33 and 137 µg L^{-1}, respectively. These parameters were computed as $LOD = 3\sigma/S$ and $LOQ = 10\sigma/S$, where σ is the standard deviation of ten peak area measurements corresponding to the 0.25 mg L^{-1} solution and S is the slope of the analytical curve.[21] The Environmental Protection Agency (EPA) defines the maximum contaminant level (MCL) for picloram in drinking water as 0.50 mg L^{-1},[20] so that the proposed method, even using the short 25 mm column, is sensitive enough for monitoring picloram.

Selectivity

Figure 3 shows the ultraviolet absorption of picloram superposed to typical river water and spiked river water spectra, showing that the chromatographic separation is needed because natural organic matter (humic substances, polysaccharides etc) and inorganic ions such as nitrate and nitrite strongly absorb radiation in the ultraviolet region. On the other hand, these substances are polar and do not interact with the C_{18} stationary phase, being eluted from the column together with the unretained peak of the sample solvent (Figure 4). Chromatograms of spiked river water sample (Figure 4) shows that the mobile phase composed by 20:80 ACN:5.0 mmol L^{-1} H_3PO_4 conferred adequate selectivity toward the sample matrix. Additionally, because picloram is used in the agriculture in combination with other herbicides such as glyphosate, 2,4 D and paraquat, the potential interference of these compounds was studied at a concentration ratio of 1:20 for each compound (0.50 mg L^{-1} picloram plus 10 mg L^{-1} of the foreigner herbicide). No significant interference was observed because glyphosate and paraquat are not retained in the stationary phase and 2,4 D elutes at distinct retention time in relation to picloram. Interference of triazine herbicides such as simazine, atrazine and propazine is not expected because these compounds require mobile phase containing higher volumetric ratio of acetonitrile (35:65),[17] so that their t_R would be longer than that of picloram using the 20:80 ($v:v^{-1}$) ACN:5.0 mmol L^{-1} H_3PO_4 mobile phase. However, the presence of these triazines would require column cleaning to avoid crossover interference between analyses. Cleaning could be made by increasing either the elution time or the proportion of ACN in the mobile phase. The latter approach would require column reconditioning, which could be made by the stepwise elution approach.[19]

Table 1. Picloram recoveries obtained by the proposed SIC method and conventional HPLC-UV method with a packed particle C_{18} column for 0.50 mg L^{-1} picloram spiked waters collected at the Salto Grande (SG), Guarapiranga (G), Billings (B), Praia dos Namorados reservoirs and a Tap Water (TW) sample collected at the laboratory

Sample	SIC		HPLC		Relative Error (%)[b]
	Conc. Found (mg L^{-1})	Recovery (%)	Conc. Found (mg L^{-1})	Recovery (%)	
SG	0.40 ± 0.08[a]	80	0.441 ± 0.003[a]	88.2	–9.2
G	0.51 ± 0.02	102	0.471 ± 0.003	94.2	8.3
B	0.49 ± 0.01	98	0.47 ± 0.01	94	4.3
PN	0.50 ± 0.09	100	0.51 ± 0.02	102	–2.0
TW	0.45 ± 0.02	90	0.43 ± 0.01	86	4.7

[a] Standard deviation of 3 measurements; [b] Relative error computed as $(\frac{SIC_{conc.} - HPLC_{conc.}}{HPLC_{conc.}})\times 100$.

Application to spiked waters

Five river water samples free of detectable amounts of picloram were spiked with 0.50 mg L^{-1} of the herbicide, and let to stand for 24 h before analyses. Recoveries between 80 and 102% were found (Table 1). From the F test, no evidence of statistically significant differences was observed in the precision of the two methods at the 95% confidence level. Relative errors of –9.2 to +8.3% were observed by comparing the results obtained by SIC with those ones obtained by HPLC.[10] The *t* test at 95% confidence level for comparison of mean results for each sample did not show evidences of statistically significant differences between the two methods.

The main drawback of the SIC system is the short life time of the guard columns (up to about 100-150 injections), which are often clogged, even filtering sample and mobile phases. The clogging increases the pressure needed for mobile phase pumping, leading to leakage through either the relief valve or through the rotary selection valve, altering the retention times of the analyte. Instrumental improvements are still needed for this relatively new and promising liquid chromatography technique to enhance its robustness. For instance, to overcome leakage problems, the change of the low pressure Cheminert® Valco10-port multi-position valve by another one capable to work at pressures up to 5000 psi is recommended.

Conclusion

Sequential injection chromatography with UV detection was feasible to determine picloram concentrations in natural spiked waters at concentrations > 137 μg L^{-1} at a sampling frequency of about 60 analyses *per* hour. Consumption of ACN is 300 μL *per* analysis, which is a significant advantage over conventional HPLC fitted to conventional 5 μm packed particle column. This is an interesting feature, attending the demand for clean analytical methods that consume less reagents and solvents.[22]

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