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Evaluation of comprehensive two-dimensional gas chromatography with micro-electron capture detection for the analysis of seven pesticides in sediment samples

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ABSTRACT

A GC-μECD and a GC × GC-μECD method were developed for the analysis of pesticides in sediments. For 1D-GC, instrumental LOD and LOQ were found in the range from 0.60 to 2.31 μg L^{-1} and 1.83 to 5.62 μg L^{-1}, respectively. For GC × GC method development two sets of columns were tested (DB-5/DB-17ms, and HP-50+/DB-1ms), and the best results were obtained with the set of columns DB-5/DB-17ms. Instrumental LOD and LOQ were found in the range from 0.08 to 1.07 μg L^{-1} and 0.25 to 3.23 μg L^{-1}, respectively. The LOD for the GC × GC was about 36% lower than those obtained for the 1D-GC. Concentrations of 21.18 μg kg^{-1} through 1D-GC method and 3.34 μg kg^{-1} for GC × GC for trifloxystrobin were found in a sediment sample which was collected close to an area of rice plantation.

1. Introduction

Comprehensive two-dimensional gas chromatography (GC × GC) is a relatively new technique, developed in 1991 by Liu and Phillips [1], and provides great separation power and sensitivity. For complex samples containing 150–250 compounds, for example, the separation obtained by 1D-GC with a single column is not sufficient for separation of all compounds [2]. Other multidimensional techniques may not provide effective separation of all analytes either. Some examples may be mentioned, such as high-performance liquid chromatography coupled to gas chromatography (HPLC–GC), supercritical fluid chromatography coupled to gas chromatography (SFC–GC), and heart-cut multidimensional gas chromatography (GC–GC) [2,3]. Among these techniques, HPLC may be advantageous, whenever removal of interferent materials or pre-separation of the sample into chemical classes by different polarities are needed [4]. SFC permits the separation of components that cannot be analyzed by HPLC and GC, such as non-volatile, of high molecular mass, reactive or thermally labile compounds. In the large majority of cases, GC–GC applies to a few narrow fractions of the sample. Whenever screening of an entire sample is required, MDGC becomes an extremely laborious and time consuming technique, with very careful fractionation and lengthy re-analysis of all fractions [4,5].

Selective detectors, such as nitrogen phosphorous detector (NPD) and electron capture detector (ECD) are usually preferential choices as they minimize matrix interferences and provide higher sensitivity for heteroatom containing compounds. This type of detector may also be cost effective when compared to mass spectrometry detectors (MSD), even though MSD is necessary for qualitative analysis and may be employed in the selective ion monitoring (SIM) mode for attaining enhanced sensitivity [6,7].

In GC × GC two chromatographic columns of different polarities are coupled in series, typically one of conventional dimensions (^{1}D, commonly 30 m in length and internal diameter of 0.25–0.32 mm), and another one shorter in the ^{2}D (the latter capable of generating fast-GC analysis) such that all, or a representative sub-sample of the effluent coming from the first column is driven to the second through a modulator [8]. The modulator is considered the "heart" of the technique, and its main functions are cutting and refocusing narrow adjacent fractions of the first column eluate and releasing them rapidly into the second column [9]. In general, the GC × GC has four advantages over 1D-GC: (1) peak capacity is much higher, which may provide a distinctly improved separation of the analytes in a sample, from each other, and from interfering matrix constituents; (2) chemically related compounds show up as ordered structures, which greatly facilitates group-type analysis and the provisional classification of unknowns; (3) greater sensitivity due to the focusing obtained through modulation and the use of sufficiently fast acquisition detectors which favors the detection of trace levels; (4) provides two different sets of retention data for all constituents of a sample, which yields and additional tool for their identification [2,10,11].

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The number of scientific publications related to the determination of pesticides by GC × GC is still relatively small but sufficient to demonstrate its potential advantages over 1D-GC. Dallüge et al. [12] studied the determination of 58 pesticides in vegetable extracts (carrots and celeriac), and showed that GC × GC dramatically improved the separation of analytes from the matrix, providing also superior quality of mass spectra. All pesticides could be identified using their full-scan mass spectra, which was not possible when using GC/TOFMS. Banerjee et al. [13] optimized a GC × GC/TOFMS method for multiresidue analysis of 51 pesticides in grapes and has observed that some GC/MS co-elution problems were solved by GC × GC. S/N ratio obtained with GC × GC was five times greater than that obtained with 1D-GC, rendering a detection limit 2 a 12 times lower for GC × GC/TOFMS, due to sharper and narrower peak shapes. GC × GC-NPD has been investigated for the separation and quantification of fungicides in vegetable samples by Khummueng et al. [14]. The results showed that GC × GC-NPD generated narrow ^{2}D peaks, which were approximately 18–20 times narrower than peaks generated in the conventional GC-NPD analysis method. The summation of peak height response from GC × GC was approximately 20-times larger than that of 1D-GC analysis, confirming the increase of analytical sensitivity. The potential of GC × GC-μECD was also verified by Bordajandi et al. [15] for the determination of five toxaphene congeners in fish oil, with a ^{1}D enantioselective column and a ^{2}D medium polarity column. A complete separation of the isomers was provided by GC × GC, while the same enantioselective column in 1D-GC led to coelution of the two compounds. Moreover, the GC × GC allowed excellent separation of the analytes from the complex matrix interferences.

Ramos et al. [16] demonstrated the feasibility of a MSPD method with GC/qMS and GC × GC-μECD analysis for the determination of 31 pesticides from three different chemical classes (organophosphorus, triazines and pyrethroids) in orange, apple, pear and grape. The limits of detection calculated for orange after sample preparation ranged from 250 to 9 μg kg^{-1} with GC/qMS (scan mode for organophosphorus and triazines, and SIM mode for pyrethroids) and 3.6–0.005 μg kg^{-1} with GC × GC-μECD analysis. The enhanced sensitivity and separation among the pesticides and the coextracted matrix components provided GC × GC-μECD allowed accurate determination of the analytes at levels far below the MRL set in current EU legislations.

Even though the use of pesticides provides several benefits, such as the enhancement of crop production and pest control, residues may remain in the environment for long periods of time, causing negative impacts to different ecosystems. Minimizing the occurrence of pesticides in air, water, soil and foodstuff is a very important goal as an environmentally correct management of the use of pesticides helps hindering public health problems. The investigation of pollutants, such as organic compounds and metals in sediments is of great environmental importance, because this matrix may be prone to accumulation of this type of compounds. Besides that, the occurrence of pesticides in sediments provides information about the quality of the water body [17].

The state of Rio Grande do Sul (RS), located in the South part of Brazil, is responsible for 61% of the rice production of the country [18]. According to research works carried out by the Environmental Agency of RS [19] and by the Rice Institute of RS (IRGA) [18], some pesticides are considered to be of major importance regarding rice production. Among them, seven compounds of different chemical classes were chosen for this study: propanil, fipronil, propiconazole, trifloxystrobin, permethrin, difenoconazole and azoxystrobin. Our aim is to assess the feasibility of the use of GC × GC-μECD and GC-μECD for the determination of these seven analytes in sediment samples collected from three regions under the influence of rice cultivation in Rio Grande do Sul.

2. Experimental

2.1. Chemicals and reagents

Standard pesticides (propanil, fipronil, propiconazole, trifloxystrobin, permethrin, difenoconazole and azoxystrobin) were chosen because they are heavily used in agriculture; specifically in rice cultivation in the region of the Santa Maria river in the state of Rio Grande do Sul, Brazil. These pesticides with purities >97% were from Sigma–Aldrich (Seelze, Germany). Acetone, and dichloromethane (analytical grade) were purchased from Vetec (Rio de Janeiro, RJ, Brazil), and ethyl acetate (HPLC grade) from Mallinckrodt (Paris, KY, USA). Acetone and dichloromethane were bidistilled before use. The anhydrous sodium sulphate was obtained from Merck (Darmstadt, Germany) and was activated prior to use. All materials employed in the extraction process were rigorously washed with soap and water, distilled water, acetone, and heptane. Single and mixed standard stock solutions and their further dilutions were prepared in ethyl acetate. Solutions were stored at −18 °C in a freezer.

2.2. Instrumentation

2.2.1. Chromatographic conditions GC-μECD

One-dimensional GC analyses were performed on an Agilent 6890N (Agilent Technologies, Palo Alto, USA) equipped with a micro electron-capture detector (μECD) system. Injections were performed by the Combi PAL (CTC Analytics, Zwingen, Switzerland) in the pulsed splitless mode (1 μL, 60 psi). Hydrogen (purity 99,999%) was used as carried gas at a flow rate of 2 mL min^{-1} and nitrogen (purity 99,999%) was the make-up gas at a flow rate of 30 mL min^{-1}. Two columns (Agilent Technologies – J&W Scientific, Palo Alto, CA, USA) were tested with a standard mixture of 100 μg L^{-1}: HP-50+ (50%-phenyl-methylpolysiloxane, 30 m × 0.25 mm I.D. × 0.25 μm film thickness) and BB-5 (5% phenyl, 95% methylpolysiloxane, 30 m × 0.25 mm I.D. × 0.25 μm film thickness). Best chromatographic oven conditions for HP-50+ were: 50 °C (1.5 min) to 190 °C at 30 °C min^{-1} and to 220 °C at 5 °C min^{-1} and to 255 °C (44 min) at 7 °C min^{-1} and for DB-5 were 60 °C (2.5 min) to 180 °C at 35 °C min^{-1} and to 240 °C at 4 °C min^{-1} and to 300 °C (2 min) at 12 °C min^{-1}. Injector temperature was 280 °C and detector temperatures were 280 °C and 325 °C for HP-50+ and DB-5, respectively.

2.2.2. Chromatographic conditions GC × GC-μECD

The same GC system (Agilent 6890N) was equipped with a secondary column oven and non-moving quadjet dual stage thermal modulator. During modulation, cold pulses were generated using dry nitrogen gas cooled by liquid nitrogen, whereas heated dry air was used for hot pulses. Injections were performed by the Combi PAL (CTC Analytics, Zwingen, Switzerland) in the pulsed splitless mode (1 μL, 60 psi, 280 °C). Hydrogen was used as carrier gas at a flow rate of 2 mL min^{-1} and nitrogen was the make-up gas at a flow rate of 150 mL min^{-1}. Two column sets were tested with the standard mixture of pesticides in a concentration of 100 μg L^{-1}: DB-5 (30 m × 0.25 mm I.D. × 0.25 μm) for ^{1}D coupled to a DB-17ms column as ^{2}D (50% phenyl, 50% methylpolysiloxane, 1.70 m × 0.18 mm I.D. × 0.18 μm), and HP-50+ (30 m × 0.25 mm I.D. × 0.25 μm film thickness) for ^{1}D coupled to a DB-1ms as ^{2}D column (100% dimethylpolysiloxane, 1.70 m × 0.10 mm I.D. × 0.10 μm film thickness). The final selected column set for all subsequent studies was DB-5/DB-17ms. Columns were connected to the modulator via mini press-fits (Siltek®, Restek). Injector and modulator temperature offset were 280 °C and 20 °C, respectively. Modulation period was 7s, while hot pulse duration and detector temperature were 2.1 s and 0.8 s and 320 and 335 for DB-5/DB-17ms and HP-50+/DB-1ms, respectively. The modulator temperature offset was

20 °C and data acquisition rate was set at 50 Hz (25–150 scans per peak). ChromaTOF version 3.32 (Leco, St. Joseph, MI, USA) software was used for acquiring the raw data, data processing, evaluation and visualization. The GC oven temperature program for DB-5/DB-17ms column set was 60 °C (2.53 min) to 180 °C at 35 °C min^{-1} and to 240 °C at 4 °C min^{-1} and to 295 °C (3 min) at 10 °C min^{-1}. For HP-50+/DB-1ms column set was 50 °C (1.5 min) to 190 °C at 50 °C min^{-1} and to 280 °C at 5 °C min^{-1} (12 min). Identification of analytes in real samples was established through comparison of retention times, using HP-50+/DB-1ms columns set.

2.3. Collection of sediments samples

Sediment samples were collected from three sites in the state of Rio Grande do Sul, Brazil, two in the Santa Maria river (SD_1 and SD_2), and one in the Gravataí river (control sample), where compounds of interest were not present. The region of Santa Maria river presents intense activity of rice cultivation and industrial sites are virtually absent, although sewage waste discharge also impacts the quality of water resources. Approximately 1 kg of sediment samples were collected near the shore of the water body and placed in glass bottles. Sample temperature was kept at 4 °C during transportation and was frozen at −18 °C upon arrival at the laboratory. They were maintained at this temperature until extraction process.

2.4. Sample preparation

Sediment sample was extracted through sonication, and parts of this method was based on information reported by You et al. [20]. Frozen sediment was thawed and the residual water present in it was removed. The sediment sample was homogenized and partially dried at room temperature. Ten grams of sodium sulphate was added to 20 g of dried sediment and, after homogenization, an aliquot of 50 mL of acetone:dichloromethane (1:1 v/v) was added to the sample. This mixture underwent extraction for 15 min in an ultrasonic bath Maxiclean (Unique, Indaiatuba, Brazil) and this procedure was repeated three times. Extracts were filtered through filter paper (Quanty, Germany) containing roughly 2 g of sodium sulphate to remove residual humidity. After combining the extracts and evaporating them to dryness, under ambient temperature, 1.5 mL of ethyl acetate was added to the residual material. One gram of homogenized sediment was dried at 90 °C overnight and subsequently weighed in order to determine water content.

2.5. Calibration curve and linearity

Calibration curves were evaluated using solutions of the pesticides in ethyl acetate in five different concentrations, ranging from 5 to 400 μg L^{-1}. Each solution was chromatographically analyzed five times. The 3,4,5-trichloroguaiacol was employed as internal standard resulting in a concentration of 100 μg L^{-1} in the final solutions. For each pesticide a calibration curve equation, regression coefficient (r^2), and linear range were calculated.

2.6. Limits of detection (LOD), and quantification (LOQ)

LOD and LOQ were determined according to the ICH (International Conference on Harmonization) guideline which suggests calculation based as 3.3 and 10 times the ratio between the standard deviation of the intercept (response), *s*, and the slope estimated, *S*, from the calibration curve of the analytes [21].

2.7. Precision (repeatability and intermediate precision), and accuracy

The precision of the method was evaluated in terms of repeatability and intermediate precision expressed as relative standard deviation (% RSD). The repeatability was verified by carrying out 8 injections of the standard solutions in the concentration of 100 μg L^{-1} in a single day while maintaining constant all the operational conditions. The intermediate precision was obtained performing 8 injections of the same standard solution in two different days. The RSD was calculated using the average of the areas and also the height for each pesticide chromatographic peak. The accuracy of a method was evaluated by carrying out recovery assays [21,22]. To evaluate the recovery of the method, analyses were carried out on seven replicates at three different spike levels (15, 30 and 150 μg kg^{-1}), using blank sediment samples (control sample, free of analytes). Volumes of 300, 60 and 30 μL of a 10 mg L^{-1} solution were employed to obtain the final spike levels. Samples were left in contact with sediment for 24 h after homogenization and sample evaporation.

Table 1
Retention time, asymmetry factors (A_s), and standard deviation (SD) obtained with the DB-5, and HP-50+ columns ($n = 3$).

Analytes (class)[a]	HP-50+		DB-5	
	t_R (min)[b]	A_s/SD	t_R (min)	A_s/SD
3,4,5-Tricloroguaiacol (I)	7.74	1.5/0.29	7.46	1.0/0.24
Propanil (II)	10.93	1.0/0.00	9.90	1.5/0.29
Fipronil (III)	11.77	0.8/0.14	12.59	1.0/0.19
Propiconazole I (IV)	16.54	1.0/0.19	16.75	1.0/0.00
Propiconazole II (IV)	16.63	1.3/0.34	16.98	1.3/0.33
Trifloxystrobin (V)	16.87	1.0/0.14	17.14	1.1/0.23
cis Permethrin (VI)	21.79	0.9/0.08	22.43	1.0/0.14
trans Permethrin (VI)	22.19	1.0/0.07	22.65	1.0/0.19
Difenoconazole I (IV)	36.80	–	25.24	1.5/0.00
Difenoconazole II (IV)	–	–	25.31	1.0/0.24
Azoxystrobin (V)	57.35	1.1/0.07	25.89	0.7/0.19

[a] Class designations: (I) organochlorine, (II) acetanilide, (III) phenylpyrazole, (IV) triazole, (V) strobilurin, (VI) pyrethroid.
[b] SD in the range of 0.009–0.047%.

3. Results and discussion

3.1. GC-μECD

Table 1 shows results for asymmetry factors, standard deviation, retention times for the two columns, and also chemical classes of pesticides. Two columns with different polarities were tested for the analysis of seven pesticides selected for the present study. The DB-5 column (lower polarity phase) showed better analytical results when compared to HP-50+ (medium polarity phase) due to the shorter analysis time (27.9 min and 61.1 min, respectively), better resolution with the exception of propiconazole II and trifloxystrobin showing resolution of 1.26 and 2.04 for DB-5 and HP-50+, respectively. The resolution obtained for the peaks of the permethrin isomers, and propiconazole isomers on HP-50+ column was 1.76 and 0.84, respectively. For the DB-5, the resolution obtained for these isomers was 2.23 and 1.64, respectively. Also, it was possible to separate the two isomers of difenoconazole ($R = 0.89$) while they co-elute in HP-50+. These results are due differences in interactions between analytes and stationary phase and also because DB-5 allows the use of higher temperature (300 °C) than HP-50+ (255 °C). The two columns showed satisfactory values of peak asymmetry (between 0.8 and 1.2).

3.2. GC × GC-μECD

Two column sets were tested for the analysis of seven pesticides under study. The first column set (DB-5/DB-17ms) resembles the typical type normally used in GC × GC – lower polarity phase as ^{1}D with higher polarity phase ^{2}D, and the second column set (HP-50+/DB-1ms) employs a more polar phase as ^{1}D and a lower

Table 2
Asymmetry factors (^2A_s, $n = 3$) for different durations of hot jet obtained with the DB-5/DB-17ms and HP-50+/DB-1ms column sets.

DB-5/DB-17ms						HP-50+/DB-1ms				
Hot jets duration (s)										
Analytes	^1t_R (min)	^2t_R (s)	1.4	1.75	2.1	0.8	1.0	1.2	^1t_R (min)	^2t_R (s)
			Asymmetry factor/standard deviation							
3,4,5-Tricloroguaiacol	7.93	3.16	1.8/0.11	2.1/0.38	1.4/0.21	2.7/0.87	3.4/0.87	2.9/0.62	7.60	1.80
Propanil	10.61	5.94	1.5/0.03	1.6/0.03	1.1/0.31	3.6/0.29	3.9/0.29	3.6/0.48	10.80	2.12
Fipronil	13.41	4.94	1.3/0.07	1.3/0.07	1.3/0.03	1.5/0.00	1.5/0.00	2.0/0.28	11.66	2.76
Propiconazole I	17.85	1.36	1.3/0.11	1.4/0.07	1.2/0.12	1.9/0.36	1.9/0.36	2.0/0.12	16.60	2.40
Propiconazole II	18.08	1.30	1.3/0.10	1.4/0.03	1.2/0.06	–	–	–	–	–
Trifloxystrobin	18.2	0.90	1.1/0.07	1.1/0.11	1.2/0.10	2.1/0.36	2.0/0.36	1.8/0.21	16.86	2.16
cis Permethrin	23.33	6.00	1.3/0.03	1.3/0.10	1.2/0.09	1.8/0.18	1.9/0.18	1.9/0.16	20.53	2.26
trans Permethrin	23.56	5.84	1.1/0.03	1.2/0.03	1.2/0.08	2.4/0.00	2.4/0.00	1.9/0.13	20.73	2.24
Difenoconazole	26.36	5.94	1.3/0.03	1.5/0.08	1.3/0.04	2.1/0.17	1.9/0.17	2.2/0.26	26.80	3.22
Azoxystrobin	27.18	1.40	1.3/0.12	1.3/0.06	1.2/0.11	2.2/0.23	2.3/0.36	2.2/0.20	34.06	3.44

polarity phase as ^{2}D. According Kristenson et al. [23], the flow of gas make up should be as high as possible to obtain narrow peak widths in GC × GC-μECD. However, in this case, the dilution effect in the detector is higher. Based on the results of these researchers as well as others [15,24], nitrogen flow used for all experiments was 150 mL min^{-1}, which is the maximum flow allowed by the equipment.

The best results obtained for injector temperature and oven temperature program in 1D-GC were used for GC × GC. Parameters such as modulation period and hot jet span were optimized for GC × GC-μECD analysis. The modulation temperature offset was kept at 20 °C. In a GC × GC system, the modulator plays a major role in improving peak shape, sensitivity and separation through cryofocusing. Achieving a good peak distribution in the two dimensional space is an important goal when dealing with complex matrices as it minimizes the probability of coelution of analytes with matrix interfering compounds. In the case of DB-5/DB-17ms column set, a 7 s modulation period was chosen among several tested (2, 4, 6, 7, and 8 s), as it gave the best peak distribution in the color plot. Hot jet span may influence the shape and intensity of peaks [25]. The hot jet was kept on for different durations: 1.4, 1.75, and 2.1 s, corresponding to 20, 25, and 30% of the modulation period. Peak tailing may show up as a consequence of a shorter or longer hot jet span, depending on analyte characteristics. The duration of cold jet is automatically determined by the choice of the hot jet span. For most volatile analytes, it is important to set these periods of time, so the cold jet should be long enough to sample and concentrate the chromatographic bands of this type of compound. In this context, few papers report the use of different hot jets for optimum chromatographic condition [13]. Keeping the hot jet on for 2.1 s provided, in general, the best peak shapes, although different hot jet spans did not contribute to increase the analytical signal of compounds.

For the HP-50+/DB-1ms column set the following parameters were tested: modulation periods of 2, 4, and 6 s and temperature offset of 10, 20 and 30 °C. A modulation period of 4 s and a temperature offset of 5 °C provided the best peak distribution in the chromatographic space. Final temperature for the primary oven was modified to 280 °C for 30 min.

Three hot pulse span were tested (0.8; 1.0, and 1.2 s) but there was no significant peak symmetry improvement and 0.8 s was chosen, as it provided a lower standard deviation for peak asymmetry values. The highest peak intensity was obtained with hot pulse duration of 0.8 and 1.2 s. Table 2 shows data for the two column sets: retention times in ^{1}D and ^{2}D, asymmetry factors corresponding to each hot jet duration, and standard deviations. Fig. 1 shows the color plots obtained for the analytes with the DB-5/DB-17ms and HP-50+/DB-1ms column sets.

The DB-5/DB-17ms column provided better analytical results compared to HP-50+/DB-1ms as analysis time was shorter (29.4 min and 35.7 min, respectively) and resolution was better for permethrin isomers (6) ($R_s = 0.72$ versus $R_s = 0.36$) Resolution between propiconazole (4) and trifloxystrobin (5) was 0.6 for both column sets. Resolution was calculated using definition reported by Adam et al. [26,27]. Peak symmetry was significantly better for the first column set than for the second (Table 2), as ideally, the asymmetry factor should be between 0.8 and 1.2 [28]. For the DB-5/DB-17ms the values were between 1.1 and 1.8 for a hot jet of 2.1 s, and only three compounds showed asymmetry values above 1.2 (3,4,5-tricloroguaiacol, fipronil, and difenoconazole). Asymmetry factors were not satisfactory for HP-50+/DB-1ms column set, as their values were between 1.5 and 3.6, using the best hot jet span (0.8 s). Even though DB-5/DB-17ms column set had provided better use of the separation space, the HP-50+/DB-1ms column set showed narrower peaks in ^{2}D, resulting in enhanced peak capacity and sensitivity due to increasing of the analytical signal intensity. The modulated peaks were around 8–62% narrower than those

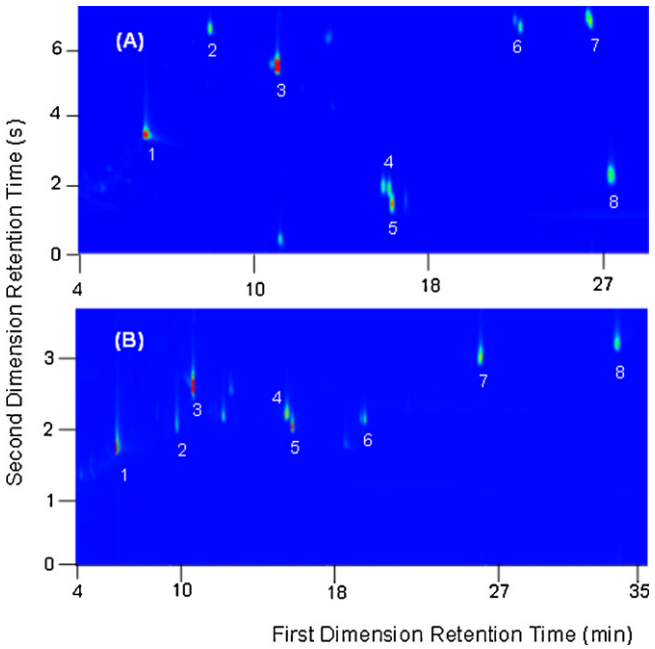

Fig. 1. GC × GC-μECD color plots for a 100 μg L^{-1} solution of the pesticides. (A) DB-5/DB-17ms column set, (B) HP-50+/DB-1ms column set. Target compounds are numbered as follows: (1) 3,4,5-trichloroguaiacol; (2) propanil; (3) fipronil; (4) propiconazole I and II; (5) trifloxystrobin; (6) permethrin *cis* and *trans*; (7) difenoconazole; (8) azoxystrobin.

Table 3
Values of the equation of linear regression, r^2 and linear range for all the pesticides studied from 1D-GC and GC × GC ($n = 3$).

Pesticides	1D-GC			GC × GC		
	Equation	r^2	Linear range ($\mu g\,L^{-1}$)	Equation	r^2	Linear range ($\mu g\,L^{-1}$)
Propanil	$y = 0.0023x + 0.0387$	0.9778	15–150	$y = 0.0023x + 0,0186$	0.9992	5–295
	$y = 0.0036x - 0.3308$	0.9765	175–415			
Fipronil	$y = 0.0114x + 0.0526$	0.9984	5–150	$y = 0.0061x + 0.0756$	0.9945	5–415
	$y = 0.016x - 1.6674$	0.9934	175–415			
Propiconazole[a]	$y = 0.0064x - 0.0248$	0.9931	5–95	$y = 0.0044x - 0.0234$	0.9920	15–295
	$y = 0.0069x - 0.4449$	0.9799	150–355			
Trifloxystrobin	$y = 0.0048x + 0.0229$	0.9945	5–115	$y = 0.0029x + 0.0061$	0.9993	5–415
	$y = 0.0045x - 0.1565$	0.9961	175–415			
Permethrin[b]	$y = 0.0019x + 0.0052$	0.9988	5–95	$y = 0.001x + 0.0013$	0.9990	5–295
	$y = 0.0013x + 0.0233$	0.9904	115–415			
Difenoconazole[a]	$y = 0.0018x - 0.0044$	0.9960	15–175	$y = 0.0014x - 0.0058$	0.9942	5–415
	$y = 0.0028x - 0.2981$	0.9557	150–355			
Azoxystrobin	$y = 0.0042x + 0.0217$	0.9917	5–150	$y = 0.0027x + 0.0092$	0.9963	5–415
	$y = 0.0055x - 0.4425$	0.9931	175–415			

[a] Sum of the stereoisomers.
[b] Sum of the *cis* and *trans* isomers.

obtained for the first column set, except for propanil, which showed peaks that were 15% larger. Overall, DB-5/DB-17ms column set was chosen for subsequent quantitative studies, since it presented the best use of separation space, with a broad distribution of analytes in ^{2}D (which is an important, and desirable feature in the analysis of complex samples), less analysis time and good symmetry for the chromatographic peaks.

3.3. Figures of merit

3.3.1. Calibration curve

Table 3 summarizes the values of the equation of linear regression, r^2 and linear range for all the pesticides studied.

Due to the wide range of concentrations studied, it was not possible to obtain linearity with only a single analytical curve for 1D-GC. Thus, it was necessary to divide the curve, producing two ranges of concentration. The proposed method showed good linearity with r^2 above 0.98 (0.9800), except for some analytes: propanil, which had an r^2 of 0.9778 and 0.9765 for both concentration ranges; propiconazole, with an r^2 of 0.9799 in the concentration range from 150 to 355 $\mu g\,L^{-1}$ and difenoconazole, for which it was not possible to reach linearity in the concentration range from 150 to 355 $\mu g\,L^{-1}$ ($r^2 = 0.9557$). The GC × GC proposed method showed good linearity in a wide range of concentration, with r^2 between 0.9920 and 0.9993 for all pesticides.

3.3.2. Limits of detection (LOD), and quantification (LOQ)

Instrumental LOD and LOQ were found in the range from 0.60 to 2.31 $\mu g\,L^{-1}$ and 1.83 to 5.62 $\mu g\,L^{-1}$, respectively for 1D-GC. For GC × GC, instrumental LOD and LOQ were found in the range from 0.08 to 1.07 $\mu g\,L^{-1}$ and 0.25 to 3.23 $\mu g\,L^{-1}$, respectively (Table 4).

Table 4
Values of LOD, and LOQ ($\mu g\,L^{-1}$).

Pesticides	1D-GC		GC × GC	
	LOD	LOQ	LOD	LOQ
Propanil	2.31	3.99	1.07	3.23
Fipronil	0.66	2.00	0.26	0.77
Propiconazole[a]	0.64	1.94	0.08	0.25
Trifloxystrobin	0.60	1.83	0.25	0.74
Permethrin[b]	0.86	2.60	0.19	0.58
Difenoconazole[a]	1.85	5.62	0.64	1.94
Azoxystrobin	0.63	1.92	0.35	1.06

[a] Sum of the stereoisomers.
[b] Sum of the *cis* and *trans* isomers.

3.3.3. Precision (repeatability and intermediate precision), and accuracy

Repeatability values for chromatographic peak areas were in the range of 1.58 and 4.17%, and for heights between 1.75 and 4.38%. The intermediate precision for the areas lay between 0.90 and 3.89% and for heights between 1.18 and 3.56% for 1D-GC. For GC × GC, the repeatability of the chromatographic peak areas was in the range of 0.64 and 3.78% and for heights, it was between 0.77 and 3.16%. The intermediate precision of the areas stayed between 0.54 and 3.50% and for heights, between 0.81 and 3.74%. For the analysis of constituents at trace levels, a maximum of 20% standard deviation is recommended for analytical precision data, depending on the sample complexity [29]. Therefore, the proposed chromatographic methods for the determination of seven compounds can be considered precise. Results for accuracy for GC × GC method are shown in Table 5 and they may be considered satisfactory (recoveries between 50 and 120% and RSD ≤ 20%) for all pesticides in the concentration level of 30 $\mu g\,kg^{-1}$. Despite the low recovery

Table 5
Mean recovery (%) and RSD obtained by extraction method of sediment, analyzed by GC × GC-μECD ($n = 9$).

Spike level	15 $\mu g\,kg^{-1}$		30 $\mu g\,kg^{-1}$		150 $\mu g\,kg^{-1}$	
Pesticides	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
Propanil	74	13	67	9	60	20
Fipronil	64	14	67	11	65	18
Propiconazole[a]	54	7	61	9	47	18
Trifloxystrobin	40	9	52	9	42	16
Permethrin[b]	133	20	52	30	39	23
Difenoconazole	254	15	115	9	92	24
Azoxystrobin	127	14	78	9	62	20

[a] Sum of the stereoisomers.
[b] Sum of the *cis* and *trans* isomers.

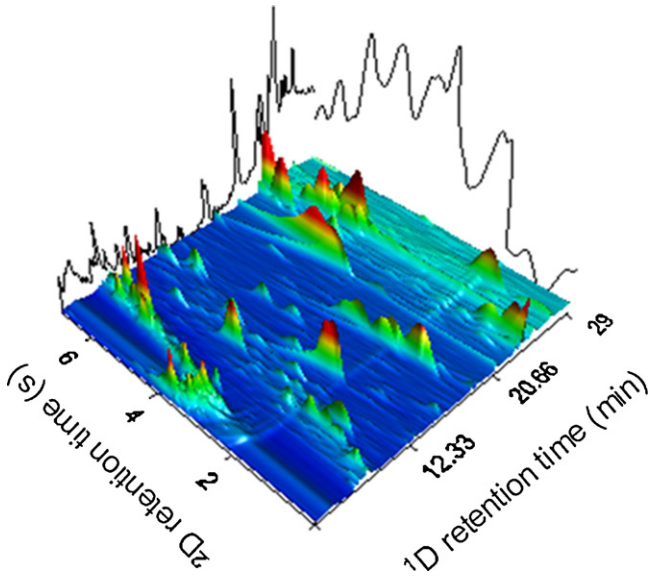

Fig. 2. 3D plot of the extract of sediment sample spiked at 15 μg kg^{-1} and the reconstructed ^{1}D and ^{2}D chromatograms.

obtained for trifloxystrobin in the concentration levels of 15 and 150 μg kg^{-1} and for propiconazole at 150 μg kg^{-1}, the RSD was less than 20%, showing good precision. Recovery for azoxystrobin and difenoconazole was higher (up 120%), as coelution with matrix components may have contributed to their peak areas at spike level of 15 μg kg^{-1}. For difenoconazole at 150 μg kg^{-1} spike level, and permethrin at 30 μg kg^{-1} level, a good recovery was reached, but precision was not satisfactory (>20%).

The ECD relative response to some compounds such as hydrocarbons is low (approximately 0.01 compared to the response of chlorobenzene as one) [30]. Coelution of this type of compounds with pesticides imply in a negative contribution to the analytical response followed by a decrease of signal intensity. The use of ultrasonic bath as extraction device may generate a heterogeneous extraction environment, as the ultrasonic waves are transmitted through the water bath and dispersion of energy takes place, providing sites of higher and lower intensity of energy [31]. Synergism of both factors (the negative effect caused by ECD lower response of coeluting interferents and the high variability of the ultrasonic extraction process) might have contributed to some of the non satisfactory recovery values found. The ultrasonic probe would probably be a good alternative for improving the repeatability of the extraction process, as it was already found out by You et al. [20]. Fig. 2 shows the 3D plot with the reconstruction of the ^{1}D and ^{2}D chromatogram of the sediment sample spiked at 15 μg kg^{-1}. 1D-GC method was satisfactory only for two compounds, permethrin and azoxystrobin at spike levels of 15 and 30 μg kg^{-1} (recoveries between 60 and 72% with RSD ≤ 20%).

The many peaks sprinkled throughout the 3D plot (Fig. 2) is an indication of the complexity of the sample, and gives hint to why 1D-GC analyses can fail for trace level determinations of pesticides. The color plot also demonstrates that the GC × GC peak capacity is significantly improved when compared to 1D-GC and this consequently enhances the possibility of successful analysis of complex samples. In this case, the use of 1D-GC would not be sufficient to obtain separation between the analytes and matrix constituents.

3.4. Determination of pesticides in sediment samples

The methods were applied for analysis of two sediments samples, designated as SD_1 and SD_2. Residues of trifloxystrobin and azoxystrobin were found only in sample SD_2. Trifloxystrobin concentration was 3.34 μg kg^{-1} and azoxystrobin concentration was below the LOD for GC × GC method. Using 1D-GC, trifloxystrobin concentration was 21.18 μg kg^{-1} and azoxystrobin concentration was below the LOD. Co-elution of matrix compounds with trifloxystrobin on 1D-GC was especially significant for the high concentration found.

4. Conclusions

This study showed the potential of the application of GC × GC-μECD to the analysis of pesticide residues in sediments, since it provided better separation between analyte and matrix interferences, minimizing the possibility of co-elutions and allowing the use of a selective detector instead of the use of a more expensive time-of-flight mass spectrometry detector, which is commonly employed for complex matrices. GC × GC-μECD developed method also resulted in low LOD and LOQ values, besides good precision. The LOD for GC × GC method were about 36% lower than those obtained for the 1D-GC. Accuracy also indicated better results for GC × GC, possibly due to its higher sensitivity and lower contribution of co-eluting matrix components, which could be minimized by increased peak capacity. Determination of pesticides and other pollutants in sediments is of great importance, as they are indicators of environmental quality of the water body where they are located. Information regarding the concentration of pesticides in sediments may also provide relevant information related to their environmental fate, since they may cause health problems to human beings and also to the biota.

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