Application Note

Instrument: Pegasus® BT 4D

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Analysis of Phytosanitary Products in Surface Water and Groundwater Using GCxGC-TOFMS

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ABSTRACT

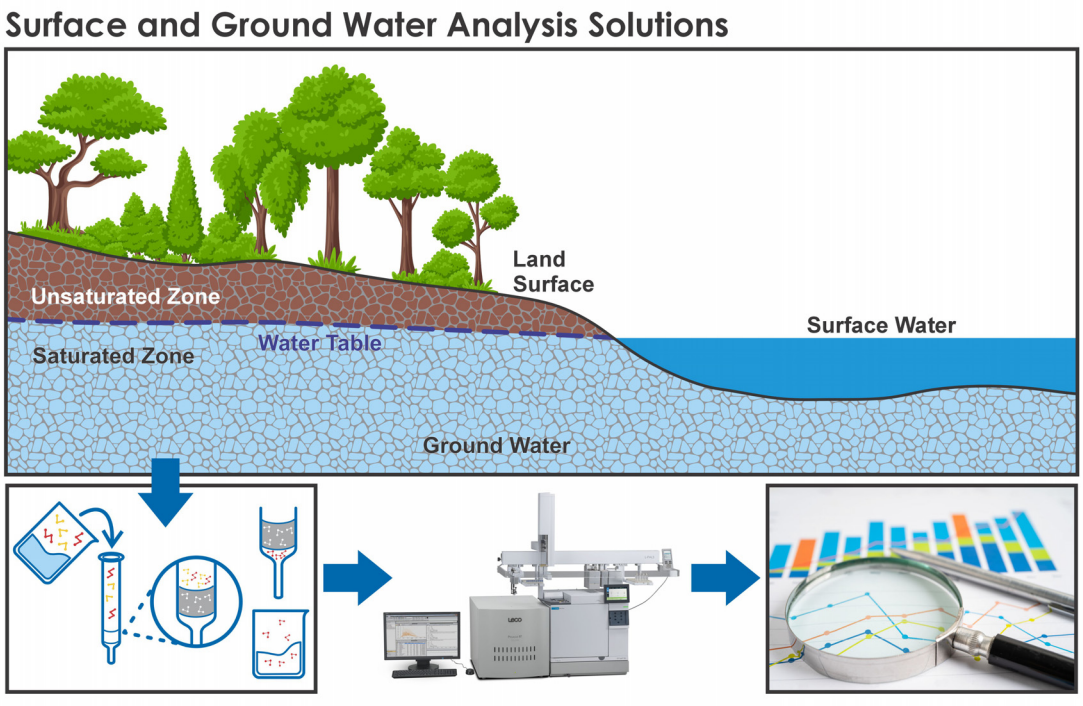
The overuse of phytosanitary products for agricultural and non-agricultural purposes have resulted in these compounds and their derivatives, leaching into surface water and groundwater resources.

The presence of phytosanitary products in water is regulated through different directives, allowing a maximum concentration of 0.1 µg/L for individual components and 0.5 µg/L for the total sum of their concentrations.

In this study, environmental water samples were extracted using Solid-Phase Extraction (SPE) and characterized and quantified for the presence of 51 phytosanitary products. Method development and validation was performed using comprehensive two-dimensional gas chromatography coupled to time-of-flight mass spectrometry (GCxGC-TOFMS). We show that these types of analytes are difficult to separate chromatographically by one-dimensional approaches. With a multidimensional system, separation is significantly improved, while also increasing sensitivity, which is important for trace analysis.

INTRODUCTION

Pesticides are chemical compounds that are used mainly in agriculture to control pest infestations (rodents, insects, or plants), and diseases.[1] The residues of these chemicals can reach water bodies through different pathways such as spray drift, runoff, wind, erosion events, leaching, vaporization and subsequent dry deposition. The European Union has set guidelines for safe, drinking water for human consumption. The maximum allowable concentration of pesticides and their degradation products in surface and groundwater is 0.1 µg/L and 0.5 µg/L for individual and the total sum of their concentrations, respectively.[2–4] In this study, the purification and extraction of the phytosanitary compounds were performed using SPE,[5] and thereafter GCxGC-TOFMS[6] was used to characterize and quantify the components present in the sample. This application note describes the development and validation of a GCxGC-TOFMS method for the quantification of 51 phytosanitary products of different chemical structures.



EXPERIMENTAL

SPE was used to extract the target analytes from samples as follows: 500 mL of starting sample was added to 250 µL of process standard (atrazine-d5, 100 µg/L in methanol), loaded onto an OASIS HLB 6 cc 200 mg SPE cartridge (Waters Corporation, Milford, MA, USA) and eluted with 2.5 mL of ethyl acetate. The dried residue was dissolved in 250 µL of an ethyl acetate solution containing 250 µg/L of internal standard (azobenzene). For quantitation, calibration curve samples were prepared by performing dilutions of a standard mixture (this also contained the process standard), drying 250 µL of it and dissolving the residue in 250 µL of internal standard solution (250 µg/L in ethyl acetate). The final concentrations of these standards were 0.01, 0.025, 0.05, 0.075, 0.1 and 0.15 µg/L. The standards were obtained from O2Si Smart Solution (North Charleston, SC, USA), Dr. Ehrenstorfer (Augsburg, Germany), and A2S Analytical Standard Solutions (Saint Jean d'Illac, France). The solvents were purchased from Merck (Darmstadt, Germany).

Method development and validation of the GCxGC-TOFMS method were conducted on a Pegasus® BT4D GCxGC-TOFMS system (LECO Corporation, MI, USA) equipped with an Agilent 7890 GC and an Automatic Liquid Sampler (Agilent Technologies, Santa Clara, CA, USA). Data was collected and analyzed using ChromaTOF® software with the Target Analyte Finding (TAF) method to rapidly process and determine the target phytosanitary compounds. Table 1 reports the instrumental and data processing parameters used in this study.

Table 1. Instrumental and data processing parameters.

Gas Chromatograph	**Agilent 7890 with LECO Dual Stage QuadJet Modulator**
Injection	2 µl Split 10:1, 250 °C
Carrier Gas	Helium, 1.3 mL/min, constant flow
Primary Column	Rxi-5MS, 30 m × 0.25 mm i.d. × 0.25 µm *df* (Restek, Bellefonte, PA, USA)
Secondary Column	Rxi-17Sil MS, 2 m × 0.25 mm i.d. × 0.25 µm df (Restek, Bellefonte, PA, USA)
Oven Temperature Program	140 °C (hold 1 min), ramped 6 °C/min to 270 °C, ramped 20 °C/min to 320 °C (hold 2 min)
Secondary Oven	+25 °C (relative to the main oven temperature)
Modulator	+15 °C (relative to the secondary oven temperature)
Modulation Period	2.60 s (hot jet: 0.78 s, cold jet: 0.52 s)
Transfer Line	250 °C
Mass Spectrometer	**LECO Pegasus BT 4D**
Ion Source Temperature	250 °C
Mass Range	40 - 500 m/z
Ionization Mode	EI, electron energy: 70 eV
Acquisition Rate	150 Hz (32 kHz extr. freq.)
Acquisition Delay	300 s
Non-Target Data Processing	**LECO ChromaTOF Software**
Minimum S/N	100
Minimum Stick Count	3
Quantitation Mass Tolerance	0.10 Da
Target Analyte Finding (TAF)	**LECO ChromaTOF Software**
Retention Time Window	1.25xFWHH
Tolerance	0.10 Da
Min Area	100
Min Height	25
Signal Mode	Centroid

The method developed in our study was validated (linearity, sensitivity, trueness, precision, and extraction recovery) following the Eurachem Guidelines.[7] The analytical curves were constructed by six calibration levels, each analyzed a total of nine times over three different days (3 replicates per day). The least squares method was applied to estimate the regression lines, and linearity was further assessed using Mandel's fitting test. The limits of detection and quantification were estimated from the calibration curve. Precision was evaluated at the lowest, and highest calibration levels (0.01 and 0.15 µg/L), both intra- and inter-day. Trueness was assessed on two levels (0.03 and 0.125 µg/L) by calculating the bias. Extraction recovery was estimated at 0.01 and 0.15 µg/L, spiking a tap water blank sample and quantifying the target analytes after the SPE process using the final GCxGC-TOFMS method.

RESULTS AND DISCUSSION

Initial chromatographic separation was performed with a primary non-polar column using GC-TOFMS. However, after a 20-minute run time, it was visible that there was a lot of coelutions occurring in the samples. The sample was then re-analyzed using two-dimensional chromatography (GCxGC-TOFMS). The combination of a non-polar primary column coupled to a polar secondary column allowed for the successful separation of the 51 targeted analytes. The differences in the separation of the targeted phytosanitary compounds when the samples were analyzed using 1D GC-TOFMS and 2D GCxGC-TOFMS is demonstrated (Figure 1).

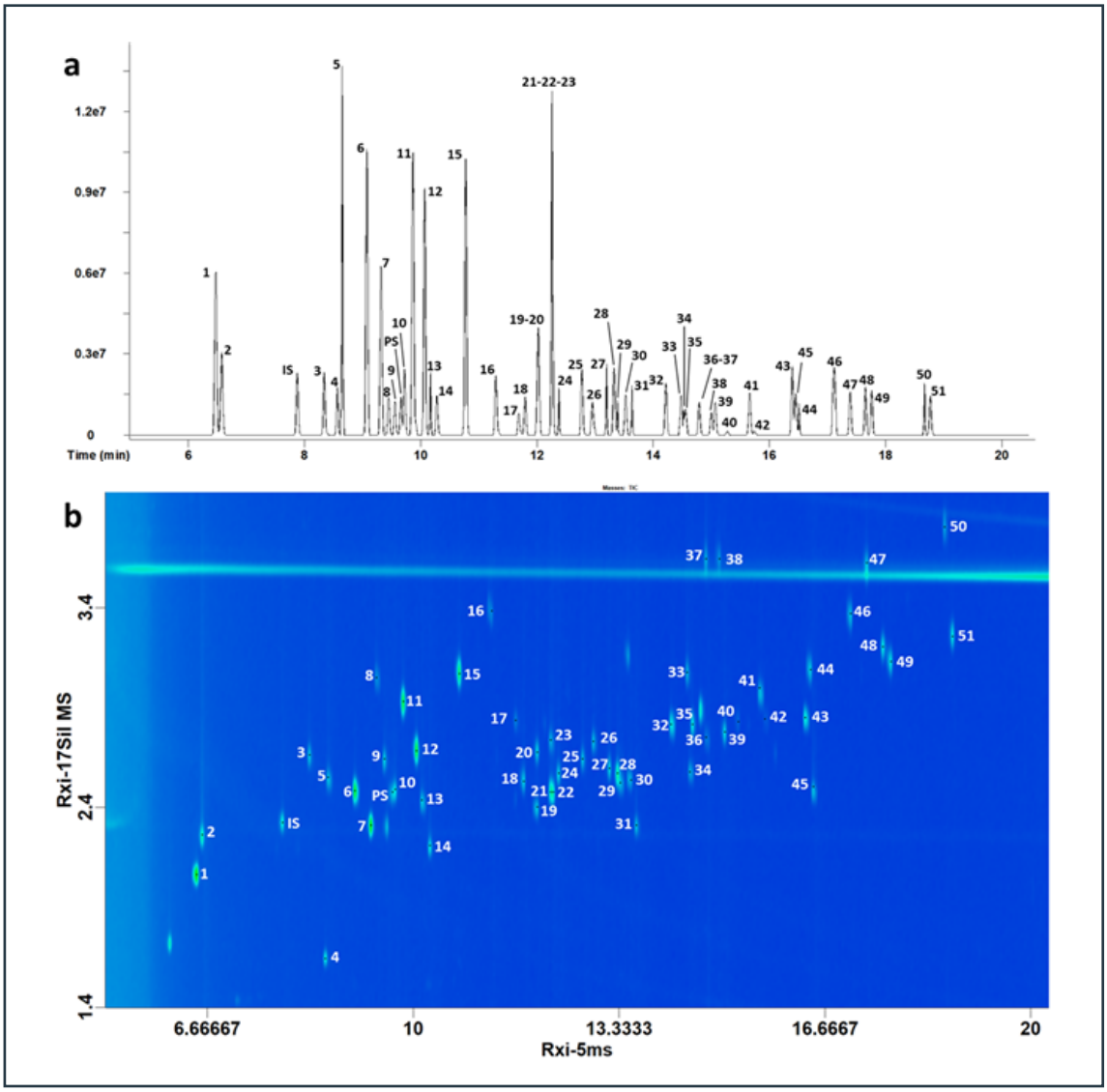


Figure 1. a. Zoomed in 1D chromatogram of standard phytosanitary compounds mix (1-51), internal standard (IS) and process standard (PS); b. Zoomed in 2D chromatogram of the same standard mix. For peak numbers, refer to Table 2.

Some compounds which are not resolved chromatographically in 1D separation, can be spectrally deconvoluted using *ChromaTOF* software. The analytes 1) chlorpyrifos-methyl and 2) vinclozolin are eluting at almost the exact same 1st dimension retention time (Figure 2a). Due to full mass range data collection at fast acquisition rates, deconvolution allowed these 2 components to be resolved and identified with good mass spectral library[8] similarity matches (806/1000 and 841/1000 respectively). The use of GCxGC allowed complete separation of these two analytes, in the 2nd dimension (Figure 2b). This also resulted in improved mass spectral similarity matches (875/1000 and 899/1000 respectively).

An additional example is provided (Figure 3) where 3 species are eluting very closely together. In this case, deconvolution does allow these species to be resolved in 1D (Figure 3a), however the use of GCxGC enabled much improved separation (Figure 3b), completely resolving Ametryn and significantly improving the mass spectral clarity of all 3 analytes. Similarity scores improved from 663/1000, 670/1000, and 706/1000 to 809/100, 812/100, and 907/1000 for Ametryn, Alachlor, and Heptachlor, respectively.

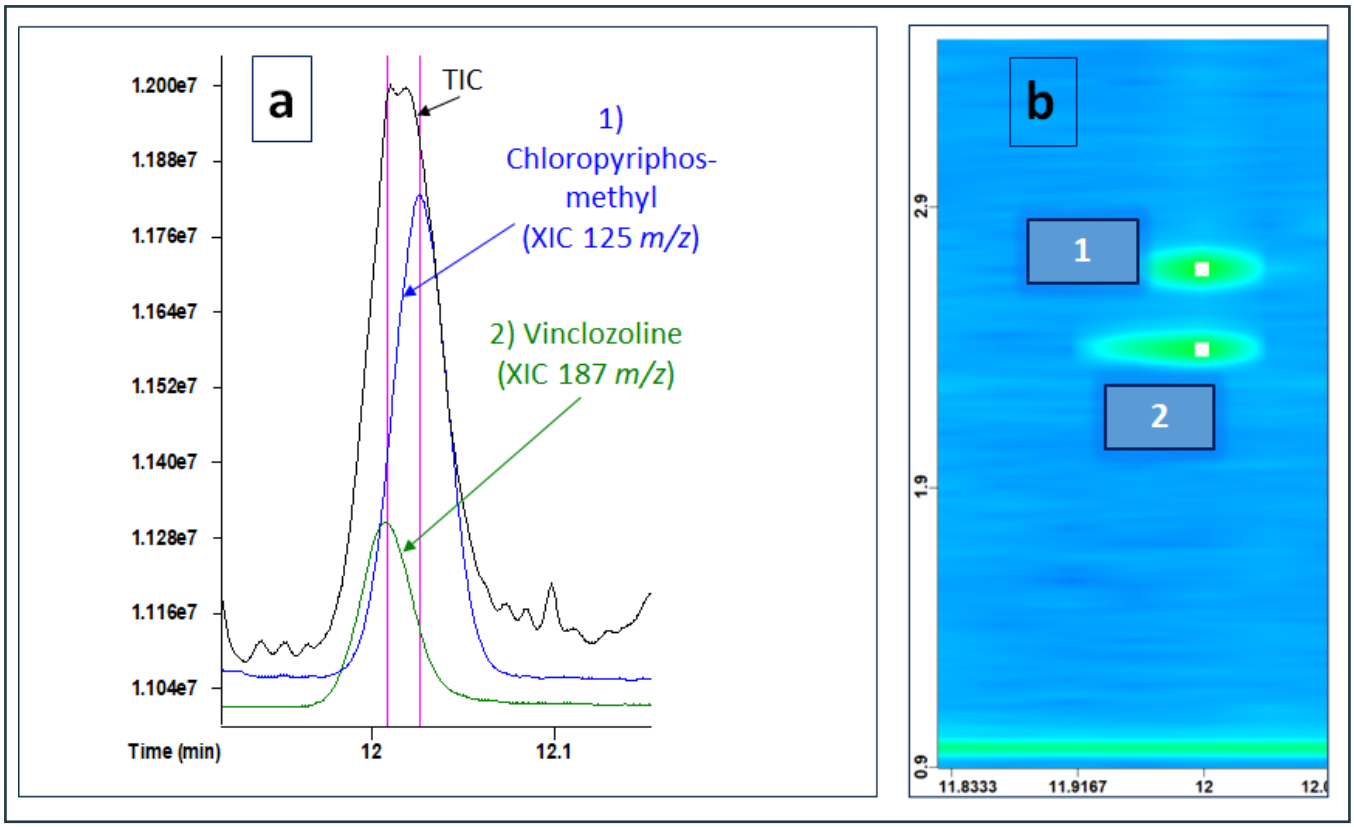


Figure 2. a. Zoomed in 1D coelution with Total Ion Chromatogram (TIC) (black line) and Extracted Ion Chromatogram (XIC) of 1) chlorpyrifos-methyl (blue line, m/z 125) and 2) vinclozolin (green line, m/z 187). b. Complete 2D separation of the same components

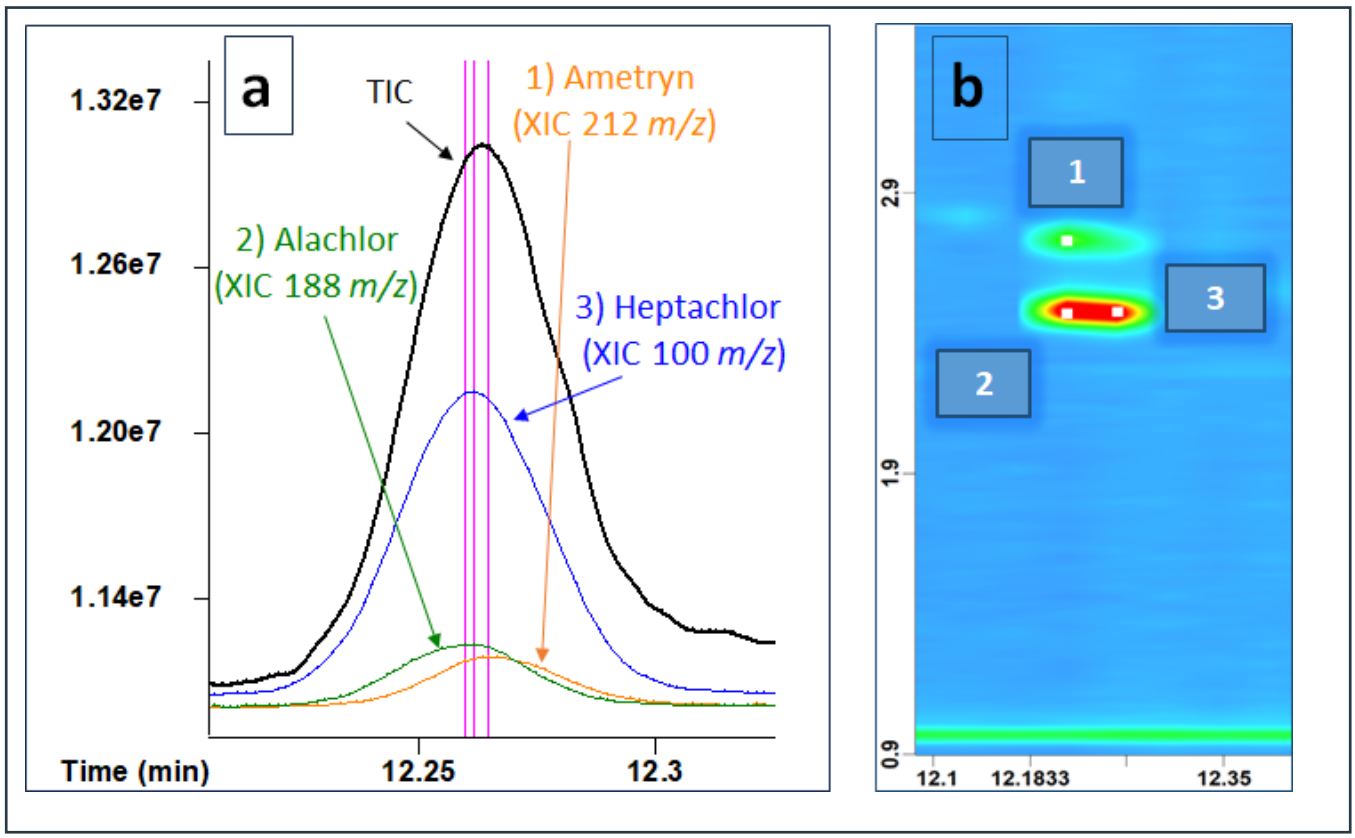


Figure 3. a. Zoomed in 1D coelution with Total Ion Chromatogram (TIC) (black line) and Extracted Ion Chromatogram (XIC) of 1) Ametryn (orange line, m/z 212), 2) Alachlor (green line, m/z 188) and 3) Heptachlor (blue line, m/z 100). b. Enhanced 2D separation of the same components

Table 2 lists the targeted phytosanitary compounds, along with their quantifier ions and relative signal-to-noise ratio (S/N) at the lowest calibration level, for both one-dimensional and two-dimensional analysis. An additional benefit of performing two-dimensional chromatography with thermal modulation is that it is more sensitive than one-dimensional chromatography, due to the cold (cryo) focusing during modulation. This is also demonstrated in Table 2 where higher S/N values using the 2D technique, ranged from a 1.7 (heptachlor epoxide) to 6.2 (trans-chlordane) -fold increase, compared with running in 1D.

Table 2. Phytosanitary compounds list with retention indices (RI from 2D separation and difference from database),[8] quantifier ions, and their S/N ratios in 1D and 2D analyses.

Peak #	Name	Quant Mass	Exp. RI	DRI	S/N 1D	S/N 2D
1	Benzene, pentachloro-	250	1519	8	789	2516
2	Molinate	126	1539	6	132	258
IS	Azobenzene	77	1615	8	864	1916
3	Desethylatrazine	172	1640	2	130	335
4	Trifluralin	306	1661	8	174	320
5	Desethylterbutylazine	186	1681	10	145	424
6	α-BHC	180	1678	15	20	71
7	Benzene, hexachloro-	284	1703	4	781	2238
8	Dimethoate	87	1712	8	53	100
9	Simazine	201	1732	9	37	92
PS	Atrazine-d5	205	1735	11	19	48
10	Atrazine	200	1735	11	63	148
11	β-BHC	111	1727	14	140	283
12	Lindane	181	1688	5	130	412
13	Terbuthylazine	214	1768	3	76	315
14	Propyzamide	173	1776	4	170	333
15	δ-BHC	181	1796	5	98	259
16	Caffeine	194	1828	9	29	59
17	Propanil	161	1858	8	33	56
18	Dimethenamid	154	1869	3	140	382
19	Vinclozolin	187	1875	1	75	172
20	Chlorpyrifos-methyl	125	1868	6	84	208
21	Alachlor	45	1886	8	24	64
22	Heptachlor	100	1876	11	145	370
23	Ametryn	58	1886	12	11	49
24	Prometryn	184	1890	15	55	136
25	Terbutryn	226	1918	10	44	93
26	Ethofumesate	161	1925	13	27	78
27	Malathion	173	1950	5	36	89
28	Aldrin	66	1937	18	67	152
29	Metolachlor	162	1951	17	120	319
30	Chlorpyrifos-ethyl	97	1964	11	48	90
31	Flufenacet	151	1983	12	37	123
32	Isodrin	193	2003	10	22	52
33	Metazachlor	81	2035	8	54	102
34	Pendimethalin	252	2032	6	28	75
35	Heptachlor epoxide	81	2055	17	43	71
36	Chlofenvinphos	267	2034	14	11	28
37	Captan	79	2053	7	30	74
38	Folpet	104	2060	11	11	30
39	Procymidone	96	2071	6	34	63
40	trans-Chlordane	373	2069	9	13	81
41	α-Endosulfan	64	2100	14	52	98
42	cis-Chlordane	373	2094	17	25	65
43	p,p'-DDE	246	2165	4	325	1423
44	Oxadiazon	175	2176	12	66	162
45	Dieldrin	79	2151	16	52	116
46	Endrin	81	2197	14	22	38
47	β-Endosulfan	64	2226	10	56	109
48	p,p'-DDD	235	2250	7	132	402
49	o,p'-DDT	235	2250	11	109	412
50	Endosulfan sulfate	272	2321	8	49	122
51	p,p'-DDT	235	2328	6	109	318

The method developed for this project was validated in terms of linearity, precision, trueness, limits of detection and quantification and these method validation figures-of-merit are summarized in Table 3.

Table 3. Figures-of-merit of method validation.

Figure-of-Merit	Range	Average value
R^2	0.9998 - 0.9919	0.9980
LOD (µg/L)	0.0005 (heptachlor epoxide) - 0.0033 (chlorpyrifos-methyl)	0.0012
LOQ (µg/L)	0.0016 - 0.0099	0.0035
Intraday precision (CV%)	At 0.01 µg/L: 2.1% (metolachlor) - 15.7% (ethofumesate)	7.4%
	At 0.15 µg /L: 0.7% (aldrin) - 5.7% (oxadiazon)	3.0%
Inter-day precision (CV%)	At 0.01 µg /L: 3.1% (metolachlor) - 20.4% (hexachlorobenzene)	10.2%
	At 0.15 µg /L: 1.7% (chlorpyrifos-ethyl) - 18.8% (hexachlorobenzene)	6.8%
Trueness (|bias%|)	At 0.03 µg /L: 0.01% (metazachlor) - 25.8% (hexachlorobenzene)	6.6%
	At 0.125 µg /L: 0.30% (chlorpyrifos-ethyl) - 30.3% (hexachlorobenzene)	7.8%
Extraction recovery (%)	At 0.01 µg /L: 82% - 109%	96%
	At 0.15 µg /L: 65% - 99%	83%

After validation, fourteen water samples (7 surface water and 7 groundwater) were extracted and analyzed. The quantitative results are shown in Figure 4. As expected, groundwater samples are less prone to phytosanitary product contamination than the surface water samples, where their average concentration is 0.070 µg/L and 0.314 µg/L, respectively.

'The analysis of the total concentration of all the phytosanitary analytes present in the water samples revealed that only two of these samples exceeded the threshold value (0.5 µg/L). However, after a comprehensive analysis and quantification of each sample, it was discovered that several of the targeted analytes were above the limit of 0.1 µg/L.

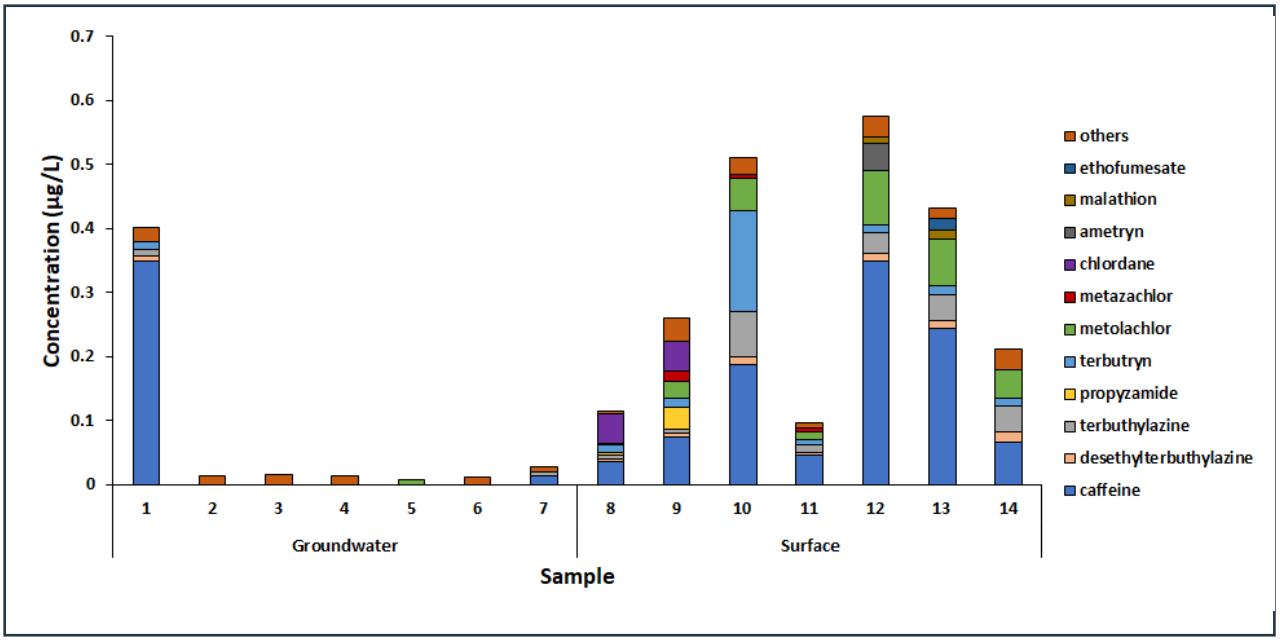


Figure 4. Quantification of phytosanitary products in the 14 environmental waters. The analytes reported individually have, in at least one sample, a concentration greater than 0.01 µg/L (the lowest calibration level).

CONCLUSION

A method involving SPE purification followed by GCxGC-TOFMS analysis was herein applied and validated for the determination of 51 phytosanitary compounds in surface water and groundwater. In one-dimensional chromatography, it was observed that there were many coelutions present in the sample. This was resolved using comprehensive two-dimensional gas chromatography, which allowed all the targeted analytes to be chromatographically separated. Another important advantage of using GCxGC-TOFMS is the gain in sensitivity as observed from 1.7 to 6.2 S/N increase, which is beneficial for trace determinations.

In this application, water extracts were data processed using the target analysis finding (TAF) method. This allowed for quick identification of the targeted analytes.

In addition to increased separation power and sensitivity, the GCxGC-TOFMS method herein developed provides post-targeted capabilities, a feature which can be exploited to screen previously acquired samples *a posteriori* for newly-regulated or emerging contaminants.

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