Determination of phytosanitary products in surface waters and groundwater by GC×GC-TOFMS

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ABSTRACT

The widespread use of phytosanitary products for agricultural and non-agricultural purposes has resulted in the presence of their residues in surface water and groundwater resources.
Their presence in water is regulated through different directives that setting a maximum concentration of 0.1 µg/L and 0.5 µg/L for individual and total sum.
In the present application, 51 phytosanitary products belonging to different chemical families were extracted using solid-phase extraction (SPE) in environmental water samples and quantified after the development and validation of a comprehensive two-dimensional gas chromatography coupled to time-of-flight mass spectrometry (GC×GC-TOF MS). We show that these type of analytes are difficult to separate chromatographically by one-dimensional analysis, and sensitivity also increases with multidimensional system, which is important for trace analysis.

INTRODUCTION

Pesticides are chemical compounds used to prevent or reduce problems caused by pests [1]. Their main use is in agriculture, and they can reach water bodies through different pathways: spray drift, runoff, and wind erosion events, leaching and vaporization and subsequent dry deposition. Since water is the most important source that humans need, to safeguard their health, the European Union set threshold values of pesticides and their degradation products in surface and groundwater of 0.1 µg/L and 0.5 µg/L for individual and total sum, respectively [2–4]. We used and applied SPE [5] for the purification and extraction of the phytosanitary compounds, and GC×GC-TOF MS [6] for the target analytes from the extracts.
This application describes the development and validation of a GC×GC-TOFMS method for the quantification of 51 phytosanitary products of different chemical structures.

EXPERIMENTAL

SPE was used to extract the target analytes from samples: 500 mL of starting sample were added to 250 µL of process standard (atrazine-d_5, 100 µg/L in methanol), loaded to OASIS HLB 6 cc 200 mg cartridge (Waters Corporation, Milford, MA, USA) and eluted through 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). The analytical curve levels were prepared without the SPE step, but by diluting a mixture of standard also containing 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), obtaining final concentration of 0.01, 0.025, 0.05, 0.075, 0.1 and 0.15 µg/L. The standards were 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 from Merck (Darmstadt, Gemany).
The development and validation of the GC×GC-MS method were conducted on a Pegasus BT 4D (LECO Corporation, Mönchengladbach, Germany) equipped with an Agilent 8890 GC and an Automatic Liquid Sampler (Agilent Technologies, Santa Clara, CA, USA). Data were collected and analyzed using ChromaTOF® BT software with the Target Analyte Finding (TAF) strategy to rapidly process and determine the target phytosanitary compounds. In **Table 1** instrumental and data processing parameters are reported.

Table 1. *Instrumental and data processing parameters.*

Gas chromatography separation: LECO GC×GC QuadJet™	
Column train	^{1}D: Rxi-5ms, 30 m × 0.25 mm × 0.25 µm d_f (Restek) ^{2}D: Rxi-17Sil MS, 2 m × 0.25 mm × 0.25 µm d_f (Restek)
Carrier gas	He; flow rate: 1.30 mL/min
Injection	2 µL in split mode (split ratio 1:10); T: 250 °C
Oven temp. program	140°C (held 1 min), ramp 6 °C/min to 270 °C, ramp 20 °C/min to 320 °C (held 2 min)
Secondary oven temp.	+25 °C (relative to the main oven temperature)
Modulator temp.	+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: 70eV
Acquisition rate	150 Hz (32 kHz extr. freq.)
Acquisition delay	300 s
Non-target data processing	
Minimum S/N	100
Minimum Stick Count	3
Quantitation mass tolerance	0.10 Da
Target Analyte Finding processing (for each target)	
Retention time window	1.25×FWHH
Tolerance	0.10 Da
Min area	100
Min height	25
Signal mode	Centroid

The developed method has been validated in terms of linearity, sensitivity, trueness, precision, and extraction recovery, following Eurachem Guide [7]. The analytical curves were constructed by six calibration levels analyzed for a total of nine times in three different days. 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, spinking a tap water blank sample and quantifying the target analytes after the SPE process using the final GC×GC-TOF MS method.

RESULTS AND DISCUSSION

The GC separation carried out using a non-polar column, while for GC×GC the same was coupled with a more polar one. In 20 min run time, some coelution were still present in 1D GC, while the successful separation of the 51 targets was obtained using GC×GC. **Figure 1** shows the GC and GC×GC chromatograms of the target phytosanitary compounds.

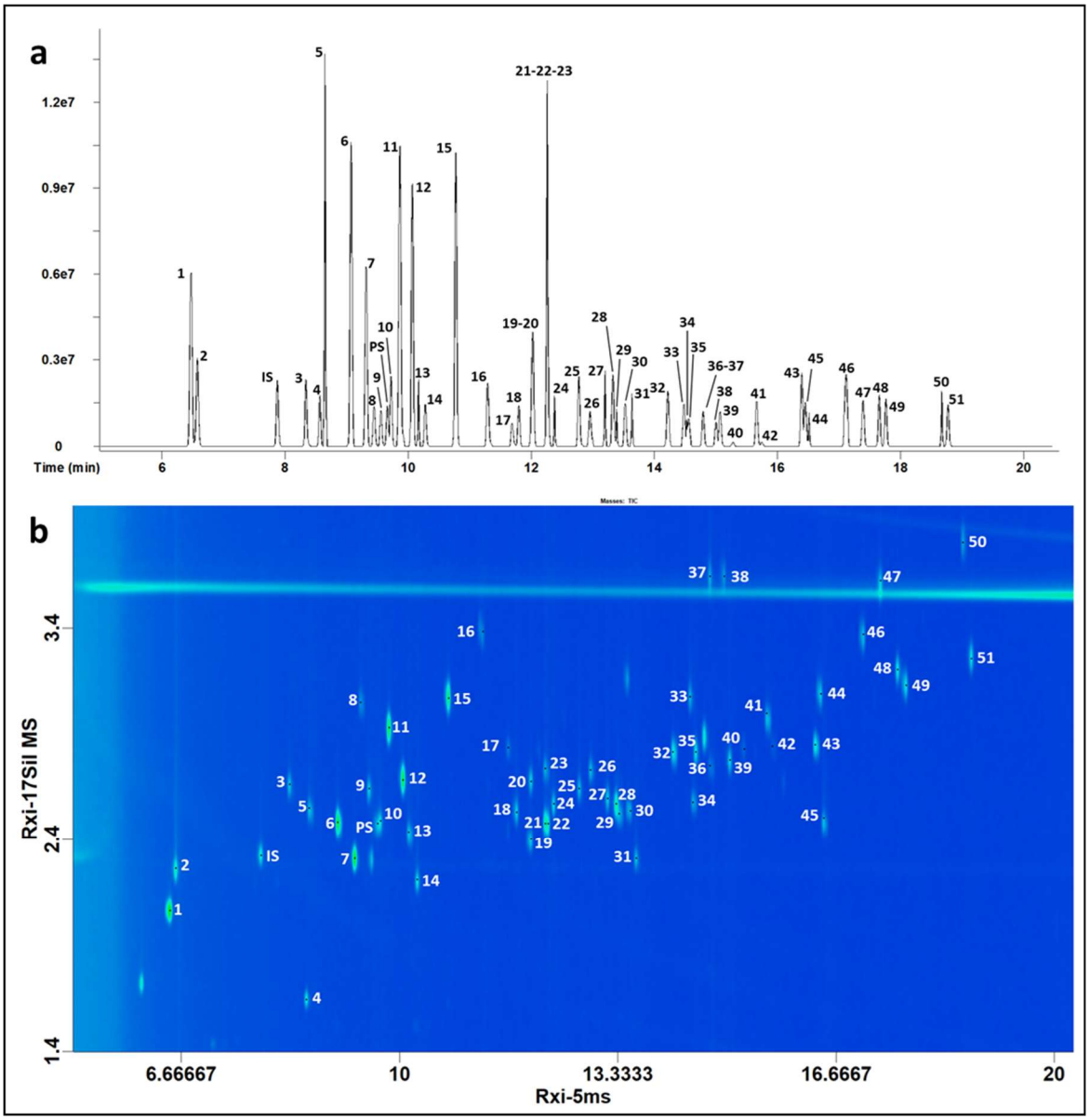

Figure 1. a*. Zoom of 1D chromatogram of standard phytosanitary compounds mix (1-51), internal standard (IS) and process standard (PS);* ***b****. Zoom of 2D chromatogram of the same standard mix. For peak numbers, refer to* ***Table 2****.*

Some compounds which are not resolved chromatographically in 1D separation, can be spectrally deconvolved thanks to the mass spectral information, as illustrated in the examples of **Figure 2**.
Thanks to the additional separation power of GC×GC, compounds that coeluted or had insufficient separation in 1D are baseline separated and are more easily identified, as the example of vinclozolin and chlorpyrifos-methyl in **Figure 2a**.

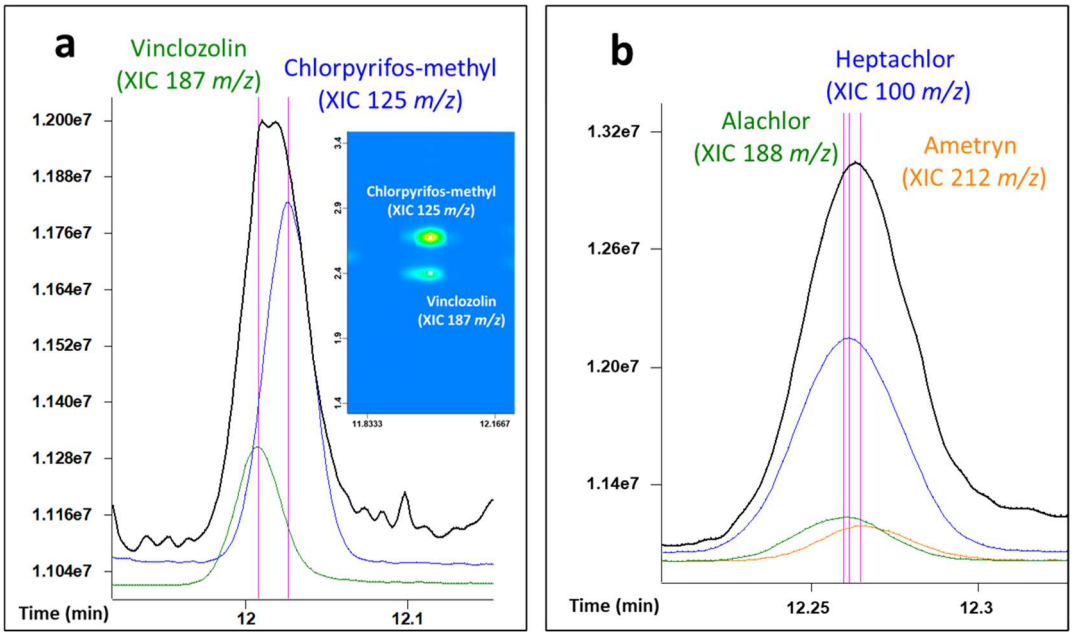

Figure 2. *Examples of 1D coelutions.* ***a****. Zoom of 1D Total Ion Chromatogram (TIC) (black line) and eXtracted Ion Chromatogram (XIC) of vinclozolin (green line,* m/z *187), and chlorpyrifos-methyl (blue line,* m/z *125). On the right their separation in 2D chromatogram.* ***b****. Zoom of 1D TIC (black line) and XIC of alachlor (green line,* m/z *188), heptachlor (blue line,* m/z *100), and ametryn (orange line,* m/z *212).*

In **Table 2** are listed the target 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 2D analysis. The data shows the higher S/N values of the 2D technique, from a 1.7 (heptachlor epoxide) to 6.2 (trans-chlordane) -fold increase, which translate to a greater overall method sensitivity compared to 1D.

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

Peak #	Name	Quant Mass	Exp. RI	ΔRI	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-d_5	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

*From NIST2020 database

The method was validated in terms of linearity, precision, trueness, limits of detection and quantification. 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 3**. As one can expect, groundwater samples are less prone to phytosanitary product contamination than the surface water samples, in fact their average concentration are 0.070 µg/L and 0.314 µg/L, respectively.

Considering the phytosanitary compounds' total concentration, only two samples exceeded the threshold value (0.5 µg/L); while regarding the quantification of single compounds, several analytes were found to be above the limit of 0.1 µg/L.

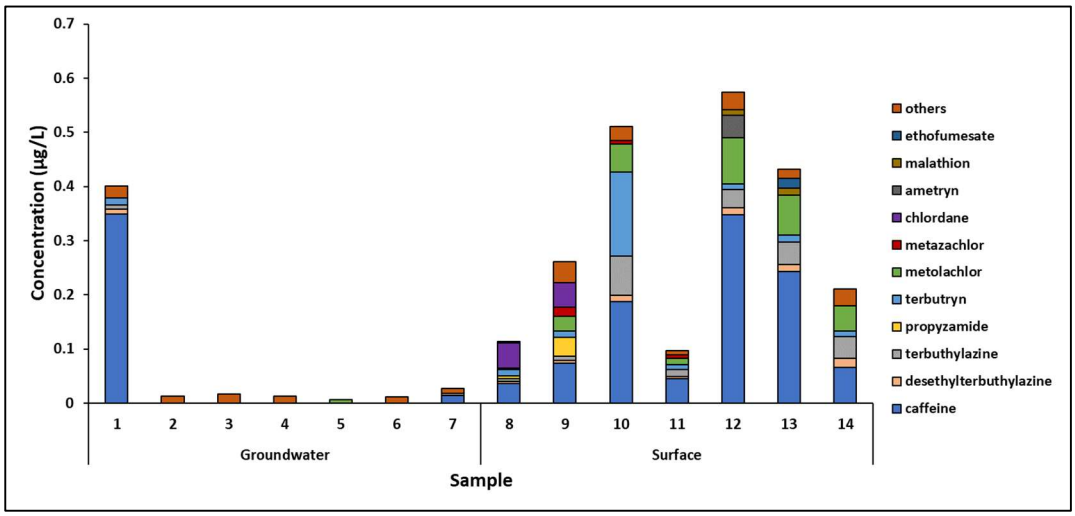

Figure 3. *Quantification of phytosanitary products in the 14 environmental waters. The analytes reported individually have, in at least one sample, a concentration greater than 0.01 µg/L (the lowest calibration level).*

CONCLUSION

A method involving SPE purification followed by GC×GC-TOFMS analysis was herein applied and validated for the determination of 51 phytosanitary compounds in surface water and groundwater. Using comprehensive two-dimensional gas chromatography, the target analytes were all chromatographically separeted, resolving some coelution issues within the pesticides or with intereferences from the sample matrix that would be otherwise present in a single column. Another important advantage of such GC×GC-TOFMS method resulted the gain in sensitivity, observed from 1.7 to 6.2 S/N increase, which is beneficial for trace determinations.

In this application, water extracts were analyzed using the target analysis finding (TAF) processing method, which make very fast, automated, and straightforward the processing, the quantification, and the reporting of a large number of samples.

In addition to increased separation power and sensitivity, the GC×GC-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.

REFERENCE

1. de Souza RM, Seibert D, Quesada HB, de Jesus Bassetti F, Fagundes-Klen MR, Bergamasco R (2020) Occurrence, impacts and general aspects of pesticides in surface water: A review. Process Safety and Environmental Protection 135:22–37. https://doi.org/10.1016/j.psep.2019.12.035
2. Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy
3. Directive 2006/118/EC of the European Parliament and of the Council of 12 December 2006 on the protection of groundwater against pollution and deterioration
4. Directive (EU) 2020/2184 of the European Parliament and of the Council of 16 December 2020 on the quality of water intended for human consumption
5. Poole CF (2021) Sample preparation for gas chromatography. In: Gas Chromatography. Elsevier, pp 615–653
6. Zanella D, Focant J, Franchina FA (2021) 30 th Anniversary of comprehensive two-dimensional gas chromatography: Latest advances. Analytical Science Advances 2:213–224. https://doi.org/10.1002/ansa.202000142
7. Magnusson B, Ornemark U (2014) Eurachem Guide: The Fitness for Purpose of Analytical Methods – A Laboratory Guide to Method Validation and Related Topics, Second Edition