Validation of a global method for the simultaneous analysis of polar and non-polar pesticides by online extraction and LC-MS/MS

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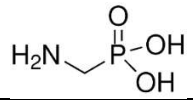
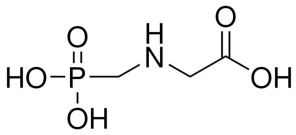
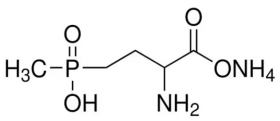
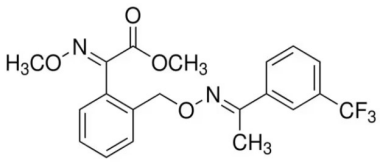
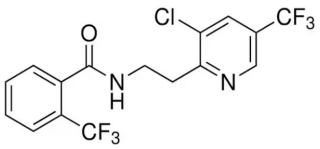
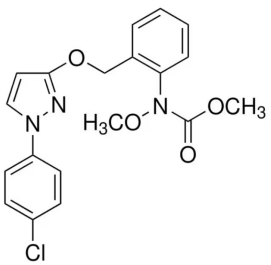
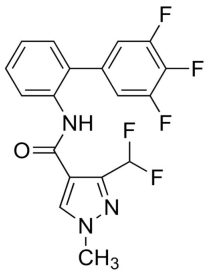
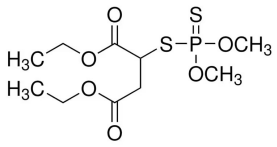
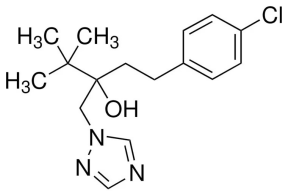
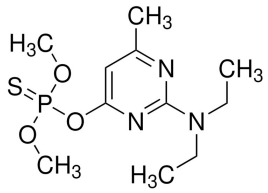
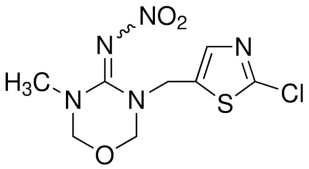
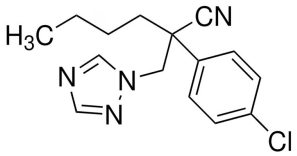
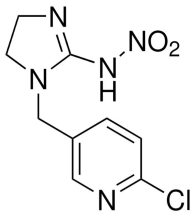
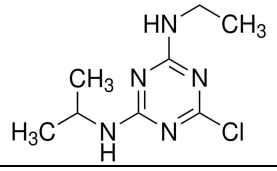
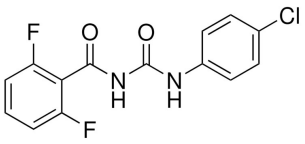
Table S1. List of analytes with the related acronyms, CAS numbers, log K_{ow}, and molecular weight.

Compound	Acronym	CAS number	log K_{ow}	Molecular weight (g mol^{-1})
(Aminomethyl)phosphonic acid 	AMPA	1066-51-9	-1.63	111.04
Glyphosate 	GLY	1071-83-6	-3.40	169.07
Glufosinate-ammonium 	GLUFO	77182-82-2	-4.4	198.16
Trifloxystrobin 	TRIFLO	141517-21-7	4.5	408.37
Fluopyram 	FLUOPY	658066-35-4	3.3	396.71
Pyraclostrobin 	PYRA	175013-18-0	3.99	387.72
Fluxapyroxad	FLUXA	907204-31-3	3.13	381.3

				
Malathion				
	MELA	121-75-5	2.36	330.36
Tebuconazole				
	TEBU	107534-96-3	3.7	307.82
Pirimiphos-methyl				
	PIRI	29232-93-7	4.12	305.33
Thiamethoxam				
	THIA	153719-23-4	-0.13	291.71
Myclobutanil				
	MYCLO	88671-89-0	2.94	288.78
Imidacloprid				
	IMIDA	138261-41-3	0.57	255.66

Atrazine 	ATRA	1912-24-9	2.61	215.68
Diflubenzuron 	DIFLU	35367-38-5	3.89	310.68

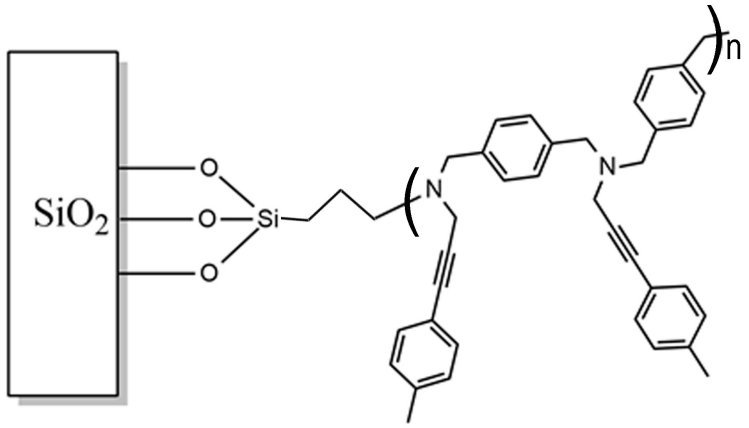


Figure S1. Picture showing the derivatization of the silica capillary within the PEEK-sil tubing with the poly(propargyl amine) monolith structure covalently bonded to the surface.

1. Adsorption experiments

The adsorption of GLY was studied as a representative example of interaction by ion exchange mechanism on the trapping device. GLY solutions were loaded using the optimized method. The amount of adsorbed analyte was determined by elution. The amount of free analyte was calculated by the difference between the loaded amount and the adsorbed amount to calculate the equilibrium concentration. The equilibrium adsorption capacity Q_e (mg m^{-1}) was calculated from Equation 1:

$$Q_e = \frac{(C_0 - C_e)V}{L} \qquad \text{Eqn. 1}$$

where L (m) is the length of the monolithic column. and C_e (mg L^{-1}) is the concentration of GLY at equilibrium. V (mL) is the volume loaded on the PEEK-sil monolithic column. The adsorption isotherm data was fitted with the Langmuir (Equation 2) and Freundlich (Equation 3) models.

$$Q_e = \frac{Q_{max} K_L C_e}{1 + K_L C_e} \qquad \text{Eqn. 2}$$

$$log Q_e = \log(\frac{C_e}{n} x K_F) \qquad \text{Eqn. 3}$$

where Q_e (mg m^{-1}) and C_e (mg L^{-1}) are the experimentally measured equilibrium adsorption capacity and the equilibrium concentration. Q_{max} (mg m^{-1}) is the maximum amount of adsorbed GLY. K_L and K_F are the Langmuir and Freundlich constants, and n is the heterogeneity factor.

The Scatchard analysis (Equation 4) was used to evaluate the theoretical binding site number.

$$\frac{Q_e}{C_e} = \frac{Q_{max} - Q_e}{K_D} \qquad \text{Eqn. 4}$$

K_D is the Scatchard constant.

Table S2. Multiple reaction monitoring (MRM) method used for acquisition of pesticide standards. For every compound, the *m*/*z* of the precursor ion and three product ions are provided, with the related collision energy (CE), S-lens settings, and polarity used for acquisition.

Compound	Acronym	Polarity	Precursor Ion (*m*/*z*)	Product Ion (*m*/*z*) and CE (V)	S-lens (V)
(Aminomethyl) phosphonic acid	AMPA	-	110	79(33), 63(21), 80(22)	48
Glyphosate	GLY	-	168	63(40), 79(41), 81(17)	44
Glufosinate-ammonium	GLUFO	-	180	63(44), 95(19), 85(21)	45
Trifloxystrobin	TRIFLO	+	409	186(21), 145(40), 206(14)	83
Fluopyram	FLUOPY	+	397	207(24), 173(31), 145(50)	106
Pyraclostrobin	PYRA	+	388	163(23), 194(12), 149(29)	108
Fluxapyroxad	FLUXA	+	382	342(23), 362 (13), 314.(24)	115
Malathion	MELA	+	331	99(23), 127(13), 125(29)	78
Tebuconazole	TEBU	+	308	70(21), 125(34), 151(25)	105
Pirimiphos-methyl	PIRI	+	306	108(33), 164(22). 67(37)	104
Thiamethoxam	THIA	+	292	211(12), 181(22), 132(23)	75
Myclobutanil	MYCLO	+	289	70(17), 125(32), 151(25)	104
Imidacloprid	IMIDA	+	256	209(17), 175(18), 84(19)	82
Atrazine	ATRA	+	216	174(17), 104(28), 68(35)	95
Diflubenzuron	DIFLU	-	309	156(13), 93(47), 42(16)	77

Table S3. Types of beer used for method development and application with brief description

Brand	**Origin**	**Beer type**	**Ingredients**	**Alcohol % vol**
Brand 1	Spain	Pilsner	barley malt	5.4
Brand 2	Netherlands	Pilsner	barley malt, hop	5
Brand 3	Denmark	Lager	Malt, hop	10
Brand 4	Scotland	Lager	barley malt, hop	9
Brand 5	Germany	Lager	Light wheat malt, dark wheat malt, Pilsner malt (light barley malt), Munich malt (dark barley malt)	5.5
Brand 6	Italy	Lager	malt	4.7
Brand 5 (unfiltered)	Germany	Weiss	Light wheat malt, dark wheat malt, Pilsner malt (light barley malt), Munich malt (dark barley malt)	5.5

Figure S2. FT-IR image of the monolithic polymer

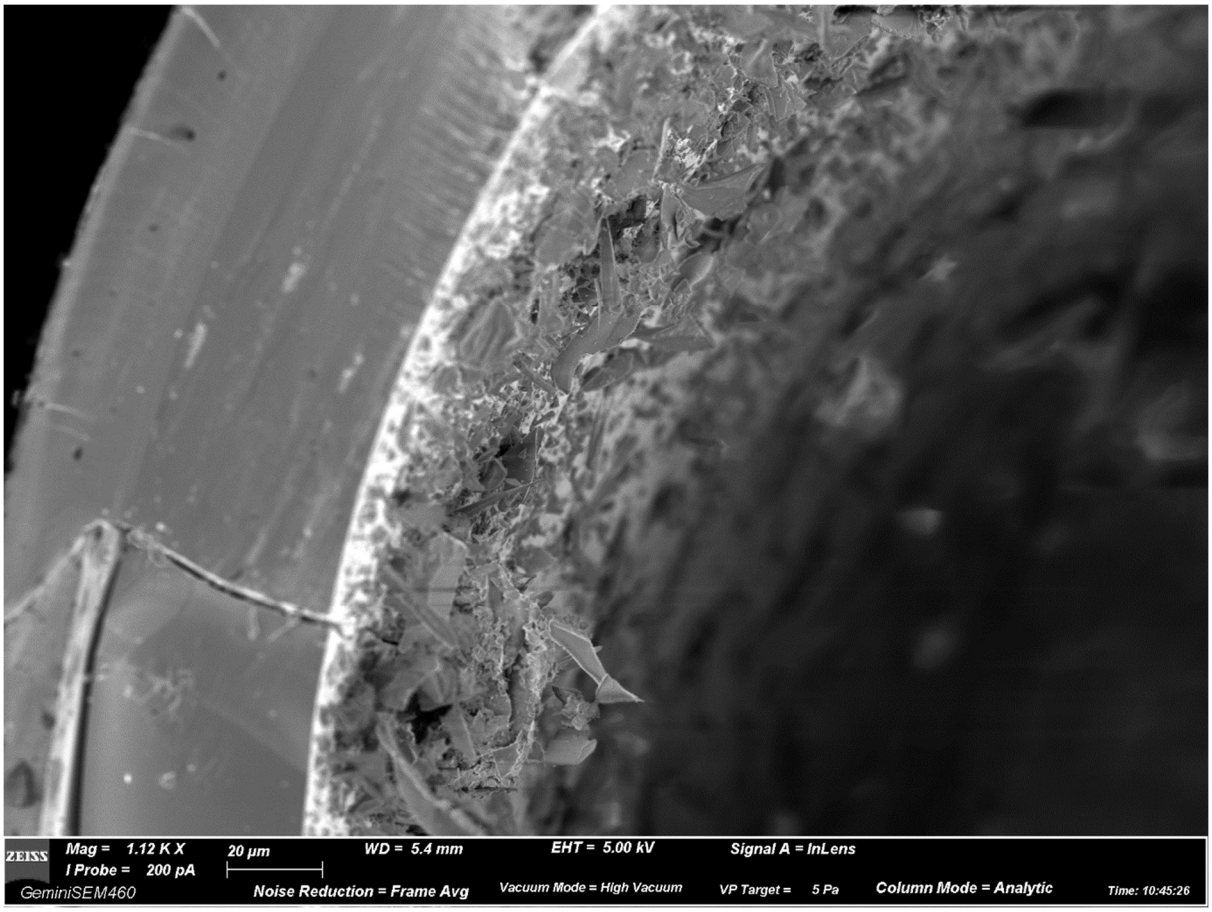


Figure S3. SEM image of the trapping device showing the layer inside the PEEK-sil tubing and hollow cavity.

Table S4. Optimization of the UHPLC separation of the pesticides for global analysis by evaluation of retention times and detection limits of the 15 pesticides.

		HILIC		C30	
Compound	***m/z***	**t_R**	**LOD (µg L^{-1})**	**t_R**	**LOD (µg L^{-1})**
AMPA	110	7.63	4	2.33	1
GLY	168	7.63	4	2.11	1
GLUFO	180	8.79	4	2.00	1
ATRA	216	1.52	7	8.69	0.1
IMIDA	256	1.63	1	7.92	0.1
MYCLO	289	1.52	3	9.09	0.1
THIA	292	1.69	5	7.60	0.2
PIRI	306	1.42	1	9.65	0.1
TEBU	308	1.59	1	9.21	0.1
DIFLU	309	1.51	9	9.18	0.2
MELA	331	1.42	2	9.15	0.2
FLUXA	382	1.48	8	9.05	0.2
PYRA	388	1.43	5	9.49	0.1
FLUOPY	397	1.43	2	9.16	0.1
TRIFLO	409	1.38	5	9.61	0.1

Table S5. Validation of a global analytical method for the analysis of 15 pesticides with online extraction and LC-MS/MS analysis. For each compound, the coefficient of determination for the linear regression is provided, with the related linear range, LOD and LOQ values, inter-day and intra-day precision provided as relative standard deviation (RSD, %, n = 6). Recoveries (RE, %) and matrix effects (ME, %) are provided at three concentration levels (c_1: 3 µg L^{-1}; c_2: 10 µg L^{-1}, c_3: 100 µg L^{-1} for GLY, AMPA, GLUFO and c_1: 0.3 µg L^{-1}; c_2: 1 µg L^{-1}, c_3: 10 µg L^{-1} for all other pesticides).

Compound	R^2	Linear range (µg L^{-1})	LOD (µg L^{-1})	LOQ (µg L^{-1})	Intra-day RE ± RSD (%)	Inter-day RE ± RSD (%)	RE% (C_1)	RE% (C_2)	RE% (C_3)	ME% (C_1)	ME % (C_2)	ME % (C_3)
AMPA	0.992	3-100	1	3	(89 ± 10)	(95 ± 15)	88%	94%	98%	120%	87%	101%
GLY	1	3-100	1	3	(97 ± 12)	(100 ± 13)	100%	99%	95%	110%	115%	102%
GLUFO	1	3-100	1	3	(95 ± 15)	(97 ± 10)	106%	88%	107%	111%	134%	99%
TRIFLO	1	0.3-10	0.15	0.3	(88 ± 2)	(89 ± 4)	85%	102%	110%	115%	105%	98%
FLUOPY	0.999	0.3-10	0.14	0.3	(94 ± 4)	(97± 7)	84%	103%	84%	106%	104%	97%
PYRA	0.999	0.3-10	0.09	0.3	(84 ± 4)	(102 ± 7)	112%	98%	96%	105%	95%	101%
FLUXA	0.999	0.3-10	0.21	0.3	(82± 2)	(95± 4)	94%	86%	95%	150%	133%	105%
MELA	0.999	0.3-10	0.12	0.3	(92± 2)	(95± 5)	98%	95%	92%	130%	100%	110%
TEBU	0.999	0.3-10	0.11	0.3	(94± 3)	(85± 3)	86%	86%	85%	110%	108%	104%
PIRI	1	0.3-10	0.12	0.3	(87 ± 6)	(95± 8)	90%	98%	89%	97%	94%	109%
THIA	1	0.3-10	0.16	0.3	(111 ± 10)	(99± 11)	108%	86%	91%	108%	97%	110%
MYCLO	0.999	0.3-10	0.02	0.3	(95 ± 8)	(88 ± 7)	71%	100%	86%	111%	112%	115%
IMIDA	0.999	0.3-10	0.11	0.3	(94 ± 6)	(97 ± 4)	98%	94%	99%	116%	115%	114%
ATRA	0.999	0.3-10	0.11	0.3	(97 ± 2)	(95 ± 2)	92%	99%	91%	105%	103%	102%
DIFLU	1	0.3-10	0.13	0.3	(98 ± 3)	(99 ± 2)	109%	102%	109%	108%	102%	109%

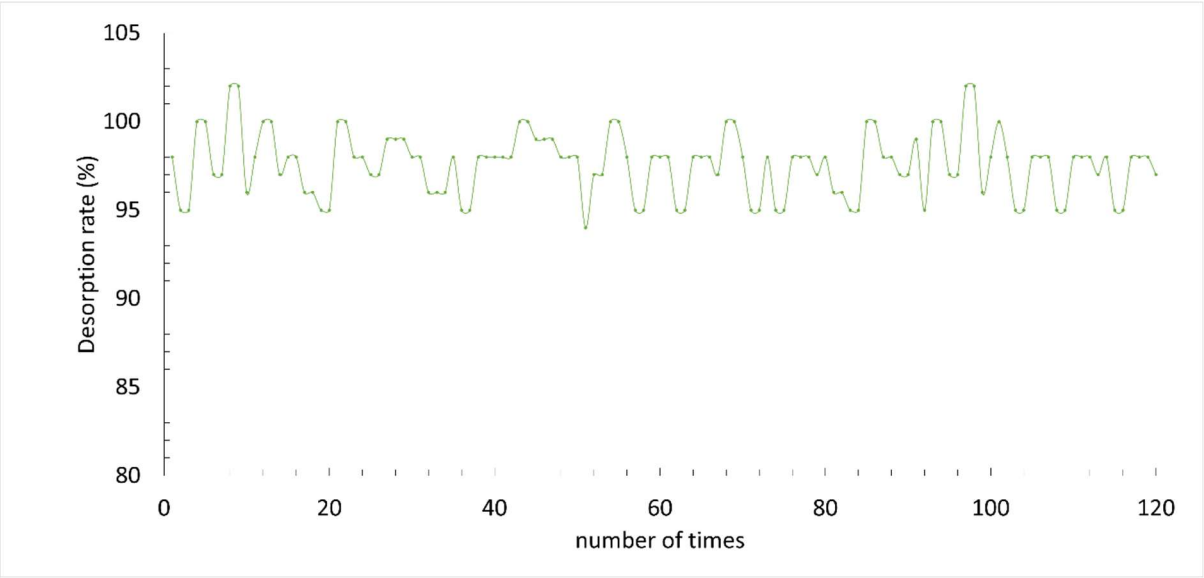


Figure S4. Graph showing the repeated loading-elution cycles of GLY from spiked beer samples (4 µg L^{-1}) on the monolithic trapping column over 120 cycles.

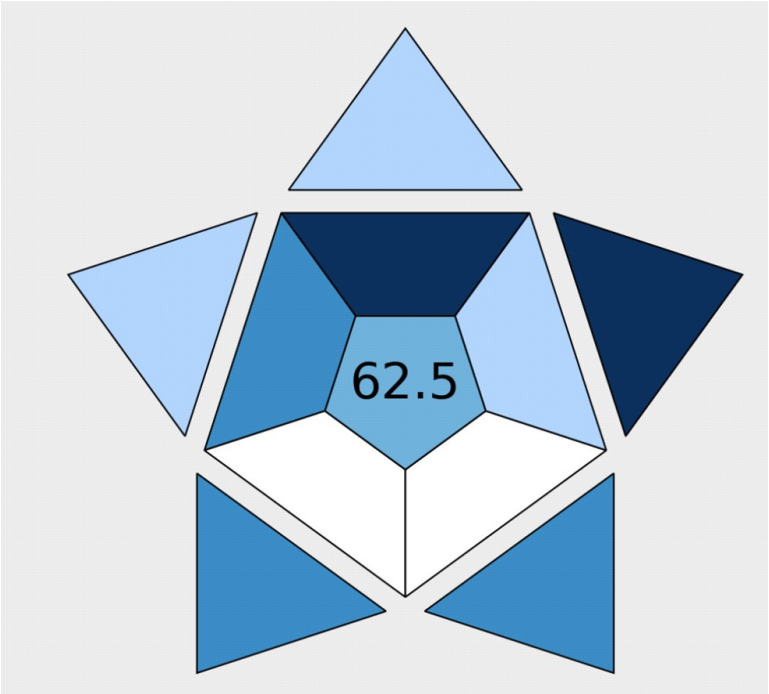


Figure S5. Asteroid pictogram produced by the online BAGI interface (https://bagi-index.anvil.app/) showing the results for the method described in this work. Colors describe the compliance of the method with the set criteria (i.e., dark blue for high compliance, blue for medium compliance, light blue for low compliance, and white for no compliance). Attributes associated with either the step of the analytical determination or sample preparation are in the

inner part of the pictogram; attributes that correspond to both steps are in the outer part. Diamond part of the pictogram, from top, counter-clockwise rotation:

1. Type of analysis: quantitative and confirmatory;
2. Multi- or single-element analysis: multi-element analysis for 6-15 compounds;
3. Analytical technique: instrumentation that is not commonly available in most lab;
4. Simultaneous sample preparation: 1;
5. Sample preparation: miniaturized extraction;

Outer triangles, from top one, counter-clockwise rotation:

6. Samples per h: 2-4;
7. Reagents and materials: need to be synthesized in the lab with common instrumentation and in a simple way;
8. Preconcentration: preconcentration required. Required sensitivity is met with one-step preconcentration;
9. Degree of automation: semi-automated with common devices (e.g HPLC autosampler);
10. Amount of sample: < 100 µL (or mg) bioanalytical samples; <10 mL (or g) food/environmental.

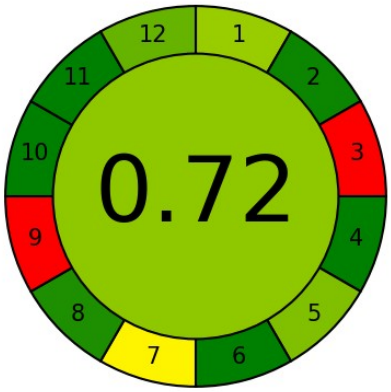


Figure S6. Image showing the results of the analysis of the method described in this study using the AGREE tool.

1. sampling procedure: on-line analysis;
2. amount of sample: 0.1 mL;
3. in situ positioning of the analytical device: off-line;
4. number of distinct steps in the sample preparation procedure: 3 or fewer;

5. degree of automation and sample preparation: semi-automatic and none or miniaturized;
6. derivatization: none;
7. amount of waste: 5 mL;
8. number of analytes and sample throughput: 15 and 4;
9. most energy-intensive technique: LC-MS;
10. no reagents;
11. no toxic reagents;
12. operator’s safety: highly flammable threats.