Analytica Chimica Acta 1329 (2024) 343231

Contents lists available at ScienceDirect

Analytica Chimica Acta

journal homepage: www.elsevier.com/locate/aca

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

Susy Piovesana, Sara Elsa Aita, Chiara Cavaliere, Andrea Cerrato, Aldo Laganà, Carmela Maria Montone *, Enrico Taglioni, Anna Laura Capriotti

Department of Chemistry, Università di Roma La Sapienza, Piazzale Aldo Moro 5, 00185 Rome, Italy

HIGHLIGHTS

- Automated online extraction of polar and non-polar pesticides from beer.
- Validation of online extraction from beer followed by HPLC-MS/MS analysis.
- Polar and non-polar pesticides analyzed by reversed-phase HPLC single run.
- Glyphosate, AMPA, glufosinate were analyzed with non-polar pesticides.

ARTICLE INFO

Keywords:
Glyphosate
AMPA
Pesticides
Online sample preparation
Beer
Reversed-phase chromatography

ABSTRACT

Background: Multi-residue methods for pesticide analysis in food are available for many compounds, but polar pesticides are not generally included due to their specific properties, which include high polarity and low molecular weight. Single residue methods are therefore needed for sample preparation, while chromatographic separation often requires derivatization, ion paring, or dedicated methods suitable for polar compounds, mostly ion chromatography and hydrophilic interaction liquid chromatography (HILIC). These challenges affect the important pesticide glyphosate and the related compounds aminomethylphosphonic acid (AMPA) and glufosinate. There are only a few methods including these compounds in large-scale analysis, mostly complex methods based on multidimensional chromatography.

Results: A new method, for the global online extraction and analysis of pesticides in beer was developed and validated. The method exploited an online trapping device, with reversed-phase (RP) and anion exchange properties, that can trap small molecules from liquid samples. The ion exchange mechanism was used to retain the very polar pesticides glyphosate, AMPA, and glufosinate. The hydrophobic properties of the trapping column were also exploited to trap pesticides suitable for multi-residue investigations. The chromatographic separation was optimized by comparison of HILIC and RP C30, which could separate pesticides, including the polar ones, with modulation by the trapping column after proper selection of the mobile phase composition and basic modifier. The validation for beer provided recoveries in the range 71–112 %, with <15 % RSD, and LOD and LOQ values of 0.02–1 and 0.3–3 μg L^{-1}, respectively. The result was competitive with previous methods on polar pesticide analysis in beer.

Significance: The method was validated for 15 pesticides, over the log K_{ow} range from −4.4 to 4.5, using a methodology with single and fast chromatographic separation under conditions compatible with multi-residue analysis by RP-LC-MS/MS. In the case of beer, for which the method was validated, the sample preparation was also performed online, after simple degassing, and sample dilution.

1. Introduction

Glyphosate (GLY) is the most-used herbicide worldwide in the history of agriculture due to the introduction of genetically modified GLY-resistant crops. The metabolite aminomethylphosphonic acid (AMPA) is also of concern due to its long-term contamination of

* Corresponding author.
E-mail address: carmelamaria.montone@uniroma1.it (C.M. Montone).

https://doi.org/10.1016/j.aca.2024.343231
Received 7 June 2024; Received in revised form 6 September 2024; Accepted 9 September 2024
Available online 10 September 2024

sediments, surface water, and groundwater and potential health problems for people and the environment [1]. In 2015, the International Agency for Research on Cancer (IARC) classified GLY as a probable human carcinogen (Group 2A) [1,2]. Nonetheless, the US Environmental Protection Agency and the European Food Safety Authority have not found significant evidence linking GLY to human cancer [3,4].

For the extraction of GLY and AMPA in environmental, plant, food, and human samples, solid-phase extraction (SPE) is mostly employed with cation and anion exchange sorbents or mixed-mode sorbents [5]. In addition, reversed-phase (RP) sorbents, immobilized metal affinity chromatography, metal oxides, and molecularly imprinted polymers have also found significant applications [5]. When RP sorbents are used, derivatization is often required to improve retention [5]. Specifically in food, common multi-residue methods, such as QuEChERS (quick, easy, cheap, effective, rugged, and safe), are not applicable. Indeed, polar pesticide analysis requires specific single-residue methods, such as the Quick Polar Pesticides Method (QuPPe), which is used for food of plant and animal origin and honey [6]. The inclusion of GLY and related compounds in the few multiclass methods developed for other acidic pesticide classes was also generally not achieved [7,8], except in a report analyzing ionic and acidic pesticides, including GLY, where QuPPe was used for oat and beer extraction [9].

Derivatization is necessary for GC analysis due to the zwitterionic nature, high polarity, and poor volatility of GLY and AMPA [5,10]. Moreover, due to their physicochemical properties, polar pesticides are not retained by classical RP C18 or C8 columns used in the multi-residue analysis [11,12]. On LC platforms, derivatization can be used to improve the detector sensitivity and chromatographic retention [5,10]. However, these approaches need extensive sample preparation, introduce isobaric interferences, and can dirty the instrument, thus providing signal suppression and sensitivity reduction [12]. Therefore, ion chromatography (IC), hydrophilic interaction liquid chromatography (HILIC), hydrophilic/weak exchange, RP/weak exchange mixed-mode chromatography, and porous graphitic carbon (PGC) are used for direct separation and detection methods in LC-MS [12–14]. However, IC and HILIC have the drawbacks of retention time-shifts, matrix effects, and high dilution factors and may need specific instrumentation instead of standard LC instrumentation [12,15]. HILIC is less common in multi-residue methods, compared to RP C18 columns, for the analysis of contaminants in foodstuffs, with limited exceptions [16]. A wider polarity range can be covered using orthogonal multidimensional chromatographic strategies. For instance, a combination of HILIC and RP-LC, employing the heart-cutting methodology on the void fraction from the first dimension, was used for the analysis of 20 pesticides with log K_{ow} from −3.2 to 4.3, except GLY, AMPA, and GLUFO. The method was not fully validated, although a representative application to tomato was reported [17]. A previous concept report, which also exploited 2D-LC-MS/MS for analysis, described the HILIC-RP-LC separation of 325 QuEChERS amenable compounds and 9 polar pesticides, including GLY and metabolites, but no samples were analyzed using this method [18]. The injection of two sample aliquots simultaneously on two orthogonal separation columns was also described for the analysis of 41 multiclass pesticides over log K_{ow} from −4 to +5.5 with application to leek [19].

Among the foodstuffs that suffer from pesticide contamination, beer is not an exception. Pesticide residues in beer come from barley [20,21] and hop [22,23] (mostly sulfonyl urea, pyrethroids, and triazoles), soil contamination, and water used for beer production [24]. From a survey of the fate of 368 pesticides during beer brewing, hydrophilic pesticides were found to be more persistent [25]. For the extraction of GLY and polar pesticides, single residue methods are used [9,26], while ion exchange chromatography [9] and capillary electrophoresis [27] were used for analysis. Derivatization by nitrosation was also described, coupled with anion-exchange chromatography and chemiluminescence determination [28].

It is desirable to develop new comprehensive methods for both polar and non-polar pesticide analysis to improve the throughput and reduce time and costs while reducing the environmental impact, especially when high energy-consuming techniques are employed. In this work, a new mixed-mode sorbent based on poly (propargyl amine) polymer was exploited for the global retention of pesticides commonly found in beer. The sorbent, which was already proven good for the retention of β-estradiol from serum samples by RP mechanism [29], was used for extraction and clean-up of non-polar pesticides. Simultaneously, the presence of tertiary amine moieties further allowed for an anion exchange mechanism, which we envisioned would elegantly suit the retention of polar pesticides, especially GLY. The method was validated for the analysis of 15 pesticides in beer, which was chosen as a representative matrix to demonstrate the scope of analyte trapping and clean-up without dedicated sample extraction.

2. Material and methods

2.1. Chemicals and reagents

Pure standards of GLY, AMPA, glufosinate-ammonium (GLUFO), trifloxystrobin (TRIFLO), fluopyram (FLUOPY), pyraclostrobin (PYRA), fluxapyroxad (FLUXA), malathion (MELA), tebuconazole (TEBU), pirimiphos-methyl (PIRI), thiamethoxam (THIA), myclobutanil (MYCLO), imidacloprid (IMIDA), atrazine (ATRA), and diflubenzuron (DIFLU) were purchased from Merck Life Science (Darmstadt, Germany). The list of chemicals and the related information is provided in Table S1. Stock solutions were prepared at 1 mg mL^{-1} in acetonitrile, except GLY, AMPA, and GLUFO, for which water was used; working solutions were prepared in water/acetonitrile, 90:10 (*v/v*) every month to prevent degradation, stored at 4 °C, and brought to room temperature before use.

2.2. Preparation and characterization of the poly (propargyl amine) monolithic trapping column

The online monolithic trapping column was prepared as described previously [29]. Briefly, the monolith was prepared inside an activated PEEK-sil tubing (150 × 0.530 mm I.D., 1.59 mm × O.D.) by a one-pot multicomponent reaction of 1,4-bis(aminomethyl)-benzene (0.10 mmol), benzene-1,4-diboronic acid (0.10 mmol), 3-(*p*-tolyl)propiolic acid (0.2 mmol), and formaldehyde (0.4 mmol) in 0.5 mL of dichloroethane at 90 °C to produce a covalently bonded poly (propargyl amine) polymer (Fig. 1a and S1). The presence of several aromatic rings and tertiary amine groups allows the polymer to be used both as RP and anion exchange material, as described in the following sections. To assess the reproducible preparation of the trapping devices, they were characterized using scanning electron microscopy (SEM) for morphological analysis and Fourier transform infrared (FT-IR) spectroscopy for chemical derivatization analysis. High-resolution SEM images were obtained after gold coating using FEG SEM Zeiss Gemini 460; FT-IR spectra were obtained on an IR spectrophotometer Nicolet iS50 coupled with a Nicolet Continuum FT-IR microscope (Thermo Scientific) with diamond cell and acquiring 40 spectra in the range 4000–650 cm^{-1}, with a spatial resolution of 8 cm^{-1}. Background spectra were subtracted for each measure.

Adsorption experiments were used to investigate the interaction of the stationary phase with GLY, which was used as a representative molecule for the ion exchange interaction, by application of the Langmuir, Freundlich, and Scatchards models [30,31]. Details are provided in the Electronic Supplementary Material (1. Adsorption experiments).

2.3. Optimization of the sample preparation method

The extraction program for polar and non-polar pesticides in beer was optimized based on previous work with some modifications [29]. The monolithic trapping column was placed inside an Ultimate 3000 UHPLC autosampler (Thermo Fisher Scientific, Milan, Italy) equipped

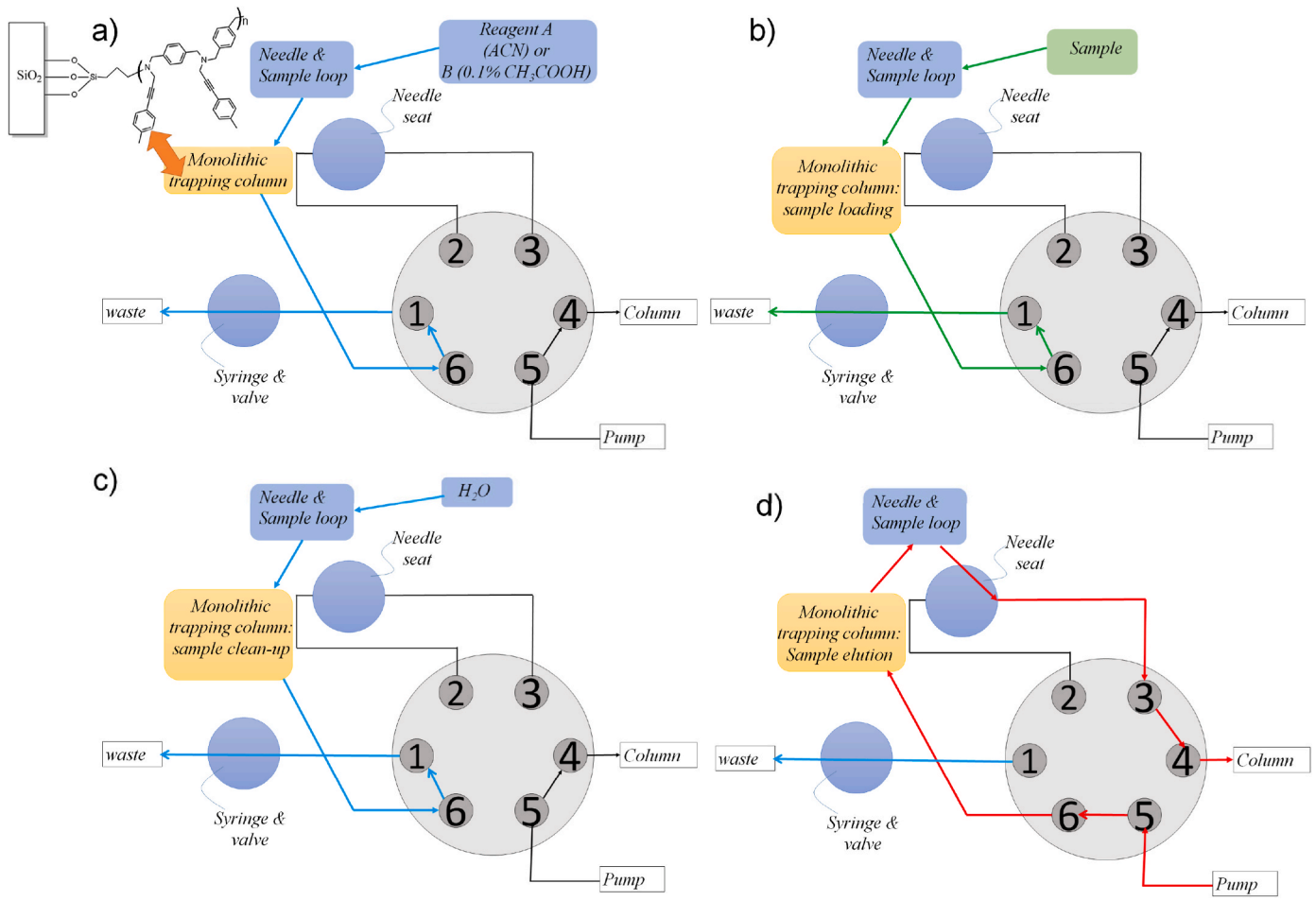

Fig. 1. Graphical representation of the method used in the autosampler program for automated use of the monolithic trapping column. a) Activation of the trapping column; b) loading of sample onto the trapping column; c) washing step for analyte clean-up; d) elution of the analytes from the trapping column by the mobile phase and analyte separation.

with a degasser, a thermostatted column compartment, a 100 µL injection loop, and a programmable 6-port Rheodyne switching valve.

The optimization of the trapping conditions was performed using 100 µL of a mix solution of GLY, AMPA, and GLUFO at 4 ng mL^{-1}. The sample was loaded using an offline syringe pump at 50 µL min^{-1}. Loading was tested in 0.1 % (*v/v*) acetic acid or 0.5 % (*v/v*) NH_4OH. The flow-through was dried down in a speed-vac system (Speed-Vac SC250 Express, Thermo Savant, Holbrook, NY, USA) and reconstituted in 100 µL of water/acetonitrile (80:20, *v/v*). The analysis provided the amount of GLY, AMPA, and GLUFO that were not retained on the trapping column during the loading step. The samples were analyzed by LC-MS/MS using an iHILIC®-Fusion UHPLC Column, SS (100 × 2.1 mm i.d., 1.8 µm particle size, Hilicon, Umeå, Sweden) with an iHILIC-Fusion Guard Column, SS, (20 × 2.1 mm, 1.8 µm particle size). Mobile phase A was acetonitrile with 0.2 % (*v/v*) formic acid; mobile phase B was water with 100 mmol L^{-1} ammonium formate and 0.2 % (*v/v*) formic acid. The following gradient was used for analysis: t0, B = 3 %; t3, B = 3 %; t5, B = 80 %; t8, B = 80 %; t10, B = 3 %; equilibration for 2 min at 3 % phase B. The HESI source was operated in switching polarity mode with a spray voltage of±4 kV. The vaporizer temperature was 300 °C; the capillary temperature was 275 °C. The gas pressures were set to 45 (arbitrary units) for sheath gas, 0 for ion sweep gas, and 4 for auxiliary gas. The analytes were detected by multiple reaction monitoring (MRM) using three selected transitions, according to the details reported in Table S2.

The elution, washing steps after analyte trapping, the draw and dispense speed, and the mobile phase flow rate were studied using the autosampler equipped with the trapping column and GLY, AMPA, and GLUFO mix solutions at three concentration levels (4, 10, 20 ng mL^{-1}) in 0.1 % (*v/v*) acetic acid. The elution was studied using the mobile phase as the eluent. The following compositions and mobile phase matchings were tested: a) acetonitrile with 0.2 % (*v/v*) formic acid (phase A) and water with 100 mmol L^{-1} ammonium formate and 0.2 % (*v/v*) formic acid (phase B); b) acetonitrile (phase A) and water with 0.1 % (*v/v*) NH_4OH (phase B); c) acetonitrile with 0.2 % (*v/v*) formic acid (phase A) and water with 100 mmol L^{-1} ammonium formate (phase B). The optimization of the washing conditions after analyte trapping was studied using 100 µL of: a) water; b) acetonitrile; c) 2 steps with water; or d) 2 steps with acetonitrile. Finally, the draw and dispense speed, and the mobile phase flow rate were investigated at 0.1, 0.2, 0.3, 0.5, and 1.2 min^{-1}. Recoveries were calculated on three experimental replicates with three technical replicates.

2.4. Optimized online sample preparation program

Under the optimized protocol, the trapping column was first activated and conditioned, setting the valve to the load position (6–1). One hundred µL of Reagent A (acetonitrile) was passed through the trapping column and discarded twice (Fig. 1a). The same procedure was then repeated with Reagent B (water with 0.1 % acetic acid) for conditioning. Then, 100 µL of the sample was loaded and discarded (Fig. 1b). The trapping column was then washed with 100 µL of water (Fig. 1c), then the valve was switched to the inject position (1–2), and the mobile phase was used for elution (Fig. 1d). The valve and autosampler were operated

according to the steps graphically displayed in Fig. 1.

2.5. UHPLC separation and MS/MS method

In the final conditions, the samples were analyzed by UHPLC–MS/MS using an Ultimate 3000 binary pump (Thermo Fisher Scientific in Bremen, Germany) and a triple quadrupole mass spectrometer (TSQ Vantage EMR, Thermo Fisher Scientific, Bremen, Germany) connected via a heated electrospray ionization source (HESI). Xcalibur software, version 2.2 (Thermo Fisher Scientific, Bremen, Germany) was employed to manage, acquire, and process LC–MS data. Samples were injected using the program described previously with the trapping column connected after the loop. Analyte separation was performed using an Accucore C30 column (2.1 × 150 mm, particle size 2.6 μm, Thermo Scientific) equipped with an Accucore C30 precolumn (2.1 × 4 mm, particle size 5 μm, Thermo Scientific) at 45 °C and 0.3 mL min^{-1} flow rate. The mobile phase A was water with 0.025 % (*v/v*) NH_4OH (pH 8); mobile phase B was acetonitrile. The following gradient was used (t in min): t0, B = 0 %; t4, B = 0 %; t8, B = 100 %; t12, B = 100 %; t13, B = 0 %; t16, B = 0 %. The HESI source was operated in switching polarity mode with a spray voltage of±4 kV. The vaporizer temperature was 350 °C; the capillary temperature was 270 °C. The gas pressures were set to 50 (arbitrary units) for sheath gas, 0 for ion sweep gas, and 30 for auxiliary gas. The analytes were detected by multiple reaction monitoring (MRM) using three selected transitions, according to the details reported in Table S2.

2.6. Beer samples

Beer samples were degassed by placing them in an ultrasonic bath for 15 min. Then, an aliquot of 1 mL of sample was used for analysis. If the ethanol content in the beer was >5 %, the sample was diluted with UHPLC-MS water to reach 4 % ethanol content. Finally, the sample pH was adjusted to 3–4 with acetic acid, then it was filtered through Acrodisc 13 mm minispike 0.2 μm GHP membranes (Pall Corporation, Puerto Rico) and placed in autosampler vials. For method development, the samples were spiked at the appropriate concentration before placing them in the autosampler vials for injection. Six beer brands were purchased at a local supermarket for the application of the developed method. The types included two brands of Pilsner, four Lager, and one Weiss (Table S3).

2.7. Method validation

The optimized method was validated for the analysis of 15 pesticides in beer, according to FDA guidelines [32]. The recovery (RE, %), matrix effect (ME, %), precision, linear dynamic range, and the limit of detection and quantification (LOD and LOQ) were used for validation, and matrix effect was evaluated for further performance investigation.

Recovery of pesticides from spiked beer samples was calculated 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, and GLUFO, and at c_1: 0.3 μg L^{-1}; c_2: 1 μg L^{-1}, c_3: 10 μg L^{-1} for all other analytes) by comparing the areas according to Equation (1).

$$RE = \frac{AreaS_1 - AreaS_0}{Area\ S_2} \times 100 \qquad \text{Eqn. 1}$$

S_1 is the beer sample spiked before injection with the trapping column inserted in the autosampler, S_0 is the beer sample without spiking (endogenous amount), and S_2 is the spiked neat solvent analyzed by direct injection without the trapping column.

The ME was calculated 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, and GLUFO, and at c_1: 0.3 μg L^{-1}; c_2: 1 μg L^{-1}, c_3: 10 μg L^{-1} for all other analytes) by comparing areas according to Equation (2).

$$ME = \frac{AreaS_1 - AreaS_0}{Area\ S_3} \times 100 \qquad \text{Eqn. 2}$$

S_3 is the spiked neat solvent injected using the setup with the trapping column.

Calibration curves were prepared in beer by adding a known amount of analytes in the range of 3–100 μg L^{-1} for GLY, AMPA, and GLUFO and in the range of 0.3–10 μg L^{-1} for all other analytes. All samples were analyzed in triplicate, and the results were averaged.

Six replicate extractions and recovery analysis were performed within a day to calculate intra-day precision as relative standard deviation (RSD, %). Analyses were repeated in the following 5 days to evaluate the inter-day precision.

LOD was evaluated by analyzing different concentrations and determining the minimum concentration that produced a signal-to-noise ratio (S/N) ≥3. This LOD value was experimentally determined and not simply calculated by the S/N ratio due to the very low noise level of MRM transitions [33]. The LOQ was set at the lowest limit of the linear dynamic range.

3. Results and discussion

3.1. Characterization of the poly (propargyl amine) monolith and static binding under ion-exchange mechanism

The monolith was prepared inside PEEK-sil tubing. The tubing was 15 cm long, and the length of the tubing was not optimized. The tubing is commercially available and it cannot be cut, but it has the advantage of being easily modifiable on the inside by activation of the silica lining and functionalization, which in this study was achieved by the multicomponent reaction. The monolith was characterized by the presence of several aromatic rings, a C ≡ C, tertiary amine moieties, and methyl moieties according to the picture provided in Fig. S1. To ensure that the preparation of the monolithic trapping device was reproducible in a new batch, the material was characterized by FT-IR analysis (Fig. S2) to check for signals associated with the structure of the product and assess the absence of signals associated with the reagents. The weak signal of the C ≡ C stretching (2212 cm^{-1}) was observed, according to a previous report [29]. No signal for the B–O stretching and O–H in-plane bending (1032 and 1002 cm^{-1}, respectively) was observed, which confirmed the reaction of the borane reagent; the same applied to the signals associated with the N–H moiety, indicating the reaction proceeded as expected. The spectra acquired had the same pattern as in our previous work [29], especially in the fingerprint region, which was indicative of the successful preparation of the material. Spectra included the signals of aromatics C–H (stretch at 3200 cm^{-1} and 3028 cm^{-1}) and of aromatics C–C (stretch at 1510 cm^{-1}), alkyne C–H stretch (3376 cm^{-1}) and C ≡ C stretch (2212 cm^{-1}), alkane C–H stretch (2920 cm^{-1}) and bending (1400 cm^{-1}), and signals associated with the C–N stretch (1112 cm^{-1}). The SEM images showed a thin layer of monolith inside the PEEK-sil tubing, according to our previous report (Fig. S3) [29]. The characterization confirmed the successful preparation of the trapping column.

The static adsorption of GLY was studied as a representative example of interaction by anion exchange mechanism on the trapping device. GLY solutions were loaded using the optimized method, as explained in our previous work [29] and Electronic Supplementary Material (section 1). The results are summarized in Fig. 2 and Table 1.

The Langmuir fitting exhibited a better correlation than the Freundlich one (R^2 of 1.0 vs 0.59), suggesting a sorption mechanism involving interaction in a monolayer with uniform active sites rather than a multilayer with heterogeneous active sites. The Scatchard model also showed a good fit (R^2 0.98). It was consistent with the results previously described for the RP interaction [29] and indicated that the binding sites were very similar but not identical. The fitting provided values for K_D from the slope and Q_{Max} from the intercepts. The values of Q_{Max} extracted by applying the Langmuir and Scatchard models were

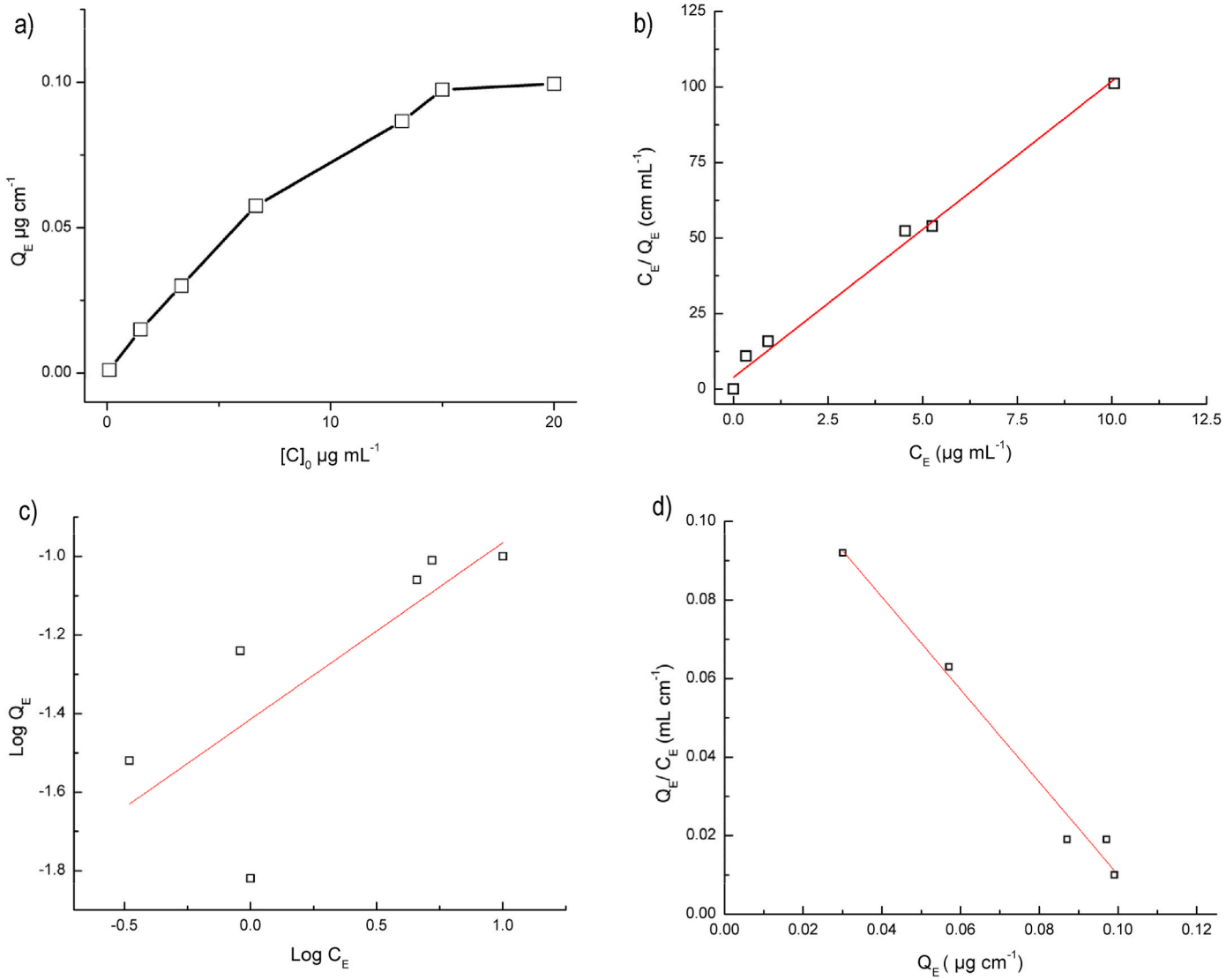

Fig. 2. a) binding of GLY to the trapping column to saturation; fitting of static binding data by the b) Langmuir, c) Freundlich, or d) Scatchard models.

Table 1
Parameters calculated by fitting the experimental data using the Langmuir, Freundlich, and Scatchard models.

Langmuir isotherm	
Q_{Max} (µg cm^{-1})	0.11
K_L (mL µg^{-1})	1.2
R^2	1.0
Freundlich isotherm	
K_F (mL cm^{-1})	0.04
n	2.2
R^2	0.59
Scatchard	
K_D	0.85
R^2	0.98
Q_{Max}	0.11

consistent and indicated that the total amount of adsorbed analyte on the developed monolithic column was approx. 1.6 µg for a 15 cm long tubing. This value was significantly larger than the one obtained for β-estradiol [29]. The loading amount of polar analytes by ion exchange mechanism was compatible with the specific application to GLY, AMPA, and GLUFO analysis in beer, where the linearity was explored up to 10 ng of the total amount of polar analytes.

3.2. Method development

3.2.1. Optimization of the online extraction and clean-up of polar pesticides

The trapping conditions were studied in detail for GLY, AMPA, and GLUFO (Fig. 3). The loading on the trapping column was investigated considering the effect of pH from a water mixture to exploit the ion exchange properties of the sorbent (details on the conditions are reported in section 2.3). The loading in 0.1 % acetic acid was far better than the one in 0.5 % ammonia solution, with residual analyte amounts between 1 and 6% in the former case and 70–90 % in the latter one (Fig. 3a). Loading under acidic conditions improved the binding by activation of the amine moieties of the stationary phase by protonation and subsequent interaction with the negatively charged phosphonate moiety of the analytes. At the same time, the experiments indicated that analyte binding was inhibited under basic pH values.

The elution was studied using the online setup and three mobile phase compositions (details are reported in section 2.3). The best recovery was achieved using condition c, namely acetonitrile with 0.2 % (*v/v*) formic acid (phase A) and water with 100 mmol L^{-1} ammonium formate (phase B, Fig. 3b). The mobile phase was used for elution from the trapping column and analyte separation on the iHILIC column;

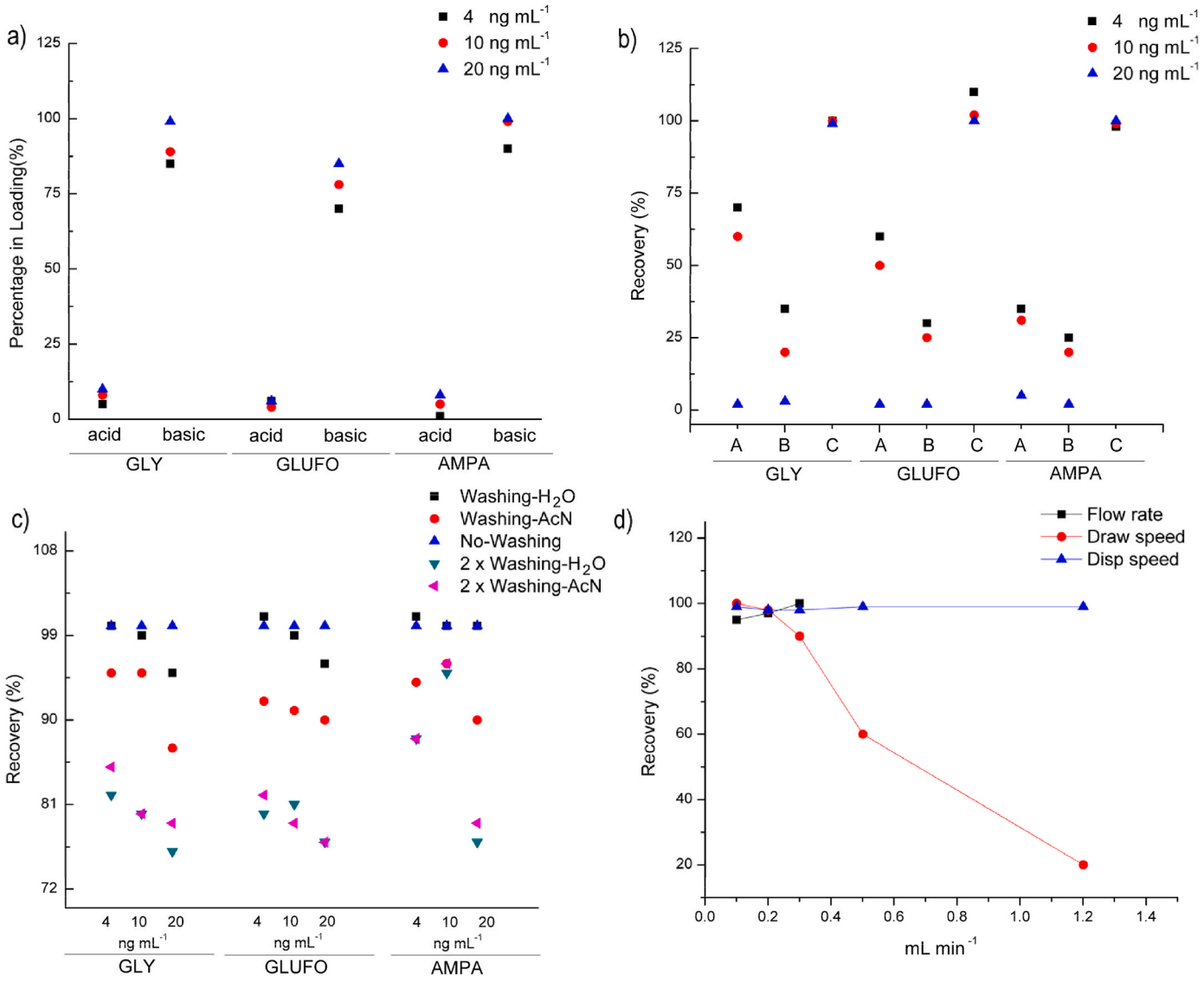

Fig. 3. Results of the optimization of GLY, AMPA, and GLUFO trapping conditions on the monolithic trapping column. Optimization described for a) loading using acid (0.1 % acetic acid) or basic (0.5 % ammonia) water; b) elution using the mobile phase pairs of A) acetonitrile with 0.2 % formic acid and water with 100 mmol L^{-1} ammonium formate and 0.2 % formic acid; B) acetonitrile and water with 0.1 % ammonia; C) acetonitrile with 0.2 % formic acid and water with 100 mmol L^{-1} ammonium formate; c) test with no washing step or single (100 μL) or double (200 μL) washing with either water or acetonitrile; d) effect of the mobile phase flow rate, draw and dispense speeds.

therefore, a compromise was necessary. Acetonitrile was needed to allow retention on the iHILIC column and, consequently, analyte separation before MS detection; ammonium formate was used to elute the analytes, which in this optimization included only the polar GLY, AMPA, and GLUFO compounds retained on the trapping column by anion exchange mechanism.

Next, a washing step was considered for analyte clean-up (section 2.3). Recoveries were >95 % using a single wash with water and decreased to 76–82 % when two washing steps were used. Similarly, in acetonitrile, a single washing step did not affect the recovery (87–95 %), but two steps decreased it significantly (79–85 %, Fig. 3c). The recoveries for GLY, GLUFO, and AMPA were almost quantitative also without any washing, with a performance compatible with the single washing with water. Nonetheless, a single washing step with water was chosen as the best condition to prevent unwanted components from the much more complex beer matrix from reaching the column and MS instrumentation.

Finally, the effect of the draw speed and dispense speed for loading and washing was evaluated, along with the effect of the mobile phase flow rate for elution (section 2.3). The recoveries were >95 % for mobile phase flow rates <0.3 mL min^{-1}. Higher flow rates were not compatible with the trapping column due to excess back pressure that would cause the disconnection of the trapping column. The flow rate of 0.3 mL min^{-1} was chosen to reduce the run time (Fig. 3d). The dispense speed had no significant effect on the recovery (>98 % for all conditions). It was set to 0.15 mL min^{-1}. The draw speed was the most important factor, as it affected the contact between the sample and the stationary phase during the loading. The recovery was good for speeds <0.3 mL min^{-1}, whereas all other conditions provided unacceptable recoveries <60 %. The best condition was found at 0.15 mL min^{-1}.

3.2.2. Extension of the trapping conditions to non-polar pesticides and optimization of the chromatographic conditions for global analysis of pesticides

The RP mechanism was expected to be the main interaction type for the non-polar pesticides, according to what was previously demonstrated for β-estradiol [29]. As such, the loading from the 0.1 % acetic acid solution was expected to be compatible with the retention of the

non-polar pesticides. HILIC was initially employed during the optimization of the trapping conditions, but it was found unsuitable for the other pesticides included in the study. HILIC employs an acetonitrile-rich mobile phase at the start of the gradient, which would disrupt the hydrophobic interaction between most pesticides and the trapping column. Consequently, non-polar pesticides eluted at the beginning of the gradient, close to the dead volume with no good separation between them. The result agreed with the published literature, as it is one drawback limiting the development of global methods for simultaneous analysis of polar and non-polar pesticides [17]. The end of the gradient, which had a mobile phase rich in water with the basic modifier, allowed the elution of the compounds retained by the ion exchange mechanism (Fig. 4).

Given the result, RP with a C30 stationary phase was tested, to maximize the interaction with small polar molecules and avoid their elution with the dead volume of the system. The chromatographic conditions of the gradient and mobile phase composition on the C30 column were evaluated and optimized with the rationale that the basic

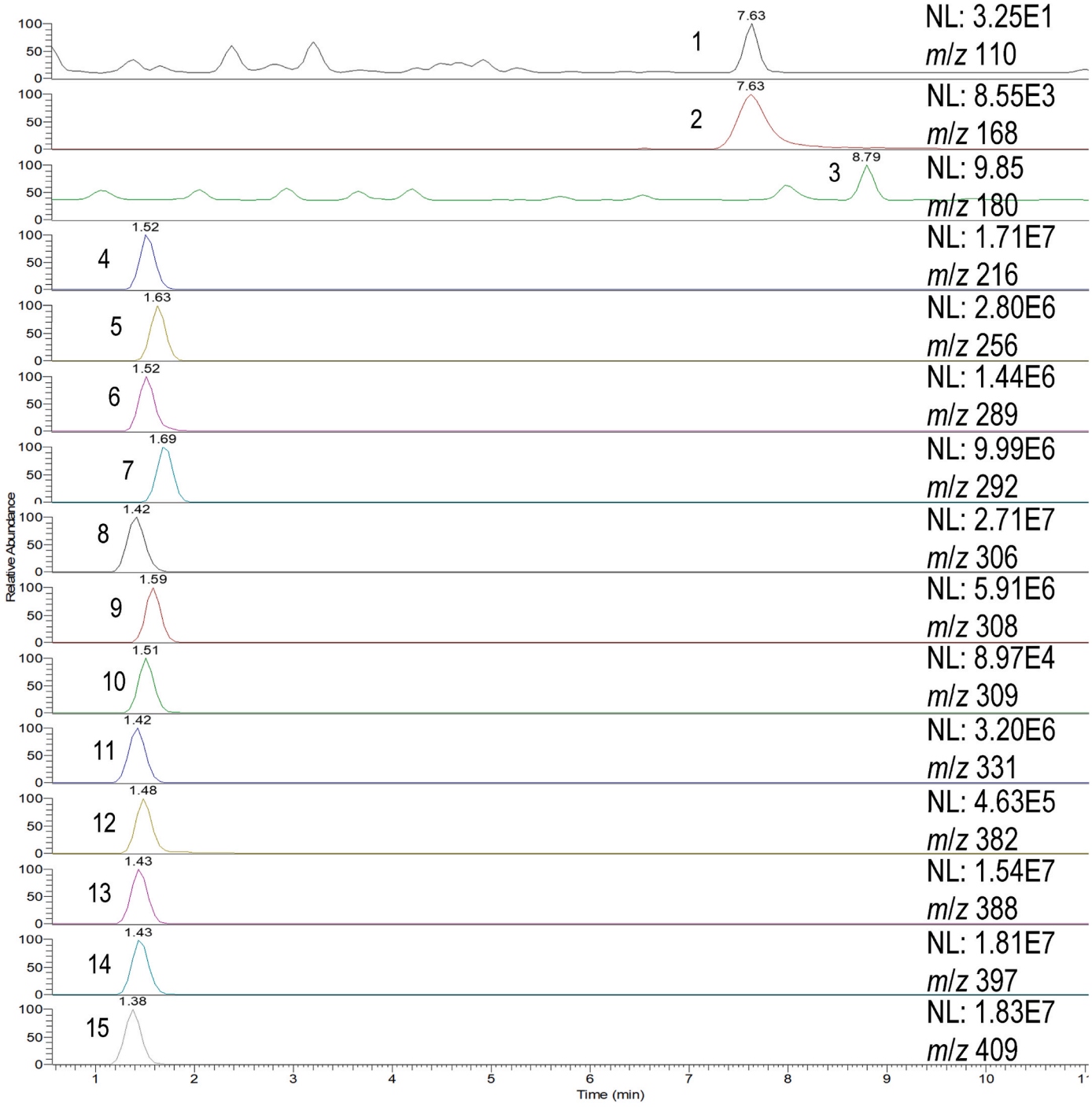

Fig. 4. Chromatograms showing the sum of the extracted transitions ($\Delta m/z \pm 0.5$, the specific transitions for each m/z are reported in Table S2) of the m/z precursors with the related intensities for the 15 pesticides using HILIC. Conditions: Mobile phase A was 0.2 % (*v/v*) formic acid in acetonitrile; mobile phase B was 100 mmol L^{-1} ammonium formate and 0.2 % (*v/v*) formic acid in water. The following gradient was used for analysis: t0, B = 3 %; t3, B = 3 %; t5, B = 80 %; t8, B = 80 %; t10, B = 3 %. Equilibration for 2 min at 3 % phase B. Peak numbers: 1-AMPA, 2-GLY, 3-GLUFO, 4-ATRA, 5-IMIDA, 6-MYCLO, 7-THIA, 8-PIRI, 9-TEBU, 10-DIFLU, 11-MELA, 12-FLUXA, 13-PYRA, 14-FLUOPY, 15-TRIFLO.

modifier would trigger the elution of the polar analytes retained by ion exchange, and that could be achieved either using it in the mobile phase B, in mobile phase A, or in both of them.

The use of 0.1 % (*v/v*) NH_4OH in acetonitrile as mobile phase B allowed the retention of the polar GLY, AMPA, and GLUFO, and their elution at the end of the chromatographic gradient. However, the other pesticides were strongly suppressed, and sensitivity was lowered (intensities for these analytes were 30–58 % lower than the intensities measured using a mobile phase without the basic modifier). The use of the basic modifier in mobile phase A allowed the elution of GLY, AMPA, and GLUFO at the beginning of the chromatographic run with a retention time different from the dead volume of the system (Fig. 5). All other compounds were efficiently separated after the polar ones, with no suppression. This condition was chosen for further method validation.

The retention times and LOD values of the 15 analytes were also calculated for both HILIC and C30 separations (Table S4). In both cases, no compound eluted with the dead volume, although the HILIC separation had 12 pesticides very close to it. The C30 column performed better in retaining the target analytes but also provided a better sensitivity over the HILIC column, with LOD values in the range of 0.2–1.0 μg L^{-1} vs 1–9 μg L^{-1}. The C30 was finally selected for method validation.

3.3. Method validation

Beer is a water-rich matrix with a low protein content; therefore, a dilution was considered only for those samples with an ethanol content >5 % to ensure the binding of the non-polar pesticides. The binding of polar pesticides was ensured by checking the pH and adjusting it if needed, according to the optimization described in the previous sections. CO_2 was removed by degassing to avoid forming bubbles in the UHPLC system. Finally, possible particulate matter in the samples was removed by filtration to avoid clogging. Beer samples were then directly injected into the UHPLC-MS system using the maximum volume capacity of the installed loop, which was 100 μL. The volume was sufficient for method development and validation and was not subjected to further investigation.

The method for analysis of polar and non-polar pesticides was validated for beer based on RE, inter-day and intra-day precision, linear regression parameters, and experimentally confirmed LOD and LOQ values. All parameters and validation results are summarized in Table S5.

The linear dynamic range was investigated between 3 and 100 μg L^{-1} for the polar pesticides AMPA, GLY, and GLUFO, as they are particularly abundant in a polar matrix like beer, whereas for all other pesticides, the range was 0.3–10 μg L^{-1}. The linearity was found to be very good for both types of analytes, with coefficients of determination for the linear regression (R^2) between 0.992 and 1.0 for the polar compounds and 0.99–1.0 for all other analytes (Table S5).

The RE values were calculated at three concentration levels. At the lowest concentration level, recoveries were 84–112 %, except for MYCLO, which was 71 %. At the intermediate concentration level, the recoveries were 86–103 %. Recoveries at the highest concentration level were 85–110 %. Recoveries were reproducible within the same day and between consecutive days. In the first case, recoveries were 82–111 %, with an RSD <15 %. In the second case, recoveries were 85–102 %, with RSD <15 %. The most remarkable part of these results was that they were satisfying for both classes of polar and non-polar pesticides, which could all be efficiently retained and recovered.

The LOD was experimentally found at 1 μg L^{-1} for the polar compounds GLY, AMPA, and GLUFO. These values matched the ones found in spiked neat solvents (Table S4), which is very valuable and indicated an efficient clean-up and reduction of noise due to the online extraction. The results were confirmed by the study of the matrix effect at three concentration levels. The matrix effect was a mild signal enhancement (105–120 %) for most compounds, except TEBU, which was slightly suppressed (97 %), and FLUXA and MELA, which were enhanced (150 and 130 %, respectively). At the intermediate concentration level, the matrix effect was between 94 and 115 %, except for GLUFO (134 %) and FLUXA (133 %), which were enhanced. At the highest concentration level, the matrix effect was negligible (97–115 %). The LOQ was set at the lower limit of the linear dynamic range.

Before proceeding to the analysis of the beer samples, the repeatability of analyte binding and elution from the monolithic column was investigated for GLY over 120 cycles in spiked beer samples at a concentration of 4 μg L^{-1} (Fig. S4). The results indicated that the same column could be used several times with only 8 % RSD of the measured area of the target analyte, which is sufficient robustness for the analysis of commercial samples without loss of capture performance.

3.4. Comparison of the validated method to other systems

Compared to the few methods previously described for analysis of pesticides over a wide range of polarity and employing multidimensional chromatography [17], the method described in this study includes GLY, AMPA, and GLUFO and is fully validated. Compared to another paper [18], the analytes included in our study are fewer, but the method works with standard solutions at concentrations significant for the application (Table 2), and there is the application to beer as a representative example. Finally, a multidimensional method applied to leek [19] had recoveries in the range of 70–120 %, with RSD <15 % and LOQ at 0.2–210 μg kg^{-1}. Leek and beer are different types of samples, and a direct comparison was not possible; however, the method described in our study can be implemented in ordinary LC systems and exploits a single chromatographic run instead of dedicated instrumentation. Our method was competitive also in terms of recovery and sensitivity and included both GLY and AMPA, which were not included in the previous study [19].

As compared to other methods for the analysis of beer, only two works described a direct method without dedicated sample preparation and included the important analyte GLY (Table 2) [27,34]. In all other cases, extensive sample preparation was required. All works but one were focused on GLY and related compounds or polar compounds, and dedicated chromatographic conditions were used. When other analytes were addressed, dedicated methods, such as QuPPe and chromatography on PGC, were used [26]. From this point of view, the method developed in our study was innovative, with a wide scope of analyte applicability and automated sample preparation. The LOD and LOQ values were generally lower than the ones reported in previous works, which means an improved sensitivity was achieved under these conditions and clean-up was still sufficient for proper quantitative analysis. Recoveries matched previous reports, which made the described method competitive with more elaborate sample preparation strategies.

For a general comparison to multi-residue methods applied to beer, a recent paper investigating 184 compounds was considered [35]. The sample preparation was similar, as a dilute and shoot method was used, and the number of target analytes was significantly larger than ours. The performance is nonetheless comparable in terms of recovery, with some improvement in the sensitivity with the method described in this study, possibly due to the lack of dilution and preconcentration achieved on the trapping column.

The Blue Applicability Grade Index (BAGI) [36] was used to evaluate the practicality of the developed method. It scored 62.5 (Fig. S5), which is an intermediate value in the BAGI scale above the threshold for a "practical" method (i.e., 60). The strength involved that the method was quantitative and confirmatory, that 6–15 compounds were investigated in 2–4 samples per h by a semi-automated analytical system (HPLC with autosampler) starting from low amounts of sample (<10 mL). The drawbacks were associated with the use of instrumentation that is not commonly available in most laboratories (LC-MS/MS), no simultaneous sample preparation, and the need for a miniaturized extraction for sample preconcentration employing a material that was synthesized for the purpose. The other methods compared in this work (Table 2) scored

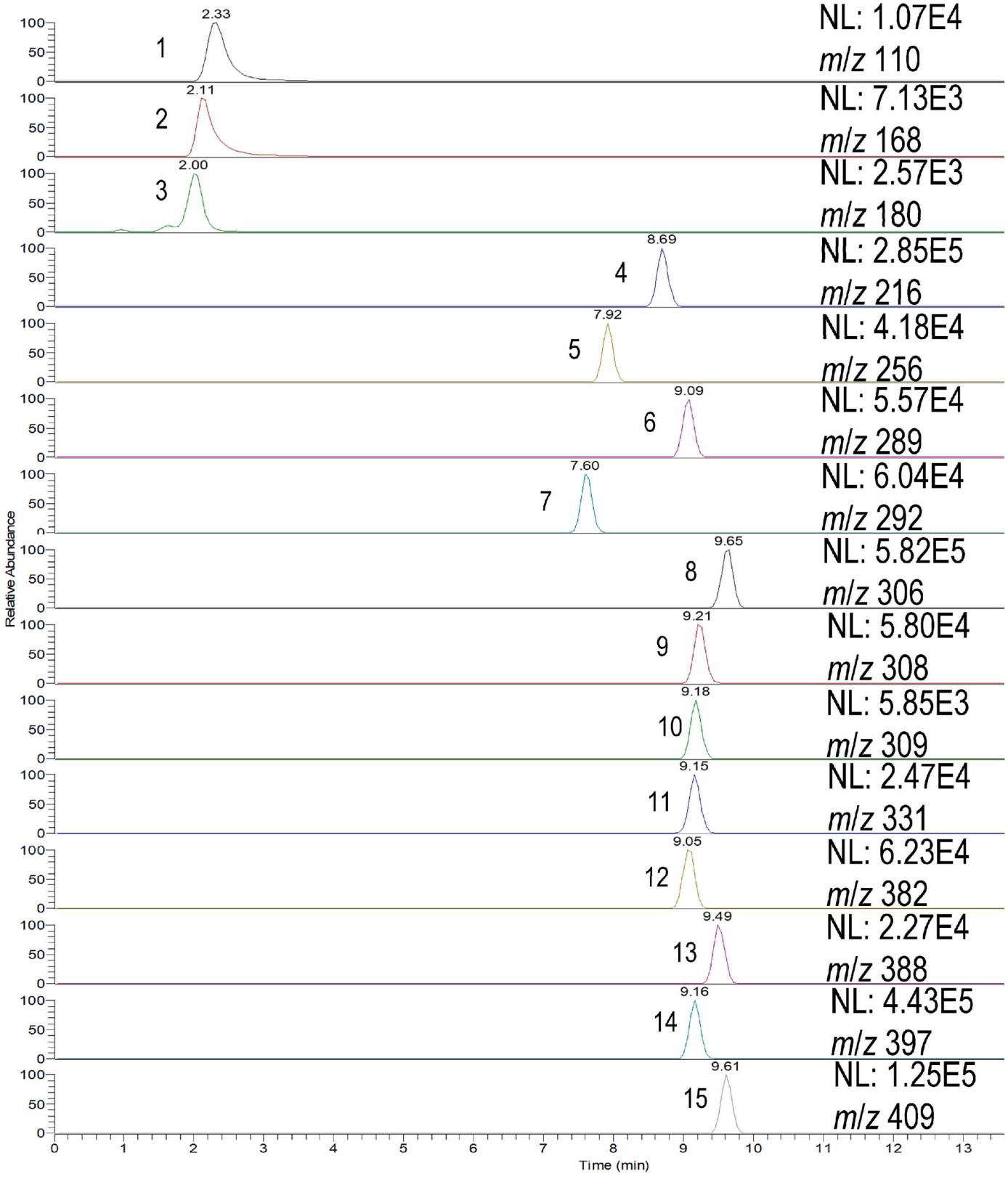

Fig. 5. Chromatograms showing the sum of the extracted transitions ($\Delta m/z \pm 0.5$, the specific transitions for each m/z are reported in Table S2) of the m/z precursors with the related intensities for the 15 pesticides using RP C30 chromatography. Conditions: Mobile phase A was 0.025 % (*v/v*) NH_4OH; mobile phase B was acetonitrile. The optimized gradient was used for analyte separation. Peak numbers: 1-AMPA, 2-GLY, 3-GLUFO, 4-ATRA, 5-IMIDA, 6-MYCLO, 7-THIA, 8-PIRI, 9-TEBU, 10-DIFLU, 11-MELA, 12-FLUXA, 13-PYRA, 14-FLUOPY, 15-TRIFLO.

Table 2
Comparison with previously developed methods for extraction and clean-up of GLY, AMPA, polar pesticides, and multi-residue analysis of pesticides from beer. The main features of the study are reported, including type of extraction, investigated analytes, if the online analysis is performed, type of chromatography-detector used, method results (LOD, LOQ, recovery, RE %), BAGI, and AGREE values.

Extraction	Analytes	online	Platform	LOD	LOQ	RE %	BAGI	AGREE	ref
SAX-SPE	GLY, GLUFO, AMPA (+2 metabolites)	no	IC-MS/MS	–	10 μg kg^{-1}	74–103 %	55	0.56	[37]
RP-SPE + SAX-SPE	GLY	no	HILIC-MS/MS	0.2 μg kg^{-1}	0.50 μg kg^{-1}	87–123 %	52.5	0.50	[38]
–	GLY, AMPA, GLUFO (+ other 11 polar pesticides)	–	HILIC-MS/MS	–	10–500 μg kg^{-1} (10 μg kg^{-1})	76–119 %	72.5	0.68	[34]
QuPPe	ionic and acidic pesticides	no	SAX-MS/MS	–	10–20 μg kg^{-1}	80–110 %	72.5	0.58	[9]
modified QuEChERS/ SRM	GLY, 16 compounds	no	GC-MS/MS; RP-MS/MS; PGC-MS/MS	–	2–50 μg kg^{-1}	70–120 %	65	0.55	[26]
–	GLY, AMPA	–	CE-MS/MS	5–30 μg L^{-1}	10–50 μg L^{-1}	80–111 %	70	0.69	[27]
Dilute and shoot	184 pesticides and mycotoxins	–	RP-MS/MS	0.001–0.002 mg kg^{-1}	0.01 mg kg^{-1}	70–103.6 %	72.5	0.67	[35]
Multicomponent monolith	3 polar, 12 non-polar pesticides	yes	RP-MS/MS	0.02–1 μg L^{-1}	0.3–3 μg L^{-1}	71–112 %	62.5	0.72	This work

between 52.5 and 72.5. All had in common the type of analysis (quantitative and confirmatory), the degree of automation (semi-automated with common devices), and the low amount of sample (<10 mL). Methods with lower scores than 62.5 were classical approaches using complex off-line sample preparations for <5 target analytes [37,38]. Methods that scored better targeted >15 analytes [9,26,35], involved simple, low-cost sample preparation methods [9,27,34,35], using commercially available reagents and with no need for preconcentration [9,26,34,35].

The compliance with the Green analytical chemistry was evaluated using the AGREE tool [39]. The method described in this study scored 0.72 (Fig. S6). The advantages involved the online analysis, the low amount of sample, the simple procedure, the semi-automated and miniaturized sample preparation, the lack of derivatization reagents, the low amount of waste, the number of target analytes quantified in a single run, and the operator's safety with reagents and solvents. The drawbacks involved the distance from the sampling site of the analytical device, and the use of high-energy consuming LC-MS instrumentation and related wastes. The use of hyphenated MS and no analysis performed in situ were drawbacks of all methods considered in the comparison, which scored lower than the method described in this study (0.50–0.69). Classical traditional methods employing SPE and offline analyses had low scores [37,38]. Intermediate values were obtained for traditional approaches using simple extractions [9,26], followed by procedures employing simple dilution/centrifugation with direct analysis [27,34,35]. The use of online sample preparation contributed to improving the scores, along with lower sample amounts and wastes. From this point of view, the miniaturization of the procedures described in this work was the main contribution to the better score obtained using AGREE.

3.5. Application to commercial beer samples

The validated method was finally applied to the analysis of 7 types of commercial beer samples with different productions (Table 3). The target analytes were mostly below the LOD or detected below the LOQ values. As indicated in previous literature, polar pesticides are more detected in beer than non-polar ones. GLY was detected in all but one sample and was measured at 3.1–5.1 μg L^{-1} concentration. AMPA and GLUFO were also detected in 4 samples, although < LOQ. As for the other analytes, DIFLU was quantified in brands number 2, 5 (unfiltered), and 6; TEBU was quantified in brands number 5 and 6; the most contaminated beer with pesticide residues was brand 5 in the unfiltered version, in which 12 pesticides were at least detected out of 15.

4. Conclusions

The work described the simultaneous analysis of polar and non-polar pesticides over a wide range of log K_{ow} from −4.4 to 4.5, using a simple methodology exploiting a trapping column that can retain small molecules by both RP and anion exchange mechanisms, with the latter allowing elution by modulation of the basic modifier in the mobile phase. By this approach, GLY, AMPA, and GLUFO were analyzed with 12 pesticides that are usually addressed in multi-residue analysis. The method was validated for analysis of beer and included online analyte extraction and clean-up, using the same trapping column for automated

Table 3
Quantitative analysis using the validated method for online extraction of 15 polar and non-polar pesticides from 7 commercial beer samples. For each beer type, the amount of each analyte is provided as a concentration in mg L^{-1}. When analytes were detected but not quantifiable, <LOQ is provided. – indicates the analyte was not detected (<LOD).

Compound	Brand 1	Brand 2	Brand 3	Brand 4	Brand 5	Brand 6	Brand 5 (unfiltered)
AMPA	–	–	<LOQ	–	<LOQ	<LOQ	<LOQ
GLY	<LOQ	0.0032 ± 0.0001	<LOQ	<LOQ	0.0031 ± 0.0002	–	0.0051 ± 0.0001
GLUFO	–	–	<LOQ	–	<LOQ	<LOQ	<LOQ
TRIFLO	–	<LOQ	–	–	<LOQ	–	0.0027 ± 0.0002
FLUOPY	–	–	–	–	<LOQ	–	<LOQ
PYRA	–	<LOQ	<LOQ	<LOQ	<LOQ	–	0.0012 ± 0.0009
FLUXA	–	–	–	–	–	–	–
MELA	–	–	–	–	<LOQ	–	0.0011 ± 0.0021
TEBU	–	<LOQ	–	–	0.0041 ± 0.0009	0.001 ± 0.0002	0.0081 ± 0.0001
PIRI	–	–	–	–	–	–	–
THIA	<LOQ	–	–	<LOQ	<LOQ	<LOQ	<LOQ
MYCLO	–	–	–	–	–	–	–
IMIDA	–	–	–	<LOQ	–	<LOQ	0.0013 ± 0.0008
ATRA	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.0011 ± 0.0001
DIFLU	–	0.0005 ± 0.0001	–	–	–	0.0042 ± 0.0001	0.0008 ± 0.0001

sample preparation. The method proved competitive with few previous reports addressing the global analysis of polar and non-polar pesticides by multidimensional chromatography while providing a simpler and general approach, compatible with most HPLC instrumentation, and fast single chromatographic separation. The method was also competitive with previous applications in beer. The work demonstrated the scope of the trapping device and the potential of analyzing small molecules over a wide range of polarity under simple conditions.

CRediT authorship contribution statement

Susy Piovesana: Writing – original draft, Supervision. **Sara Elsa Aita:** Validation, Methodology, Investigation. **Chiara Cavaliere:** Writing – review & editing, Supervision. **Andrea Cerrato:** Visualization, Data curation. **Aldo Laganà:** Resources, Funding acquisition, Conceptualization. **Carmela Maria Montone:** Visualization, Validation, Methodology, Investigation, Formal analysis, Conceptualization. **Enrico Taglioni:** Validation, Methodology, Investigation. **Anna Laura Capriotti:** Supervision, Resources, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aca.2024.343231.

References

[1] M. Ferrante, P. Rapisarda, A. Grasso, C. Favara, G. Oliveri Conti, Glyphosate and environmental toxicity with "One Health" approach, a review, Environ. Res. 235 (2023) 116678, https://doi.org/10.1016/j.envres.2023.116678.

[2] IARC monograph on glyphosate. https://www.iarc.who.int/featured-news/media-centre-iarc-news-glyphosate/. (Accessed 23 May 2024).

[3] F. Álvarez, M. Arena, D. Auteri, M. Binaglia, A.F. Castoldi, A. Chiusolo, F. Crivellente, M. Egsmose, G. Fait, F. Ferilli, V. Gouliarmou, L.H. Nogareda, A. Ippolito, F. Istace, S. Jarrah, D. Kardassi, A. Kienzler, A. Lanzoni, R. Lava, A. Linguadoca, C. Lythgo, I. Mangas, L. Padovani, M. Panzarea, J.M. Parra Morte, S. Rizzuto, A. Romac, A. Rortais, R. Serafimova, R. Sharp, C. Szentes, A. Terron, A. Theobald, M. Tiramani, G. Vianello, L. Villamar-Bouza, Peer review of the pesticide risk assessment of the active substance glyphosate, EFSA J. 21 (2023) 8164, https://doi.org/10.2903/j.efsa.2023.8164.

[4] Draft human health and ecological risk assessments for glyphosate. https://www.epa.gov/ingredients-used-pesticide-products/draft-human-health-and-ecological-risk-assessments-glyphosate. (Accessed 23 May 2024).

[5] M. Rigobello-Masini, E.A.O. Pereira, G. Abate, J.C. Masini, Solid-phase extraction of glyphosate in the analyses of environmental, plant, and food samples, Chromatographia 82 (2019) 1121–1138, https://doi.org/10.1007/s10337-019-03748-3.

[6] QuPPe document. https://www.quppe.eu/quppe_doc.asp. (Accessed 23 May 2024).

[7] S. Saito-Shida, S. Nemoto, H. Akiyama, Multiresidue method for determining multiclass acidic pesticides in agricultural foods by liquid chromatography-tandem mass spectrometry, Anal. Methods 13 (2021) 894–902, https://doi.org/10.1039/D0AY02101F.

[8] R. Raina-Fulton, A review of methods for the analysis of orphan and difficult pesticides: glyphosate, glufosinate, quaternary ammonium and phenoxy acid herbicides, and dithiocarbamate and phthalimide fungicides, J. AOAC Int. 97 (2014) 965–977, https://doi.org/10.5740/jaoacint.SGERaina-Fulton.

[9] M. Jansons, I. Pugajeva, V. Bartkevics, Evaluation of selected buffers for simultaneous determination of ionic and acidic pesticides including glyphosate using anion exchange chromatography with mass spectrometric detection, J. Separ. Sci. 42 (2019) 3077–3085, https://doi.org/10.1002/jssc.201900308.

[10] X. Wei, Y. Pan, Z. Zhang, J. Cui, R. Yin, H. Li, J. Qin, A.J. Li, R. Qiu, Biomonitoring of glyphosate and aminomethylphosphonic acid: current insights and future perspectives, J. Hazard Mater. 463 (2024) 132814, https://doi.org/10.1016/j.jhazmat.2023.132814.

[11] E. Verdini, I. Pecorelli, The current status of analytical methods applied to the determination of polar pesticides in food of animal origin: a brief review, Foods 11 (2022) 1527, https://doi.org/10.3390/foods11101527.

[12] P. Rocío-Bautista, D. Moreno-González, A.B. Martínez-Piernas, J.F. García-Reyes, A. Molina-Díaz, Novel liquid chromatography/mass spectrometry-based approaches for the determination of glyphosate and related compounds: a review, Trends Environ. Anal. Chem. 36 (2022) e00186, https://doi.org/10.1016/j.teac.2022.e00186.

[13] M. Gasparini, B. Angelone, E. Ferretti, Glyphosate and other highly polar pesticides in fruit, vegetables and honey using ion chromatography coupled with high resolution mass spectrometry: method validation and its applicability in an official laboratory, J. Mass Spectrom. 55 (2020) e4624, https://doi.org/10.1002/jms.4624.

[14] S. Piovesana, A. Laganà, A.L. Capriotti, Current advances in carbonaceous materials for analytical applications in liquid phase, TrAC, Trends Anal. Chem. 168 (2023) 117297, https://doi.org/10.1016/j.trac.2023.117297.

[15] S. Herrera López, J. Scholten, B. Kiedrowska, A. de Kok, Method validation and application of a selective multiresidue analysis of highly polar pesticides in food matrices using hydrophilic interaction liquid chromatography and mass spectrometry, J. Chromatogr., A 1594 (2019) 93–104, https://doi.org/10.1016/j.chroma.2019.02.024.

[16] G.P. Danezis, C.J. Anagnostopoulos, K. Liapis, M.A. Koupparis, Multi-residue analysis of pesticides, plant hormones, veterinary drugs and mycotoxins using HILIC chromatography – MS/MS in various food matrices, Anal. Chim. Acta 942 (2016) 121–138, https://doi.org/10.1016/j.aca.2016.09.011.

[17] I. Caño-Carrillo, A.B. Martínez-Piernas, B. Gilbert-López, A. Molina-Díaz, J. F. García-Reyes, Simultaneous analysis of highly polar and multi-residue-type pesticides by heart-cutting 2D-LC-MS, Talanta 266 (2024) 124918, https://doi.org/10.1016/j.talanta.2023.124918.

[18] U. Jost, F. Habedank, Two-dimensional hydrophilic interaction and reversed phase liquid chromatography easily extracted pesticides and polar pesticides multi-residue method—A concept, J. Chromatogr., A 1621 (2020) 461040, https://doi.org/10.1016/j.chroma.2020.461040.

[19] J. Robles-Molina, B. Gilbert-López, J.F. García-Reyes, A. Molina-Díaz, Simultaneous liquid chromatography/mass spectrometry determination of both polar and "multiresidue" pesticides in food using parallel hydrophilic interaction/reversed-phase liquid chromatography and a hybrid sample preparation approach, J. Chromatogr., A 1517 (2017) 108–116, https://doi.org/10.1016/j.chroma.2017.08.041.

[20] M. Majd, M. Gholami, A. Fathi, R. Sedghi, S. Nojavan, Thin-film solid-phase microextraction of pesticides from cereal samples using electrospun polyvinyl alcohol/modified chitosan/porous organic framework nanofibers, Food Chem. 444 (2024) 138647, https://doi.org/10.1016/j.foodchem.2024.138647.

[21] Current MRL values for the product, Selected product: Barley, https://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/start/screen/products/details/232. (Accessed 25 July 2024).

[22] M. Gruba, E. Jóźwik, M. Chmiel, K. Tyśkiewicz, M. Konkol, A. Watros, K. Skalicka-Woźniak, G. Woźniakowski, Multi-residue method for pesticides determination in dried hops by liquid chromatography tandem mass spectrometry, Molecules 28 (2023) 4989, https://doi.org/10.3390/molecules28134989.

[23] Current MRL values for the product, Selected product: HOPS, https://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/start/screen/products/details/265. (Accessed 25 July 2024).

[24] G. Pérez-Lucas, G. Navarro, S. Navarro, Understanding how chemical pollutants arise and evolve in the brewing supply chain: a scoping review, Foods 13 (2024) 1709, https://doi.org/10.3390/foods13111709.

[25] G. Pérez-Lucas, G. Navarro, S. Navarro, Comprehensive review on monitoring, behavior, and impact of pesticide residues during beer-making, J. Agric. Food Chem. 71 (2023) 1820–1836, https://doi.org/10.1021/acs.jafc.2c07830.

[26] E. Hakme, I. Kallehauge Nielsen, J. Fermina Madsen, L.M. Storkehave, M. Skjold Elmelund Pedersen, B.L. Schulz, M.E. Poulsen, T.J. Hobley, L. Duedahl-Olesen, Fate of pesticide residues in beer and its by-products, Food Addit. Contam. 41 (2024) 45–59, https://doi.org/10.1080/19440049.2023.2282557.

[27] B. Wimmer, M. Pattky, L.G. Zada, M. Meixner, S.B. Haderlein, H.-P. Zimmermann, C. Huhn, Capillary electrophoresis-mass spectrometry for the direct analysis of glyphosate: method development and application to beer beverages and environmental studies, Anal. Bioanal. Chem. 412 (2020) 4967–4983, https://doi.org/10.1007/s00216-020-02751-0.

[28] N.P. Sen, P.A. Baddoo, Determination of glyphosate as N -nitroso derivative by high performance liquid chromatography with chemiluminescence detection, Int. J. Environ. Anal. Chem. 63 (1996) 107–117, https://doi.org/10.1080/03067319608026256.

[29] A. Cerrato, S.E. Aita, C. Cavaliere, A. Laganà, C.M. Montone, S. Piovesana, E. Taglioni, A.L. Capriotti, Preparation of monolith for online extraction and LC–MS analysis of β-estradiol in serum via a simple multicomponent reaction, Anal. Chem. 96 (2024) 4639–4646, https://doi.org/10.1021/acs.analchem.3c05706.

[30] C. Cavaliere, A. Cerrato, A. Laganà, C.M. Montone, S. Piovesana, E. Taglioni, A. L. Capriotti, Dispersive solid phase extraction using a hydrophilic molecularly imprinted polymer for the selective extraction of patulin in apple juice samples, Microchim. Acta 190 (2023) 485, https://doi.org/10.1007/s00604-023-06056-8.

[31] Y. Yang, Y. Huang, Z. Wu, R. Shi, Z. Chen, G. Ruan, Porous capillary monolithic column coupled with ultrahigh performance liquid chromatography-tandem mass spectrometry for fast and effective separation and determination of estrogens, Anal. Chim. Acta 1227 (2022) 340270, https://doi.org/10.1016/j.aca.2022.340270.

[32] M10 bioanalytical method validation and study sample analysis. https://www.fda.gov/regulatory-information/search-fda-guidance-documents/m10-bioanalytical-method-validation-and-study-sample-analysis. (Accessed 23 May 2024).
[33] A. Kaufmann, Validation of multiresidue methods for veterinary drug residues; related problems and possible solutions, Anal. Chim. Acta 637 (2009) 144–155, https://doi.org/10.1016/j.aca.2008.09.033.
[34] S.H. Lopez, J. Dias, H. Mol, A. de Kok, Selective multiresidue determination of highly polar anionic pesticides in plant-based milk, wine and beer using hydrophilic interaction liquid chromatography combined with tandem mass spectrometry, J. Chromatogr., A 1625 (2020) 461226, https://doi.org/10.1016/j.chroma.2020.461226.
[35] N.A. Pires, M.L. Gonçalves De Oliveira, J.A. Gonçalves, A.F. Faria, Multiclass analytical method for pesticide and mycotoxin analysis in malt, brewers' spent grain, and beer: development, validation, and application, J. Agric. Food Chem. 69 (2021) 4533–4541, https://doi.org/10.1021/acs.jafc.0c07004.
[36] N. Manousi, W. Wojnowski, J. Płotka-Wasylka, V. Samanidou, Blue applicability grade index (BAGI) and software: a new tool for the evaluation of method practicality, Green Chem. 25 (2023) 7598–7604, https://doi.org/10.1039/D3GC02347H.
[37] Y. Nagatomi, T. Yoshioka, M. Yanagisawa, A. Uyama, N. Mochizuki, Simultaneous LC-MS/MS analysis of glyphosate, glufosinate, and their metabolic products in beer, barley tea, and their ingredients, Biosci. Biotechnol. Biochem. 77 (2013) 2218–2221, https://doi.org/10.1271/bbb.130433.
[38] M. Jansons, I. Pugajeva, V. Bartkevičs, Occurrence of glyphosate in beer from the Latvian market, Food Addit. Contam. 35 (2018) 1767–1775, https://doi.org/10.1080/19440049.2018.1469051.
[39] F. Pena-Pereira, W. Wojnowski, M. Tobiszewski, AGREE—analytical GREEnness metric approach and software, Anal. Chem. 92 (2020) 10076–10082, https://doi.org/10.1021/acs.analchem.0c01887.