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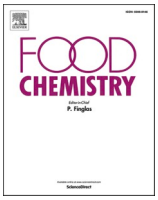

Optimization and validation of high throughput methods for the determination of 132 organic contaminants in green and roasted coffee using GC-QqQ-MS/MS and LC-QqQ-MS/MS

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ABSTRACT

Recently some major safety concerns have been raised on organic contaminants in widely consumed plants such as coffee. Hence, this study aimed to develop specifically optimized methods for determining organic contaminants, such as pesticides and polychlorinated biphenyls (PCBs), in coffee using GC–MS/MS and LC-MS/MS. QuEChERS method was used as a base extraction method, and 27 experiments were studied using design of experiments with categorical variables (extraction buffers, cleanup sorbents, and coffee roasting degree) to find the optimum method for each matrix type. The optimum method for green coffee was acetate buffer and chitosan for clean-up, while no-buffer extraction and the PSA + C18 method were ideal for light and dark-roasted coffee. The optimized methods were validated in accordance with SANTE/11312/2021. Furthermore, ten real samples (4 green, and 6 roasted) from the markets were analysed; ortho-phenylphenol was found in all the roasted coffee samples, and carbendazim was found in one green coffee sample.

1. Introduction

Coffee (Rubiaceae) is one of the most consumed beverages worldwide due to its distinctive flavor and aroma (Dias et al., 2013). Although the Rubiaceae family contains >70 distinct species, Arabica (*Coffea arabica*) and Robusta (*Coffea canephora)* are the two members with the largest economic impact (Yang et al., 2011). In addition, coffee is the second most traded commodity in the world after oil, making it a vital source of income for millions of people worldwide (Reichert et al., 2018). Furthermore, coffee is one of the richest sources of chlorogenic acid and contains a number of beneficial antioxidants (Trevisan et al., 2017; Yang et al., 2011). It is also known to have several health benefits, such as improving cognitive function and reducing the risk of certain diseases like Parkinson's and type 2 diabetes (Grosso et al., 2017). However, the presence of some organic contaminants, such as pesticides, in coffee may lead to adverse health effects for consumers (Merhi et al., 2022).

Pesticides mainly consist of organic chemicals with various physical and chemical properties (Štěpán et al., 2005). They are usually used to reduce pest damage in agriculture by controlling weeds, insect infestations, and diseases (Li et al., 2014; Sayed et al., 2022a; Thompson et al., 2017) Hence, they are frequently used at various stages of crop production and/or post-harvest storage, and their use ensures higher product yield and better storage (Malaj et al., 2012). Also, other chemicals, such as chlormequat and mepiquat, are used as plant growth regulators in the seed germination of coffee (Francesquett et al., 2019). Another sources of contamination may be due to airborne pollutants, soil and water contamination, and human industrial activities, where persistent organic pollutants such as PCBs accumulate in food and drinks such as coffee (Fernandes et al., 2023).

Unfortunately, organic contaminants are associated with a large number of diseases, including diabetes, endocrine disruption, respiratory, neurological, and cardiovascular diseases (Taiwo, 2019). Hence, international and regional entities have set tolerance limits for their presence, commonly known as the Maximum Residue Limits (MRLs), for ensuring the customers' safety (Maximum Residue Limits | CODEX-ALIMENTARIUS, 2023), (European Commission, 2021). In Egypt, the National Food Safety Agency has set the MRLs of pesticides per decree number 6 for 2021 (NFSA, 2021).

Pollutant monitoring is critical for delivering quantitative data on

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their occurrence, identifying their sources and environmental fate, adhering to legislation, and addressing human health concerns (Bakr et al., 2023; Eissa et al., 2021). For example, a study surveyed the coffee traded in Indonesia and found that 40% of the analysed samples were contaminated with pesticides (Harmoko et al., 2015). Mepiquat was also reported in roasted coffee previously (Nardin et al., 2017). Consequently, there should be stricter regulations and monitoring programs of residues in coffee, because of the increased use of pesticides in agriculture.

Previously, several chromatographic methods have been developed for identifying and quantifying pesticides in food and the environment using gas chromatography (GC) or high-performance liquid chromatography (HPLC) with the following extraction techniques: liquid-liquid extraction, solid-phase extraction (SPE), single-drop microextraction, and solid-phase microextraction (Merhi et al., 2022). Among these methods was a method developed in 2003 by (Anastassiades et al., 2003), commonly known as the Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) method. Over the years, further development on QuEChERS was carried out (Mekonen et al., 2014; Payá et al., 2007), adding buffers such as citrate buffer, ammonium and sodium acetate buffers in the extraction process to cover a wider range of analytes and various commodities.

This method proved effective for numerous types of vegetables, fruits, and herbs. However, when applied to coffee beans, the presence of co-extracted caffeine poses challenges, particularly when using GC–MS. Where, the high caffeine content injected poses integrity issues for the chromatographic system, resulting in compromised quantitative analysis around the elution region of caffeine and hindering qualitative analysis due to the broad and significant caffeine peak (Bresin et al., 2015).

Several studies have been done on the modification of the QuEChERS method for the determination of organic contaminants in green coffee. Pizzutti et al. (2012) developed the QuEChERS method for the determination of 51 pesticide residues by using the negative chemical ionization mode in gas chromatography–mass spectrometry (GC–MS) analysis. In another study, QuEChERS was developed coupled to a dispersive liquid-liquid micro-extraction (DLLME) by Bresin et al. (2015) for determining of 16 organochlorine pesticide residues by using gas chromatography-tandem mass spectrometry (GC–MS/MS), also used analyte protectants (APs) to reduce the matrix effect when injecting samples through a GC–MS/MS, where the utilization of APs proved effective in reducing the matrix effect found in coffee samples. Another study developed the QuEChERS method by Dias et al. (2013) for identifying 123 pesticide residues by using liquid chromatography-tandem mass spectrometry (LC-MS/MS). Also, Reichert et al. (2018) optimized the QuEChERS method to determine the 117 pesticide residues by using LC-MS/MS and the cleanup procedure using C18.

Till our best of knowledge, there hasn't been a study for the optimization of the QuEChERS method for the multiclass determination of organic contaminants in roasted coffee beans. In a study by Da Silva Souza & Navickiene, 2019, they determined seven pesticides in roasted coffee using the liquid-liquid microextraction (LLME) technique alongside an ultrasonic solvent, employing LC-MS/MS. Additionally, Zhang et al. (2017) utilized ionic liquid-based in situ dispersive liquid-liquid microextraction coupled to headspace gas chromatography (HS-GC) for the determination of 21 PCBs in green coffee.

Furthermore, it is known that GC is used to analyse contaminants that are nonpolar, volatile, and thermally stable. Conversely, liquid chromatography (LC) is used to analyse contaminants that are polar, nonvolatile, and thermally unstable (Alder et al., 2006). Therefore, it is important to use both GC and LC instruments for the determination of a wide range of compounds in most commodities, such as green and roasted coffee beans. Finally, specific methods for analysing pesticides in coffee must be developed to address the analytical challenges that come with their analysis.

Therefore, the aim of this study was to develop several optimized methods that are most suitable for the extraction of 132 organic contaminants (123 pesticides, two growth regulators, and seven PCBs) from green, light, and dark coffee, followed by GC–MS/MS (95 analytes) and LC-MS/MS (92 analytes) analysis. Also, this work aims to address the question of whether the change of the variety in the same commodity have an effect on the optimum extraction procedure, taking into consideration green and roasted coffee as an example. The extraction process conditions included the extraction buffer and the cleanup sorbents. The optimization was done using design of experiments with categorical variables as further discussed in the results and discussion.

2. Experimental

2.1. Chemicals and reagents

Acetonitrile (ultra-gradient grade) and formic acid (purity 99%) were purchased from Carlo Erba Reagents (Milano, Italy). Acetone (HPLC grad), n-hexane (HPLC grad), methanol (LC-MS grad), toluene (analytical grad), anhydrous magnesium sulphate (purity 98.0%), and sodium acetate (purity 99.0%) were obtained from Merck (Darmstadt, Germany).

Anhydrous sodium chloride (99.9%) was purchased from Alfa Chemical (Cairo, Egypt). Citrate buffer based QuEChERS extraction kits were supplied by Agilent (Santa Clara, USA). Ammonium acetate (analytical grad) was supplied by Honeywell (North Carolina, USA).

Water was deionized using a water purification system from Millipore Milli-Q (Darmstadt, Germany) in the laboratory. SPEX™ Sample Prep 2010 Geno/Grinder (vertical shaker) and acetic acid glacial 99% were supplied by Fisher Scientific Fisher Scientific (Pittsburgh, USA). Dispersive solid-phase extraction (d-SPE) sorbents, including graphitized carbon black (GCB), Envicarb, octadecyl (C18), and primary secondary amine (PSA) were purchased from Supleco (Pennsylvania, USA). Chitosan and all the analyte protectants (APs) (3-O ethylglycerol, 2,3-Butanediol, D-Fructose, l-Gulonic acid γ-lactone, D-Gluconic acid γ-lactone, D-Glucose, D-Ribonic acid γ-lactone, D-Ribose, D-Sorbitol, Menthol, Triglycerol, Vanillinin, and Polyethylene glycol) were purchased from Sigma-Aldrich (Oakville, Canada). The 123 pesticides, two growth regulators, Aldrin, and seven PCBs (purity ≥99%) were obtained from Dr. Ehrenstorfer (Augsburg, Germany).

2.2. Standard preparation

All standard stock solutions were prepared in toluene at 1000 μg/mL, except for carbendazim in acetonitrile at 500 μg/mL, and stored in the freezer at −20 °C. An intermediate composite standard solution mixture of 10 μg/mL was prepared by diluting all stock standard solutions in toluene. This mixture was stored in the refrigerator at 4 °C to be used as a spiking solution mixture and to prepare the calibration mixtures. A stock solution of aldrin (100 μg/mL) was prepared in n-hexane to be used as an internal injection standard (IIS). The injection standard solution was prepared with n-hexane:acetone (9:1 *v*/v), and 0.1 μg/mL of aldrin was added (Soliman et al., 2019), for peak normalization and better quantitation accuracy. The used APs mixture was prepared in acetone as described in Soliman et al. (2020).

The calibration solutions for LC-MS/MS and GC–MS/MS were prepared by diluting the spiking solution mixture in methanol for LC-MS/MS and in n-hexane: acetone (9:1 v/v) for GC–MS/MS at concentrations of 0.002, 0.01, 0.05, 0.1, and 0.5 μg/mL. A concentration of 0.1 μg/mL aldrin was added to the GC–MS/MS calibration. The calibration solutions for both LC-MS/MS and GC–MS/MS were stored in the refrigerator at 4 °C.

2.3. Sample preparation

2.3.1. Sample processing

Samples of green coffee beans were purchased from local markets.

The green coffee beans were stored at room temperature until they were used. The green coffee beans were roasted to different degrees of roasting using a muffle furnace Thermolyne™ (Massachusetts, U.S).

The light roasted coffee beans were obtained by placing them in the muffle furnace for 8 min at 200 °C, while the dark roasted coffee beans were obtained by placing them in the muffle furnace for 12 min at the same temperature (Fachruddin et al., 2021).

Two kg from each of the green, light, and dark coffees were milled in a 160 UPZ mill (Alpine, Germany). All samples were tested as blanks using the developed methods and were found to contain no residue of the targeted pesticides, except for ortho-phenylphenol (OPP) in the roasted coffee. OPP is known to occur in coffee due to roasting (Menzio et al., 2023; Theurillat et al., 2022).

2.3.2. Sample extraction

The optimization was done using design of experiments with categorical variables. Three different extraction buffers, three different sorbents, and three different types of matrices were used, as shown in Fig. 1. In general, the extraction protocol was as follows:

A weight of 2.0 ± 0.02 g of the sample was transferred into a 50 mL tube. Then, 10 mL of deionized water (DIW) was added and shaken for 1 min at 500 rounds per min (rpm) using a vertical shaker to wet the coffee samples and facilitate the extraction steps. After that, 10 mL of acetonitrile were added, and three different buffers were used, each individually, with the same salt. Buffer 1 consisted of 1 g NaCl, 4 g $MgSO_4$, sodium citrate dihydrate, and trisodium citrate (Payá et al., 2007). Buffer 2 consisted of 1 g NaCl, 4 g $MgSO_4$, and no buffer (Anastassiades et al., 2003). Buffer 3 consisted of 1 g NaCl, 4 g $MgSO_4$, and ammonium acetate buffer and 10 mL of acetonitrile 1% acetic acid were used (Dias et al., 2013; Francesquett et al., 2019; Gao et al., 2015). Then samples were shaken for 1 min at 500 rpm and centrifuged at 4500 rpm for five minutes. A syringe filter was used to filter around one mL of the supernatant directly into a vial for LC-MS/MS analysis.

The d-SPE cleanup step was performed using different sorbents. Sorbent 1 consisted of 0.25 g PSA, 0.25 g C18, and 0.6 g $MgSO_4$ (Pizzutti et al., 2012). Sorbent 2 consisted of 0.02 g envicarb, 0.5 g C18, and 0.6 g $MgSO_4$ (Reichert et al., 2018). Sorbent 3 consisted of 0.075 g Chitosan and 0.6 g $MgSO_4$ (Barci et al., 2020; Francesquett et al., 2019; Senes et al., 2020). Afterwards, three mL of supernatant were transferred to another 15 mL polypropylene centrifuge tube, shaken, and then centrifuged at 4500 rpm for two minutes. A rotary evaporator was then used to evaporate two mL of the extract in a 100 mL glass flask at 280 rpm and 39 °C till dryness. The final residue was reconstituted to a two mL injection standard solution for GC–MS/MS. Table 1 shows the different methods tested in this study. The final concentration used in the spike samples was 0.01 μg/mL.

2.4. LC-MS/MS

The HPLC (Agilent) 1200 Series instrument was coupled to an API 4000 Qtrap MS/MS from AB Sciex (Toronto, Canada) with an electrospray ionization (ESI) interface in the positive mode. The ion source

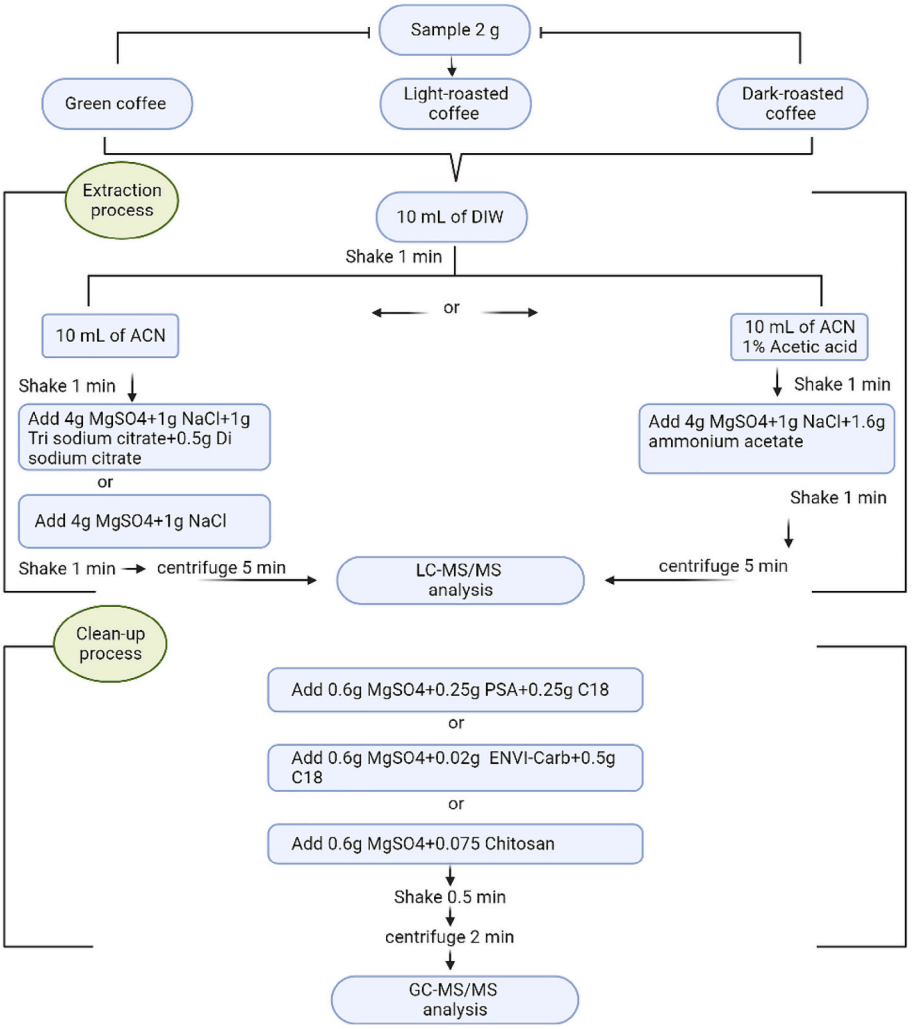

Fig. 1. Schematic figure of the different studied extraction methods (QuEChERS approach) and d-SPE sorbents.

Table 1
Buffers and sorbents were used in this study.

Methods		Solvent	Salts & Buffers	Clean-up
Z1	Z1.1	Acetonitrile	4 g MgSO4 + 1 g NaCl +1 g tri-sodium citrate +0.5 g Citrate di-sodium sesquihydrate	0.25 g PSA + 0.25 g C18 + 0.6 g MgSO4
	Z1.2	Acetonitrile	4 g MgSO4 + 1 g NaCl +1 g tri-sodium citrate +0.5 g Citrate di-sodium sesquihydrate	0.5 g C18 + 0.02 g Envicarb +0.6 g MgSo4
	Z1.3	Acetonitrile	4 g MgSO4 + 1 g NaCl +1 g tri-sodium citrate +0.5 g Citrate di-sodium sesquihydrate	0.075 Chitosan +0.6 g MgSo4
Z2	Z2.1	Acetonitrile +1% Acetic acid	4 g MgSO4 + 1 g NaCl +1.7 g ammonium acetate	0.25 g PSA + 0.25 g C18 + 0.6 g MgSO4
	Z2.2	Acetonitrile +1% Acetic acid	4 g MgSO4 + 1 g NaCl +1.7 g ammonium acetate	0.5 g C18 + 0.02 g Envicarb +0.6 g MgSo4
	Z2.3	Acetonitrile +1% Acetic acid	4 g MgSO4 + 1 g NaCl +1.7 g ammonium acetate	0.075 Chitosan +0.6 g MgSo4
Z3	Z3.1	Acetonitrile	4 g MgSO4 + 1 g NaCl + No buffer	0.25 g PSA + 0.25 g C18 + 0.6 g MgSO4
	Z3.2	Acetonitrile	4 g MgSO4 + 1 g NaCl + No buffer	0.5 g C18 + 0.02 g Envicarb +0.6 g MgSo4
	Z3.3	Acetonitrile	4 g MgSO4 + 1 g NaCl + No buffer	0.075 Chitosan +0.6 g MgSo4

temperature was 400 °C, and the ESI voltage was 5500 V. Separation was performed on an Agilent C18 column (ZORBAX Eclipse XDB, 4.6 × 150 mm with a 5.0 μm particle size). The injection volume was 5.0 μL.

As shown in Table S1, a gradient elution program was used at 500 μL min^{-1} flow rate, where one reservoir contained 10 mM ammonium formate solution in methanol: water (1:9 *v*/v) and the other contained LC-MS grade methanol. The run time was 20 min. The multiple reaction monitoring (MRM) transitions are demonstrated in Table S2 (Attallah et al., 2018; Wageed et al., 2024).

2.5. GC–MS/MS

The GC–MS/MS analysis was done using an 8890 GC Agilent gas chromatography system equipped with a 7010B triple quadrupole Agilent mass spectrometer. The chromatographic separations were accomplished using two HP-5 ms Ultra Inert capillary columns (5%-phenyl)-methylpolysiloxane, 15 m column length × 0.25 mm id × Film thickness 0.25 μm). The two columns are linked by a mid-point column back flush and were purchased from Agilent Technologies.

The key parameters for the GC method are summarized in Table S3. The total run time was 21 min at a constant flow rate of 0.7 mL/min for the first column and 0.9 mL/min for the second column. The inlet temperature was 250 °C and the injection volume was 1 μL. The sandwich injection was done in the reversed 2-layer (L2,L1) mode, where the sample is drawn first, then the APs mixture (the APs are ejected first, then the sample) (Soliman, 2021). The carrier gas used was high-purity helium (purity: 99.999%), while the collision gas was nitrogen (purity: 99.9999%) (Enia et al., 2022). The electron impact ionization mode was used, with an ionization energy of 70 eV. The ion source temperature was 320 °C and the GC–MS/MS interface temperature was 320 °C, while the Quadrupole temperature was 180 °C. The MRM transitions are demonstrated in Table S4.

2.6. Method validation

The developed methods were validated according to the method validation criteria stated in the European guidelines for analytical quality control and method validation procedures for pesticide residues analysis in food and feed (SANTE/11312/2021) (EURL, 2021). The matrix effect was measured by comparing standard in matrix injections to calibration solutions. Two different spiking levels (0.01 and 0.05 μg/mL) were done on the green, light, and dark roasted coffee. The following parameters were studied: trueness, precision, linearity, and limit of quantification (LOQ). Where trueness was expressed as average recovery of the spiked samples for each level, precision was expressed as the relative standard deviation (RSD) for the same spikes, linearity was expressed as the deviation of back-calculated from the expected concentration and determination coefficient (R^2), and LOQ was expressed as the lowest validated spike level with acceptable criteria for trueness and precision.

3. Results and discussion

3.1. Extraction methods optimization

When optimizing the QuEChERS extraction protocol, several factors can be taken in consideration, such as extraction solvent, extraction buffer, cleanup sorbents, sample homogenization, sample size and extraction time. The optimization of the extraction methods was done based on design of experiments with categorical variables on two significant categorical variables (extraction buffer and cleanup sorbent) as suggested by (García-Vara et al., 2023), additionally the coffee roasting degree was chosen as another categorical. The optimum amount of extraction buffer (Anastassiades et al., 2003; Dias et al., 2013; Francesquett et al., 2019; Gao et al., 2015; Payá et al., 2007), and cleanup sorbents (Barci et al., 2020; Francesquett et al., 2019; Pizzutti et al., 2012; Reichert et al., 2018; Senes et al., 2020) were taken from previous published works as described in the sample extraction section in the materials and methods.

The optimum extraction conditions for the studied contaminants from green coffee beans were Z1.3, while Z3.1 showed more promise for both roasted coffee types. Two criteria, spiking recovery, and matrix effect, were assessed to choose the optimal methods for each commodity, as follows:

In general, method Z1.3 was better than the others as it provided the highest acceptable recovery rate according to SANTE/11312/2021. This method achieved a recovery rate for 88 out of 92 compounds between 70 and 120% in the LC-MS/MS and for 87 out of 95 compounds in the GC–MS/MS, as shown in Fig. 2. The Z3.1 method showed an acceptable recovery range of analytes for both types of roasted coffee, since it recovered 90 out of 92 compounds in LC-MS/MS and 72 out of 95 compounds in GC–MS/MS, as well as 88 out of 92 compounds in LC-MS/MS and 64 out of 95 compounds in GC–MS/MS for analysing light and dark roasted coffee, respectively, as shown in Figs. 3 and 4. It was observed that the recovery of the spiked samples decreased as the roasting degree increased. Roasting increases the amount of lipids, fats, and basic chemicals while decreasing the percentage of certain chemical components, such as caffeine and chlorogenic acid. This caused challenges in the extraction process. Consequently, the extraction techniques needed to be adjusted depending on the degree of coffee roasting (Poisson et al., 2018; Sunarharum et al., 2014).

Additionally, this affected the cleanup process. For example, chitosan was found to be the optimal sorbent for the clean-up process of green coffee samples for GC–MS/MS analysis. The hydroxyl groups and amino acids in chitosan can interact with the acidic chemicals found in green coffee and remove them (Arias et al., 2018; Crini, 2005; Francesquett et al., 2019). Moreover, chitosan can be considered as a greener alternative to conventional sorbents such as PSA (Sayed et al., 2022b; Soliman et al., 2022). Conversely, C18 sorbent was necessary to remove high-fat content and basic compounds in roasted coffee (Anastassiades et al., 2003; Dias et al., 2013; Enia et al., 2022; Pizzutti et al., 2012; Reichert et al., 2018; Soliman et al., 2019; Soliman et al., 2020; Trevisan et al., 2017).

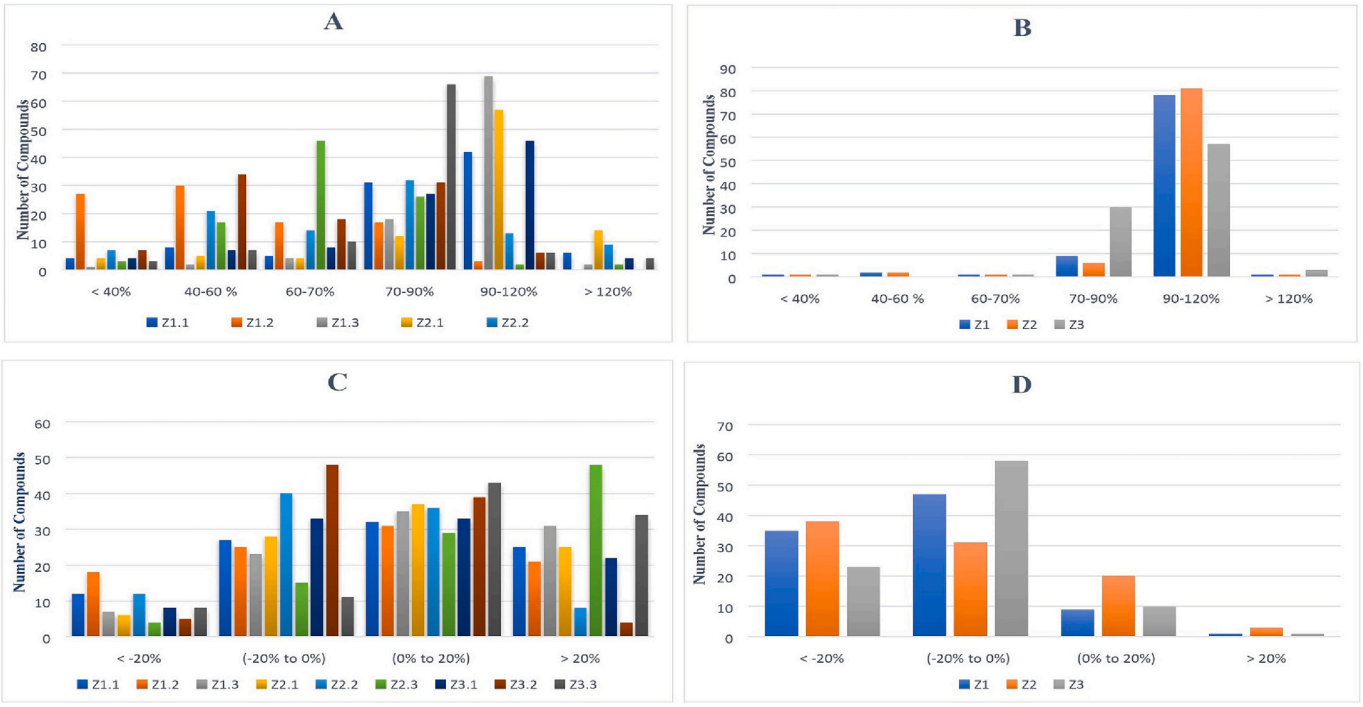

Fig. 2. Percentage of compounds recovery and the matrix effect in green coffee. A: spike recovery for GC–MS/MS. B: spike recovery for LC-MS/MS. C: matrix effect for GC–MS/MS. D: matrix effect for LC-MS/MS. The final concentration used in the spike sample was 0.01 μg/mL, while the final concentration used in the matrix effect was 0.05 μg/mL. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

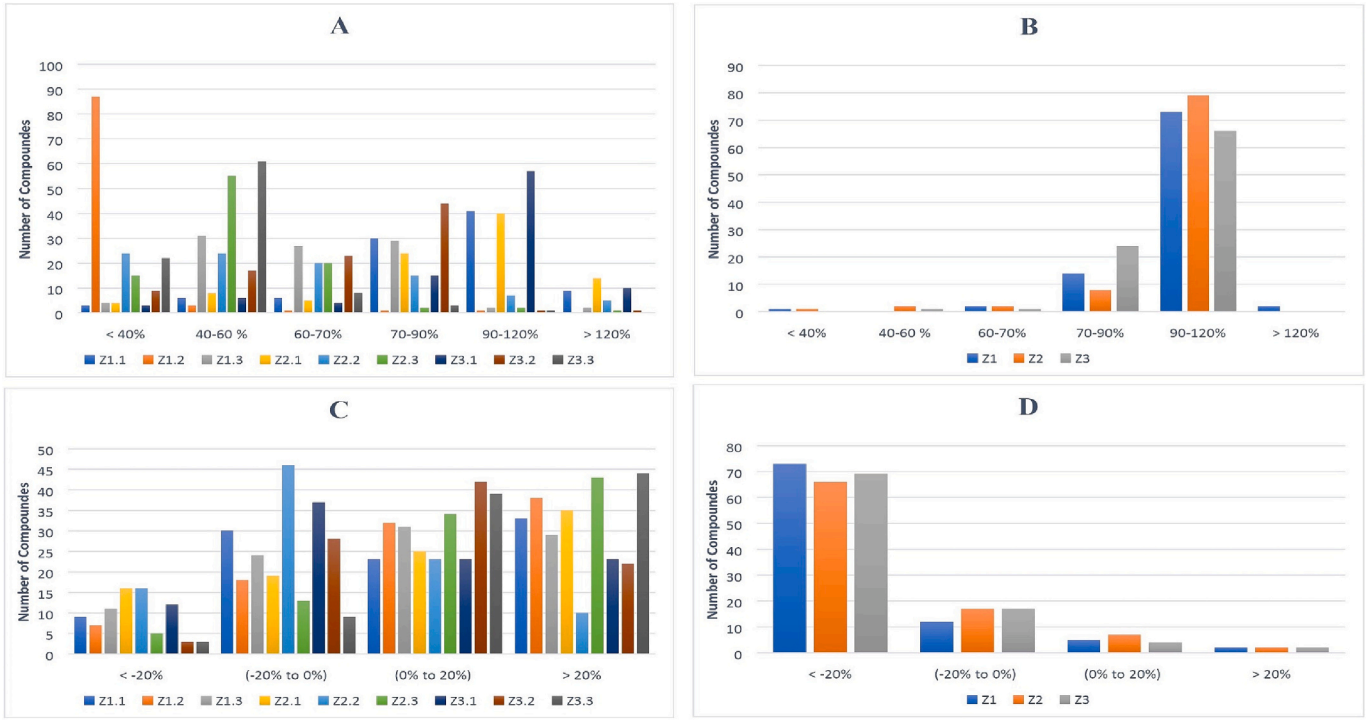

Fig. 3. Percentage of compounds recovery and the matrix effect in light-roasted coffee. A: spike recovery for GC–MS/MS. B: spike recovery for LC-MS/MS. C: matrix effect for GC–MS/MS. D: matrix effect for LC-MS/MS. The final concentration used in the spike sample was 0.01 μg/mL, while the final concentration used in the matrix effect was 0.05 μg/mL.

3.2. Method validation

3.2.1. Linearity of calibration curves

The linearity of the calibration curve for each compound was evaluated using four calibration standard preparations ranging from 0.002 to 0.1 μg/mL. The initial calibration standard level of 0.002 μg/mL was chosen due to the dilution factor of 5 and the lowest MRL for pesticides and other studied contaminants in coffee, which is 0.01 μg/

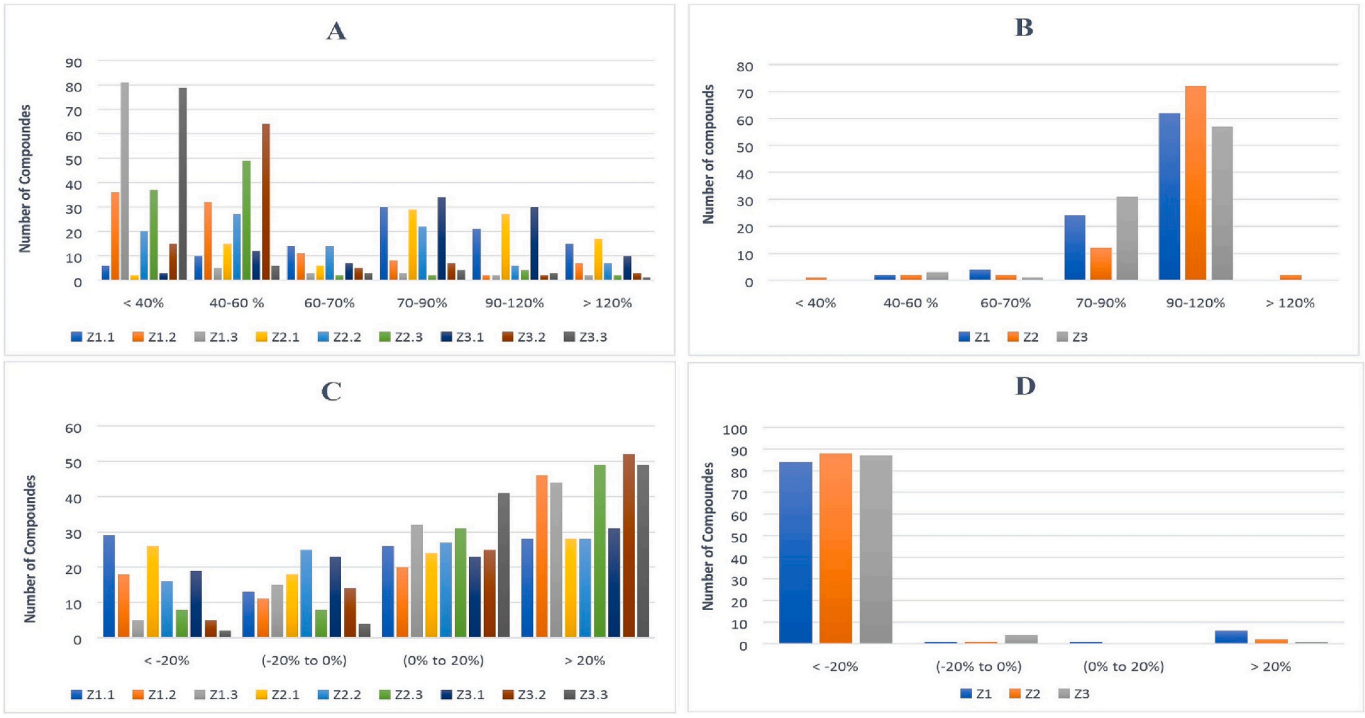

Fig. 4. Percentage of compounds recovery and the matrix effect in dark-roasted coffee. A: spike recovery for GC–MS/MS. B: spike recovery for LC-MS/MS. C: matrix effect for GC–MS/MS. D: matrix effect for LC-MS/MS. The final concentration used in the spike sample was 0.01 μg/mL, while the final concentration used in the matrix effect was 0.05 μg/mL.

mL. The calibration concentrations were increased with constant factors to the third level, ranging from 0.002, 0.01, 0.05, and 0.1 μg/mL (Enia et al., 2022).

Linear regression calibration curves for the studied analytes were plotted by considering the detector response area against the expected concentration of the standard solution. The internal standard response was taken into account for GC–MS/MS. The analytes showed linear behaviors at the studied concentration standard levels, with a deviation of back-calculated concentration from true concentration of ±20% in most cases and R^2 >0.99. The results for the linearity studies are presented in Table S5.

Two approaches were used for quantification: interpolation-based calibration using two successive calibration levels with a maximum factor of five difference between them, where the response factors for the correction calibration criteria shouldn't deviate by >20% (the highest response is taken as 100%). The other method is single-point calibration, which assumes that the analyte's response in the obtained extract will be close to its response to the single-level calibration (within 30%).

3.2.2. *Matrix effect*

The matrix effect was measured by adding pesticide standard solutions to blank green and roasted coffee samples, using a 0.05 μg/mL standard to compensate for suppression in LC-MS/MS and GC–MS/MS. The matrix-matched standard calculations for the concentrations described above are shown in Table S5. The matrix effect was calculated using the following formula:

$$\text{Matrix effect\%} = [(\text{peak area of the standard in the matrix}/\text{peak area of the standard in the solvent}) - 1] \times 100.$$

To ensure that there is no change of the matrix effect on different concentrations, a lower level (0.005 μg/mL) was injected in matrix and calculated using the previous eq. *t*-test was employed to assess whether there was a significant difference between the results obtained from these two concentrations. The analysis revealed no significant difference between the outcomes, leading to the decision to use a single one-point (0.05 μg/mL) for calculating the matrix effect.

If the matrix effect was within a range of 20% from the expected concentration, it was neglected in the calculations. The results also showed that LC-MS/MS was more affected by the matrix compared to GC–MS/MS, where APs were used, which have a known role in reducing the effect of the matrix on the analytes (Soliman, 2021).

3.2.3. *Trueness and precision*

Table 2, and Table S7 shows the results of the recovery tests utilizing the final-optimized approach for each commodity. The spike recovery tests were conducted at two levels of 0.01 and 0.05 μg/g ($n = 5$). The precision was estimated based on the corresponding RSD (RSDwr and RSDr) which is considered acceptable if ≤20%, and the trueness was computed based on the mean recoveries, with approved range of 70–120%. Where, 90% of the analytes fall within 0.01 μg/g of the acceptable range of 70–120%. While all analytes fall within 0.05 μg/g of the acceptable range of 70–120%, except chlormequat and mepiquat. Also, all analytes were within the acceptable range of RSD (≤ 20%).

It was worth noticing that some compounds detected by both instruments exhibited significantly better trueness and precision in LC-

Table 2
Mean recoveries (%) and RSDr% for organic contaminants for each matrix.

Analytes	Technique	Green coffee				Light-roasted coffee				Dark-roasted coffee			
		Level 0.01 μg/mL		Level 0.05 μg/mL		Level 0.01 μg/mL		Level 0.05 μg/mL		Level 0.01 μg/mL		Level 0.05 μg/mL	
		Mean Rec%	RSDr %	Mean Rec%	RSDr %	Mean Rec%	RSDr %	Mean Rec%	RSDr %	Mean Rec%	RSDr %	Mean Rec%	RSDr %
Ortho-Phenylphenol (OPP)	GC–MS/MS	81%	12%	76%	9%	89%	19%	87%	7%	114%	12%	93%	3%
Acetamiprid	LC-MS/MS	93%	9%	82%	2%	115%	13%	93%	3%	82%	13%	111%	7%
Acrinathrin	GC–MS/MS	107%	12%	88%	6%	70%	6%	110%	4%	126%	5%	105%	14%
	LC-MS/MS	106%	13%	103%	16%	115%	14%	96%	7%	128%	0%	119%	12%
Alachlor	GC–MS/MS	107%	4%	103%	10%	120%	10%	119%	2%	109%	6%	88%	5%
Ametoctradin	LC-MS/MS	95%	2%	92%	2%	100%	5%	92%	4%	98%	5%	96%	1%
Atrazine	GC–MS/MS	79%	19%	92%	7%	98%	1%	106%	1%	120%	13%	92%	5%
	LC-MS/MS	91%	5%	86%	2%	99%	11%	88%	6%	101%	10%	98%	4%
Azoxystrobin	LC-MS/MS	91%	3%	91%	3%	114%	6%	108%	1%	107%	5%	98%	4%
Benalaxyl	GC–MS/MS	105%	5%	101%	8%	81%	3%	112%	3%	99%	8%	88%	2%
	LC-MS/MS	103%	4%	96%	4%	99%	6%	97%	2%	95%	4%	94%	7%
Bendiocarb	GC–MS/MS	99%	19%	102%	20%	94%	7%	83%	4%	108%	18%	97%	6%
	LC-MS/MS	120%	4%	75%	4%	119%	4%	98%	2%	106%	4%	105%	1%
Bifenazate	GC–MS/MS	105%	19%	76%	9%	112%	5%	101%	9%	111%	6%	89%	7%
	LC-MS/MS	75%	20%	71%	7%	107%	5%	95%	4%	110%	6%	97%	12%
Biphenyl	GC–MS/MS	99%	20%	101%	8%	116%	16%	92%	15%	113%	20%	91%	7%
Bitertanol	GC–MS/MS	119%	5%	85%	4%	115%	19%	96%	0%	92%	0%	90%	5%
	LC-MS/MS	104%	12%	106%	5%	116%	12%	91%	5%	96%	20%	100%	6%
Boscalid	GC–MS/MS	95%	8%	93%	10%	111%	5%	91%	3%	90%	0%	93%	3%
	LC-MS/MS	95%	3%	85%	5%	119%	20%	101%	4%	73%	15%	95%	12%
Bromophos	GC–MS/MS	115%	6%	96%	8%	108%	4%	94%	3%	86%	7%	82%	8%
Bromophos-ethyl	GC–MS/MS	120%	3%	97%	9%	118%	3%	92%	4%	91%	7%	74%	10%
Bromopropylate	GC–MS/MS	115%	5%	104%	8%	113%	4%	104%	4%	99%	5%	85%	4%
Bromuconazole	GC–MS/MS	97%	5%	87%	8%	110%	7%	95%	1%	80%	5%	80%	4%
	LC-MS/MS	98%	8%	95%	3%	101%	5%	86%	4%	120%	10%	102%	14%
Bupirimate	GC–MS/MS	111%	13%	98%	9%	91%	19%	110%	2%	76%	15%	72%	3%
	LC-MS/MS	96%	5%	89%	6%	98%	2%	97%	2%	96%	5%	95%	4%
Buprofezin	GC–MS/MS	109%	20%	99%	6%	94%	18%	87%	6%	104%	10%	101%	8%
	LC-MS/MS	113%	7%	97%	6%	109%	6%	85%	5%	99%	2%	106%	2%
Butachlor	GC–MS/MS	99%	20%	96%	5%	105%	4%	91%	5%	102%	6%	109%	8%
	LC-MS/MS	79%	6%	113%	4%	120%	8%	94%	2%	118%	20%	112%	7%
Butralin	GC–MS/MS	100%	10%	83%	12%	103%	6%	89%	1%	70%	20%	72%	1%
	LC-MS/MS	108%	7%	112%	4%	88%	1%	79%	6%	104%	7%	97%	2%
Cadusafos	GC–MS/MS	74%	7%	96%	8%	85%	12%	104%	2%	77%	0%	79%	1%
Carbendazim	LC-MS/MS	93%	4%	85%	3%	97%	7%	87%	3%	120%	6%	88%	3%
Carbofuran 3OH	LC-MS/MS	81%	7%	71%	6%	120%	5%	95%	7%	120%	20%	75%	4%
Carbofuran	GC–MS/MS	132%	11%	111%	7%	100%	2%	90%	0%	101%	5%	94%	1%
	LC-MS/MS	103%	2%	102%	4%	129%	4%	101%	6%	97%	16%	107%	6%
Carbosulfan	GC–MS/MS	130%	8%	111%	20%	106%	15%	106%	16%	126%	13%	88%	15%
	LC-MS/MS	75%	12%	73%	20%	99%	10%	71%	4%	84%	15%	100%	18%
Chlorantraniliprole	LC-MS/MS	90%	3%	87%	4%	98%	2%	87%	5%	101%	8%	93%	5%
Chlordane-cis	GC–MS/MS	112%	3%	95%	10%	103%	5%	87%	5%	119%	9%	72%	4%
Chlordane-trans	GC–MS/MS	111%	10%	98%	9%	73%	0%	93%	5%	74%	20%	71%	4%
Chlorfenapyr	GC–MS/MS	95%	11%	83%	12%	71%	18%	109%	9%	78%	19%	92%	2%
Chlormequat	LC-MS/MS	48%	12%	40%	6%	41%	7%	34%	4%	48%	9%	31%	6%

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Table 2 (*continued*)

Analytes	Technique	Green coffee				Light-roasted coffee				Dark-roasted coffee			
		Level 0.01 μg/mL		Level 0.05 μg/mL		Level 0.01 μg/mL		Level 0.05 μg/mL		Level 0.01 μg/mL		Level 0.05 μg/mL	
		Mean Rec%	RSDr %	Mean Rec%	RSDr %	Mean Rec%	RSDr %	Mean Rec%	RSDr %	Mean Rec%	RSDr %	Mean Rec%	RSDr %
Chlorothalonil	GC–MS/MS	72%	16%	75%	9%	95%	7%	74%	3%	100%	5%	92%	10%
Chlorpropham	GC–MS/MS	99%	8%	83%	6%	81%	0%	110%	4%	107%	12%	84%	1%
Chlorpyrifos	GC–MS/MS	99%	13%	103%	9%	93%	0%	91%	0%	148%	15%	90%	6%
	LC-MS/MS	121%	6%	98%	4%	122%	5%	97%	6%	115%	2%	91%	8%
Chlorpyrifos-methyl	GC–MS/MS	116%	7%	98%	3%	108%	6%	94%	5%	120%	7%	88%	3%
	LC-MS/MS	100%	20%	92%	9%	95%	10%	87%	0%	94%	5%	89%	3%
Cyfluthrin	GC–MS/MS	120%	2%	93%	12%	116%	5%	92%	3%	84%	11%	78%	4%
	LC-MS/MS	110%	20%	118%	14%	110%	12%	111%	12%	80%	10%	115%	14%
Cyhalothrin (Lambda)	GC–MS/MS	93%	11%	119%	9%	115%	5%	93%	4%	114%	4%	74%	5%
	LC-MS/MS	118%	17%	96%	9%	111%	14%	103%	4%	84%	14%	97%	17%
Cymoxanil	LC-MS/MS	80%	6%	80%	10%	125%	14%	90%	5%	119%	10%	95%	16%
Cypermethrin	GC–MS/MS	111%	7%	92%	7%	81%	0%	93%	4%	93%	8%	76%	3%
	LC-MS/MS	116%	19%	94%	17%	120%	12%	83%	15%	69%	11%	81%	12%
Cyproconazole	GC–MS/MS	82%	7%	94%	10%	93%	18%	90%	3%	92%	7%	88%	3%
	LC-MS/MS	99%	11%	91%	5%	108%	5%	93%	4%	122%	5%	104%	1%
Cyprodinil	GC–MS/MS	120%	6%	112%	7%	111%	6%	109%	6%	94%	6%	74%	3%
	LC-MS/MS	107%	5%	102%	17%	82%	8%	79%	4%	94%	3%	87%	1%
DDD-o,p'	GC–MS/MS	94%	7%	120%	8%	89%	12%	113%	6%	93%	3%	80%	2%
DDD-p,p'	GC–MS/MS	102%	6%	106%	8%	78%	13%	83%	5%	72%	11%	90%	3%
DDE-p,p'	GC–MS/MS	87%	7%	100%	8%	76%	10%	73%	7%	58%	0%	73%	3%
Deltamethrin	GC–MS/MS	105%	6%	75%	11%	115%	6%	80%	8%	108%	10%	76%	1%
	LC-MS/MS	103%	10%	94%	12%	102%	10%	116%	11%	114%	20%	104%	18%
Desmedipham	LC-MS/MS	103%	4%	100%	1%	96%	5%	90%	5%	101%	5%	102%	5%
Diafenthiuron	LC-MS/MS	71%	12%	72%	15%	82%	9%	85%	2%	89%	11%	111%	6%
Diazinon	GC–MS/MS	98%	6%	96%	8%	112%	6%	109%	1%	71%	9%	87%	5%
	LC-MS/MS	101%	4%	99%	3%	98%	4%	86%	1%	91%	3%	93%	2%
Difenoconazole	GC–MS/MS	93%	7%	99%	11%	96%	0%	102%	4%	115%	8%	77%	5%
	LC-MS/MS	102%	4%	100%	2%	101%	5%	93%	2%	116%	14%	109%	3%
Dimethoate	GC–MS/MS	72%	20%	99%	9%	101%	17%	92%	9%	68%	13%	86%	10%
	LC-MS/MS	86%	7%	78%	7%	100%	2%	92%	3%	101%	2%	90%	4%
Diniconazole	GC–MS/MS	90%	8%	97%	9%	99%	6%	99%	4%	70%	1%	79%	5%
	LC-MS/MS	101%	6%	94%	3%	102%	14%	101%	4%	119%	13%	99%	5%
Dinotefuran	LC-MS/MS	120%	5%	74%	13%	115%	20%	105%	7%	117%	10%	80%	14%
Diphenylamine	GC–MS/MS	118%	13%	96%	11%	117%	0%	120%	7%	117%	20%	115%	3%
Endosulfan-alpha	GC–MS/MS	73%	5%	76%	13%	72%	12%	84%	2%	75%	13%	92%	20%
Endosulfan-beta	GC–MS/MS	75%	7%	94%	9%	94%	0%	95%	5%	109%	10%	83%	8%
Endosulfan sulfate	GC–MS/MS	117%	7%	102%	8%	108%	0%	106%	3%	90%	17%	90%	3%
Epoxiconazole	GC–MS/MS	94%	9%	95%	8%	74%	0%	108%	3%	78%	17%	89%	2%
	LC-MS/MS	98%	9%	96%	2%	113%	6%	93%	2%	101%	4%	92%	4%
Ethion	GC–MS/MS	102%	9%	98%	7%	78%	20%	99%	4%	94%	15%	99%	1%
	LC-MS/MS	105%	3%	100%	3%	90%	7%	87%	5%	101%	4%	102%	2%
Fenpropathrin	GC–MS/MS	107%	12%	103%	7%	135%	20%	120%	0%	79%	19%	70%	12%
	LC-MS/MS	116%	13%	107%	13%	89%	14%	86%	9%	117%	2%	95%	3%
Fenvalerate	GC–MS/MS	105%	8%	95%	9%	119%	6%	87%	6%	69%	10%	74%	2%
	LC-MS/MS	72%	13%	117%	15%	91%	17%	114%	12%	86%	14%	75%	20%

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Table 2 (*continued*)

Analytes	Technique	Green coffee				Light-roasted coffee				Dark-roasted coffee			
		Level 0.01 μg/mL		Level 0.05 μg/mL		Level 0.01 μg/mL		Level 0.05 μg/mL		Level 0.01 μg/mL		Level 0.05 μg/mL	
		Mean Rec%	RSDr %	Mean Rec%	RSDr %	Mean Rec%	RSDr %	Mean Rec%	RSDr %	Mean Rec%	RSDr %	Mean Rec%	RSDr %
Fluazifop-p-butyl	GC–MS/MS	109%	2%	104%	8%	107%	3%	104%	3%	84%	4%	88%	0%
	LC-MS/MS	110%	3%	101%	3%	100%	18%	95%	1%	100%	11%	97%	5%
Fludioxonil	GC–MS/MS	101%	7%	94%	10%	106%	20%	106%	3%	116%	4%	91%	6%
Flutolanil	LC-MS/MS	99%	4%	97%	4%	115%	7%	100%	3%	102%	2%	101%	3%
HCH-alpha	GC–MS/MS	109%	6%	98%	8%	94%	13%	102%	2%	74%	6%	82%	1%
HCH-beta	GC–MS/MS	84%	9%	78%	12%	116%	8%	95%	14%	106%	6%	96%	4%
HCH-delta	GC–MS/MS	122%	3%	94%	8%	102%	3%	107%	4%	95%	18%	83%	1%
HCH-gamma	GC–MS/MS	100%	11%	106%	7%	104%	11%	94%	4%	69%	19%	82%	9%
Heptachlor	GC–MS/MS	100%	19%	102%	8%	77%	11%	75%	6%	64%	8%	71%	13%
Hexachlorobenzene	GC–MS/MS	105%	12%	78%	13%	78%	7%	75%	4%	66%	6%	76%	4%
Imazalil	GC–MS/MS	71%	19%	77%	10%	106%	19%	74%	7%	71%	17%	77%	11%
	LC-MS/MS	92%	6%	90%	6%	87%	3%	84%	6%	95%	10%	86%	6%
Indoxacarb	LC-MS/MS	92%	5%	91%	7%	98%	3%	90%	2%	99%	7%	100%	2%
Malaoxon	LC-MS/MS	92%	2%	87%	1%	97%	2%	94%	1%	102%	7%	98%	3%
Malathion	GC–MS/MS	71%	8%	72%	6%	73%	6%	109%	2%	76%	17%	91%	9%
	LC-MS/MS	98%	3%	92%	3%	116%	7%	98%	4%	102%	4%	100%	3%
Mepiquat	LC-MS/MS	58%	12%	46%	7%	156%	6%	95%	20%	60%	19%	36%	20%
Metalaxyl	GC–MS/MS	99%	8%	96%	9%	86%	20%	104%	6%	86%	7%	93%	1%
	LC-MS/MS	102%	6%	95%	5%	91%	3%	87%	4%	99%	1%	98%	2%
Methomyl	LC-MS/MS	94%	14%	86%	9%	79%	11%	81%	13%	64%	16%	86%	8%
Metrafenone	GC–MS/MS	98%	8%	91%	10%	108%	0%	105%	9%	97%	17%	86%	10%
Myclobutanil	GC–MS/MS	90%	6%	94%	9%	73%	13%	99%	3%	83%	8%	85%	1%
	LC-MS/MS	97%	6%	87%	4%	103%	10%	95%	3%	105%	8%	98%	5%
Omethoate	GC–MS/MS	97%	4%	75%	7%	167%	12%	110%	6%	161%	14%	78%	12%
	LC-MS/MS	95%	9%	91%	6%	83%	14%	80%	17%	124%	20%	118%	9%
Oxamyl	LC-MS/MS	90%	18%	82%	16%	76%	5%	89%	12%	96%	20%	86%	16%
PCBs 101	GC–MS/MS	84%	8%	87%	15%	78%	7%	78%	6%	60%	11%	77%	4%
PCBs 118	GC–MS/MS	91%	7%	99%	8%	84%	5%	77%	8%	52%	8%	72%	2%
PCBs 138	GC–MS/MS	92%	5%	101%	7%	71%	1%	81%	7%	77%	8%	77%	5%
PCBs 153	GC–MS/MS	99%	6%	104%	8%	71%	4%	75%	7%	80%	9%	75%	3%
PCBs 180	GC–MS/MS	96%	6%	99%	9%	71%	5%	78%	9%	86%	4%	88%	2%
PCBs 28	GC–MS/MS	80%	20%	106%	19%	99%	18%	77%	11%	43%	12%	70%	1%
PCBs 52	GC–MS/MS	120%	6%	95%	10%	82%	9%	70%	5%	94%	4%	88%	9%
Penconazole	GC–MS/MS	61%	6%	76%	5%	89%	11%	95%	5%	102%	17%	85%	5%
	LC-MS/MS	100%	3%	93%	4%	97%	5%	87%	5%	104%	9%	93%	0%
Pencycuron	LC-MS/MS	98%	8%	97%	3%	92%	8%	87%	0%	95%	2%	98%	1%
Pendimethalin	GC–MS/MS	89%	5%	88%	8%	80%	0%	99%	6%	81%	18%	71%	2%
	LC-MS/MS	117%	5%	111%	2%	95%	7%	76%	8%	120%	7%	100%	4%
Pentachloroaniline	GC–MS/MS	99%	3%	88%	5%	75%	0%	106%	13%	120%	19%	107%	20%
Pentachloroanisole	GC–MS/MS	75%	17%	99%	15%	98%	18%	82%	4%	92%	5%	70%	4%
Pentachlorobenzene	GC–MS/MS	97%	4%	83%	9%	97%	13%	77%	3%	108%	6%	72%	1%
Permethrin	GC–MS/MS	111%	13%	88%	11%	104%	7%	87%	7%	90%	14%	70%	5%
	LC-MS/MS	108%	5%	102%	1%	111%	4%	102%	2%	114%	15%	120%	5%

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Table 2 (*continued*)

Analytes	Technique	Green coffee				Light-roasted coffee				Dark-roasted coffee			
		Level 0.01 μg/mL		Level 0.05 μg/mL		Level 0.01 μg/mL		Level 0.05 μg/mL		Level 0.01 μg/mL		Level 0.05 μg/mL	
		Mean Rec%	RSDr %	Mean Rec%	RSDr %	Mean Rec%	RSDr %	Mean Rec%	RSDr %	Mean Rec%	RSDr %	Mean Rec%	RSDr %
Phenthoate	GC–MS/MS	116%	6%	82%	10%	96%	9%	108%	4%	71%	2%	88%	8%
	LC-MS/MS	93%	2%	92%	3%	90%	6%	80%	5%	110%	9%	100%	5%
Phoxim	LC-MS/MS	90%	7%	90%	9%	89%	6%	82%	4%	97%	6%	91%	5%
Picolinafen	LC-MS/MS	100%	5%	101%	2%	103%	14%	87%	6%	100%	0%	89%	3%
Picoxystrobin	LC-MS/MS	87%	7%	77%	10%	108%	9%	93%	8%	122%	20%	118%	16%
Piperonyl butoxide	GC–MS/MS	116%	5%	97%	7%	105%	12%	117%	3%	71%	6%	89%	2%
	LC-MS/MS	99%	4%	89%	4%	100%	6%	92%	4%	92%	1%	97%	7%
Profenofos	GC–MS/MS	105%	9%	87%	8%	106%	0%	103%	6%	81%	10%	79%	2%
	LC-MS/MS	104%	1%	93%	3%	97%	7%	89%	2%	92%	7%	88%	4%
Propamocarb HCl	LC-MS/MS	70%	10%	72%	5%	75%	12%	79%	5%	77%	6%	70%	2%
Propaquizafop	LC-MS/MS	98%	2%	93%	5%	98%	6%	94%	3%	97%	8%	94%	3%
Propetamphos	LC-MS/MS	100%	12%	87%	4%	113%	9%	100%	7%	114%	20%	90%	13%
Propiconazole	GC–MS/MS	105%	6%	101%	9%	99%	5%	99%	3%	82%	4%	84%	1%
	LC-MS/MS	114%	4%	98%	5%	96%	9%	92%	1%	103%	13%	98%	3%
Propoxur	LC-MS/MS	105%	2%	105%	3%	95%	2%	97%	1%	108%	1%	88%	3%
Prothioconazole	LC-MS/MS	79%	11%	88%	8%	90%	6%	85%	5%	105%	7%	87%	6%
Pymetrozine	LC-MS/MS	100%	19%	86%	7%	75%	15%	91%	12%	120%	18%	113%	12%
Pyraclostrobin	LC-MS/MS	100%	6%	94%	2%	120%	1%	96%	3%	94%	4%	95%	1%
Pyridaben	GC–MS/MS	93%	8%	104%	8%	119%	19%	87%	5%	79%	20%	80%	6%
	LC-MS/MS	110%	5%	110%	4%	86%	2%	87%	7%	113%	15%	120%	5%
Pyrimethanil	GC–MS/MS	97%	9%	90%	10%	118%	0%	102%	3%	97%	4%	82%	2%
	LC-MS/MS	96%	3%	91%	4%	90%	5%	81%	3%	94%	1%	86%	1%
Pyriproxyfen	GC–MS/MS	110%	7%	97%	8%	110%	0%	114%	1%	115%	8%	81%	14%
	LC-MS/MS	95%	5%	92%	7%	93%	7%	89%	2%	102%	4%	102%	2%
Spinetoram	LC-MS/MS	106%	14%	101%	11%	109%	10%	100%	3%	115%	17%	120%	11%
Spinosad	LC-MS/MS	87%	8%	87%	5%	103%	5%	95%	2%	97%	4%	98%	5%
Spiromesifen	GC–MS/MS	101%	7%	111%	9%	97%	20%	105%	9%	84%	16%	79%	3%
Spirotetramate	LC-MS/MS	95%	3%	96%	4%	107%	2%	101%	3%	99%	1%	96%	1%
Sulfoxaflor	LC-MS/MS	107%	20%	88%	10%	120%	5%	102%	4%	63%	16%	73%	14%
Tebuconazole	GC–MS/MS	98%	6%	95%	8%	85%	8%	98%	3%	78%	6%	79%	4%
	LC-MS/MS	101%	8%	98%	6%	99%	7%	92%	7%	101%	9%	92%	4%
Tebufenpyrad	GC–MS/MS	109%	3%	104%	9%	79%	6%	98%	6%	74%	4%	81%	6%
	LC-MS/MS	96%	6%	100%	8%	94%	13%	86%	4%	103%	3%	96%	3%
Tetraconazole	GC–MS/MS	95%	8%	93%	9%	102%	15%	93%	5%	105%	16%	98%	4%
	LC-MS/MS	108%	8%	98%	4%	96%	6%	103%	1%	95%	5%	115%	5%
Tetradifon	GC–MS/MS	111%	4%	93%	7%	93%	11%	97%	5%	74%	16%	72%	3%
Tetramethrin	GC–MS/MS	101%	20%	102%	5%	89%	19%	85%	0%	88%	6%	94%	3%
	LC-MS/MS	163%	12%	114%	7%	81%	11%	98%	1%	120%	4%	108%	5%
Thiabendazole	LC-MS/MS	73%	14%	90%	5%	84%	2%	80%	3%	59%	4%	78%	3%
Thiamethoxam	LC-MS/MS	93%	10%	73%	5%	93%	5%	111%	5%	112%	12%	92%	1%
Thiobencarb	LC-MS/MS	107%	5%	106%	4%	96%	9%	92%	4%	96%	6%	89%	3%
Thiodicarb	LC-MS/MS	94%	2%	91%	5%	96%	6%	92%	4%	90%	3%	93%	3%
Thiometon	GC–MS/MS	113%	11%	89%	6%	113%	5%	98%	7%	94%	3%	93%	2%
Thiophanate-Methyl	LC-MS/MS	74%	12%	77%	6%	92%	5%	83%	1%	78%	9%	70%	4%
Tolclofos-methyl	GC–MS/MS	104%	17%	91%	7%	130%	9%	113%	6%	154%	6%	84%	4%
	LC-MS/MS	95%	5%	103%	4%	94%	8%	82%	2%	111%	6%	93%	5%
Tolfenpyrad	LC-MS/MS	106%	4%	101%	5%	100%	10%	95%	2%	99%	4%	96%	7%
Triadimefon	GC–MS/MS	102%	11%	95%	7%	92%	7%	106%	4%	110%	2%	91%	2%
	LC-MS/MS	96%	5%	91%	3%	91%	3%	91%	3%	105%	5%	100%	1%
Triadimenol	GC–MS/MS	100%	12%	92%	12%	106%	10%	100%	4%	119%	5%	90%	4%
	LC-MS/MS	105%	18%	89%	8%	72%	9%	102%	5%	106%	8%	104%	3%
Trifloxystrobin	GC–MS/MS	77%	8%	105%	10%	117%	12%	119%	2%	102%	17%	91%	0%
	LC-MS/MS	105%	5%	102%	6%	99%	5%	93%	3%	101%	3%	98%	4%

MS/MS, such as carbofuran, carbosulfan, imazalil, and malathion, while others showed significantly better trueness and precision in GC–MS/MS, such as fenvalerate, deltamethrin, cypermethrin, and chlorpyrifos. It is recommended to report the results for those compounds from the instrument that had better accuracy and use the other instrument's result as a confirmation.

3.2.4. Limits of quantification

The LOQ was determined as the lowest verified level with accepted trueness and accuracy in accordance with the requirements of SANTE/11312/2021. For green coffee, 124 out of 132 (88/92 in LC-MS/MS, 91/95 in GC–MS/MS) tested compounds achieved LOQ of 0.01 μg/g. For light and dark roasted coffee, 121 out of 132 (87/92 in LC-MS/MS, 92/95 in GC–MS/MS) and 112 out of 132 (82/92 in LC-MS/MS, 81/95 in GC–MS/MS) compounds had LOQ of 0.01 μg/g, respectively. However, some other analytes were within the range of 0.05 μg/g.

The LOQ for this validation covered all MRL regulations established by the Codex and the European Union. Unfortunately, some compounds didn't meet the desired criteria at both tested spiking levels. Compensation will be made for real samples with positive results for compounds whose average recoveries were proven to be between 30% and 70%, or 120% and 140%, with an RSD% within the acceptable range (<20%), according to SANTE/11312/2021.

3.3. Real samples

Ten real coffee samples from local markets were analysed, consisting of four green coffee samples and six roasted coffee samples. Initially, the samples were monitored for the occurrence of 132 organic pollutants, and quantification was conducted in the case of positive peaks. Carbendazim was found in just one sample (green coffee), while OPP was detected in all six samples of roasted coffee, which are in agreement with the obtained results of Menzio et al. (2023), as shown in Table S6. It should be noted that the presence and the levels of organic pollutants in coffee might vary based on geographical region, farming practices, airborne pollutants, soil and water contamination, post-harvest processing, and local legislation. To reduce the presence of pollutants, it is critical to encourage sustainable agricultural practices, educate farmers on proper pesticide use, create effective monitoring systems, and enforce severe pesticide residue regulations in coffee.

4. Conclusion

Two effective methods have been developed for determining 132 organic contaminants in green and roasted coffee. The extraction method for organic contaminants varies depending on the degree of coffee roasting, because the thermal effect changes the chemical and physical properties of coffee beans. Not using a buffer in the extraction process of roasted coffee yielded the best outcomes and acceptable recovery rates compared to other methods. On the other hand, citrate buffer resulted in a better outcome for green coffee. Also, Chitosan was effective in cleaning up green coffee samples before analysis on the contrary for roasted coffee where PSA and C18 were optimum. Both methods were validated according to SANTE/11312/2021 guidelines.

Overall, the methods demonstrated good linearity, reasonable recovery rates, and low LOQ values, indicating that they are appropriate for determining these pollutants in coffee samples. Ten real samples were obtained from markets and analysed. However, the number of real samples was limited, so a larger sample size of real samples will be collected to monitor the contamination level of organic contaminants in coffee in the Egyptian market.

CRediT authorship contribution statement

Abdulrhman Gamal: Formal analysis, Investigation, Resources, Validation, Visualization, Writing – original draft. **Mostafa Soliman:** Conceptualization, Investigation, Methodology, Supervision, Visualization, Writing – review & editing. **Mohamed S. Al-Anany:** Supervision, Writing – review & editing. **Fawzy Eissa:** Supervision, Writing – review & editing.

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

No data was used for the research described in the article.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2024.139223.

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