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Green solvents in dispersive liquid-liquid microextraction for the determination of carbonyl compounds in coffee extracts

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ABSTRACT

This work presents a greener approach for ultrasound-assisted (UA) dispersive liquid-liquid microextraction (DLLME) of carbonyl compounds from coffee samples, before GC–MS determination. This work aims to substitute the solvents used in the traditional DLLME procedures with greener alternatives and to decrease the volume of solvents used. Low-density solvents, 1-octanol and isooctane, were evaluated as the extraction solvent. Optimization of critical experimental parameters was conducted in two stages: an asymmetrical screening design $2^3 3^1$ // 8, followed by a Doehlert experimental design. In the first experimental design 4 parameters were optimized: the volume of aqueous phase volume (1.5 mL), the concentration of the derivatization reagent solution pentafluorophenylhydrazine (1.12 g/L) and the volume and type of extraction solvent (60 μL of isooctane). In the second experimental design, 15 min of derivatization at 50 °C were selected as optimized conditions. The enrichment factor associated with the DLLME procedure enabled the efficient extraction of nine carbonyl compounds (acetaldehyde, acrolein, benzaldehyde, diacetyl, formaldehyde, furfural, glyoxal, malondialdehyde, and methylglyoxal) from coffee samples. The method demonstrated strong analytical performance, with figures of merit including $r^2 \geq 0.9990$, limits of detection between 289 and 436 μg/L, intraday, and interday precisions < 9.5 %. Recovery values for all nine carbonyl compounds ranged from 90.0 to 110.0 %. The greenness of the developed methodology was assessed using the AGREEprep tool, yielding a score of 0.59. Acetaldehyde, benzaldehyde and furfural were quantified in most coffee samples analyzed, with no significant differences observed in carbonyl compounds composition.

1. Introduction

Coffee is one of the most consumed beverages worldwide, valued for its unique flavor, aroma and beneficial health effects [1]. Its complex composition contains >1000 different compounds, which contribute to the taste, smell, and overall quality of the final product [2]. This composition varies depending on the coffee variety, origin, roasting degree, and decaffeination, among other factors [3].

Among the wide range of chemical compounds found in coffee, carbonyl compounds are particularly significant due to their role as key markers of lipid oxidation [4]. Previous studies have highlighted the negative impact of lipid oxidation on coffee during storage, leading to the development of rancid odors, a decline in product quality, and the formation of secondary oxidation products harmful to human health [5, 6]. Compounds such as formaldehyde (FCHO), acrolein (ACRL), and acetaldehyde (ACE), which are among these oxidation products, are classified by the International Agency for Research on Cancer (IARC) as carcinogenic or probably carcinogenic to humans. [7].

The importance of quantifying these and other carbonyl compounds has led to the development of numerous methodologies with different sample preparation techniques. For instance, Daglia et al. [8] extracted α-dicarbonyl compounds from coffee using solid phase extraction (SPE) to remove interfering compounds, followed by HPLC-DAD analysis of the derivatized analytes. Cordeiro et al. [9] proposed a methodology based on gas diffusion microextraction (GDME) followed by HPLC-DAD-MS/MS for extracting and determining 27 carbonyl

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compounds. Santos et al. [10] developed an innovative extraction technique based on fan-assisted air circulation, followed by HPLC-UV, for the extraction of 19 carbonyl compounds. Other published works have also applied dispersive liquid-liquid microextraction (DLLME) based methodologies to extract these compounds from coffee samples, such as Galuch et al. [11], for the determination of acrylamide, and, more recently, Custodio-Mendoza et al. [12], for the determination of malondialdehyde (MDA), ACRL and 4-hydroxy-2-nonenal.

Dispersive liquid-liquid microextraction (DLLME) is a widely used sample preparation technique that, despite its simplicity and efficiency, typically requires hazardous solvents in small quantities [13]. In a simplified version, DLLME is a ternary solvent system consisting of a majoritarian aqueous phase (containing the analytes), a miniaturized volume of extraction solvent (immiscible with water), and a dispersive solvent (miscible in both phases). Micro-emulsification occurs when the dispersive and extraction solvents are rapidly added to the aqueous phase, enhancing the contact surface between the extraction solvent and the aqueous matrix [14]. Phase separation is achieved through centrifugation, with the extraction solvent forming a droplet at the bottom of the conical tube containing the target analytes extracted from the aqueous matrix. The extraction and pre-concentration of the target analytes, present in the aqueous phase, is achieved through DLLME [15]. This technique is favored for its rapidity, simplicity, high enrichment factor, and efficiency [16]. However, toxic solvents, such as chloroform (CH_3Cl) and dichloromethane (CH_2Cl_2), are often used in conventional DLLME methods [15].

Recent efforts have focused on developing greener DLLME approaches in response to environmental and safety concerns. With green chemistry rapidly becoming the forefront of scientific innovation, driven by the growing demand for sustainable and environmentally friendly solutions, there is an overwhelming need to develop greener alternatives for sample preparation. The principles of green chemistry emphasize the minimization of hazardous substances, reduction of waste, and optimization of energy efficiency [17]. Green chemistry encourages the development of innovative, sustainable methodologies and the incorporation of environmentally friendly practices into existing techniques [18,19].

Efforts to align the DLLME technique with these principles include the use of less toxic extraction solvents, such as ionic liquids [20], and low-density solvents [21], as well as the integration of other sample preparation steps to reduce overall time and reagent consumption [22]. A key advancement in this field was introduced in 2013 [23], comprising ultrasound-assisted dispersive liquid-liquid microextraction (UA-DLLME) and combining the analytes derivatization and extraction into a single step. Derivatization is a widely employed additional step in sample preparation, particularly for chromatographic methods using mass spectrometry (MS), as it enhances analyte detectability by forming high molecular weight and stable compounds, thereby improving precision and sensitivity of their identification by MS [24,25]. Ultrasound-assisted extraction (UAE) is an established technique for the extraction of organic compounds from solid samples [26] and has been increasingly applied in recent years for liquid samples [27]. One key mechanism of UAW is the formation and collapse of cavitation bubbles, which continuously occur as the medium is exposed to ultrasound waves. This process enhances solvent penetration into the sample matrix, increasing the mass transfer of analytes to the solvent [28]. When UAE is combined with derivatization, increases the kinetic energy of the reaction, reducing both derivatization time and overall sample preparation time [29]. Despite the efficiency demonstrated by these DLLME methodologies, one main drawback, in terms of green chemistry principles, is the use of toxic solvents [30] such as acetonitrile (ACN) and chloroform ($CHCl_3$) [31].

The present study evaluates low-density solvents, 1-octanol and isooctane, as greener alternatives to $CHCl_3$ as an extraction solvent, and ethanol (EtOH), a more environmentally friendly solvent [32], as a substitute for ACN as a dispersive solvent. Furthermore, ortho-phosphoric acid (H_3PO_4) and hydrochloric acid (HCl) were studied, in the preparation of the derivatization reagent solution, previous to the optimization studies. Reduced aqueous and organic phase volumes were also examined, aligning with *Green Chemistry Principles*. Validation of the methodology followed the Food and Drug Administration (FDA) guidelines [33,34]. A greenness assessment was conducted by comparing recently published DLLME-based methodologies for extracting carbonyl compounds, particularly those targeted in the present work, from food matrices with the developed methodology using the AGREEprep software. Thirteen coffee samples were analyzed using the developed methodology, and the results were discussed.

2. Materials and methods

2.1. Reagents

Ethanol (CAS. 64–17–5, HPLC grade) was obtained from Scharlab (Quezon City, Philippines). 1-Octanol (CAS. 111–87–5, >99 %), isooctane (CAS. 540–84–1, for gas chromatography) and pentafluorophenylhydrazine (PFPH, CAS. 828–73–9, 97 %) were obtained from Merck (Darmstadt, Germany). Ortho-phosphoric acid (CAS. 7664–38–2, 85 % w/w) and hydrochloric acid (CAS. 7647–01–0, 32 % w/w) were obtained from VWR Chemicals (Pennsylvania, USA). Ultrapure water was obtained from a Millipore purification system (Millipore, Billerica, MA, USA).

Analytical standards used, acetaldehyde (CAS. 75–07–0, ≥99.5 %), benzaldehyde (PhCHO, CAS. 100–52–7, ≥99.5 %), diacetyl (DA, CAS. 431–03–8, ≥ 99 %), deuterated acetaldehyde (ACEd4, CAS. 1632–89–9, ≥99 atom %), formaldehyde (CAS. 50–00–0, 37 %), furfural (FUR, CAS. 98–01–1, ≥98.5 %), glyoxal (GO, CAS. 107–22–2, 40 %), malondialdehyde tetrabutylammonium salt (MDA, CAS. 100,683–54–3, ≥97 %) and methylglyoxal (MGO, CAS. 78–98–8, 40 %), all from Merck. Acrolein (107–02–8, 5000 μg/mL in methanol) was obtained from Dr. Ehrenstorfer (Augsburg, Germany).

The derivatization reagent PFPH solutions were prepared at 3 concentrations (2.8 g/L, 5.6 g/L and 11.2 g/L), using either HCl (2.0 mol/dm^3) or H_3PO_4, (10.4 mol/dm^3). These solutions were freshly prepared at the begin of each working day.

Individual standard stock solutions were prepared in methanol (CAS. 67–56–1, hypergrade for LC-MS), obtained from Merck, and stored at 4 °C. Working and internal standard (IS), ACEd4, solutions were freshly prepared with ultrapure water to the desired concentrations.

2.2. Coffee samples

Thirteen roasted ground coffee samples purchased at a local market in Santiago de Compostela, Spain, were selected for analysis. The samples were divided into 4 types of coffee: 6 samples of regular coffee (RC); 5 samples of coffee mixture (regular and torrefacto (sugar-roasted) coffee) (MC); 1 sample of regular decaffeinated coffee (RDC); and 1 sample of decaffeinated coffee mixture (MDC).

Samples were prepared for analysis by adding 0.15 g of ground coffee beans (accurately weighed) to 25.0 mL of boiling water in a flask. The flask was closed and kept under agitation for 5 min. The mixture was then allowed to cool to room temperature before analysis.

2.3. Dispersive liquid-liquid microextraction

For the preliminary studies aimed at evaluating the acid used in the derivatization reagent solution preparation, the DLLME procedure started by adding the aqueous phase (5.0 mL) to the conical-bottom centrifuge tube. This aqueous phase contained 4.5 mL of the standard solution of carbonyl compounds and the internal standard at 1 mg/L, along with 500 μL of the derivatization reagent PFPH (5.6 g/L in acid). Following, 1.3 mL of ethanol (dispersive solvent) and 90 μL of 1-octanol (extraction solvent) were mixed and rapidly added to the aqueous phase.

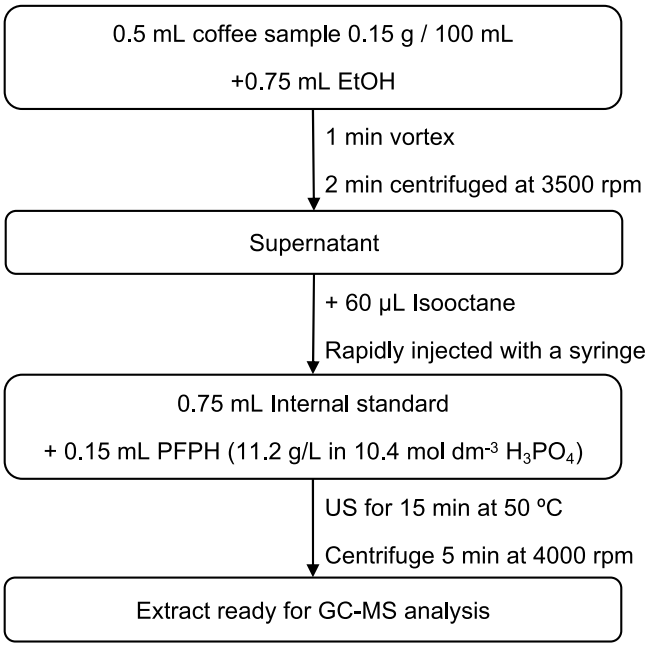

Fig. 1. Scheme of UA-DLLME-GC–MS procedure for extracting and derivatizing carbonyl compounds from coffee extracts.

The tube was then closed and placed in the ultrasonic bath (Fisherbrand, FB15055, Fisher Scientific) at 40 °C for 30 min, to allow simultaneous extraction and derivatization. The tube was then centrifuged at 4000 rpm for 5 min (P Selecta, CENTROMIX-II model 700,255, Spain), resulting in the formation of an extract drop at the top of the tube. The drop, containing the hydrazone-analytes, was collected using a 100 µL microsyringe (Hamilton, USA) and transferred to a separated vial before being analyzed by GC–MS.

Some modifications were performed in the DLLME procedure for the subsequent studies involving coffee samples. A scheme of the optimized DLLME procedure is presented in Fig. 1. In summary, a sample volume of 0.5 mL (prepared as described in Section 2.2) was added to 0.75 mL of EtOH (dispersive solvent) and vortex mixed (Hei-MIX multi Reax, Heidolph, Germany) for 1 min. The mixture was centrifuged for 2 min at 3500 rpm. The entire supernatant was collected and mixed with 60 µL of isooctane (extraction solvent). The mixture was immediately and rapidly introduced into a glass conical centrifuge tube containing 150 µL of the derivatization reagent PFPH (11.2 g/L in 10.4 mol/dm^3 H_3PO_4), and the IS, ACEd4, at 1 mg/L. The tube was closed and placed in the ultrasonic bath at 50 °C for 15 min. All subsequent steps were identical to those in the previous DLLME procedure.

2.4. GC–MS analysis

Chromatographic separation and analyte identification were carried out using 7890B-5977B GC–MS system (Agilent Technologies, CA, USA). The injector, equipped with an ultra-inert double taper liner, was operated in splitless mode at a temperature of 270 °C. A J&W HP-5MS column, Agilent Technologies, CA, US (30 m × 0.25 mm id × 0.25 µm) was used and the carrier gas used was helium, 1.0 mL/min.

The oven temperature program began at 80 °C, increasing at a rate of 10 °C/min to 150 °C, then at 30 °C/min to 280 °C, where it was held for 3 min, resulting in a total analysis time of 15.3 min. The transfer line, set at 280 °C, connected the column to an electron ionization source operating in positive mode (EI +), at 230 °C and 70 eV. A single quadrupole mass analyzer, set at 150 °C, performed a full scan (100–500 m/z) of each extracted hydrazone-analyte to identify the most intense ions. Based on these data, the selected ion monitoring (SIM) mode was used for the determination of hydrazone-analytes. A volume of 1 µL of the extract was injected for each analysis.

2.5. Method validation

The analytical method was validated following the FDA guidelines for the validation of chemical methods in food, cosmetics, and veterinary products [33], and the bioanalytical method validation guidance for industry [34], using regular coffee as the blank sample.

For each analyte and the IS, a quantifier and two qualifier ions with distinct retention times were selected. The absence of interferents within the analyte elution region confirmed the specificity of the method.

To obtain the limit of detection (LOD) and lower limit of quantification (LLOQ), a regular coffee sample with low analyte concentration was used as blank, to which three levels of concentration were added. The limits were calculated by the mean blank signal plus 3.3 (for LOD) or 10 (for LOQ) times the standard deviation of the blank signal. The obtained signal values were converted into the corresponding concentrations.

The upper limit of quantification (ULOQ) was set at 3 mg/L. Calibration curves with six concentration levels ($n = 3$) between the LLOQ and ULOQ were generated to assess the linearity of the method.

2.6. Statistical analysis

Experimental design plays an important role in optimizing research efficiency and effectiveness. By simultaneously varying multiple factors at different levels, it is possible to explore these factors' main effects and interactions with fewer experiments. NemrodW ® statistical software (LPRAI, Marseille, France) was used to generate the experimental designs, evaluate the results, and plot the effects and response.

First, an asymmetrical factorial design ($2^3 3^1$ // 8) was implemented to screen four factors at varying levels. Three variables (volume of the aqueous phase, dispersive solvent, and volume of the dispersive solvent) were studied at 2 levels, and one variable (derivatization reagent concentration) was studied at 3 levels, resulting in 8 runs. Next, a Doehlert experimental design was applied to investigate the influence of two other variables (derivatization time and temperature in the ultrasonic bath) in greater detail. For two variables, the Doehlert design includes six points forming a regular hexagon pattern, plus 3 central points [35]. This design presents advantages compared to other experimental designs, such as the ability to study variables with different levels while reducing the number of required experiments and allowing a more thorough exploration of the experimental domain [36]. Desirability functions were incorporated into the experimental design methodology to identify optimal experiment conditions [37].

3. Results and discussion

3.1. Optimization of dispersive liquid-liquid microextraction

In UA-DLLME, both the dispersive and extraction solvents are crucial for efficient analyte extraction from complex matrices and must meet specific criteria. The dispersive solvent, which facilitates the dispersion of the extraction solvent in the aqueous sample, must be miscible with both aqueous and organic solvents [38]. EtOH, a green solvent previously used by Jain et al. [39] as a dispersive solvent for the extraction of parabens from different matrices (food, cosmetic, and water), was chosen as a greener alternative to other conventional dispersive solvents, such as ACN. The extraction solvent must be either immiscible or have low miscibility with water, have a high extraction capability for the target analytes, and be able to form an emulsion with the mixture of dispersive solvent and aqueous phase [40]. Traditionally, these solvents were required to have a higher density than water. However, with the growing emphasis on greener methodologies, low-density and low toxicity solvents are becoming preferred for DLLME, such as n-hexane, 1-octanol, toluene, and isooctane [21,41]. Among these solvents,

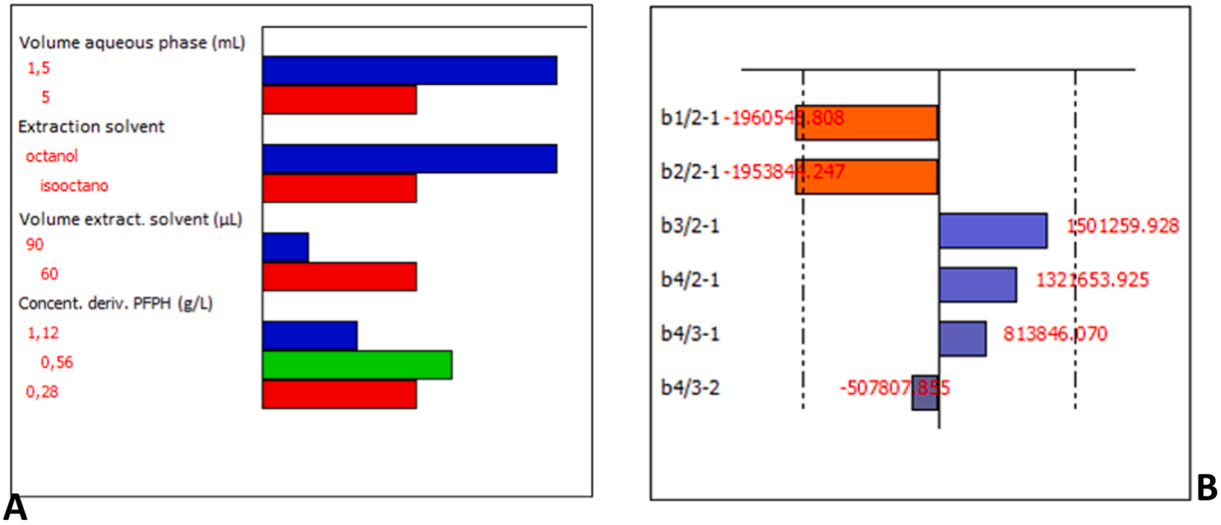

Fig. 2. A) Total effects and B) Delta Weight plots of the asymmetrical screening design $2^{3}3^{1}$ // 8 for diacetyl.

1-octanol and isooctane were selected for evaluation as extraction solvents in this study.

1-Octanol has already been demonstrated as a viable alternative to chlorinated solvents [42], offering low toxicity [43], FDA approval for human use [44], and high biodegradability (as reported in the Sigma Aldrich safety data sheet). Additionally, it can be sourced from renewable materials [42]. Isooctane also exhibits low toxicity [45]. When assessed against other commonly used solvents, Tobiszewski et al. [46] ranked isooctane 43rd out of 78 solvents (six out of sixteen non-polar solvents included) in their Environmental Risk Ranking of Solvents, indicating that although not entirely ideal, its environmental impact remains significantly lower than that of any chlorinated solvent.

3.1.1. *Derivatization reagent solution composition*

A preliminary study was conducted to evaluate the impact of the acid in the derivatization reagent solution (PFPH) by comparing HCl and H_3PO_4. Stock solutions of PFPH were prepared using 2.0 mol/dm^3 HCl and in 10.4 mol/dm^3 H_3PO_4 (as described in Section 2.1), to maintain an equal pH value (0.7), during the simultaneous extraction-derivatization process. For this initial study, specific experimental conditions were selected based on prior experience. These included a total aqueous phase volume of 5.0 mL, 90 µL of 1-octanol, 1.3 mL of EtOH, 0.56 g/L of PFPH, and 40 °C and 30 min for derivatization. The derivatization process was conducted in an ultrasonic bath under these temperature and time conditions. Carbonyl compounds were extracted from a model solution of carbonyl compounds, 1 mg/L, using PFPH solutions prepared with either HCl or H_3PO_4. The resulting extracts were analyzed by GC–MS, and the chromatographic peak areas obtained for each carbonyl compound were compared.

Not all analytes exhibited the same behavior. GO and FUR showed lower responses when using H_3PO_4, with a peak reduction of 20–50 % compared to HCl. In contrast, ACRL and ACE yielded better results with H_3PO_4, displaying a 15–30 % increase in peak area compared to HCl. For the remaining carbonyl compounds, the peak area increased by >200 % when using H_3PO_4 instead of HCl. Based on these findings, H_3PO_4 was selected as the acid for preparing the derivatization reagent solution.

3.1.2. *Experimental designs*

The optimization of UA-DLLME conditions to enhance the simultaneous extraction-derivatization efficiency of carbonyl compounds from coffee samples was performed in two steps, employing two different experimental designs. Optimization studies were carried out using a sample of regular coffee (0,60 g / 100 mL) prepared as described in Section 2.2, spiked with 1 mg/L of the 9 target analytes and 1 mg/L of IS.

An asymmetrical screening design $2^{3}3^{1}$ // 8 was applied to evaluate the influence of four parameters on the simultaneous extraction-derivatization efficiency: b_1 – volume of the aqueous phase (1.5 mL and 5.0 mL); b_2 – type of extraction solvent (1-octanol and isooctane); b_3 – volume of the extraction solvent (90 µL and 60 µL); and b_4 – PFPH concentration (1.12 g/L, 0.56 g/L and 0.28 g/L). The sample volume remained constant at 0.5 mL across all experiments. The volume of EtOH was adjusted in proportion to the aqueous phase volume to maintain a consistent aqueous-to-dispersant solvent ratio. Specifically, 0.75 mL of EtOH was used for a 1.5 mL aqueous phase, while 1.3 mL of EtOH was used when the aqueous phase volume increased to 5.0 mL.

The results for each carbonyl compound were plotted in Total Effect Plots and Delta Weight (Fig. 1S, Supplementary Information). In the Total Effect Plots, bar length is proportional to the response (chromatographic peak area) obtained for each parameter. For example, Fig. 2A illustrates that increasing the aqueous phase volume in DA extraction reduced the peak area by half. The Delta Weight plot depicts the effect of changing a parameter's level on the chromatographic response. represents the influence of changing a level of a parameter in the given response. For instance, $b_{1/2-1}$ represents the impact of changing the volume of aqueous phase (b_1) from level 1 (1.5 mL) to level 2 (5.0 mL) in the chromatographic peak area. This response is also proportional to the bar length, and the side to which the bar goes shows a negative (left) or positive (right) effect. The dotted lines represent the statistical significance level (95 % confidence level), so an effect is considered statistically significant if the bar length exceeds the dotted lines. In Fig. 2B, the increase of the aqueous volume phase ($b_{1/2-1}$), from 1.5 mL to 5.0 mL, presents a negative effect in the simultaneous extraction of DA. In contrast, a positive effect is presented with the reduction of extraction solvent volume ($b_{3/2-1}$), from 90 to 60 µL. This reduction in extraction solvent volume enhanced the enrichment factor, improving analyte extraction efficiency within the smaller extraction solvent droplet collected at the end of the procedure.

Based on the Delta Weight and Total Effect plots, the optimal extraction solvent volume for all carbonyl compounds is 60 µL. A lower aqueous phase volume (1.5 mL) yielded higher responses for most analytes except for GO. Regarding the type of extraction solvent used, isooctane performed better for 5 (ACEd4, PhCHO, FCHO, GO, and MDA), while ACE, ACRL, and FUR showed similar results with both extraction solvents. For the remaining two compounds (MGO and DA), 1-octanol provided superior results. Additionally, PFPH concentration did not have a uniform effect accross all analytes. However, 1.12 g/L produced the best overall performance.

Ultimately, based on the presented plots and obtained results, the

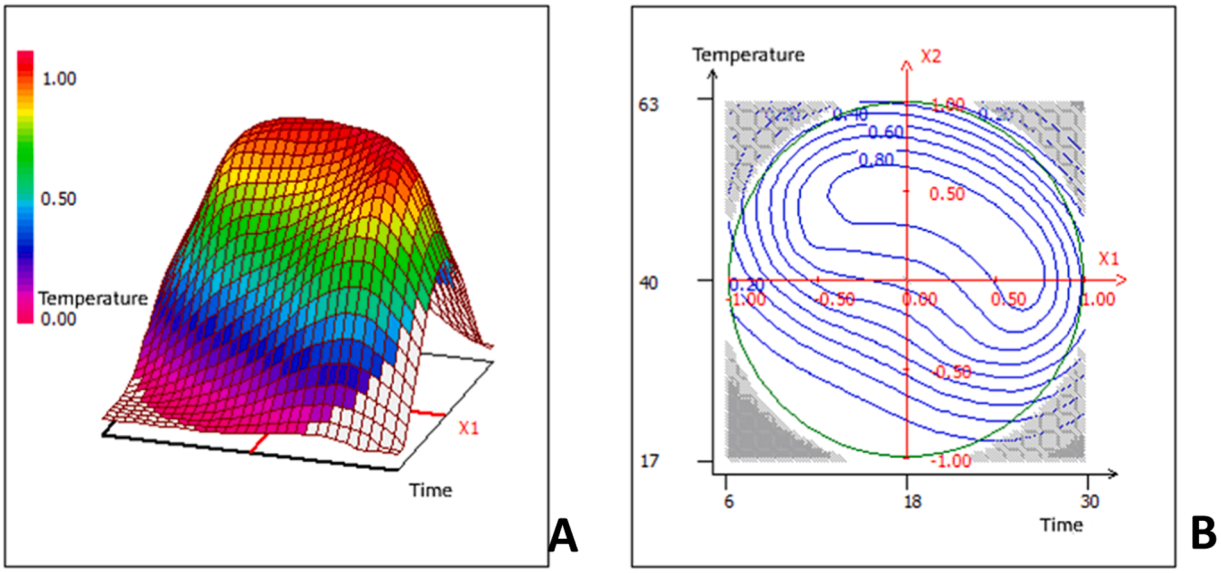

Fig. 3. Surface response plot: A – three-dimensional, B – Two-dimensional; for a two-variable Doehlert design regarding time and temperature of derivatization for all the target carbonyl compounds.

Table 1
Parameters to evaluate the method's specificity, sensitivity and linearity, and the limits of detection and quantification, for the determination of carbonyl compounds in coffee samples.

Analyte	RT	Quantifier ion	Qualifier ion		r^2	LOD	LLOQ
	min	m/z	m/z	m/z		µg/L	µg/L
FCHO	5.266	210	211	162	0.9997	377.9	388.7
MDA	5.462	234	117	180	0.9995	348.3	348.4
ACEd4*	6.346	228	227	229	——-	——-	——-
ACE	6.395	224	225	223	0.9993	383.0	421.9
ACRL	7.671	236	115	235	0.9991	304.1	304.5
DA	9.586	266	267	155	0.9991	299.7	300.9
MGO	9.783	252	155	182	0.9993	271.1	289.0
FUR	10.841	276	277	181	0.9998	362.2	370.4
PhCHO	11.452	286	405	327	0.9990	374.6	375.6
GO	12.483	235	131	183	0.9991	435.8	436.1

* Used as IS.

following optimized parameters were selected: 60 µL of isooctane (type and volume of extraction solvent), 1,5 mL (volume of aqueous phase), and 1.12 g/L (PFPH concentration).

Following the optimization of extraction parameters, a two-variable Doehlert experimental design was employed to refine the derivatization conditions for carbonyl compounds with PFPH. Derivatization time was examined at 5 levels (6, 12, 18, 24, and 30 min), while derivatization temperature was studied at 3 levels (20, 40, and 60 °C). The software generated 9 experimental runs, 3 of which were performed under identical conditions as replicated of the central point of the design. All experiments were conducted in a randomized order, as instructed by the software.

A surface response plot was generated for each carbonyl compound (Fig. 2S). Most analytes showed similar behaviors, yielding optimal results near the central point (18 min at 40 °C). However, MGO, ACE, and ACEd4 showed different trends. The surface response plot in Fig. 3 illustrates the multi-criteria decision analysis, based on desirability functions, used to predict the optimal conditions. The best results produced a plateau in the surface response plot, reveling a correlation between time and temperature; a shorter derivatization time was required at higher temperatures to achieve similar results. For instance, derivatization at 50 °C for 15 min yielded comparable results to derivatization at 40 °C for 25 min. Based on these findings, the optimized derivatization conditions were set at 15 min at 50 °C for further studies.

3.2. Analytical validation

The developed methodology was validated according to the FDA guidelines, and the results of the evaluated parameters are summarized in Table 1 and Table 2. Method specificity and selectivity were evaluated

Table 2
Accuracy, precision and matrix effect studies of UA-DLLME-GC–MS methodology for the determination of 9 carbonyl compounds in coffee samples.

Analyte	Accuracy			Precision						Matrix effect	
	% Recovery ($n = 3$)			Intraday ($n = 5$)			Interday ($n = 5$)				
	500 µg/L	1000 µg/L	2000 µg/L	500 µg/L	1000 µg/L	2000 µg/L	500 µg/L	1000 µg/L	2000 µg/L	F-test	*t*-test
FCHO	99.69	105.47	95.52	5.71	3.54	3.25	4.24	4.74	3.10	1.75	2.57
MDA	90.02	101.09	106.41	3.57	6.28	4.27	4.84	7.82	4.20	2.14	2.19
ACE	107.41	104.17	98.53	8.37	4.39	4.91	9.56	5.37	6.91	3.27	1.25
ACRL	108.43	103.08	96.79	6.08	5.85	5.64	6.80	9.03	8.23	3.80	0.14
DA	104.64	100.53	102.19	5.49	3.42	2.67	7.78	4.30	2.59	2.99	0.54
MGO	94.24	93.92	101.62	5.52	7.83	5.17	5.80	8.78	9.03	1.62	0.76
FUR	90.18	96.92	96.71	2.85	6.24	4.75	7.35	7.77	5.40	4.56	1.62
PhCHO	108.49	109.47	106.20	8.05	3.90	5.63	8.49	8.79	9.43	1.23	1.04
GO	106.93	99.40	105.48	6.46	6.98	4.24	8.78	8.35	3.26	2.49	0.51

Intraday and interday studies are presented as relative standard deviation (%).
$t_{critic} = 3.18$ (95 %, $U = 4$); $F_{critic} = 18.51$ (95 %, $U_1 = 1$ and $U_2 = 2$).

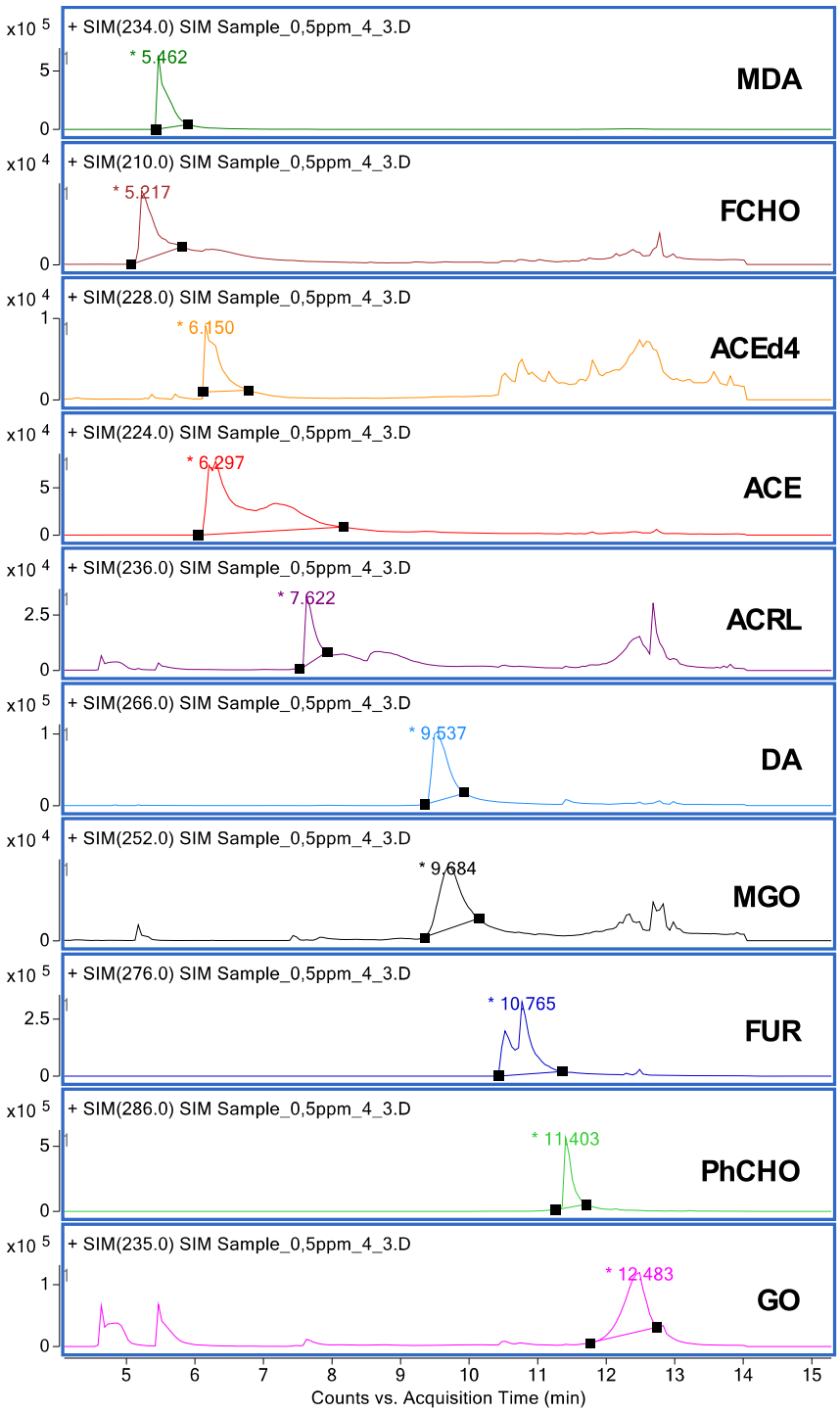

Fig. 4. Chromatogram of 0.5 mg/L of MDA, FCHO, ACE, ACRL, DA, MGO, FUR, PhCHO and GO extracted by UA-DLLME from coffee. ACEd4 was used as IS at of 1 mg/L.

by selecting a quantifier and two qualifier ions for each analyte, each with different retention times. As shown in Fig. 4 and Table 1, the carbonyl compounds have different quantifier ions and retention times. No interferences were observed in the GC–MS chromatograms of the coffee samples.

Linearity, limits of detection and quantification, and sensitivity for the target analytes are summarized in Table 1. ACEd4 was used as IS at a concentration of 1000 μg/L. Using the standard addition method with IS, a concentration range of 300 μg/L to 3000 μg/L was evaluated for all target analytes, except for ACE, which had a starting concentration of 500 μg/L. The calibration curves exhibed excellent linearity, with $r^2 \geq 0.9990$. Table 1 provides the LOD and LLOQ concentrations for all target analytes, while the ULOQ was set at 3000 μg/L.

Matrix effect, accuracy, and precision are summarized in Table 2. The matrix effect was evaluated by performing the statistical comparison of the slopes obtained for the calibration curve and the standard addition method [47], for each analyte, using three concentration levels ($n = 3$). The values obtained for the F-test and *t*-test, compared to their respective critical values, indicated that the developed methodology showed no significant matrix effect for carbonyl compound extraction

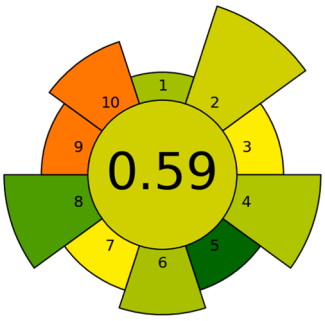

Fig. 5. Graph of AGREEprep assessment of the environmental impact associated with the methodology's sample preparation.

from coffee samples. The standard addition method was chosen, as coffee samples with different and more complex compositions were analyzed. The standard addition method was also used to evaluate the recovery ($n = 3$) and intraday and interday precision, measured as % relative standard deviation (RSD) ($n = 5$). Both were assessed at 3 concentration levels. Recovery values ranged from 90 % and 110 %. Intraday precision was below 8.4 %, while interday precision did not exceed 9.6 %.

3.3. Greenness assessment

The developed methodology offers a more environmentally friendly alternative for extracting carbonyl compounds from coffee samples using the US-DLLME technique.

AGREEprep open access software [48] was employed to evaluate the environmental and safety impact of the developed methodology. This tool assesses the methodologies according to the principles of green chemistry, considering parameters such as reagents' toxicity, waste generation, energy consumption, and sustainability. The methodology is evaluated across 10 criteria, each receiving a score from 0 (poorest performance) to 1 (optimal performance). A final score (ranging from 0 to 1) is calculated using predefined weights for each criterion. The developed UA-DLLME-GC–MS methodology achieved an AGREEprep score of 0.59 (Fig. 5). The individual criterion scores are detailed in the AGREEprep report (Supplementary information). The overall positive score highlights the methodology's favorable environmental and safety performance, with six of the ten criteria scoring above 0.5. Notably, criterion 8 received a high score due to the low energy consumption per analysis, attributed to the use of an ultrasonic bath, which enables the simultaneous preparation of multiple samples. This underscores the method's alignment with green chemistry principles. Only two criteria presented scores below 0.5, post-sample preparation configuration for analysis (criterion 9) and operator's safety (criterion 10).

To contextualize the current methodology, Table 3 summarizes key features from other methodologies reported in the literature, employing the DLLME technique for carbonyl compounds extraction from food matrices, providing a comparative framework.

Firstly, it is important to highlight that many reported methodologies require additional sample preparation steps before or after the DLLME process. These steps include pH adjustment, derivatization, or dilution in an organic solvent. However, two reviewed methodologies

Table 3
Published methodologies for the extraction of carbonyl compounds targeted in this work, from food matrices, using DLLME technique.

Carbonyl compound	Food matrix	Sample preparation pre-DLLME	Steps included in DLLME		Extraction solvent		Dispersive solvent		Instrument for analysis	Ref
			Steps	Time	Solvent	Volume (µL)	Solvent	Volume (µL)		
MDA DA FCHO MGO ACE FUR ACRL PhCHO GO	Coffee	Interferent precipitation with dispersive solvent	Ultrasound-assisted derivatization and extraction Centrifugation	15 min 5 min	Isooctane	60	EtOH	750	GC–MS	Current work
FCHO ACE (5 more aldehydes)	Drinking water Alcoholic beverages	Filtration pH adjustment Derivatization[a]	Centrifugation	1 min	DES	100	ACN	700	HPLC-UV/Vis	[52]
ACE ACRL FCHO MDA (2 more aldehydes)	Edible oils	GDME with derivatization[a] and ultrasound agitation	Sonification Centrifugation	5 min 2 min	$CHCl_3$	70	ACN	750	GC–MS	[24]
FCHO	Beverages	Dilution pH adjustment Filtration	Microwave-assisted derivatization[a] and extraction Centrifugation[#]	90 s 10 min	Ionic liquid	70	ACN	400	HPLC-UV/Vis	[49]
MDA ACRL 4-Hydroxy-2-nonenal	Beverages	Interferent precipitation with dispersive solvent	Ultrasound-assisted derivatization[a] and extraction Centrifugation	5 min 2 min	$CHCl_3$	90	ACN	1300	GC–MS	[12]
GO[$]	Alcoholic beverages	pH adjustment Interferent precipitation with dispersive and extraction solvents	Vortex Centrifugation[$]	1 min 5 min	CH_2Cl_2	150	Butan-1-ol	750	HPLC-FD	[54]
FUR Hydroxymethylfurfural	Baby formula	Hydrolyzation Saponification pH adjustment Protein precipitation	Shaking Centrifugation	2 min 2 min	1-octanol	60	EtOH	650	HPLC-UV/Vis	[53]

[a] DNPH as derivatization reagent.
[#] After extraction the ionic liquid phase was dissolved in 100 µL ACN.
[$] After extraction the upper phase was collected, the pH adjusted, and methanol was added before analysis.

Table 4
Quantification of the studied analytes in different coffee samples. All results are presented in μg/L.

Coffee sample	FCHO	MDA	FCHO	ACE	ACRL	DA	MGO	FUR	PhCHO	GO
RC1	NQ	NQ	NQ	602 ± 33	NQ	ND	ND	1623 ± 62	ND	NQ
RC2	NQ	NQ	NQ	613 ± 31	NQ	ND	ND	NQ	ND	NQ
RC3	NQ	NQ	NQ	591 ± 38	NQ	ND	ND	NQ	ND	NQ
RC4	NQ	NQ	NQ	538 ± 33	NQ	ND	ND	NQ	834 ± 1	NQ
RC5	NQ	NQ	NQ	541 ± 34	NQ	ND	ND	NQ	764 ± 31	NQ
RC6	NQ	NQ	NQ	631 ± 31	NQ	ND	ND	2659 ± 122	583 ± 50	NQ
MC1	NQ	NQ	NQ	601 ± 35	NQ	ND	ND	2650 ± 147	ND	NQ
MC2	NQ	NQ	NQ	597 ± 36	NQ	ND	ND	1890 ± 146	ND	NQ
MC3	NQ	NQ	NQ	574 ± 32	NQ	ND	ND	2250 ± 125	418 ± 24	NQ
MC4	NQ	NQ	NQ	622 ± 31	NQ	ND	ND	NQ	585 ± 37	NQ
MC5	NQ	NQ	NQ	597 ± 36	NQ	ND	ND	NQ	930 ± 57	NQ
RDC1	NQ	NQ	NQ	611 ± 31	NQ	ND	ND	NQ	NQ	NQ
MDC1	NQ	NQ	NQ	600 ± 37	NQ	ND	ND	NQ	ND	NQ

NQ – detected, but not quantifiable.
ND – not detected.

incorporate the derivatization reaction in the extraction procedure [12,49], as does the present work, thereby decreasing the overall sample preparation time.

Some of the presented methodologies use high-density solvents, such as $CHCl_3$ and CH_2Cl_2 as extraction solvents and ACN as the dispersive solvent, but none of them are included in the green solvents category [50,51]. Nevertheless, other studies present promising green alternatives [49,52,53], introducing deep eutectic solvents (DES), ionic liquids, and 1-octanol as extraction solvents. Regarding the dispersive solvent, only two methodologies use green solvents [53,54], namely butan-1-ol and EtOH. Of the presented methods, only one [53], employs both extraction and dispersive solvents, categorized as green solvents.

Regarding the analysis following the DLLME process, most methodologies use liquid chromatography coupled with spectrophotometric detection [49,52,53]. An exception is the analysis of GO, which employs fluorometric detection [54]. Meanwhile, two works use GC–MS [12,24], as does the present study. GC–MS is considered a greener chromatographic technique for solvent consumption and waste generation compared to liquid chromatography.

Compared to the presented methodologies, the developed methodology offers notable advantages for determining carbonyl compounds in food matrices. It allows the extraction and determination of a broader range of carbonyl compounds, including PhCHO, for which no DLLME-based methods for food matrices have been reported. Furthermore, this work highlights the feasibility of extracting carbonyl compounds using green solvents in the DLLME procedure.

3.4. Carbonyl compounds occurrence in coffee samples

The developed methodology was applied to determine the target carbonyl compounds in 13 coffee samples. The samples were analyzed in triplicate and the standard addition method with IS was used for analyte quantification. The results are presented in Table 4.

ACE was detected at similar concentrations (541 to 631 μg/L) across all samples. PhCHO was quantifiable in 3 regular coffee samples (583 to 834 μg/L) and 3 of the mixture coffee samples (418 to 930 μg/L), but its concentration in decaffeinated coffee samples was below the LLOQ. FUR quantification was possible in 2 of the regular coffee samples (1623 to 2659 μg/L) and 3 of the mixture coffee samples (1890 to 2650 μg/L). GO, FCHO, MDA, and ACRL were detected in all samples; however, their concentrations were below the LLOQ. MGO and DA were not detected in any sample.

Table 1S, in the Supplementary Material, compares the concentrations of carbonyl and dicarbonyl compounds reported in previous studies. The results suggest that higher levels of GO, MGO, and DA were observed in earlier studies compared to this work.

Chen et al. [55] reported GO (1.2–1.7 μg/g), MGO (0.4–18.7 μg/g), and DA (10.3–23.7 μg/g) in green coffee beans with varying roasting degrees, using liquid-liquid extraction (LLE) and dinitrophenylhydrazine (DNPH) derivatization, followed by UHPLC—Orbitrap-MS analysis. Similarly, Papetti et al. [56] found GO (0.23–2.66 μg/g), MGO (0.07–2.4 μg/g), and DA (0.03–0.36 μg/g) in roasted coffee, while Lee et al. [57] reported GO (0.81–7.74 μg/g), MGO (18.53–89.08 μg/g), and DA (0.43–6.74 μg/g) in Arabica coffee beans subjected to acidic soaking before preparation.

Furfural concentrations were also higher in roasted coffee (109–200 μg/g, [58]) than in this study. However, similar levels of FCHO and ACE were detected in coffee beverages by Jeong et al. [59], while Liu et al. [60] reported comparable FUR concentrations in Chinese coffee products, aligning with the present findings.

The elevated levels of carbonyl and dicarbonyl compounds observed in previous studies are likely attributed to processing conditions, such as coffee bean roasting and acidic treatments before preparation, which facilitate the formation and release of these compounds. The consistency of this study's results with those from previous research supports the robustness of the developed methodology for the simultaneous determination of carbonyl compounds in coffee extracts.

4. Conclusion

The developed UA-DLLME-GC–MS method enabled the simultaneous extraction-derivatization of nine carbonyl compounds (malondialdehyde, formaldehyde, acetaldehyde, acrolein, diacetyl, methylglyoxal, furfural, benzaldehyde, and glyoxal) from coffee extracts. Optimization of the sample preparation step was achieved through studies of key parameters using two experimental designs.

The developed methodology provides a greener alternative to previously reported methods, utilizing isooctane and ethanol as extraction and dispersive solvents, respectively, and achieving an AGREEprep score of 0.59.

Validation conducted according to FDA guidelines confirmed excellent selectivity, specificity, sensitivity, linearity and accuracy, with no observed matrix effect and recovery values ranging between 90 and110 % for all analytes.

Among the studied analytes, three were quantified in all coffee samples: acetaldehyde, benzaldehyde, and furfural. Benzaldehyde was quantifiable in only 6 of the analyzed coffee samples, while furfural was quantifiable in 5.

CRediT authorship contribution statement

Alexandra Rangel Silva: Writing – original draft, Validation, Investigation, Formal analysis, Data curation. **Jorge A. Custodio-Mendonza:** Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **João**

Rodrigo Santos: Writing – review & editing, Supervision, Funding acquisition, Formal analysis, Conceptualization. **Paulo J. Almeida:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization. **José A. Rodrigues:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization. **Antonia M. Carro:** Writing – review & editing, Supervision, Software, Resources, Project administration, Methodology, Funding acquisition, Conceptualization.

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.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2025.465743.

Data availability

Data will be made available on request.

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