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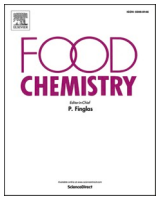

Analytical Methods

Development and validation of a green analytical method for simultaneously determining plasticizers residues in honeys from different botanical origins

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ABSTRACT

A novel method has been proposed to determine nine plasticizers in honey samples by gas chromatography–mass spectrometry. An efficient sample treatment was proposed (average analyte recoveries between 77% and 118%) involving a double solvent extraction with ethyl acetate, followed by a clean-up step with florisil. Chromatographic analysis (< 21 min) was performed in an Agilent HP-5MS column under programmed temperature conditions. The greenness of the method was assessed with different tools that classified it as environmentally friendly. The method was validated in terms of selectivity, limits of detection (0.1–3.1 μg kg^{-1}) and quantification (0.2–10.3 μg kg^{-1}), linearity, matrix effect, trueness, and precision (relative standard deviation <9%). An analysis of thirty samples from different sources (commercial or experimental apiaries) revealed the presence of residues of five plasticizers in most of the samples. Finally, health risk assessment was evaluated, and the results indicated no associated health risks for consumers.

1. Introduction

Honey, a universally sweet food, is consumed worldwide. Due to its widespread popularity, it necessitates standards and regulations ensuring its identity, quality and safety. These measures are crucial for enabling its unrestricted circulation in both domestic and international markets (Food and Agriculture Organization of the United Nations, 2022). Numerous studies have focused on assessing the presence of contaminants in honey like metals, pesticides, antibiotics and veterinary drugs (Bonerba et al., 2021; Brugnerotto et al., 2023; Fuente-Ballesteros et al., 2023; Makni, Diallo, Areskoug, Guérin, & Parinet, 2023; Yang, Lin, Liu, & Lin, 2022; Valverde et al., 2018). However, nowadays, the investigation of plasticizers is a trending research topic, and there is a remarkable interest in examining plastic pollution due to the widespread use of these materials and their ubiquity. Plasticizers, which are employed to augment the elasticity, flexibility, color, resistance, and longevity of diverse plastic polymers employed in food packaging applications, lack chemical bonds with the polymer chains present in the plastics, thereby enabling the potential migration from the packaging materials into the food substances they are in contact with, gradually over time (Peñalver, Arroyo-Manzanares, Campillo, & Viñas, 2021). A plethora of plasticizers, among them phthalate esters (PAEs), adipates, and related chemical compounds like ethers, are presently employed in food packaging (Cohen, Richardson, March, Gosliner, & Hauser, 2023;

Abbreviations: AF, samples spiked after sample treatment; AGREE, analytical greenness calculator; BBP, benzyl butyl phthalate; 4-BDE, 4-bromodiphenyl ether; BF, samples spiked before sample treatment; bw, body weight; 4-CDE, 4-chlorodiphenyl ether; CI, mean plasticizer concentration in honey samples; DBP, dibutyl phthalate; DBP-d_4, dibutyl phthalate-3,4,5,6-d_4; DEHA, bis(2-ethylhexyl)adipate; DEHP, bis(2-ethylhexyl)phthalate; DEP, diethyl phthalate; DLLME, dispersive liquid-liquid microextraction; DMP, dimethyl phthalate; DNOP, di-n-octyl phthalate; EDI, estimated the daily intake; EI, electronic impact; GAC, green analytical chemistry; GAPI, green analytical procedure index; GC, gas chromatography; GC–MS, gas chromatography–mass spectrometry; HDEs, halodiphenyl ethers; HI, hazard index; HPLC, high performance liquid chromatography; HQ, hazard quotient; IR, ingestion rate; IS, internal standard; LOD, limit of detection; LOQ, limit of quantification; m/z, mass-to-charge; MS, mass spectrometry; MS/MS, tandem mass spectrometry; PAEs, phthalates; PET, polyethylene terephthalate; PVC, polyvinyl chloride; QuEChERS, quick, easy, cheap, effective, rugged & safe; R^2, determination coefficient; RfD, reference dose; RSD, relative standard deviation; S/N, signal-to-noise; SCI, slope confidence intervals; SE, solvent extraction; SIM, selected ion monitoring; SMLs, specific migration limits; SPE, solid phase extraction; TDI, tolerable daily intake values..

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Lu et al., 2023; Özgür, Aktürk, Köseoğlu, Onac, & Akdoğan, 2023). PAEs, notably dimethyl phthalate (DMP), diethyl phthalate (DEP), dibutyl phthalate (DBP), benzyl butyl phthalate (BBP), bis(2-ethylhexyl) phthalate (DEHP) and di-n-octyl phthalate (DNOP) have a prominent role within the polymer industry. These PAEs find extensive use in various food-packaging materials, including paper, cardboard, plastics, metal closures of glass jars, cans, tetra bricks, and in food processing (Dobaradaran et al., 2020). Bis(2-ethylhexyl)adipate (DEHA), a widely used plasticizer, is found in food contact materials such as polyvinyl chloride (PVC) food wrapping film and polyethylene terephthalate (PET) bottles (Lo Turco et al., 2016). In addition, during the plastic production process, brominated flame retardants serve as essential safety components. Among these, halodiphenyl ethers (HDEs), such as 4-bromodiphenyl ether (4-BDE) and 4-chlorodiphenyl ether (4-CDE) are a commonly utilized type, found in various plastic products like electronic thermoplastics and textiles (Alabi, Ologbonjaye, Awosolu, & Alalade, 2019). These three groups of compounds, PAEs, adipates, and HDEs, pose detrimental effects on human health. They are linked to fertility issues and adverse impacts on newborn development, and they also act as endocrine disruptors with pronounced effects on women's health due to their physiology (Giuliani, Zuccarini, Cichelli, Khan, & Reale, 2020; Songue Same et al., 2023). According to that, the European Commission defined specific migration limits (SMLs) on plastic materials and articles intended to come into contact with food (European Commission, 2011, 2023).

These substances have been detected in various food matrices, such as rice, cereals, mill, sweets, fruits, vegetables, eggs, meat, fish, or oil (Giuliani et al., 2020; González-Sálamo, Socas-Rodríguez, & Hernández-Borges, 2018; Martínez-Gómez, Elmore, Valverde, Ares, & Bernal, 2024). However, previous research examining the presence of plasticizers in honey remains relatively limited (Cao, Zhao, & Dabeka, 2015; Di Fiore et al., 2023; Kartalovic, Vranešević, Petrović, Đurđević, & Ratajac, 2021; Koo, Yahaya, & Omar, 2017; Lo Turco et al., 2016; Massous et al., 2023; Notardonato et al., 2020a, 2020b; Peñalver et al., 2021; von Eyken, Ramachandran, & Bayen, 2020; Zhou et al., 2014; see Supplementary Material, Table 1S), and authors do not make a distinction regarding the botanical origin of the honey samples when optimizing and validating the method. Gas chromatography (GC) and high performance liquid chromatography (HPLC) are the most widely employed techniques for the analysis of these substances in food samples, since they provide high sensitivity and selectivity (Martínez-Gómez et al., 2024). However, as shown in Table 1S (see Supplementary Material) the most employed separation technique when analyzing honey samples is GC. This preference stems from the thermal stability and volatile characteristics exhibited especially for PAEs. Regarding to the detectors, mass spectrometry (MS) or tandem mass spectrometry (MS/MS) has been selected in all publications regardless of the chromatographic technique used (see Supplementary Material, Table 1S). This choice in mainly related to specificity and high sensitivity of these detectors that are required when analyzing these compounds at low concentration levels in complex matrices, like honey. Concerning the most commonly employed sample treatments to determine the selected compounds in honey, an analysis of the existing literature (see Supplementary Material, Table 1S) indicates that solvent extraction with different solvents (hexane, acetonitrile, acetone, dichloromethane and ethyl acetate) and dispersive liquid-liquid microextraction (DLLME) alone or in combination with other technique, ultrasound (Notardonato et al., 2020a, 2020b) predominate. Other alternatives, like solid phase extraction (SPE) employing a polymeric sorbent (Lo Turco et al., 2016), and QuEChERS (quick, easy, cheap, effective, rugged and safe) protocol (Massous et al., 2023), were also employed in some studies. It should be specified that out of the total papers available in the databases focusing on the analysis of PAEs, adipates, or ethers in honey samples, 92% examined PAEs and 34% investigated adipates. However, none of these studies investigated HDEs (see Supplementary Material, Table 1S).

Therefore, the main goal of the present study is to propose a method for determining simultaneously of six PAEs (BBP, DBP, DEHP, DEP, DMP, DNOP), one adipate (DEHA), and two HDEs (4-BDE and 4-CDE) in honeys from three different botanical origins (multifloral, rosemary, and heather) using GC–MS. It must be specified that in this work we have decided to select honey from three botanical origins (multifloral, rosemary, heather), to develop and validate the method for the following reasons: **i**) they have very different compositions and physicochemical characteristics. So, if the method is valid for these botanical origins, it would be probably valid for other types of honey. This allows increasing the relevance, validity, and interest of the proposed method; **ii**) these are three of the botanical origins that are most found in supermarkets and produced by beekeepers. Another objective of this work is to propose an efficient sample treatment applicable to honeys from different origins. These conditions aim to ensure good recoveries, minimize the potential matrix effect, and adhere as closely as possible to the principles of green chemistry. Indeed, analytical greenness calculator (AGREE), AGREEprep and complex green analytical procedure index (GAPI) metrics were applied to evaluate the greenness of the developed method. To the best of our knowledge, this is the first study in which an analytical methodology has been proposed for determining these substances in different types of honey, and that it has been evaluated in accordance with various green analytical metrics. Our study also aims to validate the proposed method in accordance with current European legislation (EURACHEM, 2014) and analyze experimental and commercial honey samples from the aforementioned origins.

Table 1
Ions (quantification and confirmation), limits of detection (LODs), quantification (LOQs), and specific migration limits (SMLs) of the studied plasticizers.

Compound	QI (*m/z*)	CI (*m/z*)	MH		HH		RH		SMLs* (mg kg^{-1})
			LOD (μg kg^{-1})	LOQ (μg kg^{-1})	LOD (μg kg^{-1})	LOQ (μg kg^{-1})	LOD (μg kg^{-1})	LOQ (μg kg^{-1})	
Dimethyl phthalate (DMP)	163	77, 92	0.1	0.2	0.4	1.2	0.1	0.2	60
Diethyl phthalate (DEP)	149	177, 176	0.1	0.2	0.1	0.2	0.1	0.3	60
4-Chlorodiphenyl ether (4-CDE)	204	206; 141	0.1	0.4	0.2	0.7	0.1	0.4	60
4-Bromodiphenyl ether (4-BDE)	248	141, 77	0.1	0.5	0.2	0.8	0.1	0.4	60
Dibutyl phthalate (DBP)	149	150, 205	0.1	0.1	0.5	1.8	0.2	0.6	0.12
Benzyl butyl phthalate (BBP)	149	91, 206	0.1	0.2	0.1	0.2	0.1	0.2	6
Bis(2-ethylhexyl)adipate (DEHA)	129	112, 147	0.1	0.5	0.1	0.4	0.1	0.4	18
Bis(2-ethylhexyl)phthalate (DEHP)	149	167, 279	0.1	0.3	0.3	0.9	0.1	0.4	0.6
Di-n-octyl phthalate (DNOP)	149	279, 150	0.2	0.7	3.1	10.3	0.5	1.7	60
Dibutyl phthalate-3,4,5,6-d_4 (DBP-d_4)	153	227							

* According to. European Commission, 2011, 2023. **CI,** confirmation ion; **HH**, heather honey; **LOD**, limit of detection; **LOQ**, limit of quantification; **MH**, multifloral honey; **QI**, quantification ion; **RH**, rosemary honey.

2. Materials and methods

2.1. Reagents and materials

PAEs, adipate, and HDEs standards (BBP, DBP, dibutyl phthalate-3,4,5,6-d_4 (DBP-d_4), DEHP, DEP, DMP, DNOP, DEHA, 4-BDE and 4-CDE; see structures in Supplementary Material, Table 2S), all of analytical-grade and with purity >98%, were purchased from Sigma-Aldrich Chemie Gbmh (Steinheim, Germany). An isotope labeled standard (DBP-d_4) was chosen as internal standard (IS), since it has the same physical and chemical properties as the unlabeled analyte. Dichloromethane (HPLC grade) was supplied by Lab Scan Ltd. (Dublin, Ireland), chloroform (HPLC grade) by Scharlab S. L. (Barcelona, Spain), acetone (HPLC grade) by Carlo Erba Reagents-SA (Milan, Italy), nitric acid (Analytical-grade) by ITW Reagents (Monza, Italy) and methyl tert-butyl ether by Sigma-Aldrich Chemie Gbmh (Steinheim, Germany). The remaining solvents (ethyl acetate, cyclohexane, hexane, and acetonitrile) were of chromatographic grade and obtained from VWR Prolabo Chemicals (Fontenay-sous-Bois, France). Ultrapure water was obtained using Millipore Milli-RO plus and Milli-Q systems (Bedford, MA, USA). A vortex mechanical mixer from Heidolph (Schwabach, Germany), a drying oven, and a vibromatic mechanical shaker, all supplied by J.P. Selecta S.A. (Barcelona, Spain), a 5810 R refrigerated bench-top centrifuge from Eppendorf (Hamburg, Germany), and PTFE syringe filters (17 mm, 0.22 μm; Branchia, Spain) were employed for sample treatment. In addition, sodium chloride, florisil and alumina were supplied by Merck (Germany) and C_{18} was provided by Supelco (Bellefonte, PA, USA).

It should be noted that, owing to the common presence of plasticizers, especially PAEs, in the laboratory environment and materials, it is crucial to control their potential presence in the background signal values of blanks (González-Sálamo et al., 2018; Kartalovic et al., 2021; Lo Turco et al., 2016; Massous et al., 2023; Peñalver et al., 2021). A cleaning procedure was implemented to prevent contamination caused by reagents, materials, and laboratory equipment. Firstly, the glassware was soaked in ultrapure water, washed with 0.2 mol L^{-1} nitric acid, and sonicated for 20 min. Then, it was rinsed again with ultrapure water and subjected to the same ultrasonic conditions. Subsequently, it was washed with an acetone and methanol (1:1, *v/v*) mixture, dried at 150 °C for at least 1 h, and finally covered with aluminum foil until analysis. A laminar flow cabinet was utilized, and no laboratory gloves were used during sample weighing. Procedural blanks, in which ultrapure water replaced honey, were systematically run between sets of samples to monitor potential abnormal background values.

Table 2
GC–MS parameters.

GC parameter	Final setting
Programmed temperature conditions	From 60 °C (1 min) to 125 °C (3.6 min) at 25 °C min^{-1}, then to 310 °C (22.1 min) at 10 °C min^{-1} and finally keep at 310 °C (25.1 min)
Carrier gas	Helium
Flowrate (mL min^{-1})	1.2
Injection mode	Pulsed splitless
Injector temperature (°C)	280
Injection volume (μL)	1
MS parameter	**Final setting**
Operating mode	Electron impact
Ionization energy (eV)	70
Scan range (*m/z*)	50–550
Ion source temperature (°C)	230
Quadrupole temperature (°C)	150
Nebulizer gas (N_2) pressure (psi)	40

2.2. Standards

Standard stock (≈ 1000 mg L^{-1}) and working solutions of the studied compounds were prepared in acetone. Two stock solutions were prepared according to commercially available standards. One mixture contained six PAEs (BBP, DBP, DEHP, DEP, DMP and DNOP) and DEHA, while the other was composed by the six PAEs along with the two HDEs (4-BDE and 4-CDE). Honey samples, in which the absence of plasticizers residues had been previously confirmed using GC–MS (blank samples) were spiked with different amounts of the analytes before (BF samples) or after (AF samples) sample treatment (see **subsection 2.3**). The spiking protocol was adapted from a previous work (Fuente-Ballesteros et al., 2023; see Supplementary Material, Table 3S). The IS was always added at the same concentration (0.1 mg L^{-1}) to all samples. These samples were used for validation (spiked samples (low, medium, and high) and calibration curves), as well as sample treatment studies. The study involved the preparation of three replicates, each of which was injected three times. Each spiked sample was prepared with a blank sample fortified with three different concentrations of the plasticizers within the linear range. These were as follows: low-LOQ (see Table 1); medium-500 μg kg^{-1}; high-1000 μg kg^{-1}. The standard stock solutions were stored in glass containers in darkness at −20 °C, and working and standard matrix solutions were stored in glass containers and kept in the dark at 4 °C until the analysis.

2.3. Sample procurement and treatment

2.3.1. Samples

Honey samples (n = 30) were kindly donated by the Center for Agroenvironmetal and Apicultural Investigation (Marchamalo, Guadalajara, Spain; n = 14, EA1-EA14) or acquired from local supermarkets in different countries (Brazil, Spain, Finland, and Estonia; n = 16, LS1-LS16). Selection criteria included diverse factors such as their botanical origin, Pfund values, packaging, and overall composition. It is noteworthy that the botanical sources of all samples were verified through melissopalynological analysis (see Supplementary Material, Table 4S). The color of honey was assessed using a portable photometer (HI96785 honey color, Hanna Instruments). Each sample underwent individual homogenization through stirring with a glass rod and was subsequently stored in separate tubes in darkness at 4 °C until analysis.

2.3.2. Sample treatment

Briefly, 1.0 g of homogenized honey sample was weighed in a 10 mL screw-cap glass centrifuge tube, after which 2 mL of ultrapure water was added, and the tube was shaken for 1 min in a vortex device. Next, 2 mL of ethyl acetate was added to the tube and then shaken again in a vibromatic device (10 min; 750 rpm) and centrifugated for 10 min at 4000 rpm. An aliquot of 2 mL was collected and kept in a vial while a second solvent extraction was performed as previously described. Both supernatants (4 mL) were collected and transferred to a screw-cap glass centrifuge tube containing 50 mg of Florisil. Then, the tube was shaken in a vibromatic device (5 min; 750 rpm) and centrifuged (5 min; 4000 rpm). Two milliliters were evaporated to dryness using a gentle nitrogen steam. Finally, the dry residue was reconstituted with 1 mL of an IS solution (0.1 mg L^{-1}), and it was passed through a 0.22 μm PTFE filter prior GC–MS analysis. Fig. 1 summarizes the steps of the selected sample treatment.

2.4. GC–MS conditions

An Agilent Technologies (Palo Alto, CA, USA) 7890 A GC coupled to an Agilent Technologies 5975C MS equipped with an ALS 7693B autosampler and a MS ChemStation E 01.00.237 software (Agilent Technologies) was used. The chromatographic column was an Agilent HP-5MS (30 m × 0.25 mm × 0.25 μm). The GC-parameters, which were optimized (see **subsection 3.2**), are summarized in Table 2. Analyses

Table 3
Evaluation of the extraction efficiency (recovery percentages ± %RSD) of the sample treatment and the matrix effect (mean values ± %RSD). Data obtained as described in Sections 2.2, 3.4, and the results were obtained from three replicates were injected in triplicate.

Compound	MH						HH						RH					
	EE			ME			EE			ME			EE			ME		
	LL	ML	HL	LL	ML	HL	LL	ML	HL	LL	ML	HL	LL	ML	HL	LL	ML	HL
DMP	77 ± 2	81 ± 3	83 ± 1	−24 ± 2	−21 ± 1	−23 ± 2	79 ± 2	83 ± 3	78 ± 3	−31 ± 2	30 ± 2	−33 ± 3	98 ± 2	99 ± 5	91 ± 1	−30 ± 3	−32 ± 4	−37 ± 3
DEP	116 ± 3	118 ± 4	111 ± 3	27 ± 2	23 ± 2	20 ± 3	84 ± 3	91 ± 3	86 ± 3	21 ± 1	26 ± 4	23 ± 2	87 ± 3	88 ± 3	80 ± 3	28 ± 2	26 ± 2	30 ± 4
4-CDE	105 ± 4	103 ± 1	99 ± 4	−34 ± 1	−32 ± 1	−30 ± 3	96 ± 4	95 ± 3	98 ± 1	−34 ± 3	−38 ± 4	−37 ± 2	87 ± 1	87 ± 2	81 ± 4	−37 ± 3	−38 ± 1	−35 ± 6
4-BDE	100 ± 2	97 ± 2	105 ± 5	−24 ± 3	−23 ± 3	−25 ± 2	92 ± 4	99 ± 4	98 ± 1	−15 ± 3	−12 ± 1	−21 ± 2	80 ± 1	94 ± 5	96 ± 5	−29 ± 4	−33 ± 2	−33 ± 1
DBP	97 ± 2	94 ± 1	92 ± 5	−36 ± 3	−37 ± 4	−37 ± 3	98 ± 1	99 ± 4	102 ± 1	−22 ± 3	−26 ± 5	−27 ± 3	104 ± 1	108 ± 4	105 ± 5	−27 ± 3	−31 ± 2	−33 ± 2
BBP	105 ± 1	106 ± 4	101 ± 2	29 ± 2	26 ± 3	24 ± 4	102 ± 2	105 ± 1	101 ± 2	17 ± 2	22 ± 3	25 ± 3	98 ± 2	107 ± 3	106 ± 3	20 ± 3	24 ± 3	29 ± 2
DEHA	107 ± 1	108 ± 4	104 ± 2	27 ± 2	24 ± 3	25 ± 2	99 ± 2	93 ± 1	102 ± 3	20 ± 2	22 ± 3	28 ± 1	97 ± 1	101 ± 3	96 ± 2	23 ± 3	24 ± 2	26 ± 2
DEHP	77 ± 1	79 ± 3	84 ± 1	22 ± 2	25 ± 1	26 ± 1	95 ± 2	100 ± 2	95 ± 2	26 ± 1	24 ± 2	29 ± 2	101 ± 2	94 ± 2	99 ± 2	19 ± 3	22 ± 1	27 ± 2
DNOP	83 ± 1	78 ± 3	83 ± 3	24 ± 2	18 ± 2	25 ± 2	90 ± 2	92 ± 3	85 ± 2	18 ± 3	17 ± 1	21 ± 3	89 ± 3	92 ± 2	96 ± 5	29 ± 1	28 ± 1	36 ± 3

EE, extraction efficiency; **HH**, heather honey; **HL,** high level (1000 μg kg^{-1}); **LL**, low level (LOQ, see Table 1); **ME**, matrix effect; **ML**, medium level (500 μg kg^{-1}); **MH,** multifloral honey; **RH**, rosemary honey.

Table 4
Results (means of triplicate analyses (μg kg^{-1}); %RSD < 9% in all cases) of the plasticizers found in honeys. The other bisphenols under study were below LOD in the samples.

Sample	DEP	BBP	DEHA	DBP	DEHP
EA1	< LOQ	572	173	< LOQ	< LOQ
EA2	249	457	157	< LOQ	< LOQ
EA3	< LOQ	354	< LOQ	< LOQ	< LOQ
EA4	201	415	< LOQ	< LOQ	< LOQ
EA5	237	481	137	< LOQ	< LOQ
EA6	< LOQ	406	107	< LOQ	< LOQ
EA7	< LOQ	435	110	< LOQ	< LOQ
EA8	< LOQ	372	< LOQ	< LOD	< LOQ
EA9	< LOQ	476	147	< LOQ	< LOQ
EA10	640	435	100	< LOQ	< LOQ
EA11	< LOQ	455	105	< LOQ	< LOQ
EA12	247	286	< LOQ	< LOQ	< LOQ
EA13	< LOQ	342	137	< LOQ	< LOQ
EA14	< LOQ	481	152	< LOQ	< LOQ
LS1	< LOQ	372	< LOQ	< LOQ	< LOQ
LS2	< LOQ	290	102	< LOD	< LOQ
LS3	< LOQ	503	137	< LOQ	< LOQ
LS4	< LOQ	420	193	< LOQ	< LOQ
LS5	276	692	124	< LOQ	< LOQ
LS6	< LOQ	420	189	< LOQ	< LOQ
LS7	219	365	132	< LOD	< LOQ
LS8	< LOQ	513	238	< LOQ	< LOQ
LS9	< LOQ	452	230	< LOQ	< LOQ
LS10	704	404	302	< LOD	< LOQ
LS11	< LOQ	173	< LOD	< LOQ	< LOQ
LS12	< LOQ	380	< LOQ	< LOQ	< LOQ
LS13	186	471	220	< LOQ	< LOQ
LS14	< LOQ	729	143	< LOQ	< LOQ
LS15	< LOQ	473	< LOQ	< LOQ	< LOQ
LS16	< LOQ	506	< LOD	< LOD	< LOQ

BBP, benzyl butyl phthalate; **DBP**, dibutyl phthalate; **DEHA**, di-2-ethylhexyladipate; **DEHP**, bis(2-ethylhexyl)phthalate; **DEP**, diethyl phthalate; **EA**, honey samples from experimental apiaries; **LOD**, limit of detection (see Table 1); **LOQ**, limit of quantification (see Table 1); **LS**, honey samples from local supermarkets.

were performed in selected ion monitoring (SIM) mode, with one target/quantification ion and one or two qualifier/confirmation ions for each analyte (see Table 1). Under these optimal GC–MS conditions, all compounds eluted in <21 min (see Fig. 2).

3. Results and discussion

3.1. Optimization of the sample treatment

The first consideration was given to evaluate some of the procedures that have been already proposed in the literature (see Supplementary Material, Table 1S). It should be specified that 1.0 g of multifloral honey (analyte free-blank, AF and BF) and 2 mL of water to dissolve the honey (Valverde et al., 2018) were set in these preliminary experiments. In addition, and intermediate spiked concentration of 0.25 mg L^{-1} was selected for performing the initial tests. Firstly, it was tested the suitability of DLLME by evaluating the performance of three different extractant solvents (chloroform, acetone, and dichloromethane), as this technique was employed in several publications (Koo et al., 2017; Notardonato et al., 2020a, 2020b; Peñalver et al., 2021). Extractant solvents were rapidly injected into the disperser solvent (acetonitrile) by means of a micro-syringe, but no cloudy solution was formed. Consequently, its use was discarded. Then, it was decided to apply SE-based methodologies that provided a good performance for determining some of the studied plasticizers in honeys (see Supplementary Material, Table 1S). Several SE tests were performed, involving both solubilization and non-solubilization of the honey prior to analysis. The tests included variations in the solvent extraction (methyl tert-butyl ether, dichloromethane and acetone (1:1, *v/v*) mixture; ethyl acetate and cyclohexane (1:1, v/v) mixture). Unfortunately, acceptable recoveries for the majority of analytes were not achieved (< 40%; data not shown). In order to enhance both extraction efficacy and recovery values for all analytes, the method was modified from a single SE to a double SE using the solvents previously mentioned. A remarkable enhancement of the extraction efficiency was observed when utilizing the ethyl acetate, which is considered as a green solvent (see Supplementary Material, Fig. 1S-A), and subsequently it was selected as extraction solvent for continuing with the optimization procedure. Then, the following extraction parameters were optimized: sample amount (0.5–2.0 g), extraction volume (1–5 mL), agitation time (5–15 min), centrifugation time (5–15 min), and supernatant volume (1–3 mL). Among all the tests performed, the best values in terms of recovery percentages and matrix effect were obtained when using 1 g of sample, 2 mL of water to aid in the solubilization of honey, 2 mL of extractant solvent, 10 min of shaking time, 10 min of centrifugation, and 2 mL of supernatant for each

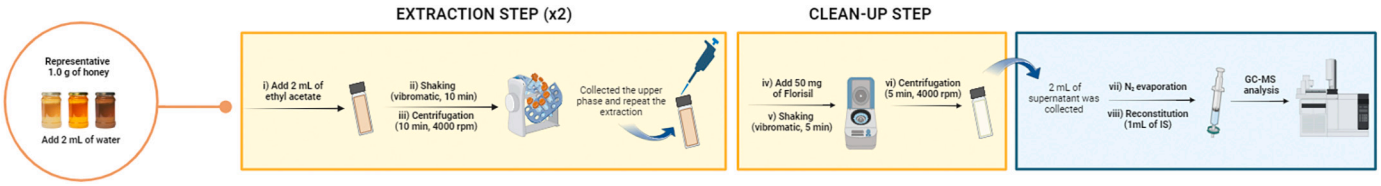

Fig. 1. Analytical procedure work-up flow chart.

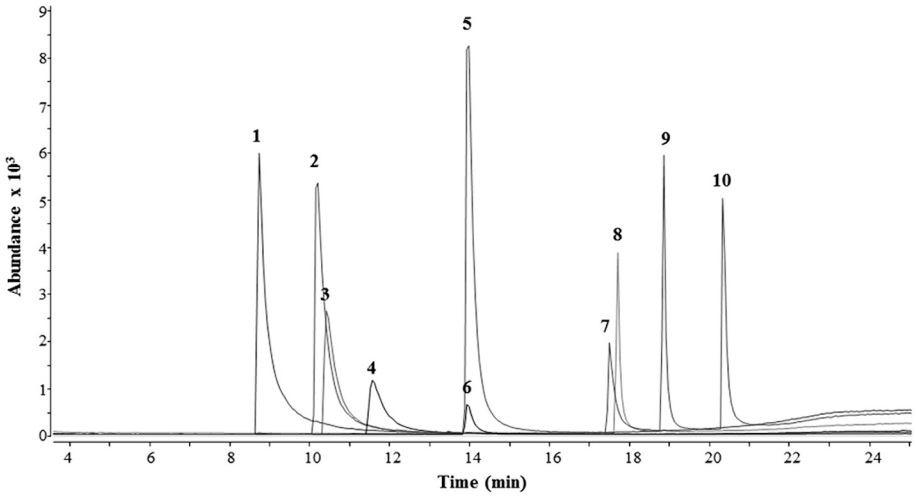

Fig. 2. Representative GC–MS chromatograms (SIM mode using the quantification ions; see Table 1) obtained from a standard in solvent mixture (1.0 mg L^{-1}; IS, 0.1 mg L^{-1}). GC–MS conditions are summarized in subsection 2.4 and **Tables 1 and 2**. **1**, dimethyl phthalate (DMP); **2**, diethyl phthalate (DEP); **3**, 4-chlorodiphenyl ether (4-CDE); **4**, 4-bromodiphenyl ether (4-BDE); **5**, dibutyl phthalate (DBP); **6**, dibutyl phthalate-3,4,5,6-d4 (DBP-d4); **7**, benzyl butyl phthalate (BBP); **8**, bis(2-ethylhexyl)adipate (DEHA); **9**, bis(2-ethylhexyl)phthalate (DEHP); **10**, di-n-octyl phthalate (DNOP).

extraction (data not shown). Consequently, it was decided to continue the optimization procedure with a double SE by testing the influence of some crucial parameters: shaking device, ionic strength, and pH. In the first instance, the use of ultrasound-assisted shaking instead of vibromatic was evaluated, resulting in a decrease in the recovery percentages that was especially significant for DNOP (≈ 50%; data not shown). Next, the effect of ionic strength was investigated by using different solutions of sodium chloride (0.9 mM–3.4 mM; Cao et al., 2015; Peñalver et al., 2021), but no significant improvement in terms of recovery percentages was obtained (see Supplementary Material, Fig. 1S-B). After that, the effect of pH was evaluated as it had been identified as an important parameter in previous studies (Notardonato et al., 2020a; Sewwandi, Wijesekara, Rajapaksha, Soysa, & Vithanage, 2023). We conducted trials adjusting the pH within the range of 4.0–8.0 (pH values of the analyzed honeys were comprised between 4.0 and 5.8) but again, no significant improvements were observed in the extraction efficiency and matrix effect (see Supplementary Material, Fig. 1S-C). Thus, we decided to evaluate a further clean-up step as an alternative for reducing the matrix effect through. Some sorbents were studied in different amounts and combinations to reduce the interferences from the matrix (alumina, C_{18}, and florisil). Alumina can adsorb a wide range of compounds, including, fatty acids, steroids, and other organic molecules. Florisil removes lipids and polar compounds, while C_{18} efficiently eliminates certain lipids. As can be seen in Fig. 1S-D (see Supplementary Material), the use of 50 mg of florisil provided quite good results for reducing the matrix without affecting the extraction efficiency of the analytes. Thus, it was decided to incorporate the clean-up step with florisil into the sample treatment method. It should be specified that the matrix effect was minimized to the maximum extent using the mentioned sorbent, but it could not be completely eliminated for most of the compounds. Therefore, matrix-matched calibration curves would be required for quantification purposes. Once the sample treatment for multifloral honey was evaluated at three different concentration levels (low-LOQ (see Table 1); medium-500 μg kg^{-1}; high-1000 μg kg^{-1}), the suitability of the proposed sample treatment for two other botanical origins (heather and rosemary) was evaluated. Results showed that it can be successfully employed to both types of honey, as similar results were obtained (extraction efficiency and matrix effect) for all plasticizers at the three concentration levels studied (see Table 3).

To the best of our knowledge, this is the first time that a sample method has been developed for determining plasticizers in honey samples from different botanical origins. Moreover, existing literature tends to investigate these compounds by groups (PAEs, adipates or esters). Some of the advantages of our proposal compared to previous works are related to the solvent consumption (Kartalovic et al., 2021; Zhou et al., 2014), sample amount (Di Fiore et al., 2023; Kartalovic et al., 2021; Lo Turco et al., 2016; Massous et al., 2023; Notardonato et al., 2020b, 2020a; Zhou et al., 2014), solvent toxicity (Kartalovic et al., 2021; Notardonato et al., 2020b, 2020a; Peñalver et al., 2021; Zhou et al., 2014), or sample preparation times (Kartalovic et al., 2021; Zhou et al., 2014). Moreover, the proposed method provided satisfactory recovery values for all compounds and honey, which are comparable to the best values reported in previous works (see Supplementary Material, Table 1S). On the other hand, although it is true that the matrix effect is significant for most compounds, this parameter has not been considered in previous works, and therefore our work is also pioneering in this aspect.

3.2. Optimization of GC–MS conditions

A non/low-polar stationary HP-5MS phase was used, as it has been widely employed for the GC analyses of plasticizers in food (Martínez-Gómez et al., 2024). Different initial oven temperatures and temperature programs were initially examined to attain suitable retention time

and satisfactory peak resolution according to previous studies. The initial column oven temperature was set as follows: starting at 60 °C, increasing from 60 to 170 °C at a rate of 40 °C min^{-1}, and then from 170 to 310 °C at a rate of 8 °C min^{-1} (3 min hold). Despite this setup, effective separation of the nine compounds was not achieved due to the overlapping observed between 4-BDE and 4-CDE, owing to their closely aligned retention times. Consequently, it was decided to modify the chromatographic ramp conditions as follows: from 60 °C (for 1 min) to 125 °C (for 3.6 min) at a rate of 25 °C min^{-1}, followed by an increase to 310 °C (for 22.1 min) at a rate of 10 °C min^{-1}, with a final hold at 310 °C. This adjustment yielded a 25-min runtime, including 5 min for initial setup and conditioning, and all analytes were eluted in <21 min (see Fig. 2). Once the retention time of each analyte had been determined in full-scan mode, the subsequent step involved optimizing the conditions for the SIM mode to ensure effective identification and high signal intensity for each target analyte. A full scan of the standard solution for each analyte was conducted to display its mass spectrum and compare it with the standard. Molecular ions, highly abundant fragment ions, and characteristic ions with selectivity to minimize cross-interferences were selected and fine-tuned to enable the qualification and quantification of the analytes. Following the criteria set by these parameters, we ultimately designated one specific ion for quantification purposes and two other ions for qualitative assessment for each analyte (see Table 1).

3.3. Greenness evaluation

Analytical greenness calculator (AGREE), AGREEprep and complex green analytical procedure index (GAPI) metrics were applied to rate the greenness of the developed method, giving a similar final score. Considering all the metrics available in the literature, they were selected for being the most commonly used in the last years (see Fig. 3). All of them are commonly applied, and free software is available along with detailed description of their application (Pena-Pereira, Tobiszewski, Wojnowski, & Psillakis, 2022; Pena-Pereira, Wojnowski, & Tobiszewski, 2020; Płotka-Wasylka & Wojnowski, 2021; Shi et al., 2023; Wojnowski, Tobiszewski, Pena-Pereira, & Psillakis, 2022). In general, AGREE offers an overall assessment of process sustainability, whereas AGREEprep focuses primarily on the sample preparation step considering factors like the quantity and type of solvents used, extraction efficiency, and waste generation. Complex GAPI is a more intricate metric, incorporating a broader array of parameters, often more specific and stringent. Its purpose is to evaluate the eco-efficiency and environmental impact of an analytical procedure in greater detail. AGREE and AGREEprep metrics are based on twelve criteria (Gałuszka, Migaszewski, & Namieśnik, 2013), transformed into a 0–1 scale, where the final score is determined by the collective contribution of all these criteria (see **Fig. 3**A and **3**B). Offline measurements (parameter 3), eight or more steps in the analytical process (parameter 4), and the use of energy expenditure by GC–MS (parameter 9) were negatively weighted by the AGREE metric. A low degree of automation was also penalized (highlighted in yellow; parameter 5). In contrast, the rest of the parameters in green color (sample treatment activities, type of analysis, derivatization status, amount of waste, number of analytes per hour, toxic reagents, and operator safety) were aligned with the green analytical chemistry (GAC) principles. Focusing on the AGREEprep, the ex-situ sample preparation placement and the high number of sample preparation steps for double extraction with solvents were the parameters that had the most negative influence on the final score. Hazardous solvents/reagents and operator safety were underscored by both metrics as the most significant contributions to the green values. As expected, the greenness scores calculated using both metrics were quite similar and close to 1, indicating good compliance with the green principles. Both metrics are more flexible and yield results that are easier to interpret compared to Complex GAPI. It is an extensive and semi-quantitative GAC metric that can be utilized for assessing the greenness of each step within the analytical procedure, providing a comprehensive and detailed overview of the process. Multiple parameters were categorized as green, like reagents, solvents, compounds considering its health and safety hazard, instrumentation, transport, yield, and conditions (see Fig. 3C). Conversely, sample collection and preservation, the necessity of an extraction step, and its scalability were rated with a low degree of greenness (highlighted in red). The nature of solvents/reagents, additional treatments, storage, and energy consumption were classified with a medium environmental impact. Moreover, a value of E = 0 was obtained, indicating that no waste is generated during the analytical process concerning the quantity of obtained products. However, it is essential to consider that the Complex GAPI metrics consider a significantly high number of restrictive parameters, the slight variation of which can significantly affect the environmental impact. Specific considerations make some metrics genuinely challenging to measure and assess. To summarize, the sample preparation and method developed can be considered a promising alternative to existing methods summarized in Table 1S (see Supplementary Material), because it can be considered as an environmentally friendly method aligned with the GAC principles. In addition, it should be noted that it is the first study with these compounds and matrix supported by the AGREE, AGREEprep, and Complex GAPI metrics (Gałuszka et al., 2013; Shi et al., 2023), which demonstrated a consistent correlation among the metrics.

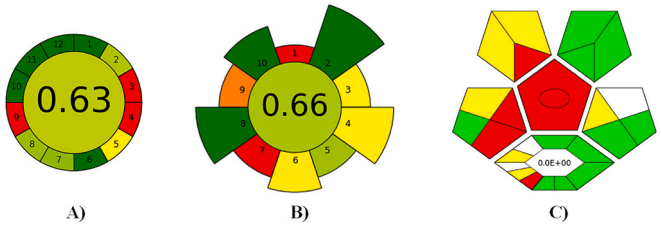

Fig. 3. Greenness evaluation using **A**) AGREE, **B**) AGREEprep, and **C**) Complex GAPI metrics.

3.4. Method validation

The method validation was conducted in accordance with current regulation (EURACHEM, 2014) and recent publications from our group (Fuente-Ballesteros et al., 2023). In addition, several of the main elements of uncertainty (Konieczka & Namieśnik, 2010) were considered when optimizing and validating this method (amount of sample used; recovery value of the analytical procedure; precision, and repeatability). Validation was performed with blank honeys, standards in the solvent, and standards in matrix extracts obtained according to the selected sample treatment (see **subsection 2.3.2**).

3.4.1. Selectivity

Selectivity was evaluated by comparing the chromatograms and mass spectra of standards in solvents, standard in matrix extracts and blank honeys of the three different botanical origins ($n = 6$). As showed in Fig. 2 and Fig. 2S (see Supplementary Material), no matrix interferences were observed at analytes retention times. Moreover, similar mass spectra of the analytes under study in solvent and standards in matrix extracts were obtained. The relative intensities of the selected ions for each analyte in both types of standards were compared and, for all cases, they were within ±11% of the relative intensity (data not shown).

3.4.2. Limits of detection and quantification

The limits of detection (LODs) and quantification (LOQs) were determined by the injection of several blank sample measurement noise at the elution times for the studied plasticizers and comparing this response (mean values) with the signal (peak heights) of compounds at low concentration levels. The LODs and LOQs were estimated to be three and ten times the S/N ratio, respectively. In addition, it was checked that

LOQs met the identification and method performance criteria for recovery (70%–120%) and precision (< 20%). LODs values ranged from 0.1 to 0.2 μg kg^{-1} for multifloral honeys, from 0.1 to 3.1 μg kg^{-1} for heather honeys, and from 0.1 to 0.5 μg kg^{-1} for rosemary honeys (see Table 1). Meanwhile, LOQs values were comprised between 0.2 and 0.7 μg kg^{-1} for thyme honeys, 0.2 and 10.3 μg kg^{-1} to heather honeys, and 0.2 and 1.7 μg kg^{-1} for rosemary honeys (see Table 1). All values were below to the established SMLs (see Table 1; European Commission, 2011, 2023). Moreover, they were comparable or better in most cases that the reported values (see Supplementary Material, Table 1S).

3.4.3. Matrix effect

To ascertain how the matrix influenced the ionization for the analytes a comparison was made of the detector responses (analyte peak area/IS area) of standard in solvent ($R_{solvent}$) and standard in matrix extracts ($R_{matrix;}$ AF samples) of the different botanical origins spiked at three different concentrations. It was calculated using the following formula: Matrix effect (%) = $[(R_{matrix}/R_{solvent})\ -1] \times 100$. Analyte responses at the three levels assayed in each matrix ranged in all cases between −37% of signal suppression to +29% or signal enhancement (see Table 3). In addition, the slope confidence intervals (SCIs) with standards in solvent and standards in matrix extracts were also compared, and it was not found an overlapping of the SCI (see Supplementary Material, Table 5S). Therefore, the use of calibration standards in solvent only would lead to an overestimate of the analyte concentrations in the analyzed samples, and matrix-match calibration curves are required. The addition of a clean-up step with florisil in the sample treatment was not enough to avoid a significant influence of the matrix effect onto the analyte signals in most cases. However, it should be remarked that this parameter was not studied in previous publications (see Supplementary Material, Table 1S).

3.4.4. Linearity/working range

Matrix-matched calibration curves (BF samples) were used to quantify the analytes in honey samples due to the significant matrix effect. Calibration curves ($n = 6$) were constructed by plotting the signal on the y-axis (analyte peak area/IS area) against the analyte concentration on the x-axis. Linearity was evaluated by visual analysis of the plots, a calculation being made of the determination coefficient (R^2), and by our back calculation of the concentrations of the individual calibration standards. The concentration of the analytical curves varied between LOQ and 1000 μg L^{-1} (LOQ, 50, 100, 250, 500, and 1000 μg L^{-1}), which corresponds to concentrations between LOQ and 2000 μg kg^{-1}. The graphs obtained in all the calibration curves were straight lines, with R^2 values higher than 0.99 in all cases (see Supplementary Material, Table 5S). Moreover, the deviation of back-calculation concentration from true concentration was lower than 15% (data not shown).

3.4.5. Precision

Precision was expressed as relative standard deviation (%RSD) and performed concurrently by repeated sample analysis using BF samples, at three different concentrations levels (low-LOQ (see Table 1); medium-500 μg kg^{-1}; high-1000 μg kg^{-1}). These took place either on the same day (repeatability), or over three consecutive days (partial reproducibility). %RSD values were lower than 9% in all cases (see Supplementary Material, Table 6S), which is consistent with the current European legislation.

3.4.6. Trueness

Trueness was evaluated by means of recovery experiments (as a measure of trueness), by comparing the results (analyte peak area/IS area) obtained from blank honey samples spiked at three different concentrations (low-LOQ (see Table 1); medium-500 μg kg^{-1}; high-1000 μg kg^{-1}), either prior to (BF samples) or following (AF samples) sample treatment. Mean recoveries for the studied plasticizers ranged from 77% to 118%, while %RSD values were lower than 6% in all cases (see Table 3). Those values are similar or better than the recoveries obtained in previous works (see Supplementary Material, Table 1S).

3.5. Application of the method

The validated method was applied for the study of nine plasticizers in different honey samples. As described before, all of these were analyzed in triplicate, and the IS was added at the same concentration in the samples. Honeys were handle based on the sample procedures outlined in **subsection 2.3.2**. Five analytes were detected in some of the analyzed samples (DEP, DBP, BBP, DEHA, and DEHP; see Table 4), while residues of three of them were found in all samples (DEP, BBP, and DEHP). However, only three of the plasticizers could be quantified (DEP, BBP, and DEHA) in a wide concentration range (100–720 μg kg^{-1}; see Table 4). It could be also remarked that no correlation patterns were found among the detected residues, packaging, and botanic origins, and that relatively high concentrations of these substances were found in both groups of honeys (commercial and experimental origins). Nevertheless, all these concentrations were below the established SMLs (European Commission, 2011, 2023), and are similar to those previously reported (see Supplementary Material, Table 1S). This does not indicate a limitation in the method's applicability since international organizations such as the European Commission have already defined SMLs for these compounds in food products.

The occurrence of plasticizer residues, particularly PAEs and adipates, in honey is not a new concern, as various cases of contamination have been previously documented (see Supplementary Material, Table 1S). For instance, plasticizer concentrations in honeys higher than 3000 μg kg^{-1} were detected (Notardonato et al., 2020b) and DBP concentrations levels 1.5–3 times higher than the SML were reported in Moroccan honeys (Massous et al., 2023). The presence of plasticizers in honey may originate from contaminated pollen and nectar (Massous et al., 2023; Notardonato et al., 2020b). Indeed, these substances could arise from environmental pollution (Di Fiore et al., 2023; Lo Turco et al., 2016), potentially due to the proximity of beehives to a large urban/industrial area (Notardonato et al., 2020a). In addition, production processes that involve direct contact with unsuitable plastic cups (Koo et al., 2017), containers (Zhou et al., 2014), honeycombs (Notardonato et al., 2020b), extractors, or uncappers (Lo Turco et al., 2016; Massous et al., 2023) can contribute to contamination. Additionally, crystallized honey is typically heated to return it to a liquid state, which enhances the release of plasticizers (Kartalovic et al., 2021; Zhou et al., 2014). Moreover, the acidity and high moisture content of honey might influence the transfer of harmful organic additives from the plastic cup to the honey, contributing to migration (Koo et al., 2017; Peñalver et al., 2021). Extended storage under specific conditions of temperature, humidity, and light can not only impact the distinctive physicochemical properties of honey, but also lead to gradual polymer degradation. Consequently, this degradation can result in the migration of plastic additives from the packaging into the honey (Massous et al., 2023).

Finally, the identification of contaminant residues associated with plastics in honey underscores the importance of developing analytical methods to ensure the safety of these products and preserve human health since some of them had been classified as endocrine disruptors (Notardonato et al., 2020b; Zhou et al., 2014). While this situation has been widely studied in different types of food, beverages and food stimulants, however, to the best of our knowledge, there is a little information regarding honey samples. It would be also recommended that in future studies, there should be an increase in the number of samples analyzed (often less than twelve in previous works), and the testing of honeys from different geographical and botanical origins to attain a more comprehensive perspective. Furthermore, in accordance with the European Regulation, the limitation of 60 mg kg^{-1} for those compounds without a defined SML requires careful consideration.

3.6. Risk assessment

To evaluate potential hazards associated with the honey samples under study, the average concentration of each plasticizer was used for calculating the estimated daily intake (EDI) with the following formula: EDI = CI × IR / bw (Ekici, Isci, & Bicer, 2024; Isci & Dagdemir, 2024; Xie, Zhang, Wu, & Wu, 2023). EDI expresses the plasticizer exposure per unit of body weight (bw) resulting from honey consumption (μg kg^{-1}, bw/day), where CI represents the mean plasticizer concentration in honey samples (μg kg^{-1}), and IR is the ingestion rate of honey set at 50 g/day for adults (Sadeghi, Akhlaghi, & Salehi, 2020) with a corresponding reference body weight (bw) of 70 kg. The assessment of non-carcinogenic health risks was conducted through the hazard quotient (HQ) for individual plasticizers, calculated as follows: HQ = EDI / RfD. The reference dose (RfD) values for DEP, BBP and DEHA were 8×10^{-2}, 2×10^{-1}, 6×10^{-2} mg kg^{-1} bw/day, respectively (Environmental Protection Agency, 2004; European Chemical Agency, 2012). The hazard index (HI) was determined as the total sum of individual HQ contributions (HI = $\sum$ HQ DEP + HQ BBP + HQ DEHA; Environmental Protection Agency, 2019). HQ and HI values below 1 imply no significant non-carcinogenic risks to human health.

This study evaluated, for the first time, the potential non-carcinogenic health risk associated with plasticizer consumption in honey samples sourced from various botanical origins employing parameters such as EDI, HQ and HI (see Supplementary Material, Table 7S). The sum EDI values for DEP, BBP and DEHA ranged from 0.3 to 1.0 μg kg^{-1} bw/day, significantly lower than tolerable daily intake values (TDI) of 50 μg kg^{-1} bw/day for DEP and BBP, and 300 μg kg^{-1} bw/day for DEHA (Nehring et al., 2020; Silano et al., 2019; Weiss et al., 2018). BBP exhibited the highest EDI values for all samples, peaking at 0.521 μg kg^{-1} bw/day. The assessment of HQ and HI (see Supplementary Material, Fig. 3S and Table 7S) revealed these values to be at least 100 times lower than the threshold value of 1, as reported by the Environmental Protection Agency (2019) suggesting a negligible non-carcinogenic health risk association. Consequently, our results indicate a lack of apparent risk to consumer health. However, the scarcity of existing literature concerning direct comparisons between honey consumption and plasticizer exposure underscores the necessity for further research in this area. These circumstances highlight the urgency for continued investigation in this field.

4. Conclusions

In this study, an analytical methodology has been developed and optimized to determine nine plasticizers (six PAEs, one adipate, and two HDEs) in honey samples from different origins by GC–MS. It was proposed an efficient, simple, and environmentally friendly sample treatment involving a double extraction with ethyl acetate followed by a clean-up step with florisil. This procedure allowed the obtention of good recovery percentages for all compounds and honeys, but it was not enough to minimize completely the matrix effect in all cases, a situation not commonly investigated when analyzing honey. In addition, the GC–MS method has been specifically developed for this study, and under the proposed conditions, all analytes were eluted in <21 min. The proposed method has been validated, and the results showed that the analytical performance of the method was good enough. The LODs and LOQs were significantly lower than the established SMLs. Additionally, a greenness assessment was conducted, resulting in the formulation of an environmentally friendly method. This evaluation is novel as the consideration of green methodologies has not been previously integrated into the study of plasticizers in honey. The proposed and validated method was applied to analyze several honey samples from different origins (botanical and geographical). Five analytes were detected in some of the analyzed samples (DEP, DBP, BBP, DEHA, and DEHP), while residues of three of them were found in all samples (DEP, BBP, and DEHP). However, only three of the plasticizers could be quantified (DEP, BBP, and DEHA) in a wide concentration range. Finally, an evaluation of the risk assessment revealed that the analyzed honey samples according to the presence of plasticizers do not pose an apparent risk to human health.

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CRediT authorship contribution statement

Adrián Fuente-Ballesteros: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation. **José Bernal:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Methodology, Investigation, Conceptualization. **Ana M. Ares:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Silvia Valverde:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Methodology, Investigation, 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.

Data availability

Data will be made available on request.

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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.2024.139888.

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