Development of novel methods based on GC-HRMS and LC-HRMS for the determination of non-phthalate plasticizers in soil

Raquel Capilla-Flores, Gonzalo Egea-Castro, Rosalía López-Ruíz, Roberto Romero-González and Antonia Garrido Frenich*

Research Group "Analytical Chemistry of Contaminants", Department of Chemistry and Physics, Research Centre for Mediterranean Intensive Agrosystems and Agri-Food Biotechnology (CIAIMBITAL), University of Almeria, Agrifood Campus of International Excellence, ceiA3, E-04120 Almeria, Spain.

ORCID CODES and e-mail addresses:
Raquel Capilla-Flores: 0009-0002-8029-2754; rcf307@ual.es
Gonzalo Egea-Castro: 0009-0007-8231-0860; gec844@inlumine.ual.es
Rosalía López-Ruíz: 0000-0003-0806-9013; rosalialr@ual.es
Roberto Romero-González: 0000-0002-2505-2056; rromero@ual.es
Antonia Garrido Frenich: 0000-0002-7904-7842; agarrido@ual.es

* Corresponding author: agarrido@ual.es.

Abstract

Non-phthalate plasticizers (NPPs) are a suitable alternative to phthalates, which are harmful compounds for human, animal health, and the environment. In this study, 28 commercial non-phthalate plasticizers (NPPs) from different families, including adipates, citrates, phosphates, sebacates, trimellitates, benzoates and cyclohexanoates, were determined. Two novel methods for determining these alternative compounds in soil were developed using gas chromatography coupled to high-resolution mass spectrometry (GC-HRMS-Q-Orbitrap) and liquid chromatography coupled to high-resolution mass spectrometry (LC-HRMS-Q-Orbitrap). Solid-liquid extraction (SLE) with ethyl acetate or acetonitrile, along with water as extraction solvents, were employed. In most cases, the GC method exhibited recoveries ranging from 84.9 % to 110.8 % at 20, 40 and 200 µg/kg, while the LC method achieved recoveries between 73.1 % and 115.4 % at 10, 20, 40 and 200 µg/kg. Most of the relative standard deviation (RSD) values were below 20 % for both methods. The validated methods were then applied to analyse soil samples collected from four different areas in Almeria. The results indicated that the compounds detected most frequently at high concentrations were 1-hydroxycyclohexyl phenyl ketone (HCPK) using GC, in the range 29.1 – 67.4 µg/kg and 2,2,4-trimethyl-1,3-pentanediol diisobutyrate (TXIB) using LC, in the range 39.9 – 51.5 µg/kg. Additionally, suspect and unknown analysis were carried out, and other plasticizers as phthalates, were also detected, in addition to other substances present in the analysed samples. All the soils exhibited the presence of a few plasticizers, either phthalic and/or non-phthalic.

KEYWORDS: emerging plasticizers, environmental analysis, chromatography, high resolution mass spectrometry, non-targeted analysis.

1. INTRODUCTION

Despite being an ideal material with several advantages and applications, plastics have negative effects on the environment and human health. Between 10 and 70 % *w/w* of the plastic are made of plasticizers. These are chemical additives that modify some specific characteristics of polymers, such as increasing their flexibility or their heat resistance. Due to the absence of any covalent bond between the polymer and plasticizers, these compounds can be released into the environment through desorption, leading to their migration into the surroundings.[1] This indicates that plasticizers are as present as plastics, making them ubiquitous emerging contaminants in the environment.[2] Approximately 8.4 million tons of plasticizers are consumed every year around the world.[3] The presence of these chemicals in the environment is concerning due to the potential ecological impacts they may cause. Furthermore, they pose risks to human health due to their carcinogenic, hepatoxic and teratogenic characteristics.[4]

Phthalates (phthalic acid esters, PAEs) have historically been the most recognized and widely used plasticizers, constituting approximately 85 % of the total plasticizers in the market.[5] These plasticizers have been associated with various health and environmental concerns, including carcinogenesis, hepatotoxicity, nephrotoxicity, reprotoxicity, cardiotoxicity and pollution.[6–8] Among the PAEs, bis(2-ethylhexyl) phthalate (DEHP) is the most commonly used, which is of particular importance because of the negative health effects of its metabolites. These metabolites include di-n-butyl phthalate (DBP), benzyl butyl phthalate (BBP), diethyl phthalate, di-n-octyl phthalate, mono-(2-ethyl-5-hydroxyphenyl) phthalate, mono-(2-ethyl-5-oxohexyl) phthalate, mono-(2-ethyl-5-carboxypentyl) phthalate and mono-[2-(carboxymethyl) hexyl] phthalate,[9] which exhibit more detrimental health effects than the parent compound.[10] Additionally, 14 phthalates are included in the REACH Authorisation List,[11] indicating their regulatory concerns. Furthermore, the European Union had imposed restrictions on the four most hazardous

phthalates: DEHP, DBP, BBP and diisobutyl phthalate (DiBP).[12] Another group of harmful plasticizers are associated with bisphenol A (BPA), a highly toxic endocrine disruptor and contaminant. In 2022, 10.6 million tons of BPA were produced.[13] Due to the restrictions and concerns about the PAEs, the use of alternative plasticizers has been increasing over the past 15 years.[3] For this reason, there is a growing need to replace phthalates and their metabolites with other types of compounds known as non-phthalate plasticizers (NPPs) or alternative plasticizers, being adipates, citrates, sebacates, benzoates, trimellitates, cyclohexanoates the most used.[14]

Bearing in mind that NPPs serve the same purpose as PAEs, they also exhibit similar characteristics. Even among NPPs within the same group, compounds show significant variation in their properties. One notable property is their high octanol/water partition coefficient (log Kow), the studied compounds have log Kow ranging from -1.00 to 10.40, corresponding to trimethyl citrate (TMC) and tris(2-ethylhexyl) trimellitate (TOTM), respectively. This difference in polarity makes both GC and LC techniques necessary for the analysis of all target compounds, with both techniques acting in a complementary manner, due to LC is employed to analyse the more polar compounds, while GC is used for the less polar ones. As a result of the limited water solubility, certain plasticizers tend to accumulate in soil, sediment, and aquatic biota.[15] This accumulation can persist in soil for extended periods, posing risks to organisms within this ecosystem and human health. Plasticizers indirectly contribute to the degradation of soil microbiota, leading to ecosystem disturbances and subsequent consequences that can contribute to climate change. Recognizing the importance of preserving and restoring healthy soils, the EU Horizon Europe initiative includes specific tasks related to innovation and research in this area.[16,17]

To date, no studies have specifically focused on the presence of NPPs in soil. However, numerous investigations have explored the occurrence of NPPs in other matrices, such as polyvinyl chloride (PVC) used in food contact materials,[14] medical devices,[18] rivers,[19] air and dust,[20] coffee,[21] fish fillets and squid,[22] gloves,[23] and foodstuffs,[24] among others.

The previous studies on NPPs have applied several extraction methods, including solid-liquid extraction (SLE) followed by reflux,[18] SLE with clean-up,[19,20] Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS),[21,22] and ultrasound assisted extraction (USE).[23,24] Among these methods, SLE with clean-up was used to the extraction of NPPs in dust.[20] However, its application for the extraction of NPPs from soil has not been previously explored.

The analytical techniques employed for the determination of NPPs mainly involve gas chromatography coupled to low-resolution mass spectrometry (GC-LRMS).[14,18–23] Additionally, liquid chromatography (LC) coupled to various detectors has been used, although to a lesser extent.[19,23,24] Classical detectors such as Diode-Array detector (DAD) have been coupled to high-pressure liquid chromatography (HPLC),[23] while more advanced detectors were also utilised as low-resolution mass spectrometers, including single quadrupole (Q)[14,18–23] coupled to GC and triple quadrupole (QqQ) coupled to LC.[19] Furthermore, high-resolution mass spectrometers as Orbitrap have been used coupled to HPLC.[24] In two of the aforementioned articles,[19,23] both GC and LC were used simultaneously, indicating the complementarity nature of these techniques.

A distinctive feature of our study, apart from two previous research, is the use of HRMS, specifically Q-Orbitrap.[25,26] This advanced technology enables the possibility of conducting non-targeted analysis, which combined with adequate software allows for the detection of additional compounds in the sample that were not included in the analytical

method as it provides exact masses with 5 decimal numbers, increasing the scope of the analysis.

Due to the significant variation in properties among the studied NPPs and their analogues or similar compounds from the same families, there is a challenging situation where a comprehensive analytical method for their simultaneous determination does not exist yet. The main objective of this study is to develop a reliable and robust analytical methodology for the analysis of a large number of NPPs in soil. By achieving this goal, the proposed method will contribute to expanding the scope of NPP analysis, thereby enhancing our understanding of their presence and behaviour in soil environments. To address this aim, two novel and efficient methods based on GC-HRMS-Q-Orbitrap and LC-HRMS-Q-Orbitrap were developed for the simultaneous determination of 28 NPPs in soil. Furthermore, non-targeted analysis was performed to tentatively identify the presence of phthalates and other compounds in soils.

2. Material and methods

2.1. Reagents, standards, and materials

The 28 analytical standards belonging to different families, including: (i) adipates such as dibutyl adipate (DBA), bis(2-butoxyethyl) adipate (DBEA), diethyl adipate (DEA), bis(2-ethylhexyl) adipate (DEHA), diisobutyl adipate (DiBA), diisodecyl adipate (DiDA), diisononyl adipate (DiNA) and dimethyl adipate (DMA); (ii) citrates such as acetyl tributyl citrate (ATBC), acetyl triethyl citrate (ATEC), butyryl trihexyl citrate (BTHC), triethyl citrate (TEC), tributyl citrate (TBC) and trimethyl citrate (TMC); (iii) phosphates such as 2-ethylhexyl diphenyl phosphate (EHDP), tricresyl phosphate (TcP) and tris(2-ethylhexyl) phosphate (TEHP); (iv) sebacates such as bis(2-ethylhexyl) sebacate (DEHSb) and dibutyl sebacate (DBSb), and (v) others such as benzyl salicylate

(BeS), diisononyl cyclohexane-1,2-dicarboxylate (DINCH), diethylene glycol dibenzoate (DGB), 1-hydroxycyclohexyl phenyl ketone (HCPK), methyl 3-(3,5-di-tert-butyl-4-hydroxyphenyl) propionate (MBPP), N-butylbenzenesulfonamide (NBBS), triacetin (TA), tris(2-ethylhexyl) trimellitate (TOTM) and 2,2,4-trimethyl-1,3-pentanediol diisobutyrate (TXIB). All of these standards were supplied by Cymit Quimica (Barcelona, Spain) with a purity of ≥ 95 %.

Primary standard solutions of each plasticizer were prepared in acetonitrile (ACN) at the concentration of 1000 mg/L. Intermediate standard mix solutions at a concentration of 10 mg/L were prepared in ethyl acetate (EtOAc) and in ACN. Additionally, standard mix solutions at a concentration of 1 mg/L were also prepared in ACN and EtOAc. All the solutions were stored in amber containers closed with plastic screw caps wrapped with parafilm at – 21 ºC.

For internal standards, high purity standard of bis(2-ethylhexyl) phthalate-d_4 (DEHP-d_4), obtained from Cymit Quimica was used as a procedure internal standard (P-IS) to verify the extraction performance. Two injection internal standards (I-IS) were purchased from Sigma-Aldrich (St. Louis, MO, USA): high purity anthracene-d_{10} for GC and high purity diisobutyl phthalate-d_4 (DiBP-d_4) for LC.

HPLC-MS grade EtOAc (purity ≥ 99.8 %) was obtained from Chem-Lab (Zedelgem, Belgium). ACN, H_2O and methanol (MeOH) of LC-MS grade were obtained from Honeywell Riedel-de-Haën (Seelze, Germany). Formic acid with a purity higher than 98 % and anhydrous magnesium sulphate ($MgSO_4$) of high purity grade were purchased from PanReac AppliChem (Barcelona, Spain). Technical grade acetone was used for the cleaning of the material, purchased from PanReac AppliChem.

Experimental procedures and sample preparation used a J.P. Selecta lab's oven (Barcelona, Spain), an Ika 4 Basic Vortex from IKA (Staufen, Germany), a J.P. Selecta

Centronic-BL-II centrifuge (Barcelona, Spain) and a rotary shaker Reax 2 from Heidolph, (Schwabach, Germany).

Syringe filters with a diameter of 13 mm and a pore size of 0.20 µm, made of polytetrafluoroethylene (PTFE), were used for filtering the sample extracts. These filters were purchased from Agilent Technologies (Santa Clara, CA, USA).

For the GC-Q-Orbitrap analyser, perfluorotributylamine was used as the mass calibrant. The mass calibration for the LC-Q-Orbitrap analyser included a mixture of acetic acid, caffeine, Met-Arg-Phe-Ala-acetate salt, and Ultramark 1621 (ProteoMass LTQ/FT-hybrid ESI positive), as well as a mixture of Ultramark 1621, sodium dodecyl sulphate, taurocholic acid sodium salt hydrate, and acetic acid (ProteoMass LTQ/FT-hybrid ESI negative). Mass-lock calibration was performed in both positive and negative modes with specific mass values: *m/z* 112.98559; 214.08963; 279.15909; 391.24429; 414.98098 (positive mode) and *m/z* 212.07489; 265.14790 (negative mode). These three mass calibrants were purchased from Thermo Fisher Scientific (Waltham, MD, USA).

2.2. Instrumentation

2.2.1. GC-Q-Orbitrap analysis

The GC-MS analysis was performed using a Trace 1310 gas chromatograph equipped with a split/splitless (S/SL) injector, and TriPlus RSH autosampler coupled to a Q-Exactive Orbitrap hybrid mass analyser (Thermo Fisher Scientific, Waltham, MA). A BP5-MS capillary analytical column (30 mm x 0.25 mm i.d, 0.25 µm particle size) from SGE Analytical Science (Victoria, Australia) was employed for the separation of NPPs. The GC oven temperature was programmed with an initial temperature of 40 °C, held for 2 min, followed by an increase to 310 °C at a rate of 35 °C/minute, and maintained for 5 min. The total running per sample was 15 min. Helium (99.999 %) obtained from Linde

(Valencia, Spain) was used as the carrier gas at a constant flow rate of 1 mL/min. The injector temperature was set to 250 °C and 1 µL of the sample extract was injected using the splitless mode (splitless time of 1 min).

The GC-Orbitrap-HRMS operated in full scan-MS acquisition mode with a scan range of *m/z* 90-500, scan time of 200 ms and the Automatic Gain Control (AGC) target value was set to 1e6. Electron ionisation (EI) at 70 electron volts (eV) was employed. A solvent delay of 5 min was implemented to prevent detector overload. The resolution power was set to 60,000 Full Width at Half Maximum (FWHM) at *m*/*z* 200. Data processing was performed using Xcalibur™ version 4.1 software, specifically the Qual Browser and Quan Browser software from Thermo Fisher Scientific.

2.2.2. LC-Q-Orbitrap-HRMS analysis

The LC-Q-Orbitrap analysis was performed using a Thermo Fisher Scientific Vanquish Flex Quaternary LC chromatograph (Thermo Scientific, San Jose, CA, USA) coupled to a hybrid mass spectrometer Q-Exactive Orbitrap Thermo Fisher Scientific (Q-Exactive™, Thermo Fisher Scientific, Bremen, Germany). The chromatographic separation was carried out using as stationary phase a Hypersil GOLD™ aQ C18 column (100 mm x 2.1 mm x 1.9 µm particle size) from Thermo Fisher Scientific. The mobile phase consisted of MeOH (eluent A) and an aqueous solution of formic acid, 0.1 % (eluent B). The gradient profile started with 5 % of eluent A, which was maintained for 1 min. Then, it linearly increased to 100 % over 2 min and kept constant during 9 min. Afterward, the composition was returned to the initial conditions within 0.5 min, followed by a re-equilibration time of 2.5 min so, the total running time was 15 min. The column temperature was maintained at 30 °C, the flow rate was set to 0.25 mL/min and the injection volume was 10 µL.

An electrospray interface (ESI) (HESI-II, Thermo Fisher Scientific, San Jose, CA, USA) was used in positive and negative modes. The following parameters were applied: spray voltage of 4 kV, N_2 > 95 % as sheath gas at a flow rate of 35 (adimensional), N_2 as auxiliary gas with a flow rate of 10 (adimensional), S-lens RF level 50 (adimensional), heater temperature set to 305 ºC and capillary temperature at 300 ºC.

The mass spectra were acquired using two alternating acquisition functions in two acquisition modes (ESI + and ESI -). On one hand, full MS mode was utilized without fragmentation (HCD was off), with a mass resolving power of 70,000, an AGC target of 1e6, and the scan range was *m/z* 50 - 750. On the other hand, data-dependent mass spectrometry fragmentation (dd-MS/MS) mode was implemented involving fragmentation using HCD with a collision energy (CE) of 30 eV, a mass resolving power of 35,000 FWHM, an AGC target of 1e5.

The acquired data were processed using Xcalibur™ version 4.3.73 with Quan Browser and Qual Browser software (Thermo Fisher Scientific, Les Ulis, France). For the compound identification, the spectral libraries *mzCloud*, *HMBD* and *MassFrontier* were used.

2.3. Sample extraction procedure

Soil samples were extracted by SLE. Thus, 2.00 ± 0.01 g were weighed and transferred to a 50 mL centrifuge tube. Two extraction methods were performed for the 28 NPPs.

For GC-amenable compounds, the soil spiked at 200 µg/kg of the P-IS standard was hydrated with 2 mL of H_2O. The mixture was homogenised on a vortex for 30 s, and after 5 min, 8 mL of EtOAc were added. The extracts were vortexed again during 30 s and then, on a rotary shaker for 20 min. Afterward, the tubes were centrifuged at 3700 rpm (2760 Relative Centrifugal Force, RCF) for 10 min. To remove residual water, the

extracts were dried by mixing 1.5 mL of the supernatant with 150 mg of $MgSO_4$ during 30 s on the vortex and then, centrifuged at 3700 rpm during 10 min.

For LC-amenable compounds, the soil spiked at 200 µg/kg of P-IS was hydrated with 2 mL of H_2O. The mixture was vortexed during 30 s and after 5 min, 8 mL of ACN was added. The system was mixed again in vortex during 30 s and then placed on a rotary shaker for 20 min, followed by 10 min in the centrifuge at 3700 rpm.

The supernatants from both extraction methods were filtered with a PTFE filter and, 1 mL of the final supernatant was transferred to 2 mL amber vials for analysis by GC-Q-Orbitrap and LC-Q-Orbitrap, respectively. To verify the correct performance of the analytical system, the extracts were fortified with the corresponding I-IS at 50 µg/L before the analysis.

2.4. Method validation

To ensure the reliability of the two optimised methods, several parameters were assessed according to SANTE 11312/2021 guidelines: linearity, matrix effect (ME), limits of quantification (LOQ), mean recovery (trueness), intra- and inter-day precision and selectivity.[27] For that purpose, a loam soil was used for the validation of the method.

Linearity was evaluated by solvent and matrix-matched calibration ranging from 2 - 200 µg/L for GC and LC. The linearity was assessed by least-squares regression of relative peak area (analyte/I-IS) versus concentration, and the determination coefficient (R^2) had to be higher than 0.99 to meet the linearity criteria. Besides, the deviation of the residuals must be $\leq \pm$ 20 %.

ME refers to the influence of matrix components on the analytes. It was determined by comparing the slopes of the solvent calibration curve (EtOAc for GC and ACN for LC)

with a matrix-matched calibration curve prepared using blank soil. The ME was calculated employing the Equation 1:

$$\text{Matrix effect (\%)} = \left(\frac{\text{Slope in matrix}}{\text{Slope in solvent}} - 1\right) \cdot 100 \quad (1)$$

Consequently, ME values higher than 20 % indicated matrix enhancement, while values lower than -20 % indicated matrix suppression. LOQs were determined as the lowest concentration with valid mean recovery and precision criteria (expressed as relative standard deviation, % RSD) studied for each NPP. The mean recovery percentage (expressed as trueness) was determined by spiking samples at different concentration levels and analysing them in triplicate. Precision was evaluated under two conditions (n=5): intra-day (repeatability) and inter-day (reproducibility). Intra-day precision was determined by analysing spiked samples on the same day, while inter-day precision was determined by analysing spiked sample on five different days. Recovery and precision were evaluated at three spiking levels for GC (LOQ level at 20 µg/kg; 2xLOQ level at 40 µg/kg and 10xLOQ level at 200 µg/kg) and at four spiking levels for LC (LOQ level at 10 µg/kg; 2xLOQ level at 20 µg/kg; 4xLOQ level at 40 µg/kg and 20xLOQ level at 200 µg/kg). According to the SANTE guidelines, mean recoveries must be between the range 70 – 120 %, with a precision ≤ 20 %.

2.5. Analysis of soil samples

Seven urban, three agricultural and one environmental soil samples were collected from four municipalities in Almería: El Ejido, Dalías, Senés and La Cañada. The sampling locations are represented in **Figure S1**. The soil samples were collected and transferred to glass bottles, and then stored at room temperature. Before analysis, the soil samples were dried either in the sun or in an oven, depending on their moisture content. The dried

soil was then sieved to remove any particles larger than 2 mm in size. This preparation process ensured that the soil samples were suitable for subsequent analysis.

2.6. Quality control and quality assurance

Due to the ubiquitous presence of plasticizers, several precautions need to be taken in their analysis:

- Use glass material instead of plastic material.
- Wash the non-plastic material with water and acetone before use.
- Wear nitrile gloves by the analyst.
- Avoid the use of personal care products as creams or cosmetics.
- Check for the presence of targeted compounds in the case that the use of plastic material cannot be avoided.
- Do not share solvents with colleagues to prevent cross-contamination, as this could lead to potential contamination issues.
- Use PTFE filters instead of nylon filters, as PTFE filters are less likely to introduce additional contaminants.

Given the ubiquitous nature of plasticizers, special care was taken during method development to avoid misconceptions and false positive results. Blanks from the plastic facilities were analysed to ensure no contamination occurred.

Regarding the quality control used, a known amount of two types of internal standards were added to the samples: (i) P-IS was used as a reference compound that behaves similarly to the analyte of interest during the analytical process. In this case, for both analyses, DEHP-d_4 was used as P-IS because it has similar properties than the targeted compounds, and (ii) I-IS is similar than P-IS but specifically added before the

chromatographic analysis. For GC, anthracene-d_{10} was employed and for LC DiBP-d_4 was used.

In addition, procedure and sample blanks in triplicate were utilized to account for any background contamination originating from the solvents or laboratory environment during the analysis and the sample, respectively. If the blanks gave a signal for a compound, the mean of the three replicates was subtracted from the signal corresponding to the sample.[28,29]

2.7. Data processing in non-targeted analysis

The obtained sample raw files used to identify the targeted compounds were also processed using Compound Discoverer®, and two types of non-targeted analysis were carried out: (i) suspect screening and (ii) unknown analysis. This was done with the aim of looking for suspect compounds, such as phthalates or bisphenols, as well as unknown compounds.

(i) Data processing in suspect analysis

Compound Discoverer ® processing utilized a home-made database with data from bibliography for each technique (LC and GC), which contained approximately 100 compounds for each one. All the suspect compounds including in the databases, such as phthalates and bisphenols, are collected in **Table S1**. The database contained details such as compound name, and CAS or NIST number. For the identification of the mentioned compounds, a mass error of 5 ppm was applied in both cases.

Besides, raw data obtained by GC-HRMS and LC-HRMS analysis were manually processed with Xcalibur Qual Browser in order to monitor the spectra of the detected compounds and confirm the characteristic ions.

(ii) Data processing in unknown analysis

The raw files were also processed with Compound Discoverer®. The databases employed were *ChemSpider* and *mzCloud* for LC and NIST MS Search 2.2 (National Institute of Standards and Technology, Gaithersburg, MD) for GC. In this case, the identification criteria were more complex: suitable peak shape signals; in case noise was absent, a signal should be present in at least five subsequent scans per peak of each ion, mass error lower than or equal to 5 ppm; and at least two fragment ions of each compound should be detected. These criteria were defined according to SANTE guidance. [27]

When these settings were used, more than 1000 features were achieved. To decrease the false positives and the features, some filters were applied: in the case of GC, a Relative Heavy Reference Factor (RHRF) Score greater than 95.00; Retention Score Index (RSI) greater than 700 and peak areas greater than 1e5 were used. These filters helped in the selection and prioritization of reliable and significant compounds. For LC, the result filter applied was *mzCloud* best match greater than 85.00.

In addition, *ChemSpider* and NIST databases were employed and a threshold filter of 1e5 was set for peak intensity.

3. RESULTS AND DISCUSSION

3.1. Optimization of the GC-Q-Orbitrap method

In order to select the characteristic ions of the analytes, individual standard solutions (100 µg/L) in EtOAc of the 28 analytes were injected into the GC-Q-Orbitrap. For the identification of these analytes, experimental mass spectra of each compound need to match with the corresponding one included in the mass spectral library NIST, both acquired in EI mode at 70 eV. At these conditions, the ion with high *m/z* and higher relative abundance was selected as the quantifier ion and the next two most abundant ions

were chosen as qualifier ions. Quantifier and qualifier ions should have an associated mass error lower than 5 ppm and retention time (RT) differences lower than 0.1 min.

During the optimisation of the 28 targeted compounds through GC-Q-Orbitrap, only 20 of them gave a positive response. The optimised parameters as the quantifier and qualifier ions, exact mass, molecular formula, and associated mass errors corresponding to these 20 NPPs are shown in **Table 1**. In addition, the exact masses used for the ISs were *m/z* 149.02335 (corresponding to DEHP-d_4) and *m/z* 188.14102 (corresponding to anthracene-d_{10}).

The chromatographic conditions were adjusted analysing a mix solution (100 μg/L) of the 20 NPPs in EtOAc. Initially, the compounds were analysed using a 23 min method with full-scan MS acquisition mode, and a scan range *m/z* 90 – 500. The temperature program used for this analysis was the following: the initial temperature was set to 40 °C and held for 2 min, followed by an increase to 310 °C at a rate of 20 °C/min, and maintained for 8 min. To reduce the overall analysis time, the temperature program was modified as follows: the temperature increase rate was changed to 35 ºC/min from the initial 20 ºC/min, and the hold time was reduced from 8 to 5 min. As a result, the modified temperature program was 8 min faster, allowing for the development of a 15 min GC-HRMS method. Notably, this newly proposed method is faster than the methods previously reported in the literature for NPPs in other matrixes.[14,18–23]

In addition, two of the studied NPPs, DiBA and DBA are isomers, presenting the same ions. Both had *m/z* 111.04400 as quantifier ion and *m/z* 129.05455 and 185.11715 as qualifier ions. Despite this, DiBA (8.07 min) and DBA (8.35 min) had different RTs, when the individual solutions for each one at 100 μg/L are injected, as it is shown in **Figure 1a** and the determination of each analyte can be performed avoiding the interference from the other, involving one of the biggest challenges of this technique.

Besides, the experimental mass spectra were reflected in **Figure 1b**. Although there are not isomers, the pairs of compounds TBC/ATBC, as well as TEC/ATEC, have the same quantifier and qualifier ions in GC-MS analysis (Table 1) because they belong to the same family. However, TBC and ATBC have distinct structures and molecular masses from each other, as do TEC and ATEC, and therefore, the retention times are different, and they can be determined individually.

3.2. Optimization of the LC-Q-Orbitrap method

To carry out the spectrometric characterization of NPPs by LC, characteristic ions of each analyte were selected: the precursor ion (protonated or deprotonated molecule as the quantification ion) and two fragment ions (as confirmation ions). For this purpose, spectrometric conditions were adjusted by injecting individual standard solutions at 100 µg/L in ACN using positive and negative ionisation mode (ESI + and ESI -). The precursor ions ($[M+H]^+$ or $[M-H]^-$) were acquired from full scan mass spectra considering that the mass error must be lower than 5 ppm. It is important to note that all the LC-amenable compounds were ionised by ESI + except for TMC, which was the only one ionised by ESI -. After that, MS/MS spectra (CE = 30 eV) were studied to select two fragment ions for each plasticizer. The criteria used to choose the fragments was based on the relative abundance of the ion and the RT, which must be equal to the corresponding of the precursor ion with a maximum difference of ± 0.1 min.

The optimised parameters for the 21 compounds that gave positive response of the total 28 NPPs for the LC analysis, as the precursor and fragment ions, their exact mass, molecular formula, and their associated mass errors, are shown in **Table 2**. The software *MassFrontier,* and the databases *mzCloud* and *HMBD* were used to determine the ions mentioned before. The isomers indicated in the previous section, DBA and DiBA had the

same characteristic ions and RTs, so it was not possible their chromatographic separation by LC as reflected in **Figure 2a**, showing a disadvantage compared to GC. Moreover, in **Figure 2b** the experimental mass spectra of these two isomers are shown.

For compounds of the same family, the fragmentation pathways were similar so, common fragments can be used to search for compounds of the same family not included in the method. For instance, adipates have *m/z* 129.05462 as a common fragment that corresponds to $[C_6H_8O_3 + H]^+$ (belonging to protonated adipic anhydride), citrates have a characteristic fragment at *m/z* 157.01315, which belongs to the formula $[C_6H_5O_5]^+$ (a derivate of the citrate group), and phosphates have a characteristic ion at *m/z* 98.98417 $[H_3PO_4 + H]^+$ (belonging to the protonated phosphoric acid).

Optimal separation was achieved by injecting a standard solution of the 21 analytes in ACN at 100 µg/L. For this, a mobile phase composed of water with 0.1 % formic acid and MeOH was used, and the total analysis time was 15 min (faster than previous methods).[19,23] With these conditions, different elution gradient profiles and flow rates, which are indicated in **Table S2,** were tested. As shown in **Table S3**, for the first gradient studied, some compounds eluted at the column re-equilibration time (after 12.5 min). To reduce the elution times of the most retained analytes, gradient 1 is changed and the organic phase composition increased from 5 to 100 % in 2 min instead of 7 min (gradient 2). The objective was almost achieved except for the most retained compound (TOTM), so a new elution gradient was checked by modifying the initial composition of the mobile phase, exhibited in **Table S2**. The conditions of gradient 3 instead of improving the elution of the compounds in comparison with gradient 2, worsened them, so the best gradient conditions were the second ones but with a higher flow rate, from 0.20 to 0.25 mL/min, reducing the elution time of TOTM. Therefore, option 4 (gradient 2 with a flow rate of 0.25 mL/min) was chosen because the most retained compounds had elution times

shorter than the re-equilibration time. The RTs of the 21 target compounds for each studied gradient were shown in **Table S3.**

Taking into account these results, the following data can be observed in the **Table S4**: 20 compounds were suitable for GC and 21 for LC analysis. More specifically, 7 of the compounds were only detected by GC-MS and 8 by LC-MS, while the remaining 13 compounds were detected by both techniques. From these results it can be said that both techniques can be used in a complementary way for a comprehensive determination of NPPs.

3.3. Optimization of NPPs extraction

3.3.1. Optimization of SLE by GC-HRMS

Considering the wide range of polarity of the analytes (**Table S5**), an extraction method of NPPs from blank loam soil was optimized. For that, 2 mL of H_2O followed by 8 mL of EtOAc with an intermediate waiting of 5 min was used as extraction solvent, and after that, the extractant was injected in GC-HRMS. Besides, two parameters were investigated to achieve the highest extraction efficiency: (i) extraction time and (ii) dehydration mode. (i) The soil was extracted with H_2O:EtOAc (20:80, *v*/*v*) using a rotary shaker for two different extraction times: 10 and 20 min. **Table S6** demonstrated that increasing the extraction time from 10 to 20 min led to an improvement in the number of targeted compounds with acceptable recoveries, increasing from 11 to 20. When the extracts were shaken during 10 min, only 11 of the studied analytes exhibited recoveries between 72.5 and 120.0 %. Furthermore, three compounds were not extracted at all (recoveries < 10 %) and the other six presented recoveries out of range (three of them with recoveries between 50.9 and 61.9 % and the other three in the range 146.6 – 171.0 %). However, when the extraction was extended to 20 min, all the GC-amenable compounds presented recoveries

between 89.3 and 107.6 %. Based on these results, it can be concluded that using the mixture H_2O:EtOAc (20:80, *v/v*) as extraction solvent with an extraction time of 20 minutes, acceptable recoveries for all 20 target compounds, when GC-HRMS was used, were achieved.

(ii) The water present in the extraction solvent needed to be removed from the final extract after centrifugation due to its incompatibility with the GC equipment. Therefore, two methods for drying the extracts were employed (**Table S7**): (1) freezing the aqueous phase at a low temperature (-20 ºC) for 90 min,[30] and (2) utilizing drying agents, such as $MgSO_4$. When the low-temperature method was employed after extraction, all GC-amenable compounds exhibited recoveries between 80.2 and 107.5 %. On the other hand, when $MgSO_4$ was used, the recoveries ranged from 89.3 to 107.6 %. Since the extraction time required for the low-temperature method was longer (90 min in the freezer), it was preferred the use of $MgSO_4$ as the drying agent due to its shorter extraction time.

3.3.2. Optimization of SLE by LC-HRMS

To develop an unified extraction method suitable for both GC-HRMS and LC-HRMS analysis, the extraction solvent assessed in the previous section was employed for the extraction of LC-amenable plasticizers in soil. However, this approach required an additional step involving evaporation, and redissolution before injecting into the LCsystem, which posed challenges for equipment compatibility. Thus, 2 mL of H_2O followed by 8 mL of EtOAc with an intermediate waiting time of 5 min was added to the soil and it was evaluated using LC-HRMS, and after evaporation, the resulting dry extract was redissolved with ACN. Evaluation of solvent efficiency was conducted through mean recovery and intra-day precision studies in triplicate on blank soils spiked at two concentrations levels, 40 and 200 µg/kg. The summarized results in **Table S7** indicate the

analyte response was low, and recoveries were out of the optimal range (70 – 120 %). Specifically, only 13 out of the 21 targeted compounds exhibited recoveries between 75.9 and 109.5 %, five compounds were not detected, and the remaining 3 NPPs displayed recoveries of 53.8, 55.7 and 142.0 %.

Therefore, an alternative extraction solvent was tested to eliminate the evaporation step. For that purpose, 2 mL of H_2O followed by 8 mL of ACN were employed for the extraction of LC-amenable NPPs from soil. This modification resulted in satisfactory recoveries ranging from 82.9 % to 107.7 % for all 21 analytes. These findings indicate that the initial approach utilizing H_2O:EtOAc, which was suitable for GC-HRMS, proved insufficient for extracting the LC-amenable plasticizers. Consequently, a single extraction method was not feasible, and each analytical technique required its own specific extraction method.

In order to simplify the extraction procedure, a new way of hydrating the soil was studied as well as the hydration step was avoid, comparing three extraction approaches: a) a two-step process consisting of adding 2 mL of H_2O followed by 8 mL of ACN, with a 5 min waiting period between the two steps previously tested, b) directly adding 10 mL of a mixture of H_2O:ACN in a 20:80 (*v*/*v*) ratio, and c) directly adding 10 mL of ACN. The obtained results for these three alternatives are presented in **Figure 3**, and the findings can be summarized as follows: option a) provided recoveries between 82.9 and 107.5 % for all 21 target compounds, option b) successfully extracted only 9 analytes, with recoveries falling within the range from 70.0 to 116.9 %, and option c) yielded satisfactory recoveries ranging from 76.9 to 120.0 % for 15 of the 21 analytes. However, the remaining 6 analytes exhibited recoveries in the range of 126.0 to 158.2 %.

Based on the observed results, it can be concluded that the most effective extraction solvent for UHPLC-HRMS analysis was option a), with the two-steps process.

3.4. Analytical method validation

Validation parameters for the method employed for GC-HRMS are shown in **Table 3**, while the parameters for the LC-HRMS method are in **Table 4**.

First, linearity was assessed in the matrix extract (loam soil), which was previously obtained by SLE from non-spiked soil (blank matrix). Due to a soil free of NPPs was very difficult to find, the analytical signals of the blank matrix corresponding to the targeted analytes were subtracted from the standard solutions, when matrix-matched calibration was employed. As it is shown in **Table 3** (GC parameters) and **Table 4** (LC parameters), the linear calibration prepared in matrix extract provided determination coefficients (R^2) higher than 0.9900 for both techniques.

Then, ME was evaluated and it was observed that the ME of the 20 analytes detected by GC-HRMS fall within the range between 84.7 % and 277.1 %, whereas the MEs of the 21 compounds detected by LC-HRMS ranged between -60.5 and 234.6 %. Due to the excessive ME, it was essential to use the matrix-matched calibration to quantify the analytes. Additionally, the correction of ME was carried out by utilising the signal of the I-IS (see **Table 3** and **4**). This correction involved determining the relative signal, which was achieved by diving the signal of the analyte by the one of the I-IS. To create the calibration lines for this correction, different I-IS were employed depending on the analytical technique used. In the case of GC, anthrace-d_{10} was used as I-IS, while for LC, DiBP-d_4 was utilized for this purpose. As a result of these corrections, the corrected ME values were within 0.78 – 2.39 % for GC and 0.57 – 1.05 % for LC. Given that negligible values of ME were obtained when I-IS were used, solvent calibration was employed as a more practical and effective approach for both analytical methods.

The results achieved for intra- and inter-day precision and trueness (mean recovery) through GC and LC are shown in **Table 3** and **Table 4**, respectively. Most of the

compounds for GC meet the requirements of the SANTE guidelines at the three concentration levels studied, except three of them that only meet them for one or two levels: TMC (200 μg/kg), TXIB (40 and 200 μg/kg) and DBEA (200 μg/kg). As it can be seen at **Table 3**, for 17 of the 20 targeted compounds the range of mean recovery values was 77.1 – 119.9 %, and its associated inter-day and inter-day precision values (RSD ≤ 19.9 %) were acceptable.

For LC analysis, the intra-day precision and trueness were studied at four different concentration levels for all compounds. At 40 and 200 μg/kg mean recoveries were between 77.4 and 105.3 % and RSD inter-day associated values were lower than or equal to 12.6 %. At 20 μg/kg, DINCH and TOTM were not detected but the other 19 compounds exhibited mean recoveries from 73.1 % to 115.3 %, with RSD intra-day associated values lower than 13.2 %. Finally, for the lower concentration studied (10 μg/kg) five compounds were not detected (TMC, TOTM, DINCH, DiNA and BTHC), and the mean recovery for TXIB, ATBC, EHDP and TEHP were 167.2, 166.0, <10.0 and 145.6 % respectively, with RSD intra-day values of >25 in all the cases, except for TEHP (RSD < 20 %). Regarding the compounds with valid recoveries and RSD values, the recovery range was 81.2 – 115.4 % and the intra-day and inter-day precision values were lower than 20.0 %.

Besides, any variations or losses that may occur during sample preparation, extraction, or analysis can be accounted by adding a known amount of P-IS to the sample. This compound helped correct for these variations and provided a more accurate measurement of the analyte concentration. I-IS was also used, and this was injected along with the sample onto the chromatographic system. I-IS aided in monitoring the performance of the chromatographic system by providing a reliable and consistent peak for comparison. In

this case, I-ISs help corrected of any variations in the injection process, instrument response, or retention time shifts, ensuring the accuracy and precision of the analysis.

For GC, the studied range was 20 – 200 µg/kg and **Table 3** shows the LOQs of the 20 targeted compounds. While for LC the range studied was 10 – 200 µg/kg and **Table 4** shows the LOQs for the 21 suitable analytes. The studied range for LC is wider compared to GC, this is attributed to the higher sensitivity of LC, enabling it to accurately quantify lower concentration limits. The maximum residue limits (MRLs) set by the EU for these compounds in soil are not defined.

Considering the scope of the method, the nature of the analytes and the results obtained for LOQs by both approaches, LC and GC, a comparison between them can be done. In the case of the compounds analyzed by both LC and GC, it can be noted that LOQs were higher in GC than in LC. This could mean that for the common compounds, LC provided better sensitivity. These analytes were DEA, TMC, TXIB, DiBA, DBA, DBSb, TBC, ATBC and DBEA, most of them from the adipate and citrate families. This difference might be related to the interferences, bearing in mind that in GC, the data was acquired via Full-MS, but in the case of LC, the quadrupole acted as a mass filter, so the presence of other compounds apart from the targeted one was fewer.

In comparison with the work of Khosravi et al., [31]focused on the determination of PAEs in soils, the LOQ values achieved with the developed extraction and analytical methods in this study were lower. Regarding the recoveries and precision values, both the values obtained in Khosravi's study and those obtained in the present investigation fall within the acceptable limits, achieving recovery values between 70 – 120 % and RSD values lower than 20 %.

According to the validation results of the two proposed methods, **Table S4** displays the most suitable method for each family or specific compound. LC-HRMS is deemed the

most suitable technique for adipates (except for DMA and the isomers DiBA and DBA, as they coeluted in LC-HRMS). Additionally, LC-HRMS is preferred for citrates due to its capability to detect more compounds of this family compared to GC. The same rationale applied to phosphates, as LC-HRMS was also able to detect a greater number of compounds of this family than GC. In addition, for compounds that can be detected by both techniques, such as DEA, TMC, TEC, DiBA, DBA, TBC, DBEA and DEHA, LC-HRMS was capable of quantifying them at lower levels compared to GC. On the other hand, for sebacates, HCPK, NBBS, BeS, and MBPP, GC-HRMS was found to be the most appropriate technique. Overall, for the analysis of the 28 total compounds in this study, the complementary use of GC-HRMS and LC-HRMS was necessary.

Finally, the selectivity of the method was studied as the ability of the method to distinguish and selectively quantify the analyte in the presence of other components. To address this, procedure blanks of EtOAc (for GC) and ACN (for LC), and sample blanks were prepared and analysed alongside the samples. These blanks were subjected to the same extraction and analysis procedures as the samples.

Furthermore, all experiments were performed in triplicate, allowing the assessment of the precision and reproducibility of the results.

3.5. Application to soil samples

After validating the developed and optimised methods for GC-Q-Orbitrap and LC-Q-Orbitrap, they were applied to a set of eleven soil samples. The samples were classified in three groups according to the origin of the soil: urban, agricultural, and environmental soil. By analysing these different soil types, the study aimed to assess the distribution and potential sources of plasticizers in these soil samples, providing insights into their presence and migration patterns.

The first group consisted of seven samples of garden soil collected from different locations at the University of Almeria. These samples were chosen to assess the variation in plasticizer concentrations based on their location and proximity to potential sources of plastic, such as irrigation pipelines, car or bicycle tires, shoe soles, and other sources. Three greenhouse soil samples, representing agricultural soils, were analysed to investigate the migration of the compounds from the greenhouse cover and other agricultural inputs into the soil. The last type of soil studied was environmental soil collected from a beach. This last type of soil was analysed to determine the presence and number of plastic additives that should not be present in a potential blank soil, indicating potential contamination from external sources.

To ensure the reliability of the results, as mentioned in Section 2.6, internal quality control measurements were implemented. The concentration of the studied plasticizers, determined by GC and LC techniques, are presented in **Table 5** (expressed in μg/kg).

By analysing the eleven soils, a total of 12 compounds were quantified using both techniques in a complementary manner. Seven compounds were quantified using GC-HRMS and LC-HRMS. When GC-HRMS was used, 11 compounds were quantified, whereas when LC-HRMS was employed, 8 compounds were quantified. To assess the complementarity of both techniques, the concentrations of the 7 common compounds (DiBA, TXIB, TBC, DEHA, ATBC, EHDP and ATEC) shown in **Table 5** were compared. In this comparison, it can be seen that both techniques provided similar concentrations for the same analyte. Therefore, either of the two techniques can be used for their quantification in soil. Regarding the compounds detected in the studied soils, it was observed that GC-amenable compounds were more detected than LC-amenable substances.

In terms of the presence of NPPs in the different types of soil, urban and agricultural soils had the same number of compounds (9) at concentrations higher than their LOQ, whereas the environmental soil had the least number of NPPs (5 compounds). Urban and agricultural soils have in common the following six compounds: TXIB, TBC, DEHA, ATBC, HCPK and MBPP. The other three compounds detected in each soil were type-specific: BeS, ATEC and DiBA for agricultural soil and EHDP, DMA and DINCH for urban soils. The compounds with the higher concentration in urban soils were HCPK (53.3 – 79.9 μg/kg) and DINCH (67.7 μg/kg) but the first one was quantified in almost all the soils, while the later one was only detected in urban soil 7, being TXIB the compound detected in most urban soils at high concentrations. In agricultural soils, the most concentrated compounds were HCPK (29.1 – 73.4 μg/kg) and MBPP (46.1 – 52.9 μg/kg), and finally the most concentrated NPP detected in the environmental soil was HCPK (60.9 μg/kg). TXIB, TBC, DEHA, HCPK and MBPP were found in almost all the samples, meaning the common use of these plasticizers. In **Figure 4**, the extracted-ion *chromatograms* (XIC) of TXIB, corresponding to precursor and fragment ions are shown, observing that the peak corresponding to the fragment ion elutes at the same retention time than the precursor ion, ensuring the reliability of the identification process.

All the compounds detected in the soils have a log Kow higher than 1 with the exception of ATEC, which means that they have a greater tendency to remain in the soil than to migrate with water. The presence of these compounds in the soil can be attributed to various factors, including plastic litter, such as food packaging films, which can be degraded and fragmented in the environment, leading to the migration of plasticizers into the soil. It is important to note that most of the compounds detected in this study were commonly used in the manufacturing of plastic-based materials, such as food packaging, coatings, storage, and toys. Besides, DiBA, TBC, DEHA, ATBC, EHDP, ATEC, MBPP

and DMA are commonly employed in the agricultural industry for various applications, such as greenhouse films, mulch films, irrigation pipelines, and agricultural packaging. It is also important to note that one of the compounds only detected in agricultural soils was also used to protect crops, and BeS is employed in the production of biocides. [32]

3.6. Toxicity evaluation of plasticizers

In addition, the toxicity data of the NPPs detected in soil samples, except for DiBA, can be found in the safety data sheet of each compound. [3233] The toxicity of DiBA is still lacking in literature, so its corresponding oral rats LD_{50} (Lethal Dosis, 50 %) was predicted using the T.E.S.T (Toxicity Estimation Software Tool) software. [34] The oral rats LD_{50} values for the other twelve NPPs are shown in **Table 6**. Furthermore, due to the lack of appropriate toxic human data, the LD_{50} for human, also known as the estimated human dose (EHD), was estimated from the LD_{50} for rats. This conversion was accomplished by dividing the animal dose by a factor of 6.2 as suggested by Jacob et al.[35]

In addition to the EHD, employing the parameters for daily intake via dust dermal contact as indicated by Tan et al.[36], estimated daily intake via soil was calculated.

$$E_{DI}\text{-dermal} = \frac{BSA \; x \; C \; x \; SAS \; x \; FA \; x \; IEF}{BW} \quad (2)$$

where E_{DI} is the estimated daily intake (ng/kg body weight/day), C is the detected concentration of the NPP in soil (ng/g = μg/kg), IEF is the indoor exposure fraction (hours/days), BSA is body surface area (cm^2/day), SAS is the amount of solid particles adhered onto skin (mg/cm^2), and FA is the fraction of the NPP absorbed through the skin. The value of E_{DI}-dermal for each NPP detected in soil, was estimated using the parameters included in **Table S8,** showing the values presented in **Table S9**.

Based on lethal dose data for human and the results of this study, it was found that the compounds HCPK (LD_{50} > 322.6 mg/kg), detected by GC-HRMS, and TXIB (LD_{50} > 322.6 mg/kg), detected by LC-HRMS and GC-HRMS, exhibited the highest toxicity

levels in all soils, except for urban soil 1, where their concentrations were above the LOQ. These two compounds are considered the most lethal among the NPPs studied, as they required a smaller amount of material to cause the death of 50 % of a group of humans. Besides, the majority of the NPPs detected in the studied soils displayed high levels of toxicity when compared to other toxic substances. For instance, glyphosate (LD_{50} rats = 10537 mg/kg; LD_{50} humans = 1669.5 mg/kg) was only more toxic than 3 of the 12 detected compounds (EHDP, TBC and ATBC). Similarly, cyanuric acid (LD_{50} rats = 7700 mg/kg; LD_{50} humans = 1241.9 mg/kg) was only more toxic than 4 of the 12 detected compounds (DiBA, EHDP, TBC and ATBC). On the contrary, EHDP was the least dangerous among the 12 compounds detected, as it required 79 times more than the deadliest compound, and it was only present in urban soil 6 at a concentration close to 40.0 µg/kg. In terms of toxicity comparison, EHDP was almost as lethal as white sugar (sucrose LD_{50} rats > 29700 mg/kg; LD_{50} humans = 4790.3 mg/kg). [37] [37]

In terms of daily dermal exposure, the NPPs to which we are most exposed are DINCH (1437.2 ng/g under high exposure for toddlers), followed by HCPK (1208.8 ng/g) and BeS (1111.3 ng/g). On contrary, we are least dermally exposed to DMA (484.0 ng/g) and ATEC (501.0 ng/g).Finally, the toxicities of the detected compounds were compared with phthalates. As mentioned in the introduction, the most harmful phthalates are DEHP, DiBP, DBP and BPP with oral LD_{50} for humans of 3225.8 mg/kg (DEHP), 2419.4 mg/kg (DiBP), 1016.1 mg/kg (DBP) and 375.8 mg/kg (BPP). In terms of LD_{50} values, the most harmful phthalate by far is BBP. The LD_{50} of BBP was as low as the lethal doses of the most harmful NPPs detected in this study: HCPK, TXIB and BeS. Some NPPs can indeed be more toxic than certain PAEs. So, it is important to consider these toxicity data in the context of the potential risks associated with the presence of these compounds in soils. Further research and studies are necessary to fully understand the toxicity and

environmental impact of these compounds and to explore alternatives that are less harmful to human health and the environment. In this regard, the compounds TBC, ATBC, and EDHP, which were identified as having the lowest toxicity based on their LD_{50} values, are potential candidates to be considered as fewer toxic alternatives to phthalates. The fact that these compounds require relatively high doses (more than 31.5 g for TBC and ATBC and 158 g for EDHP) to cause harm to 50 % of the tested rats, indicates a lower level of acute toxicity compared to other compounds studied. On the contrary, the other 9 detected compounds in the study had LD_{50} values lower than 20000 mg/kg (DEHP), being more toxic than the most PAE commonly used.

However, it is important to note that the assessment of toxicity should not solely rely on LD_{50} values, as chronic effects and sublethal impacts also need to be considered. Furthermore, the potential environmental persistence, bioaccumulation, and long-term effects of these alternative compounds should be thoroughly evaluated to ensure that they do not pose unintended risks.

The research conducted so far sheds light on the need for continued investigation into the toxicity of these compounds and the exploration of alternative options. This will enable informed decision-making, regulatory actions, and the development of safer practices in agriculture and other relevant industries to protect both human health and the environment.

3.7. Non-targeted analysis

Both suspect and unknown analyses were carried out, using the workflows used by López-Ruiz et al. [28] and those indicated in the Section 2.7.

(i) Suspect analysis

Three phthalates were tentatively identified in the eleven samples analysed by GC-HRMS: dimethyl phthalate (DMP); DBP and DEHP. On the other hand, only one phthalate, DBP, was detected by LC-HRMS analysis. As shown in **Table S10**, the phthalate detected at higher peak area by GC-HRMS was DMP followed by DBP, whereas DEHP was the compound detected with lower peak area. Therefore, the compound with the highest relative concentration detected was DMP. **Table S11** shown the area of the unique phthalate detected by suspect analysis through LC-HRMS. DBP was detected with a higher peak area in urban soil 6, whereas agricultural soils 1 and 2 showed the lower peak area for DBP.

When comparing the peak area of the common detected compounds by the two techniques, it was observed that LC-HRMS provided higher peak areas than GC-HRMS. This indicated that LC-HRMS exhibited higher sensitivity, allowing for better detection and quantification of the compounds present in the samples.

(ii) Unknown analysis

To identify other compounds not included in the home-made databases, an unknown analysis was performed for both GC and LC by processing the raw files using Compound Discoverer and applying an 'unknown analysis mode'. The workflows included *ChemSpider* databases for LC (including BLDpharm, ChEBI, FDA Structured Product Labeling index data, FooDB, MDPI and Phenol- Explorer) and NIST databases for GC.

As result, vitamin E and 1-methylnaphthalene (1-MNp) were detected by GC-HRMS. Additionally, ciclophenyl diphenol (CPhDPh) and phenyl 2-[bis(4-hydroxyphenyl)methyl]benzenesulfonate (PDbnz) were found using LC-HRMS. The origin of vitamin E in agricultural soils is derived from the crops that grow in those soils, while planted seeds in urban soil are considered to be the best sources of vitamin E. The

presence of 1-MNp is attributed to various anthropogenic sources, such as vehicle emissions or chemical usage. On the other hand, PDbnz can be found in certain cleaning formulation and when these products are used and disposed of, the mentioned compound can be released into the environment and potentially find its way into soils. Regarding CPhDPh, its presence in soils can occur due to various sources and pathways. Industrial activities, landfill leachate and runoff, and land application are some of the primary sources. The characteristic parameters of these identified compounds by unknown analysis are shown in **Table S12** and **Table S13**, respectively.

All these compounds can be used in the manufacturing of PVC materials, so this can be the possible origin of them in the soil due to migration from irrigation pipelines in case of urban and agricultural soils. In the case of environmental soils, the origin of the phthalates can be from bottle coatings.

4. CONCLUSION

Two new methods for the analysis of NPPs in soils using GC-HRMS and LC-HRMS were developed and validated. Both methods, GC-HRMS and LC-HRMS, allows for the determination of 20 and 21 out of the 28 plasticizers studied, respectively. This indicated the complementary nature of the two techniques to identify and quantify the different NPPs, allowing a more comprehensive analysis and ensuring a broader coverage of the plasticizers present in the samples.

The results showed that the combination of both techniques provided a more comprehensive analysis. However, when used individually, GC-HRMS was more effective in detecting a higher number of compounds compared to LC-HRMS due to the targeted compounds present in the studied soils were more suitable for the former analytical technique.

The three most hazardous compounds found in soils were identified as HCPK, BeS, and TXIB. This suggested that the alternatives to phthalates that are being used, including NPPs, may be equally or even more toxic than some phthalates. For instance, HCPK was the most frequently detected compound in the studied soils, and it was also the most toxic among the compounds studied. On the other hand, this study identified TBC, ATBC, and EHDP as the best alternatives with low toxicity values.

It is not surprising that for urban soils the concentration of the targeted compounds and presence of non-targeted ones is greater than for agricultural soils, since the exposure to these kinds of pollutants is greater in urbanizations, where plastics are ubiquitous (e.g., shoes soles, wheel rubbers, plumbing systems, etc).

The non-targeted analysis found that phthalates and derivatives are still being detected in soils due to their use and ubiquity. Based on these results, it can be concluded that GC is more suitable for plasticizers analysis, along with the developed extraction method. Conversely, LC and the extraction method was more convenient for bisphenols, as indicated by the results of both targeted and non-targeted analysis. Furthermore, LC-HRMS presented higher sensitivity than GC-HRMS for the studied analytes.

Supplementary material

Supplementary material associated with this paper can be found in the online version.

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Declaration of interest statement

The authors declare that they have no conflict of interest.

CRediT authorship contribution statement

RCF: Methodology, Formal Analysis, Data curation, Validation, Writing – original draft & editing. **GEC**: Methodology, Formal analysis, Data curation, Validation, Writing – original draft & editing. **RLR:** Conceptualization, Supervision, Writing – review & editing. **RRG:** Conceptualization, Supervision, Writing – review & editing. **AGF:** Conceptualization, Resources, Writing – review & editing, Funding acquisition, Project administration.

REFERENCES

(1) Hansen, E.; Nilsson, N. H.; Lithner, D.; Lassen, C. *Hazardous substances in plastic materials*. Hazardous substances in plastic materials. http://www.miljodirektoratet.no/no/Publikasjoner/Publikasjoner/2013/Februar/Hazardous_substances_in_plastic_materials/ (accessed 2023-07-04).

(2) Huo, C. Y.; Li, W. L.; Liu, L. Y.; Sun, Y.; Guo, J. Q.; Wang, L.; Hung, H.; Li, Y. F. Seasonal Variations of Airborne Phthalates and Novel Non-Phthalate Plasticizers in a Test Residence in Cold Regions: Effects of Temperature, Humidity, Total Suspended Particulate Matter, and Sources. *Science of the Total Environment* **2023**, *863*, 160852. https://doi.org/10.1016/j.scitotenv.2022.160852.

(3) CEFIC. *PLASTICISERS (factsheet)*. https://www.plasticisers.org/wp-content/uploads/2019/08/Plasticisers_Factsheet_EN_MAY2020.pdf (accessed 2023-07-07).

(4) ECHA. *Ftalatos*. https://echa.europa.eu/es/hot-topics/phthalates (accessed 2023-07-07).

(5) Qadeer, A.; Kirsten, K. L.; Ajmal, Z.; Jiang, X.; Zhao, X. Alternative Plasticizers As Emerging Global Environmental and Health Threat: Another Regrettable Substitution? *Environ Sci Technol* **2022**, *56* (3), 1482–1488. https://doi.org/10.1021/acs.est.1c08365.

(6) Mariana, M.; Feiteiro, J.; Verde, I.; Cairrao, E. The Effects of Phthalates in the Cardiovascular and Reproductive Systems: A Review. *Environ Int* **2016**, *94*, 758–776. https://doi.org/10.1016/j.envint.2016.07.004.

(7) Singh, S.; Li, S. S. L. Phthalates: Toxicogenomics and Inferred Human Diseases. *Genomics* **2011**, *97* (3), 148–157. https://doi.org/10.1016/j.ygeno.2010.11.008.

(8) Lu, M.; Jones, S.; McKinney, M.; Kandow, A.; Donahoe, R.; Faulk, B. C.; Chen, S.; Lu, Y. Assessment of Phthalic Acid Esters Plasticizers in Sediments of Coastal Alabama, USA: Occurrence, Source, and Ecological Risk. *Science of The Total Environment* **2023**, 165345. https://doi.org/10.1016/j.scitotenv.2023.165345.

(9) Matsumoto, M.; Hirata-Koizumi, M.; Ema, M. Potential Adverse Effects of Phthalic Acid Esters on Human Health: A Review of Recent Studies on Reproduction. *Regulatory Toxicology and Pharmacology* **2008**, *50* (1), 37–49. https://doi.org/10.1016/j.yrtph.2007.09.004.

(10) Rowdhwal, S. S. S.; Chen, J. Toxic Effects of Di-2-Ethylhexyl Phthalate: An Overview. *Biomed Res Int* **2018**, *2018*, 1750368. https://doi.org/https://doi.org/10.1155/2018/175036.

(11) ECHA. *Authorisation List*. https://echa.europa.eu/es/authorisation-list?p_p_id=disslists_WAR_disslistsportlet&p_p_lifecycle=0&p_p_state=normal&p_p_mode=view&_disslists_WAR_disslistsportlet_orderByCol=prc_entry_no&_disslists_WAR_disslistsportlet_substance_identifier_field_key=&_diss (accessed 2023-06-28).

(12) European Union. *ECHA 2018 (ANNEX XVII TO REACH - Conditions of restriction)*. https://echa.europa.eu/documents/10162/aaa92146-a005-1dc2-debe-93c80b57c5ee (accessed 2023-06-28).

(13) James, K.; Vasant, K.; Padua, S.; Gopinath, V.; Abilash, K. S.; Jeyabaskaran, R.; Babu, A.; John, S. An Assessment of Microplastics in the Ecosystem and Selected Commercially Important Fishes off Kochi, South Eastern Arabian Sea, India. *Mar Pollut Bull* **2020**, *154*, 132997. https://doi.org/10.1016/j.marpolbul.2020.111027.

(14) Carlos, K. S.; de Jager, L. S.; Begley, T. H. Investigation of the Primary Plasticisers Present in Polyvinyl Chloride (PVC) Products Currently Authorised as Food Contact Materials. *Food Addit Contam Part A Chem Anal Control Expo Risk Assess* **2018**, *35* (6), 1214–1222. https://doi.org/10.1080/19440049.2018.1447695.

(15) Fromme, H.; Küchler, T.; Otto, T.; Pilz, K.; Müller, J.; Wenzel, A. Occurrence of Phthalates and Bisphenol A and F in the Environment. *Water Res* **2002**, *36* (6), 1429–1438. https://doi.org/10.1016/S0043-1354(01)00367-0.

(16) Horizon European. *MISSIONS*. https://www.horizonteeuropa.es/misiones (accessed 2023-06-28).

(17) European Commission. *Caring for soil is caring for life. Ensure 75% of soils are healthy by 2030 for food, people, nature and climate*. https://op.europa.eu/en/publication-detail/-/publication/4ebd2586-fc85-11ea-b44f-01aa75ed71a1/ (accessed 2023-06-28).

(18) Gimeno, P.; Thomas, S.; Bousquet, C.; Maggio, A. F.; Civade, C.; Brenier, C.; Bonnet, P. A. Identification and Quantification of 14 Phthalates and 5 Non-Phthalate Plasticizers in PVC Medical Devices by GC-MS. *J Chromatogr B Analyt Technol Biomed Life Sci* **2014**, *949–950*, 99–108. https://doi.org/10.1016/j.jchromb.2013.12.037.

(19) Liu, Y. E.; Luo, X. J.; Huang, C. C.; Zeng, Y. H.; Lu, Q.; Wang, S.; Mai, B. X. Legacy and Alternative Plasticizers in Surface Sediment of Black-Odorous Urban Rivers across China: Occurrence, Spatial Distribution, and Ecological Risk Assessment. *Chemosphere* **2021**, *283*, 131206. https://doi.org/10.1016/j.chemosphere.2021.131206.

(20) Takeuchi, S.; Tanaka-Kagawa, T.; Saito, I.; Kojima, H.; Jin, K.; Satoh, M.; Kobayashi, S.; Jinno, H. Differential Determination of Plasticizers and Organophosphorus Flame Retardants in Residential Indoor Air in Japan. *Environmental Science and Pollution Research* **2018**, *25* (8), 7113–7120. https://doi.org/10.1007/s11356-015-4858-z.

(21) Domínguez-Hernández, C.; Ortega-Zamora, C.; González-Sálamo, J.; Hernández-Borges, J. Determination of Phthalic Acid Esters and Di(2-Ethylhexyl) Adipate in Coffee Obtained from Capsules. *Food Chem* **2022**, *388*, 132997. https://doi.org/10.1016/j.foodchem.2022.132997.

(22) Sambolino, A.; Ortega-Zamora, C.; González-Sálamo, J.; Dinis, A.; Cordeiro, N.; Canning-Clode, J.; Hernández-Borges, J. Determination of Phthalic Acid Esters and Di(2-Ethylhexyl) Adipate in Fish and Squid Using the Ammonium Formate Version of the QuEChERS Method Combined with Gas Chromatography Mass Spectrometry. *Food Chem* **2022**, *380*, 132174. https://doi.org/10.1016/j.foodchem.2022.132174.

(23) Poitou, K.; Rogez-Florent, T.; Lecoeur, M.; Danel, C.; Regnault, R.; Vérité, P.; Monteil, C.; Foulon, C. Analysis of Phthalates and Alternative Plasticizers in Gloves by Gas Chromatography–Mass Spectrometry and Liquid Chromatography–Uv Detection: A Comparative Study. *Toxics* **2021**, *9*, 200. https://doi.org/10.3390/toxics9090200.

(24) Zhang, Y.; Li, J.; Su, G. Comprehensively Screening of Citric Acid Ester (CAE) Plasticizers in Chinese Foodstuffs, and the Food-Based Assessment of Human Exposure Risk of CAEs. *Science of the Total Environment* **2022**, *817*, 152933. https://doi.org/10.1016/j.scitotenv.2022.152933.

(25) Zhang, Y.; Li, J.; Su, G. Comprehensively Screening of Citric Acid Ester (CAE) Plasticizers in Chinese Foodstuffs, and the Food-Based Assessment of Human Exposure Risk of CAEs. *Science of the Total Environment* **2022**, *817*. https://doi.org/10.1016/j.scitotenv.2022.152933.

(26) Carrizo, J. C.; Vo Duy, S.; Munoz, G.; Marconi, G.; Amé, M. V.; Sauvé, S. Suspect Screening of Pharmaceuticals, Illicit Drugs, Pesticides, and Other Emerging Contaminants in Argentinean Piaractus Mesopotamicus, a Fish Species Used for Local Consumption and Export. *Chemosphere* **2022**, *309*. https://doi.org/10.1016/j.chemosphere.2022.136769.

(27) Comission DG-SANTE, E. *SANTE 11312/2021. Guidance Document on Analytical Quality Control and Method Validation Procedures for Pesticide Residues Analysis in Food and Feed.*; 2021.

(28) López-Ruiz, R.; Maldonado-Reina, A. J.; Marín-Sáez, J.; Romero-González, R.; Martínez-Vidal, J. L.; Garrido Frenich, A. Unravelling Plant Protection Product Analysis: Use of Chromatography Techniques (GC and LC) and High Resolution Mass Spectrometry. *Trends in Environmental Analytical Chemistry* **2023**, *37*, e00191. https://doi.org/10.1016/j.teac.2022.e00191.

(29) López-Ruiz, R.; Romero-González, R.; Garrido Frenich, A. Metabolomics Approaches for the Determination of Multiple Contaminants in Food. *Curr Opin Food Sci* **2019**, *28*, 49–57. https://doi.org/10.1016/j.cofs.2019.08.006.

(30) Pereira, N. G. F.; Silvério, F. O.; Pinho, G. P. Optimisation, Validation and Application of the Solid-Liquid Extraction with Low-Temperature Purification Followed by Gas Chromatography-Mass Spectrometry for Determination of Phthalates in Sewage Sludge. *Int J Environ Anal Chem* **2020**, *100* (9), 968–980. https://doi.org/10.1080/03067319.2019.1646735.

(31) Khosravi, K.; Price, G. W. Determination of Phthalates in Soils and Biosolids Using Accelerated Solvent Extraction Coupled with SPE Cleanup and GC-MS Quantification. *Microchemical Journal* **2015**, *121*, 205–212. https://doi.org/10.1016/j.microc.2015.03.013.

(32) European Chemical Agency. *Benzyl salicylate*. https://echa.europa.eu/es/substance-information/-/substanceinfo/100.003.876 (accessed 2023-06-28).

(33) *Cymit Quimica S.L.* https://cymitquimica.com/es/ (accessed 2023-06-28).

(34) EPA. *Toxicity Estimation Software Tool (TEST)*. https://www.epa.gov/chemical-research/toxicity-estimation-software-tool-test (accessed 2023-05-09).

(35) Jacob, S.; Nair, A. B.; Morsy, M. A. Dose Conversion Between Animals and Humans: A Practical Solution. *Indian Journal of Pharmaceutical Education and Research*. Association of Pharmaceutical Teachers of India July 1, 2022, pp 600–607. https://doi.org/10.5530/ijper.56.3.108.

(36) Tan, H.; Yang, L.; Liang, X.; Huang, D.; Qiao, X.; Dai, Q.; Chen, D.; Cai, Z. Nonphthalate Plasticizers in House Dust from Multiple Countries: An Increasing Threat to Humans. *Environ Sci Technol* **2023**, *57* (9), 3634–3644. https://doi.org/10.1021/acs.est.2c08110.

(37) *Safety data for sucrose*. https://web.archive.org/web/20110612032043/http://msds.chem.ox.ac.uk/SU/sucrose.html (accessed 2023-06-28).

Figure Captions

Figure 1. a) Extracted Ion Chromatograms for 100 µg/L of DBA and DiBA analysed by GC-HRMS, and b) Experimental mass spectra for 100 µg/L of DBA and DiBA analysed by GC-HRMS.

Figure 2. a) Extracted Ion Chromatograms for 100 µg/L of DBA and DiBA analysed by LC-HRMS, and b) Experimental mass spectra for 100 µg/L of DBA and DiBA analysed by LC-HRMS.

Figure 3. Three different alternatives of solvent extraction to the optimisation of the extraction method by analysing 200 µg/kg of NPPs in soil: a) H_2O followed by ACN, b) mixture of H_2O and ACN and c) ACN.

Figure 4. Extracted Ion Chromatograms of TXIB (precursor and fragment ions) identified in sample 6 at 50.1 µg/kg.

HIGHLIGHTS

- First study that comprehensively analysed non-phthalate plasticizers (NPP) in soils
- Two methods based on GC and LC-HRMS were developed for the analysis of 28 NPPs
- Twelve NPPs were detected in soil samples above their LOQs
- TBC, ATBC and EHDP are less toxic than conventional phthalates
- Identification of phthalates was carried out when non-targeted analysis was applied

Table 1. GC-HRMS parameters used for the identification of targeted NPPs.[a]

NPP	Quantifier ion			Qualifier ions			RT (min)
	Molecular formula	Exact mass (*m/z*)	Error mass (ppm)	Molecular formula	Exact mass (*m/z*)	Error mass (ppm)	
DMA	$C_6H_7O_2$	111.04460	0.52	$C_7H_{11}O_3$	143.07082	0.24	6.40
				$C_6H_{10}O_2$	114.06808	-0.36	
DEA	$C_8H_{13}O_3$	157.08647	0.57	$C_7H_{12}O_2$	128.08373	0.40	6.98
				$C_6H_{11}O_2$	115.07590	-0.14	
TA	$C_4H_7O_3$	103.03952	0.31	$C_7H_{10}O_4$	158.05791	0.15	7.24
				$C_6H_9O_4$	145.05008	-0.09	
TMC	$C_6H_7O_4$	143.03443	0.06	$C_7H_{11}O_5$	175.06065	0.27	7.30
				$C_7H_5O_4$	153.01878	-0.40	
TXIB	$C_{13}H_{23}O_4$	243.15963	0.07	C_8H_{15}	111.11737	0.09	7.77
				$C_8H_{15}O_3$	159.10212	-0.20	
TEC	$C_7H_9O_4$	157.05008	0.62	$C_9H_{15}O_5$	203.09195	0.52	7.98
				$C_8H_9O_5$	185.04500	-0.37	
DiBA	$C_6H_9O_3$	129.05517	0.55	$C_{10}H_{17}O_3$	185.11777	0.43	8.07
				$C_6H_{16}O_2$	156.11503	-0.16	
ATEC	$C_7H_9O_4$	157.05008	0.72	$C_9H_{15}O_5$	203.09195	0.63	8.24
				$C_8H_9O_5$	185.04500	-0.59	
HCPK	$C_6H_{10}O$	99.08099	0.34	$C_{13}H_{13}O$	185.09664	0.86	8.26
				C_7H_5O	105.03404	-0.08	
DBA	$C_6H_9O_3$	129.05517	0.55	$C_{10}H_{17}O_3$	185.11777	0.43	8.35
				$C_6H_{16}O_2$	156.11503	0.06	
NBBS	$C_6H_5O_2S$	141.00103	-0.36	$C_7H_8O_2NS$	170.02757	-0.38	8.61
				$C_8H_8O_2NS$	158.02757	-0.45	

BeS	$C_7H_5O_2$	121.02895	0.07	$C_{14}H_{10}O_2$	210.06808	0.20	8.82
				C_7H_7	91.05478	-0.24	
MBPP	$C_{17}H_{25}O_3$	277.18037	0.07	$C_{14}H_{19}O_3$	235.13342	0.05	8.89
				$C_{15}H_{23}O$	219.17489	-0.37	
DBSb	$C_{14}H_{27}O_4$	259.19093	0.09	$C_{14}H_{25}O_3$	241.18037	0.04	9.53
				$C_{10}H_{17}O_3$	185.11777	-0.29	
TBC	$C_9H_{13}O_4$	185.08138	0.63	$C_{13}H_{23}O_5$	259.15455	0.06	9.56
				$C_6H_5O_5$	157.01370	-0.62	
ATBC	$C_9H_{13}O_4$	185.08138	0.77	$C_{13}H_{23}O_5$	259.15455	0.02	9.71
				$C_6H_5O_5$	157.01370	-0.59	
DBEA	$C_8H_{13}O_4$	173.08183	0.50	$C_{14}H_{25}O_5$	273.17020	0.01	9.81
				$C_{12}H_{21}O_4$	229.14398	-0.51	
DEHA	$C_6H_9O_3$	129.05517	0.19	$C_6H_7O_2$	111.04460	0.12	10.10
				$C_5H_9O_2$	101.06025	0.21	
EHDP	$C_{12}H_{12}O_4P$	251.04732	0.26	$C_6H_8O_4P$	175.01602	0.44	10.26
				$C_{12}H_{10}O$	170.07316	-0.88	
DEHSb	$C_{10}H_{19}O_4$	203.12833	0.60	$C_{18}H_{33}O_3$	297.24297	3.18	11.37
				$C_{10}H_{17}O_3$	185.1177	-0.51	

[a] Abbreviations: ATBC (acetyl tributyl citrate); ATEC (acetyl triethyl citrate); BeS (benzyl salicylate); DBA (dibutyl adipate); DBEA (bis(2-butoxyethyl) adipate); DBSb (dibutyl sebacate); DEA (diethyl adipate); DEHA (bis(2-ethylhexyl) adipate); DEHSb (bis(2-ethylhexyl) sebacate); DiBA (diisobutyl adipate); DMA (dimethyl adipate); EHDP (2-ethylhexyl diphenyl phosphate);); HCPK (1-hydroxycyclohexyl phenyl ketone); MBPP (methyl 3-(3,5-di-tert-butyl-4-hydroxyphenyl) propionate); NBBS (N-butylbenzenesulfonamide); TA (triacetin); TBC (tributyl citrate); TEC (triethyl citrate); TMC (trimethyl citrate); TXIB (2,2,4-trimethyl-1,3-pentanediol diisobutyrate).

Table 2. LC-HRMS parameters used for the identification of targeted NPPs.[b]

NPP	Precursor ion			Fragment ions			RT (min)
	Molecular formula	Exact mass (m/z)	Mass error (ppm)	Molecular formula	Exact mass (m/z)	Mass error (ppm)	
TMC	$C_9H_{14}O_7$	235.08123	-4.69	$C_{11}H_{22}O_4$	218.15236	-0.62	5.34
				$C_6H_7O_4$	143.03389	-1.57	
TEC	$C_{12}H_{20}O_7$	277.12818	-4.86	$C_8H_9O_5$	185.04445	-2.37	6.25
				$C_7H_9O_4$	157.04954	-1.05	
ATEC	$C_{14}H_{22}O_8$	319.13874	-4.52	$C_9H_{15}O_5$	203.09250	-2.10	6.51
				$C_7H_9O_4$	157.04954	-0.62	
DEA	$C_{10}H_{18}O_4$	203.12779	-4.90	$C_7H_{13}O_2$	129.09101	-1.64	6.56
				$C_8H_{13}O_3$	157.08592	-1.80	
DGB	$C_{18}H_{18}O_5$	315.12270	-4.82	$C_9H_9O_2$	149.05971	-2.24	6.91
				C_7H_5O	105.03349	-1.32	
DBEA	$C_{18}H_{34}O_6$	347.24828	-4.45	$C_{14}H_{25}O_5$	273.06519	-2.94	7.13
				$C_8H_{11}O_3$	155.07027	-1.71	
TBC	$C_{18}H_{32}O_7$	361.22208	-4.26	$C_9H_{13}O_4$	185.08184	-1.45	7.22
				$C_5H_5O_4$	129.01824	0.11	
DiBA	$C_{14}H_{26}O_4$	259.19039	-4.50	$C_{10}H_{17}O_3$	185.11722	-3.03	7.26
				$C_6H_{11}O_4$	147.06519	-2.57	
DBA	$C_{14}H_{26}O_4$	259.19039	-4.40	$C_{10}H_{17}O_3$	185.11722	-1.08	7.26
				$C_6H_9O_3$	129.05462	-1.12	
ATBC	$C_{20}H_{34}O_8$	403.23264	-3.90	$C_9H_{13}O_4$	185.08084	-1.12	7.39
				$C_6H_5O_5$	157.01315	-1.12	
TXIB	$C_{16}H_{30}O_4$	287.22169	-4.86	$C_{12}H_{23}O_2$	199.16926	-1.41	7.43
				C_8H_{15}	111.11683	0.98	
TcP	$C_{21}H_{21}O_4P$	369.12502		$C_{21}H_{20}O_3P$	351.11555	-1.32	7.52

			-5.00	$C_{14}H_{15}O$	199.11284	-2.31	
EHDP	$C_{20}H_{27}O_4P$	363.17197	-4.61	$C_{12}H_{12}O_4P$	251.04677	-2.32	7.65
				$C_{12}H_9$	153.06988	-1.74	
DBSb	$C_{18}H_{34}O_4$	315.25299	-4.86	$C_{10}H_{19}O_4$	203.12779	-1.92	7.91
				$C_9H_{15}O$	139.11174	-1.55	
DEHA	$C_{22}H_{42}O_4$	371.31559	-4.95	$C_6H_9O_3$	129.05462	-2.39	8.73
				$C_6H_{11}O_4$	147.06519	-0.31	
BTHC	$C_{28}H_{50}O_8$	515.35784	-4.93	$C_{11}H_{17}O_4$	213.11214	-2.36	8.80
				$C_6H_5O_5$	157.01315	-1.83	
DiNA	$C_{24}H_{46}O_4$	399.34689	-3.6	$C_6H_{11}O_4$	147.06519	-2.41	9.17
				$C_6H_9O_3$	129.05462	-0.98	
TEHP	$C_{24}H_{51}O_4P$	435.35977	-2.59	H_4O_4P	98.98417	2.90	9.31
				C_5H_{11}	71.08553	1.33	
DiDA	$C_{26}H_{50}O_4$	427.37819	-1.95	$C_6H_9O_3$	129.05462	0.52	9.57
				$C_6H_7O_2$	111.04406	2.23	
DINCH	$C_{26}H_{48}O_4$	425.36254	-2.13	$C_8H_{11}O_3$	155.07027	-0.52	9.81
				C_6H_{13}	85.10118	5.00	
TOTM	$C_{33}H_{54}O_6$	547.39932	-1.38	$C_{17}H_{21}O_5$	305.13835	-0.45	10.63
				$C_9H_5O_5$	193.01315	-0.62	

[b] Abbreviations: ATBC (acetyl tributyl citrate); ATEC (acetyl triethyl citrate); BTHC (butyryl trihexyl citrate); DBA (dibutyl citrate); DBEA (bis(2-butoxyethyl) adipate); DBSb (dibutyl sebacate); DEA (diethyl adipate); DEHA (bis(2-ethylhexyl) adipate); DGB (diethylene gycol dibenzonato); DiBA (diisobutyl citrate); DiDA (diisodecyl adipate); DiNA (diisononyl adipate); DINCH (diisononyl cyclohexane-1,2-dicarboxylate); EHDP (2-ethylhexyl diphenyl phosphate); TBC (tributyl citrate); TcP (tricresyl phosphate); TEC (triethyl citrate); TEHP (tris(2-ethylhexyl) phosphate); TMC (trimethyl citrate); TOTM (tris(2-ethylhexyl) trimellitate); TXIB (2,2,4-trimethyl-1,3-pentanediol diisobutyrate).

Table 3. Validation parameters for the 20 NPPs analysed by GC-HRMS in soil samples.[a]

NPP	LOQ (µg/kg)	Linear range (µg/L)	R^2 (n = 7)	Matrix effect (%)	Matrix effect with I-IS correction[b] (%)	Mean recovery (%)			Intra-day precision, %RSD (inter-day precision, %RSD)		
						20 µg/kg	40 µg/kg	200 µg/kg	20 µg/kg	40 µg/kg	200 µg/kg
DMA	20	2 - 200	0.9982	84.7	0.80	118.9	106.4	95.9	13.3 (15.3)	3.9 (9.5)	2.7 (4.4)
DEA	20	2 - 200	0.9900	82.8	0.78	89.7	85.4	95.7	9.2 (10.1)	8.7 (7.6)	2.1 (4.8)
TA	40	2 - 200	0.9989	94.0	0.88	98.8	100.6	89.5	11.9 (19.9)	11.4 (15.8)	4.5 (7.0)
TMC	200	10 - 200	0.9963	102.2	0.96	ND	ND	85.8	ND	ND	5.7 (15.5)
TXIB	40	2 - 200	0.9982	98.8	0.92	40.0	99.1	96.1	17.4 (36.4)	4.5 (13.1)	2.9 (10.1)
TEC	20	2 - 200	0.9993	101.1	0.95	119.9	102.5	97.5	6.8 (19.9)	6.1 (6.9)	4.1 (4.8)
DiBA	20	5 - 200	0.9988	100.5	0.94	110.8	94.6	91.2	2.9 (5.9)	0.8 (9.3)	2.2 (8.1)
ATEC	20	2 - 200	0.9987	98.4	0.92	108.3	98.6	94.3	5.3 (11.3)	2.1 (6.6)	1.6 (3.4)
HCPK	20	2 - 200	0.9981	111.4	1.02	105.1	90.8	93.4	3.5 (13.1)	2.8 (12.2)	1.0 (6.7)
DBA	20	5 - 200	0.9981	100.5	0.94	98.3	100.9	92.8	4.6 (11.6)	2.3 (0.6)	2.1 (7.7)
NBBS	10	2 - 200	0.9998	97.9	0.91	107.4	100.7	96.7	5.2 (13.0)	4.3 (2.8)	1.2 (2.7)
BeS	20	5 - 200	0.9946	118.1	1.11	95.3	98.6	91.7	18.0 (18.1)	8.2 (13.1)	3.0 (7.9)
MBPP	20	2 - 200	0.9988	96.9	0.90	92.8	77.1	78.6	0.6 (6.7)	2.1 (3.9)	1.5 (5.1)
DBSb	10	5 - 200	0.9975	108.1	1.01	94.4	94.3	90.7	9.5 (15.6)	9.0 (11.7)	7.1 (11.6)

TBC	20	2 - 200	0.9985	277.1	2.39	109.5	102.6	95.1	3.4 (8.7)	1.6 (8.5)	0.8 (5.6)
ATBC	20	2 - 200	0.9979	104.3	0.98	97.3	97.8	97.1	4.1 (7.6)	1.7 (4.0)	0.2 (3.7)
DBEA	200	50 - 200	0.9906	106.6	1.00	ND	ND	97.1	ND	ND	2.4 (4.8)
DEHA	20	2 - 200	0.9955	119.8	1.15	92.8	89.7	94.9	4.3 (8.1)	2.9 (4.5)	0.4 (3.0)
EHDP	20	2 - 200	0.9993	120.0	1.13	98.1	93.6	96.7	2.4 (4.5)	2.3 (3.0)	2.6 (3.0)
DEHSb	20	10 - 200	0.9983	104.1	0.97	84.9	99.4	75.6	6.0 (11.6)	6.2 (9.7)	3.6 (4.9)

[a] Abbreviations of the targeted compounds: see Table 1. ND = not detected

[b]Matrix effect corrected with anthracene-d_{10} signal as I-IS.

Table 4. Validation parameters for the 21 NPPs analysed by LC-HRMS in soil samples.[a]

NPP	LOQ µg/kg	Linear range (µg/L)	R^2	Matrix effect (%)	Matrix effect with I-IS correction[b] (%)	Mean recovery %				Intra-day precision, %RSD (inter-day precision, %RSD)			
						10 µg/kg	20 µg/kg	40 µg/kg	200 µg/kg	10 µg/kg	20 µg/kg	40 µg/kg	200 µg/kg
TMC	20	2 - 200	0.9999	250.6	0.93	ND	109.1	80.3	101.6	ND	9.0 (7.4)	6.0 (4.5)	3.8 (3.3)
DEA	10	2 - 200	0.9927	16.5	0.77	85.6	85.8	95.0	102.2	8.8 (14.8)	2.3 (14.1)	1.7 (4.8)	3.3 (0.5)
TXIB	40	2 - 200	0.9984	103.6	0.57	167.2	73.1	96.9	99.1	9.0 (>25)	7.7 (0.9)	4.9 (15.8)	2.6 (13.7)
TEC	10	2 - 200	0.9978	200.2	1.05	109.4	92.2	100.8	101.8	2.2 (4.4)	2.3 (5.0)	0.8 (4.0)	2.4 (3.5)
DiBA	10	2 - 200	0.9902	119.6	0.73	100.0	78.8	93.5	89.3	5.7 (10.4)	0.5 (4.4)	5.3 (4.6)	2.8 (4.9)
ATEC	20	2 - 200	0.9945	137.7	0.68	81.2	99.5	104.1	104.0	6.7 (11.0)	0.5 (5.9)	4.1 (4.0)	4.3 (14.1)
DBA	10	2 - 200	0.9936	119.2	0.67	94.2	91.3	88.9	88.8	5.9 (17.5)	3.3 (17.3)	5.0 (7.4)	2.4 (6.6)
DBSb	10	2 - 100	0.9940	193.0	0.71	103.6	107.0	77.4	75.2	13.0 (7.5)	10.3 (4.3)	8.6 (7.3)	7.8 (7.5)
TBC	10	2 - 200	0.9936	197.6	0.91	87.1	96.7	95.7	92.6	9.8 (11.7)	0.9 (5.0)	4.9 (7.7)	4.2 (6.5)
ATBC	20	2 - 200	0.9985	221.0	0.57	166.0	99.7	81.0	99.5	4.0 (>25)	5.2 (18.6)	4.7 (12.7)	0.5 (11.3)
DBEA	10	2 - 200	0.9989	4.3	0.66	94.4	90.9	87.3	85.2	7.8 (19.9)	5.9 (17.7)	1.7 (15.8)	7.2 (10.9)

DEHA	10	2 - 200	0.9949	81.9	0.63	115.4	96.7	105.3	93.6	2.3 (2.9)	7.1 (19.5)	4.8 (15.0)	10.8 (14.3)
EHDP	20	2 - 100	0.9904	234.6	0.72	< 10	115.3	91.7	91.0	8.9 (>25)	3.6 (3.7)	7.2 (9.1)	5.1 (6.4)
DiDA	10	2 - 200	0.9977	20.4	0.94	89.3	85.3	91.7	89.2	2.6 (16.0)	4.5 (8.0)	3.7 (12.2)	0.9 (11.8)
TOTM	40	4 - 200	0.9941	12.0	0.82	ND	ND	92.2	78.0	ND	ND	2.3 (9.5)	1.5 (8.6)
DINCH	40	8 - 200	0.9929	17.1	0.86	ND	ND	108.9	87.4	ND	ND	12.6 (16.3)	3.8 (7.8)
DiNA	20	4 - 200	0.9932	31.3	0.83	ND	101.6	85.1	88.8	ND	12.5 (10.9)	5.3 (10.6)	5.2 (7.7)
BTHC	20	8 - 200	0.9928	47.3	0.94	ND	85.1	97.9	102.5	ND	8.9 (16.5)	7.1 (10.4)	2.3 (5.9)
TcP	20	2 - 200	0.9928	9.7	0.80	95.6	88.5	97.6	88.2	17.7 (19.0)	13.9 (14.2)	9.2 (9.8)	8.7 (10.2)
TEHP	20	2 - 40	0.9983	69.4	0.78	145.6	95.9	86.3	93.6	6.23 (0.2)	8.5 (14.1)	5.7 (12.9)	0.7 (9.5)
DGB	10	4 - 200	0.9924	223.2	0.79	88.6	92.8	98.3	103.8	18.1 (11.5)	13.2 (11.2)	5.4 (6.3)	1.1 (5.4)

[a]Abbreviations of the targeted: see Table 2. ND = not detected

[b]Matrix effect corrected with DiBP-d_4 signal as I-IS.

Table 5. Mean concentration (n = 3) in μg/kg and RSD (%) of NPPs found in soil.[a]

Compounds	Urban soil[b]							Agricultural soil			Environmental soil
	1c	2c	3c	4d	5c	6c	7c	1a	2b	3c	1c
DiBA	ND[e]	ND	ND	ND	ND	ND	ND	29.8 (7.8)	ND	ND	ND
	ND[f]	ND	ND	ND	ND	ND	ND	30.0 (9.8)	ND	ND	ND
TXIB	ND[e]	41.2 (12.7)	52.5 (11.9)	48.6 (12.5)	52.0 (13.1)	45.5 (12.9)	47.8 (12.1)	40.3 (13.4)	41.8 (12.7)	53.3 (13.2)	40.2 (12.0)
	ND[f]	40.9 (13.5)	51.5 (12.5)	50.1 (12.3)	53.1 (13.9)	50.1 (12.4)	47.6 (12.5)	39.9 (14.4)	40.8 (12.7)	51.3 (12.2)	42.1 (13.0)
TBC	31.1 (8.0)[e]	24.2 (10.0)	25.2 (9.5)	24.8 (10.6)	28.0 (8.2)	24.6 (10.1)	26.4 (9.0)	23.8 (10.1)	24.0 (10.0)	28.1 (8.1)	25.5 (9.8)
	30.1 (10.3) [f]	22.4 (11.1)	22.5 (8.9)	23.4 (9.2)	26.8 (8.7)	23.4 (10.5)	27.6 (8.3)	21.5 (9.7)	22.5 (10.3)	27.5 (8.5)	23.3 (9.1)
DEHA	22.3 (6.0)[e]	26.0 (5.0)	<LOQ	30.8 (4.5)	21.2 (6.2)	33.6 (4.0)	30.4 (4.2)	20.5 (6.5)	24.7 (5.9)	31.0 (4.0)	39.7 (3.0)
	23.2 (6.4)[f]	25.5 (6.3)	<LOQ	31.7 (4.9)	22.4 (6.7)	31.4 (4.7)	29.4 (3.5)	18.4 (7.0)	24.5 (5.1)	29.8 (3.9)	38.0 (4.5)
ATBC	<LOQ[e]	21.1 (7.0)	26.0 (6.5)	33.4 (5.5)	<LOQ	26.7 (6.3)	<LOQ	<LOQ	24.1 (5.8)	24.5 (6.0)	<LOQ
	<LOQ[f]	20.2 (6.7)	25.5 (5.9)	35.4 (5.1)	<LOQ	25.4 (6.8)	<LOQ	<LOQ	23.8 (5.2)	24.0 (5.7)	<LOQ
EHDP	<LOQ[e]	<LOQ	<LOQ	<LOQ	<LOQ	39.1 (4.5)	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
	<LOQ[f]	<LOQ	<LOQ	<LOQ	<LOQ	38.8 (3.9)	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
ATEC	ND[e]	ND	ND	ND	ND	ND	ND	23.6 (7.0)	ND	ND	ND
	ND[f]	ND	ND	ND	ND	ND	ND	23.4 (7.5)	ND	ND	ND
HCPK	<LOQ[e]	55.2 (12.0)	59.4 (11.2)	67.5 (9.9)	53.3 (12.5)	79.9 (9.5)	54.8 (12.4)	35.9 (13.5)	29.1 (14.0)	73.4 (9.1)	60.9 (10.3)
MBPP	44.5 (4.5)	45.3 (4.7)	52.6 (4.1)	52.1 (4.8)	50.1 (5.6)	55.6 (5.1)	47.2 (4.1)	46.1 (4.7)	47.6 (3.9)	52.9 (3.6)	52.2 (3.5)

BeS	ND[e]	ND	ND	ND	ND	ND	ND	52.4 (10.5)	52.3 (11.0)	ND	ND
DMA	<LOQ[e]	<LOQ	<LOQ	<LOQ	<LOQ	22.8 (12.0)	<LOQ	<LOD	<LOQ	<LOQ	<LOQ
DINCH	ND[f]	ND	ND	ND	ND	ND	67.7 (10.0)	ND	ND	ND	ND

[a]Abbreviations: see Table 1 and Table 2; ND = no detected; LOQ = limit of quantification

[b]Sample location: “a” = Dalías; “b” = El Ejido; “c” = La Cañada; “d” = Senés

[e]Analytes quantified by GC-HRMS

[f]Analytes quantified by UHPLC-HRMS

Table 6. Predicted toxicity of target plasticizers detected in samples of soil.[a]

Compound	Toxicity according to literature	Extrapolated toxicity to humans[c]
HCPK	LD_{50} > 2000 mg/kg	LD_{50} > 322.6 mg/kg
TXIB	LD_{50} > 2000 mg/kg	LD_{50} > 322.6 mg/kg
BeS	LD_{50} = 2227 mg/kg	LD_{50} = 359.2 mg/kg
MBPP	LD_{50} > 4170 mg/kg	LD_{50} > 672.6 mg/kg
DMA	LD_{50} > 5000 mg/kg	LD_{50} > 806.5 mg/kg
DINCH	LD_{50} > 5000 mg/kg	LD_{50} > 808.5 mg/kg
DEHA	LD_{50} = 5600 mg/kg	LD_{50} = 903.2 mg/kg
ATEC	LD_{50} = 7000 mg/kg	LD_{50} = 1129.0 mg/kg
DiBA	LD_{50} = 8550.33 mg/kg[b]	LD_{50} = 1379.1 mg/kg
EHDP	LD_{50} = 15800 mg/kg	LD_{50} = 2548.4 mg/kg
TBC	LD_{50} = 31400 mg/kg	LD_{50} = 5064.5 mg/kg
ATBC	LD_{50} > 31500 mg/kg	LD_{50} > 5080.1 mg/kg

[a]Abbreviations: see Table 1

[b]Predicted toxicity by T.E.S.T.

[c]Predicted toxicity values for humans obtained by dividing the LD_{50} for rats by 6.0.

Figure 1

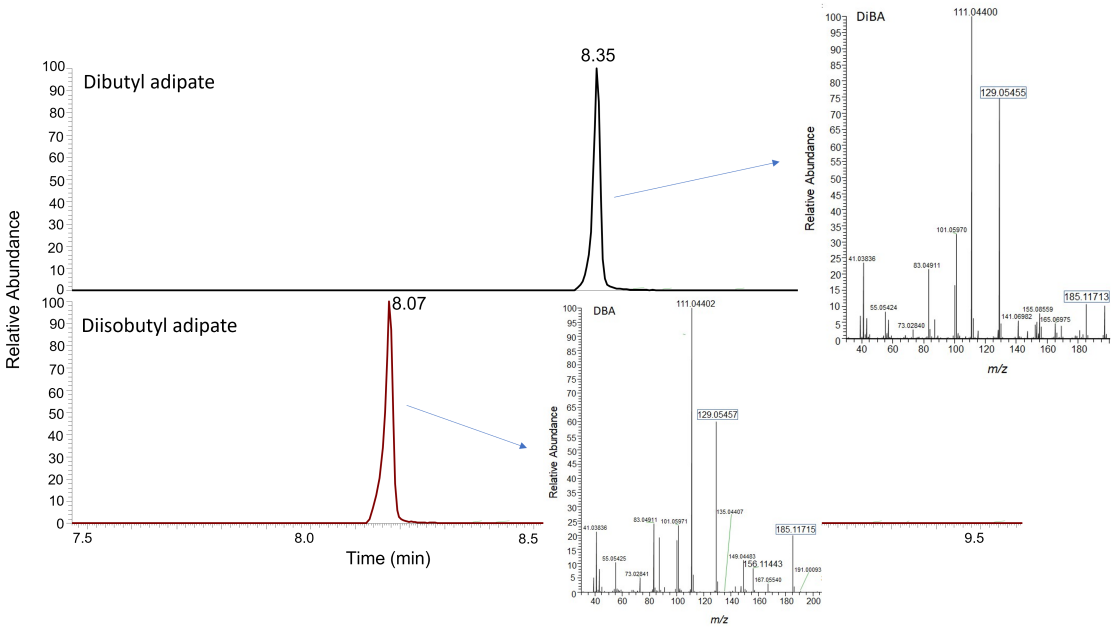

Figure 2

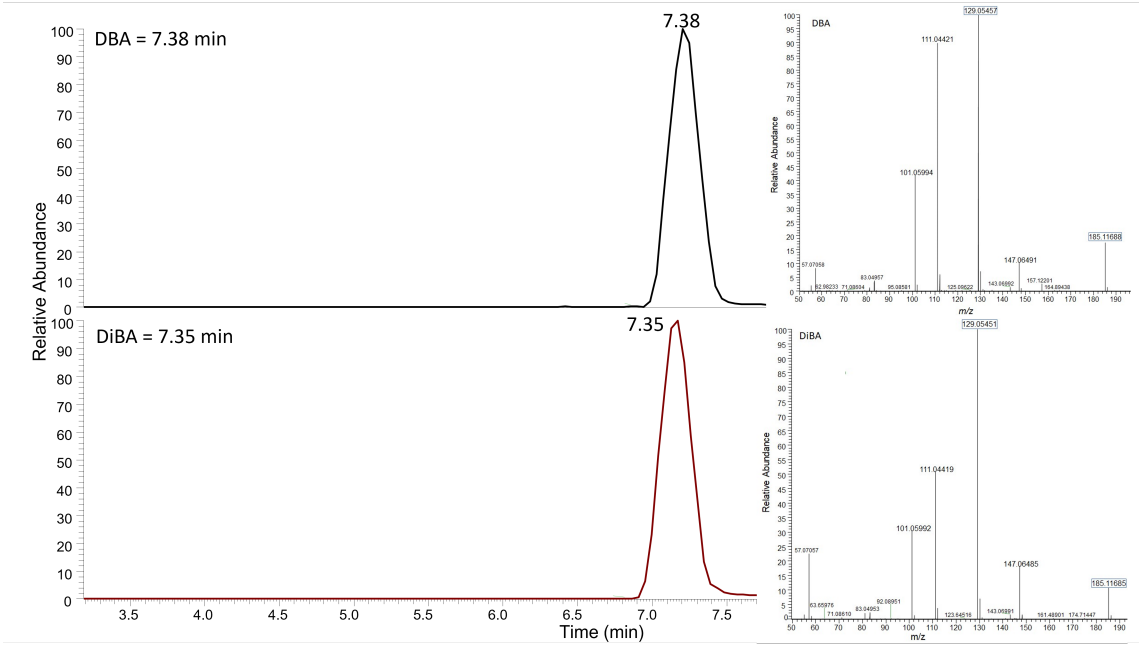

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Figure 3

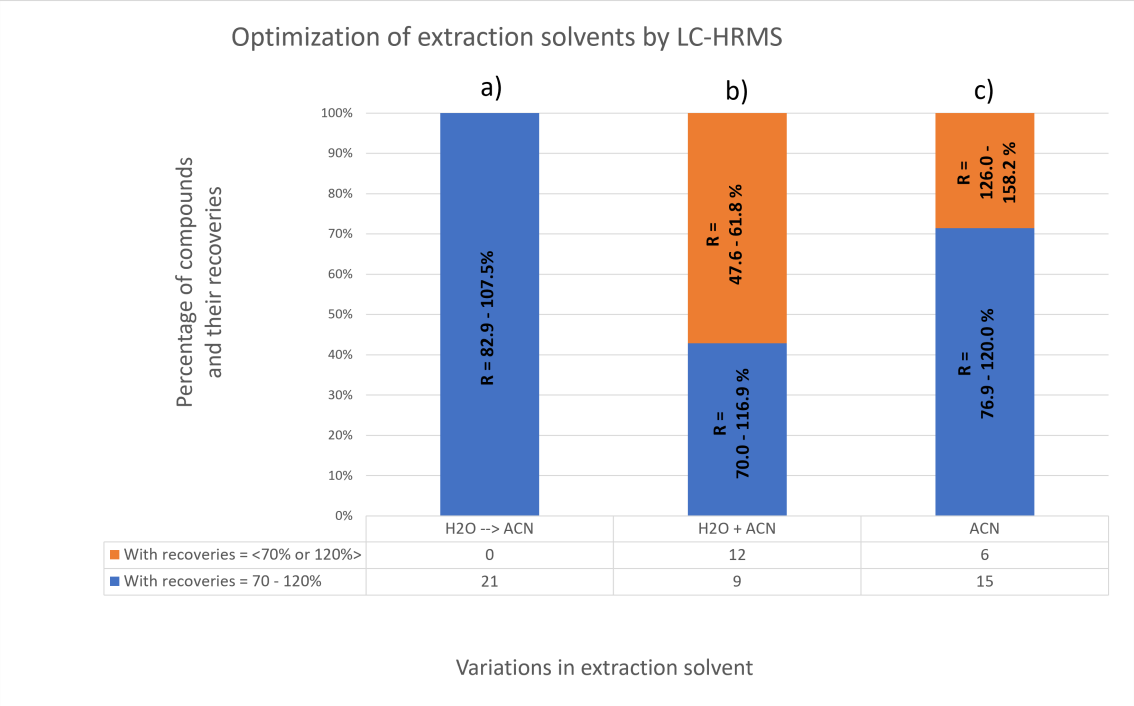

Figure 4