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Advantages of online supercritical fluid extraction and chromatography hyphenated to mass spectrometry to analyse plastic additives in laboratory gloves

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Abstract

Plastic additives are introduced in plastic material formulations, along with organic polymers, to offer different properties such as stability, plasticity or colour. However, plastic additives may migrate from the plastic material to the content (in case of plastic containers) or to the material in contact with the plastic, like human skin. In the case of plastic medical devices, this migration is of particular interest, as plastic additives may be deleterious to health. In the present paper, we examined the interest of combining supercritical fluid extraction (SFE) to supercritical fluid chromatography (SFC) hyphenated to mass spectrometry (MS) in an online system to characterize plastic additives in laboratory gloves, taken as samples of medical devices. A set of target compounds comprising 18 plasticizers, 4 antioxidants and 2 lubricants was defined and their detectability with MS was examined, where it appeared that electrospray ionization (ESI) provided better detectability than atmospheric pressure chemical ionization (APCI). After examining possible stationary phases with the help of Derringer desirability function, an isocratic chromatographic method (CO₂:methanol 95:5) was developed on Shim-pack UC Phenyl column. The extraction method was examined with a 3-level full factorial design of experiments to optimize the extraction temperature (40°C) and pressure (200 bar). The online SFE-SFC-MS method was compared to offline methods where the samples were extracted with liquid solvents at atmospheric pressure or high pressure then analysed with SFC-MS. In all cases, offline methods showed significant contaminants (like the oleamide lubricant) issuing from laboratory plastic materials as nitrogen drying station, syringes and filters, while the online method allowed a complete elimination of laboratory contaminations. Furthermore, the online method saved time,

solvents and laboratory consumables. It will also show that transferring a compressible fluid from a loading loop is favourable to high efficiency, as the resulting chromatographic peaks are much thinner than when transferring a liquid. Compared to injecting liquid heptane, the efficiency increase was 3.4-fold, while compared to injecting liquid methanol (a common practice in SFC), the efficiency increase was 13-fold. Finally, the additive composition of different laboratory gloves was compared.

Keywords: mass spectrometry; medical devices; plastic additives; supercritical fluid chromatography; supercritical fluid extraction

1. Introduction

Plastics are among the most widely used materials in our society across many fields. They are composed of a mixture of organic polymers and small-molecule additives to obtain specific physico-chemical properties in the final product. Each type of plastic composition has different properties and is suitable for different applications [1]. One of the important applications for plastics is in the hospital sector, where they are used in medical devices such as infusion tubing, blood bags, nutritive bags, syringes, medical gloves, and more [2].

The introduction of additives is essential during plastic formulation to obtain specific physico-chemical properties of the final product. It allows controlling the properties of the material, as flexibility or colour, but may also increase the product's lifetime by enhancing resistance to oxidation, thermal stability and aging degradation [3]. Other additives are simply introduced to facilitate manufacturing of the material, *e.g.*, when it must be formed into a specific shape. To achieve these properties, various families of plastic additives such as plasticizers, antioxidants, slip agents or lubricants, are incorporated during the manufacturing process [4]. However, one of the major issues with plastic additives is their possible migration. Migration effects involve a mechanism where additives, which are not chemically bonded to polymers, transfer from the containers to the content, or to the material in contact with plastic [5–7]. For medical devices, it may be blood, nutritive liquid, water, human skin, *etc* [8–10]. These mechanisms are accelerated under certain conditions such as high temperature, humidity, low or high pH, or due to plastic aging [11,12]. Unfortunately, these migrations can be harmful for health depending on migrant compounds [13–15]. Indeed, numerous plastic additives are described as migrant compounds and some of them are already established as toxic or CMR (carcinogenic, mutagenic and reprotoxic substances) [16]. Among these compounds, plasticizers were most often investigated, but in some cases, lubricants or slip agents used during plastic manufacturing were also identified in the content matrices [17]. As a result, the use of some plastic additives in medical devices is now regulated [18–20]. However, in most cases, manufacturers do not provide any information on the plastic additive content of finished products, prompting the need for reliable extraction and analysis methods to determine the composition of plastic materials.

Plastic additives include a wide variety of molecules from different chemical families and with different polarities. As a result, several different extraction and analysis methods were described [21]. On the one hand, the plastic material may be totally dissolved in a strong solvent as chloroform or tetrahydrofuran, to characterize and quantify the whole plastic formulation, in simple solvent dissolution [21,22] or Soxhlet device [23,24]. On the other hand, when only plastic additives are of interest and not the polymer composition, or when only migrating compounds are of interest (which may be additives and/or monomers),

extraction methods can be implemented as pressurized liquid extraction (PLE) [25], supercritical fluid extraction (SFE) [26] or solvent extraction with a weak solvent that would not dissolve the polymer [14,27]. In this case, solvents with different polarities such as methanol, ethanol or acetone have been described to extract a large range of possible molecules. When the extract is obtained, a wide range of analysis methods can be employed to analyze plastic additives, typically including spectroscopic methods such as vibrational spectroscopy (Fourier-transform infra-red, Raman) [28] or nuclear magnetic resonance (NMR) [29,30], or chromatographic methods in the gaseous (GC), liquid (LC) [27,31–33] or supercritical phase (SFC) [21,34].

One of the main issues of analysis and quantification of plastic additives is the laboratory contaminations during sample preparation [35]. Indeed, lubricant agents or plasticizers such as phthalates are present in most laboratory consumables, such as syringes, pipettes and cones [36,37]. To avoid potential contaminations, the online combination of extraction, separation and detection is highly desirable. In addition, it limits compound degradation of unstable analytes (like oxidizable compounds) and reduces the overall sample preparation time. Hyphenated systems including SFE and SFC have already been described in the literature with different hyphenation devices [38]. This online combination, often added with mass spectrometry (MS), has already been used in many fields such as metabolites analysis [39], environmental analysis [40] or, even polymer analysis a few decades ago with capillary SFC [41]. To achieve such online method, not only the SFE and SFC-MS methods must be optimized, possibly in a joint fashion [42], but also the best way to hyphenate the two. The aim of this paper is to demonstrate the advantages of using online supercritical fluid combination for the extraction and analysis of plastic additives in medical devices. In particular, the full development of both methods and the interest of a transfer loop are described, using laboratory gloves as a reference material. A sample application is proposed with the characterization of additives in different types of laboratory gloves.

2. Material and methods

2.1. Chemicals

Carbon dioxide with a purity of 99.7% was delivered by Air Liquide (Paris, France). HPLC-grade methanol (MeOH) used as a diluent in standard solutions of the plastic additives, as a chromatographic co-solvent and as extracting solvent for PLE; HPLC-grade ethanol (EtOH) and HPLC-grade n-heptane, used as extraction solvent for PLE and HPLC-grade acetone used for solvent extraction were provided by VWR Chemicals (Fontenay-sous-Bois, France). HPLC-grade ethyl acetate used as extraction solvent for PLE was provided by Sigma-Aldrich (Saint-Quentin-Fallavier, France). Ultra-pure water was supplied by a Milli-Q® IQ 7000

system from Merck (Darmstadt, Germany). Ammonium formate (>99.995%) used as additive in the make-up fluid to assist mass spectrometry ionization was provided by Sigma-Aldrich (Saint-Quentin-Fallavier, France). The 21 following plastic additives standards were used to develop the method: *bis*(2-ethylhexyl) phthalate (DEHP), diisononyl phthalate (DINP, >99.0%), diisodecyl phthalate (DIDP, >99.0%), dipentyl phthalate (DPP, >99.0%), diisobutyl phthalate (DiBP, 99%), *bis*(methylglycol) phthalate (DMEP), dioctyl terephthalate (DEHT, >96%), dimethyl phthalate (DMP, >99.0%), diethylphthalate (DEP, 99.5%), benzylbutyl phthalate (BBP, 97.6%), dicyclohexyl phthalate (DCHP, 99.7%), *bis*(2-ethylhexyl) adipate (DEHA, 99%), tributyl-O-acetylcitrate (ATBC, 98%), trioctyltrimellitate (TOTM, 99%), dibutyl sebacate (DBS, >97.0%), 2,2'-methylene-*bis*(6-*tert*-butyl-4-methylphenol) (Cyanox 2246), octadecyl-3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)-propionate (Irganox 1076, 99%), *tris*(2,4-di-*tert*-butylphenyl)-phosphite (Irgafos 168), erucamide (>99%), Diisononyl cyclohexane-1,2-dicarboxylate (DINCH), Didodecyl 3,3'-thiodipropionate (Irganox PS800), and three more compounds added after the method development to complete the characterization: oleamide (>99%), dibutyl phthalate (DBP), dioctyl sebacate (DOS), were all provided by Sigma-Aldrich (Saint-Quentin-Fallavier, France). Compound names, abbreviations, CAS number, molecular weight, log P, topological polar surface area (TPSA), number of hydrogen-bond acceptors (HBA) and donors (HBD), number of rotatable bonds (*n*_rot) and boiling point are described in Table S1 (supplementary information). Molecular descriptors were determined with MolDesc open-access tool (<http://moldesc.icoa.fr/>) using the Simplified Molecular Input Line Entry Specification (SMILES) of each molecule. A normalized principal component analysis (PCA) using molecular descriptors as variables was computed with XLStat 19.03 software by Addinsoft (New York, United States of America).

2.2. Samples

To develop and optimize the online combination method, laboratory gloves were used as reference samples. Laboratory gloves are considered as medical devices, they are easily available and contain different plastic additives such as plasticizers or slip agents. In addition, they were previously described in the literature as sample material to examine offline extraction and analysis [43], thus were considered ideal to compare the efficiency and accuracy of our online method. Three types of laboratory gloves were examined: two types of nitrile gloves, (i) purple and (ii) blue nitrile laboratory gloves, certified as category 3, and (iii) latex laboratory gloves. Purple nitrile gloves were used for the different steps of the method optimization, while the other two samples were extracted and analysed only with the fully developed SFE-SFC-MS method. For PLE and SFE experiments, diatomaceous earth beads (Hydromatrix Bulk Material) from Agilent Technologies (Santa Clara, United States of

America) were introduced with the sample in the extraction vessel to fill the cell and avoid sample agglomeration.

2.3. Instruments

All experiments were performed on a Nexera UC system from Shimadzu Corporation (Kyoto, Japan). The instrument was composed of the following parts: a CO₂ pump (LC-30ADsf), a co-solvent pump (LC-30AD) used for chromatographic separation and another solvent pump (LC-20ADXR) used to introduce make-up fluid prior to entering the mass spectrometer; an autosampler (SIL-30AC) for direct injection in the chromatographic system; an extractor (SFE-30A) connected with a rack-changer containing 48 5-mL extraction vessel slots (Rack Changer II); two column ovens (CTO-20AC) with 6 column positions each; photodiode array (PDA) detector (SPD-M20A); two back-pressure regulators (BPR) (SFC-30A), one to regulate the pressure in the auto-extractor (SFE BPR) and the second one for the chromatographic system (SFC BPR). The SFE-SFC system was hyphenated to a single-quadrupole mass spectrometer (LCMS-2020) from Shimadzu Corporation, with two ionization sources: electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI). Offline extractions (PLE and SFE) were performed using the same instrument but directly hyphenating the extraction system to a fraction collector (FRC-40SF). Instrument control, data acquisition and reprocessing were achieved with LabSolutions LC-MS version 5.120 from Shimadzu Corporation (Kyoto, Japan).

2.4. Mass spectrometry method

Initially, all plastic additives standards were analysed separately using flow-injection analysis (FIA), with both ionization sources (ESI and APCI). Solutions were prepared in methanol at concentrations ranging from 10 to 20 ppm. ESI and APCI-MS used the following parameters: interface temperature 350°C, desolvation line temperature 250°C, heat block temperature 250°C, nebulizing gas flow 1.5 L/min, drying gas flow 5 L/min, interface tension 4.5 kV. Detection was done in positive and negative scan mode ranging from m/z 100 to 1200. When target ions were identified (most intense ions for each analyte), the following experiments were done with single ion monitoring (SIM) (see selected ions in Table S2). Because the column effluent was mainly composed of CO₂, a make-up solvent at 0.1 mL/min was introduced before the back-pressure regulator and thus before entering the MS, to limit solubility issues upon CO₂ depressurization and assist analyte ionization. The composition was MeOH-H₂O 98/2 (v/v), containing 20 mM ammonium formate.

2.5. Development of the chromatographic method

To determine the best chromatographic column, a preliminary column screening was performed. Seven columns from the Shim-pack UC series (Shimadzu Corporation) were examined (Figure S1). All columns had the same dimensions of 250 x 4.6 mm, 5 μ m. To compare them, a 10 min-gradient method was used, with a flow rate of 3 mL/min. Methanol concentration was set at 5% during 1 min, then increased to 50% during 7 min, and maintained there during 1 more minute, followed by a return to initial conditions during 1 min. Oven temperature was kept at 25°C. The BPR was set at 150 bar and heated at 60°C to assist depressurization. The CO₂ pump chiller was set at 5°C.

The obtained chromatograms were compared with Derringer desirability functions [44]. Different desirability criteria were defined to select the best column (Figure S2): (d₁) number of eluted compounds, (d₂) range of retention times of the eluted compounds, (d₃) peak width at half height, (d₄) peak asymmetry at 5% peak height, (d₅) average peak width at half height of the peaks resulting from standards that are composed of isomers (for DIDP, DIBP and DINCH) and (d₆) resolution of isobaric compounds (DEHP and DEHT). The overall desirability score was computed as the geometric mean of the six individual desirability functions, using identical weight for each function.

When the best column was selected (Shim-pack UC Phenyl), the first experiments done to compare extraction modes still used the gradient elution method. However, for the final online method, an isocratic elution mode was preferred in order to simplify online analyses and reduce solvent consumption. The final method for online combination was then as follows: mobile phase composed of CO₂:MeOH 95:5, flow rate 3 mL/min; BPR was set at 150 bar and the column oven temperature at 25°C.

2.6. Extraction methods

2.6.1. Simple solvent extraction

Simple solvent extraction was performed according to literature [43]. 1 g of purple nitrile gloves was cut into small pieces and transferred to a glass tube. 10 mL of acetone were added, and the stoppered tube was placed in ultrasonic bath during 1 hour. The solvent was transferred into another glass tube and dried under nitrogen flow. The dry extracts were solubilized with 500 μ L of methanol, filtered on a PTFE filter (13 mm, 0.45 μ m provided by Agilent Technologies (Santa Clara, United States of America) and transferred into a glass vial for injection. SFC analyses were performed using the gradient method described in section 2.5.

2.6.2. Pressurized liquid extraction

Pressurized liquid extractions were performed with the same Nexera UC system from Shimadzu Corporation. 1 g of purple nitrile gloves were cut into small pieces and mixed with

diatomaceous earth beads in a 5-mL extraction vessel. Static extraction was performed during 10 min followed by a dynamic extraction step during 20 min, with a flow rate of 3 mL/min, at 40°C and 200 bar. Different extraction solvents were used (methanol, ethanol, ethyl acetate and heptane), to compare the impact of solvent polarity on additives extraction. Three successive extractions were performed on each sample to examine extraction kinetics. Each fraction was collected in a glass tube with the fraction collector. Extracts were dried under nitrogen flow and transferred to a glass vial after filtration on a PTFE filter. SFC analyses were performed using the gradient method described in section 2.5.

2.6.3. Offline supercritical fluid extraction

1 g of purple nitrile gloves were cut into small pieces and mixed with diatomaceous earth beads in a 5-mL extraction vessel. Static extraction was performed during 10 min followed by a dynamic extraction step during 20 min, at 40°C and 200 bar with pure supercritical CO₂ (scCO₂) at 3 mL/min. Two successive extractions were performed on each sample to follow extraction kinetics. Extracts were collected in glass tubes with the fraction collector using a co-solvent pump with ethanol at 0.5 mL/min. Extracts were dried under nitrogen flow and transferred to a glass vial after filtration on a PTFE filter. SFC analyses were performed using the gradient method described in section 2.5.

2.7. Hyphenation of SFE and SFC

To achieve online combination as described in Figure 1, a transfer loop was added between the extraction step and the separation step. The volume of the loop is an important criterion to consider. To measure the loop volume precisely, experiments were done using the delay volume and flow rate. Delay volumes between the pump and UV detector were measured with and without the loop. To measure these delay volumes, MeOH with 0.1% acetone were used as mobile phase. Different isocratic plateaus were performed (at 5, 10, and 15% MeOH in CO₂), the time difference between the percentage of MeOH at the pump and the baseline jump observed with UV detection was measured. This indicated that the loop volume was 60 µL.

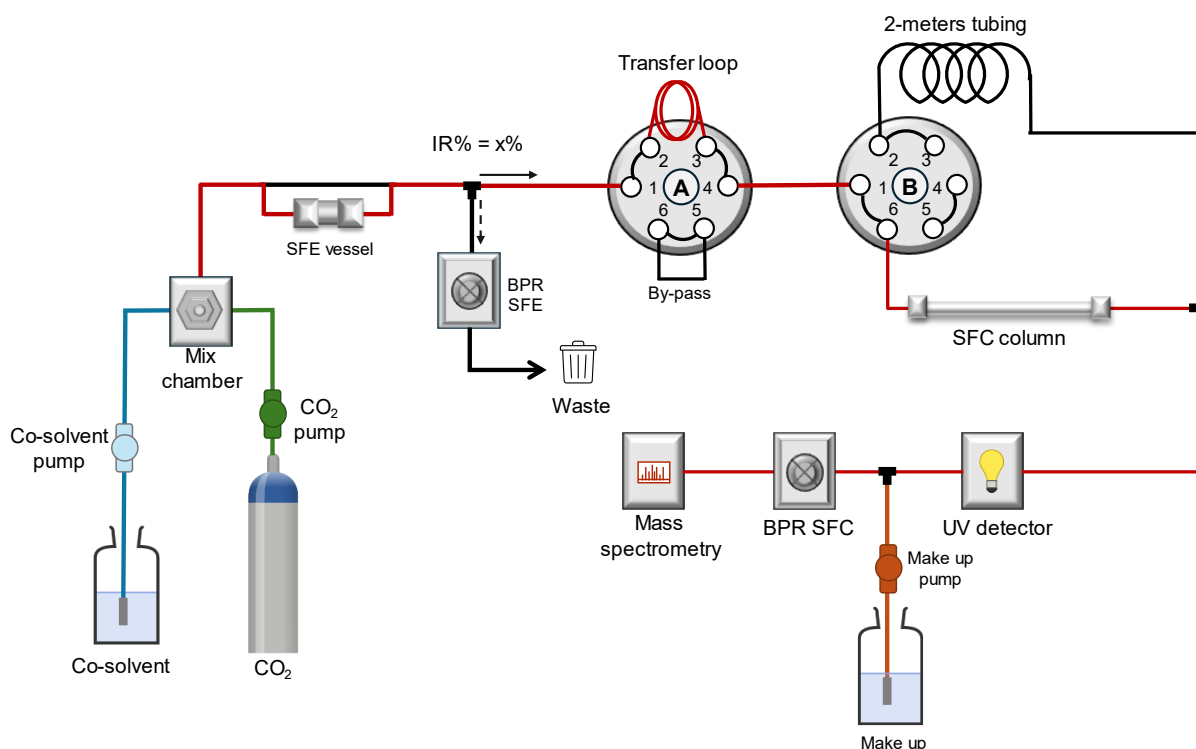


Figure 1. Scheme of the online SFE-SFC-MS system using a transfer loop to hyphenate extraction and analysis

The online combination method was divided into different successive steps as previously described by Gros *et al.* [45]. After a system conditioning (Figure 2A), the method started with a static extraction during 20 min (Figure 2B) with pure scCO₂ at 3 mL/min and 40°C with BPR of SFE set up to 200 bar to bring the extracting solvent in contact with the plastic matrix. Static extraction was followed by a dynamic extraction (Figure 2C) during 0.80 min with a flow rate at 3 mL/min to flush approximately 50% of the 5-mL vessel. During this step, the BPR of SFE was set up at 200 bar and the BPR of SFC at 198 bar. According to calculations of Sakai *et al.* [46] (S3) and in the present conditions, a difference of 2 bar corresponds to an introduction ratio (IR%) of 3%, meaning that 3% of the extract should be transferred to the chromatographic system, while 97% of the extract was discarded (or could be recovered with a fraction collector, whenever it is desirable). A 2-meters metal tubing was added in parallel of the column to maintain close pressure during the extraction step. The transfer loop was then filled during 0.80 min (Figure 2D). A rinsing step was necessary (Figure 2E) to avoid contaminations before switching to the analysis step. Then the system was switched to separation method (Figure 2F) with an isocratic mobile phase composed of 5% MeOH in CO₂, at 3 mL/min.

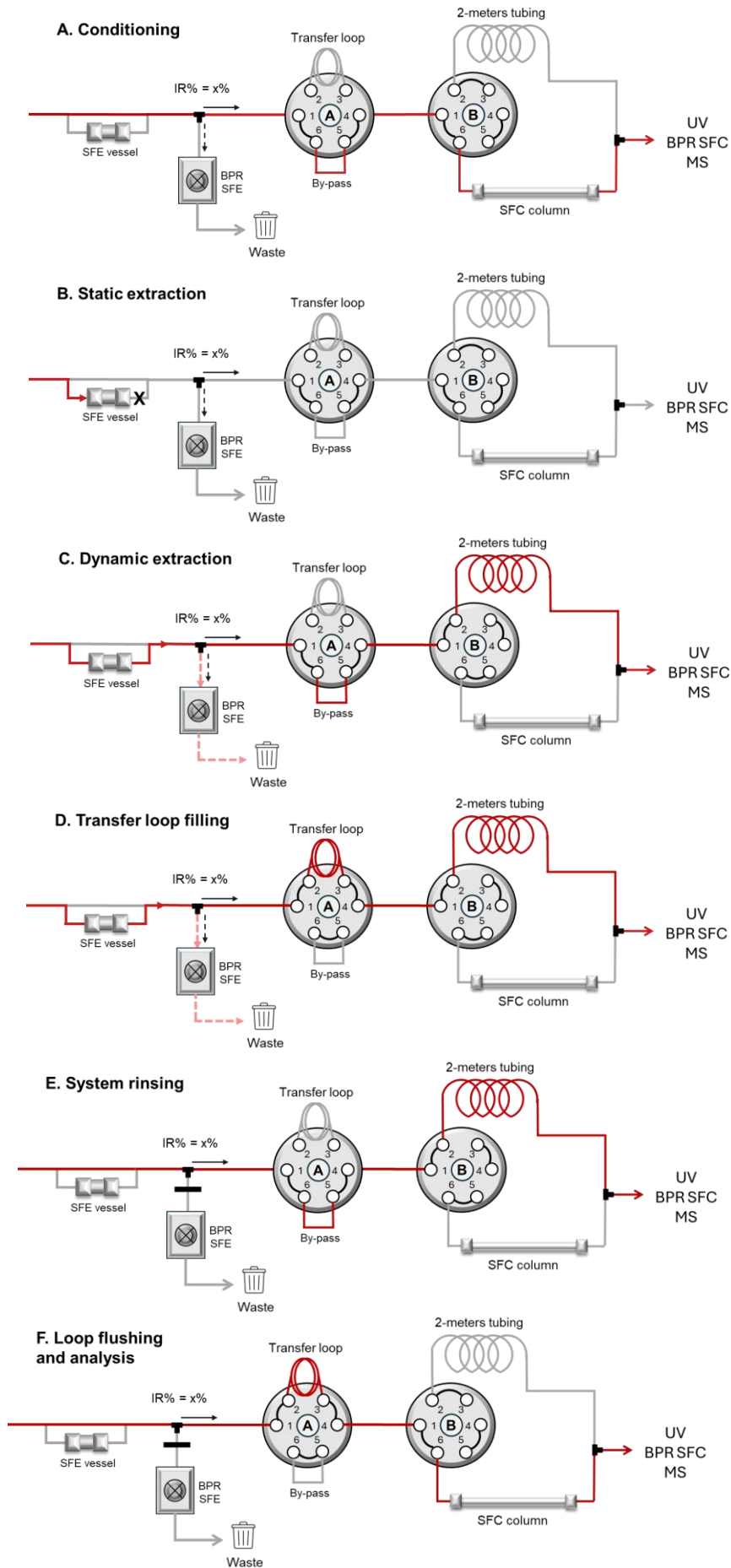


Figure 2. Scheme of SFE-SFC system operation during different steps of extraction and analysis.

The oven temperature was set at 25°C. The SFE BPR was then switched off and the SFC BPR was set at 150 bar, corresponding to an IR% of 100% (*i.e.*, 100% of the loop volume will be transferred to the chromatographic column). The system was equilibrated for a few minutes, then the loop was flushed and the content was sent to the chromatographic column during 15 min. Finally, the system was re-conditioned for the next online extraction and analysis (Figure 2A).

2.8. SFE Design of experiments

To optimize the pressure and temperature of scCO₂ during the SFE extraction, a design of experiments (DoE) was developed. The design type was a 3-level full factorial design, with 2 factors: extraction temperature varying from 30°C to 80°C and extraction pressure varying from 110 bar to 230 bar. The pressure of the SFC BPR was adapted to each extraction pressure, in order to keep the IR% constant to 3%. 14 experiments were done randomly, including 5 replicates at the centre point. 5-mL extraction vessels were prepared with 10 mg of purple nitrile gloves cut into small pieces and mixed with diatomaceous earth beads to fill the extraction vessel. 100 µL of internal standard (DMEP, which was not detected in purple nitrile gloves) were added directly on the top of the extraction vessel before closing it. Data reprocessing, calculation and graph visualization were performed with the StatEase software (Minneapolis, United States of America).

2.9. Rinsing method

To avoid any contaminations issuing from the extraction vessel and to ensure the absence of carry-over, a rinsing method was developed. Extraction vessels were rinsed twice with ultra-pure water, then rinsed during 30-45 min with fresh ethanol in ultrasonic bath, then naturally dried in open air (not under nitrogen flow). Before characterization and quantification, rinsing using online combination method is essential to remove the remains of contamination which could still be present in extraction vessels. These contaminants may come from ambient air, rinsing solvent, or other laboratory contamination. Extraction vessels were flushed during 15 min with dynamic extraction using scCO₂ and then analysed with the online method to check for contamination. In order to highlight the effectiveness of the flushing method with the online combination to avoid contamination, two empty 5-mL extraction vessels were extracted and analysed with the online combination method after the rinsing method (using pure water and ethanol). One of these vessels was previously dried under nitrogen and the

other one was naturally dried in open air. One of these vessels was analysed again after flushing with scCO₂.

3. Results and discussions

3.1. Selection of target compounds

Numerous compounds may be found in plastic formulations, and our purpose was not to be exhaustive. Thus, a selection of target compounds was defined, based on literature survey, to include the additives that were most often cited, those appearing to be frequently present, those that were already or should soon be regulated (like DEHP), their existing or intended replacements (e.g., TOTM, ATBC, DINCH) and well-known analysis contaminants like lubricants. We also looked for some molecular diversity, as exemplified in Figure 3.

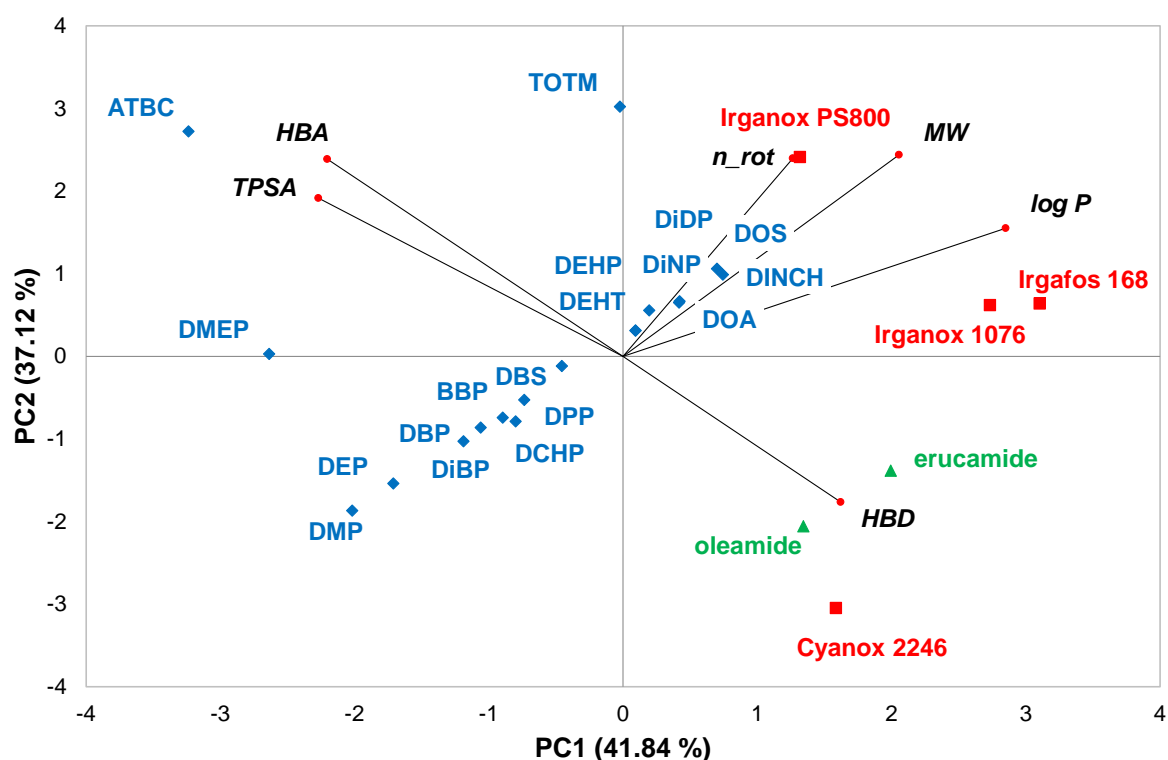


Figure 3. Principal component analysis (PCA) showing the target compounds and their principal features, as described in Table S1. Blue diamonds are plasticizers, red squares are antioxidants, green triangles are lubricants.

The latter represents the biplot of a principal component analysis (PCA) computed with the 24 target analytes as observations and 6 molecular descriptors as variables. The PC1-PC2 plane represents about 79% of the initial variance and is thus amenable to interpretation. It appears that most plasticizers fall on a diagonal line in this figure, in the direction of log P,

molecular weight (MW) and the number of rotatable bonds (n_{rot}). This is not surprising because these plasticizers are phthalate or phthalate-like, with varying chain length. So, the increase of alkyl chain length should cause increased molecular weight, but also increased hydrophobicity ($\log P$) and increased flexibility (n_{rot}). Three plasticizers fall out of this trend, in the upper left part of the biplot: DMEP, TOTM and ATBC, all characterized by a larger polar surface area (TPSA) due to a larger number of hydrogen-bond acceptor groups. DMEP is *bis*(2-methoxyethyl)-phthalate so the alkyl chains contain two more oxygen atoms, compared to usual phthalates; TOTM has three ester groups attached to the central aromatic ring, thus also includes two more oxygen atoms; ATBC is acetyltributylcitrate, containing no aromatic ring but four ester functional groups, thus four more oxygen atoms than most phthalates in this list. On the other side of the diagonal line, at the bottom right of the figure, are the two lubricants oleamide and erucamide. The amide function is providing hydrogen-bond donating (HBD) capability. HBD feature is also present in some antioxidant compounds: Cyanox 2246 that possesses two phenolic functions, and Irganox 1076 that possesses one phenolic function. The other two antioxidants in this list, Irganox 168 and Irganox PS800, have a hydrocarbon skeleton with oxygen and sulphur (Irganox PS800) atoms. From these preliminary observations, it can be expected that most of the plasticizers examined will have only small differences in hydrophobicity, while the other target compounds will differ somewhat in polarity through HBA and HBD groups. Based on this, the columns selected for method development were mostly hydrophobic ligands.

3.2. Development of the SFC-MS method

3.2.1. Mass spectrometry ionization

First, the determination of optimal ionization source for each target compound was necessary. Both ionization sources were compared (ESI and APCI). The intensities of most intense ions were compared for each ionization sources in positive scan mode, except for Cyanox 2246 which was better ionized in negative mode. The results can be observed in Figure S3. It appears that ESI offered much better ionization than APCI for a large majority of the target compounds. This result could be surprising since ESI is generally useful to ionize polar and moderately polar compounds, while the plastic additives examined here are mostly hydrophobic, exhibiting a range of polarities with $\log P$ values varying from 1.30 to 13.20. Judging from these observations, the method was further developed with the ESI source.

3.2.2. Development of the chromatographic method

The development of a separation method was the next necessary step. The goal was to obtain a rapid separation method that would provide sufficient resolution, particularly for critical pairs of isobaric compounds. To determine the best column for this task, a column

screening of seven columns from Shim-pack UC series was performed. The data obtained from the column screening were compared using Derringer desirability functions, based on six different criteria, as described in section 2.5. The desirability functions used for each criterion can be seen in Figure S2. Briefly, each function was defined with scores varying from zero to one. A score close to one corresponds to a most desirable behaviour of the column for the target criterion. For instance, elution of all target compounds that could be detected with ESI-MS is expected and should yield a score of $d_1 = 1$, indicating that no compounds remained stuck in the column. Conversely, a score close to zero indicates that the column does not match the desired expectations. For instance, in the case where no compounds could be eluted within the gradient time, the d_1 score would be 0.

	Choles	SIL II	Phenyl	GIS II	PBr	PyE	RP
d1 - Eluted compounds	1.00	1.00	1.00	1.00	0.90	1.00	1.00
d2 – Retention range	0.37	0.05	0.31	0.37	0.58	0.41	0.31
d3 - Peak width (50%)	0.95	0.97	0.91	0.95	0.81	0.92	0.91
d4 - Asymmetry	0.27	0.31	0.39	0.27	0.06	0.13	0.28
d5 - Isomers separation	0.62	1.00	0.71	0.69	0.40	0.40	0.56
d6 - Isobars separation	1.00	1.00	1.00	0.34	1.00	0.00	0.00
D - Overall desirability	0.62	0.49	0.65	0.53	0.46	0.00	0.00

Table 1. Derringer desirability scores (d values) for each criterion and overall desirability (D) for each column tested

As can be seen in Table 1, showing all values of d scores and overall desirability D (geometric mean of individual d values), this case of course never happened. Indeed, only one column was discriminated by this criterion, the highly retentive PBr column (pentabromobenzyloxy-bonded silica), on which some compounds could not be eluted within the gradient time. The d_2 function was related to the retention range of standard compounds, based on boxplots as shown in Figure S4, enabling the observation of the distribution of target compounds on a specific column. A wider distribution generally corresponds to a better separation of compounds on the stationary phase. The extent of this retention range may be computed based on different values. For instance, the retention of most and least retained compounds could be used. However, extreme retention behaviours are not necessarily representative of the general behaviour of a majority of compounds. So, the d_2 score was based on the interquartile value (Q3-Q1): a d_2 score equal to one corresponded to 4.5 min,

which would be an ideal interquartile value for this gradient profile (9 min of total analysis time, including both isocratic plateaus at start and end of the gradient). In this case, the PBr column appeared to be the best column in terms of compound distribution with an interquartile value close to 2.48 min, followed by the Choles (cholesteryl-bonded silica), PyE (pyrenyl-bonded silica), and RP (polar-embedded octadecyl-bonded silica) columns with values around 1.7 min. The column with the smallest retention range, resulting from low retention of most target compounds, was Sil II (bare silica gel). This was not unexpected as most of the target compounds are rather hydrophobic.

The d3 score was based on the average peak width at half height measured for all eluted compounds. The d4 score was based on the measurement of the average asymmetry at 5% peak height (according to European Pharmacopeia definition), measured for all eluted compounds.

The d5 score was based on the band width observed for some plastic additives that are available as mixtures of isomers and not as a pure compound: DINP, DINCH and DIDP. However, to better identify them and quantify them as a single entity, it is desirable to avoid separating these isomers as much as possible. Figure 4 shows representative chromatograms obtained on two different columns: on the Phenyl column, the isomer mixtures eluted as a single peak, although some structure is visible and the line is not entirely smooth. However, they should allow a reasonable quantitation, unlike the partly resolved broad peaks observed on the Choles column. Therefore, a large d5 score was attributed to the thinnest band widths and a zero score was attributed to a hypothetical large band width that was never observed in practice, to avoid a complete discrimination of a column that would not perform well on this criterion. Three different functions were defined for the three mixtures of isomers, and the mean value was computed as the d5 score. The best scores were obtained on the Sil II column, which does not separate isomers at all because it hardly retains them. The second-best column was Phenyl, followed by GIS II and Choles columns. However, as appears on Figure 4, the partial separation observed on Choles was already considered unacceptable.

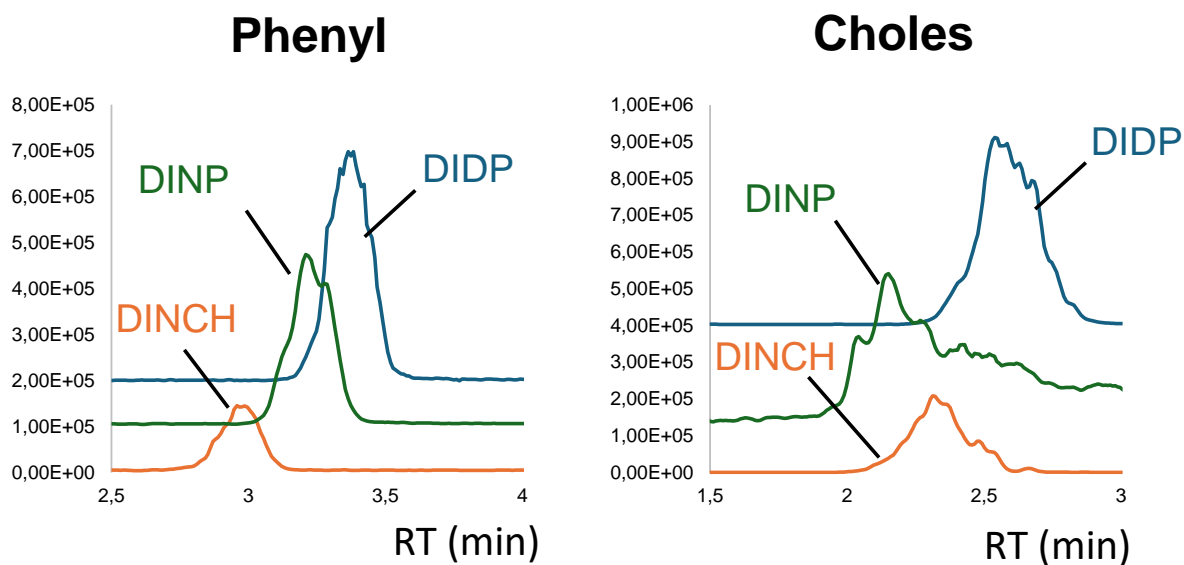


Figure 4. Chromatograms of DINCH, DINP and DDP standards available as a mixture of isomers, analyzed on the ShimPack UC Phenyl and Choles columns.

Finally, the last important criterion was the resolution of isobaric compounds. Two target compounds have the same molecular mass of: DEHP and DEHT, which are *ortho* and *para* isomers of diethyl-hexylphthalate. Their molecular weight is 390 g/mol and their identifier ions were the same (m/z 391 and 408, corresponding respectively to $[M+H]^+$ and $[M+NH_4]^+$). In addition, DEHP is strictly regulated, while DEHT is increasingly introduced in plastic formulations to substitute DEHP, thus a good resolution of this critical pair was essential. A d_6 score of 1 was attributed to the best resolution observed, which was 1.5. Among the selected columns, four of them allowed a total separation of DEHP and DEHT: Choles, Sil II, Phenyl and PBr, while GIS II offered partial resolution and PyE and RP could not resolve these isomers.

The overall desirability (D value) was calculated using the geometric mean of the d values. A geometric mean is more discriminating than an arithmetic mean, as it would exclude a column for which a d value is equal to zero. D values are shown at the bottom of Table 1. An overall desirability close to 1 thus corresponds to columns matching all expectations. Two columns seem to be acceptable, Choles and Phenyl columns with respectively an overall desirability score close to 0.62 and 0.65. The Choles and Phenyl had very close overall performance, with the Phenyl column showing some superiority in the elution of isomeric mixtures, as observed in Figure 4. Finally, the Phenyl column was selected for further analyses. The chromatogram of all standards can be seen in Figure 5A.

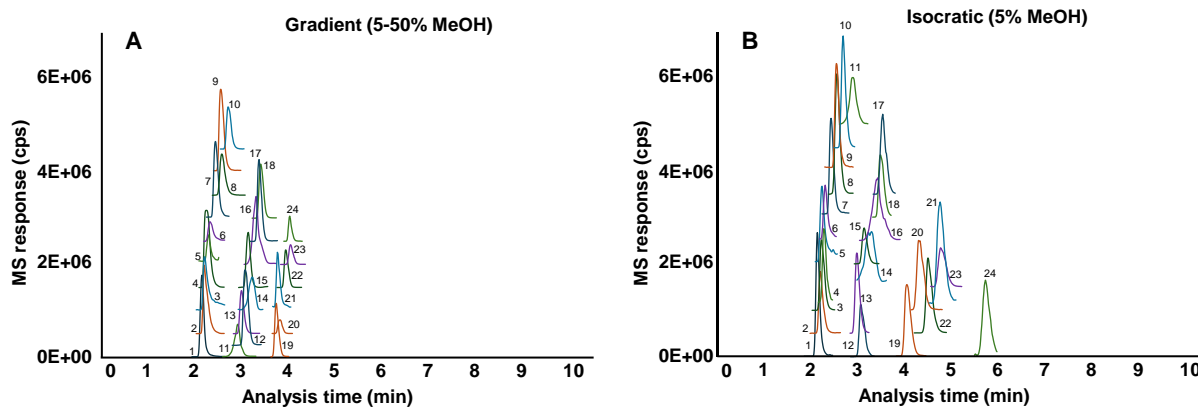


Figure 5. Reconstituted chromatogram with SIM ions of plastic additives standards using the ShimPack UC-Phenyl column (250 x 4.6 mm, 5 μ m). Chromatogram A was obtained with gradient elution (5 to 50% MeOH in CO₂) and chromatogram B with isocratic elution (5% MeOH in CO₂). See Table S3 for analyte identification.

However, as appears on this figure, resolution could be improved, which could be obtained with lower elution strength. As appears in Figure 5B, a constant mobile phase composition comprising only 5% MeOH provided satisfying resolution in a short analysis time (6 min). The retention times and peak widths at half-height are detailed in Table S3 for both modes. As shown in Figure S5, which shows the retention time distributions and peak widths of the compounds, the use of an isocratic mode with 5% methanol gives a better distribution on the chromatogram, and therefore better separation. However, as expected, peak widths are slightly increased, especially for compounds that are mixtures of isomers, but this did not interfere with the separation and did not prevent correct identification of all target compounds. Finally, the isocratic mode facilitated the online SFE-SFC-MS operation, reduced solvent consumption, and reduced the solvent flow entering the mass spectrometer, which was favourable to low background noise and improved ionization.

It must be noted that the column selected here (Shim-pack UC Phenyl) has a low retentivity, so it is unlikely to cause excessive retention of undesirable sample components. However, to avoid any carry-over or long-term contamination of the column, it could be advised to add a cleaning step, typically including 20 to 50% methanol in CO₂. Note that no such problem was observed in the present study, but different samples might yield different contaminations.

3.3. Offline extraction method comparison

Different offline extraction methods were examined to be compared to the online method. Based on frequent observations in the literature, three types of extraction methods were compared: simple solvent extraction using acetone, pressurized liquid extraction (PLE) with

different polarities of solvent (methanol, ethanol, ethyl acetate and heptane) and supercritical fluid extraction (SFE) with pure scCO₂. Figure S6 represents chromatograms obtained for each extraction method and with each solvent, while Table S4 indicates the compounds that could be detected in purple nitrile gloves. In accordance with literature [43], solvent extraction with acetone allowed extracting different plastic additives such as DMP, DEP, DIBP, DEHP, DEHT and DINP. In addition to these compounds, other plastic additives were extracted: ATBC, DIDP, DEHA, and TOTM; these plasticizers are used as DEHP substitutes. Some antioxidants were also extracted such as Cyanox 2246 or Irganox PS800 in high proportion, and two lubricants that may be used during manufacturing, oleamide and erucamide. Almost all compounds extracted with acetone were also extracted using PLE (EtOH and MeOH) or with scCO₂, except for DEHT.

As could be expected, the polarity of the extraction solvent influenced the nature and quantity of compounds extracted. Indeed, some compounds, such as ATBC, DBP, DIDP, DEHA, DEP, DINP and DMP were not extracted in PLE with ethyl acetate or heptane. However, these two solvents had the advantage to avoid extraction of oligomers at the first extraction of the polymer. Figure S7 shows the chromatograms of extraction kinetics with heptane and ethyl acetate. With ethyl acetate, after 40 min extraction, the polymer was starting to dissolve and oligomers were extracted with the plastic additives. Ethyl acetate and heptane appeared to have a lesser penetration of the plastic matrix and did not allow a total extraction of plastic additives in one step.

Histograms in Figure S8 show the peak area obtained for the extracted compounds, depending on extraction method and solvent. For all extraction methods the most abundant was Irganox PS800, which is an antioxidant used in the manufacturing of polymers. Another antioxidant was also found in large proportions, Cyanox 2246, which is used in non-food packaging, medical devices or pharmaceutical applications. Also, two plasticizers, DINP and DEHP, were extracted in significant amount. These two compounds are already widely described in the literature for the manufacturing of medical devices, especially for DEHP which is a controversial compound considered as CMR.

Oleamide (lubricant) also yielded an intense signal for all extraction methods. However, the presence of oleamide in the sample is debatable. Indeed, oleamide and erucamide are often encountered as contaminants issuing from laboratory plastic consumables [36], resulting in contaminant ions in mass spectrometry. But these compounds may also result from the plastic production, since the machines used during manufacturing are lubricated to facilitate production. Therefore, these compounds can be found in the formulation of finished products. In the present case, it was difficult to confirm the origin of lubricants, if they issued from laboratory contaminations were originally present in the glove sample. This point required further investigation (*vide infra*).

To conclude on this section, the comparison of these extraction methods confirmed that the use of scCO₂ was appropriate to extract plastic additives. In addition, it allowed a significant reduction of solvent consumption.

3.4. Laboratory contaminations

One of the major issues in the analysis of plastic additives is to avoid laboratory contaminations. Indeed, many consumables and devices are made with plastic and would then contain plastic additives. Migration of these additives from their plastic matrix to the samples under examination or to the instrument has already been described in the literature [35]. First, blank reference analyses were performed with methanol. To examine all possible sources of contamination in offline analysis, methanol was processed with the same preparation methods as the samples. Figure 6 represents chromatograms of different blank analyses.

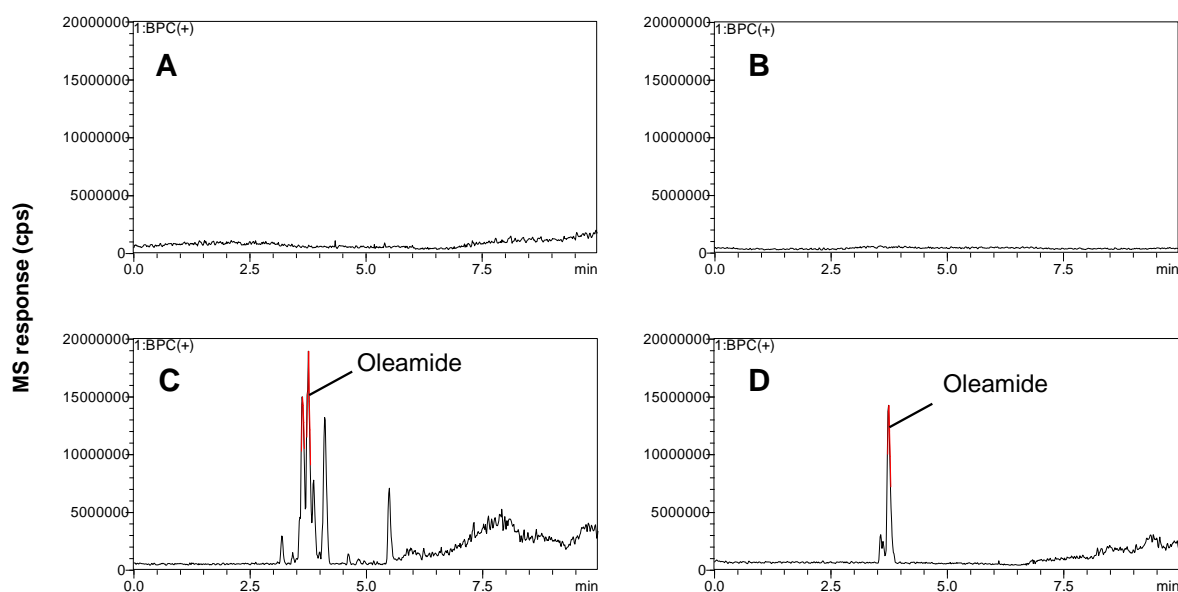


Figure 6. Base peak chromatograms of (A) blank analysis of the system, (B) blank analysis of the HPLC-grade methanol used as solvent for sample preparation, (C) blank analysis of the same HPLC-grade methanol after drying step and reconstitution, (D) blank analysis of the same HPLC-grade methanol after filtration step with plastic syringe and PTFE filter.

Figure 6A is simply an analysis run to test the system, without any sample introduction: it shows a rather flat baseline, devoid of contamination, indicating that all parts of the SFC-MS instrument were clean. Figure 6B shows the analysis of the HPLC-grade methanol that was used for sample preparation: it also shows a clean trace, indicating that no contaminants were present in the solvent. Figure 6C is the analysis of the same methanol solvent, that was first evaporated under nitrogen stream, then reconstituted to be injected in the system: it

shows strong contamination, with many peaks observed, including oleamide. This contamination can be explained by the presence of plastic tubings in which the nitrogen flow is directed towards the sample to evaporate. Finally, Figure 6D is the analysis of the same methanol solvent that has been introduced in a plastic syringe and filtered on a PTFE filter: it also shows some contamination, particularly a strong peak of oleamide. The latter was previously described as contaminant in plastic consumables [47].

It is important to note that the presence of these contamination is not necessarily repeatable and depends on the consumables and systems used in the laboratory. Although this does not prevent the characterization or quantification of other additives in the sample, it does show that sample preparation steps can induce significant contamination. On-line extraction and analysis, avoiding these sample preparation steps, should avoid these contaminants and thereby offer more reliable results.

To ensure that the online method would be free of contaminants, also the extraction vessels would need to be perfectly clean. The extraction vessels were cleaned with EtOH and water, but required drying. We had first considered drying them under a nitrogen stream, but, as could be expected from the above results, contaminations were visible (Figure 7). Again, plasticizers (DIDP, DEHP, TOTM, DBP and DINP) and lubricants (erucamide) were observed. Secondly, we tried simply leaving the extraction vessels to naturally dry in the open air, but here again, some contaminants remained, although in smaller amounts. We must conclude that these contaminants were present in the ambient air of the laboratory, or were perhaps deposited by the rinsing solvents.

Finally, we considered flushing the extraction vessels with scCO₂ before sample introduction. Figure 7 shows that none of the contaminants observed with the other two methods were visible after this scCO₂ cleaning. This procedure of vessel flushing prior to sample introduction was therefore retained for all subsequent experiments.

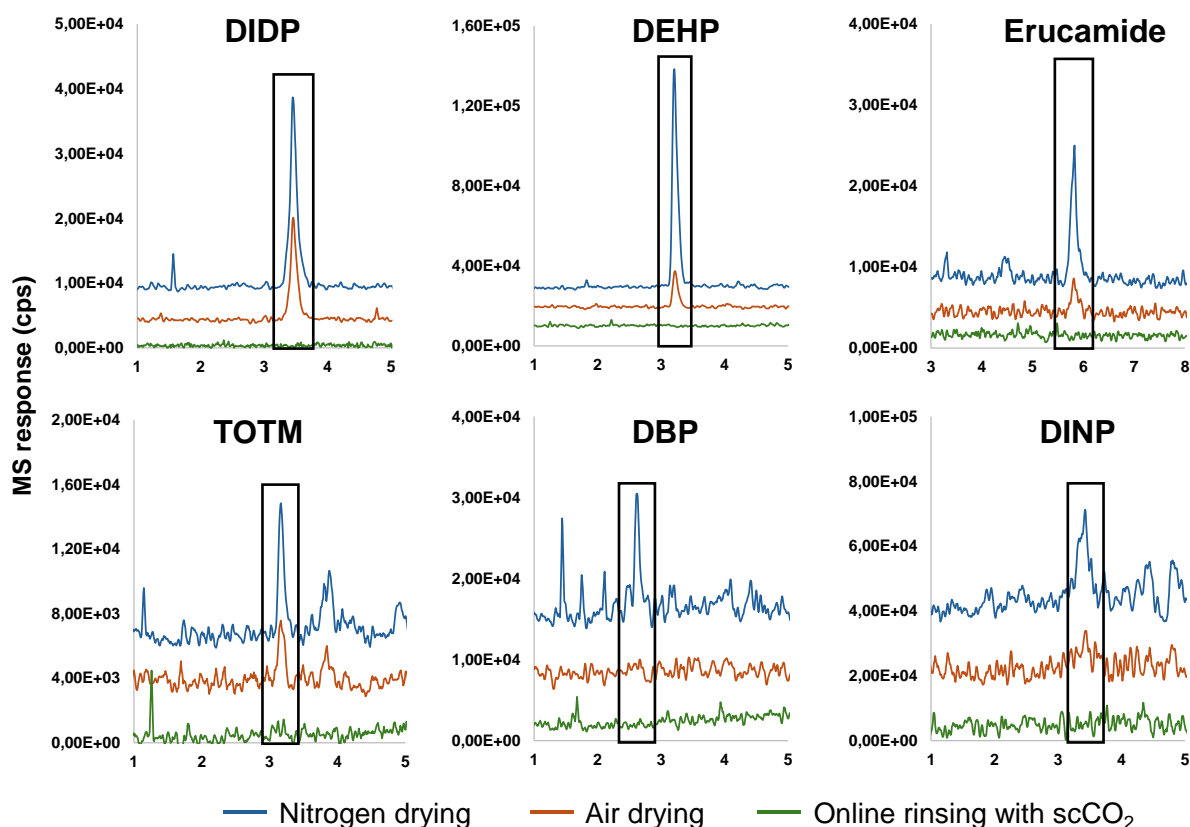


Figure 7. SIM of target compounds that can be detected in extraction vessel after rinsing step with different drying methods. Blue: nitrogen drying station; orange: open air drying; green: scCO_2 flushing.

To conclude on this important section, it should be clear that the online SFE-SFC-MS method should be free of contaminants, and that any additive detected would result solely from the sample. In addition, it would reduce sample handling time, the consumption of solvents and the consumption of plastic consumables.

3.4 Development of the online SFE-SFC-MS method

3.4.1. Determination of loop filling time

Hyphenating SFE to SFC can be achieved with different devices but a transfer loop has significant advantages [38]. First, it allows independent control of extraction and separation steps, so the separation method needs not be developed with the constraint of the extraction method. Second, it allows transferring a defined and controlled volume of the extract to the analysis system. Third, this transfer is done only when the start of analysis is desired, avoiding early elution of low-retention compounds from the column, which would result from direct hyphenation of SFE to SFC. Indeed, as demonstrated by the above-described chromatographic observations and by the extraction method, neat CO_2 is well capable to elute most of the target plastic additives, which are mostly low-polarity compounds. If column

head-trapping was used as hyphenating method, the extracted compounds would start progressing in the column during the dynamic extraction step, possibly deteriorating the separation and impairing compound identification, especially for isobaric compounds. Further information and strategies to achieve efficient trapping of sample plug can be found in a detailed study from B. Berger and co-workers [48]. As a result from the above considerations, a transfer loop was selected in the present study.

However, the filling time of the loop is an important parameter to assess. To ensure good reproducibility of the method and accurate quantitation, the loop should be completely filled with compounds transferred from the extraction process. However, because scCO₂ is a compressible fluid, assessing the volume is not a trivial question. Indeed, the amount of scCO₂ that can fill a defined geometrical volume will vary with the fluid compressibility. The latter is lower for high-density scCO₂ (typically at high pressures and low temperatures) and is higher for low-density scCO₂ (at low pressures and high temperatures).

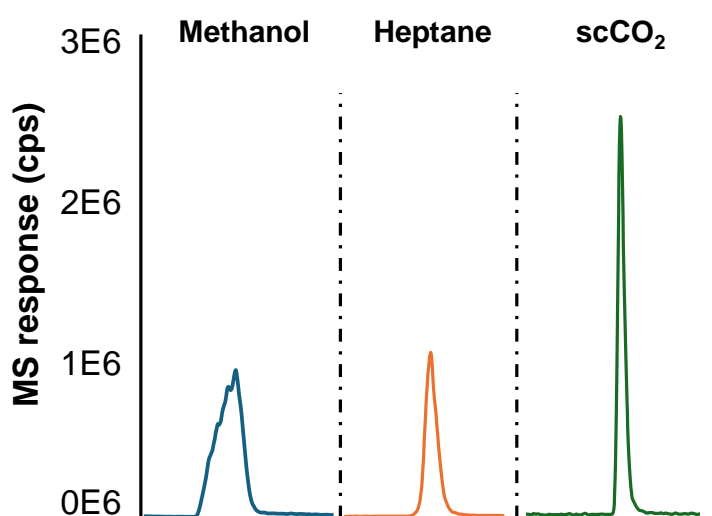
As the (geometrical) loop volume was 60 µL, with a 3 mL/min flow rate and an introduction ratio of 3%, the theoretical time to entirely fill the loop was expected to be 0.7 min. To confirm this value, we examined the peak areas observed for one target compound (Irganox PS800, abundantly present in laboratory gloves) and an internal standard (DMEP) spiked in extraction vessels, depending on loop filling time. The results are presented in Figure S9. As expected from theoretical volume, a filling time of 0.4 min showed low peak areas for both compounds, together with high RSD values (12 and 22%). From 0.8 min to 2.0 min filling time, peak areas were not increasing significantly while the average RSD values were rather low (5% and 9%). Considering the variability that may be expected from the extraction step and from the dynamic online transfer, this was considered as acceptable. Therefore, the minimum time to fill the loop was 0.8 min, which was well fitting with the theoretical estimation.

3.4.2. Effect of injecting a scCO₂ sample

Under normal circumstances, the injection of a large sample volume in a chromatographic column can induce peak distortion. This is especially true when the eluting strength of the dilution solvent is larger than the eluting strength of the mobile phase, or when the viscosity of the former is significantly different from the viscosity of the latter (viscous fingering effect). In addition, the dilution solvent may adsorb on the stationary phase, causing retention variation and/or peak distortion[49]. Considering such previous knowledge, the introduction of 60 µL sample might have been worrying. However, in the present case, the sample injected is not in the liquid form, as it is diluted in 100% scCO₂.

To demonstrate the advantage of scCO₂ in this case, we compared the online SFE-SFC transfer to the direct injection of a liquid sample in the SFC system. Two liquid solvents were

considered: methanol, which is often used as a dilution solvent in SFC analysis, and heptane, which polarity is most similar to that of scCO₂. The results are presented in Figure 8, showing the introduction of DMEP in identical quantity for all three experiments. In the table below the chromatograms, the measured peak areas indicate that the quantity introduced in the column was indeed identical. However, as appears from the chromatograms and the measurement of peak width at half height and efficiency (N), the contribution of the dilution solvent on chromatographic quality was huge, as the injection of 60 µL scCO₂ was far superior to the injection of 60 µL liquid.



	Methanol	Heptane	scCO ₂
Peak Area	1.72E+07	1.08E+07	1.24E+07
Peak width (50%) in minutes	0.322	0.164	0.089
N	490	1891	6421

Figure 8. Chromatograms of DMEP with the same amount injected with a full loop of 60 µL using different solvents: liquid methanol, liquid heptane or supercritical CO₂. The table below shows the area, the peak width at half height and corresponding number of theoretical plates (N) for each injection.

Several reasons may explain this result. First, the diluent polarity has some contribution, since the chromatogram obtained with heptane diluent was much better than the chromatogram obtained with methanol diluent, which was strongly distorted. The measurement of theoretical plates shows that replacing methanol with heptane resulted in a nearly 4-times increase in efficiency. As scCO₂ has a comparable polarity to heptane, it

should also benefit from the low polarity effect in focusing the analytes at the column entrance. Secondly, the viscosity mismatch should be much lower when neat scCO₂ is injected in a CO₂-MeOH 95-5 stream than when a liquid sample is injected, thereby minimizing the impact of viscous fingering. Thirdly, the injection of scCO₂ is not likely to cause unwanted solvent adsorption on the stationary phase, thus not disturbing the adsorption of the analytes [49]. Last but not least, we believe that the compressibility of the scCO₂ sample is probably an important factor to explain the sample focusing at the column entrance. Indeed, when the loop is loaded, the pump pressure was about 200 bar, while it increases to 235 bar when the loop content was transferred to the column, thereby further re-focusing the analytes.

Whatever the reason, the peak obtained in scCO₂ diluent was 3.4-fold more efficient than the one obtained in heptane diluent, and 13 times more efficient than the one obtained in methanol diluent, thereby favouring resolution and detection. This is an interesting observation because it suggests that bigger loops could be considered when low-concentration compounds need to be detected, without risking deteriorating chromatographic quality.

3.4.3. Determination of sample weight

Manufacturers usually do not disclose the composition of plastic additives used in formulated products. In addition, the concentration range from one additive to another can vary to a large extent. Therefore, it is not possible to predict the additive concentrations from one sample to another and it is best to optimize the amount of sample introduced in the extraction vessel for each sample individually. The appropriate amount of sample should allow to detect and quantify target compounds without saturating the mass spectrometer, so a compromise must be found.

Figure S10 represents chromatograms with different weighted amounts of the nitrile gloves introduced in extraction vessel and analysed with the online SFE-SFC-MS method. From chromatogram A, it appears that around 10 mg of nitrile gloves should be sufficient: some peaks are already saturated, but minor compounds are still detected. A small quantity of sample is beneficial to avoid saturation but also to reduce extraction time. Compared with offline extraction methods reported in previous works [14,21,50] (between 0.5 and 1g were used), a lower sample weight was used without impairing the capability to detect even the minor target compounds. In addition, it also indicates that small amounts of samples could be amenable to this method, *e.g.*, to analyse microplastics.

3.4.4. Influence of the extraction time

Two extraction modes are possible, static and dynamic. Dynamic extraction time were optimized in section 3.4.1, allowing compounds to be transferred from the extraction vessel into the transfer loop. The static extraction time corresponds to the contact time between the extraction solvent (pure scCO₂) and the sample. At this stage, the same volume of solvent remains in the extraction cell in constant conditions of temperature and pressure, and is not changed. Next, the dynamic extraction step is renewing the solvent in the extraction cell but also eluting the extract out of the cell, towards the analysis system and/or the fraction collector.

Depending on sample features, it is useful to optimize static time to ensure sufficient time for the extraction solvent to penetrate the sample matrix and extract analytes. Laboratory nitrile gloves were extracted with different static times using the online combination method described in part 2.5. Area and extraction yield for two successive extractions of 20 min each for one major compound (Irganox PS800) and one minor compound (DEHP) are compared in function of the static extraction time, described in Figure S11. For both compounds, the static extraction time was not strongly impacting the total peak area. However, for the major compound Irganox PS800, 5 min of static extraction were not sufficient to perform a single extraction. The extraction yield was around 30% while it reached 65% when the first static extraction lasted 20 min. For the minor compound DEHP, the static time only had a moderate impact on the extraction yield. However, the values of RSD(%) were much higher for the minor compound than for the major one, which can be explained by the fact that the DEHP peak height was close to the detection limit. Even if the static time appeared to have only a moderate impact for this matrix, the following experiments were performed with a single static extraction lasting 20 min, to ensure measurable extracted quantities without excessively long extraction times. For quantification, to obtain a better yield and exact values, two successive extractions are probably recommended.

3.4.5. Design of experiments

In order to optimize the extraction conditions, an experimental design was performed with a 2-factor 3-level full factorial design. The results obtained are presented as a heat map in Figure 9 for the same two identified compounds, the major Irganox PS800 and minor DEHP. Corrected areas were calculated considering sample weight and peak area measured for the internal standard. The relative standard deviation for the corrected peak area at the centre point (5 replicates) was around 6% for Irganox PS800, and around 28% for DEHP. Again, the variability is higher for DEHP, which is a minor compound with a low concentration, close to the limit of quantification. However, the two figures show the same trend: the combination of high pressure and low temperature allows for a better extraction yield for these compounds. According to Tarafder *et al.* [51] the density of scCO₂ for a higher pressure and low

temperature is higher than with high temperature. In addition, high density making a less compressible fluid is usually favourable to reproducibility because the fluid density will vary less with small variations of pressure and temperature. However, high pressure can cause system issues during extraction, and a low temperature (although above the room temperature) may be difficult to stabilize with the SFE extractor. Finally, considering all the above, we set the pressure at 200 bar and the temperature around 40°C.

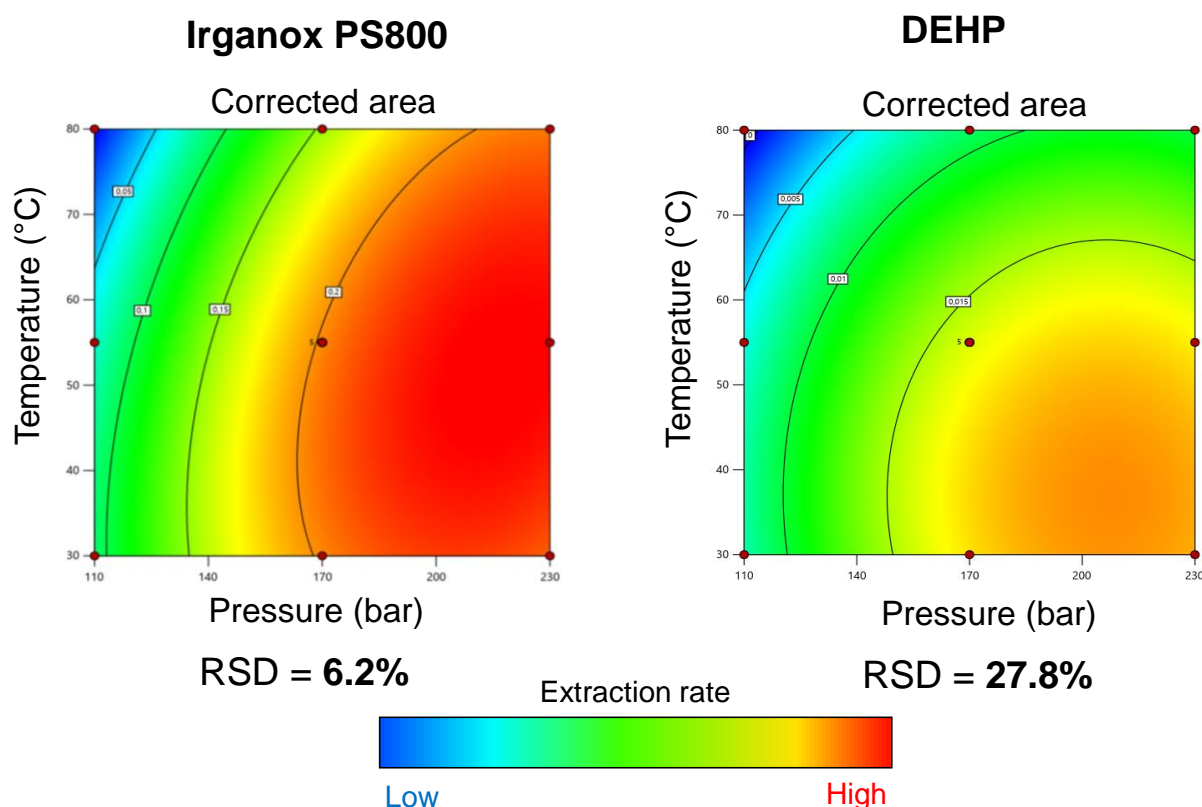


Figure 9. Heat maps showing the variations of corrected peak area depending on extraction temperature and pressure, for the major compound (Irganox PS800) and minor compound (DEHP) found in nitrile gloves. Relative standard deviation (RSD) is given for five replicates at the center point. Calculation and heat maps visualization were done with StatEase software.

3.5. Characterization of plastic additives for different types of laboratory gloves

The final online combination method allows to extract different plastic additives using pure scCO₂ and simple, isocratic elution conditions using only 5% MeOH in CO₂. The MS chromatograms (TIC and SIM) obtained for purple nitrile gloves are represented in Figure 10.

Compounds	Solvent extraction	PLE				SFE	SFE-SFC
	Acetone	Ethanol	Methanol	Ethyl acetate	Heptane	Pure scCO ₂	Pure scCO ₂
ATBC							
BBP							
Cyanox 2246							
DBP							
DBS							
DCHP							
DIDP							
DEHA							
DEHP							
DEHT							
DEP							
DIBP							
DINCH							
DINP							
DMEP							
DMP							
DOS							
DPP							
Erucamide							
Irgafos 168							
Irganox 1076							
Irganox PS800							
Oleamide							
TOTM							

Compound extracted and detected

Compound not extracted or detected for a specific extraction method

Compound not extracted or detected for all extraction methods

Table 2. Summary of compounds extracted and detected for each extraction method for purple nitrile laboratory gloves

The compounds detected were also compared between the different offline and online methods examined (Table 2), and it appears that the online combination method enabled to extract and characterize the same compounds as the offline methods, such as plasticizers (ATBC, DBP, DIDP, DEHA, DEHP, DEP, DIBP, DINP, DMP and TOTM), antioxidants (Cyanox 2246 and Irganox PS800, in high proportion) and lubricants (oleamide and erucamide). The only target compound that could not be extracted or detected with the online method was DEHT. It would be surprising that scCO₂ would not be able to extract this molecule as it is an isomer of DEHP, which was well extracted. Perhaps DEHT was extracted in a concentration below the detection limit.

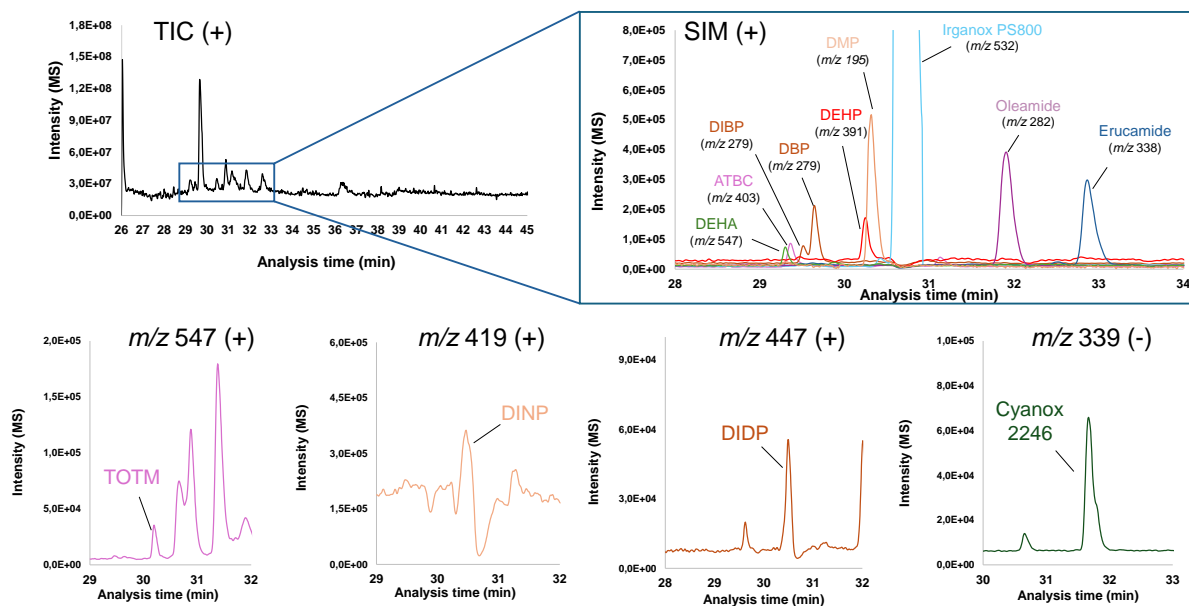


Figure 10. Total Ion Chromatogram (TIC) in positive scan mode for purple nitrile gloves using the online SFE-SFC-MS method and Single Ion Monitoring (SIM) of target ions in positive and negative modes.

Other types of laboratory gloves (blue nitrile and latex laboratory gloves) were also extracted and analyzed using the online method in order to characterize the composition of plastic additives. Figure S12 shows histograms of corrected area of the compounds that could be detected in the three different types of gloves. Corrected areas were calculated using the peak area of the compounds divided by the sample weight and corrected with internal standard. Relative standard deviation values (RSD%) were calculated based on three replicates for each sample. Many plastic additives that were extracted and characterized appeared to be the same in the three types of gloves. Among these compounds some plasticizers were detected, such as DMP, DINP, DBP, DIBP, DEHP, TOTM, DIDP, the two lubricants erucamide and oleamide, and also two antioxidants Irganox PS800 and Cyanox 2246. Irganox PS800 was detected in higher proportion in purple and blue nitrile gloves than in latex gloves. Oleamide and DIBP were more concentrated in latex laboratory gloves than in nitrile gloves. In some cases, RSD% were very large, such as for Irganox PS800, DMP, DINP or DBP. These variabilities can be due to different factors, such as the low sample weight (10 mg), which may not be sufficiently representative of the whole sample composition if the plastic glove is somewhat heterogeneous. The lubricants may be detected in the glove composition, even if they were not introduced in the plastic formulation, but perhaps only at the glove forming stage. This type of contaminant compounds may well vary from one sample to another, thereby increasing the analysis variability.

4. Conclusions

The experiments presented in this paper demonstrate that an online SFE-SFC-MS method is well able to characterize plastic additives from small amounts (10 mg) of plastic samples. The most significant advantage over offline methods is the possibility to avoid laboratory contaminations, to ensure that the compounds detected really issued from the sample. Indeed, in accordance with previous literature on the topic, it appeared that obtaining a real blank analysis was difficult with offline methods. Other significant advantages are the reduction of sample amount, making it possible to examine very small quantities of sample (as microplastics); the reduction of sample preparation steps; the reduction of solvent consumption (for extraction, separation and rinsing) and laboratory consumables as syringes and filters. Overall, the online method lasts only 45 minutes for each sample. Finally, to better understand variability issues, quantitation and method validation will be addressed in future works, where the method will be applied to diverse medical devices.

CRedit Author Contributions

Benjamin Caux: Conceptualization, Investigation, Methodology, Validation, Visualization, Writing – Original Draft; Clément De Saint Jores, Writing – Review & Editing, Conceptualization; Ramy Abou-Naccoul: Resources, Supervision; Shinnosuke Horie: Resources, Supervision; Caroline West: Conceptualization, Visualization, Writing – Original Draft, Review & Editing, Supervision, Project administration, Funding acquisition.

Conflicts of interest

Benjamin Caux, Ramy Abou-Naccoul and Shinnosuke Horie report financial support provided by Shimadzu Corporation. Clément De Saint Jores and Caroline West report a relationship with Shimadzu Corporation that includes non-financial support.

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Supplementary Information to:

Advantages of online supercritical fluid extraction and chromatography hyphenated to mass spectrometry to analyse plastic additives in laboratory gloves

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Figure S1. Structures of the 7 stationary phases from the Shim-pack UC series that were examined at the screening stage. Dimensions were the same for all columns (250 x 4.6 mm, 5 μ m).

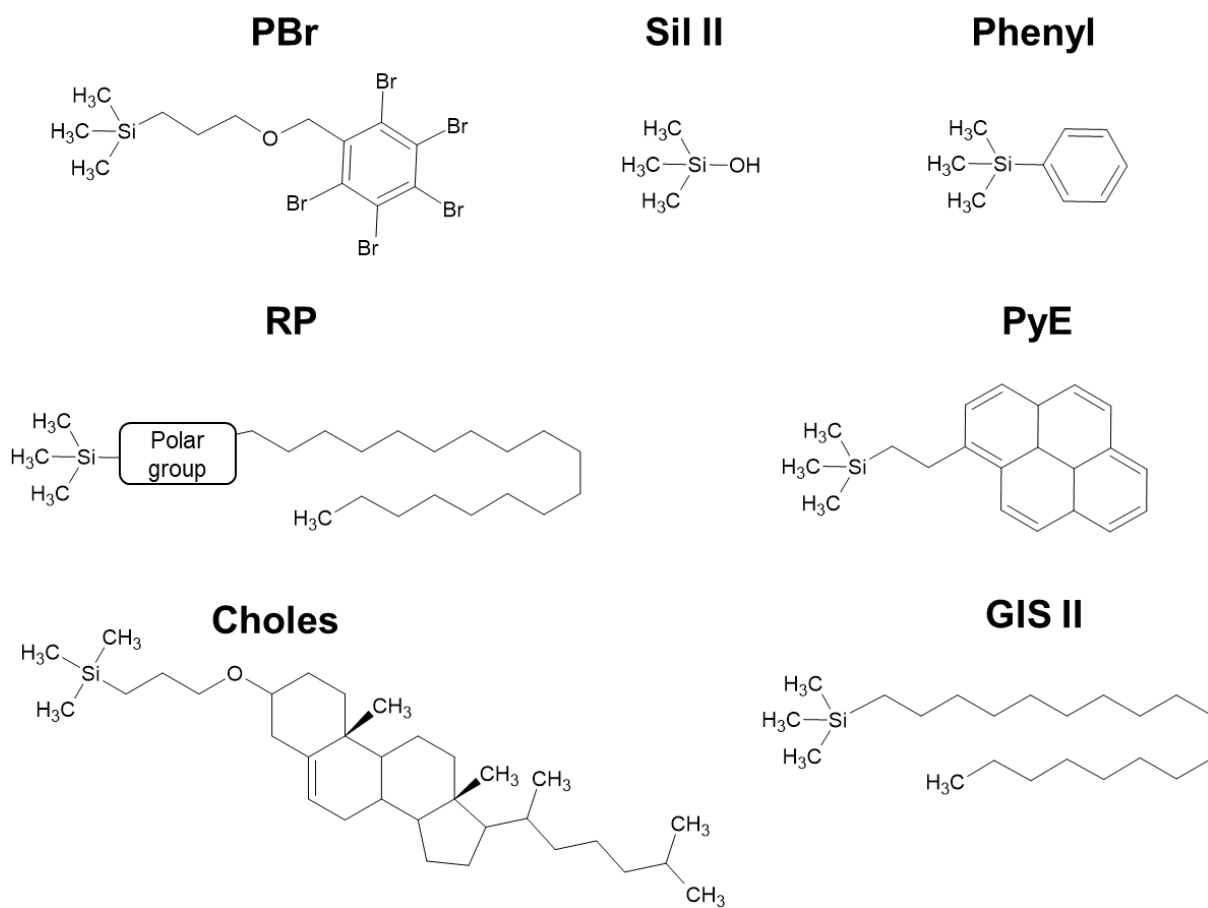


Figure S2. Derringer desirability functions used to rank the 7 columns tested for SFC analysis. See section 3.2.2. for details.

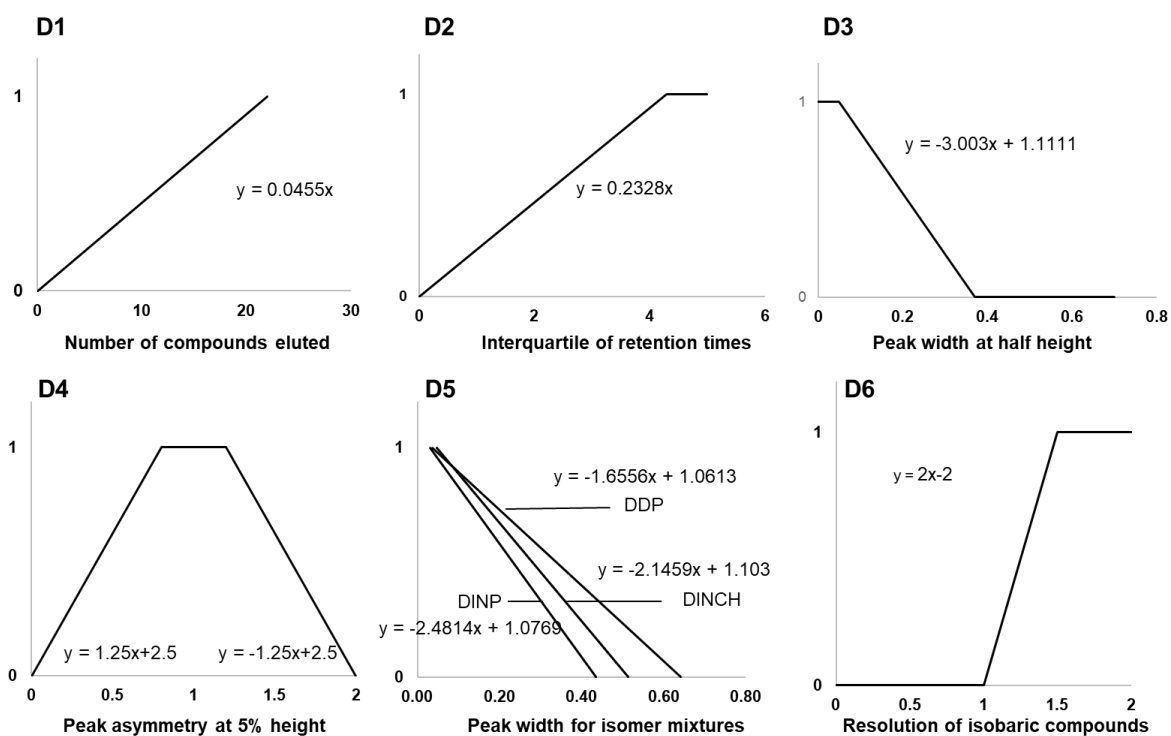


Figure S3. Histogram of ion intensities measured with ESI and APCI in scan mode for each standard compound. All ions were observed in positive ionization mode for all compounds except Cyanox 2246 observed in negative mode.

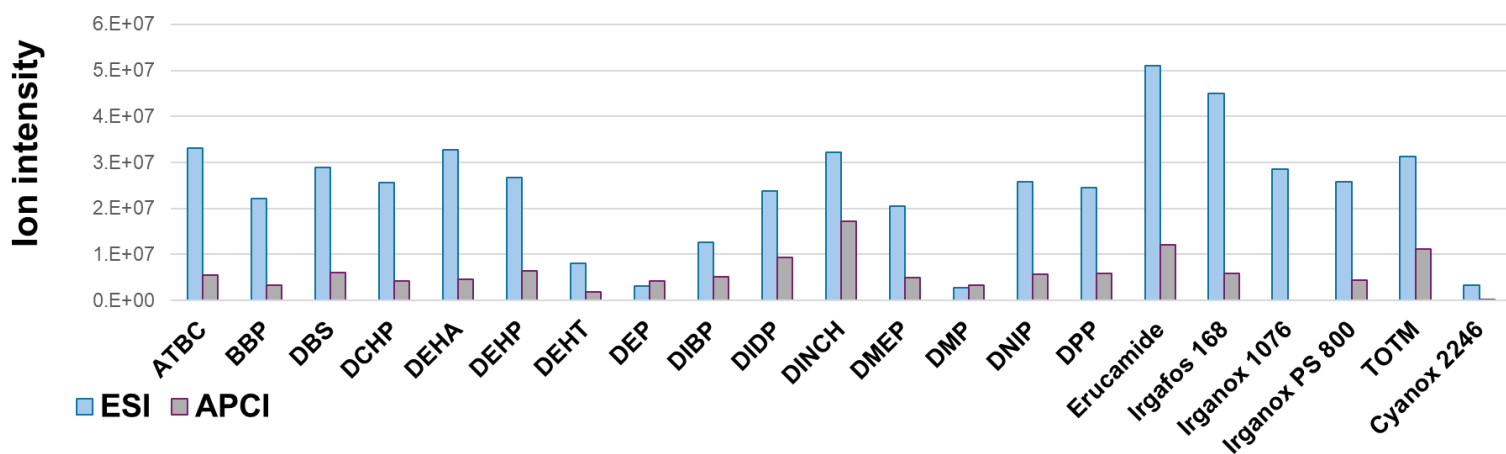


Figure S4. Box plot representing the retention time compound distribution for each column tested. The numbers correspond to the interquartile value (Q3 – Q1) expressed in minutes.

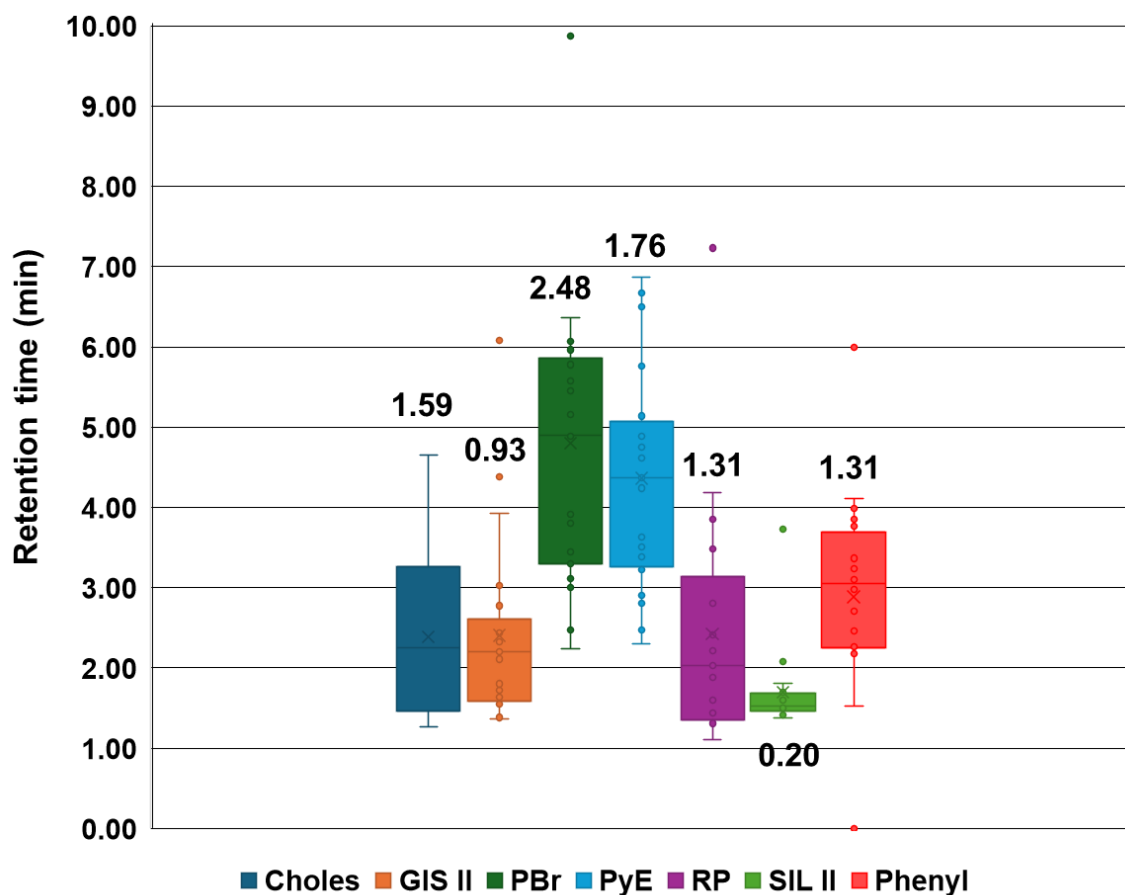


Figure S5. Box plot representing (A) the retention time and (B) the peak width at half height of the standard compounds, for gradient and isocratic mode. The numbers correspond to the interquartile value (Q3 – Q1) expressed in minutes.

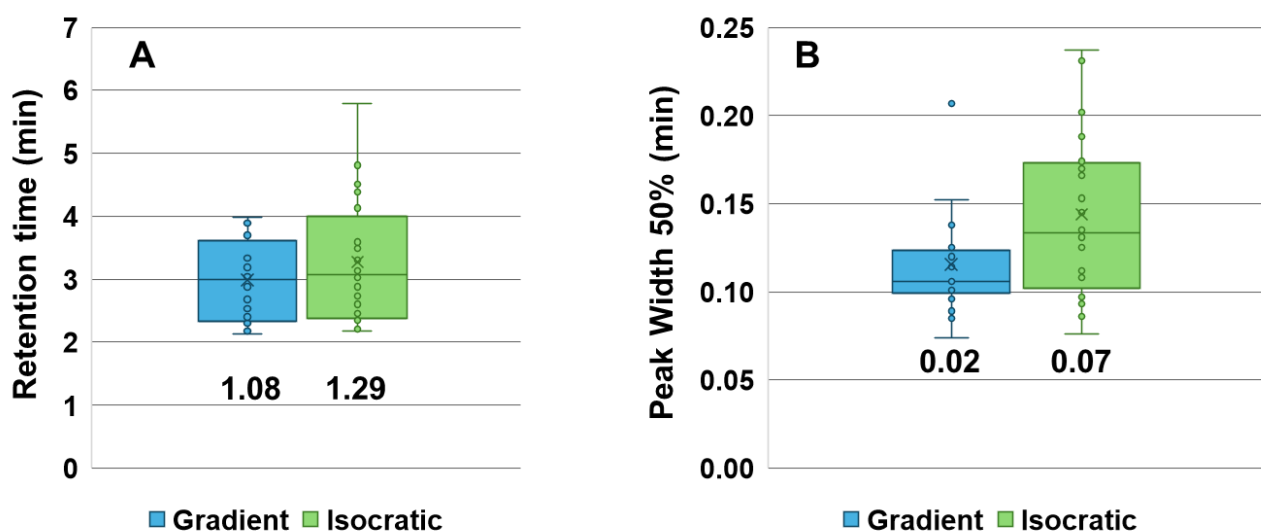


Figure S6. SFC-MS chromatograms obtained for different offline extraction methods on purple nitrile gloves. (A) Solvent extraction with acetone. (B, C, D, E) Pressurized liquid extraction with methanol, ethanol, ethyl acetate and heptane respectively. (F) Supercritical fluid extraction with pure scCO₂. See section 2.6 for detailed extraction conditions.

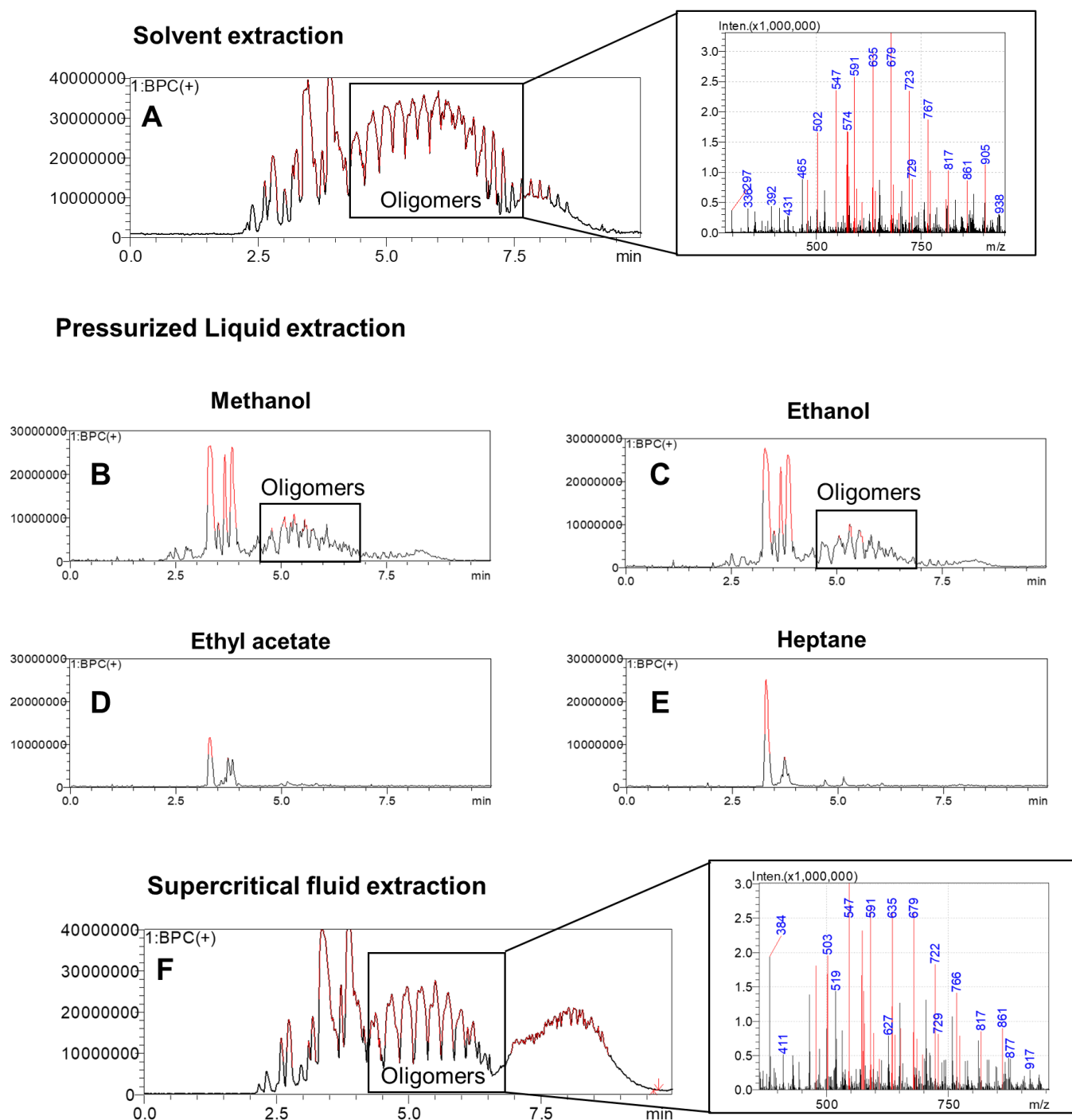


Figure S7. Successive PLE extractions done with methanol, ethanol, ethyl acetate and heptane on the same sample of purple nitrile gloves. Chromatograms (1), (2) and (3) correspond to the first, second and third extraction cycles respectively, lasting 20 min each.

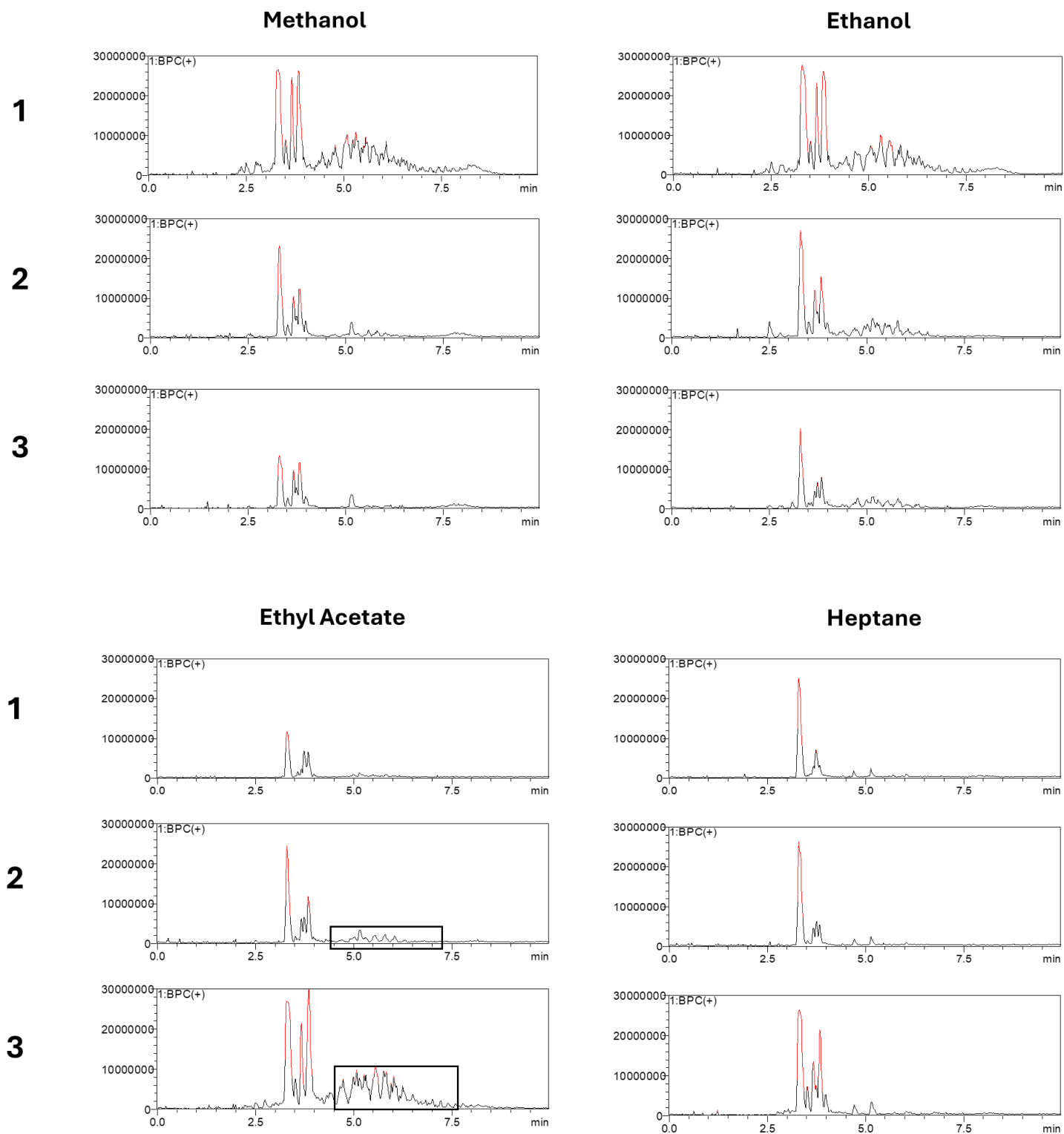


Figure S8. Histograms representing relative proportions of the areas of each compound based on the most intense compound (Irganox PS800) for solvent extraction with acetone, PLE with ethanol, methanol, ethylacetate and heptane and for SFE with pure scCO₂.

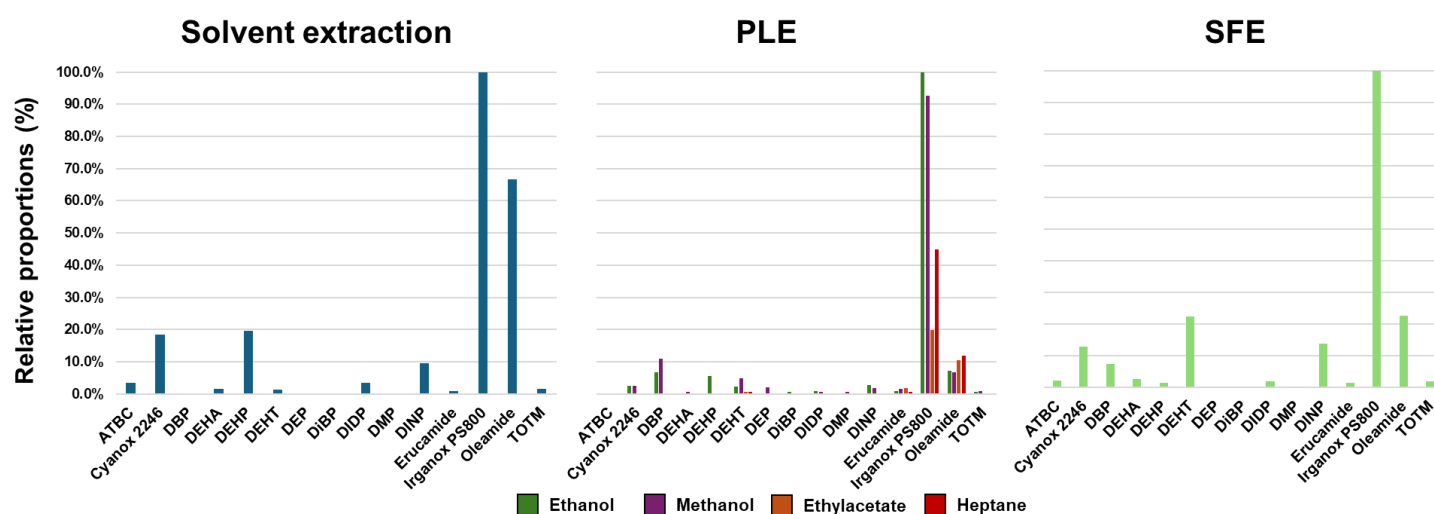


Figure S9. Peak areas measured for one compound of laboratory gloves (Irganox PS 800) and the internal standard (DMEP) depending on loop filling time. RSD(%) values based on 3 replicates.

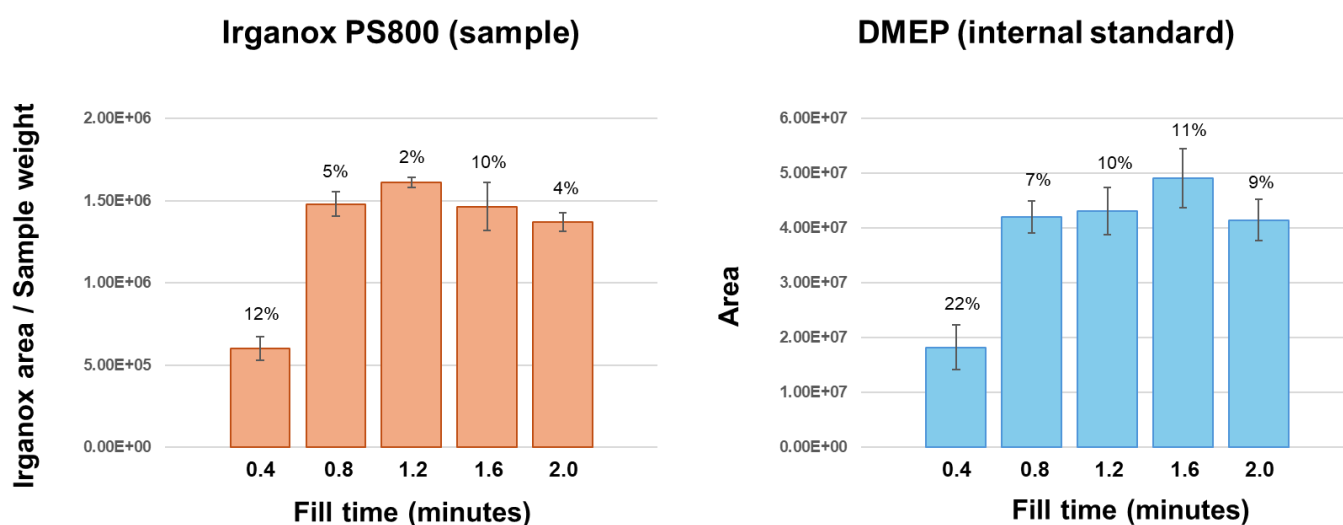


Figure S10. Base peak chromatograms of online SFE-SFC-MS of different amounts of nitrile glove samples: 10 mg (A), 30 mg (B), 50 mg (C) and 70 mg (D). Red part of chromatogram corresponds to a saturated signal.

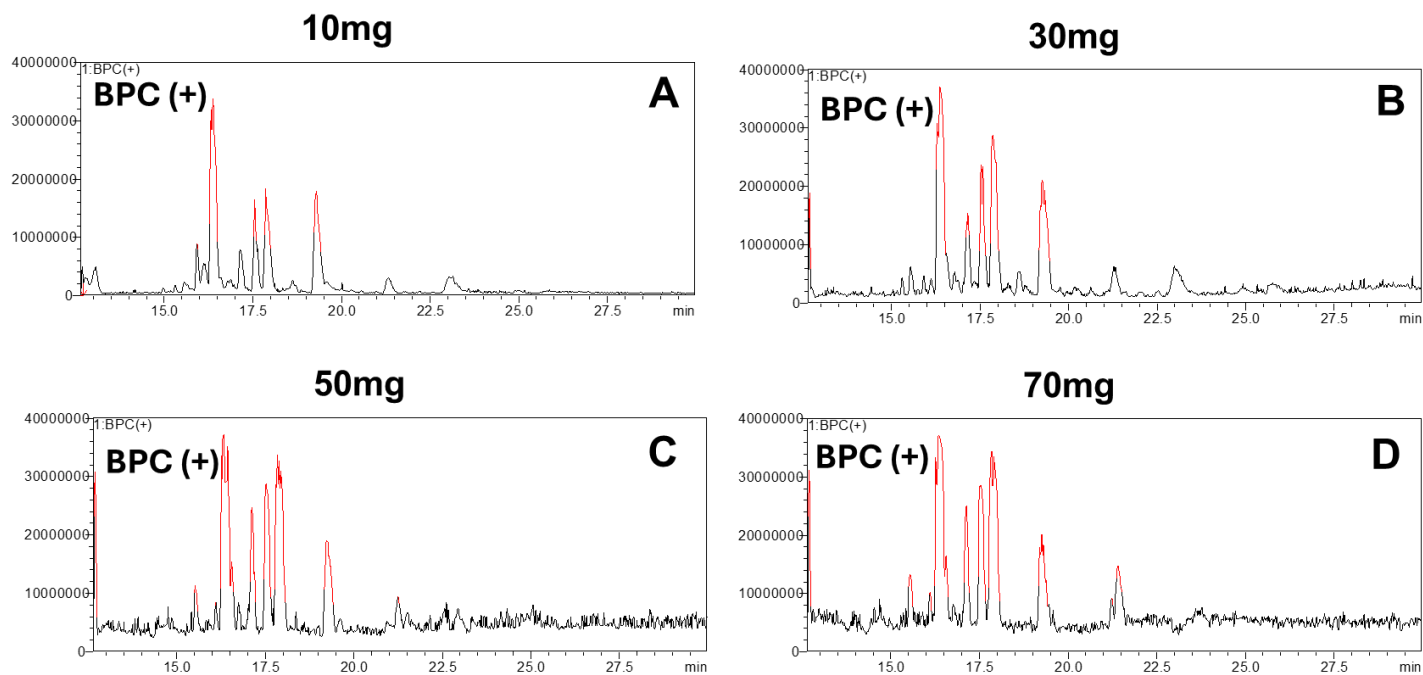


Figure S11. Histograms representing area in function of sample weight for different static time. Extraction yield (%) of the first extraction was calculated for two successive extractions of the same sample. DEHP and Irganox PS800 are compounds that are present in laboratory nitrile gloves used as reference. RSD(%) values indicated above the error bars were based on 3 replicates.

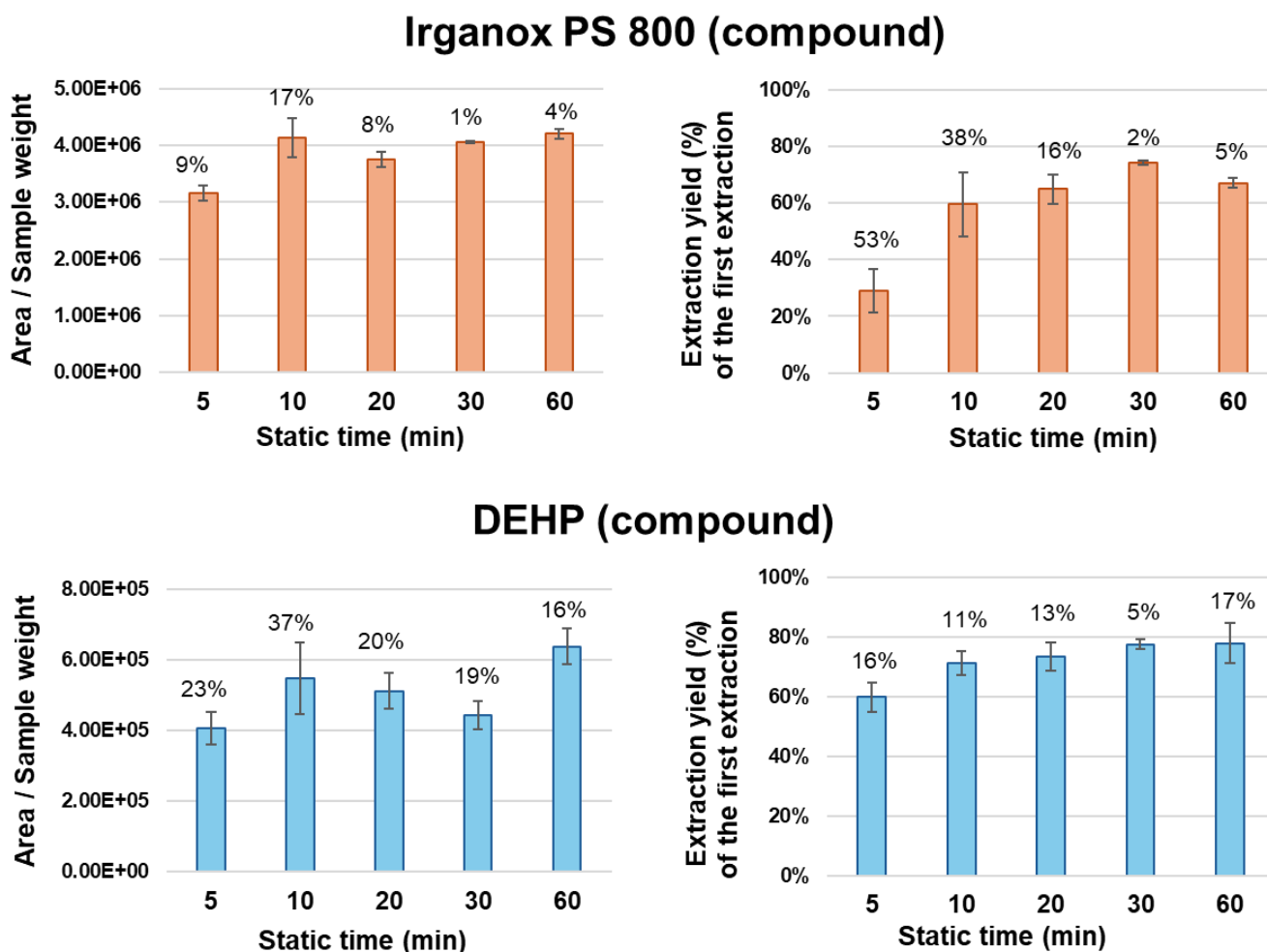
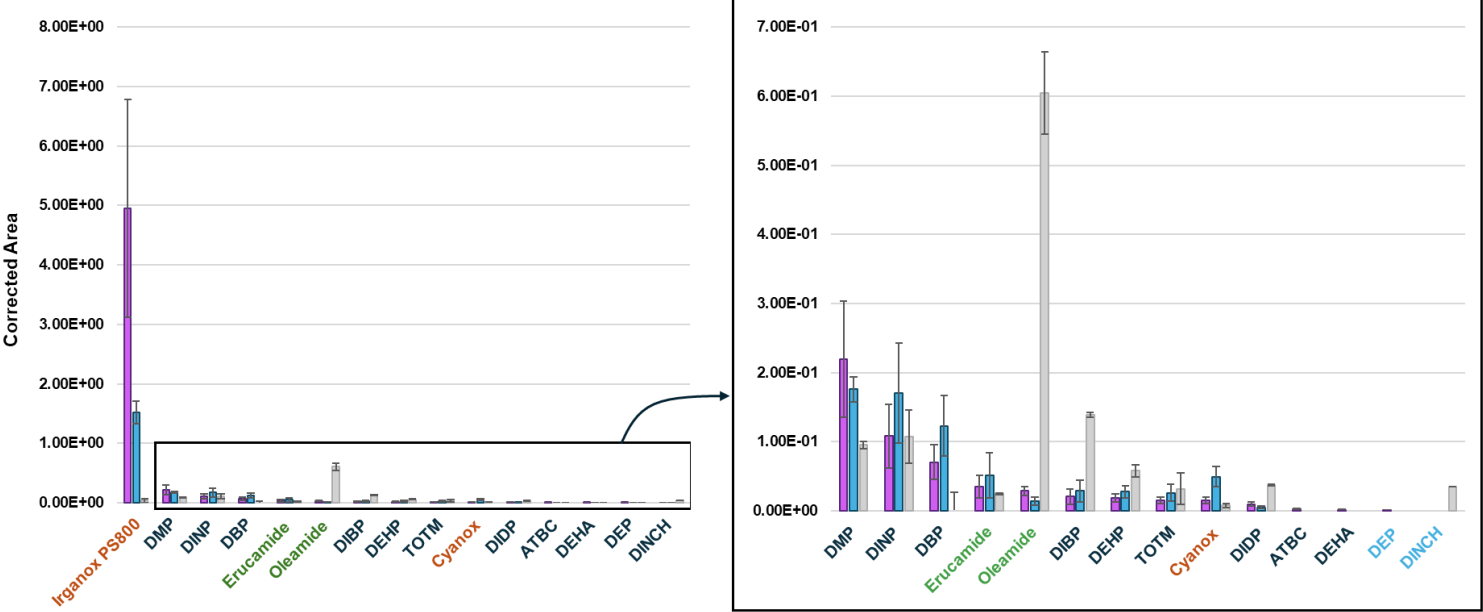


Figure S12. Histograms of extracted and characterized compounds for each type of laboratory gloves (purple nitrile, blue nitrile and latex). Corrected area corresponds to the area of the compound divided by the sample weight and the area of ISTD (DMEP). Histograms on the right correspond of a zoom on minor compounds.



- Purple nitrile laboratory gloves
- Blue nitrile laboratory gloves
- Latex nitrile laboratory gloves
- Label Antioxidants
- Label Plasticizers
- Label Lubricants

Table S1. Physicochemical properties of additives compounds described in this publication.

Categories	Trivial name	Chemical name	CAS n°	MW	log P	TPSA	HBA	HBD	n_rot	bp
Plasticizers	ATBC	Acetyltributylcitrate	77-90-7	402.2	3.1	105.2	8	0	15	326°C
	BBP	Benzylbutyl phthalate	85-68-7	312.1	4	52.6	4	0	7	370°C
	DBP*	Dibutyl phthalate	84-74-2	278.2	3.6	52.6	4	0	8	340°C
	DBS	Dibutylsebacate	109-43-3	314.2	4.8	52.6	4	0	15	344°C
	DCHP	dicyclohexyl phthalate	84-61-7	330.2	4.7	52.6	4	0	4	224°C
	DEHP	Bis (2-ethylhexyl)phthalate	123-79-5	390.3	6.4	52.6	4	0	14	405°C
	DEHT	Dioctyl Terephthalate	117-81-7	390.3	6.4	52.6	4	0	14	385°C
	DEP	diethyl phthalates	6422-86-2	222.1	2	52.6	4	0	4	383°C
	DiBP	diisobutylphthalate	84-66-2	278.2	3.3	52.6	4	0	6	295°C
	DiDP	Di-isodecylphthalate	84-69-5	446.3	8	52.6	4	0	18	320°C
	DINCH	di-isononyl cyclohexane-1,2-dicarboxylate	26761-40-0	424.4	7.1	52.6	4	0	16	253°C
	DINP	Di-isononylphthalate (complexes de phthalates dialkyles)	166412-78-8	418.3	7.2	52.6	4	0	16	394°C
	DMEP	Bis(2-methoxyethyl) phthalate	28553-12-0	282.1	1.3	71.1	6	0	8	250°C
	DMP	Dimethylphthalate	117-82-8	194.1	1.3	52.6	4	0	2	308°C
	DOA	di-(2-ethylhexyl) adipate	131-11-3	370.3	6.4	52.6	4	0	19	283°C
	DOS*	Dioctylesebacate	122-62-3	426.4	7.6	52.6	4	0	21	256°C
DPP	dipentyl phthalate	131-18-0	306.2	4.4	52.6	4	0	10	342°C	
TOTM	Tri-octyl trimellitate	3319-31-1	546.4	8.8	78.9	6	0	21	414°C	
Antioxidants	Cyanox 2246	Phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl-	119-47-1	340.2	5.9	40.5	2	2	2	125°C
	Irgafos 168	Tris -(2,4-di -tert -butyl -phenyl)-phosphite 2,4-ditert-butylphenyl) phosphate (forme oxydée)	31570-04-4	646.5	13.2	27.7	3	0	6	594°C

	Irganox 1076	2,6-Di-tert-butyl-4-(octadecanoxycarbonylethyl)phenol	123-28-4	530.5	10.7	46.5	3	1	20	570°C
	Irganox PS800	Dilauryl thiodipropionate	2082-79-3	514.4	9.4	52.6	5	0	28	568°C
Lubricants	erucamide	(Z)-docos-13-énamide	112-84-5	337.3	7.1	43.1	1	1	19	573°C
	Oleamide*	9-Octadecenamide	301-02-0	281.3	5.5	43.1	1	1	15	433°C

MW molecular weight in g/mol, log P is octanol-water partition coefficient, TPSA is the topological polar surface area expressed in Å², HBA and HBD are the numbers of H-bond acceptors and donors respectively, n_rot is the number of rotatable bonds and bp is the boiling point.

**Compounds not used for column screening and method development.*

Table S2. *m/z* values and corresponding ions for 24 target compounds observed with ESI-MS.

Compounds	MW (g.mol ⁻¹)	Ion 1		Ion 2	
		<i>m/z</i>	ions	<i>m/z</i>	ions
ATBC	402.2	403	[M+H] ⁺	420	[M+NH ₄] ⁺
BBP	312.1	313	[M+H] ⁺	330	[M+NH ₄] ⁺
Cyanox 2246	340.2	339	[M-H] ⁻	358	[M+NH ₄] ⁺
DBP*	278.2	279	[M+H] ⁺	/	/
DBS	314.2	332	[M+NH ₄] ⁺	315	[M+H] ⁺
DCHP	330.2	331	[M+H] ⁺	/	/
DEHA	370.3	371	[M+H] ⁺	388	[M+NH ₄] ⁺
DEHP	390.3	391	[M+H] ⁺	408	[M+NH ₄] ⁺
DEHT	390.3	408	[M+NH ₄] ⁺	391	[M+H] ⁺
DEP	222.1	223	[M+H] ⁺	/	/
DIBP	278.2	279	[M+H] ⁺	296	[M+NH ₄] ⁺
DIDP	446.3	447	[M+H] ⁺	/	/
DINCH	424.4	425	[M+H] ⁺	442	[M+NH ₄] ⁺
DMEP	282.1	300	[M+NH ₄] ⁺	283	[M+H] ⁺
DMP	194.1	195	[M+H] ⁺	/	/
DINP	418.3	419	[M+H] ⁺	433	[M+NH ₄] ⁺
DOS	426.4	444	[M+NH ₄] ⁺	427	[M+H] ⁺
DPP	306.2	307	[M+H] ⁺	324	[M+NH ₄] ⁺
Erucamide	337.3	338	[M+H] ⁺	/	/
Irgafos 168	646.5	647	[M+H] ⁺	/	/
Irganox 1076	530.5	549	[M+NH ₄] ⁺	529	[M-H] ⁻
Irganox PS800	514.4	532	[M+NH ₄] ⁺	515	[M+H] ⁺
Oleamide*	281.3	282	[M+H] ⁺	/	/
TOTM	546.4	547	[M+H] ⁺	564	[M+NH ₄] ⁺

*Compounds not used for column screening and method development.

Table S3. Retention times, m/z ions for each standard compound in gradient and isocratic mode chromatograms shown in Figure 5.

Peak number	Compounds	m/z	RT		Peak Width (50%)	
			Gradient	Isocratic	Gradient	Isocratic
1	DBS	332	2.13	2.18	0.09	0.09
2	DEHA	371	2.18	2.24	0.11	0.10
3	DMP	195	2.19	2.21	0.11	0.10
4	ATBC	403	2.22	2.25	0.14	0.11
5	DEHT	408	2.24	2.3	0.09	0.08
6	DEP	223	2.3	2.35	0.11	0.09
7	DIBP	279	2.53	2.6	0.21	0.23
8	DOS*	444	2.55	2.61	0.12	0.11
9	DBP*	279	2.4	2.46	0.21	0.23
10	DPP	307	2.68	2.73	0.11	0.10
11	DINCH	425	2.88	2.88	0.13	0.15
12	TOTM	547	3.04	3.13	0.12	0.13
13	DMEP	300	2.96	3.03	0.10	0.11
14	DINP	419	3.19	3.3	0.15	0.24
15	DEHP	391	3.1	3.19	0.10	0.13
16	DIDP	447	3.27	3.49	0.13	0.20
17	Irganox PS800	532	3.33	3.59	0.10	0.14
18	BBP	313	3.37	3.57	0.11	0.13
19	DCHP	331	3.7	4.13	0.09	0.15
20	Irganox 1076	549	3.78	4.38	0.11	0.17
21	Oleamide*	282	3.72	4.81	0.10	0.17
22	Cyanox 2246	339	3.89	4.51	0.10	0.15
23	Irgafos 168	647	3.99	4.87	0.10	0.19
24	Erucamide	338	3.98	5.79	0.07	0.17

**Compounds not used for column screening and method development.*

Table S4. Identified compounds for each type of laboratory gloves with online combination method

Compounds	Purple Nitrile		Blue Nitrile		Latex	
	Average Corrected area	Delta RT (min)	Average Corrected area	Delta RT (min)	Average Corrected area	Delta RT (min)
ATBC	1.87E-03	-0.05	<LOD	<LOD	<LOD	<LOD
BBP	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Cyanox 2246	1.46E-02	-0.05	4.93E-02	-0.10	7.37E-03	-0.09
DBP	7.00E-02	-0.04	1.23E-01	-0.07	<LOD	<LOD
DBS	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
DCHP	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
DIDP	9.24E-03	-0.11	4.88E-03	-0.13	3.70E-02	0.16
DEHA	7.77E-04	0.08	<LOD	<LOD	<LOD	<LOD
DEHP	1.80E-02	-0.06	2.72E-02	-0.09	5.77E-02	-0.09
DEHT	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
DEP	3.93E-04	-0.05	<LOD	<LOD	<LOD	<LOD
DIBP	2.02E-02	-0.05	2.87E-02	-0.10	1.39E-01	0.06
DINCH	<LOD	<LOD	<LOD	<LOD	3.47E-02	-0.06
DINP	1.08E-01	-0.01	1.70E-01	-0.03	1.07E-01	-0.04
DMEP (ISTD)	1.00E-01	-0.06	1.02E-01	-0.08	9.32E-02	-0.08
DMP	2.19E-01	-0.07	1.76E-01	-0.09	9.50E-02	-0.09
DOS	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
DPP	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Erucamide	3.46E-02	-0.06	5.07E-02	-0.12	2.38E-02	-0.13
Irgafos 168	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Irganox 1076	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Irganox PS800	4.95E+00	-0.07	1.52E+00	-0.10	3.56E-02	-0.10
Oleamide	2.85E-02	-0.03	1.36E-02	-0.09	6.04E-01	-0.11
TOTM	1.53E-02	-0.05	2.55E-02	-0.08	3.15E-02	-0.08

<LOD corresponds to a non-detected compound.

Delta RT corresponds to the difference of retention time between the compound observed in sample and the standard reference

Average Corrected area is the average peak area of 3 replicates divided by sample weight and internal standard (DMEP)