separations

MDPI

Article

Combination of Natural Deep Eutectic Solvents and Nano-Liquid Chromatography towards White Analytical Chemistry: A Practical Application

Álvaro Santana-Mayor [1,2], Giovanni D'Orazio [2,*], Salvatore Fanali [3], Miguel Ángel Rodríguez-Delgado [1] and Bárbara Socas-Rodríguez [1,*]

[1] Departamento de Química, Área de Química Analítica, Facultad de Ciencias, Universidad de La Laguna (ULL), Avenida Astrofísico Francisco Sánchez s/n, 38206 San Cristóbal de La Laguna, Tenerife, Spain; asantanm@ull.edu.es (Á.S.-M.); mrguez@ull.edu.es (M.Á.R.-D.)
[2] Istituto per i Sistemi Biologici (ISB), CNR—Consiglio Nazionale delle Ricerche, Montelibretti, 00185 Rome, Italy
[3] School in Nanoscience and Advanced Technologies, University of Verona, 37129 Verona, Italy; salvatore.fanali@gmail.com
* Correspondence: giovanni.dorazio@cnr.it (G.D.); bsocasro@ull.edu.es (B.S.-R.); Tel.: +34-922-318-036 (B.S.-R.)

Abstract: In this work, a green and practical analytical method based on natural deep eutectic solvents (NADES) as extraction agents and nano-liquid chromatography as a separation technique was developed. To demonstrate the applicability of the methodology, alkylphenols and bisphenol A were evaluated as model compounds in olive and sunflower oils as model fatty samples by liquid–liquid microextraction. With this aim, several NADES based on mixtures of choline chloride with glycerol, lactic, ascorbic, and citric acids or glycerol with amino acids were evaluated as potential extraction solvents. In addition, to select the most suitable stationary phase for the separation of this group of contaminants, some stationary phases were tested, including Pinnacle II phenyl, Cogent Bidentate C_{18}™, and XBridge® C_{18}. The last one provided the best performance with an analysis time of 11 min. To solve the problem of the compatibility of hydrophilic NADES with chromatographic systems without harming the solubility of analytes, different aqueous organic mixtures were tested. Methanol/water mixtures were the most suitable as an injection solvent. Finally, following the White Analytical Chemistry principles, different tools were used to evaluate the greenness, the practicality, and applicability of the method based on the Analytical Eco-Scale, the Analytical GREEnness metric approach, and the Blue Applicability Grade Index.

Keywords: sustainability; white analytical chemistry; green solvent; nano-liquid chromatography; green chemistry; miniaturization; natural compound; oil; phenol

Citation: Santana-Mayor, Á.; D'Orazio, G.; Fanali, S.; Rodríguez-Delgado, M.Á.; Socas-Rodríguez, B. Combination of Natural Deep Eutectic Solvents and Nano-Liquid Chromatography towards White Analytical Chemistry: A Practical Application. *Separations* **2024**, *11*, 119. https://doi.org/10.3390/separations11040119

Academic Editors: Victoria Samanidou and Evroula Hapeshi

Received: 15 March 2024
Revised: 10 April 2024
Accepted: 12 April 2024
Published: 16 April 2024

1. Introduction

The fulfilment of the Sustainable Development Goals has become a hot topic [1]. One of the areas which can have a great influence on the achievement of sustainability is the chemistry sector. Sustainability in chemistry serves as a foundational principle, aiming to integrate ecological, economic, and social dimensions [2–4]. This involves the responsible use of resources, minimization of the environmental impact, and the development of chemical products and processes that contribute positively to both human well-being and the health of the planet. It sets the stage for subsequent disciplines to align with global efforts towards a more sustainable future [1,3].

Within the scope of analytical chemistry, green analytical chemistry emerges as a pivotal concept [5]. This approach focuses on minimizing the environmental footprint of analytical techniques. Key strategies include the reduction or elimination of hazardous reagents, the optimization of energy consumption, and the development of rapid and

efficient analytical methods while optimizing resource utilization. Green analytical chemistry emphasizes the selection of environmentally friendly solvents, the reduction of waste generation, and the incorporation of innovative technologies to achieve more efficient and eco-friendly analyses [5]. By adopting these green principles, analytical chemists contribute to the overall sustainability of chemical analysis without compromising the quality of the results. The combination of green principles with analytical methodologies functionality characterizes a novel approach called white analytical chemistry [2]. This involves well-balanced methods that adopt cleaner sample preparation methods, which reduce solvent usage and waste generation, and the use of analytical instrumentation that enables the detection and quantification of target compounds at lower concentrations, minimizing the amount of sample and reagents required while retaining their functionality and usefulness [2].

In this regard, nano-liquid chromatography (nano-LC) shows many advantages as a separation technique. The use of capillary columns with internal diameters typically lower than 100 μm added to flow rates in the nanoliter per minute range results in an enhanced sensitivity and chromatographic resolution. In addition, the reduced flow rates, even when compared to ultra-high performance liquid chromatography, allow for solvent consumption and waste generation to diminish substantially [6–8]. The low flow rate is also an improvement, since it allows for good compatibility with mass spectrometry systems in which the effluent is fully transferred to the detector [9,10]. These features make nano-LC systems suitable for the analysis of small amounts of sample and/or compounds found at trace levels in a shorter analysis time than in conventional systems. Despite packaging materials for nano-LC capillary columns being often expensive, the small amounts required make it an advantage in terms of cost/durability [6–8]. Based on that, nano-LC is considered a more environmentally friendly technique.

The great popularity of deep eutectic solvents (DES) is due to, among other things, their intrinsic properties and versatility, making them promising candidates for a more environmentally friendly alternative to conventional solvents in many fields of application, including extraction and separation processes, electrochemistry, synthesis, biomass treatment, biocatalysis, metal processing, drug delivery, CO_2 capture, food industry, and energy storage, among others [11–19]. Among these characteristics is its good biodegradability, especially in the solvents formed only by compounds found in nature. These DES are known as natural DES (NADES). This fact makes them more environmentally friendly than many conventional solvents. Likewise, the possible use of compounds that come from renewable sources for the preparation of NADES is aligned with the principles of sustainable chemistry, reducing their environmental impact. Compared to some traditional solvents, NADES generally exhibit lower toxicity, minimizing potential health risks for both researchers and end-users [18,19]. In the field of analytical chemistry processes, other characteristics stand out, including a broad range of solubility, tunable properties through the adjustment of the components and their molar ratios, offering a customizable platform for specific applications, and great thermal stability. Thus, it has been widely demonstrated in several publications that they possess great potential as extraction agents [18–21]. Nevertheless, its high viscosity, although effective in avoiding processes such as oxidation or degradation of certain compounds, hinders its fluidic properties, handling, and mass transfer between sample and solvent. In addition, its compatibility with analysis systems represents an analytical challenge [19,20]. Therefore, studying the different ways of combining NADES with chromatographic systems has become a necessity. It suggests, for instance, the evaluation of new combinations of NADES components to reduce the viscosity and improve their properties or improve their volatility.

Here, a novel analytical methodology is proposed based on the use of hydrophilic NADES as extraction solvents for a liquid–liquid microextraction (LLME) and nano-LC–UV-Vis as determination technique. As hydrophobic NADES are ideal for the extraction of organic compounds from aqueous samples, hydrophilic ones are very promising, among others, for the analysis of fatty samples. In this regard, a wide group of alkylphenols (phe-

nol, 2,4-dimethylphenol (2,4-DMP), 2,3,6-trimethylphenol (2,3,6-TMP), 4-tert-butylphenol (4-TBP), 4-sec-butylphenol (4-SBP), 4-tert-amylphenol (4-TAP), 4-n-hexylphenol (4-HexP), 4-tert-octylphenol (4-TOP), 4-n-heptylphenol (4-HepP), 4-n-octylphenol (4-OP), and 4-n-nonylphenol (4-NP)) and bisphenol A (BPA) were used as representative analytes, whereas olive and sunflower oils were selected as model matrices representing oily samples. Finally, different tools were applied to evaluate the greenness and blueness of the method.

2. Materials and Methods

2.1. Chemicals and Materials

Standards and solvents were used as received (without any additional purification process). The analytical standards of phenol (CAS 108-95-2), BPA (CAS 80-05-7), 2,4-DMP (CAS 105-67-9), 2,3,6-TMP (2416-94-6), 4-TAP (CAS 80-46-6), 4-OP (CAS 1806-26-4), 4-TOP (CAS 140-66-9), 4-NP (CAS 104-40-5) with a purity higher than 96.3% were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany), while 4-TBP (CAS 98-54-4), 4-HexP (CAS 2446-69-7), and 4-HepP (CAS 1987-50-4) with a purity higher than 99.9% were supplied by Sigma-Aldrich Chemie (Madrid, Spain) and 4-SBP (CAS 99-71-8) with 98.0% of purity was acquired from Combi-Blocks, Inc. (San Diego, CA, USA). Stock solutions of studied compounds were prepared in methanol at 1000 mg/L and stored at −18 °C in darkness. Working solutions were prepared through appropriate dilutions.

Water, methanol, acetonitrile (can) for HPLC, and acetone (HiPerSolv CHROMANORM®) were from VWR International, Part of Avantor (Milan, Italy). Choline chloride (≥98.0%), citric acid (99.0%), glycerol (≥99.5%), and DL-lactic acid (≥85.5%) were from Sigma-Aldrich (Milan, Italy), whereas ascorbic acid (≥99.7%) was from Carlo Erba Reagents (Milan, Italy). L-Alanine (≥99.0%), L-histidine (≥99.0%), and L-lysine (≥97.0%) were from Fluka (Milan, Italy), while L-proline (99.0%), benzene, ethylbenzene, and propylbenzene were from Merck (Darmstadt, Germany).

Capillary columns were prepared by utilizing polyimide-coated fused silica capillaries purchased from Polymicro Technologies (West Yorkshire, UK), featuring OD of 375 μm and an ID of 100 μm. Different stationary phases were investigated, including bulk-acquired Pinnacle II phenyl (3 μm, 110 Å pore size, 6% carbon load) from Restek (Bellefonte, PA, USA), Cogent Bidentate C_{18}™ (4 μm, 100 Å pore size, 18–19% carbon load) obtained from MicroSolv (Leland, NC, USA), and XBridge® C_{18} (3.5 μm, 130 Å pore size, 18% carbon load) sourced by unpacking a guard cartridge from Waters (Milford, MA, USA).

2.2. Sample Selection

In this work, commercially available olive and sunflower oils were purchased in local supermarkets in Rome, Italy, with the aim of using them as model samples of fatty foods.

2.3. Apparatus and Software

An FS 100b Decon ultrasonic bath (Hove, UK) was used to sonicate mobile phases, analyte solutions, and stationary phase slurry during the packing procedure. An optical transmission microscope, Stereozoom 4 Microscope with an illuminator device (Bausch & Lomb, Rochester, NY, USA) was used to check the status of the stationary phase into the column in the packing step. Finally, a Perkin Elmer HPLC Series 10 pump (Palo Alto, CA, USA) was employed for moving the stationary phase slurry into the fused silica capillary.

A nano-/capillary-LC pump, Ultimate™ Capillary HPLC unit from LC Packings Dionex (Amsterdam, The Netherlands), equipped with a manual sample injector and an internal flow splitting unit, was employed for nano-LC experiments. The injector device was a low dispersion six-port valve from VICI VALCO Instrumentations (Houston, TX, USA) equipped with a 15 μL external loop. To reduce dead volumes, thus minimizing the chromatographic band broadening effect, the capillary columns were directly connected to the injector valve.

The target compounds were detected by a laboratory-made on-column detection cell at 214 nm. The temperature of the chromatographic system was controlled by continuous

room conditioning in the range of 18–22 °C. The pump and UV detector were controlled by Chromeleon™ Chromatography Management System Software (Version 6.6, LC Packings).

The separation process was carried out using a step gradient mode [22], regulated through the injection valve. The external loop was employed for both, loading analytes/samples and introducing the mobile phase. The injection volume was adjusted based on the injection time at the column workflow. In this setup, ACN, as the pump solvent, was exclusively used to thrust forward the injection plug into the column. The waste solvent was recycled into the pump reservoir [8]. Under the final chromatographic conditions, the step gradient mode was achieved by plug injection of aqueous/organic ratios with increased elution strength. The applied gradient is reported in Table 1. At the end of the day, the column was flushed with ACN. Forty nanoliters was the injection volume applied to all studied columns.

Table 1. Step gradients developed for the different stationary phases.

Stationary Phase	Column Conditioning	Step gradient (Time; Composition)
Pinnacle II phenyl	50/50, ACN/H_2O, *v*/*v*	8.1 min; 70/30, ACN/H_2O, *v*/*v*
Cogent Bidentate C_{18}™	50/50, ACN/H_2O, *v*/*v*	9.0 min; 80/20, ACN/H_2O, *v*/*v*
XBridge® C_{18}	70/30, ACN/H_2O, *v*/*v*	5.5 min; 90/10, ACN/H_2O, *v*/*v*

Injection volume: 40 nL; flow rate: 240 nL/min.

2.4. Preparation of Packed Capillaries

All capillary columns were prepared in our laboratory following a slurry packaging method [6] and packed with an effective and total length of 25.0 cm and 35.0 cm, respectively, as shown in Figure 1.

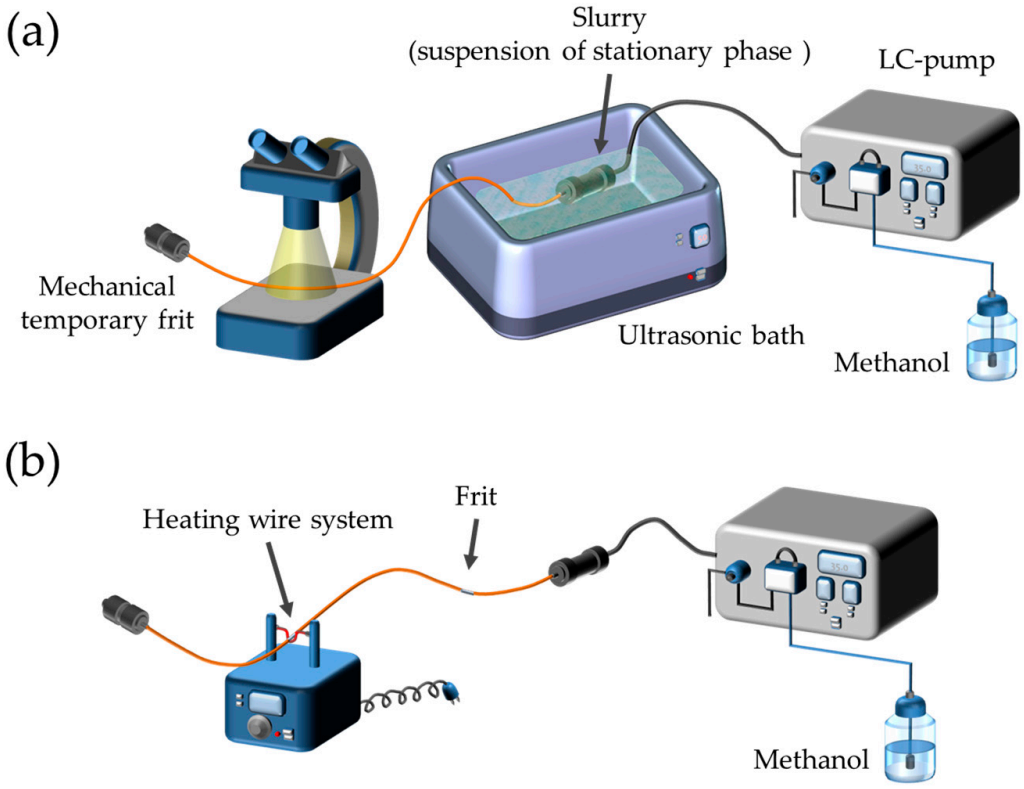


Figure 1. Scheme of (**a**) the system used for capillary packaging using the slurry method and (**b**) the preparation procedure for sintered silica frits.

Briefly, each end of 35–40 cm of fused silica capillary was joined both to a mechanical temporary frit (Valco, Houston, TX, USA) used to retain the packing material and to a 10 cm × 4.1 mm ID stainless steel HPLC pre-column (Valco) used as a reservoir for the slurry, respectively. Each slurry was prepared suspending about 50 mg of the selected stationary phase in 1 mL of acetone. After a brief sonic bath, the homogenous suspension obtained was transferred to the reservoir and pumped through the empty capillary by using an LC pump. At a maximum of 35 MPa (350 bar, 5000 psi), the capillary was packed for a length of about 30 cm. During this step, each capillary was immersed in the ultrasound bath in order to obtain a homogeneous stationary-phase bed structure. After a thorough washing of the phase with distilled water, the stationary phase was raised with 5 mM NaCl. A laboratory-made electrical heated wire was used to prepare inlet and outlet frits by simply heating a small capillary section at about 700 °C for 7–8 s. The temporary frit was removed, and the excess of stationary phase was eliminated in the back-flush mode.

The detection windows were prepared by removing the outer polyimide layer with a razor for a length of about 0.5 cm, close to the outlet frit (effective length, 26.6 cm). An optical microscope was used to monitor the whole procedure. Finally, each capillary was cut to the desired length and was equilibrated using a 50/50 (*v*/*v*) H_2O/ACN mixture.

A mixture of three alkylbenzenes, namely benzene, ethylbenzene, and propylbenzene (0.1% (*v*/*v*) each in water), was used as a test sample and periodically injected to evaluate the chromatographic performance of each column. Injected at 60 nL, the test mixture was separated in isocratic mode by using a mobile phase consisting of an ACN/water (80/20, *v*/*v*) mixture.

A comparative study of the main chromatographic parameters was the tool to assess the robustness of the system and the goodness of the prepared column.

2.5. NADES Preparation

The preparation of NADESs involved the magnetically assisted agitation of the components at specific molar ratios, according to previous publications, with a few modifications [7,23–25], as shown in Table 2. Glass tubes were then subjected to heating at 80 °C for 30 min, resulting in a homogeneous and clear liquid mixture. The resulting solvent was stored in a vacuum desiccator to prevent moisture until ready for use.

Table 2. Components for the synthesis of the different NADES used.

Component 1	Component 2	Component 3	Molar Ratio	Reference
Choline chloride	Glycerol	-	1:2	[23]
Choline chloride	Lactic acid	-	1:2	[24]
Choline chloride	Ascorbic acid	H_2O	2:1:4	[7]
Choline chloride	Citric acid	H_2O	2:1:4	[24]
Glycerol	L-Alanine	-	3:1	[25]
Glycerol	L-Histidine	-	3:1	[25]
Glycerol	L-Proline	-	3:1	[25]
Glycerol	L-Lysine	-	4.5:1	[25]

2.6. Microextraction Tests

One hundred microliters of NADES were rapidly added to 1 mL of oil sample. Then, the solvent was dispersed using one minute of vortex agitation. Later, the sample was centrifuged for one minute. An aliquot of the NADES-enriched phase placed at the bottom of the centrifuged tube (20 µL) was collected and diluted four-fold with a methanol/water mixture (50/50, *v*/*v*) to a final volume of 80 µL. The diluted sample extract was finally blended prior to its injection into the nano-LC system (Figure 2).

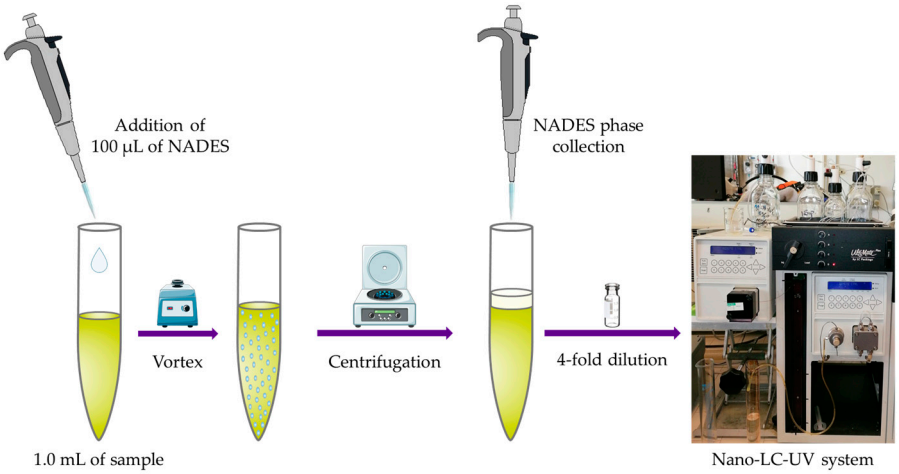


Figure 2. Schematic illustration of the NADES-LLME-nano-LC-UV procedure.

2.7. Sustainability Assessment Tools

For the evaluation of the environmental friendliness of the method, different tools were used. It included the Analytical Eco-Scale [26], the Analytical GREEnness (AGREE) metric approach [27], and, finally, the more recent Blue Applicability Grade Index (BAGI) [28], introducing a new metric tool for assessing the practicability of a method.

3. Results and Discussion

A significant effort has been undertaken to steer analytical chemistry toward a more sustainable paradigm aligned with the 12 principles of sustainable chemistry proposed by Anastas and Warner [5]. Key criteria, such as "preventing waste, using safer solvents and reaction conditions, and designing safer chemicals and products" are effectively addressed through the miniaturization of analytical methodologies. Novel proposals, for instance, include the application of nano-LC systems combined with miniaturized extraction techniques and the utilization of NADES as alternative extraction solvents.

3.1. Optimization of the Nano-LC-UV Method

For the development of a methodology that allowed the determination of alkylphenols and bisphenols in the samples of interest, firstly it was necessary to optimize the chromatographic separation.

The alkylphenols exhibit characteristics of being non-charged compounds in a wide range of pH with pKas higher than 9.5 and a hydrophobic molecular structure characterized by partition coefficients (log P) in the range 1.46–5.76 [29]. This final consideration suggests that the nature of the molecular structure imposes restrictions on the selection of the stationary phase, particularly in relation to the solvent used to dilute the standard mixture. From the perspective of environmental sustainability, an approach based on a reverse-phase partitioning mechanism was adopted, choosing stationary phases designed to enhance interaction with the target compounds. Based on this consideration, three reverse-phase stationary phases, two C_{18}, and one phenyl moieties linked to silica particles, totally end capped, were studied. Different parameters (i.e., flow rate, mobile phase composition, injection solvent) were changed in order to reach the best chromatographic separation in terms of performance and analysis time.

The miniaturization of the separation system using capillary columns provides two significant chromatographic advantages. Firstly, it promotes a greater homogeneity of the packing bed, thus reducing the effect of chromatographic band broadening [30]. Additionally, it contributes to a lower chromatographic dilution [31]. Given that the downscaling effect requires an injection volume of a few nanoliters, the extremely low analytical sensitivity it entails is certainly not compatible with the development of a novel analytical methodology. This limitation can be overcome by increasing the injection volume

and by working under overloading conditions. Although this is a chromatographically disadvantageous condition, it can be controlled and transformed into a valid online pre-concentration technique defined as on-column focusing. This approach allows for the concentration/focusing of the analyte at the column head without affecting the column's performance and chromatographic profile [32].

Generally, in reverse-phase chromatography, during the injection step, when non-polar compounds are dissolved in mixtures with a lower elution strength compared to the initial mobile phase, thinning of the injection plug occurs at the head of the column. Increasing the water content in the solvent dilution has been found to be an easy solution to pre-concentrate and focus the on-column analytes, maintaining a chromatographic profile characterized by a controlled band broadening effect [33]. On the contrary, the injection of organic solvent (ACN, methanol) was found to result in a worse chromatographic profile despite the small volume used in this case (60 nL). In addition, the maximum effect of injection plug compression was achieved using water as the dilution solvent [33]. In this context, due to the low solubility in the aqueous phase, especially for the more hydrophobic alkylphenols (such as 4-TOP, 4-HepP, 4-OP, 4-NP), an aqueous organic mixture was necessary to obtain an appropriate chromatographic profile.

Initial experiments focused on enhancing solubility by exploring different aqueous organic mixtures. The analytical standards were diluted in basic aqueous mixtures of alcohol and ACN. Equivalent injection volumes resulted in higher chromatographic peaks, indicating increased sensitivity, when using ACN/water and methanol/water mixtures. Additionally, it was observed that the use of methanol allowed for improved chromatographic separation profiles on all studied columns in terms of performance and repeatability, as it has also been observed in previous works [34,35].

Following the step gradient described in Table 1, Figure 3 shows a comparative study of the selectivity of the stationary phases studied that provided the best baseline separation, but under different conditions and analysis times.

For silica C_{18}-packed columns, the elution order was maintained, but significant differences in selectivity were observed. Although XBridge C_{18} and the Bidentate C_{18} exhibit a similar carbon load (18–19%), the latter showed notably higher retention for 4-TAP, 4-HexP, 4-HepP, 4-OP, 4-NP (11–17 min). However, by using the XBridge C_{18} column, analytes could be eluted in 7–11 min by a final elution phase with 90% ACN. It may be ascribed to the slightly reduced particle size of XBridge C_{18} compared to the Bidentate C_{18}, resulting in a larger available surface area and, consequently, enhanced retentive properties influencing peak width. Furthermore, the greater selectivity of the XBridge stationary phase, resolving 12 out of 13 peaks at baseline compared to Bidentate, which resolved 10 out of 13 peaks, may be attributed to the different pore sizes. A pore size of 130 Å for XBridge compared to 110 Å for the Bidentate C_{18} likely allowed for better accommodation of steric bulkier alkylphenols, improving interaction and expediting the partitioning mechanism.

Finally, despite the low carbon load, the Pinnacle II Phenyl phase exhibited exceptional selectivity for alkylphenols, facilitated by the presence of unsaturated moieties promoting π–π interactions with the phenyl moieties of the stationary phase. Due to the different partitioning mechanisms, a reversal in the elution order of 2,4-DMP and BPA was observed compared to C_{18}, particularly in the separation of 4-MP and BPA, which coeluted in the XBridge column.

To achieve the optimal compromise between on-column focusing effects and analytical solubility, under the most suitable chromatographic separation conditions achieved, the mixture of alkylphenols was injected with different ratios of water/methanol mixture. The 50/50 (v/v) water/methanol ratio allowed for good sensitivity for each studied column but with changing effects in terms of chromatographic efficiency. The greater the elution strength difference between the dilution solvent and the conditioning mobile phase, the smaller the observed band broadening effect. The high selectivity and carbon load of

XBridge C_{18} enabled elution with a higher organic phase content, simultaneously achieving a thin injection plug even with a high methanol content.

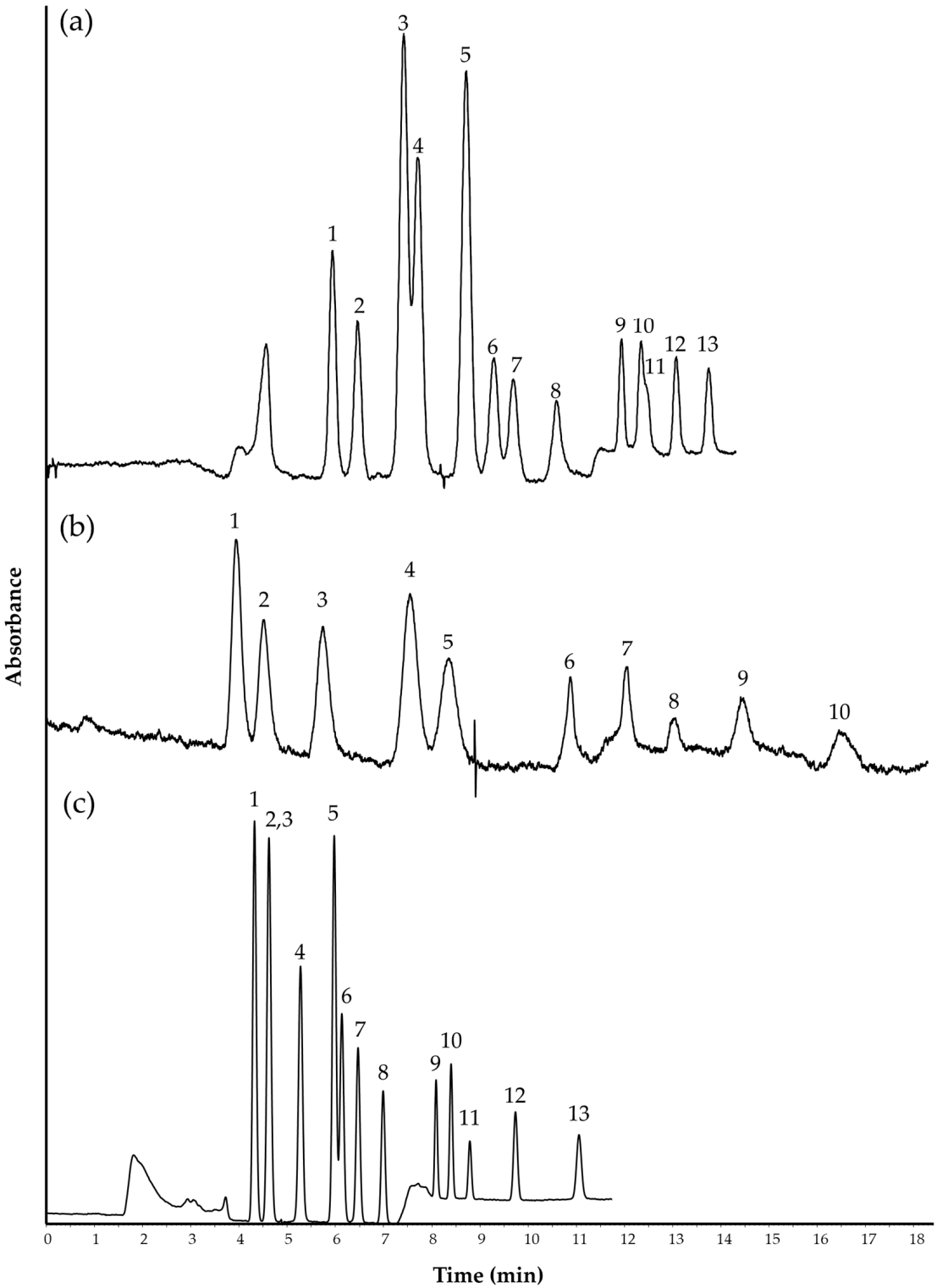


Figure 3. Selectivity of different stationary phases toward the studied compounds. Comparison of nano-LC-UV chromatograms (λ: 214 nm). Experimental conditions: analytes concentration at 16 µg/mL. (**a**) Pinnacle II Phenyl; (**b**) Cogent Bidentate C_{18}; (**c**) XBridge C_{18}. For gradient conditions, see Table 2. Peak identification: (1) Phenol, (2) 4-MP, (3) BPA, (4) 2,4-DMP, (5) 2,3,6-TMP, (6) 4-TBP, (7) 4-SBP, (8) 4-TAP, (9) 4-HexP, (10) 4-TOP, (11) 4-HepP, (12) 4-OP, (13) 4-NP.

Based on these results, as depicted in Figure 3, the XBridge C_{18} capillary column achieved the best outcomes in terms of selectivity, analysis time, and peak shape, and, thus, it was selected for further experiments.

3.2. NADES-LLME-Nano-LC-UV Method for the Analysis of Vegetable Oil Samples

Once the separation conditions were established, the method was applied to evaluate target analytes in two vegetable oils as model samples. Intending to develop a more sustainable methodology and considering the fact that the determination system complies with several points of the white analytical chemistry, the next step consisted of the performance of the extraction procedure. Based on that, nowadays, NADES are one of the alternatives that best fit the trends for the sake of more sustainable analytical chemistry approaches, including LLME methods [36]. Therefore, different NADES based on choline chloride with glycerol or organic acids (lactic, ascorbic, citric acids) and glycerol with different amino acids (L-alanine, L-histidine, L-proline, L-lysine) were prepared as described in Section 2.5.

Despite the many advantages that characterize NADES, sometimes these go against the development of the analytical methods. Among them, their viscosity, which makes the handling and direct injection of NADES extract difficult, and their low immiscibility with conventional organic solvents that are used both to dilute the extract and as components of the chromatographic system are two examples [19,20]. In this sense, in general terms, the NADES prepared in the present work are characterized by a low miscibility in solvents such as ACN. This implies that the dilution solvent must contain a high percentage of water when this type of NADES is used ($\geq$60%, v/v). On the one hand, this aspect benefits the chromatographic performance, since the elution strength of the injection solvent is lower. To achieve a good chromatographic separation, the injection solvent must have a lower elution strength than that of the mobile phase. Otherwise, not only is chromatographic efficiency and resolution lost, but it also does not favor the on-column focusing. On the other hand, the low solubility of target compounds in aqueous phases hindered the use of a high-water content mixture of solvents. Due to all of that, the optimization of the chromatographic system in combination with hydrophilic extracts and non-polar compounds represents a very difficult challenge.

In this case, and as previously mentioned, although alkylphenols are easily soluble in organic solvents, the NADES-based extracts are not miscible with those. For this reason, different dilution solvents were tested to find a compromise between all the factors that could be detrimental to the development of the method: chromatographic efficiency and resolution, sensitivity, solubility of the analytes, and compatibility of solvents, among others. Thus, it was found that the smaller alcohols, i.e., methanol, ethanol, and isopropanol, in proportions of 50/50, v/v, with water, are not miscible with NADES but also with solubilize alkylphenols. Furthermore, the chromatographic efficiency, resolution, and sensitivity were comparable to those of solvent standards, which greatly benefited chromatographic development. Despite this, and as expected, as the viscosity of the alcohol increases in the order isopropanol > ethanol > methanol, the viscosity of the injection solvent also increases; therefore, the chromatographic efficiency and resolution worsen. Therefore, the mixture formed by methanol and water in a 50/50 (v/v) proportion, was selected as the most suitable for further experiments.

After the optimization of the injection conditions, the different synthesized NADES were applied to the extraction of alkylphenols and BPA from olive and sunflower oils, as representative samples of oily nature, obtaining promising results. As it can be seen in Figure 4a, the chromatogram of a standard containing 25% (v/v) of NADES of choline chloride and glycerol in methanol/water (50/50, v/v) is represented. At the wavelength used, the NADES does not present the absorption band characteristic of the hydrophilic NADES, which usually elutes at the first part of the chromatogram [37,38]. However, in the oil extracts, shown in Figure 4b–d, a large band of more than a minute wide can be seen, and its intensity is much higher than that of the compounds of interest. As it can be observed, all tested NADES were able to extract most of the target compounds from the model matrix,

which demonstrates their potential to extract polar compounds from fatty matrices, as has also been remarked in other works [39–41]. The most polar analytes included in this study (phenol and BPA) are very affected by the tail of the co-extracted matrix components. Nevertheless, in general terms, the application of the different evaluated NADES resulted in very good results. Indeed, their chromatographic performance is comparable with that of a solvent standard. Similar results were obtained for the other NADES described in Table 2, as well as for the sunflower matrix.

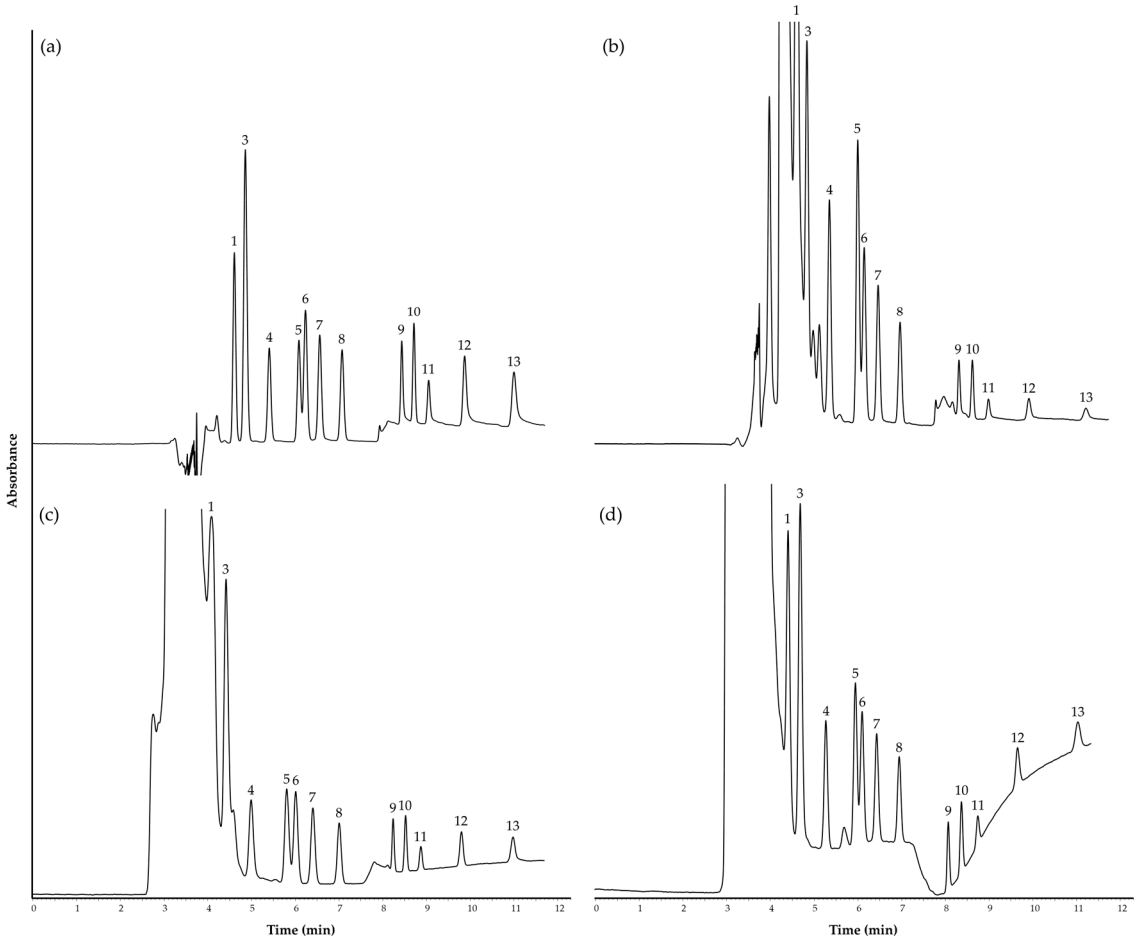


Figure 4. Nano-LC-UV separation of (**a**) a solvent standard containing 25% (*v*/*v*) of choline chloride:glycerol NADES in methanol:water (50/50, *v*/*v*) and olive oil after the microextraction step using a NADES of choline chloride and (**b**) glycerol, (**c**) lactic acid, and (**d**) ascorbic acid. For NADES composition and gradient conditions, see Tables 1 and 2. Peak identification: (1) Phenol, (3) BPA, (4) 2,4-DMP, (5) 2,3,6-TMP, (6) 4-TBP, (7) 4-SBP, (8) 4-TAP, (9) 4-HexP, (10) 4-TOP, (11) 4-HepP, (12) 4-OP, (13) 4-NP.

Additionally, it is worth mentioning that, although in this case there exists overlap between the matrix band and the most polar analytes that hinders their correct determination when a UV detector is used, the application of these kinds of conventional instruments increases the accessibility of the methodology to a greater number of laboratories. Apart from that, this approach could be improved for those laboratories that have a mass spectrometry detector, in which the resolution is based on the mass/charge ratio as well as on the fragmentation of the compounds, in the case of tandem mass spectrometry, as this approach could be of great importance in solving this type of problem.

3.3. Sustainability Assessment

Several tools have been suggested for evaluating the compliance of metric tools with the principles of the green analytical chemistry, including the National Environmental Methods Index (NEMI) [42], the Analytical Eco-Scale [19], the (Complementary) Green Analytical Procedure Index (GAPI) [43,44], the AGREE [27] and its version for sample preparation procedures [45], and, more recently, the BAGI [28] approaches. These methods have unique considerations and distinct frameworks for assessing their eco-friendly profile and, therefore, their results and interpretations can lead to debate and controversies about the environmental, useful, and practical validity of a methodology. To easily compare the different tools and evaluate the information of the results obtained, the Analytical Eco-Scale [26], AGREE [27], and BAGI [28] tools were compared.

The Analytical Eco-Scale [26] aims to evaluate the green nature of an analytical procedure in a "semiquantitative" way by assigning penalty points to those parameters of the analytical process that are detrimental to the ideal green analysis (represented by a score of 100). The AGREE method [27] evaluates the greenness of an analytical methodology based on the 12 principles of green analytical chemistry. The result is reflected in a pictogram divided into 12 parts whose color ranges from dark green (green principle) to red (non-green principle). In the center of the pictogram, the result appears reflected on a scale from 0 to 1, in which 1 represents the ideal analytical procedure. Finally, the BAGI [28] measures the practicality and applicability of the method. It was performed as a complementary metric tool to the green metrics considering the principles of white analytical chemistry. The response obtained is shown in a blue-colored asteroid pictogram divided into ten parts with the final score in the central section (on a scale of 25 to 100). In these terms, white analytical chemistry first appeared in 2021 as an alternative approach to green analytical chemistry [2]. This new approach, in addition to the green character of a method (greenness), proposes a red principle that reflects the validity of the analytical parameters of a method (redness) and a blue principle because of its practical attributes (blueness). Notwithstanding, the red part depends, to a large extent, on the analysis system used and the ranges evaluated relative to the figure of merits. Therefore, this aspect has not been included in the comparison. The results obtained are shown in Figure 5.

Based on the Analytical Eco-Scale tool (Figure 5a) [26], a score of 84 out of 100 was obtained for those procedures in which lactic- or citric-acid-based NADES were used. For the other NADES used as extraction solvents, 85 out of 100 points were obtained since no pictograms or signs of danger were presented. This final score represents an excellent green analysis. Nevertheless, some aspects must be explained in this sense. For instance, although the use of methanol is limited to 30 µL as a diluent of the final extract of the sample, the penalty points are the same for those volumes lower than 10 mL. Therefore, this score is overstated.

In the case of AGREE (Figure 5b) [27], a final score of 0.66 out of 1.00 was obtained for all cases. This result is in good agreement with some of the principles of green analytical chemistry. However, some aspects are difficult to comply with when attempting to analyze a large number of compounds in complex samples such as vegetable oils. Among these aspects is the unreal situation of analyzing this type of sample without prior treatment (point 1). This point is closely related to the type of analysis, which also makes direct analysis of the sample impossible (point 3). Furthermore, the need for systems that allow determinations in a selective and specific manner, such as that based on chromatographic systems, is detrimental to what is considered to be a green procedure (point 9). Likewise, as in the Analytical Eco-Scale, the use of solvents with hazard pictograms or that are not bio-based, even in very small quantities, severely impairs the evaluation. All these aspects present an unrealistic approach to analytical procedures; despite the greatest number of determinations possible being sought with families of compounds of different types in samples of a diverse nature, the only way to do this is with systems that use high energy and prior sample treatment.

(a)

Analytical Eco-Scale	
Reagents	**Penalty points**
Lactic/citric acid-based NADES (< 10 g)	1*
Methanol (< 10 mL)	6
Instruments	
Centrifuge	1
Vortex	1
LC (≤ 1.5 kWh/sample)	1
Occupational hazard	0
Waste	6
	∑16*
Total penalty points	16
Analytical Eco-Scale total score	84*

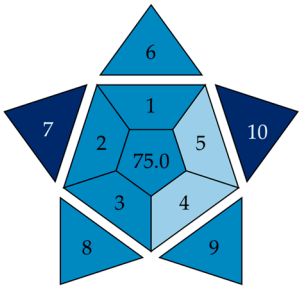
*Same results were obtained for the NADES formed by lactic or citric acids. In the case of other NADES, no penalty points were assigned to NADES components, and the final score obtained was 85.

(b)

Analytical Greenness Calculator (AGREE)

1. Sample treatment (external and batch analysis)
2. Sample amount (1.0 mL)
3. Devise positioning (off-line)
4. Sample preparation stages (3 or fewer)
5. Automation, miniaturization (semi-automatic and miniaturized)
6. Derivatization (none)
7. Waste (1.160 mL)
8. Analysis throughput (12 analytes; 5 samples/hour)
9. Energy consumption (LC, most energy-intensive technique)
10. Source of reagents (some bio-based reagents)
11. Toxicity (Yes, methanol)
12. Operator's safety (highly flammable solvent)

(c)



Blue Applicability Grade Index (BAGI)

1. Type of analysis (quantitative)
2. Multi-element analysis (12 compounds)
3. Analytical technique (simple instrumentation available in most labs)
4. Simultaneous sample preparation (2-12 samples)
5. Sample preparation (miniaturized extraction)
6. Samples per hour (5 samples)
7. Reagents and materials (common commercially available reagents)
8. Preconcentration (required; one-step preconcentration)
9. Degree of automation (semi-automated with home-made systems)
10. Amount of sample (< 10 mL food/environmental)

Figure 5. Results obtained by the (**a**) Analytical Eco-Scale, (**b**) AGREE, and (**c**) BAGI metric tools of the developed NADES-LLME-nano-LC-UV method.

Finally, the functionality and applicability of the developed method, based on the blueness of the white analytical chemistry, was assessed using the BAGI tool [28]. Figure 5c shows the weak and strong points of the method in these terms in a blue asteroid-shaped pictogram. The final score of 75.0 out of 100 shows that the method is practical (recommended result is higher than 60 points). The same result was obtained for all NADES used, considering the easy commercial availability of NADES components and solvents. The main improvements to the method are the type of analysis that requires the use of a mass spectrometry system that allows the confirmation of the results obtained (point 1), the preparation of a higher number of simultaneous samples (point 4), and the miniaturization of extraction system versus the possibility of simpler systems or no sample preparation (point 5). Moreover, the use of common, commercially available reagents benefits simplicity relative to the method application (point 7). Therefore, despite these improvable elements of the developed methodology, in general terms, it is a simple and cheap analysis method.

4. Conclusions

For the first time, a method based on NADES combined with nano-LC-UV has been developed for the evaluation of alkylphenols and BPA in vegetable oil samples (olive and sunflower) as model compounds and matrices, respectively. The separation was studied in terms of stationary phase, gradient mode, mobile phase, and injection solvent compositions. The best results were obtained using an XBridge C_{18} capillary column, achieving a good separation in 11 min in step gradient mode. The injection solvent was based on a methanol/water mixture as the dilution solvent for hydrophilic NADES.

The green and blue profile of the proposed method was calculated to evaluate its environmental and practical scope. In this context, the Analytical Eco-Scale, the AGREE, and the BAGI metric tools returned scores of 84, 0.66, and 75.0, respectively. These data demonstrate good alignment with the green principles whilst showing good usability.

The proposed methodology, based on the results obtained, could be applied to the analysis of other polar and non-polar compounds in several oily samples. In addition, the improvement of the detection system for a more selective and sensible one could allow a better combination with NADES that present UV absorption, thus increasing the scope of application of these solvents. In this sense, the future path of DES must be directed toward a better understanding of their properties and how the components interact with each other, so that the compatibility of DES in different analytical processes can be improved.

Author Contributions: Conceptualization, Á.S.-M., G.D. and B.S.-R.; methodology, Á.S.-M. and G.D.; validation, Á.S.-M.; formal analysis, Á.S.-M.; investigation, Á.S.-M., G.D. and B.S.-R.; writing—original draft preparation, Á.S.-M., G.D., B.S.-R., S.F. and M.Á.R.-D.; supervision, G.D. and B.S.-R.; project administration, G.D. and M.Á.R.-D.; funding acquisition, G.D. and M.Á.R.-D. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the Spanish Ministry of Economy, Industry and Competitiveness (project AGL2017-89257-P) as well as by the University of La Laguna, and the Spanish Ministry of Science, Innovation and Universities, and the National Recovery and Resilience Plan (NRRP), Mission 4 Component 2 Investment 1.3—Call for proposals No. 341 of 15 March 2022 of Italian Ministry of University and Research funded by the European Union—NextGenerationEU; Award Number: Project code PE00000003, Concession Decree No. 1550 of 11 October 2022 adopted by the Italian Ministry of University and Research, CUP D93C22000890001, Project title "ON Foods—Research and innovation network on food and nutrition Sustainability, Safety and Security—Working ON Foods" (ONFOODS).

Data Availability Statement: Data are contained within the article.

Acknowledgments: Á.S.-M. would like to thank his FPI fellowship and his grant for short stays abroad at the CNR-ISB (Rome) cofinanced by Agencia Canaria de Investigación, Innovación y Sociedad de la Información de la Consejería de Economía, Conocimiento y Empleo y Fondo Social Europeo (FSE) Programa Operativo Integrado de Canarias 2014–2020, Eje 3 Tema Prioritario (85%).

Conflicts of Interest: The authors declare no conflict of interest.

References

1. United Nations A/RES/70/1. Resolution Adopted by the General Assembly on 25 September 2015. Transforming Our World: The 2030 Agenda for Sustainable Development. General Assembly of the United Nations 2015. Available online: https://undocs.org/en/A/RES/70/1 (accessed on 11 April 2024).
2. Nowak, P.M.; Wietecha-Posłuszny, R.; Pawliszyn, J. White Analytical Chemistry: An Approach to Reconcile the Principles of Green Analytical Chemistry and Functionality. *TrAC—Trends Anal. Chem.* **2021**, *138*, 116223. [CrossRef]
3. Ballesteros-Vivas, D.; Socas-Rodríguez, B.; Mendiola, J.A.; Ibáñez, E.; Cifuentes, A. Green Food Analysis: Current Trends and Perspectives. *Curr. Opin. Green Sustain. Chem.* **2021**, *31*, 100522. [CrossRef]
4. Koel, M. Do We Need Green Analytical Chemistry? *Green Chem.* **2016**, *18*, 923–931. [CrossRef]
5. Anastas, P.T.; Warner, J.C. *Green Chemistry: Theory and Practice*; Online; Oxford University Press: Oxford, UK, 2000; ISBN 9780197704059.
6. Fanali, S.; D'Orazio, G.; Quaglia, M.G. Separation of Aryl Propionic Acids by Capillary Liquid Chromatography. *Chromatographia* **2004**, *60*, S239–S243. [CrossRef]

7. Liu, W.; Zhang, K.; Chen, J.; Yu, J. Ascorbic Acid and Choline Chloride: A New Natural Deep Eutectic Solvent for Extracting Tert-Butylhydroquinone Antioxidant. *J. Mol. Liq.* **2018**, *260*, 173–179. [CrossRef]
8. Asensio-ramos, M.; Fanali, C.; D'Orazio, G.; Fanali, S. Nano-Liquid Chromatography. In *Liquid Chromatography: Fundamentals and Instrumentation*; Fanali, S., Haddad, P.R., Poole, C.F., Riekkola, M.-L., Eds.; Elsevier: Amsterdam, The Netherlands, 2017; pp. 637–695. ISBN 9780128053935.
9. dos Santos, N.G.P.; Maciel, E.V.S.; Medina, D.A.V.; Lanças, F.M. NanoLC-EI-MS: Perspectives in Biochemical Analysis. *Int. J. Mol. Sci.* **2023**, *24*, 11746. [CrossRef]
10. Cestaro, B.I.; Machado, K.C.; Batista, M.; da Silva, B.J.G. Hollow-Fiber Liquid Phase Microextraction for Determination of Fluoxetine in Human Serum by Nano-Liquid Chromatography Coupled to High Resolution Mass Spectrometry. *J. Chromatogr. B* **2024**, *1234*, 124018. [CrossRef]
11. Perna, F.M.; Vitale, P.; Capriati, V. Deep Eutectic Solvents and Their Applications as Green Solvents. *Curr. Opin. Green Sustain. Chem.* **2020**, *21*, 27–33. [CrossRef]
12. Prabhune, A.; Dey, R. Green and Sustainable Solvents of the Future: Deep Eutectic Solvents. *J. Mol. Liq.* **2023**, *379*, 121676. [CrossRef]
13. Álvarez, M.S.; Longo, M.A.; Rodríguez, A.; Deive, F.J. The Role of Deep Eutectic Solvents in Catalysis. A Vision on Their Contribution to Homogeneous, Heterogeneous and Electrocatalytic Processes. *J. Ind. Eng. Chem.* **2024**, *132*, 36–49. [CrossRef]
14. Al-Farsi, R.; Hayyan, M. Paving the Way for Advancement of Renewable Energy Technologies Using Deep Eutectic Solvents: A Review. *Renew. Sustain. Energy Rev.* **2023**, *184*, 113505. [CrossRef]
15. Jafari, K.; Fatemi, M.H.; Estellé, P. Deep Eutectic Solvents (DESs): A Short Overview of the Thermophysical Properties and Current Use as Base Fluid for Heat Transfer Nanofluids. *J. Mol. Liq.* **2021**, *321*, 114752. [CrossRef]
16. Ge, X.; Gu, C.; Wang, X.; Tu, J. Deep Eutectic Solvents (DESs)-Derived Advanced Functional Materials for Energy and Environmental Applications: Challenges, Opportunities, and Future Vision. *J. Mater. Chem. A* **2017**, *5*, 8209–8229. [CrossRef]
17. Mannucci, G.; Teixeira, G.; Sosa, F.H.B.; Palluzzi, M.; Busato, M.; Coutinho, J.A.P.; D'Angelo, P. Predicting the Thermal Behavior in the Design of Type V Deep Eutectic Solvents: The Combined Role of Polarity and Steric Asymmetry. *ACS Sustain. Chem. Eng.* **2024**, *12*, 2862–2870. [CrossRef]
18. Santana-Mayor, Á.; Rodríguez-ramos, R.; Herrera-herrera, A.V.; Socas-Rodríguez, B.; Rodríguez-Delgado, M.Á. Trends in Analytical Chemistry Deep Eutectic Solvents. The New Generation of Green Solvents in Analytical Chemistry Alvaro. *TrAC—Trends Anal. Chem.* **2021**, *134*, 116108. [CrossRef]
19. Cunha, S.C.; Lapa, R.; Fernandes, O. Trends in Analytical Chemistry Deep Eutectic Solvents for Microextraction of Endocrine Disruptor Contaminants from Water and Food: Review. *TrAC—Trends Anal. Chem.* **2024**, *171*, 117495. [CrossRef]
20. Mansour, F.R.; Bedair, A.; Hamed, M.; Magdy, G.; Ali, I.; Locatelli, M. Applications of (Natural) Deep Eutectic Solvents in Liquid Phase Microextraction: A Review. *Microchem. J.* **2024**, *198*, 110178. [CrossRef]
21. Molnar, M.; Gašo-Sokač, D.; Komar, M.; Jakovljević Kovač, M.; Bušić, V. Potential of Deep Eutectic Solvents in the Extraction of Organic Compounds from Food Industry By-Products and Agro-Industrial Waste. *Separations* **2024**, *11*, 35. [CrossRef]
22. Fanali, S.; Aturki, Z.; D'Orazio, G.; Rocco, A.; Ferranti, A.; Mercolini, L.; Raggi, M.A. Analysis of Aloe-Based Phytotherapeutic Products by Using Nano-LC-MS. *J. Sep. Sci.* **2010**, *33*, 2663–2670. [CrossRef] [PubMed]
23. Khezeli, T.; Daneshfar, A.; Sahraei, R. A Green Ultrasonic-Assisted Liquid-Liquid Microextraction Based on Deep Eutectic Solvent for the HPLC-UV Determination of Ferulic, Caffeic and Cinnamic Acid from Olive, Almond, Sesame and Cinnamon Oil. *Talanta* **2016**, *150*, 577–585. [CrossRef]
24. Sang, J.; Li, B.; Huang, Y.Y.; Ma, Q.; Liu, K.; Li, C.Q. Deep Eutectic Solvent-Based Extraction Coupled with Green Two-Dimensional HPLC-DAD-ESI-MS/MS for the Determination of Anthocyanins from *Lycium ruthenicum* Murr. *Fruit. Anal. Methods* **2018**, *10*, 1247–1257. [CrossRef]
25. Huang, Y.; Feng, F.; Jiang, J.; Qiao, Y.; Wu, T.; Voglmeir, J.; Chen, Z.G. Green and Efficient Extraction of Rutin from Tartary Buckwheat Hull by Using Natural Deep Eutectic Solvents. *Food Chem.* **2017**, *221*, 1400–1405. [CrossRef]
26. Gałuszka, A.; Migaszewski, Z.M.; Konieczka, P.; Namieśnik, J. Analytical Eco-Scale for Assessing the Greenness of Analytical Procedures. *TrAC—Trends Anal. Chem.* **2012**, *37*, 61–72. [CrossRef]
27. Pena-Pereira, F.; Wojnowski, W.; Tobiszewski, M. AGREE—Analytical GREEnness Metric Approach and Software. *Anal. Chem.* **2020**, *92*, 10076–10082. [CrossRef]
28. Manousi, N.; Wojnowski, W.; Płotka-Wasylka, J.; Samanidou, V. Blue Applicability Grade Index (BAGI) and Software: A New Tool for the Evaluation of Method Practicality. *Green Chem.* **2023**, *25*, 7598–7604. [CrossRef]
29. National Library of Medicine (US). National Center for Biotechnology Information PubChem. Available online: https://pubchem.ncbi.nlm.nih.gov/ (accessed on 8 March 2024).
30. Kennedy, R.T.; Jorgenson, J.W. Preparation and Evaluation of Packed Capillary Liquid Chromatography Columns with Inner Diameters from 20 to 50 Mm. *Anal. Chem.* **1989**, *61*, 1128–1135. [CrossRef]
31. Vissers, J.P.C. Recent Developments in Microcolumn Liquid Chromatography. *J. Chromatogr. A* **1999**, *856*, 117–143. [CrossRef]
32. Vissers, J.P.C.; De Ru, A.H.; Ursem, M.; Chervet, J.P. Optimised Injection Techniques for Micro and Capillary Liquid Chromatography. *J. Chromatogr. A* **1996**, *746*, 1–7. [CrossRef]
33. Héron, S.; Tchapla, A.; Chervet, J.P. Influence of Injection Parameters on Column Performance in Nanoscale High-Performance Liquid Chromatography. *Chromatographia* **2000**, *51*, 495–499. [CrossRef]

34. Rodríguez-Ramos, R.; Herrera-Herrera, A.V.; Díaz-Romero, C.; Socas-Rodríguez, B.; Rodríguez-Delgado, M.Á. Eco-Friendly Approach Developed for the Microextraction of Xenobiotic Contaminants from Tropical Beverages Using a Camphor-Based Natural Hydrophobic Deep Eutectic Solvent. *Talanta* **2024**, *266*, 124932. [CrossRef]
35. Baute-Pérez, D.; Santana-Mayor, Á.; Herrera-Herrera, A.V.; Socas-Rodríguez, B.; Rodríguez-Delgado, M.Á. Analysis of Alkylphenols, Bisphenols and Alkylphenol Ethoxylates in Microbial-Fermented Functional Beverages and Bottled Water: Optimization of a Dispersive Liquid-Liquid Microextraction Protocol Based on Natural Hydrophobic Deep Eutectic Solvents. *Food Chem.* **2022**, *377*, 131921. [CrossRef]
36. Wang, Y.; Li, J.; Sun, D.; Yang, S.; Liu, H.; Chen, L. Strategies of Dispersive Liquid-Liquid Microextraction for Coastal Zone Environmental Pollutant Determination. *J. Chromatogr. A* **2021**, *1658*, 462615. [CrossRef]
37. Santana-Mayor, Á.; Socas-Rodríguez, B.; Rodríguez-Ramos, R.; Rodríguez-Delgado, M.Á. A Green and Simple Procedure Based on Deep Eutectic Solvents for the Extraction of Phthalates from Beverages. *Food Chem.* **2020**, *312*, 125798. [CrossRef]
38. Santana-Mayor, Á.; Rodríguez-Ramos, R.; Socas-Rodríguez, B.; Rodríguez-Delgado, M.Á.; D'Orazio, G. Nano-Liquid Chromatography Combined with a Sustainable Microextraction Based on Natural Deep Eutectic Solvents for Analysis of Phthalate Esters. *Electrophoresis* **2020**, *41*, 1768–1775. [CrossRef]
39. Rodríguez-Juan, E.; Rodríguez-Romero, C.; Fernández-Bolaños, J.; Florido, M.C.; Garcia-Borrego, A. Phenolic Compounds from Virgin Olive Oil Obtained by Natural Deep Eutectic Solvent (NADES): Effect of the Extraction and Recovery Conditions. *J. Food Sci. Technol.* **2021**, *58*, 552–561. [CrossRef]
40. Jing, X.; Cao, C.; Wu, W.; Zhao, W.; Wang, Y. Deep Eutectic Solvent-Based Vortex-Assisted Dispersive Liquid–Liquid Microextraction Combined with High Performance Liquid Chromatography for the Determination of Phenolic Acids in Vegetable Oils. *Food Sci. Technol. Res.* **2019**, *25*, 563–568. [CrossRef]
41. Coscarella, M.; Nardi, M.; Alipieva, K.; Bonacci, S.; Popova, M.; Procopio, A.; Scarpelli, R.; Simeonov, S. Alternative Assisted Extraction Methods of Phenolic Compounds Using NaDESs. *Antioxidants* **2024**, *13*, 62. [CrossRef]
42. National Water Quality Monitoring Council; U.S. Geological Survey (USGS); (USEPA), U.S.E.P.A. NEMI—National Environamental Methods Index. Available online: https://www.nemi.gov/home/ (accessed on 8 March 2024).
43. Płotka-Wasylka, J. A New Tool for the Evaluation of the Analytical Procedure: Green Analytical Procedure Index. *Talanta* **2018**, *181*, 204–209. [CrossRef]
44. Płotka-Wasylka, J.; Wojnowski, W. Complementary Green Analytical Procedure Index (ComplexGAPI) and Software. *Green Chem.* **2021**, *23*, 8657–8665. [CrossRef]
45. Wojnowski, W.; Tobiszewski, M.; Pena-pereira, F.; Psillakis, E. AGREEprep—Analytical Greenness Metric for Sample Preparation. *TrAC—Trends Anal. Chem. Anal. Chem.* **2022**, *149*, 116553. [CrossRef]