Analytica Chimica Acta 1312 (2024) 342758

Contents lists available at ScienceDirect

Analytica Chimica Acta

journal homepage: www.elsevier.com/locate/aca

Exploring sample treatment strategies for untargeted metabolomics: A comparative study of solid phase microextraction (SPME) and homogenization with solid-liquid extraction (SLE) in renal tissue

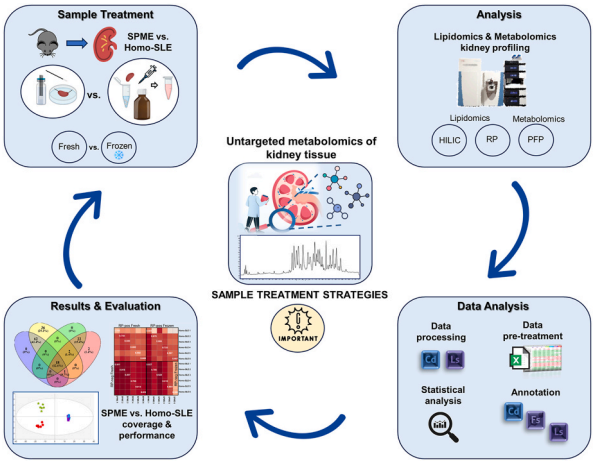
Paula Cuevas-Delgado [a], Natalia Warmuzińska [b], Kamil Łuczykowski [b], Barbara Bojko [b], Coral Barbas [a,*]

[a] Centre for Metabolomics and Bioanalysis (CEMBIO), School of Pharmacy, Universidad San Pablo-CEU, CEU Universities, Urbanización Montepríncipe, Madrid, Spain
[b] Department of Pharmacodynamics and Molecular Pharmacology, Faculty of Pharmacy, Collegium Medicum, Nicolaus Copernicus University, Bydgoszcz, Poland

HIGHLIGHTS

- In metabolomics, it is imperative to consider kidney tissue's complexity and heterogeneity.
- Appropriate sample treatment is crucial for compound coverage and analytical outcomes.
- Evaluating SPME metabolome and lipidome coverage remains necessary.
- Comparing SPME and Homo-SLE methods in murine kidney tissue gives an idea of their capabilities and limitations.

GRAPHICAL ABSTRACT



ARTICLE INFO

Handling Editor: Dr. L. Liang

Keywords:
Kidney
Lipidomics

ABSTRACT

Background: The selection of the sample treatment strategy is a crucial step in the metabolomics workflow. Solid phase microextraction (SPME) is a sample processing methodology with great potential for use in untargeted metabolomics of tissue samples. However, its utilization is not as widespread as other standard protocols involving steps of tissue collection, metabolism quenching, homogenization, and extraction of metabolites by

Abbreviations: C18, octyldecylsilane particles; C8, octyl particles; CV, coefficient of variation; FDR, false discovery rate; HILIC, hydrophilic interaction liquid chromatography; Homo, homogenization; kNN, k-nearest neighbours; LC-HRMS, liquid chromatography - high resolution mass spectrometry; MM, mix-mode mixture; MVA, multivariate statistical analysis; OPLS-DA, orthogonal partial least squared regression discriminant analysis; PCA-X, principal component analysis; PFP, pentafluorphenyl; PLS-DA, partial least squared regression discriminant analysis; QC, quality control; RP, reversed-phase; SCX, strong cation-exchange; SLE, solid liquid extraction; SPE, solid phase extraction; SPME, solid phase microextraction; UVA, univariate statistical analysis.

* Corresponding author.
E-mail address: cbarbas@ceu.es (C. Barbas).

https://doi.org/10.1016/j.aca.2024.342758
Received 8 January 2024; Received in revised form 6 May 2024; Accepted 20 May 2024
Available online 21 May 2024

Sample processing
Tissue freezing
Storage
SPME

solvents. Since SPME allows us to perform all these steps in one action in tissue samples, in addition to other advantages, it is necessary to know whether this methodology produces similar or comparable metabolome and lipidome coverage and performance to classical methods.
Results: SPME and homogenization with solid-liquid extraction (Homo-SLE) sample treatment methods were applied to healthy murine kidney tissue, followed by comprehensive metabolomics and lipidomics analyses. In addition, it has been tested whether freezing and storage of the tissue causes alterations in the renal metabolome and lipidome, so the analyses were performed on fresh and frozen tissue samples Lipidomics analysis revealed the exclusive presence of different structural membrane and intracellular lipids in the Homo-SLE group. Conversely, all annotated metabolites were detected in both groups. Notably, the freezing of the sample mainly causes a decrease in the levels of most lipid species and an increase in metabolites such as amino acids, purines, and pyrimidines. These alterations are principally detected in a statistically significant way by SPME methodology. Finally, the samples of both methodologies show a positive correlation in all the analyses.
Significance: These results demonstrate that in SPME processing, as long as the fundamentals of non-exhaustive extraction in a pre-equilibrium kinetic regime, extraction in a tissue localized area, the chemistry of the fiber coating and non-homogenization of the tissue are taken into account, is an excellent method to use in kidney tissue metabolomics; since this methodology presents an easy-to-use, efficient, and less invasive approach that simplifies the different sample processing steps.

1. Introduction

Untargeted metabolomics strives to analyze a broad spectrum of small molecules within a single experiment. The vast number and chemical diversity of metabolites, coupled with their extensive biological concentration range, necessitate the application of multiple analytical techniques. Furthermore, along with the choice of analytical platform, the selection of the sample processing strategy is crucial, as it will affect both the metabolite profile obtained and the quality of the data [1–3].

Variations in metabolomics studies often stem from different stages of the workflow, particularly pre-analytical steps. These steps, integral to study design, can greatly impact sample quality, results, and biological interpretation if not properly executed. Ensuring metabolism quenching, metabolite stabilization, and sample integrity preservation is crucial for reliable analysis [1,4].

While most metabolomics studies attempt to use non-invasive or minimally invasive biological samples, such as biofluids, feces or exudates, direct tissue analysis is sometimes required. This approach can provide valuable information by analyzing where a given disease starts and revealing the origin of disease-related metabolic changes. However, tissue heterogeneity, as seen in kidneys, and regional differences in cell composition or metabolism within the same tissue type, increase biological variability, being a critical point to consider in sample collection and processing [4].

In tissue-based metabolomics, maintaining sample homogeneity is critical. This involves collecting samples from the same organ region, and of similar sizes. Contamination with blood should be avoided, possibly by rinsing tissue samples with deionized water post-collection. Moreover, sampling should be performed as quickly as possible, and tissue samples should either be processed immediately or snap-frozen in liquid nitrogen and stored at −80 °C until processing, to completely prevent the continuation of metabolism activity [1,3–5]. Freeze-thaw cycles, which can alter metabolic profiles due to increased cell and protein degradation, must also be considered [4,6].

Another crucial step in untargeted metabolomics tissue analysis is the sample preparation, which could bias the analyses since it strongly affects the metabolome coverage and the quality of the results. In this sense, more attention should be placed on selecting appropriate and robust sample preparation, bearing in mind the sample type and the study's objective [5].

For tissue sample metabolite extraction, several methodologies are nowadays available, including solid-liquid extraction (SLE) or solid-phase extraction (SPE) [1]. For instance, in kidney tissue samples, SLE is typically used with different solvents depending on the type of analysis. Mechanical disruption and homogenization are necessary to facilitate solvent penetration into the tissue, resulting in efficient extraction of intracellular metabolites [5], as previously performed in several studies [7–10].

When analyzing kidney tissue for renal disease studies through metabolomics, the inherent sample complexity and potential disease-associated factors increasing heterogeneity, such as fibrosis, must be considered [11]. The subsequent stages of sample processing are labor-intensive, involving multiple steps that must be meticulously executed. Furthermore, the procurement of renal tissue samples is particularly challenging, more so from patients than animal models, which constrains the breadth of metabolomic investigations in this area. It is therefore still necessary to extensively evaluate the metabolic and lipidomic profile of renal tissue in order to obtain more complete information that will allow further progress in the study of renal diseases.

SPME, a non-exhaustive and minimally invasive method, extracts analytes based on equilibrium principles, and which is widely accepted in bioanalysis area [12–16].

Tissue SPME technology uses special fibers coated with biocompatible sorbents that can be inserted directly into the tissue to extract small molecules in amounts proportional to their unbound biologically active concentrations (free form) [17–19]. Furthermore, with the appropriate calibration method, SPME can quantify both the amount of free analyte extracted and the total concentration of the analyte in the biological sample [20]. As in SPME technology, if the sample volume is significantly larger than the extraction coating volume, the extracted amount of analyte is independent of the sample volume. Therefore, it would not be necessary to collect a defined amount of sample before analysis. In this way, the fiber can be exposed directly in the tissue, either extracted, collected, and homogenized, directly in the tissue *ex vivo*, or even *in vivo*, as the amount of analyte extracted in the fiber will be a negligible portion relative to what is present in the sample, and will not alter its environment. Crucial parameters in tissue SPME, such as the type of fiber coating, its thickness, and extraction time, must be well established prior to the experiment [13,15].

In addition, SPME is a non-exhaustive sample preparation procedure based on chemical biopsy, combining sampling, sample preparation, metabolite quenching and metabolite extraction in a single step, which allows for capturing unstable and short-lived metabolites, often undetectable by traditional methods [21]. This methodology has been successfully applied in *ex vivo* animal studies as an alternative to standard protocols [22,23], and has also demonstrated its potential for *in vivo* animal studies [21,24,25].

Given SPME's, it could be a valuable sample processing methodology for the metabolomic study of renal damage directly in the kidney. SPME simplifies the sample treatment process, allows precise location of kidney areas for sample extraction, and avoids the problems associated with the heterogeneity of the kidney itself or that generated by damage and fibrosis. Furthermore, SPME could be applied in *ex vivo* animal models,

either in homogenates or directly in the kidney, but also *in vivo*, allowing samples to be taken at different times to check the organ's state at the metabolic level, as in the case of transplants [23,26].

However, to establish SPME as a routine protocol in renal tissue sample treatment for metabolomic analysis, it is necessary to verify that the results obtained are comparable to those from classical sample treatment protocols, such as renal tissue homogenization and metabolite extraction by SLE, in terms of metabolome coverage, sensitivity and reproducibility.

In this study, we have evaluated the results obtained after metabolomic and lipidomic analyses of healthy murine kidney tissue, using SPME and homogenization followed by SLE (Homo-SLE) as sample treatment methodologies. The aim was to test whether different sample processing methodologies produce a different capture of the renal tissue metabolome or comparable results.

For this purpose, we performed SPME with fibers coated with a mix-mode mixture of strong cation-exchange (SCX) and octyl (C8) particles, allowing the extraction of both polar and non-polar compounds in the same fibers, carrying out the extraction directly in the healthy renal tissue. For the Homo-SLE methodology, an extraction with methyl *tert*-butyl ether and methanol (1:4) was performed. The sample treatment was followed by lipidomics analyses using two methodologies, reversed phase chromatography (RP) and hydrophilic interaction liquid chromatography (HILIC), allowing a broad coverage of the different lipid classes and a metabolomic analysis.

Another point considered was the evaluation of the effects of tissue freezing on the lipidome and renal metabolome. As it is known that freeze-thaw cycles can affect metabolite levels [6,27], we studied the sensitivity of the two sample treatment methodologies to this effect. Therefore, analyses were performed on fresh and frozen renal tissue.

2. Materials and methods

2.1. Study design

All experimental procedures applied to animals were performed according to the Guide for the Care and Use of Laboratory Animals of the Directive 2010/63/EU and were carried out in the animals' facilities of the Faculty of Pharmacy in the Ludwik Rydygier Collegium Medicum in Bydgoszcz, Poland. C57BL/6 mice (born 14–17.08.2021) were purchased from the Experimental Medicine Centre of the Medical University in Bialystok. The experiment included nine adult males. The animals were housed in a controlled environment with the temperature at 22 ± 2 °C, 12 h light-dark cycle, humidity 55 ± 10 %, standard mouse chow, and water available ad libitum. The mice were sacrificed (October 08, 2021) by manual cervical dislocation, which resulted in euthanasia within approximately 10 s. Once euthanasia was confirmed, left and right kidneys were immediately collected. According to European Union law, permission from the Local Ethical Commission is not required for the use of animal tissue or organs for scientific purposes.

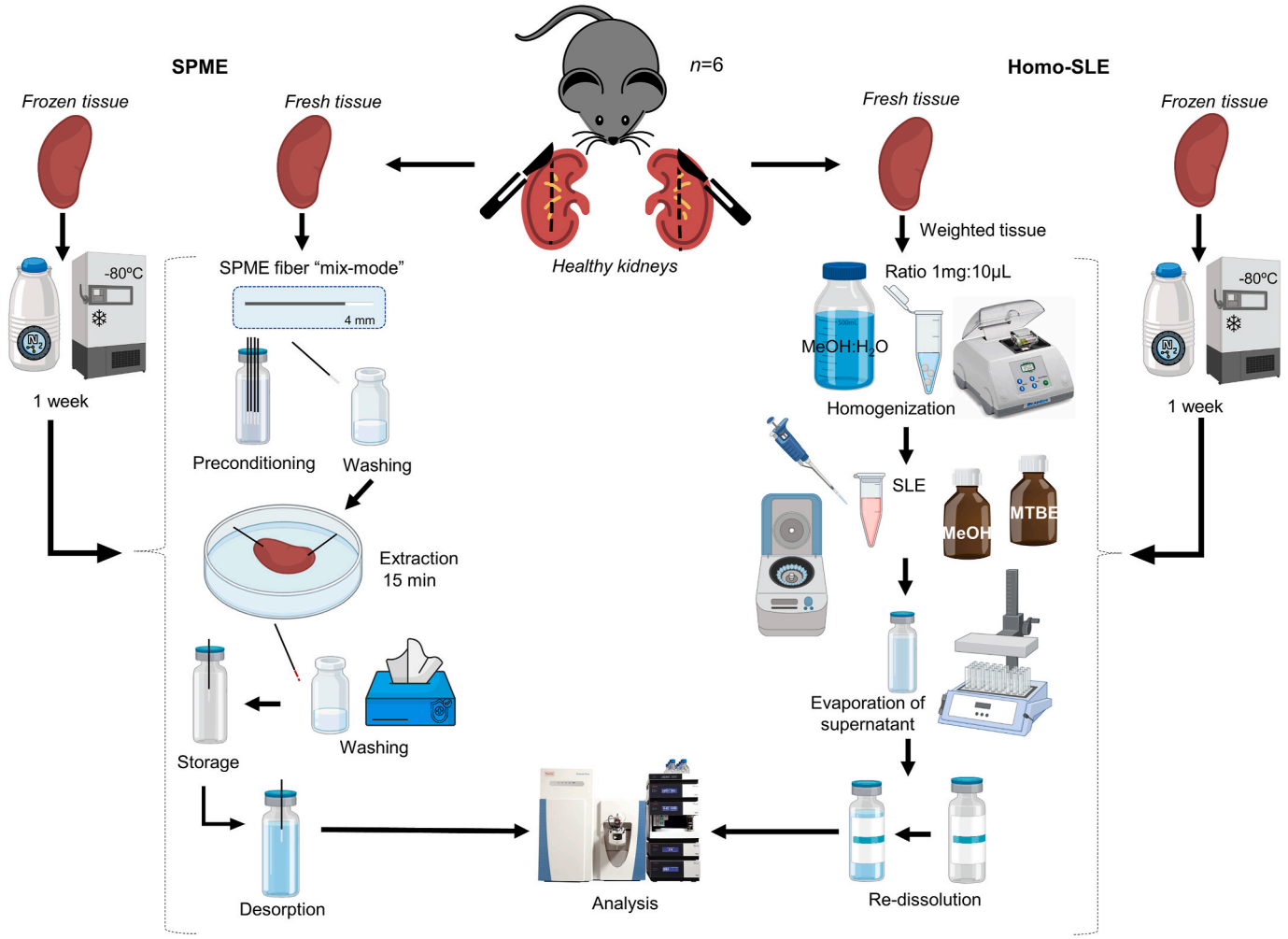


Fig. 1. Experimental design. Graphical representation of the experimental design followed in this study. The steps of the two sample treatment methodologies are represented until the analysis part. In the case of frozen tissue groups, the protocol followed was the same as the fresh tissue case, but the tissue was defrosted prior to the first step.

The six mice were controls; thus, 12 healthy kidneys were obtained and classified into four different experimental groups according to the sample treatment methodology (Fig. 1). The SPME methodology was applied in two experimental groups, and the two other groups were treated with a homogenization-SLE method. The process was carried out within each methodology in fresh and frozen tissue. Therefore, the four experimental groups are SPME fresh kidney tissue (SPME-fresh), SPME frozen kidney tissue (SPME-frozen), Homogenization-SLE fresh kidney tissue (Homo-SLE fresh), and Homogenization-SLE frozen kidney tissue (Homo-SLE frozen).

In the case of the experimental groups with fresh tissue (SPME-fresh and Homo-SLE fresh), the sample treatment procedures started following the collection of the kidneys. On the other hand, for the experimental groups with frozen tissue (SPME-frozen and Homo-SLE frozen), the kidney was collected, frozen in liquid nitrogen, and stored at −80 °C for one week before the sample treatment.

2.2. Chemicals

The LC-MS grade solvents methanol, formic acid, *tert*-butyl methyl ether (MTBE), and isopropanol were purchased from Alchem (Alchem, Poland). High-purity water, ammonium acetated and acetic acid were obtained from Merck (Merck, Poland). Pierce LTQ Velos ESI Positive External Calibrant Solution and Negative Ion Calibration Solution were purchased from Anchem (Anchem, Poland). The Mass Spec Standard mix SPLASH® Lipidomix® was obtained from Avanti (Avanti, Polar-Lipids, USA), and the Metabolomics Amino Acid Mix Standard MSK-A2-1.2 was from Cambridge Isotope Laboratories (Cambridge Isotope Laboratories, Inc, USA).

2.3. SPME fibers

Biocompatible SPME fibers coated with mix-mode ((MM) mixture of SCX and C8 particles) with 4 mm coating length, and 40 μm coating thickness were provided by Supelco. All fibers went under pre-conditioning steps before extraction process which involved overnight exposure to 1 mL of methanol:water (1:1, v/v) and rinsed with purified water for 10 s prior to insertion [26].

2.4. Sample collection and treatment

After kidney collection, a dissection in half lengthwise was made to obtain one-half of each kidney sample, which was prepared for the specific sample treatment according to the experimental group.

2.4.1. SPME methodology

The protocol followed for SPME was previously described [18,26], therefore, all the parameters and conditions were optimized. Briefly, two preconditioned fibers were inserted into each half kidney tissue sample. To control the depth at which the fiber was inserted and to avoid variability, the fibers were inserted until the part containing the extraction phase (white coating) was no longer visible, trying to cover only the part of the renal cortex, which was visually identified. The extraction time was 15 min, and after this time, the fibers were removed from the organ, quickly rinsed with water, and gently cleaned with sterile wipes to remove any possible blood or tissue contamination. The fibers were individually stored in empty vials at −80 °C until their analysis. Extraction blank samples (without the analytes of interest) were also prepared. All fibers were desorbed immediately before instrumental analysis, in their specific desorption solvents for each analysis (more info in the Supplementary Material). Quality control (QC) samples were prepared after the desorption step together with Homo-SLE samples, taking 10 μL from each sample of the four experimental groups.

2.4.2. Homo-SLE methodology

For the Homo-SLE method, the tissue disruption, homogenization, and metabolite extraction procedures were carried out using our previous protocols [28–30] with minor modifications. In summary, the two halves kidney were weighted, and one was used for the “fresh tissue method”, and the other one was frozen in liquid nitrogen and stored at −80 °C for one week until its data treatment. For the homogenization process, cold methanol:water (1:1, v/v) was added in a ratio of 1 mg:10 μL, together with three zirconium beads of 3.0 mm (mean diameter) (Benchmark Scientific, USA). The disruption of the tissue was carried out using a Beagbug™ microtube homogenizer (Benchmark Scientific, USA), in 5 cycles of 3 min at maximum power (4000 rpm, 50Hz), with 1-min breaks with samples on ice. The weight range of the half kidney tissue samples varied between 70.8 and 89.6 mg in Homo-SLE fresh group and between 74.2 and 91.3 mg in the Homo-SLE frozen group.

After obtaining the homogenate, 320 μL of methanol was added to 100 μL of homogenate, and this solution was mixed in the vortex. Next, 80 μL of MTBE was added, and the samples were vortex-mixed for 1h at room temperature, followed by centrifugation (4000 g, 20 min, 20 °C) [29,30]. From the supernatant obtained, 350 μL was transferred to a chromacol vial and evaporated to dryness using a nitrogen flow. Extraction blank samples were also considered in this methodology. The process to re-dissolve the dried samples used the same solvents as the desorption methods.

2.5. Liquid chromatography-high resolution mass spectrometry analysis (LC-HRMS)

An LC-HRMS methodology based on an ultra-high performance liquid chromatograph (Dionex UltiMate 3000 UHPLC, Thermo Scientific) coupled to a Q-Exactive Focus Orbitrap mass spectrometer (Thermo Scientific) was used for the measurement of all samples. Data acquisition was performed using Xcalibur 4.2 Thermo Scientific software (Thermo Fisher Scientific, USA). The instrument was calibrated via external calibration every 48h, obtaining a mass accuracy of $\leq$2 ppm. All the samples were randomized in the analysis sequence, and QC samples were measured regularly, every 7–10 samples, to monitor instrument performance.

2.5.1. Metabolomics analysis

The chromatographic separation for the metabolomics analysis was carried out in reversed-phase as previously described [18,26]. Samples were injected at a volume of 10 μL in a pentafluorophenyl (PFP) (Discovery HS F5 100 × 2.1 mm, 3 μm) column. Mobile phase A was water with formic acid (99.9:0.1; v/v), and mobile phase B was acetonitrile with formic acid (99.9:0.1; v/v). The complete information about the chromatographic method and the mass spectrometer parameters of the metabolomics analysis can be found in the Supplementary Material.

2.5.2. Lipidomics analysis

For lipidomics analysis, two different chromatographic separations were carried out to cover the different possible lipid classes comprehensively. The analyses were performed on a hydrophilic stationary phase (HILIC) method and in a reversed-phase (RP) method [26].

2.5.2.1. Reversed phase lipidomics analysis. For reversed-phase (RP) analysis, a C18 column (Waters, XSelect CSH C18, 3.5 μm, 2.1 × 75mm) was used, as previously described [26]. The mobile phases were, phase A consisted of water: methanol (60:40; v/v) and phase B of isopropanol: methanol (90:10; v/v), both containing 10 mM ammonium acetate and 1 mM acetic acid. All the information about this methodology is presented in the Supplementary Material.

2.5.2.2. HILIC lipidomics analysis. For this lipidomics analysis, the method and parameters used were previously described [26]. The

chromatographic separation was carried out with a hydrophilic stationary phase (HILIC) column (SeQuant ZIC-HILIC, 3 μm 100 × 2.1 mm). The mobile phases were acetonitrile (A) and 5 mM ammonium acetate in water (B), with a gradient of 0–2 min (96 % B), 2–15 min (96-80 % B), 15–15.1 min (80–96 % B), 15.1–21 min (96 % B), and the flow rate was established at 0,4 mL/min, more information in the Supplementary Material.

In the Fig. 1_Supplementary Material presents the Total Ion Chromatograms (TIC) from our analyses. This visualization enables the discernment of both the differences and similarities across the chromatographic profiles derived from the two distinct sample treatment methodologies, as well as the comparison between frozen and fresh tissue processing. In this sense, the chromatograms of untargeted HILIC lipidomics, RP lipidomics, and metabolomics analyses show a great richness, presenting numerous peaks, indicative of the various molecular species present in the samples.

2.6. Data analysis

2.6.1. Data processing, data pre-treatment and statistical analysis

The data processing step for metabolomics raw MS data was performed using Compound Discoverer 3.1 software (Thermo Fisher Scientific, USA), and in the case of lipidomic data with LipidSearch 4.1.30 software (Thermo Fisher Scientific, USA), as it was previously performed [17,18]. The parameters applied for each analysis in this step are indicated in the Supplementary Material.

The six data matrices obtained, metabolomics data positive and negative (metabo+ and metabo-), lipidomics HILIC positive and negative (HILIC +, and HILIC -), lipidomics RP positive and negative (RP + and RP -), were imported into Microsoft Excel (Microsoft Office 2016) to further calculations. Blank subtraction, curation, and removing detected duplicate features were performed in all matrices. Missing values in the experimental samples were imputed using the K-nearest neighbours (kNN) [31]. The matrices were normalized by internal standard (IS) and were filtered by the coefficient of variation (CV) of QCs samples, maintaining those features that presented a CV below 25 %. Once the matrices were normalized and filtered, they were imported into SIMCA P+16 (Umetrics®, Sweden) for multivariate statistical analysis (MVA) and to MATLAB R2018a software (Mathworks, USA) for univariate statistical analysis (UVA).

In the MVA, the orthogonal partial least square-discriminant analysis (OPLS-DA) models were cross-validated, and their CV ANOVA *p* values were obtained by the CV-ANOVA tool provided by the software. From these models, the values of variable influences on projection (VIP), correlation coefficient (*p*-corr) and jackknife confidence interval were calculated, being significant those metabolites and lipids with a VIP value ≥ 1, *p*-corr ≥ 0.5 in absolute value, and a jackknife not including 0.

A univariate statistic was also carried out within these comparisons, employing a Mann-Whitney *U* test. *P* values < 0.05 were determined as statistically significant, and the false discovery rate (FDR) for multiple hypothesis testing was controlled using a standard Benjamini-Hochberg method (level $\alpha = 0.05$).

After the different statistical analyses, the percentage of change (% Change), and the base two logarithms of the FC (Log_2FC) were calculated, comparing frozen vs. fresh for each methodology.

Venn diagrams were made with the lists of compounds detected in the different analyses (PFP, HILIC and RP) in positive and negative modes to evaluate the number of metabolites and lipids annotated in both sample treatment methodologies.

With those compounds present in both SPME and Homo-SLE samples, a correlation analysis was performed in Metaboanalyst 5.0 https://www.metaboanalyst.ca [32], obtaining correlation heatmaps between the samples of the two methodologies, with their Pearson correlation coefficients and the *p*-values of these coefficients.

2.6.2. Metabolite annotation

For metabolomics data, the annotation process was carried out in the Compound Discoverer 2.1 and FreeStyle 1.4 software (Thermo Scientific, USA), where the features were tentatively annotated based on the assignment of possible candidate metabolites to a signal based on the correspondence of their masses, retention time and spectral fragmentation, with entries from different online databases. The spectra of fragmented compounds were annotated by the software linked to the mzCloud online database [17,18]. The lipidomics annotation process was carried out in the LipidSearch 4.1.30 software (Thermo Scientific, USA) with the parameters shown in Supplementary Material. An identification grade filtering was applied to filter false positive lipid ID from LipidSearch results. Only lipids with grades A and B in the software were admitted [17].

Finally, the tentatively annotated lipids and metabolites obtained with the software were manually curated to obtain the final list. At this point, it is necessary to clarify that throughout this work a distinction is made between "lipids" and "lipid species". In this case, “lipids” refer to the number of features annotated with a sum composition nomenclature, as derived from our lipidomics analysis and data processing. In contrast, “lipid species” are groups of these annotated lipids that fall within the same categories and classes, and present the same sum composition.

3. Results

3.1. Compounds detected in SPME and HOMO-SLE groups

3.1.1. Lipidomics results

After data processing, matrix curation, annotation and statistical analysis, the results obtained from the lipidomics analyses were evaluated, observing the number of lipids detected by this methodology, and the differences present between the Homo-SLE and SPME samples, as can be seen in the Venn diagrams represented in Fig. 2.

In the HILIC results, a total of 126 lipids were annotated in the positive mode (Table 1), of which, with the tentative species sum annotation, there are 119 different lipid species annotated in total. All these 126 lipids are detected in the samples with Homo-SLE sample treatment. However, using SPME, only 87 lipids out of these 126 lipids were detected, with 81 different lipid species. Therefore, 39 lipids are detected specifically and only in the Homo-SLE samples (Fig. 2 A)). These 39 lipids belong to different classes, including 1 acylcarnitine, 8 hexosylceramides, 7 glycero-phosphocholines, 3 glycerophosphoethanolamines, 7 glycerophosphoserines, 6 glycerophosphoglycerols, 4 sphingomyelins, 1 sterol, and 2 sphingosines. In the case of the negative mode analysis, a total of 50 lipids were annotated (Table 2), yielding 45 different lipid species. All these 50 lipids were detected in the Homo-SLE samples, but only 47 were present in the SPME samples, with 42 different lipid species. therefore, 3 lipids were only detected in the Homo-SLE samples (Fig. 2 B)), 2 lipids of the glycerophosphoethanolamines class and 1 of the glycerophosphoinositols class.

In addition, in the Venn diagram of **panel C** in Fig. 2, these annotation results of HILIC positive and negative analyses have been put together. This way, it was possible to observe 18 lipids present in the SPME and Homo-SLE groups in both polarities. Moreover, 62 lipids are specific to positive and 22 to negative modes. Also, in this case, for the Homo-SLE experimental group, only 36 lipids are specific in positive mode and 2 in negative mode.

In the RP results, we see that in the positive mode analysis, 277 lipids were tentatively annotated (Table 3), giving 263 different lipid species. All these lipids have been identified in the Homo-SLE group, while 175 lipids have been detected in the SPME group, with 172 different lipid species. Of the 102 lipids (91 lipid species) were present only in the Homo-SLE samples, 2 belong to the acylcarnitine class, 11 ceramides, 8 hexosylceramides, 1 cholesterol ester, 3 diglycerides, 20

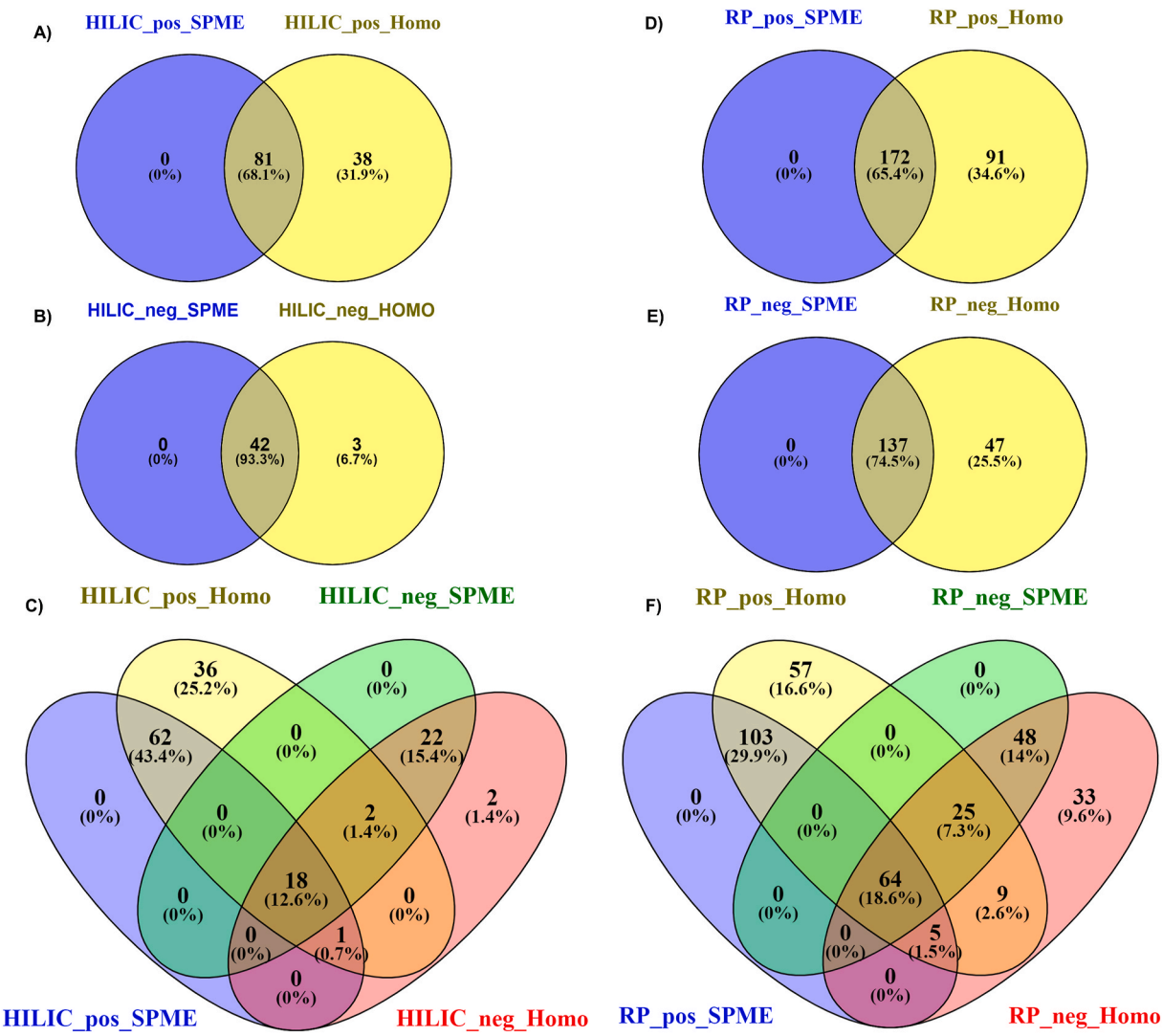


Fig. 2. Venn diagrams representing the list of compounds annotated in each analytical technology within SPME and Homo-SLE experimental groups. A) Comparison of data from the HILIC positive mode analysis, evaluating SPME and Homo-SLE groups. B) Comparison of data from the HILIC negative mode analysis, evaluating SPME and Homo-SLE groups. C) Comparison of the data from the two ionization modes in HILIC analysis. D) Comparison of data from the RP positive mode analysis, evaluating SPME and Homo-SLE groups. E) Comparison of data from the RP negative mode analysis, evaluating SPME and Homo-SLE groups. F) Comparison of the data from the two ionization modes in RP analysis.

glycerophosphocholines, 10 glycerophosphoethanolamines, 7 glycerophosphoserines, 4 glycerophosphoglycerols, 8 glycerophosphoinositols, 15 sphingomyelins, 1 sphingosine, and 12 triglycerides (Fig. 2 D)). In contrast, 191 lipids were annotated in the negative mode RP analysis results (Table 4), yielding 184 different lipid species. Of these 191 lipids present in the Homo-SLE group, 142 were detected in the SPME group (137 different species). Therefore, 48 lipids are only detected in the Homo-SLE group (Fig. 2 E). Of these 49 lipids, 4 are ceramides, 9 are glycerophosphocholines, 8 glycerophosphoethanolamines, 11 glycerophosphoserines, 6 glycerophosphoglycerols, 5 glycerophosphoinositols and 6 sphingomyelins.

Moreover, in the Venn diagram of **panel F** in Fig. 2, both annotation results of RP positive and negative modes have been represented together. In this way, 64 lipids are detected in the SPME and Homo-SLE groups in both polarities. Moreover, 103 lipids are specific to positive and 48 to negative modes. Also, in this case, for the Homo-SLE experimental group, only 57 lipids are specific in positive mode and 33 in negative mode.

3.1.2. *Metabolomics results*

In the metabolomics results, after data processing, matrix curation, annotation step and statistical analyses, it was possible to observe that in both modes, positive and negative, all the metabolites annotated were detected in both experimental groups, SPME and Homo-SLE. In total, 78 and 51 metabolites were annotated in the positive and negative polarities, respectively (Tables 5 and 6). Only 13 metabolites were common between the two ionization modes.

3.2. *Exploratory inspection of SPME and Homo-SLE data by multivariate analysis*

Firstly, PCA-X models were created with the six data matrices (Fig. 3) to evaluate the quality of the instrumental analysis through the QC samples clustering, visualize the data, and have a general idea about the trends of experimental samples.

As observed in the different panels of Fig. 3, the QC samples from the six analyses are present in tight clusters, confirming the good analytical quality of the results. Moreover, the six plots of PCA-X models showed the separation through the first component of the SPME and Homo-SLE

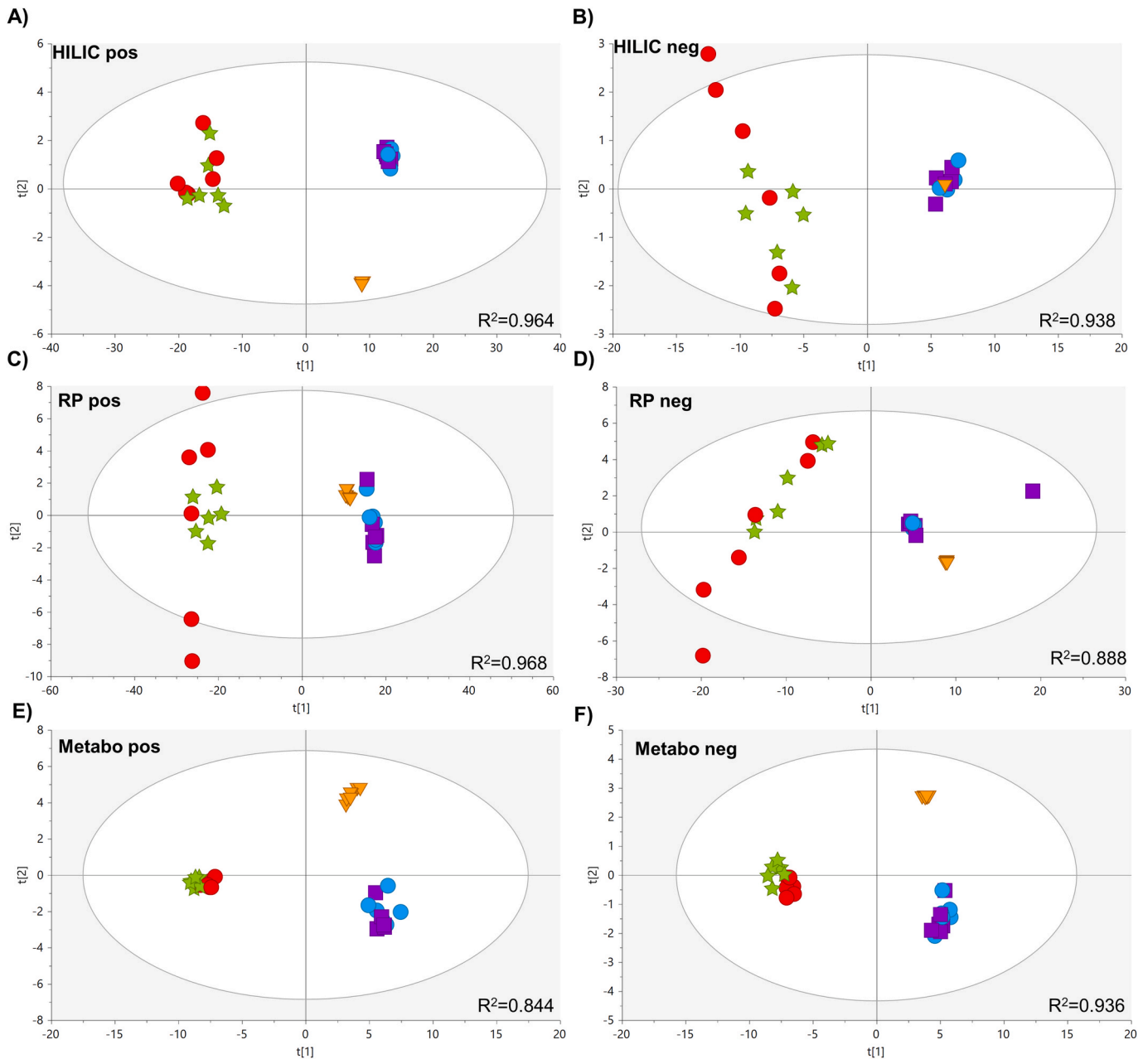


Fig. 3. PCA-X models. This figure plots the PCA-X models for the three analyses, HILIC lipidomics, RP lipidomics and metabolomics (Metabo), in positive and negative ionization modes. The legend of the models would be as follows, QCs samples are represented with orange inverted triangles, fresh Homo-SLE group blue circles, frozen Homo-SLE group purple squares, fresh SPME group green stars and frozen SPME red circles. A) PCA-X model of the positive HILIC data, with an R^2 = 0.964 and two components explaining 95.9 % (*t1*) and 0.5 % (*t2*) of model variation; B) PCA-X model of the negative HILIC data, with an R^2 = 0.938, and two components explaining 91.9 % (*t1*) and 1.8 % (*t2*) of model variation; C) PCA-X model of the positive RP data, with an R^2 = 0.968, 94.7 % (*t1*) and 2.1 % (*t2*); D) PCA-X model of the negative RP data, with an R^2 = 0.888, and 84.2 % (*t1*) and 4.6 % (*t2*); E) PCA-X model of the positive metabolomics data, with an R^2 = 0.844, and 78.9 % (*t1*) and 3.2 % (*t2*); F) PCA-X model of the negative metabolomic data, with an R^2 = 0.936, and 91.3 % (*t1*) and 1.6 % (*t2*). All models have been generated with a Pareto scaling and log transformation. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

groups. This separation was expected due to the great difference between the metabolite amount extracted in the Homo-SLE method (exhaustive method) and the SPME methodology (non-exhaustive method), with its corresponding difference in the intensity of the analytical signal. In the PCA-X models, the effect of the type of sample, frozen or fresh tissue, is represented in both experimental groups. While Homo-SLE groups (Homo-SLE fresh and Homo-SLE frozen) are represented together in a tight cluster in the six analyses with no separation, the SPME groups (SPME fresh and Homo-SLE frozen) present a higher variability, and in the HILIC positive and negative, and RP positive and negative plots show a certain degree of separation.

Hereafter, supervised models such as PLS-DA were built to highlight sample grouping and reveal compound association to the experimental conditions. In the six PLS-DA models (Fig. 2_Supplementary), the R^2 and Q^2 parameters obtained indicate that the models have moderate goodness of fit but low predictive quality. Additionally, the separation of the two SPME groups through the orthogonal component is shown in five of the six plots.

3.3. Evaluation of the differences between frozen and fresh kidney tissue samples in SPME and Homo-SLE methodologies

OPLS-DA models for each data matrix were built by group pairs to determine the main driving forces among the variables and maximize class discrimination between frozen and fresh experimental groups, and thus be able to evaluate their potential differences detected by each methodology. Given the limited sample size in our experimental model, we took precautions to prevent overfitting of the models. Initially, we assessed group separation using the unsupervised PCA-X model [33]. Once we confirmed the quality and trends, we proceeded with the supervised OPLS-DA analysis. The model was cross-validated using the "1/3 out" approach, and the quality and predictive parameters were obtained. Of all the models obtained, only the Frozen vs. Fresh comparison of the SPME data from the metabolomics analysis actually shows a separation between the groups in the PCA-X models, with a moderately high R^2, generating validated OPLS-DA models with statistical significance, as shown in Fig. 4. The models obtained with the SPME data from the HILIC and RP lipidomics analyses, despite showing some separation trend in the OPLS-DA CV-scores, clearly show no separation between the frozen and fresh groups in the unsupervised PCA-X models (Fig. 3_Supplementary). Moreover, the R^2 and Q^2 values are moderately low and show a large difference between them, casting doubt on the quality of the models obtained.

Furthermore, in our metabolomics analysis, the differences induced in the metabolome by the freezing process and detected using the SPME methodology are also reflected in the changes in metabolite levels represented in the heatmaps (Fig. 5). Notably, the natural grouping tendency correctly classifies frozen and fresh samples, with only one fresh sample deviating from its expected position. This clustering arises from the distinct change profiles exhibited by the metabolome during the analysis. Both heatmaps clearly demonstrate that the frozen SPME group (color red) exhibits elevated levels of various metabolites compared to the fresh group (color green). These differences in metabolite levels are visually delineated by dashed lines within the heatmaps. However, this clustering of the samples in both groups, or the clear difference of increase and decrease in the levels of different compounds, is not obtained in the heatmaps with the lipidomic SPME data (Fig. 4_Supplementary).

On the other hand, the MV models derived from the Homo-SLE experimental groups, as depicted in Fig. 5_Supplementary, indicate no significant distinctions between the frozen and fresh groups using this method. The unsupervised PCA-X models do not reveal any discernible trends in group separation, and the negative polarity lipidomics analysis datasets fail to yield a supervised OPLS-DA model. Correspondingly, these outcomes are mirrored in the various heatmaps presented in Fig. 6_Supplementary, where there is an absence of distinct clustering for the frozen and fresh samples. Furthermore, there is no apparent pattern of change in the levels of the different metabolites and lipids associated to the process of freezing. , Overall, all these results have been further confirmed with univariate statistics analyses, obtaining the corrected *p*-values, present in Tables 7–12 in the Supplementary Material, together with values of change, %change and Log_2FC, and VIP, JK and p(corr) values obtained from the cross-validated OPLS-DA models, for each comparison.

3.4. Evaluation of the differences between SPME and Homo-SLE groups

Correlation analyses were performed to compare the results obtained by SPME and Homo-SLE. For this purpose, a Pearson correlation analysis was performed between samples from the SPME and Homo-SLE groups, evaluating fresh and frozen tissue separately.

For the HILIC lipidomics analysis, 4 correlation analyses were performed, evaluating the trend of the 6 SPME and 6 Homo-SLE samples, in the positive and negative analysis, and selecting frozen or fresh tissue. In

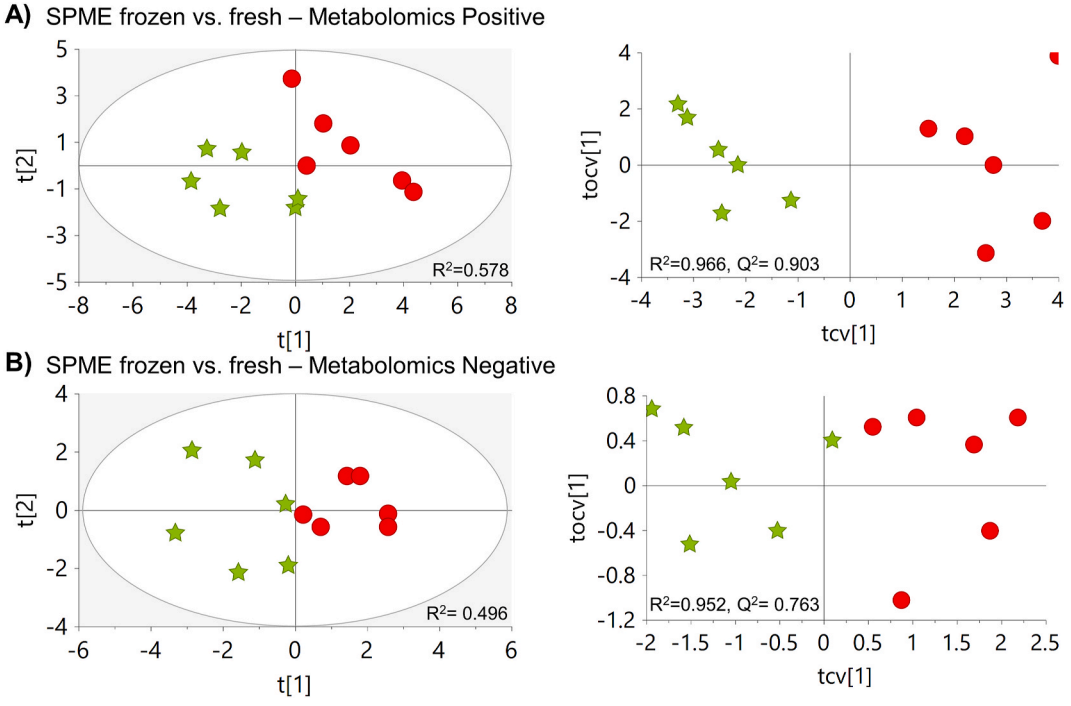


Fig. 4. SPME frozen vs. fresh comparison metabolomics data. .PCA-X and validated OPLS-DA CV-score plots of SPME data for the comparison of frozen and fresh tissue methodology. A) SPME frozen vs. fresh metabolomics positive data. PCA-X model with an $R^2 = 0.578$, and two components explaining 42 % (t1) and 16 % (t2) of model variation. Followed by the CV-score obtained in the "1/3-out" validation process of the OPLS-DA model of this comparison, with a prediction accuracy 100 %, CV-ANOVA *p* value 0.0012, and quality and prediction parameters $R^2 = 0.966$, $Q^2 = 0.903$. B) SPME frozen vs. fresh metabolomics negative data. PCA-X model with an $R^2 = 0.496$, and two components explaining 34 % (t1) and 16 % (t2) of model variation. Followed by the CV-score obtained in the "1/3-out" validation process of the OPLS-DA model of this comparison, with a prediction accuracy 91.6 %, CV-ANOVA *p* value 0.0238, and quality and prediction parameters $R^2 = 0.952$, $Q^2 = 0.763$. All models have been generated with a Pareto scaling and log transformation.

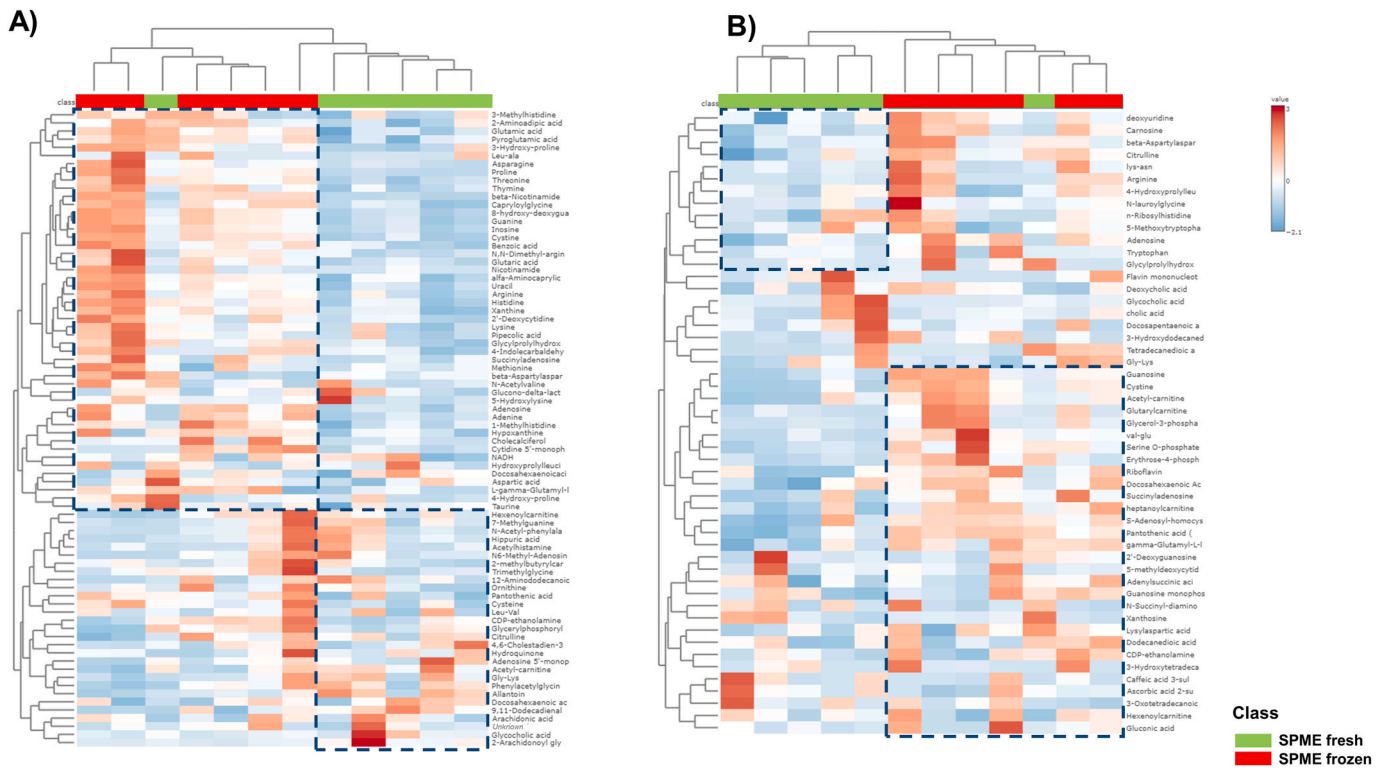


Fig. 5. Heatmaps of SPME data for the comparison of frozen and fresh tissue methodology. Heatmaps with hierarchical clustering with euclidean distance measure and complete clustering. A) SPME frozen vs. fresh metabolomics positive data. B) SPME frozen vs. fresh metabolomics negative data. The boxes marked with dashed lines are to facilitate the visualization of the changes according to the experimental groups.

the heatmaps present in Fig. 6, we can see that the HILIC analysis presents a strong-moderate positive correlation between the results obtained with SPME and Homo-SLE, as we obtain Pearson correlation coefficients greater than 0.7 in almost all samples, both in frozen and fresh tissue. In the case of the correlation analyses with the RP lipidomics data (Fig. 7_Supplementary), the same positive trend is maintained in the samples of both methodologies, as in the HILIC data. But in this case, the correlation is stronger in the negative analysis, with higher correlation coefficients.

Finally, the metabolomics data show similar results to the RP case (Fig. 8_Supplementary). Positive correlation coefficients were obtained between the samples of both experimental groups in the four analyses, but they were higher in the case of the negative ionization mode.

It should be noted that all Pearson correlation coefficients obtained in all analyses are significant, with a *p*-value of less than 0.05.

4. Discussion

The overall aim of this study was to compare the results obtained in the lipidomic and metabolomic analysis of murine kidney tissue after using two sample processing methodologies, SPME and Homo-SLE. To evaluate the possible differences in the coverage of the renal lipidome and metabolome with each technique and determine their future uses in studies of renal diseases through metabolomics.

Tissue analysis is of great value, as it provides direct information on the processes occurring in the organ [4,34]. In the case of kidney diseases, such as chronic kidney disease, it is important to assess the metabolic changes occurring in the kidney that initiate the development and progression of the disease. Therefore, metabolomic analysis of kidney tissue has great potential for studies of this type of disease [2].

As is well known, selecting the sample processing methodology is a crucial step in the metabolomics workflow, as it will determine the coverage of the metabolome to be obtained, the quality of the data, and the interpretation of the results. Despite this, there are no standardized sample processing methodologies for analyzing kidney tissue. Commonly, recollection of a part of the kidney, its homogenization, and liquid-liquid metabolite extraction is usually followed as sample treatment in metabolomics studies. However, this methodology can generate bias in the results if the heterogeneity of the renal tissue itself is not considered, as well as the heterogeneity that can be produced by some biological situations, such as fibrosis [1,4,5,35,36]. SPME methodology can be an interesting alternative in renal studies as it simplifies the sample treatment process. This methodology has been previously applied to renal tissue to evaluate the quality of the kidneys during the preservation period in transplantations [23,26,37]. However, it would be necessary to know its limitations concerning the extracted metabolome, and in comparison with Homo-SLE to establish its applicability.

For this objective, the present study did not consider quantitative analysis, only qualitative comparisons between results obtained for the different analyses were performed.

The differences in the number of lipids detected according to the sample treatment methodology may be due to different factors and facts, such as the tissue disruption and homogenization process in Homo-SLE methodology and its exhaustive extraction, the localized sampling performed in SPME, the type of coating of the SPME fibers, and the non-exhaustive nature of the SPME technology, which will be discussed below.

Firstly, tissue disruption by homogenization is a destructive process which will produce the release of intracellular components and lipids that are part of the membranes [38,39]. In our study, this can be observed with the detection of more lipids of certain classes, such as glycerophospholipids, sphingomyelins, ceramides and hexosylceramides only in the Homo-SLE group. Glycerophospholipids, such as glycerophosphocholines (PC) and glycero-phosphoethanolamines (PE), and sphingolipids, such as sphingomyelins (SM), are fundamental parts of cell membranes and are present in great abundance in these structures and not in free form [38,40]. Therefore, high coverage of these lipids is not possible by SPME since a process that produces their release is

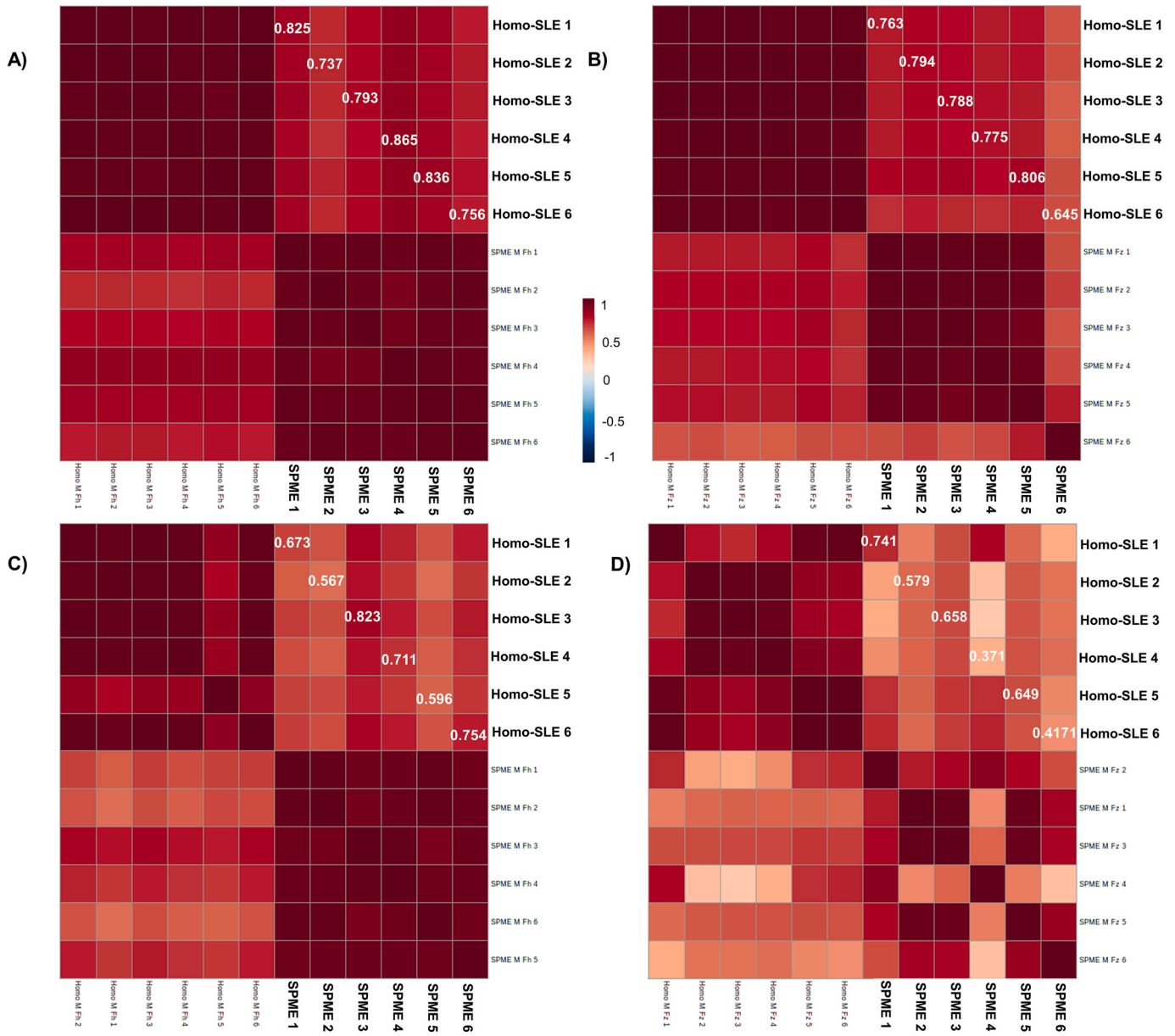


Fig. 6. Correlation heatmaps from lipidomics HILIC data. The upper panels correspond to Pearson's correlation analysis for positive HILIC SPME vs Homo-SLE A) fresh tissue and B) frozen tissue data. The lower panels correspond to Pearson's correlation analysis for negative HILIC SPME vs Homo-SLE, C) fresh tissue, and D) frozen tissue data. The white numbers indicated in each box of each comparison (Homo-SLE sample vs SPME sample) represent the correlation coefficients obtained in the analysis.

needed. The same is true for ceramides and hexosylceramides, which are found in high concentrations intracellularly, forming part of cellular structures such as the Golgi apparatus or the endoplasmic reticulum, and can only be greatly covered if they are released and extracted [41]. These results are in line with previous studies where it has been observed that tissue homogenization can drastically alter the type and amount of lipids in the sample compared to intact tissue analysis [27,38,39].

Furthermore, in the case of the SPME methodology, fibers with a small diameter (no more than 250 μm) are used, which means that their direct insertion into the tissue results in minimal damage and does not generate a large cellular rupture that causes a change in the type and concentration of free-form lipids, for this reason, it is applicable for *in vivo* studies. In this way, SPME extracts those free analytes present in the immediate area surrounding the fiber insertion site, thus providing information on the spatial resolution of the analytes within the organ [39]. This spatial information is lost in the process of homogenization and tissue disruption, which would also be involved in the difference in annotated lipids between the two groups. Therefore, this feature of SPME is a point to consider, as it can be a great advantage in heterogeneous organs and tissues such as the kidney, allowing us to obtain the lipidomic and metabolomic profile of the different regions of the organ. However, it can also be a limitation, depending on the study's objective, and knowing that if we want to obtain a representative sample of the metabolic profile of the organ, we will have to carry out the extraction with several fibers at different points of the tissue [17,39].

Regarding the fibers for the SPME methodology, the type of coating used will determine the affinity of the analyte in a given medium for the extraction device [42,43]. In this study, we have used commercial biocompatible nitinol-based SPME fibers coated with a mixture of SCX and C8 particles to extract a balance of polar and non-polar metabolites [18,26,39,44]. This way, the lipidomics and metabolomics analyses

could be carried out using a single fiber type. The use of the mix-mode coating makes the extraction in the lipidomics study less specific since the fiber coating does not have a high affinity for all lipid classes and their different polarities; for instance, in our results, we can observe that some triglycerides and ceramides, which are very hydrophobic lipids, are not detected in SPME group. A C18 particle coating could be used for specific lipidomics extraction, allowing lipids with lower polarity to be extracted [17,45]. Recent studies have shown that a hydrophilic lipophilic balance (HLB) coating shows higher recovery and a broader spectrum of metabolites compared to C8-SCX mix-mode coated fibers [46]. However, these HLB fibers are not yet commercially available, complicating their integration into routine metabolomics procedures. Therefore, in this study we have focused on the use of fibers that are commercially available and allow us to obtain a broad spectrum of compounds.

In the Homo-SLE methodology, on the other hand, we have used a solvent extraction that allows a broad coverage of the different types of lipids, including those of the cell membrane, since methanol can dissolve lipid structures, such as lipid-protein complexes, releasing some lipid classes that will then be extracted by MTBE [38,47]. As we can see, the choice of fiber coating and solvents has favored the extraction of lipids in Homo-SLE methodology, but the opposite is true for metabolites.The results obtained in the metabolomics analysis show that the mix-mode coating allows the extraction of metabolites with a polarity range very similar to the extraction solvent used in Homo-SLE as it has been shown that all the annotated metabolites are detected in both groups. Previous studies where the optimization of high-throughput SPME methods for polar and non-polar metabolites has been carried out show that other types of fiber extraction phases can achieve a wider polarity range [46,48,49]. This issue, can be overcome in the SPME approach by using multiple fibers with different coating chemistries, that would help to expand the analytes coverage, and also can improve the recovery of the compounds if the fibers are desorbed in the same aliquot of solvent, increasing the detection limit [17,39].

Another point that may affect the differences in the annotated compounds is the fundamentals of the two methodologies. SLE is classified as an exhaustive extraction, in which the analytes present in a matrix are entirely transferred to the extraction phase, in our case, the solvents. In this technique, the ratio of extractant to sample is significantly higher than in microextraction approaches, resulting in a high abundance of all lipid classes in the extraction. In contrast, SPME is a non-exhaustive method based on the equilibrium principle. In this methodology, the amount of extraction phase is much smaller than the sample, generally used to separate a small fraction of the analyte from the sample matrix [36,50]. Nevertheless, despite not being an exhaustive technique, and seeing that our results show differences between the Homo-SLE and SPME groups, it can also be observed that SPME presents relatively comprehensive coverage of lipids, and this is due to its ability to clean and concentrate the sample. A biocompatible binder in the fiber coating prevents macromolecules that interfere with the extraction of free analytes from interacting with the coating. This also allows the quenching step of metabolism to occur simultaneously with the extraction, allowing for a much cleaner background than in the SLE method. This methodology results in a more balanced recovery of analytes, with a reduction of the matrix effect in LC-MS analysis and a reduction of the baseline in chromatography so that analytes with low concentrations can be seen [39]. For these reasons, it should be considered that in the case of the SLE methodology, all lipids and metabolites present in the renal tissue would be extracted in a general way, both in free form and those bound to different structures, proteins, or reservoirs. In addition, when the homogenization process is carried out, the compounds extracted will be extracellular and intracellular, as explained above. On the other hand, with SPME, the metabolites and lipids extracted are all found free in the extracellular and interstitial space of the tissue [27].

Therefore, the differences in the extraction type are also reflected in the concentration of analytes extracted and in the analytical signal, which are represented in the unsupervised and supervised models of the multivariate analysis, with the clear separation of the Homo-SLE and SPME groups by the first component.

The multivariate analysis additionally reflects another of the points evaluated in this study, the difference between fresh and frozen kidney tissue. The effect of freezing of biological samples and their storage on the concentration of metabolites has been previously studied using separate exhaustive and non-exhaustive methods [6,27]. Therefore, we wanted to analyze the possible changes in metabolite and lipid concentration produced by one-week freezing and storage of kidney tissue using both sample treatment methodologies at the same time. While previous studies have analyzed changes produced by various freeze-thaw cycles [6], a study with SPME assessed changes in the metabolome and lipidome of tissue frozen for one year without freeze-thaw cycles [27], so we wanted to evaluate whether simply freezing the sample and storing it for a short time could generate changes in the lipidomic and metabolic profile of the tissue.

With the results obtained, we have observed that there are some alterations in the levels of the metabolites and lipids in the frozen tissue compared to the fresh control. However, these alterations are mainly present and are statistically significant in the SPME group, not in Homo-SLE. These results indicate that the SPME methodology is more sensitive and can detect these variations. The tissue, having undergone a single freeze-thaw cycle and being frozen for only a week, exhibits minimal changes due to cell degradation. When compounds are extracted extensively and abundantly, these minor alterations due to freezing become obscured. In contrast, as SPME produces non-exhaustive extraction based on pre-equilibrium in our case, it can detect all those changes in metabolite and free lipid levels produced by freezing [27,37]. The changes produced by freezing and observed in the SPME group, in the comparison of frozen vs. fresh tissue, are a general decrease of most lipid classes in the two lipidomics analyses (HILIC and RP), except for an increase of certain acylcarnitines, glycerophosphoethanolamines, glycerophosphoserines, ceramides, and triglycerides. In contrast, in the metabolomics results, we see that there is mainly an increase in the metabolites, such as amino acids, purines and pyrimidines, in which there is a statistically significant change. These changes in levels of lipids and metabolites could be related to different biological and/or physical processes; for example, most of the increased compounds are physiologically bound species, which could be released during thawing, and in the case of the decrease in lipids and metabolites may be due to cell degradation that would occur during the freezing-thawing cycle [6]. However, all these changes regarding freezing and storage should be studied in more detail to evaluate their biological relevance or physical origin. Nevertheless, also these results would indicate the possible sensitivity of SPME methodology to detect tissue damage in situ, as it could identify alterations in the levels of certain metabolites or lipids related to cellular impairment, such as the release of purines into the extracellular medium in response to lysis or apoptosis [51,52].

Finally, after evaluating the results of the correlation analyses of those lipids and metabolites present in both experimental groups, we verified that the samples of both groups show a moderate-high positive correlation. These correlation analyses would indicate that in the mean value of all the annotated compounds, the SPME and the Homo-SLE compounds follow the same trend.

Therefore, considering everything exposed in this discussion, including the limitations of SPME and the different ways of optimizing this technology to increase its coverage and sensitivity, this sample processing technique produces biological results comparable to those obtained by Homo-SLE, being a methodology with great advantages for the study of renal tissue.

5. Conclusion

In the present study, we have conducted a thorough evaluation and

comparison of renal lipid and metabolic profiling achieved through Homo-SLE and SPME sample processing methodologies. Notable differences were observed in the lipidomic analysis (HILIC and RP) of healthy murine renal tissue, with certain lipids being differentially detected, whereas the metabolomic analysis demonstrated equivalent metabolite coverage. Despite Homo-SLE's established status as a standard protocol for renal tissue sample treatment in metabolomic studies—ensuring comprehensive, uniform, and representative coverage of the entire tissue, including structural and intracellular lipids—we advocate for the selective application of the SPME methodology, which offers distinct advantages under specific circumstances. We can affirm that SPME presents specific advantages in relation to tissue, such as the reduction in the number of steps when processing renal tissue, avoiding the collection of a fragment of tissue and spatially localizing the analysis in a certain region of the organ, crucial considerations when dealing with limited renal samples or targeting localized renal disease studies.

Nevertheless, it is imperative to acknowledge the limitations and considerations unique to SPME when applied to renal tissue. The selection of fiber coating profoundly influences the obtained coverage, as expected. But in the context of renal tissue, when faced with a limited sample size, and the necessity to employ a coating that equitably extracts compounds across a broad polarity range, one must be cognizant of the resultant limitations in lipidomic analysis. Additionally, to achieve a homogenous representation of the tissue, multiple fibers must be utilized across different sample sections, bearing in mind that the lipid profile will lack structural or intracellular lipids, which are only accessible post-homogenization.

Moreover, this study has also assessed the impact of the one-week freezing and storage step on sample processing compared to fresh tissue analysis. This step is pivotal when working with SPME, as it does not introduce significant alterations in the Homo-SLE methodology.

Collectively, these findings have significantly expanded our understanding of renal tissue sample processing and analysis, thereby facilitating the advancement of renal disease studies through metabolomics, utilizing this complex biomatrix.

CRediT authorship contribution statement

Paula Cuevas-Delgado: Writing – review & editing, Writing – original draft, Investigation, Formal analysis, Data curation, Conceptualization. **Natalia Warmuzińska:** Writing – review & editing, Methodology, Investigation, Conceptualization. **Kamil Łuczykowski:** Methodology, Investigation. **Barbara Bojko:** Writing – review & editing, Supervision, Resources, Project administration, Conceptualization. **Coral Barbas:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgments

This work was supported by the Ministry of Science, Innovation and Universities of Spain (MCIN) PID2021-122490NB-I00/AEI/10.13039/501100011033 by ERDF-"A way of making Europe" and the Autonomous Community of Madrid project INNOREN-CM S2022/BMD-7221. Cuevas-Delgado, P. was supported by a fellowship from CEINDO – BANCO SANTANDER'S Mobility fellowship Aid.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aca.2024.342758.

References

[1] D. Vuckovic, Sample preparation in global metabolomics of biological fluids and tissues, in: Proteomic and Metabolomic Approaches to Biomarker Discovery, second ed., Elsevier Inc., 2020, pp. 53–83, https://doi.org/10.1016/B978-0-12-818607-7.00004-9.

[2] P. Cuevas-Delgado, V. Miguel, S. Lamas, C. Barbas, Metabolomics tools for biomarker discovery: applications in chronic kidney disease, in: S. Ozkan, N. Bakirhan, F. Mollarasouli (Eds.), The Detection of Biomarkers, first ed., Academic Press, 2022, pp. 153–181, https://doi.org/10.1016/B978-0-12-822859-3.00013-4.

[3] J. Ding, Y.Q. Feng, Mass spectrometry-based metabolomics for clinical study: recent progresses and applications, TrAC, Trends Anal. Chem. 158 (2023), https://doi.org/10.1016/j.trac.2022.116896.

[4] L. Smith, J. Villaret-Cazadamont, S.P. Claus, C. Canlet, H. Guillou, N.J. Cabaton, S. Ellero-Simatos, Important considerations for sample collection in metabolomics studies with a special focus on applications to liver functions, Metabolites 10 (2020), https://doi.org/10.3390/metabo10030104.

[5] S. Macioszek, D. Dudzik, J. Jacyna, A. Wozniak, P. Schöffski, M.J. Markuszewski, A robust method for sample preparation of gastrointestinal stromal tumour for LC/MS untargeted metabolomics, Metabolites 11 (2021), https://doi.org/10.3390/metabo11080554.

[6] F. Torell, K. Bennett, S. Cereghini, S. Rännar, K. Lundstedt-Enkel, T. Moritz, C. Haumaitre, J. Trygg, T. Lundstedt, Tissue sample stability: thawing effect on multi-organ samples, Metabolomics 12 (2016) 1–6, https://doi.org/10.1007/s11306-015-0933-1.

[7] Y.Y. Zhao, X.L. Cheng, F. Wei, X. Bai, X.J. Tan, R.C. Lin, Q. Mei, Intrarenal metabolomic investigation of chronic kidney disease and its TGF-β1 mechanism in induced-adenine rats using UPLC Q-TOF/HSMS/MSE, J. Proteome Res. (2013), https://doi.org/10.1021/pr3007792.

[8] P. Cuevas-Delgado, D. Dudzik, V. Miguel, S. Lamas, C. Barbas, Data-dependent normalization strategies for untargeted metabolomics—a case study, Anal. Bioanal. Chem. 412 (2020) 6391–6405, https://doi.org/10.1007/s00216-020-02594-9.

[9] B.B. Misra, R.P. Upadhayay, L.A. Cox, M. Olivier, Optimized GC–MS metabolomics for the analysis of kidney tissue metabolites, Metabolomics (2018), https://doi.org/10.1007/s11306-018-1373-5.

[10] R.B. Khattri, T. Thome, T.E. Ryan, Tissue-specific 1H-NMR metabolomic profiling in mice with adenine-induced chronic kidney disease, Metabolites 11 (2021) 1–15, https://doi.org/10.3390/metabo11010045.

[11] P. Cuevas-Delgado, V. Miguel, S. Lamas, C. Barbas, Metabolomics tools for biomarker discovery: applications in chronic kidney disease, The Detection of Biomarkers (2022) 153–181, https://doi.org/10.1016/B978-0-12-822859-3.00013-4.

[12] J. Pawliszyn, B. Pawliszyn, M. Pawliszyn, Solid-phase microextraction, in: The Chemical Educator, 1999, pp. 803–807, https://doi.org/10.1007/978-3-662-49054-9_2843-1.

[13] J. Pawliszyn, Theory of Solid-phase Microextraction, Elsevier Inc., 2012, https://doi.org/10.1016/B978-0-12-416017-0.00002-4.

[14] J. Pawliszyn, Solid-Phase Microextraction in Perspective, Elsevier Inc., 2012, https://doi.org/10.1016/B978-0-12-416017-0.00001-2.

[15] M. Huq, Fundamentals of SPME Extraction of Tissue and Plasma : Experimental and In-Silico Approaches, University of Waterloo, 2021.

[16] N. Reyes-Garcés, E. Gionfriddo, G.A. Gómez-Ríos, M.N. Alam, E. Boyacl, B. Bojko, V. Singh, J. Grandy, J. Pawliszyn, Advances in solid phase microextraction and perspective on future directions, Anal. Chem. 90 (2018) 302–360, https://doi.org/10.1021/acs.analchem.7b04502.

[17] J. Bogusiewicz, K. Burlikowska, K. Łuczykowski, K. Jaroch, M. Birski, J. Furtak, M. Harat, J. Pawliszyn, B. Bojko, New chemical biopsy tool for spatially resolved profiling of human brain tissue in vivo, Sci. Rep. 11 (2021) 1–10, https://doi.org/10.1038/s41598-021-98973-y.

[18] I. Stryjak, N. Warmuzińska, J. Bogusiewicz, K. Łuczykowski, B. Bojko, Monitoring of the influence of long-term oxidative stress and ischemia on the condition of kidneys using solid-phase microextraction chemical biopsy coupled with liquid chromatography–high-resolution mass spectrometry, J Sep Sci 43 (2020) 1867–1878, https://doi.org/10.1002/jssc.202000032.

[19] K. Burlikowska, I. Stryjak, J. Bogusiewicz, B. Kupcewicz, K. Jaroch, B. Bojko, Comparison of metabolomic profiles of organs in mice of different strains based on SPME-LC-HRMS, Metabolites 10 (2020) 1–15, https://doi.org/10.3390/metabo10060255.

[20] G. Ouyang, J. Pawliszyn, A critical review in calibration methods for solid-phase microextraction, Anal. Chim. Acta 627 (2008) 184–197, https://doi.org/10.1016/j.aca.2008.08.015.

[21] K. Burlikowska, I. Stryjak, J. Bogusiewicz, B. Kupcewicz, K. Jaroch, B. Bojko, Comparison of metabolomic profiles of organs in mice of different strains based on SPME-LC-HRMS, Metabolites 10 (2020) 1–15, https://doi.org/10.3390/metabo10060255.

[22] J. Bogusiewicz, B. Kupcewicz, P.Z. Goryńska, K. Jaroch, K. Goryński, M. Birski, J. Furtak, D. Paczkowski, M. Harat, B. Bojko, Investigating the potential use of

chemical biopsy devices to characterize brain tumor lipidomes, Int. J. Mol. Sci. 23 (2022), https://doi.org/10.3390/ijms23073518.
[23] I. Stryjak, N. Warmuzińska, J. Bogusiewicz, K. Łuczykowski, B. Bojko, Monitoring of the influence of long-term oxidative stress and ischemia on the condition of kidneys using solid-phase microextraction chemical biopsy coupled with liquid chromatography–high-resolution mass spectrometry, J Sep Sci 43 (2020) 1867–1878, https://doi.org/10.1002/jssc.202000032.
[24] N. Looby, A. Roszkowska, A. Ali, B. Bojko, M. Cypel, J. Pawliszyn, Metabolomic fingerprinting of porcine lung tissue during pre-clinical prolonged ex vivo lung perfusion using in vivo SPME coupled with LC-HRMS, J Pharm Anal 12 (2022) 590–600, https://doi.org/10.1016/J.JPHA.2022.06.002.
[25] A. Napylov, N. Reyes-Garces, G. Gomez-Rios, M. Olkowicz, S. Lendor, C. Monnin, B. Bojko, C. Hamani, J. Pawliszyn, D. Vuckovic, In vivo solid-phase microextraction for sampling of oxylipins in brain of awake, moving Rats_ Supporting information, Angewandte Chemie - International Edition 59 (2020) 2392–2398, https://doi.org/10.1002/anie.201909430.
[26] I. Stryjak, N. Warmuzińska, K. Łuczykowski, M. Hamar, P. Urbanellis, E. Wojtal, M. Masztalerz, M. Selzner, Z. Włodarczyk, B. Bojko, Using a chemical biopsy for graft quality assessment, JoVE 2020 (2020) 1–12, https://doi.org/10.3791/60946.
[27] A. Roszkowska, M. Yu, V. Bessonneau, L. Bragg, M. Servos, J. Pawliszyn, Tissue storage affects lipidome profiling in comparison to in vivo microsampling approach, Sci. Rep. 8 (2018) 1–10, https://doi.org/10.1038/s41598-018-25428-2.
[28] P. Cuevas-Delgado, D. Dudzik, V. Miguel, S. Lamas, C. Barbas, Data-dependent normalization strategies for untargeted metabolomics—a case study, Anal. Bioanal. Chem. (2020), https://doi.org/10.1007/S00216-020-02594-9.
[29] C. Gonzalez-Riano, S. Tapia-González, G. Perea, C. González-Arias, J. DeFelipe, C. Barbas, Metabolic changes in brain slices over time: a multiplatform metabolomics approach, Mol. Neurobiol. 58 (2021) 3224–3237, https://doi.org/10.1007/S12035-020-02264-Y.
[30] S. Naz, A. García, C. Barbas, Multiplatform analytical methodology for metabolic fingerprinting of lung tissue, Anal. Chem. 85 (2013) 10941–10948, https://doi.org/10.1021/ac402411n.
[31] E.G. Armitage, J. Godzien, V. Alonso-Herranz, Á. López-Gonzálvez, C. Barbas, Missing value imputation strategies for metabolomics data, Electrophoresis 36 (2015) 3050–3060, https://doi.org/10.1002/elps.201500352.
[32] Z. Pang, J. Chong, G. Zhou, D.A. De Lima Morais, L. Chang, M. Barrette, C. Gauthier, P.É. Jacques, S. Li, J. Xia, MetaboAnalyst 5.0: narrowing the gap between raw spectra and functional insights, Nucleic Acids Res. 49 (2021) W388–W396, https://doi.org/10.1093/NAR/GKAB382.
[33] B. Worley, R. Powers, PCA as a practical indicator of OPLS-DA model reliability, Curr Metabolomics 4 (2016) 97–103, https://doi.org/10.2174/2213235x04666160613122429.
[34] M. Saoi, P. Britz-Mckibbin, New advances in tissue metabolomics: a review, Metabolites 11 (2021), https://doi.org/10.3390/metabo11100672.
[35] M. Kaykhaii, Introductory chapter: evolution of sample prep, in: Sample Preparation Techniques for Chemical Analysis, IntechOpen, London, 2021, pp. 1–4, https://doi.org/10.5772/intechopen.101434.
[36] M. Sargazi, S.H. Hashemi, M. Kaykhaii, Modern sample preparaiton techniques: a brief introduction, in: Sample Preparation Techniques for Chemical Analysis, IntechOpen, London, 2021, pp. 1–19, https://doi.org/10.5772/intechopen.100715.
[37] I. Stryjak, N. Warmuzińska, K. Łuczykowski, K. Jaroch, P. Urbanellis, M. Selzner, B. Bojko, Metabolomic and lipidomic landscape of porcine kidney associated with kidney perfusion in heart beating donors and donors after cardiac death, Transl. Res. (2023), https://doi.org/10.1016/j.trsl.2023.12.001.
[38] A.P. Birjandi, B. Bojko, Z. Ning, D. Figeys, J. Pawliszyn, High throughput solid phase microextraction: a new alternative for analysis of cellular lipidome? J Chromatogr B Analyt Technol Biomed Life Sci 1043 (2017) 12–19, https://doi.org/10.1016/j.jchromb.2016.09.034.
[39] J. Pawliszyn, R.W. Jiang, K. Jaroch, Solid-phase microextraction of endogenous metabolites from intact tissue validated using a Biocrates standard reference method kit, J Pharm Anal (2022) 131932, https://doi.org/10.1016/j.jpha.2022.09.002.
[40] J.P. Slotte, Biological functions of sphingomyelins, Prog. Lipid Res. 52 (2013) 424–437, https://doi.org/10.1016/j.plipres.2013.05.001.
[41] Y.A. Hannun, L.M. Obeid, Many ceramides, J. Biol. Chem. 286 (2011) 27855–27862, https://doi.org/10.1074/jbc.R111.254359.
[42] N. Reyes-Garcés, E. Gionfriddo, G.A. Gómez-Ríos, M.N. Alam, E. Boyacl, B. Bojko, V. Singh, J. Grandy, J. Pawliszyn, Advances in solid phase microextraction and perspective on future directions, Anal. Chem. 90 (2018) 302–360, https://doi.org/10.1021/acs.analchem.7b04502.
[43] E. Boyaci, Á. Rodríguez-Lafuente, K. Gorynski, F. Mirnaghi, É.A. Souza-Silva, D. Hein, J. Pawliszyn, Sample preparation with solid phase microextraction and exhaustive extraction approaches: comparison for challenging cases, Anal. Chim. Acta 873 (2015) 14–30, https://doi.org/10.1016/j.aca.2014.12.051.
[44] J.C. Nadal, F. Borrull, R.M. Marcé, N. Fontanals, Novel in-house mixed-mode ion-exchange materials for sorptive phase extraction techniques, Advances in Sample Preparation 1 (2022) 100008, https://doi.org/10.1016/j.sampre.2022.100008.
[45] M. Olkowicz, H. Rosales-Solano, V. Kulasingam, J. Pawliszyn, SPME-LC/MS-based serum metabolomic phenotyping for distinguishing ovarian cancer histologic subtypes: a pilot study, Sci. Rep. 11 (2021) 1–14, https://doi.org/10.1038/s41598-021-00802-9.
[46] M. Olkowicz, H. Rosales-Solano, K. Ramadan, A. Wang, M. Cypel, J. Pawliszyn, The metabolic fate of oxaliplatin in the biological milieu investigated during in vivo lung perfusion using a unique miniaturized sampling approach based on solid-phase microextraction coupled with liquid chromatography-mass spectrometry, Front. Cell Dev. Biol. 10 (2022), https://doi.org/10.3389/fcell.2022.928152.
[47] C. Gonzalez-Riano, A. Gradillas, C. Barbas, Exploiting the formation of adducts in mobile phases with ammonium fluoride for the enhancement of annotation in liquid chromatography-high resolution mass spectrometry based lipidomics, Journal of Chromatography Open 1 (2021) 100018, https://doi.org/10.1016/j.jcoa.2021.100018.
[48] F. Mousavi, B. Bojko, J. Pawliszyn, Development of high throughput 96-blade solid phase microextraction-liquid chromatrography-mass spectrometry protocol for metabolomics, Anal. Chim. Acta 892 (2015) 95–104, https://doi.org/10.1016/j.aca.2015.08.016.
[49] D. Vuckovic, E. Cudjoe, D. Hein, J. Pawliszyn, Automation of solid-phase microextraction in high-throughput format and applications to drug analysis, Anal. Chem. 80 (2008) 6870–6880, https://doi.org/10.1021/ac800936r.
[50] F.S. Mirnaghi, K. Goryński, A. Rodriguez-Lafuente, E. Boyaci, B. Bojko, J. Pawliszyn, Microextraction versus exhaustive extraction approaches for simultaneous analysis of compounds in wide range of polarity, J. Chromatogr. A 1316 (2013) 37–43, https://doi.org/10.1016/j.chroma.2013.09.084.
[51] J. Linden, F. Koch-Nolte, G. Dahl, Purine release, metabolism, and signaling in the inflammatory response, Annu. Rev. Immunol. 37 (2019) 325–347, https://doi.org/10.1146/annurev-immunol-051116-052406.
[52] S.C. Chow, G.E.N. Kass, S. Orrenius, Purines and their roles in apoptosis, Neuropharmacology 36 (1997) 1149–1156, https://doi.org/10.1016/S0028-3908(97)00123-8.