Highlights

- Applications of liquid phase microextraction for analytes in complex samples.
- Focused on works optimized by the response surface methodology.
- Literature search of the works reported from 2009 to 2019.
- Illustrative example with information to carry out LPME.
Applications of liquid-phase microextraction procedures to complex samples assisted by response surface methodology for optimization

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Abstract

This review presents applications of liquid phase microextraction (LPME) for extracting analytes in complex samples. This process has been introduced to simplify the extraction methods, and enhance the selectivity, sample cleanup and efficiency, allowing the extraction of a wide variety of analytes. The revision was focused on those works in which the performance of the technique was optimized by the response surface methodology (RSM). Firstly, a description of the different LPME systems is presented. Then, a brief explanation of the most popular tools applied for optimization is displayed. After that, the results of a literature search of the works reported from 2009 to 2019 based on the implementation of microextraction supported by experimental design and optimization can be found summarized in a table. Finally, an illustrative example providing the necessary information to carry out this kind of work is presented. A list of the most popular software available to apply RSM is also presented.

Keywords: Liquid phase microextraction (LPME); Response surface methodology (RSM); Complex samples; Chemometrics
1. Introduction

Very recently, Miguel de la Guardia and co-workers have pointed out that there has been increasing concern in the experimental chemistry world related to environmental issues [1]. They stated that in different fields of analytical chemistry “there is a complete agreement about the need of taking care of the sustainability of analytical procedures and the need to extend the beneficial effects of the use of analytical data to the whole population”. In this context, the use of miniaturized, simplified and automatized procedures for preconcentration and cleanup of complex samples plays an interesting role in the total analytical process [2].

It should be remarked that from an ideal point of view, a green analytical chemistry application should avoid preconcentration steps. However, the low thresholds established for several environmental contaminants lead analytical chemists to apply pretreatment steps to attain accurate measurements in samples containing small amounts of target analytes [3].

In this scenario, liquid-phase microextraction (LPME), a novel miniaturized sample pretreatment method, which allows trace determination of target compounds in complex matrices, can be considered as an environmentally-friendly, simple, easy to operate, and highly sensitive process for preconcentration. Complex samples can be defined as those that require a tedious cleanup effort to isolate the analyte(s) from the interfering substances present in the matrix. Therefore, their pretreatment step in analytical determinations could be considered as the procedure bottleneck. The sample pretreatment depends on several factors, such as class and concentration of the analyte, complexity of the sample matrix, detection mode, types of interferences, etc.

The aim of the sample pretreatment methods consists in converting a real matrix into a sample suitable for analysis, in terms of having the analyte in an adequate level of concentration, eliminating possible interferences, converting an analyte into a more adequate form (e.g. derivatization) and/or dissolving the analyte in a media compatible with the
The analysis of biological samples usually requires extra filtration and precipitation steps. For example, urine and plasma samples are generally centrifuged to separate a white solid phase, which can be attributed to co-sedimentation of matrix ingredients [6]. Moreover, protein precipitation with methanol or acetonitrile is a traditional technique for preparing blood samples [7]. On the other hand, food and environmental solid samples should be finely milled and homogenized in the first phase of the analytical process. This allows achieving representative sampling and suitable dissolution in a proper solvent [8, 9].

Interestingly, during the development of an LPME procedure, there is a need for carefully optimizing significant factors that affect the quality of the results. These factors could be types and volumes of extraction and dispersant solvents, extraction time, sample amount, pH, and salt addition, among others [2]. In this situation, the response surface methodology (RSM) plays an important role in finding the best combination of factors that produces the optimum response, e.g., sensitivity, percentage of recovery, peak area in a chromatographic method, etc. [10]. The latter is a collection of statistical techniques which represents an important tool for modeling and analyzing the effects of several parameters of the process under study. It should be noted that the underlying philosophy is to reach the optimum conditions carrying out the lesser number of experiments as possible and calculating interactions among the independent variables. Interestingly, this methodology is more practical compared to the conventional experimental work, which is called “one variable at time” (univariate approach), as it is carried out from experimental data which include interactive effects among the variables, obtained from a statistical experimental design built under certain requirements (multivariate approach) [10].

It should be stressed that, regrettably, RSM is not as known and applied as it should be desirable, and many reports show that the optimization of the procedures was performed
by the univariate approach. Thus, the goal of this review is to evidence the real advantages in terms of both the reduced experimental effort and the improved quality of information that can be obtained by following this approach in the implementation of a LPME procedure.

The review is focused on applications of LPME for extracting analytes in complex samples, considering those cases in which the performance of the technique was optimized by RSM. For this purpose, the works reported between the years 2009 to 2019 were taken into account.

2. Liquid phase microextraction

The term liquid phase microextraction was firstly introduced to describe two-phase systems in solvent microextraction [11]. By definition, “microextraction” is an extraction technique in which a very small extractant solvent volume concerning the sample volume is utilized [12]. The extraction yield depends on the partition coefficient of the analyte(s) between the sample donor phase and the extractant solvent or acceptor phase.

Different LPME systems have been introduced to simplify the extraction methods, and enhance the selectivity, sample cleanup and efficiency, allowing the extraction of a wide variety of analytes. Currently, the classification of the LPME systems is carried out taking into account how the extractant solvent comes into contact with the analyte in the matrix. From the first method presented [13], several alternatives were developed with the object of improving the procedure. Until today, the researchers provide enhancements to generate the best analytical results. In this sense, Table 1 summarizes the advantages attained during the last years, describing the source of each liquid-liquid microextraction and the new improvements and automation in the procedure. In the latter table, the column “Procedure” describes the basis of each microextraction, while the column “Option” shows the different alternatives. Besides, the improvements achieved in the last years due to technological
advances such as ultrasound or microwave were listed. These tools allowed enhancing the process through the development of automation systems.

In the following subsections, a brief description of the most important procedures applied for the implementation of liquid phase microextraction is presented, focusing on their differences, advantages/disadvantages, and essential characteristics.

2.1. Liquid-liquid microextraction or liquid-liquid-liquid microextraction (LLME or LLLME)

These procedures (LLME and LLLME) require two or three liquid phases, a magnetic stirrer, a vial and an immiscible solvent which should be less dense than water. Their implementation is very simple, also being feasible to the complete automation of the process of extracting analytes from water.

The traditional LLME technique employs 10–100 μL of solvent, which is added to the center of a vortex originated in an aqueous sample during the stirring. The direct interface of solvent and water leads to rapid extraction and concentration of the analyte into the organic solvent, which is then removed with a capillary tube or syringe [14]. LLLME is similar to LLME, except for the fact that the analyte is firstly extracted into the organic solvent, and then back-extracted into an aqueous drop [14].

2.2. Single-drop microextraction (SDME)

This process is based on the use of a single drop of water-immiscible extractant solvent for the retention of the analyte(s) contained in an aqueous sample. SDME was the first developed LPME procedure and presents some advantages and disadvantages.

The first implementation was reported in 1996 and consisted in suspending a micro-drop of a water-immiscible solvent (ca. 1.3 μL) in a larger aqueous volume containing sodium docecyl sulphate (SDS) as ion-pair [13]. The external aqueous phase contains the analyte and
is continuously delivered and aspirated away throughout sampling. A negative aspect of this procedure in its different modes of implementation is that the extraction is rarely exhaustive. The major problem is that, in general, the distribution between the donor aqueous phase and the acceptor organic solvent drop is only favorable for one analyte or a group of them. Figure 1 shows the variants of the general procedure. In general, the variations are given by the immersion (direct-immersion) or not (headspace) of the drop, or the use of solvents more or less dense than water, commonly known as high-density or low-density solvents.

2.3. Dispersive liquid-liquid microextraction (DLLME)

In 2006, Rezaee et al. developed the dispersive liquid-liquid microextraction (DLLME) procedure for preconcentration of polycyclic aromatic hydrocarbons (PAHs) in water [15]. This method is based on a ternary system of solvents in which both the water-immiscible extractant solvent mixture and the dispersive solvent are injected rapidly into the aqueous solutions employing a syringe or micropipette. A cloudy solution or unstable microemulsion (water/dispersive solvent/extractant solvent) is formed in the mixture. Interestingly, high efficiency is attained in a relatively short time due to the large contact surface between the two immiscible phases. Figure 2 shows the variants of the overall procedure. Variations can be achieved using solvents with different densities than water.

2.4. Hollow-fiber-protected microextraction (HFME)

Although HFME is often mentioned in the literature as liquid-phase microextraction (LPME), this can be confusing since the same designation is also used for single-drop microextraction (SDME). HFME is based on the partition of analytes between an aqueous solution and a small quantity of organic solvent in a microporous tube (the rod configuration). Even though it is usually described as a liquid-liquid microextraction process,
this extraction should be considered as a hybrid process which does not follow the basis of the liquid-liquid microextraction, i.e. the use of two or more liquids to carry out the extraction, due the nature of the procedure, which involves the use of a solid phase (microporous tube).

2.5. New solvents used in extraction process

In line with the accomplishment of the principles of green chemistry, the development of alternative solvents has grown exponentially during the last decade [16]. Although the ideal situation is the achievement of “solvent-free” extraction schemes [17], this concept is still rather utopic. Therefore, the search for substitute solvents is of utmost importance [18].

In this sense, ionic liquids (ILs) gained great attention as green media, because of their biodegradability, biocompatibility and sustainability. ILs are non-molecular compounds, with melting points below 100 °C, typically consisting of a big asymmetric organic cation, and a smaller organic or inorganic anion. Due to their proprieties, ILs have been applied in many analytical chemistry fields as an alternative to traditional organic solvents. Considering the specification of each extraction method, the utilization of ILs may be divided in solvent-based extraction and sorption-based extraction. Liu et al. described the first use of an IL in a LPME system for the extraction of PAHs in water [19].

Later, a new kind of solvents based on the eutectic behavior of their counterparts, emerged as an alternative to ILs. Deep eutectic solvents (DESs) were introduced by Abbott et al. [20], showing a wide liquid range and interesting properties. A DES consists in a mixture prepared by complexing an ammonium halide with a hydrogen-bound donor (HBD) such as carboxylic acids, alcohols, amides, among others, under simple laboratory conditions. The main physicochemical properties of DESs responsible for their use as green solvents at room temperature are: freezing points, density, viscosity, polarity, ionic conductivity and
acidity/alkalinity. Moreover, DESs have been successfully used as effective, reliable, inexpensive, non-toxic, biodegradable and biocompatible new solvents. Besides, in 2011 a new kind of DESs, the “Natural Deed Eutectic Solvents” (NADES), formed by cellular constituents such as sugars, alcohols, amino acids, organic acids and choline derivatives were presented [21]. NADES are typically obtained by mixing a hydrogen-bond acceptor (HBA) with a hydrogen bond donor (HBD) molecule, leading to a significant depression of the melting point. The use of DESs and NADES is growing, and several reviews attaining their applications can be consulted in the literature [22-27].

2.6. Advances in LPME

Since the first applications of LPME reported in the mid-to-late 1990s, the researchers made the efforts to develop new devices, accelerate the extraction steps and automate the systems. In this context, the use of a polyethylene Pasteur pipette [28] or a special extraction vessel [29] for DLLME was reported.

Following the principles of green chemistry [16], the application of microwave, ultrasound and ultraviolet irradiation are genuine alternatives to conventional methods involving classical chemical reactions or to enhance the mass and/or heat transfer. In recent years, attempts have been made to introduce these clean energies in combination with microextraction techniques, thereby giving rise to the development of virtually reagentless and ecofriendly methods. Since the application of ultrasound to assist the extraction described by Huang et al. in 2006 [30], the use of clean energies and other strategies have been frequently reported (see Table 1).

Moreover, one of the goals of green chemistry is the automation of analytical methodologies to enhance the overall analysis. Automation of LPME procedures improves reproducibility compared to manual operation, and numerous samples can be analyzed in
unattended operation. Liu and Dasgupta [31] developed the first automated drop-based system in 1995, triggering the generation of numerous automation procedure reports. Several automation procedures based on microextraction with systems using continuous-flow (CFME) [32], syringe pump or chip device were reported. The wide variety of automation and devices can be consulted in the scientific literature [33, 34].

3. Response surface methodology

The well-known response surface methodology (RSM), based on the application of multivariate design of experiments (DOE) followed by optimization through mathematical modeling, plays an important role in sample pretreatment applications. Probably, it is due to the fact that the implementation requires fewer efforts and resources than those involved in univariate procedures, consuming less time to accomplish the same goal [10].

The application of RSM comprises the following consecutive steps: a) identification of the responses (for example, recovery percentage in the extraction of a substance), b) building the screening design to reduce the number of factors originally suspected of having influence in the response, c) building the response surface design and modeling to establish the relationship between one response (or several) and the factors, and d) application of multiple response optimization in those cases in which a large number of responses are involved in the study [35]. Hereafter, a brief description of each step will be presented.

3.1. Responses

In the field of sample pretreatment, and especially in extraction and preconcentration steps, the enhancement of the percent recovery of a substance is one of the main objectives. Since the expected situation is the complete migration of the analyte present in the sample to the final solution where the analysis will be carried out, this response should be maximized,
i.e. the combination of the factors which produces a maximum recovery must be found. On the other hand, the response related to the precision of the technique, evaluated by replicating the procedure and computing, for example, the coefficient of variation, should be minimized.

3.2. Screening designs

The screening step consists in the exploration of the factors (or variables) which could have an influence on the extraction process. In the beginning, a large number of factors are taken into account to perform experiments (following a screening design) and statistical analysis of the data (usually employing ANOVA) to finally decide which of them have a significant influence on the response (or responses).

Factors can be divided into quantitative (temperature, time, volume, etc.), qualitative (kind of agitation, material, etc.) and mixture-related (a mixture of solvents considering differences in the polarity of the analytes).

Mostly, screening designs involve factor variations in two levels (−1, +1), being the range the widest interval in which the factor can be varied in the system under study. This range is usually chosen based on the literature information or previous analyst knowledge. The most popular designs are full factorial, fractional factorial, Plackett–Burman (PBD) and Taguchi designs (TD). As mentioned, they consider two levels for each factor \((k)\). The factorial fractional design is the most used and allows the evaluation of a large number of factors in a small number of experiments by fractioning a full factorial \(2^k\) design in a \(2^{k-p}\) design, being \(p\) the number chosen to fractionate the design. As an example, if eight factors should be studied, a fractional factorial design with \(2^{8-3} = 32\) experiments is a better option against the 256 experiments required for a \(2^8\) full factorial design. On the other hand, PBD and TD are highly fractioned designs that permit studying a large number of factors through a reduced number of experiments, but with the drawback that the main effects are confounded.
with double interactions, and, consequently, their application should be extremely careful [35].

3.3. Optimization designs

Several designs are available to model a second order response surface. Generally, the election of the design is related to different goals such as performing a reduced number of experiments, excluding zones from the experimental region, reaching high quality adjusted parameters, etc. The designs mostly reported in the literature are full factorial, central composite (CCD), Box-Behnken (BBD) and mixture simplex centroid designs, which will be briefly discussed below.

The full factorial design is an extension of the full factorial $2^k$ presented for screening designs. In this case, the number of experiments is equal to $3^k$. In the case of three factors ($k=3$), 27 experiments are required, although replication of the central points is recommended to evaluate a lack of fit. Figure 3-A shows a $3^2$ full factorial design with 9 experiments.

The central composite design is among the most popular options, built with two-level (–1 and +1) factorial design points, axial or star points and center points (with all factors set to 0). All factors in the star points are set to 0, except for one factor with the value $\pm \alpha$; a number that determines the location and usually varies from 1 to $\sqrt{k}$. Figure 3-B shows the experimental points of a CCD with two factors $x_1$ and $x_2$. The number of experiments (equal to 9) results from the equation $(2^k+2k+\text{central points})$. For three factors ($k=3$) and one central point, at least fifteen experiments are required, plus several replicated points.

The number of experiments for a Box-Behnken design is computed as $[2(k–1)+\text{central points}]$. For three factors ($k=3$) and one central point design, at least 13 experiments are required. As can be appreciated, this design requires less (or equal) amount
of experiments than the two designs previously presented (see Fig. 3-C). Another fact is that this design can only be implemented for 3 or more factors.

The mixture simplex centroid design is built in those cases in which the analyzed factors are the components of a mixture, i.e. their levels are not independent of one another, allowing the study of the effect of the ratio variations among the factors. The domain is a regular figure having as many vertices as components, in a space with dimensionality equal to the number of components minus one [10]. Figure 3-D displays the graphical representation of a mixture design with three components. As can be seen, the design is an equilateral triangle whose vertices correspond to combinations containing 100 percent of a single component, each of the three sides represents a binary mixture and the internal points correspond to ternary mixtures. These designs are usually augmented with additional points in the interior of the experimental region. It should be noted that the models (known as Scheffé polynomials) used in mixture designs differ from the polynomials used in response surface for independent variables.

3.4. Modeling

3.4.1. Least squares fitting (LS)

3.4.1.1. Modeling of screening design data

The general approach to conduct screening designs to perform factor selection consists in analyzing signs and magnitudes of factors by building a preliminary model for the response, performing statistical tests, refining the model (removing any non-significant factor from the initial model), and analyzing residuals to check model adequacy and assumptions. The effect of a factor on a response is calculated as the difference between the average response of the experiments with positive signs and the average response of the experiments with negative signs (in a codified design). The coefficients of the estimated model are related
to the later value. The response \((y)\) in a screening experiment for two factors \((x_1 \text{ and } x_2)\) can be described by the following linear with interaction regression model:

\[
y = b_0 + b_1 x_1 + b_2 x_2 + b_{1-2} x_1 x_2 + e
\]  

(1)

where \(b_0\) is the overall mean effect, \(b_1\) and \(b_2\) represent the effects of the factors \(x_1\) and \(x_2\), \(b_{1-2}\) is the effect of the interaction between both factors, and \(e\) is a random error component, which represents other sources of variability not accounted for in the model.

Several graphical tools can be employed to assess the significance of the effects, in order to decide which of them should be included or excluded in the final model: a) normal and half-normal probability plots, in which it is considered that the negligible effects are normally distributed and tend to fall along a straight line on this plot, and b) the Pareto chart, where factor values are represented in descending order by bars, indicating if they are significant or not by a threshold established through a statistical test. However, the factors selected from the graph analysis should be examined by ANOVA, a statistical test used to assess the differences between group means and their associated procedures, such as variation among and between groups.

3.4.1.2. Data modeling of RSM designs by LS

For RSM modeling, a mathematical model can be built for each response fitting a second (or higher) order polynomial function, using the data collected during the optimization stage. The response \((y)\) in an RSM experiment for two factors \((x_1 \text{ and } x_2)\) can be described by the following regression model:

\[
y = b_0 + b_1 x_1 + b_2 x_2 + b_{1-2} x_1 x_2 + b_{1-1} x_1^2 + b_{2-2} x_2^2 + e
\]  

(2)
where \( b_0, b_1, b_2, b_{1,2} \) and \( e \) are similar to Eq. 1, and \( b_{1,1} \) and \( b_{2,2} \) correspond to the quadratic terms. The significance of the coefficients is investigated through ANOVA, as commented before for the screening step.

The fitted model must properly describe the data relationship to make accurate predictions inside the experimental region. For the optimization of two factors, the response can be represented as a solid surface in a three-dimensional space. For more than two factors, the graphical representation is made for two of them, maintaining the others at constant values. Another option is to use a contour plot representation, which consists of lines of constant response, corresponding to a specific height of the response surface.

As previously mentioned for the case of fitting linear models, an ANOVA test should be applied to determine the significance of the second order model. The latter can be considered satisfactory when the regression is significant, and a non-significant lack of fit is obtained for the selected confidence level (\( \alpha=0.05 \)). Nevertheless, a significant model does not necessarily mean that the variation in the data is correctly explained. Also, the analysis of the residual plots is mandatory, as well as both the coefficient of determination (\( R^2 \)) and the adjusted coefficient of determination (\( R_{adj}^2 \)) evaluations, which represent the percentage of variance explained by the model. The normal probability plot indicates that the residuals follow a normal distribution, one of the basic assumptions for the validity of ANOVA. The homogeneity of the variance (another ANOVA assumption) can be evaluated by the plot of residuals versus the ascending predicted response values.

In those cases in which lack of normality or heterocedasticity in residuals is detected, the use of non-parametric methods, as artificial neural networks (ANN), or the analysis of a transformed response is suggested [35].
3.4.1.3. Data modeling of RSM designs by artificial neural networks (ANNs)

ANNs allow modeling non-linear relationships among factors and responses, mimicking the human brain. The well-known multilayer feed-forward networks [36] or multilayer perceptron (MLP) networks are commonly used for prediction, classification and optimization purposes. In these cases, the typical architecture consists of three layers of neurons or nodes (the basic computing units). The input layer has a number of active neurons equal to the number of factors being investigated. The hidden layer has a variable number of active neurons that should be optimized. The output layer has a unit for each response. These neurons are linked hierarchically: the outputs of one layer of neurons are used as inputs for the next layer and so on. The hidden layer commonly uses a sigmoid function in its nodes. On the other hand, linear functions are generally used both in the input and output layers. The number of hidden layers and the neurons in each of them must be optimized to achieve the satisfactory fitting ability of the network, associated with a satisfactory predictive ability.

It is important to stress that the main difference between modeling by LS regression or ANNs is that for the latter there is no need to know the exact form of the function on which the model should be built. Additionally, neither the functional type nor the number of model parameters needs to be given.

Another popular ANN is based on the use of radial basis functions (RBF) [37]. These networks have a single hidden layer of neurons incorporating Gaussian transfer functions, and a linearly activated output layer. In comparison with MLP networks, RBF offers some advantages such as robustness towards noisy data, as well as a faster training phase.

3.5. Multiple response optimization by means of the desirability function

Frequently, the optimization procedure involves several responses, i.e. the attainment of acceptable conditions for many analytes and good performance in terms of time or solvent
consumption [38]. In addition, the optimization criteria are often contradictory to each other. Thus, the overall solution must be included in an optimal region, leading to a compromise solution. Among the several approaches that have been presented for solving multiple response optimizations [39], the desirability function [40], which is the most popular tool, will be discussed.

After being presented by Derringer and Suich in 1980, the desirability function has been used extensively in several applications in a wide variety of disciplines [40]. It is based on the idea that the quality of a product (or process) having several outputs is unacceptable if one of them lies outside of a desirable limit. Under the later consideration, the function searches operating conditions to ensure agreement with the criteria of all the responses and, at the same time, to provide the best compromise value for all of them.

The first step is to compute an individual desirability function $d_i(\hat{y}_i)$ for each response $\hat{y}_i(x)$ using previously fitted models (by LS or ANN) [35]. The optimization criteria can be maximization, minimization, reaching a target value or a range of values. For that, different functions should be built, depending on the criteria adopted. The desirability figures vary within an acceptable range of response values established by $[U_i (\text{upper acceptable value})−L_i (\text{lower acceptable value})]$. For maximization purposes, which is the case in which the recovery of a substance is studied, $d_i(\hat{y}_i)$ is described by the following equation:

$$
\begin{align*}
  d_i(\hat{y}_i(x)) &= \begin{cases} 
    0 & \text{if } \hat{y}_i(x) < L_i \\
    \left( \frac{\hat{y}_i(x)−L_i}{U_i−L_i} \right)^s & \text{if } L_i \leq \hat{y}_i(x) \leq U_i \\
    1 & \text{if } \hat{y}_i(x) > U_i 
  \end{cases} 
\end{align*}
$$

(3)

where $s$ is a weight establishing the importance of the closeness of $\hat{y}_i$ to the maximum.

According to Eq. (3), the individual desirability takes values between 0 and 1. If
\( d_i(\hat{y}_i) = 0 \), the response is undesirable. On the contrary, if \( d_i(\hat{y}_i) = 1 \), the response is completely desirable (ideal value). Interestingly, intermediate values of \( d_i(\hat{y}_i) \) indicate more or less desirable responses and can be accepted. It is important to remark that factor levels might also be included in the optimization procedure; for example, the use of a lower amount of certain solvent could be allowed.

Finally, once desirability functions are computed for each response (and factors if it is required), they are combined in a global desirability function \((D)\), which allows to find out the best combined responses, through the following equation:

\[
D = (d_1^n \times d_2^n \times \ldots \times d_n^n)^{1/\sum_i r_i} = \left( \prod_{i=1}^{n} d_i^n \right)^{1/\sum_i r_i} \tag{4}
\]

where \( r_i \) is the importance of each response (or factor) relative to the others, i.e. it allows to indicate if one or more responses should be more carefully taken into account than others.

The analysis of Eq. 4, which consists in a cumulative product, evidences that if \( D \) reaches a value different from zero, all the responses can be considered to have a desirable value. Whereas, if \( d_i(\hat{y}_i) = 0 \), \( D \) will be zero, thus, at least one of the responses is completely undesirable. It is important to consider that the goal of an optimization procedure is to find a good set of conditions to fulfill all the requirements, but not to get a \( D \) value equal to 1.

Let us consider the following example: a LPME procedure is being implemented for five analytes (A, B, C, D and E) whose experimental recoveries (%) obtained once the experiments (suggested by an experimental design) were carried out, vary inside the following ranges: (A), 50-90; (B), 30-60; (C), 80-100; (D), 50-80; and (E), 60-90. The goal is to obtain the best compromise values for the five analytes, but focalizing in analyte B, because it is expected maximize it recovery without affecting the others. Thus, the following
lower and upper values for the five analytes can be defined: (A), 80-90; (B), 55-60; (C), 90-100; (D), 70-80; and (E), 80-90. The question is: is it possible to obtain a combination of the factors that satisfies these conditions? The answer would be obtained after computing the individual desirabilities by applying Eq. 3, in the whole experimental space, using the models fitted for every response (the percent recovery for each analyte). The final step is to calculate the global desirability using Eq. 4. If the results show any region in which $D$ is different from zero, any combination of the factors in that region satisfies the objective. Real examples showing the application of these concepts will be presented and discussed in Section 5.

3.6. Software


Very recently, an interesting graphical interface for the application of ANN in the domain of experimental design and optimization has been presented. It is a graphical user interface for multiple surface response optimization based on radial basis functions, a kind of ANN, which has been shown to furnish good results in optimization issues [41]. The codes, manual and examples are freely available at http://www.iquir-conicet.gov.ar/descargas/opt_rbf.rar.
4. Literature search

The works reported from 2009 to 2019 based on the implementation of microextraction supported by DOE and optimization are summarized in Table 2 (A and B). This table displays information about the different procedures and options carried out for microextraction, as well as the application of screening steps and optimization of factors for the determination of an analyte or a group of analytes in complex samples. Figure 4 shows the distribution of the microextraction techniques reported in the articles reviewed in this study. As can be appreciated, DLLME was the mostly applied procedure (72%), while SDME and LLME were almost implemented with the same frequency (13 and 15%, respectively).

5. Illustrative literature example

During the development of a microextraction process, several steps should be optimized preferentially using chemometric tools. In this section, an example will be described to illustrate the steps, the variables and the type of design that can be used during the development of a new analytical method requiring a preconcentration stage. It consists in the development of a microextraction method for the determination of six veterinary drugs in eggs by high performance liquid chromatography carried out in our laboratory [42].

Firstly, the extraction process should be defined. In this work, two pretreatment procedures were studied: (A) air assisted-dispersive liquid-liquid microextraction based on solidification of organic drop (AA-SFO-DLLME), and (B) dispersive liquid-liquid microextraction (DLLME).

Secondly, the factors and responses to be analyzed must be established. In this case, for the AA-SFO-DLLME procedure, the critical step was the obtainment of the analyte in solution, thus, the following factors were analyzed: volume of acetonitrile (A), methanol (B), isopropanol (C), acetone (D), water (E), and amount of salt (F). In order to figure out the
significance of the studied factors, and due to their high number, a fractional factorial ($2^{6-2}$) design was constructed for screening (see Section 3.2). The evaluated responses were: area of chloramphenicol (CAP, R1), area of nicarbazin (DNC, R2), area of albendazole (ABZ, R3), and purity of CAP (R4) (see details in Ref. 23). Table 3 shows the combination of factors and the responses obtained after carrying out the experiments.

The following step includes the analysis of the significance of each factor and their influence (negative or positive). This can be done using the Pareto chart, which allows defining the significance by graphical inspection, and by application of the well-known statistical Bonferroni limit test. Figure 5 shows the Pareto chart analyzed in this work corresponding to the investigation of the factors affecting response number 3 (see above). As can be seen, the factor F (amount of ZnSO$_4$) overcomes the boundary and can be labeled as a highly significant factor in the system for R3. Moreover, the factor E (volume of water) and the interaction AD (volumes of acetonitrile and acetone) have influence because they have an effect value located between the $t$ and the Bonferroni limit values, and should be considered in the optimization step.

The analysis described above was repeated for each of the studied responses. Then, with the gathered information, the factors that influence the system could be defined, i.e. volumes of acetonitrile, methanol, acetone and water, and amount of salt.

The following step involves the optimization, which includes building a design considering the selected factors and using the desirability function to obtain the combination that satisfies the requirements for the four responses simultaneously. In this work, a quarter-fractional central composite design with 24 experiments and five central points was constructed to analyze the five factors defined in the previous study: volume of acetonitrile (A), methanol (B), acetone (C), water (D), and amount of ZnSO$_4$ (E). Table 4 summarizes the combination of the factors and the responses obtained after carrying out the experiments. In
this case, three responses (areas of CAP, ABZ and DNC) were analyzed, and the best fit of the model was quadratic, linear and linear with interaction for the CAP, ABZ and DNC areas, respectively. The criterion followed to simultaneously optimize the three responses was maximization, giving more importance to the one with the smallest recovery (ABZ). Finally, using the desirability function, the combination of the factors that allows the best extraction conditions was defined.

During the optimization step, the response surface plots become an important tool to analyze the results. Figure 6 shows the response surface plot for the global desirability function corresponding to the AA-DLLME-SFO procedure as a function of two factors: volume of acetonitrile (A) and water (D), while maintaining the other three factors at their optimum values. Under the optimization criterion, the experimental conditions corresponding to a maximum in the desirability function ($D = 1.00$) were: 1140 µL of water, 125 mg of ZnSO$_4$, 1175 µL of ACN, 1200 µL MeOH and 740 µL of ACE, using 1.00 g of homogenized egg and 50 µL of 1-dodecanol as extractive solvent. With this combination of factors, the prediction areas were: 5064 for CAP, 828 for ABZ and 4344 for DNC.

On the other hand, in the second studied procedure (DLLME), which includes a step of evaporation and re-suspension, the use of a mixture design allowed obtaining the best combination of solvents-aqueous solution for the extraction. In this work, a lattice mixture design was built to study the best proportion of the following factors: volume of methanol (A), acetonitrile (B) and buffer phosphate 10 mmol L$^{-1}$ pH $= 3.50$ (C). Nine responses were simultaneously optimized (area of trimethoprim, oxitetracycline, enrofloxacin, CAP, ABZ and DNC, and peak width of oxitetracycline, CAP and trimethoprim), being the criterion for the optimization their individual maximization.

Table 5 shows the combination of the solvents and the responses obtained after the execution of the experiments. As in the case of the previous optimization, the use of the
response surface plot can help establish the system. Figure 7 shows the contour plot of the response surface for the global desirability function corresponding to the lattice mixture design for the optimization of the DLLME procedure.

Under the optimization criterion, the mixture corresponding to a maximum in the desirability function \( D = 0.683 \) was ACN and sodium phosphate buffer 10 mmol L\(^{-1}\) pH = 3.50, whose proportion was 30 and 70 v/v, respectively. With this combination, the predicted areas were 570 for trimethoprim (TMP), 205 for oxitetracycline (OTC), 1299 for enrofloxacin (ENR), 562 for CAP, 449 for ABZ and 781 for DNC, and the predicted widths 0.300 for OTC, 0.128 for CAP and 0.278 for TMP.

As can be appreciated in Fig. 7, the blue parts correspond to \( D = 0 \), i.e. totally prohibited values because they do not satisfy the a priori established conditions (optimization criterion). Therefore, the experimenter should select any other combination, with a \( D \) value different from zero, located, in this case, in the green zone.

6. Conclusions

Liquid phase microextraction is a novel miniaturized sample pretreatment method to attain trace determination of target compounds in complex matrices. It can be considered as an environmentally-friendly, simple, easy to operate, and highly sensitive process for preconcentration, which has been introduced to simplify the extraction methods. Interestingly, the analysis of the reported applications shows that LPME positively impacts on selectivity, sample cleanup and efficiency, while allowing the extraction of a wide variety of analytes in complex samples.

A literature search reveals that numerous applications of LPME assisted by RSM for optimization have been reported during the last ten years. These reports evidenced that the coupling of the sample pretreatment with statistical tools allowed researchers to improve the
overall process by doing a reduced number of experiments while obtaining more reliable results.

Consequently, it could be expected that an increase in the use of the chemometric tools herein presented would become beneficial for the development of analytical procedures for the determination of analytes in complex samples.

In the present review, the LPME procedure and its different types of implementation reported during the years 2009 to 2019 were presented. The main focus was the application for the extraction of analytes in complex samples, considering those cases in which the performance of the technique was optimized by RSM.

Acknowledgements

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Author Contribution Statement

Maira Carabajal: Resources, Methodology, Investigation
Carla Teglia: Resources, Methodology, Investigation
Soledad Cerutti: Resources, Methodology, Investigation
María J. Culzoni: Methodology, Writing - Review and Editing
Héctor Goicoechea: Project administration, Visualization
**Figure Captions**

**Figure 1.** Schematic representation of the single-drop microextraction (SDME) variants: (A) headspace (HS-SDME), (B) directly-immersion (DI-SDME), (C) with low-density solvent and (D) with high-density solvent.

**Figure 2.** Schematic representation of dispersive liquid-liquid microextraction (DLLME) process. Orange: dispersant and blue: extractant.
Figure 3. Schematic representation of: (A) a two factors full factorial design with \( (2^3 = 9) \) points, (B) a two factors central composite design with \( (2^2 + 2 \times 2 + 1 = 9) \) points, (C) a three factors Box-Behnken design with \( (2 \times 3 (3-1) + 1 = 13) \) points, and (D) a three factors mixture simplex centroid design with 7 points.
Figure 4. Percent distribution of microextraction applications of the articles cited in Table 2.
Figure 5. Pareto chart for analysis of factor effects when studying the response 3 by a fractional factorial $2^{6-2}$ design. For the model to be hierarchical, it is necessary to consider both factors A and D, since their interaction is significant [23].
Figure 6. Response surface plot for the global desirability function corresponding to the AA-DLLME-SFO procedure as a function of two factors: volume of acetonitrile (A) and water (D). The other three factors are maintained at their optimum values.
Figure 7. Contour plot of the response surface for the global desirability function corresponding to the *lattice* mixture design for the optimization of the DLLME procedure. (A) methanol, (B) buffer phosphate 10 mmol L\(^{-1}\) pH = 3.50, and (C) acetonitrile.
Table 1: Description of the different procedures and options, improvements and automation for microextraction. References correspond to the first time each procedure was presented.

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Option</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liquid-liquid microextraction (LLME)</td>
<td></td>
<td>[43]</td>
</tr>
<tr>
<td></td>
<td>Headspace (HS-SDME)</td>
<td>[44]</td>
</tr>
<tr>
<td></td>
<td>Directly-immersion (DI-SDME)</td>
<td>[45]</td>
</tr>
<tr>
<td>Single-drop microextraction (SDME)</td>
<td>Drop to drop (DDME)</td>
<td>[13]</td>
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<td></td>
<td>Continuous-flow (CFME)</td>
<td>[32]</td>
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<tr>
<td></td>
<td>Solidified floating organic (SFO-DLME)</td>
<td>[46]</td>
</tr>
<tr>
<td></td>
<td>Liquid-liquid-liquid microextraction (LLLME)</td>
<td>[47]</td>
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<tr>
<td></td>
<td>Low-density solvent (LD-DLLME)</td>
<td>[48]</td>
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<tr>
<td>Dispersive liquid-liquid microextraction (DLLME)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>High-density solvent (HD-DLLME)</td>
<td>[15]</td>
</tr>
<tr>
<td></td>
<td>Solidified floating organic solvent (SFO-DLLME)</td>
<td>[46]</td>
</tr>
<tr>
<td></td>
<td>Auxiliary solvent to adjust the density (AS-DLLME)</td>
<td>[49]</td>
</tr>
<tr>
<td>Hollow-fiber protected microextraction (HFME)</td>
<td></td>
<td>[47]</td>
</tr>
<tr>
<td>Improvement</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>UV-assisted (UV)</td>
<td>[50]</td>
</tr>
<tr>
<td></td>
<td>Vortex-assisted (VA)</td>
<td>[51]</td>
</tr>
<tr>
<td></td>
<td>Air-assisted (AA)</td>
<td>[52]</td>
</tr>
</tbody>
</table>
Ultrasound-assisted (UA) [30]
Special extraction devices [29, 53]
Magnetic stirring-assisted [54]
Microwave-assisted [55, 56]
Use of ionic liquids (IL) [19]
Use of deep eutectic solvents (DES) [57]

<table>
<thead>
<tr>
<th>Automation</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Continuous-flow (CF)</td>
<td>[32]</td>
</tr>
<tr>
<td>Automated drop-based system</td>
<td>[31]</td>
</tr>
<tr>
<td>Sequential injection (SI-DLLME)</td>
<td>[58]</td>
</tr>
<tr>
<td>Automated dynamic in-syringe</td>
<td>[59]</td>
</tr>
</tbody>
</table>
Table 2A: Literature search (2009-2019) of the use of RSM for the optimization of microextraction procedures of inorganic analytes for complex matrices.

<table>
<thead>
<tr>
<th>Year</th>
<th>Microextraction</th>
<th>Screening step – variables</th>
<th>Optimization step – variables</th>
<th>Analytes</th>
<th>Instrumental analysis</th>
<th>Sample</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>2012</td>
<td>ISF-LLME</td>
<td>Full factorial design – reagent concentration, amount of IL, amount of ion-pairing agent and salt concentration.</td>
<td>CCD – reagent concentration and amount of IL.</td>
<td>Ni(II)</td>
<td>FAAS</td>
<td>Lettuce</td>
<td>[60]</td>
</tr>
<tr>
<td></td>
<td>TIL-DLLME</td>
<td>PBD – IL volume, concentration of complexing agent, pH, incubation time and temperature.</td>
<td>CCD – IL amount, pH and temperature.</td>
<td>Pb(II)</td>
<td>FAAS</td>
<td>Blood</td>
<td>[61]</td>
</tr>
<tr>
<td>2015</td>
<td>UA-IL-DLLME</td>
<td></td>
<td>Full factorial design – pH, volume of IL, CCl₄ volume and sonication time.</td>
<td>Cu(II), Ni(II) and Pb(II)</td>
<td>FAAS</td>
<td>Vegetable and fruit</td>
<td>[62]</td>
</tr>
<tr>
<td></td>
<td>MIL-DLLME</td>
<td>DPB – NaClO₃ concentration, acetonitrile volume, agitation time, MIL volume and sample volume.</td>
<td></td>
<td>As (III)</td>
<td>ETAAS</td>
<td>Honey</td>
<td>[63]</td>
</tr>
<tr>
<td></td>
<td>μS-SHS-LLME</td>
<td>DPB – volume of SHS, pH, volume of Na₂CO₃, and volume of H₂SO₄.</td>
<td>CCD – pH, volume of the SHS and volume of Na₂CO₃.</td>
<td>Vanadium</td>
<td>ETAAS electrothermal atomic absorption spectroscopy</td>
<td>Tomato, spinach, potato and drinks</td>
<td>[64]</td>
</tr>
</tbody>
</table>
Table 2B: Literature search (2009-2019) of the use of RSM for the optimization of microextraction procedures of organic analytes for complex matrices.

<table>
<thead>
<tr>
<th>Year</th>
<th>Microextraction</th>
<th>Screening step – variables</th>
<th>Optimization step – variables</th>
<th>Analytes</th>
<th>Instrumental analysis</th>
<th>Sample</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>2009</td>
<td>IL-DLLME</td>
<td>-</td>
<td>CCD – sample pH, NaCl percentage, IL amount and volume of disperser solvent.</td>
<td>Eight pesticides</td>
<td>HPLC-DAD</td>
<td>Banana</td>
<td>[65]</td>
</tr>
<tr>
<td></td>
<td>DLLME</td>
<td>-</td>
<td>CCD – extraction temperature, sample weight, acetonitrile volume, extraction time, and CCl volume.</td>
<td>Two antioxidants, (Irganox 1010 and Irgafos 168)</td>
<td>LC-DAD</td>
<td>Polymer</td>
<td>[66]</td>
</tr>
<tr>
<td></td>
<td>HD-HS-SDME</td>
<td>-</td>
<td>CCD – drop volume, extraction time, plant sample weight and cooling time after hydrodistillation.</td>
<td>Thymol and carvacrol</td>
<td>GC-FID</td>
<td>Oleum thymi essential oil</td>
<td>[67]</td>
</tr>
<tr>
<td>2010</td>
<td>UAE-DLLME</td>
<td>Fractional factorial design – sample volume, extracting agent volume, sample pH, ionic strength, cavitation time and centrifugation time.</td>
<td>CCD – extracting agent volume, sample pH, ionic strength, cavitation time and centrifugation time.</td>
<td>Seven sulfur compounds</td>
<td>GC-MS</td>
<td>White wine</td>
<td>[68]</td>
</tr>
<tr>
<td></td>
<td>UAE-SFO-SDME</td>
<td>Full factorial design – extraction solvent volume, salt effect, extraction time and centrifugation time.</td>
<td>BBD – extraction solvent volume, salt effect and extraction time.</td>
<td>Six phthalate esters</td>
<td>HPLC-DAD</td>
<td>Shampoo, after shave gel and hair spray samples</td>
<td>[69]</td>
</tr>
<tr>
<td></td>
<td>DLLME</td>
<td>-</td>
<td>CCD – volume of extraction solvent, NaCl percentage and water volume.</td>
<td>Aflatoxins B1, B2, G1 and G2</td>
<td>HPLC-FLD</td>
<td>Cereal products (maize, rice and wheat)</td>
<td>[8]</td>
</tr>
<tr>
<td>2011</td>
<td>DLLME</td>
<td>-</td>
<td>CCD – volume of dispersive solvent, extracting solvent, sample solution volume and pH.</td>
<td>Three organophosphorus pesticides</td>
<td>HPLC-UV</td>
<td>Water, fruit juice and fruits</td>
<td>[70]</td>
</tr>
<tr>
<td></td>
<td>UA-DLLME</td>
<td>PBD – sample volume, solvent volume, extraction temperature, extraction time, centrifugation speed and time.</td>
<td>CCD – sample volume and solvent volume.</td>
<td>Geosmin and 2 – methylisoborneol</td>
<td>GC-MS</td>
<td>Water and wine (red, rose and white)</td>
<td>[71]</td>
</tr>
<tr>
<td></td>
<td>RP-DLLME</td>
<td>-</td>
<td>CCD – disperser volume, extraction solvent volume, pH of the aqueous</td>
<td>Hydroxytyrosol and tyrosol</td>
<td>HPLC-UV</td>
<td>Virgin olive oil</td>
<td>[72]</td>
</tr>
<tr>
<td>Method</td>
<td>Parameters</td>
<td>Analytes</td>
<td>Techniques</td>
<td>Samples</td>
<td>References</td>
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<td></td>
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</tr>
<tr>
<td>IL-DLLME</td>
<td>phase and centrifugation time. CDD – sample pH, IL amount, volume of dispersion solvent and NaCl percentage.</td>
<td>Eight pesticides</td>
<td>HPLC-FLD</td>
<td>Soil extracts</td>
<td>[73]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SEV-DLLME</td>
<td>CDD – Silylation: NaOH concentration, HCl concentration, silylation agent solutions, NaOH, HCl and silylation agent contact times. CDD – Microextraction: extraction time, CHCl$_3$, volume, methanol volume, centrifuge rate and time, and salting-out effect.</td>
<td>Six pesticides</td>
<td>GC-FID</td>
<td>Wastewater, well water, and fruit juice (apple and grape)</td>
<td>[74]</td>
<td></td>
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<tr>
<td>DLLME</td>
<td>Full Factorial design – dispersive solvent and extraction solvent. CDD – dispersive volume, extraction volume, pH, and NaCl concentration.</td>
<td>Sorbic and benzoic acids</td>
<td>GC-FID</td>
<td>Beverages (carbonated soft drinks)</td>
<td>[75]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2012 DLLME</td>
<td>Full factorial design – volume of extracting solvent, disperser solvent, amount of salt and pH. CDD – volume of extracting solvent and amount of salt.</td>
<td>Five organochlorine pesticides</td>
<td>GC-MS</td>
<td>Honey</td>
<td>[76]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HS-SDME</td>
<td>PBD – water volume used for honey dilution, NaCl content (w/v) in the donor solution, volume of the donor solution, and stirring rate. CDD – volume of the donor solution and extraction temperature.</td>
<td>Six pesticide contaminants</td>
<td>GC-ECD</td>
<td>Honey</td>
<td>[77]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DLLME</td>
<td>CDD – extraction solvent dichloromethane and dispersive solvent acetonitrile volumes.</td>
<td>Seven neonicotinoid insecticides</td>
<td>LC-MS/MS</td>
<td>Honey</td>
<td>[78]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2013 DLLME</td>
<td>CDD – extractor volume, disperser volume, ionic strength, and pH. Chlordiazepoxide</td>
<td>HPLC-UV</td>
<td>Water, urine, plasma, and chlordiazepoxide tablet</td>
<td>[79]</td>
<td></td>
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<tr>
<td>Method</td>
<td>Variables</td>
<td>Analytes</td>
<td>Instruments</td>
<td>Applications</td>
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</tr>
<tr>
<td>DLLME</td>
<td>TD – extractant organic volume, disperser volume, aqueous phase volume, aqueous phase pH, NaCl concentration and centrifugation time.</td>
<td>Vitamins D_{2}, D_{3}, K_{1}, K_{2}, and K_{3}</td>
<td>LC-DAD, LC-APCI-MS</td>
<td>Spinach, cos lettuce, iceberg lettuce, lamb's lettuce and infant foods</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DLLME</td>
<td>CCD - disperser solvent volume, extraction solvent volume, salt amount and sample pH.</td>
<td>Benzaldehyde and sorbic acid salts</td>
<td>HPLC-UV</td>
<td>Yogurt</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EAE-IL-DLLME</td>
<td>CCD - pH, volume of extraction solvent, volume of disperser solvent, and ionic strength.</td>
<td>Pantothenic acid</td>
<td>HPLC-UV</td>
<td>Apple juice</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>UA-DLLME</td>
<td>CCD – temperature, sonication time, volume of preconcentration solvent and salt concentration.</td>
<td>Volatile components</td>
<td>GC-MS</td>
<td>Tea plants</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HS-SDME</td>
<td>CCD – weight salt, extraction time, extraction temperature and stirring rate.</td>
<td>Six furanic compounds</td>
<td>GC-MS</td>
<td>Coffee</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>ILAM-HS-SDME</td>
<td>BBD – mass ratio of ILs, sample mass, extraction temperature and extraction time.</td>
<td>Monoterpane hydrocarbons and oxygenated monoterpenes from essential oil</td>
<td>GC-MS</td>
<td>Forsythia suspense</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RP-DLLME</td>
<td>CCD – volume and ratio of disperser and extracting solvents</td>
<td>Eighteen phenolic compounds</td>
<td>LC-DAD-MS</td>
<td>Virgin olive oil</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PLE-DLLME</td>
<td>TD – CCl_{4} volume, aqueous phase volume, acetonitrile volume, NaCl concentration and centrifugation time.</td>
<td>Tocopherols, tocotrienols and tocopherol acetate</td>
<td>Capillary LC-DAD</td>
<td>Cosmetic products</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PLE-DLLME</td>
<td>TD – CCl_{4} volume, methanol volume, aqueous sample volume, sample pH, NaCl concentration and centrifugation time.</td>
<td>Tocopherols and tocotrienols</td>
<td>LC-APCI-MS</td>
<td>Spinach, corn, cranberry, pomegranate, mango juice</td>
<td></td>
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<td></td>
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<tr>
<td>UA-RM-DLLME</td>
<td>BBD – surfactant and modifier volume, sonication and centrifugation time.</td>
<td>Acetoin</td>
<td>HPLC-UV</td>
<td>Butter</td>
<td></td>
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</table>

2014

2015
<table>
<thead>
<tr>
<th>Method</th>
<th>Design</th>
<th>Analysis</th>
<th>Samples</th>
<th>References</th>
</tr>
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<tbody>
<tr>
<td>DMAE-SDME</td>
<td>–</td>
<td>BBD – microwave power, extraction time and extraction solvent flow rate.</td>
<td>Seven organophosphorus pesticides</td>
<td>Tea samples</td>
</tr>
<tr>
<td>MSA-SI-LLME</td>
<td>–</td>
<td>CCD – stirring time, pH, extraction solvent volume and centrifugation time.</td>
<td>Five fluoroquinolones</td>
<td>HPLC-FLD Milk, eggs and honey</td>
</tr>
<tr>
<td>UA-SI-LLME</td>
<td>–</td>
<td>CCD – solvent volume, pH, extraction time and weight of salt.</td>
<td>Five fluoroquinolones</td>
<td>HPLC-FLD Fish, chicken, pork and beef</td>
</tr>
<tr>
<td>DLLME</td>
<td>–</td>
<td>CCD – extraction solvent, disperser solvent, pH of sample solution, centrifugation time and ionic strength.</td>
<td>UV Spectrophotometry</td>
<td>Cinnamon syrup and Cinnamon tea</td>
</tr>
<tr>
<td>MA-DLLME</td>
<td>–</td>
<td>CCD – volume of extraction and extraction solvent, salt amount and ethanol ratio.</td>
<td>Sixteen PAHs</td>
<td>GC-MS Grilled meat</td>
</tr>
<tr>
<td>MSA-DLLME</td>
<td>Fractional factorial design – extraction solvent volume, disperser solvent volume, pH of sample, salt addition, temperature, stirring rate and time of extraction.</td>
<td>CCD – extraction solvent volume, pH of sample, temperature and stirring rate.</td>
<td>Rhodamine B and rhodamine 6G</td>
<td>HPLC-Vis Water samples, soft drinks and cosmetic products</td>
</tr>
<tr>
<td>UA-SFO-DLLME</td>
<td>Fractional factorial design – extraction time, extraction temperature, volume of dispersant and salt addition.</td>
<td>CCD – IL amount, volume of dispersant solvent, pH, and KCl concentration.</td>
<td>Benzimidazole and nifurtimox</td>
<td>HPLC-UV Human breast milk</td>
</tr>
<tr>
<td>IL-DLLME</td>
<td>–</td>
<td>CCD – extractant volume, dispersant volume, and extraction time.</td>
<td>Six triazole fungicides</td>
<td>HPLC-PDA Pear, apple, and grapefruit</td>
</tr>
<tr>
<td>QuEChERS-IL-DLLME</td>
<td>–</td>
<td>CCD – ionic strength, pH and sample volume.</td>
<td>Triflumuron, hexaflumuron, lufenuron and chlorfluazuron</td>
<td>HPLC-UV Honey</td>
</tr>
</tbody>
</table>

2016
<table>
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<tr>
<th>Method</th>
<th>Design Type</th>
<th>Parameters</th>
<th>Detection Method</th>
<th>Sample Types</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>DLLME</td>
<td></td>
<td>derivatization temperature, derivatization time and the molar ratio of BCEC-Cl to the EDCs. DLLME: extraction solvent volume, disperser solvent volume and ionic strength.</td>
<td>HPLC-FLD</td>
<td>Fish, chicken and pond water</td>
<td></td>
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<tr>
<td>DLLME</td>
<td></td>
<td>Full Factorial design – type of extraction solvent, type of disperser solvent and protein precipitation.</td>
<td>CE</td>
<td>Porcine blood</td>
<td>[7]</td>
</tr>
<tr>
<td>DLLME</td>
<td>Reduced factorial design – extracting solvent volume and dispersing solvent volume.</td>
<td>CCD – dispersing solvent volume and extracting solvent volume.</td>
<td>HPLC-DAD</td>
<td>Serum</td>
<td>[101]</td>
</tr>
<tr>
<td>AA-LLME</td>
<td></td>
<td>CCD – pH value for the donor phase, volume of the organic solvent, pH value for the acceptor phase, and volume of the acceptor phase.</td>
<td>HPLC-UV</td>
<td>Human plasma and wastewater</td>
<td>[102]</td>
</tr>
<tr>
<td>UA-DE-LLME</td>
<td></td>
<td>BBD – volume of DES$_1$, the ultrasonic time and the temperature of ultrasonic bath.</td>
<td>HPLC-UV</td>
<td>Vegetable oils</td>
<td>[103]</td>
</tr>
<tr>
<td>TAA-SFO-LLME</td>
<td></td>
<td>CCD – pH value for the donor phase, volume of the organic solvent, pH value for the acceptor phase and volume of the acceptor phase.</td>
<td>HPLC-UV</td>
<td>Human plasma and wastewater</td>
<td>[104]</td>
</tr>
<tr>
<td>UA-DLLME</td>
<td></td>
<td>Fractional factorial design – the dispersion solvent volume, the extraction solvent volume, the pH and the UA stirring time.</td>
<td>UPLC-PDA</td>
<td>Human plasma</td>
<td>[105]</td>
</tr>
<tr>
<td>DL-SDME</td>
<td></td>
<td>Fractional factorial design (the factors were divided in different groups) – sample weight, extraction solution volume, sonication time,</td>
<td>GC-MS</td>
<td>Mango</td>
<td>[106]</td>
</tr>
</tbody>
</table>
extractant solvent, drop volume, stirring rate, ionic strength, time, pH and temperature of extraction.

**DLLME**
Fractional factorial design – ethylation time, addition of NaCl, volumes of methanol and tetrachloroethylene.  
CCD – volumes of the disperser and extraction solvents.  
Three organotin compounds  
GC-PFPD Marine sediment [107]

**IL-VA-LLME**
DPB – pH, ionic salt, extraction solvent volume, vortex time, vortex speed, centrifuge speed and centrifuge time.  
CCD – pH, vortex time, vortex speed and extraction solvent volume.  
 Bisphenol A and Bisphenol S  
 LC-MS/MS Thermal paper receipts  
 [108]

**AA-SFO-LLME**
–  
CCD – volume of the organic solvent used, pH value for the sample solution, amount of salt solution (% w/v), and number of air-agitation cycle.  
 Amantadine and imipramine  
 GC-FID Human plasma and wastewater  
 [109]

**AA-DLLME**
–  
BBD – volume of extractant, number of extraction, pH, and rate of centrifugation.  
 Deoxynivalenol  
 HPLC-DAD Rice  
 [9]

**2018**  
HFIP/Brij-35 SUPRAS-LLME
DPB – concentration of Brij-35, concentration of HFIP, pH, vortex time, centrifugation time, cooling time, standing time, ionic strength and sample volume.  
CCD – concentration of Brij-35 and concentration of HFIP.  
 Six parabens  
 HPLC-DAD Environmental waters, pharmaceuticals and personal care products (sunscreen and lotion)  
 [110]

**2019**  
IL-UA-LLME
DPB – extraction solvent volume, dispersive solvent volume, cooling time, ultrasonic time and centrifugation time.  
CCD – extraction solvent volume, dispersive solvent volume and cooling time.  
 Bisphenol A, bisphenol B and bisphenol AF  
 HPLC-FLD Milk and fruit juice  
 [111]

**DLLME**
–  
CCD – volumes of the disperser and extraction solvents.  
SLD – combinations of acetonitrile, methanol and sodium phosphate buffer.  
 Albuterol, chloramphenicol, trimethoprim, enrofloxacin, oxytetracycline and nicotine  
 HPLC-DAD HPLC-FSFD Egg  
 [42]

**UA-DLLME**
–  
BBD – amount of NaCl in honey  
 Chloramphenicol  
 UHPLC-MS/MS Honey  
 [112]
<table>
<thead>
<tr>
<th>Method</th>
<th>Parameters</th>
<th>Analytes</th>
<th>Solvent</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA-SFO-DLLME</td>
<td>Fractional factorial design – acetonitrile volume, methanol volume, isopropyl alcohol volume, propanone volume, water volume and ZnSO₄ amount</td>
<td>Albenzaflazole, chloramphenicol, trimethoprim, enrofloxacin, oxitetracycline and albendazole</td>
<td>DMSO, H₂O, MeOH, EtOH, AcN</td>
</tr>
<tr>
<td>CCD – acetonitrile volume, methanol volume, propanone volume, water volume and ZnSO₄ amount</td>
<td>HPLC-DAD, HPLC-FSD</td>
<td>Egg</td>
<td>[42]</td>
</tr>
</tbody>
</table>

**Abbreviations:**
- IL: ionic liquid
- DLLME: dispersive liquid-liquid microextraction
- HD-HS-SDME: hydrodistillation-headspace solvent microextraction
- UAE-SFO-DLME: ultrasound-assisted emulsification microextraction with solidification of floating droplet
- UA-DLLME: ultrasound-assisted dispersive liquid-liquid microextraction
- RP-DLLME: reversed-phase dispersive liquid-liquid microextraction
- SA-DLLME: surfactant-assisted dispersive liquid-liquid microextraction
- SEV-DLLME: silylated extraction vessel -dispersive liquid-liquid microextraction
- TIL-DLLME: temperature controlled ionic liquid- dispersive liquid-liquid microextraction
- UA-IL-DLLME: ionic liquid based ultrasound assisted dispersive liquid-liquid microextraction
- HS-SMSE: headspace single-drop microextraction
- ILA-LLME: ionic liquid based ultrasound assisted dispersive liquid-liquid microextraction
- EAE-LLME: enzyme-assisted extraction and ionic liquid- based dispersive liquid-liquid Microextraction
- ILAM-HS-SMSE: ionic liquids assisted microwave distillation coupled with headspace single drop microextraction
- ILPE-DLLME: pressurized liquid extraction and dispersive liquid – liquid microextraction
- UA-AM-DLLME: ultrasound-assisted reverse micelles dispersive liquid liquid microextraction
- MA-DLLME: microwave assisted – dispersive liquid-liquid microextraction
- DMAE-SMSE: dynamic microwave assisted extraction
- MSA-SI-LLME: magnetic – stirring salt-induced liquid-liquid microextraction
- UA-SI-LLME: ultrasound-assisted, salt-induced, liquid-liquid microextraction
- AA-DLLME: air assisted-liquid-liquid microextraction
- AA-DE-LLME: ultrasound assisted liquid-liquid microextraction method based on deep eutectic solvent
- TAA-SFO-LLME: tandem air agitated liquid-liquid microextraction based on solidification of floating droplets
- DI-SMSE: direct-immersion - single-drop microextraction
- IL-VA-DLLME: ionic liquid based vortex assisted liquid-liquid microextraction
- AA-SFO-LLME: air-agitated liquid-liquid microextraction with solidification of floating organic droplets
- AA-DLLME: air assisted-dispersive liquid-liquid microextraction
- µS-SHS-LLME: micropipette tip switchable hydrophilicity microextraction syringe system
- in-syringe DSIL-DLLME: in-syringe dispersive liquid-liquid microextraction based on the direct solidification of ionic liquids
- HFIP/Brj 35 SUPRAS-LLME: supramolecular solvent based on hexafluoropropanol-mediated Brj 35 for liquid microextraction
- ISF-LLME: in situ solvent formation microextraction
- IL: ionic liquid
- PBD: Plackett-Burman design
- CCD: central composite design
- BBD: Box-Behnken design
Table 3. Fractional factorial $2^{6-2}$ design used in the illustrative example.

<table>
<thead>
<tr>
<th>Std.</th>
<th>Run</th>
<th>A: ACN volume $c$</th>
<th>B: MeOH volume $c$</th>
<th>C: IPA volume $c$</th>
<th>D: ACE volume $c$</th>
<th>E: water volume $c$</th>
<th>F: ZnSO$_4$ $d$</th>
<th>Area 1</th>
<th>Area 2</th>
<th>Area 3</th>
<th>Purity of response 1</th>
</tr>
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<tbody>
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<td>500</td>
<td>500</td>
<td>500</td>
<td>500</td>
<td>100</td>
<td>13679.5</td>
<td>1572.9</td>
<td>4132.1</td>
<td>0.994</td>
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<td>0.731</td>
</tr>
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</table>

$^a$Std. refers to the standard order in the design.
$^b$Run refers to the experiment order.
$^c$ACN: acetonitrile, MeOH: methanol, IPA: isopropanol, ACE: acetone and water in μL.
$^d$ZnSO$_4$ in mg.
Table 4. Central composite design for AA-DLLME-SFO used in the illustrative example.

<table>
<thead>
<tr>
<th>Std</th>
<th>Run</th>
<th>Factors (k)</th>
<th>Responses</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>A: ACN volume</td>
<td>B: MeOH volume</td>
</tr>
<tr>
<td>1</td>
<td>24</td>
<td>1000</td>
<td>1000</td>
</tr>
<tr>
<td>2</td>
<td>6</td>
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<td>500</td>
</tr>
<tr>
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</tr>
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</tr>
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<tr>
<td>24</td>
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</tbody>
</table>

Std refers to the standard order in the design.
Run refers to the experiment order.
ACN: acetonitrile, MeOH: methanol, ACE: acetone and water in μL.
ZnSO₄ in mg.
Table 5. Lattice-mixture design for DLLME used in the illustrative example.

<table>
<thead>
<tr>
<th>Stda</th>
<th>Runb</th>
<th>Factors (k)</th>
<th>Responses</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>A: % MeOH</td>
<td>B: % Buffer</td>
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<td>14</td>
<td>0.500</td>
<td>0.500</td>
</tr>
</tbody>
</table>

*aStd refers to the standard order in the design.
*bRun refers to the experiment order.
*cBuffer phosphate 10 mmol L⁻¹ pH = 3.50
References


[84] M. Chaichi, A. Mohammadi, M. Hashemi, Optimization and application of headspace liquid-phase microextraction coupled with gas chromatography–mass spectrometry for determination of

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Declaration of interests

☒ 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.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: