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Definitive Screening Designs and latent variable modelling for the optimization of solid phase microextraction (SPME): Case study - Quantification of volatile fatty acids in wines

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ABSTRACT

In the present study, we apply the recently proposed Definitive Screening Designs (DSD) to optimize HS-SPME extraction in order to analyze volatile fatty acids (VFA) present in wine samples. This is the first attempt to apply this new class of designs to one of the most well-known and widely applied extraction techniques. The latent structure of the responses is also explored for defining the optimal extraction conditions. DSD is a new screening design with the potential to significantly reduce the number of experiments required to estimate the model parameters and to establish the optimum operation conditions. Therefore, there is an obvious interest in assessing the benefits of DSD in practice. In this work, this design framework is applied to the simultaneous optimization of seven extraction parameters (responses). Both qualitative and quantitative extraction parameters are considered, in order to test the flexibility of DSD designs: a two-level qualitative variable, the fiber coating, and six quantitative variables, namely the pre-incubation time, the extraction time and temperature, the headspace/sample volume, the effect of agitation during extraction and the influence of the ethanol content (sample dilution). Optimization of analytes' chromatographic responses was carried out both individually (response by response) and altogether, by modelling the responses in the latent variable space (i.e., explicitly considering their underlying correlation structure). In the end, a consensus analysis of all perspectives was considered in the definition of the overall optimal extraction conditions for the quantification of VFA in fortified wines. The solution found was to use a DVB/Car/PDMS fiber, 10 mL of samples in 20 mL vial, 40 min of extraction at 40 °C. The analysis also revealed that the factors incubation time, agitation and sample dilution do not play a significant role in explaining the variability of extraction parameters. Therefore, they were set to the most convenient levels. The methodology followed was thoroughly validated and the following figures of merit were obtained: good linearity ($R^2 > 0.999$, for all compounds), high sensitivity (LOD and LOQ are close or below the values found in literature), recoveries of approximately 100% and suitable precision (repeatability and reproducibility lower than 7.21% and 8.61%, respectively). Finally, the optimized methodology was tested in practice. Several wine samples were analyzed and the odor activity value calculated to facilitate the identification of their importance as odor active compounds in different aged fortified wines. This work demonstrates the benefits of using DSD and latent variable modelling for the optimization of analytical techniques, contributing to the implementation of rigorous, systematic and more efficient optimization protocols.

1. Introduction

Definitive Screening Designs have been attracting a considerable interest in several communities strongly involved in the active collection and analysis of experimental data. This new class of screening designs requires only one more experiment than twice the number of factors under analysis, and still allows to estimate the main effects without any aliasing with each other or with two-factor interactions (in fact, they were incidentally discovered while searching for designs with good aliasing properties through optimal DOE computational approaches). An

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Abbreviations

DOE	Design of experiments
DSD	Definitive Screening Designs
HS-SPME	Headspace solid-phase microextraction
KI	Kovats indexes
LOD	Limit of detection
LOQ	Limit of quantification
OAV	Odor activity value
OFAT	One-factor-at-a-time
PCA	Principal Component Analysis
VFA	Volatile fatty acids
WLS	Weighted least squares regression

interesting feature of DSDs is that, contrary to classical screening designs, pure quadratic effects can also be estimated, if the number of factors is greater than 6 and their strength is large enough to be detected [1]. This is granted by the existence of center points in every row of the design and by the particular combination of levels used in the design matrix. This makes DSD a three-level screening design, but whose levels are not set following the classical Response Surface Methods (the design may not be orthogonal in general, but for some number of factors it is indeed orthogonal for the main effects). The aforementioned low number of runs required, roughly twice as many as the number of quantitative factors under optimization [2–4], is of course of significant interest to practitioners. When compared with classical fractional factorial designs, DSD's have the advantage of estimating independently the main and the quadratic effects (without confounding), as well as of being unaliased with two-factors interactions. However, the literature still lacks information on the application of DSD to real world applications and the sensitivity of these designs to capture the relevant effects. Therefore, in this work we set the goal to assess the application of DSD to HS-SPME optimization, given the relevancy of this technique.

The present case study regards the quantification of Volatile Fatty Acids in wines through the application of HS-SPME. Seven extraction factors are considered in the optimization of the HS-SPME procedure for quantification of nine volatile fatty acids (VFA) in wines (whose chromatographic responses constitute the outputs of the analysis), namely: isobutyric acid, butyric acid, isovaleric acid, valeric acid, hexanoic acid, octanoic acid, nonanoic acid, decanoic acid and dodecanoic acid. So far, only Ref. [5] reports a procedure where HS-SPME coupled to gas chromatography with a mass spectrometry detector (GC–MS) is used for the quantification of volatile fatty acid in wines. The present study, considers the analysis of two additional parameters in the extraction procedure, namely the pre-incubation time and ratio of headspace/sample volume, besides a completely different optimization and data analysis procedure. The remaining variables analyzed were fiber, time and extraction temperature, the effect of agitation during extraction and the influence of the ethanol content (sample dilution). Given the existence of multiple responses (the chromatographic profiles of the nine VFAs), which are furthermore strongly correlated, our analysis included a latent variable modelling approach for describing the dominating correlation structure of the responses and to analyze the simultaneous impact of the design factors over the chromatographic responses of the analytes. The analysis conducted led to an optimal consensus solution, which was then validated and finally applied to new samples, on order to demonstrate its effectiveness.

The reminding parts of this article are organized as follows. Section 2 revises the application of Headspace Solid-Phase Microextraction (HS-SPME) for the analysis wine. Section 3 presents the relevant experimental information of the study, including a description of the samples, methods and instrumentation used. The fourth section is dedicated to a brief description of DSD and the latent variable modelling framework used for data analysis. The results obtained are presented and discussed in Section 5. The validation of the HS-SPME method and its application to new samples are reported in Section 6. The article is closed with a summary of the main conclusions about the methodological aspects (DSD and latent variable modelling for optimizing the extraction conditions) and the results obtained in the case study (Section 7).

2. Headspace Solid-Phase Microextraction (HS-SPME) for the analysis of volatile compounds in wine

Headspace solid-phase microextraction (HS-SPME) is a state of the art extraction methodology in the analysis of volatiles compounds in wines. Its application in this context was recently reviewed in Ref. [6], where it was highlighted the good sensitivity and accuracy of HS-SPME methodologies, together with the advantages of being solvent-free and requiring only limited manipulation of the samples. The additional possibility of full automation makes it one of the first choices in this experimental domain. In the same review paper the authors underline the critical importance of conducting an appropriate optimization of the HS-SPME process, an aspect that is often overlooked and remains to be properly addressed: most of the times the parameters are optimized through simple one-factor-at-a-time (OFAT) approaches, where one factor is optimized in turn, and fixed from then on. This OFAT procedure prevents the identification of any relevant interactions among factors, a limitation that cannot be underestimated in real world applications. Therefore, the final solutions found with OFAT procedures end up being sub-optimal, at best. The disadvantages of OFAT optimization are well-known and documented in the Design of Experiments (DOE) literature, and have been thoroughly discussed elsewhere [7–15], being now widely recognized its limitations and the fact that it can lead to suboptimal results concerning the efficiency of the methodology [16].

The reluctance to adopt and applying DOE methodologies in wine volatile analysis contrasts with other application fields of HS-SPME in analytical chemistry, in which this practice is already part of method development workflow [9,10,17–23]. In these cases, the number of parameters involved in the optimization phase is in general in the range of 3–5 [24–46], being the time and temperature of extraction the parameters most frequently considered, followed by the salting out effect, the ratio of sample/headspace volume and the stirring influence. Although fewer in number, there are also other works that include in the optimization phase other HS-SPME parameters with known influence in the extraction process, namely the fiber coating [47], the pre-incubation time and temperature [47–49], the desorption time and temperature [49–51] and the derivatization conditions in situations when this process is also involved [49,52].

The choice of the particular experimental design methodology to be used depends on the objectives of the study and the nature of variables. Generally, the DOE methodologies are classified as screening, process characterization, matching target, optimization and robustness designs, being screening and optimization the prevailing classes in practical applications. Screening is focused in the identification of the main factors involved in the phenomena, separating the “critical few” from the “trivial many”. Optimization, on the other hand, addresses the definition of the factor levels that optimize a given criterion. In the scope of HS-SPME tuning, the class of full factorial [29,36] and fractional factorial designs (with resolution IV and V) [25,33–35,39,42,44] are the most often used approaches. As to optimization, the response surface methodologies (RSM), namely the Central Composite design [26,30–32,37,38,52] and the Doehlert designs [27], together with the algorithmic Optimal designs [28,45,47,48,53], are the dominant techniques for establishing the optimal settings for each factor. Other studies also adopt both categories of designs in a sequential manner, first identifying the important factors and then looking for their optimum settings [24,40,41,43,46,49,50].