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Ethanol/Water Extraction Combined with Solid-Phase Extraction and Solid-Phase Microextraction Concentration for the Determination of Chlorophenols in Cork Stoppers

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The appearance of 2,4,6-trichloroanisole (TCA) in cork stoppers is of great concern because it can cause off-flavors in bottled wine. To prevent this sensorial defect, there should not be any traces of 2,4,6-trichlorophenol (TCP), 2,3,4,6-tetrachlorophenol (TeCP), or pentachlorophenol (PCP) in the finished corks, because they are the direct precursors of TCA. In the course of this study two methodologies based upon an extraction with ethanol/water mixtures to determine the chlorophenolic content in cork matrices were developed. The cork extract is preconcentrated using both solid-phase extraction and solid-phase microextraction methodologies. The latter was optimized by applying a full two-level factorial design. Finally, spiked ground corks at nanogram per gram levels of each chlorophenol were analyzed under optimal conditions and by applying both procedures. The obtained results demonstrate that chlorophenols can be detected in corks contaminated at the nanogram per gram level and, thus, these approaches can be successfully applied as quality control measures in the cork industry.

KEYWORDS: Chlorophenols; cork stoppers; solid-phase extraction; solid-phase microextraction

INTRODUCTION

Natural cork has been used extensively to seal wine due to its unique physical properties, including long-lasting flexibility, hydrophobicity, and gas impermeability (*1*). Although other methods for sealing wine, such as synthetic stoppers, have been introduced into the market, the cork stopper is regarded as the standard for quality wines. Unfortunately, leakages, oxidative spoilages, and especially the appearance of musty/moldy taints in bottled wines, also called cork taint, which are caused by defective corks, have raised doubt about the effectiveness of cork stoppers. Cork taint is associated with several chemical substances, such as geosmin, 2-methylisoborneol, guaiacol, 1-octen-3-one, 1-octen-3-ol, pyrazines, chloroanisoles, 2,4,6-tribromoanisole, and chlorophenols, but 2,4,6-trichloroanisole (TCA) is considered to be the main cause of the defect (*2*−*4*). Because TCA has an extremely low odor threshold and has been reported to be in at least 80% of spoiled wines (*5*), rigorous controls are required to avoid this compound's getting into wine. Why TCA is produced in cork stoppers still has not been clearly explained; nevertheless, it is believed that it can form either in the cork manufacturing process or in the wine industry, chlorophenols being its direct precursors (*6*). Chlorinated phenols have been widely used as fungicides, biocides, herbicides, wood preservatives, and washing products, which is why they are found in cork oak forest and cellar environments. In particular, pentachlorophenol-based products containing different percentages of less chlorinated compounds have been applied for many decades to the cork oak to control insect plagues (*7*). The microbial conversion of chlorophenols to chloroanisoles, as a detoxification process, is a significant source of cork taint. It has been reported that filamentous fungi in cork are able to convert 2,4,6-trichlorophenol (TCP) into TCA directly by O-methylation (*8*). Once TCA is formed, it can migrate into wine, causing unpleasant aromas that are disagreeable for the consumer (*9*). As TCP, 2,3,4,6-tetrachlorophenol (TeCP), and pentachlorophenol (PCP) are implicated in this defect, a fast and reliable methodology for their determination is essential for ensuring the quality of cork stoppers.

Cork is an extremely complex matrix with many components (*10*). This makes the determination of chlorophenols in cork samples difficult. Only a few methods have been reported, and most of them are based on solvent extraction of the target analytes using *n*-hexane (*11*) or dichloromethane (*12*) as solvents and then analysis of the resulting organic extracts by gas chromatography (GC). Continuous extraction methods in a Soxhlet have been also reported (*12*, *13*).

One of the drawbacks of this methodology is that huge quantities of interferences are coextracted along with the selected analytes. For this reason, using cleanup procedures before

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injection into a gas chromatograph becomes crucial. Moreover, it is necessary to apply a preconcentration step to reach the low detection limits needed. Both analytical requirements can be accomplished by applying suitable sample-handling techniques such as solid-phase extraction (SPE) and solid-phase microextraction (SPME). According to the current literature, C_{18}, polymeric, and functionalized sorbents have been widely employed for trapping phenolic compounds, especially for water analysis (*14*). Soleas et al. (*15*), in their studies on wine and cork, applied a C_{18} sorbent after extracting TCA and TCP from the cork sample with an ethanol/water mixture.

Alternatively, SPME, which is a simple, inexpensive, solvent-free, efficient, and selective preparation technique, can be used. The application of SPME to determine chloroanisoles in aqueous matrices, such as wine, and cork stoppers (*1*, *16*, *17*) has been reported many times. SPME methods have also been developed for detecting chlorophenols in environmental matrices (*18*) and wine (*19*).

In a recent work (*20*), we have estimated the chlorophenolic concentration in cork macerates—that is, hydroalcoholic solutions containing 12% ethanol—in order to determine the releasable chlorophenols. This determination quantifies what amount of chlorophenols has been transferred to the solution but does not offer information about the total concentration in cork stoppers. To our knowledge, few papers deal with tri-, tetra-, and pentachlorophenol determination in cork samples (*11*−*13*). All of them, as a methodological approach, use organic solvents to extract these compounds from cork stoppers.

In this work, we have developed two approaches for determining the total content of tri-, tetra-, and pentachlorophenol in cork stoppers by gas chromatography with electron capture detection (GC-ECD). Both methods are based on extraction using an environmentally friendly ethanol/water mixture followed by SPE or SPME—these two methods were also used when the releasable chlorophenols were determined (*20*)—preconcentration and purification steps. In the SPE method, an Oasis HLB syringe barrel (a copolymer of polydivinylbenzene−*N*-vinylpyrrolidone) was used. In SPME a poly(dimethylsiloxane)−divinylbenzene (PDMS-DVB) fiber was selected. Screening with a two-level factorial design was performed to establish the influence of the main parameters affecting the SPME method (*21*). The aim of this approach is to find the optimal experimental conditions not for each compound separately but for all of the compounds together. The developed SPE and SPME procedures were applied to spiked samples to evaluate the efficiency of both methodologies.

MATERIALS AND METHODS

Chemicals, Reagents, and Cork Samples. The chlorophenol standards TCP (Fluka, Vienna, Austria), TeCP (Riedel-de-Haën, Seelze, Germany), and PCP (Sigma Aldrich Química, Madrid, Spain) used in this study had a purity of at least 99%. As an internal standard, 2,4,6-tribromophenol (TBP) (Supelco, Bellefonte, PA) was employed. The stock solution, which contained 100 μg/mL of each analyte (TCP, TeCP, and PCP), was prepared in methanol (Carlo Erba Reagenti, Milan, Italy). Individual standard solution of TBP was also prepared in methanol at a concentration level of 500 μg/mL. All solutions were stored in a refrigerated environment at 4 °C and kept in darkness.

n-Hexane (Panreac, Barcelona, Spain) and absolute ethanol (Carlo Erba Reagenti) were of pesticide residue grade, whereas anhydrous sodium sulfate, NaCl, and K_2CO_3 were of analytical reagent grade. The high-purity water was taken from a Milli-Qplus water system.

Natural-cork stoppers (grade a) were kindly supplied by AECORK (the trade association of the Catalan cork manufacturers). The quality of corks used for the experiments was ensured according to the usual selection process.

Preparation of Standards for Calibration. Working solutions were made daily by diluting the stock solutions either with methanol or with hydroalcoholic mixture [12% (v/v) ethanol] for calibration with SPME.

In the case of SPE, calibration standards were prepared in the 1−30 ng/mL range containing the internal standard at a concentration of 20 ng/L.

For the SPME calibration, the diluted standard solutions contained the internal standard at a concentration level of 100 ng/L ranging from 1 to 50 ng/L.

Acetylation of Standards in Methanol. The acetylation procedure to convert the chlorophenols into more suitable compounds was as in Rodríguez et al. (*22*). One milliliter of the standard solution containing the internal standard in methanol, 2 mL of 5% K_2CO_3, and 200 μL of acetic anhydride were shaken for 1 min. After that, 1 mL of *n*-hexane was added and mixed for another minute. The organic phase containing acetylated compounds was separated, and the aqueous phase was reextracted with 1 mL of *n*-hexane. The two organic fractions were mixed, dried over anhydrous sodium sulfate, and reduced to 1 mL under a gentle stream of nitrogen before their injection into the GC-ECD system.

Cork Extraction. Cork stoppers were milled to a ≤5 mm size with a conventional Moulinette D56 grinder (Moulinex España, Barcelona, Spain). The detachable parts of the grinder were washed between samples by using the standard procedure for glassware.

One gram of the sample was carefully weighed, placed in a 100 mL glass bottle, and afterward spiked with 1 mL of the diluted methanolic solution containing the target chlorophenols (at 20 ng/mL in the case of SPE and at 13.5 ng/mL for the SPME method). The spiked samples were allowed to dry, and then they were extracted by adding 80 mL of hydroalcoholic solution and shaking in the rotary mixer (Dinko Instruments, Dinter, Barcelona, Spain) for 24 h. An ethanol/water mixture was chosen as extractive solution because of its similarity to the wine. Before the preconcentration and purification by applying SPE or SPME, the extract was diluted by adding the appropriate quantity of Milli-Q water (see Results and Discussion).

SPE Procedure. Oasis HLB (macroporous polydivinylbenzene−*N*-vinylpyrrolidone copolymer, 60 mg, Waters) was selected as sorbent according to the results obtained in a previous work (*20*). The conditioning was carried out by applying 2 mL of methanol followed by 2 mL of 12% (v/v) ethanol/water at pH 2. The cork extract obtained was conveniently diluted and the pH adjusted to 2 to ensure the neutral form of chlorophenols. Then, the solution was loaded at a flow rate of 4 mL/min with the aid of a peristaltic pump (Miniplus 3, Gilson). Afterward, the syringe barrels were rinsed with 1 mL of hydroalcoholic solution and the sorbent was dried under vacuum. At the end, elution of the analytes was carried out with 3 mL of methanol, and 1 mL of the internal standard at a 20 ng/mL concentration level was added. The methanolic solution was derivatized prior to gas chromatography analysis following the same procedure used for the standards in methanol.

Headspace SPME (HS-SPME) Procedure. A manual SPME holder with a 65 μm PDMS-DVB fiber (Supelco) was tested. Before use, each fiber was conditioned by inserting it into the GC injector for 30 min at 250 °C.

Previous to the sample extraction, blank runs were carried out to confirm the absence of contaminant chlorophenols.

The hydroalcoholic extract obtained after cork extraction (see Cork Extraction) was diluted to 12% (v/v) ethanol by adding Milli-Q water, which contained the appropriate amount of the internal standard (100 ng/L final concentration level of TBP). Five milliliters of the liquid sample (either standards used for calibration or the diluted cork extract) was introduced into a 15 mL screw-cap glass vial. After the addition of NaCl, 2 mL of a 5% K_2CO_3 solution, and 200 μL of acetic anhydride, the vial was sealed and clamped over a magnetic stirrer (Variomag) in a water-thermostated bath (Bunsen). Magnetic stirring (medium speed) was applied during the extraction using a Teflon-coated stir bar. The fiber was drawn into the headspace generated in the sample vial. After the selected adsorption time, the fiber was retracted into the needle and the SPME device was removed from the vial and inserted into the injection port of the GC for thermal desorption (5 min).

Table 1. Factor Levels Considered in the Experimental Optimization

variable	low level (–)	medium level (0)	high level (+)
c^a/g	0.8	1.0	1.2
T/°C	50	60	70
t/min	40	50	60

[a] c stands for NaCl content.

The optimized SPME conditions were 0.8 g of NaCl added to the solution and headspace sampling at 50 °C for 60 min (see Study of the Effect of Temperature, Time, and Ionic Strength on SPME).

Experimental Design. A full factorial design was performed to check the influence that the parameters had on the extraction of chlorophenols from a 12% (v/v) hydroalcoholic solution. This allowed us to change all of the experimental factors studied simultaneously and also to determine the interactions between the factors. These interactions cannot be detected with the classical experimental methods that vary one factor at a time. For each analyte, three variable factors that can affect the adsorption of chlorophenols on the fiber were considered: ionic strength quantified as salt concentration (c), temperature (T), and extraction time (t). A 2^3 full factorial design was selected. **Table 1** shows the experimental range for each factor.

All eight experiments were carried out twice in a random order. The central point (1 g, 60 °C, 50 min) was measured four times and was used as a calibration point. The Minitab v14 computer program was used for data manipulation (*23*).

Chromatography. Gas chromatographic analysis was performed with a GC 8000 series (8160) gas chromatograph equipped with an ECD 80 electron capture detector (Fisons Instruments, Milan, Italy). A DB-5 capillary column (J&W Scientific, Folsom, CA) (30 m × 0.25 mm i.d., film thickness = 0.25 μm) was used. The operating conditions were as follows: injector temperature, 270 °C (250 °C in SPME); detector temperature, 330 °C; carrier gas, helium at 30 cm/s; makeup gas, N_2 at 43 cm/s; oven temperature program, 2 min at 70 °C, increasing by 5 °C/min to 180 °C and by 10 °C/min to 270°C, and finally 3 min at 270 °C. Splitless mode injections (1 μL) were performed with the purge valve opened at 1 min or at 5 min when SPME was used. The chromatographic data were analyzed by Chrom-Card software.

To identify the target compounds, their retention times were compared with those obtained for standard solutions injected separately. Calibration curves were constructed following the internal standard method. The ECD response was lineal in the range of concentrations considered (~1−30 ng/mL and ~1−50 ng/L for SPE and SPME methods, respectively).

RESULTS AND DISCUSSION

Development of SPE Method. In a previous work (*20*), we demonstrated that Oasis HLB syringe barrels are capable of extracting and preconcentrating chlorophenols (TCP, TeCP, and PCP) from cork macerates, obtained from a 12% (v/v) hydroalcoholic solution.

Here, we have evaluated a procedure based on the extraction with ethanol/water and preconcentration with Oasis HLB to quantify the chlorophenolic content of cork stoppers. For this purpose, we believe that increasing the ethanol content of the solution will also increase its extraction power. Due to the fact that Oasis HLB sorbent is a suitable hydrophilic−lipophilic polymeric material for extracting polar compounds from aqueous samples, the influence of alcohol present in the matrix on the adsorption of the target compounds must be tested. Different hydroalcoholic mixtures (100 mL) spiked with 20 ng/mL of the chlorophenolic compounds and with an ethanol content ranging from 12 to 50% (v/v) were analyzed. As can be seen in **Figure 1**, the recovery of TCP, TeCP, and PCP is quantitative for ethanol contents up to 30% (v/v). A higher alcohol concentration results in decreased efficiency for all of the analytes studied. One-factor analysis of variance (ANOVA) was performed to compare the results obtained with 20 and 30% (v/v) ethanol. No significant differences at a 95% confidence level were found.

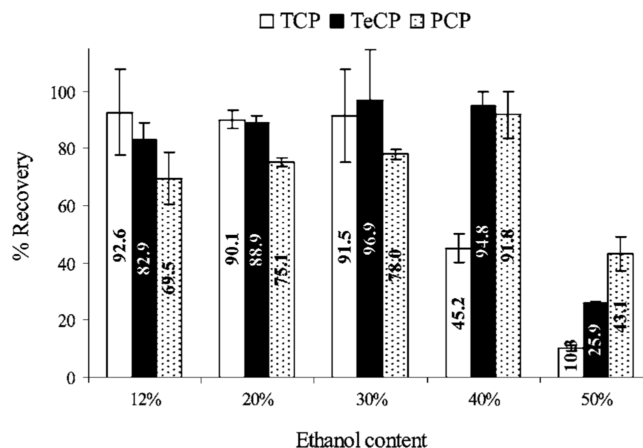

Figure 1. Recoveries for different ethanolic concentrations applied to SPE syringe barrel (Oasis HLB) containing 20 ng/mL of tri-, tetra-, and pentachlorophenol (n = 2).

In a second step we checked the extraction efficiency of ethanol/water mixtures with different compositions. Spiked cork samples (20 ng/g) were extracted with solutions containing 40 and 60% (v/v) ethanol. Previous experiments showed that <40% (v/v) ethanol gives poor results in terms of analyte recovery. After extraction, the appropriate volume of Milli-Q water was added to the extract to obtain, respectively, 20 and 30% (v/v) ethanol. In this way we ensured that compounds were not lost in the preconcentration step with Oasis HLB as demonstrated in the above paragraph. The recovery data (n = 2) when using the 40% (v/v) ethanol/water mixture as extractant were 78.6% for TCP, 59.2% for TeCP, and 33.6% for PCP. When the extraction was carried out with the 60% (v/v) ethanol/water solution, saturation of the Oasis HLB sorbent was observed.

Therefore, we can conclude that the proposed method that consists of extracting the substances using a hydroalcoholic solution containing 40% (v/v) ethanol, followed by dilution to 20% (v/v) and preconcentration with Oasis HLB sorbent, gives quantitative recoveries for TCP, which is considered to be the direct precursor of TCA, at the concentration level assayed. The limit of detection calculated for TCP in cork, taking in account the corresponding recovery result (78.6%) and for a 1 mL final volume extract (see Materials and Methods), can be estimated at ~1.5 ng/g. For the more chlorinated phenols, especially for PCP, the overall process gave poor recovery results and higher limits of detection.

Development of the HS-SPME Method. Besides SPE, SPME can also be employed to determine volatile or semi-volatile compounds at low nanograms per liter levels. Using SPME to extract analytes from solid samples generally involves the addition of some solvent to the sample in the same vial where extraction takes place (*1*, *17*, *24*). In our case and for the sake of comparison, the SPME was performed using the cork extract obtained as in the SPE method developed here. However, before the SPME process, the extract was diluted to 12% (v/v) ethanol. It has been demonstrated that increasing the ethanol content of the solution will generally decrease the partition coefficients of volatile compounds in wine and will decrease sensitivity (*25*).

Choosing a suitable coating is crucial for establishing a SPME method, and it depends on the chemical structure of the

Table 2. ANOVA Results for the Three Experimental Designs[a]

analyte	main effects: *p* value	main effects: terms	*p* value for two-way interactions	*p* value for curvature
TCP	0.001	+*t* (0.006), −*T* (0.001)	0.202	0.001
TeCP	0.009	+*t* (0.001)	0.199	0.009
PCP	0.020	+*t* (0.007)	0.658	>0.05

[a] Significance *p* values are given for main effects, double interactions and for curvature evidence. For single-variable main effects the terms are also shown: the signs indicate the optimal variable level and numbers in parentheses are the corresponding *p*-value significances

compounds (polarity and volatility). In accordance with the findings reported by Martínez-Uruñela et al. (*19*), either PDMS, PDMS-DVB, or polyacrylate (PA) fibers can be successfully employed to extract acetylated chlorophenols from wine samples. In the present work, we propose the use of PDMS-DVB fiber, which has good selectivity for extracting low-middle molecular weight and semipolar analytes. This is why it is applied to determine a wide variety of substances in complex matrices such as wine (*26*). This stationary phase includes DVB, which enhances the extraction of the target compounds by $\pi-\pi$ interactions with the aromatic ring in the sorbent (*18*).

Two types of sampling are commonly performed using SPME: direct and headspace extraction. The latter was chosen to extend the fiber's operative time and thus protect it from damage caused by matrix interferences and magnetic stirring.

Study of the Effect of Temperature, Time, and Ionic Strength on SPME. The experimental parameters affecting the SPME process (NaCl content, temperature, and extraction time) were tested by applying a factorial design. Thus, the experimental conditions, which enhance the adsorption of chlorophenols on the fiber, can be established. **Table 2** shows for each analyte the significances (*p* values) attached to the individual and double interactions and the evidence of curvature effects. All of the significances of the main effects are below the 5% level. PCP is identified as the worst case, although it is also significant at the 2% level. For TCP and TeCP the differences between the experiments were larger, even in the case of the nonsignificant double interactions.

Table 2 shows the variables that control these effects. The sign beside each variable name indicates the optimal level that maximizes the response and the numbers in parentheses are the corresponding *p*-value significances. The data show that the extraction time is a crucial variable, as it has a noticeable influence on all three analytes and maximizes the response when it is set at the highest level. Therefore, sampling time was fixed at 60 min. It is well-known that higher temperatures result in a decrease in the partition coefficients between the analytes and the fiber, and so the extraction efficiency is reduced (*27*). As the temperature is significant only for TCP and has a positive effect at the lowest level, the working temperature was kept at 50 °C. When studying the NaCl content, we can take as a general rule that adding a soluble salt to the sample increases the ionic strength of the solution: the organic substances become less soluble, and so the amount of compound released into the headspace and, therefore, into the fiber, increases. In our case, we observed the opposite. We believe that the ethanol content of the cork extract alters the partition coefficients of the chlorophenolic derivates between the aqueous phase and the headspace and, consequently, the extraction efficiency is reduced when the salt concentration increases (*25*). In conclusion, we carried out the extraction at the lowest salt level studied (0.8 g of NaCl). These results compare well with those obtained for the extraction of some volatile phenols in wine (*28*).

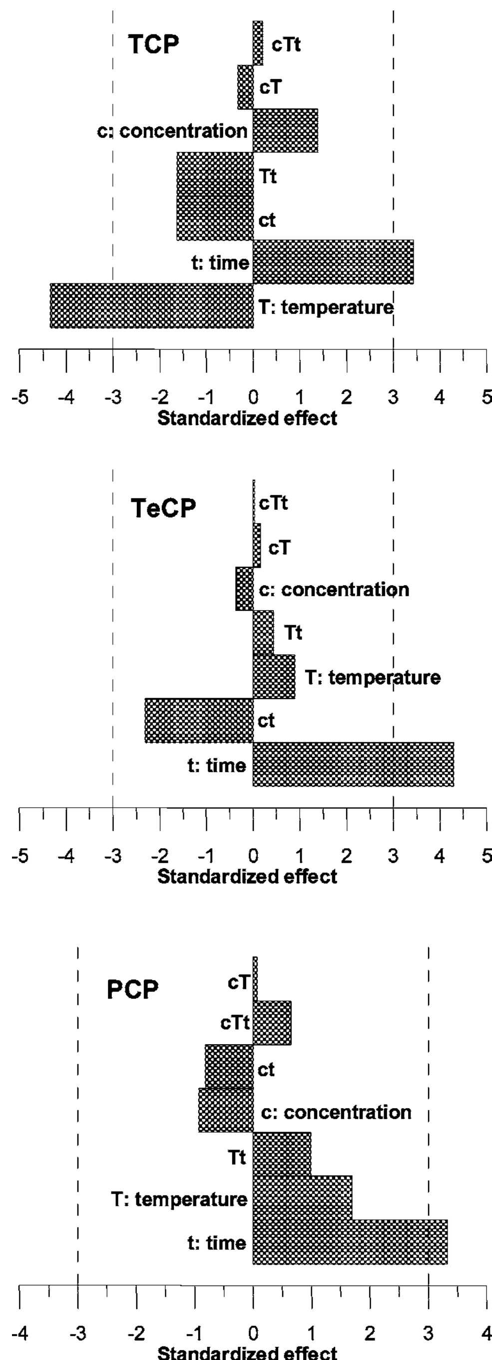

Figure 2. Pareto diagrams showing standardized effects of all variables and combinations of them in the experimental results. As a common feature, in all cases the extraction time plays a crucial role and double and triple interactions are not significant. See text for more details.

These main conclusions are visually presented using Pareto graphs shown in **Figure 2**. For the three analytes the influencing variables are arranged according to the size of their standardized effect. For TeCP a double interaction was ranked as one of the relevant factors (concentration with time), but the ANOVA results confirmed that its significance level is >5%.

From the data in **Table 2**, it is also clear that in the cases of TCP and TeCP there is evidence of curvature. A more careful inspection of the data revealed that this curvature represents a concave relationship between the response and the other control

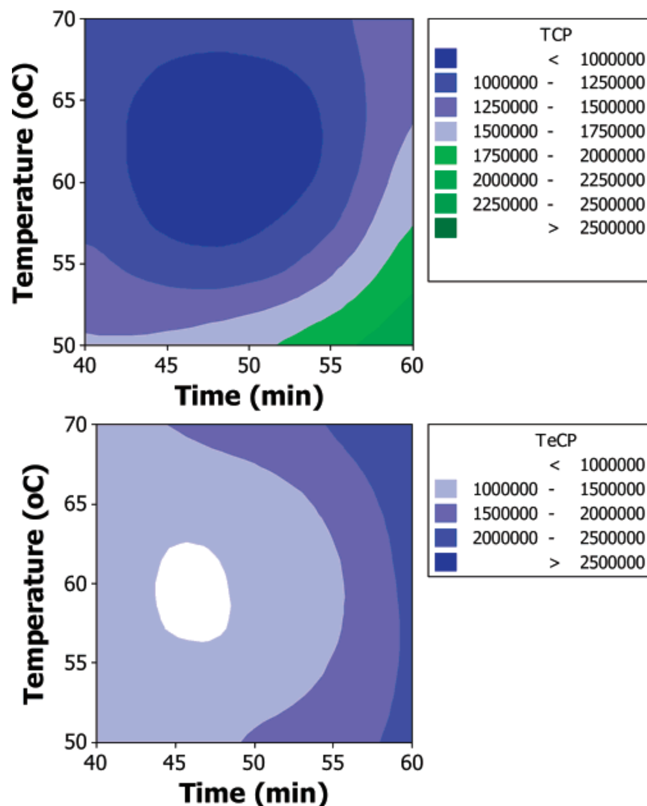

Figure 3. Contour plots for the analytes presenting evidence of curvature effects: (**a**) contour plot of TCP response versus temperature and extraction time; (**b**) contour plot of TeCP response versus temperature and extraction time. The isolevel units are chromatographic peak areas. The common optimal region is found at the 60 min and 50 °C point.

variables. This can be seen by examining the contour plots in **Figure 3**. From these qualitative pictures it can be deduced that local minimal responses are found close to the center point.

The evidence of a minimum in the two surfaces that were inspected has also been checked by constructing multilinear regression (MLR) models (*29*) with a built-in-house program which constructs statistically significant models by combining all of the variable forms (*30*). As a conclusion, a minimum has been found inside the inspected region and, consequently, the maximal response is located at the border of the search region, close to the selected experimental conditions.

Estimation of Quality Parameters. Because porous polymer fibers such as PDMS-DVB extract analytes by an adsorption mechanism, nonlinear responses are observed over broad concentration ranges (*31*). A competitive process for a limited number of active sites placed on the polymer surface has been established, and therefore some compounds with high affinity toward the coating can displace other analytes with low distribution ratios. Thus, the amount of substance extracted can be affected by the matrix composition. Taking into account that great care should be taken when performing calibration curves, we checked the linearity for the working concentration range (1−50 ng/L) using TBP as the internal standard. Calibration curves were obtained with standards in hydroalcoholic solution [12% (v/v)] using the optimized conditions. Five concentration levels for a mixture containing the target compounds were examined in duplicate. In **Table 3** we can see that the correlation coefficients were >0.97 for all analytes studied.

The precision of the procedure was also tested. To do this, eight standards at 50 ng/L were consecutively extracted under the same conditions. Relative standard deviations (RSD) were

Table 3. Linear Ranges, Parameters of Calibration Lines, Limits of Detection (LOD), and Precision from Standards Prepared in 12% Ethanol/Water (v/v) Mixture Obtained with HS-SPME Method[a]

compd	linear range (ng/L)	a (S_a)	b (S_b)	r	RSD (%)	LOD (ng/L)
TCP	1.0–47.7	0.6 (0.1)	2.4 (0.2)	0.973	11.4	0.8
TeCP	1.0–53.9	0.23 (0.02)	2.9 (0.1)	0.997	10.6	0.8
PCP	1.0–68.4	0.07 (0.03)	2.2 (0.1)	0.993	8.3	2.5

[a] a = intercept; S_a= standard deviation of a; b = slope; S_b= standard deviation of b; r = correlation coefficient; %RSD under repeatability conditions (n = 8, concentration = 50 ng/L).

≤11.4% (see **Table 3**). The sensitivity of the developed method was evaluated in terms of limit of detection (LOD), which was calculated based on a signal-to-noise ratio of 3:1. **Table 3** shows that LODs in the range of nanograms per liter were found for the three compounds studied.

To demonstrate that the entire analytical procedure based on extraction with water/ethanol mixture and HS-SPME for the determination of tri-, tetra-, and pentachlorophenol in cork samples can be applied effectively, two different experiments were carried out. First, cork ethanolic extracts were spiked at 25 ng/L and then analyzed. Recovery results were, respectively, 93.7, 80.8, and 88.0% for TCP, TeCP, and PCP.

Second, the extraction process for spiked cork (at the 13.5 ng/g level) followed by SPME was evaluated. After extraction with a 40% (v/v) hydroalcoholic solution and dilution with Milli-Q water containing the appropriate amount of the internal standard (see Cork Extraction), 5 mL of ethanolic extract was derivatized and analyzed under optimal HS-SPME conditions. The mean recoveries (n = 2) obtained were 78.5, 51.9, and 19.6% for TCP, TeCP, and PCP, respectively. Combining the values shown in **Table 3** and these recovery results, the following limits of detection for the entire HS-SPME method can be estimated: 0.25 ng/g for TCP, 0.4 ng/g for TeCP, and 3.1 ng/g for PCP.

These results show that the SPME method evaluated here is suitable for analyzing TCP in cork stoppers. Moreover, recovery levels of this compound compare well with the values obtained using the SPE method also presented here. The amount of TeCP recovered using SPME (52%) also compares well with the amount recovered using SPE (59%). However, both methodologies failed to give quantitative recoveries of PCP. We ascribe this fact to the hydroalcoholic solution not being able to completely extract pentachlorophenol from the corks.

Conclusion. In this work, we have evaluated the extraction of chlorophenolic compounds from ground cork samples with hydroalcoholic solutions as extractant combined with SPE or HS-SPME preconcentration and GC-ECD analysis. We have found that optimum conditions are as follows: an ethanol content of 40% (v/v) of the extraction solution and dilution to 20% (v/v) ethanolic content for SPE preconcentration with Oasis HLB or dilution to 12% (v/v) ethanolic content for HS-SPME (PDMS-DVB fiber, 0.8 g of NaCl added to the solution, and headspace sampling at 50 °C for 60 min). Both methodologies compare well in terms of recovery for the target analytes: 78.6% for TCP, 59.2% for TeCP, and 33.6% for PCP in SPE versus 78.5, 51.9, and 19.6% for TCP, TeCP, and PCP, respectively, in HS-SPME. We consider that the extraction seems not to be completed for the more chlorinated compounds.

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