

Determination of Chloroanisoles and Chlorophenols in Cork and Wine by using HS-SPME and GC-ECD Detection

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ABSTRACT

J. Inst. Brew. 115(1), 71–77, 2009

Cork taint is an off-flavor problem in wine, the main reason being the presence of 2,4,6-trichloroanisole (TCA) in the cork stopper. In addition to the TCA, the presence of other chloroanisole and chlorophenol family compounds (the perception limits of which are very low) can also result in, or contribute to, cork taint problem. In this study, the levels of 2,4-dichloroanisole (DCA), 2,4,6-trichloroanisole (TCA), 2,3,4,6-tetrachloroanisole (TeCA), pentachloroanisole (PCA), 2,4,6-trichlorophenol (TCP), 2,3,4,6-tetrachlorophenol (TeCP) and pentaclorophenol (PCP) were assayed in cork stoppers (natural, agglomerated and colmate) and in red wine samples from different wineries in Turkey using HS-SPME and GC-ECD detection. The performance parameters for all chloroanisole and chlorophenol compounds were as follows: recovery 92.48–102.53%, R 0.992–0.996. The LOQ values were DCA (8.4 ng/L), TCA (0.8 ng/L), TeCa (0.6 ng/L), PCP (0.8 ng/L), TCP (0.8 ng/L), TeCP (1.2 ng/L), and PCA (1.1 ng/L) respectively. In cork stoppers, the amounts of 2,4,6 TCA ranged between 5.4–130.6 ng/g. The 2,3,4,6 TeCA ranged between 1.12–8.2 ng/g and the PCA ranged between nd (not detected)–11.01 ng/g. In the wine samples, 2,4,6 TCA ranged between 1.42–70.2 ng/L. The 2,3,4,6 TeCA ranged between nd–15.1 ng/L and the PCA ranged from nd–5.16 ng/L. The results indicated that there was a significant correlation between the TCA in wines and the TCA in cork stoppers.

Key words: chloroanisoles, chlorophenols, cork, cork taint, HS-SPME (HeadSpace-Solid Phase Microextraction), GC-ECD (Gas Chromatography-Electron Capture Detection), wine.

INTRODUCTION

The organoleptic defect of the wines originating from cork called a musty/mouldy taint or traditionally known as “cork taint” represents an important problem in the wine industry. Cork is a natural product and thus is not inert. It can interact with wine, sometimes modify its flavour and give it organoleptic defects²⁷. Some of the compounds involved in this defect may originate in the cork

stopper³⁰ (for instance, 1-octen-3-ol, 1-octen-3-one, 2-methylisoborneol, geosmin, guaiacol and/or chloroanisoles⁸). However, the presence of 2,4,6-trichloroanisole⁷ in cork stoppers is primarily responsible (in at least 80% of the cases) for this defect. To a lesser extent, 2,3,4,6-tetrachloroanisole (TeCA) and 2,3,4,5,6-pentachloroanisole (PCA) play a part in the occurrence of cork taint in wine^{1,9,16,25,29}. The problem of cork taint causes economic losses every year in the wine industry because of the rejection of off-odour wines by the consumer^{1,18}. According to data derived from the literature, this problem affects between 0.1 and 10% of European bottled wines and it has been estimated that as much as US\$10 billion is lost annually as a result of the cork taint problem due to TCA¹⁸.

The mechanisms leading to the appearance of TCA in wines have been discussed by several authors^{3,5,21,22,23}. Anisoles are mainly formed due to the microbial induced methoxylation of phenols. The latter compounds can be directly or indirectly introduced in wine cellars through different sources, such as the use of wooden pallets, cartons and packing materials previously treated with polychlorophenolic biocides; the employment of chlorophenolic compounds during production of bark cork and the further elaboration of cork stoppers; the use of hypochlorite solutions in the cleaning of wooden barrels³. Polluted bark cork, cork stoppers and different wooden materials, employed in the environment of wine cellars, may transfer the native pollutants, and thus the earthy-musty defect, to wine samples^{21,22}. Nevertheless, combined effects of more than one compound (e.g., other chloroanisoles, chlorophenols and guaiacol) are often required to cause the observable defect²¹. Chlorophenols that have been used as fungicides, bactericides, herbicides and wood preservatives can be biotransformed into the corresponding chloroanisoles. Fungal activity in cork contaminated by chlorophenols that have been used as fungicides in cork tree forests can also cause this defect. Some authors report that the formation of anisoles and thioanisoles in cork is a result of the methylation of halogenated phenols due to the presence of *Rhodococcus*, *Acinetobacter* and *Pseudomonas* strains in the cork tree^{2,16,17}.

The detection of 2,4,6-trichloroanisole (TCA), which was first reported to be the main compound responsible for the cork taint, is an important task in the wine industry, since TCA has a great influence on the acceptance or rejection of wines by the consumer¹. Reported values for the sensory threshold for TCA in wine varies in the literature from 1.4 ng/L to 210 ng/L³³. According to

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some other authors, the human identification threshold for 2,4,6-TCA in cork soaks varies from 4 to 10 ng/L^{13,23}. Although olfactory and gustatory thresholds for TCA range from 0.03 to 50 ng/L depending on the age and the variety of the wine and the sensitivity and training of the judge, the TCA concentration considered as a defect in wine ranges from 10 to 40 ng/L¹.

Therefore, the detection of chloroanisoles in wines has led to extensive research over the last decade to develop methods as sensitive as the human sensory threshold. However, to avoid the economic losses due to this musty off-flavor, it is very important to prevent the occurrence of this defect with an effective control of chloroanisoles in cork³⁴. This control requires appropriate analytical methods which must provide sensitivity and selectivity as well as good repeatability and recovery.

Among the various methods developed for the analysis of chlorophenols in aqueous samples, gas chromatographic methods are most often used because of their high sensitivity and power of resolution^{4,5,14}. Gas chromatography coupled with electron capture detector (GC-ECD) and mass-spectroscopy (GC-MS) is usually used for the trace analysis of 2,4,6-TCA and other chloroanisoles^{5,18,28,33,34}. In general, due to adsorption problems, tailed peaks and detectability, chlorophenols have to be derivatized prior to separation and quantification by gas chromatography. A large number of derivatizing reagents, such as diazomethane¹², pentafluorobenzyl bromide¹¹, methyl iodide²⁶, or acetic anhydride⁶, have been used for this purpose. Acetylation is one of the procedures most widely employed to convert chlorophenols into less polar compounds, thus increasing extraction efficiency²⁰. The need to determine chlorophenols at low concentrations requires sample preparation steps prior to injection into a gas chromatograph. Appropriate sample-handling techniques such as solid-phase extraction (SPE) and solid-phase microextraction (SPME) are extensively applied by virtue of their well-known advantages^{15,35}. Recently, the development of new SPE sorbents has encouraged the use of this extraction and preconcentration technique in different types of samples^{19,28,31,36}.

SPME is a solvent-free method of extracting analytes from a variety of matrices by partitioning them from a liquid or gaseous sample into an immobilized stationary phase. It uses a very simple setup and requires no additional instrumentation other than a conventional gas chromatograph (GC). SPME eliminates preconcentration steps by directly extracting the analytes into a poly-(dimethylsiloxane)-coated fibre, which is the most suitable fibre for the analysis of 2,4,6-TCA in wines²⁸.

The aim of the present study is both to develop SPME coupled to GC-electron-capture detection (ECD) and to determine tri-, tetra- and pentachlorophenol in cork stoppers and wine samples from different Turkish wineries.

MATERIALS AND METHODS

Chemicals and reagents

The following reagents were obtained from Sigma-Aldrich (Madrid, Spain): 2,4-dichloroanisole, 2,4,6-trichloroanisole (99%), 2,3,4,6-tetrachloroanisole, pentachloroanisole (99%), 2,4,6-trichlorophenol (98%) and

pentachlorophenol (98%). Methanol was obtained from Merck (Darmstadt, Germany) and 65 µm PDMS/DVB fibres were obtained from SUPELCO.

Stock standard solutions were prepared by diluting 1 mg of each standard in methanol. The calibration solutions for the analysis were prepared from stock standard solutions by diluting in the range of 0.1 to 1000 ng/g and the calibration standard solutions were stored in the dark at 4°C.

Wine samples

In order to determine 2,4,6-TCA and other compounds which can result in cork taint off-flavor, twenty six wines (red, white and rosé) from ten different wineries in Turkey were obtained in their original bottles, with their original cork stoppers. Different wineries were classified from A to F. All wine samples were kept at 4°C until extraction.

Wine sample preparation

Wine samples were used directly for SPME extraction.

Cork sample preparation

The cork samples were classified as a “natural (n)”, “colmate (c)” and “agglomerated (a)” cork. After classification the cork was cut into small portions and ground in a blender to sizes of ~ 0.5 × 0.5 cm diameter. The cork samples were prepared as follows: 550 mg of cork stopper was extracted into 10 mL of n-hexane at 4°C, during 24 h in the dark and the extracts were used for the SPME procedure.

SPME fibres and HS-SPME procedure

Headspace sampling was conducted using 50 mL vials, each containing 20 mL of liquid sample. A suitable amount of NaCl (98%v/v) was added to the 20 mL sample to obtain a 5 M final concentration in total volume. The glass vials were tightly capped with silicone/PTFE-faced silicone septa and placed in a 25°C water bath. The extraction was carried out at 300 rpm (using a magnetic stirring bar) with constant stirring. The sample vials were pre-equilibrated for 30 minutes at 25°C. The 65 µm polydimethylsiloxane/divinylbenzene fibre (PDMS/DVB) was used for solid-phase microextraction. The stainless steel needle, in which the fibre was housed, was pushed through the vial septum, allowing the coating to be exposed to the headspace over the sample for 30 minutes. After 30 minutes the fibre was pulled into the needle sheath and the SPME device was removed from the glass vial and inserted into the GC injection port for thermal desorption at 250°C for 3 minutes²⁸.

Instrumental analysis

Chromatographic analyses were performed with a Shimadzu GC-14 B gas chromatograph equipped with a split/splitless injector, electronic pressure control in the injector and an electron capture detector (ECD).

Column: GL-Science TC-Wax 60 m × 0.32 mm, 0.25 µm fused-silica column

Carrier gas: Nitrogen (N₂) at a flow of 1 mL/min was used as a carrier gas.

Injector temperature: 250°C

Detector temperature: 300°C

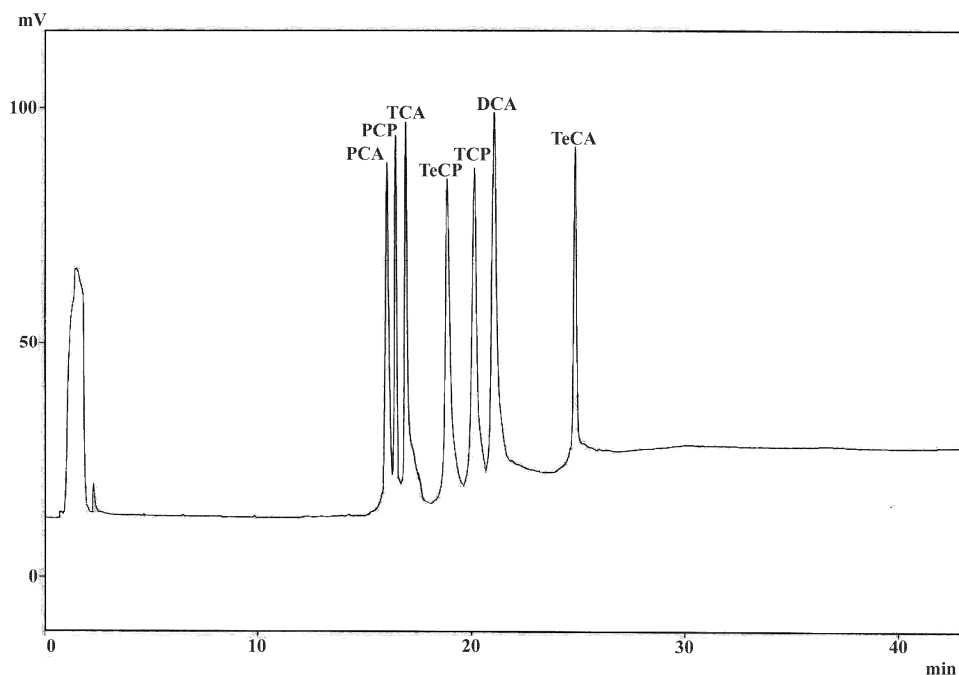


Fig. 1. The GC-ECD chromatogram of a standard solution of chlorophenols and chloroanisoles.

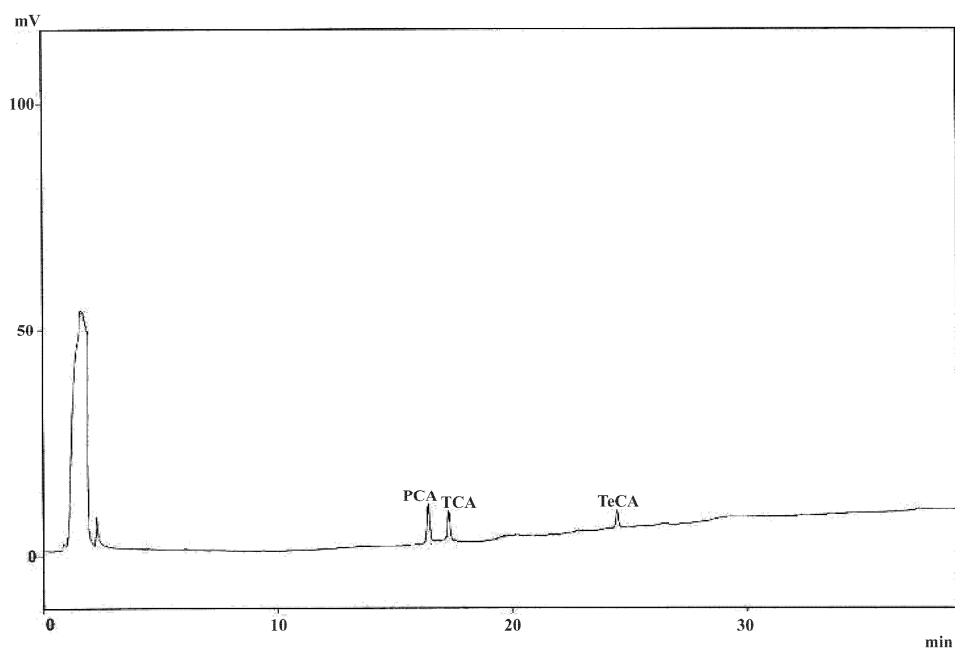


Fig. 2. The GC-ECD chromatogram of chlorophenols and chloroanisoles of cork sample (ARN1).

The oven temperature was programmed as follows: 40°C for 2 min, heated to 150°C at 4°C/min and kept for 1 minute; heated to 200°C at 4°C/minute, kept for 1 minute and raised to 220°C at 15°C/min and held for 5 min. Injection was performed in the splitless mode for 2 minutes and then the split flow was set to 30 mL/min^{12,23}.

Sensorial analysis

Sensorial analysis was performed by seven experienced tasters using a slightly modified positive notation system^{10,24} and taint scores were notated from nd (not detected) to +++ (distinctly detected). The results were

given after eliminating the maximum and minimum extreme scores of the tasters²⁴.

Statistical analysis

The importance of the means of the variance analysis were shown by the Duncan test³².

RESULTS AND DISCUSSION

In this study we investigated the HS-SPME procedures with GC-ECD detection for the quantitative determination of chloroanisoles and chlorophenols responsible for cork

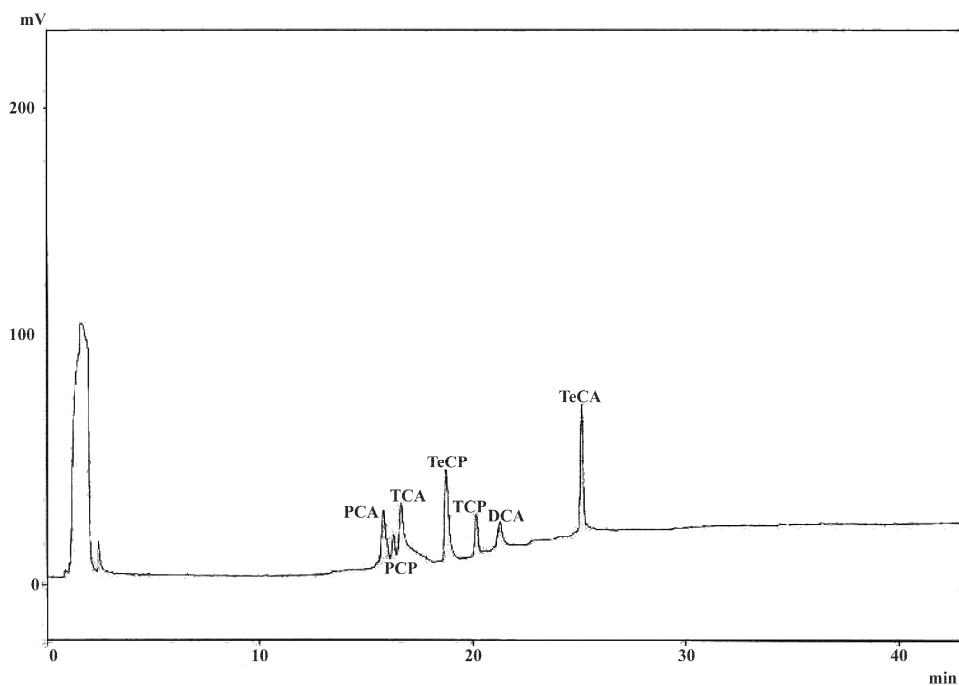


Fig. 3. The GC-ECD chromatogram of spiked cork (ARN1) at 20 ng/g concentration of each chloroanisole and chlorophenol standards.

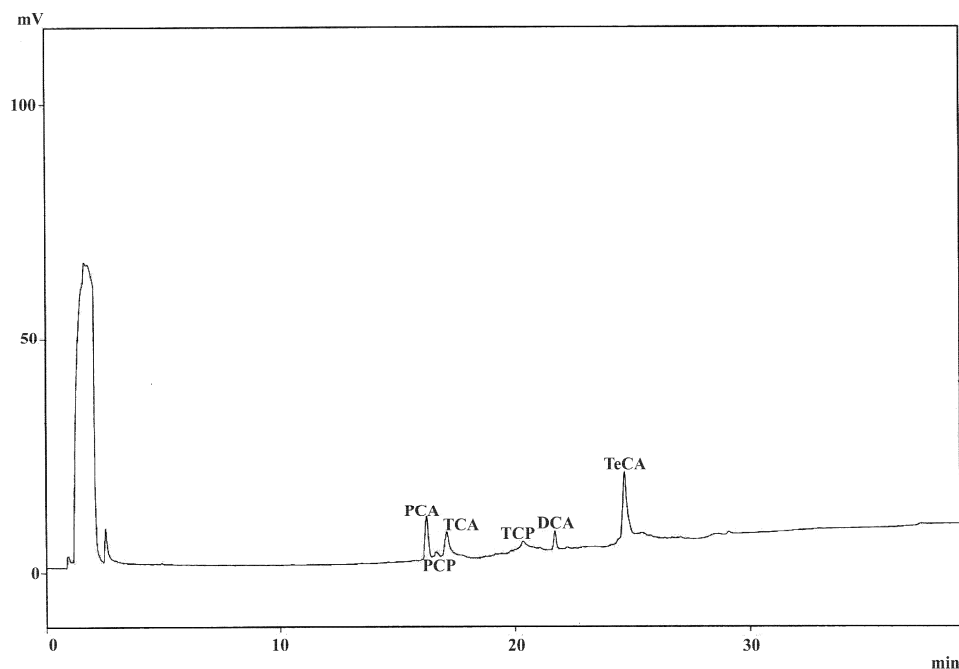


Fig. 4. The GC-ECD chromatogram of chlorophenols and chloroanisoles of red wine sample (AR1).

taint problem in wines and the identification and quantification of these compounds in Turkish wines.

The HS-SPME procedures with GC-ECD detection were successfully applied for the detection of chloroanisoles and chlorophenols in wines and in cork samples. Fig. 1 shows the GC-ECD chromatogram of a standard solution of chlorophenols and chloroanisoles. Fig. 2 shows the GC-ECD chromatogram of chloro-

phenols and chloroanisoles of cork sample (ARN1). Fig. 3 shows the GC-ECD chromatogram of spiked cork (ARN1) and Fig. 4 shows the GC-ECD chromatogram of chlorophenols and chloroanisoles in red wine sample (AR1).

The performance parameters of the HS-SPME-GC-ECD method are reported in Table I. According to these results, the performance parameters of all the investigated

Table I. Performance parameters of the HS-SPME-GC-ECD method.

Compound	Linear range (ng/g)	Correlation coefficient (r)	LOQ S/N = 10	LOD S/N = 3	Recovery (%)
2,4-Dichloroanisole (DCA)	0.8–500	0.996	8.4	2.4	96.25
2,4,6-Trichloroanisole (TCA)	0.3–100	0.992	0.8	0.2	97.54
2,3,4,6-Tetrachloroanisole (TeCA)	0.3–200	0.994	0.6	0.2	102.35
Pentachloroanisole (PCA)	0.5–500	0.994	0.8	0.2	95.35
2,4,6-Trichlorophenol (TCP)	0.3–100	0.995	0.8	0.2	92.48
2,3,4,6-Tetrachlorophenol (TeCP)	0.3–200	0.993	1.2	0.4	102.53
Pentachlorophenol (PCP)	0.5–500	0.994	1.1	0.5	98.35

Table II. The concentration of chloroanisoles and chlorophenols in bottled red wine samples (ng/L).^a

Sample ^b	N	2,4 DCA		2,4,6 TCA		2,3,4,6 TeCA		PCA		2,4,6 TCP		2,3,4,6 TeCP		PCP	
		Mean	Sd	Mean	Sd	Mean	Sd	Mean	Sd	Mean	Sd	Mean	Sd	Mean	Sd
A-R 1	3	0.83f	0.0203	2.51g	0.0321	1.60e	0.0115	3.50b	0.0145	33.03b	0.0173	-	-	66.12d	0.0115
A-R 2	3	2.00d	0.0173	2.53g	0.0260	1.51e	0.0203	3.52b	0.0208	18.07f	0.0161	-	-	46.09d	0.0160
A-R 3	3	2.14b	0.0109	5.42e	0.0401	-	-	4.45	0.0812	32.16b	0.0231	-	-	82.01c	0.0103
A-R 4	3	2.09c	0.0108	5.43e	0.0602	-	-	4.42	0.0341	21.04e	0.0309	-	-	84.12c	0.0219
A-R 5	3	1.13e	0.0230	2.50g	0.0730	2.11d	0.0253	1.16d	0.0129	17.91f	0.0402	5.2c	0.0234	96.11b	0.0108
A-R 6	3	1.11e	0.0703	2.51g	0.0415	2.09d	0.0413	1.131d	0.0902	18.09f	0.0103	6.7b	0.0128	94.42b	0.0111
A-R 7	3	-	-	70.2a	0.0532	4.32b	0.0901	-	-	41.98a	0.0109	-	-	64.12d	0.0805
A-R 8	3	-	-	16.3c	0.0632	-	-	-	-	29.14c	0.0105	5.1c	0.0145	67.19d	0.0502
B-R 9	3	-	-	1.42h	0.0830	3.33c	0.0831	-	-	13.17g	0.0107	1.9e	0.0132	66.09d	0.0731
B-R 10	3	3.94a	0.0134	2.71g	0.0911	-	-	-	-	12.96g	0.0109	-	-	24.12g	0.0739
B-R 11	3	3.92a	0.0107	3.23f	0.0203	-	-	1.33d	0.0216	18.19f	0.0206	-	-	23.32g	0.0801
B-R 12	3	1.13e	0.0801	8.12d	0.0801	3.46c	0.0718	1.35d	0.0229	26.15d	0.0304	-	-	12.11h	0.0714
B-R 13	3	1.99d	0.0220	1.52h	0.0567	15.1a	0.0514	-	-	33.03b	0.0120	-	-	63.17d	0.0936
C-R 14	3	0.86f	0.0504	2.56g	0.0319	-	-	-	-	20.81e	0.0323	-	-	66.11d	0.0103
C-R 15	3	2.14b	0.0901	3.41f	0.0109	-	-	-	-	32.19b	0.0932	-	-	123.22a	0.0203
C-R 16	3	2.13b	0.0672	3.38f	0.0105	-	-	5.13a	0.0921	42.06a	0.0734	-	-	24.34g	0.0216
C-R 17	3	1.16e	0.0941	8.12d	0.0253	1.52e	0.0812	5.16a	0.0134	13.19g	0.0108	-	-	46.23e	0.0123
C-R 18	3	-	-	8.24d	0.0203	0.92f	0.0916	4.12b	0.0409	18.05f	0.0401	10.2a0	0.0102	44.34e	0.0078
D-R 19	3	-	-	33.12b	0.0313	1.10f	0.0760	4.10b	0.0271	17.83f	0.0731	3.6d	0.0134	34.63f	0.0109
D-R 20	3	3.92a	0.0713	33.0b	0.0419	2.09d	0.0411	-	-	42.90a	0.0339	1.8e	0.0456	122.01a	0.0126
D-R 21	3	1.15e	0.0801	1.42h	0.0040	1.12f	0.0810	-	-	26.11d	0.0228	-	-	120.03a	0.0123
D-R 22	3	0.85f	0.0409	1.47h	0.0108	-	-	-	-	15.21	0.0534	-	-	12.13h	0.0342
D-R 23	3	-	-	2.34 g	0.0203	-	-	2.60c	0.0309	13.26g	0.0329	-	-	34.46f	0.0437
E-R 24	3	2.16b	0.0707	2.36g	0.0133	1.11f	0.0130	2.59c	0.0631	13.14g	0.0287	-	-	35.49f	0.0341
E-R 25	3	1.01f	0.0306	5.38e	0.0117	1.08f	0.0201	-	-	19.18f	0.0560	-	-	65.01d	0.0561
F-R 26	3	0.99	0.0145	2.62g	0.0542	3.62c	0.0173	2.62c	0.0260	25.19d	0.0317	-	-	25.13g	0.0203

^a The differences between two means shown in the same column are statistically significant ($p < 0.01$).^b A-F: The wineries, R: red wines.

compounds were found with a 92.48–102.53% recovery and with 0.992–0.996 R values.

In Tables II and III, the chloroanisoles and chlorophenols, detected in bottled wine samples and in the cork stoppers respectively, are given. Table IV shows the sensorial analysis of bottled red wines for determining the olfactory threshold level of cork taint. According to Tables II and III, the chlorophenols were detected more frequently in higher quantities than the chloroanisoles. The TCA were detected in all of the samples. The minimum olfactory threshold was 3.41 ng/L (Table IV). However, other compounds can also affect the minimum sensorial detection limit of cork taint; consequently, it is difficult to give a minimum detection limit.

PCP is the compound present at the highest concentration. However, large differences were obtained between the maximum and minimum limits. Apart from PCP, TCA is the second most abundant compound in both wine and cork. In the case of TCP, the amount of the compound differs considerably on a large scale in wine and the corresponding cork samples. It was observed that all of the chlorophenols and chloroanisoles displayed great variability in wines and their cork stoppers. The presence of

chlorinated compounds in wines are of a polluting nature and their concentrations are related to the intensity of contamination²³. Other research results also confirm the considerable variation in the distribution of chloroanisoles and chlorophenols between wines and cork stoppers^{23,31}.

Chloroanisoles usually arise from the *O*-methylation of chlorophenols, as a detoxification method by different microorganisms, especially fungi, under particular conditions of temperature and humidity²⁹. It has been observed that there is a correlation between TCA in wines and TCP in corks and between TeCP in wines and TeCP in corks. This correlation can be explained by the biomethylation of the chlorinated compounds²³. The presence of chloroanisoles in wine is also due to the fact that, if they are present in contaminated cork, they can migrate from cork to wine²⁹.

In some cases, the concentration of chlorinated compounds in cork stoppers was much higher than that in the corresponding wines. Similar results have been cited by some other researchers^{23,31}. The chemical composition of wine affects the level of absorption of chlorinated compounds. Hence, this incongruity can be attributed to the chemical composition of the wines.

Table III. Concentrations of chlorophenols and chloranisoles detected in cork stoppers (ng/g).^a

Sample ^b	N	2,4 DCA		2,4,6 TCA		2,4,6 TeCA		PCA		2,4,6 TCP		2,3,4,6 TeCP		PCP	
		Mean	Sd	Mean	Sd	Mean	Sd	Mean	Sd	Mean	Sd	Mean	Sd	Mean	Sd
A-Rnc 1	3	-	-	28.2g	0.0321	5.21d	0.0115	6.62d	0.0145	-	-	-	-	-	-
A-Rnc 2	3	-	-	38.6f	0.0260	7.43b	0.0203	4.41e	0.0208	55.03d	0.0161	-	-	15.79e	0.0160
A-Rnc 3	3	-	-	43.4e	0.0401	6.12c	0.0321	4.45e	0.0812	68.12c	0.0231	-	-	31.95c	0.0103
A-Rnc 4	3	-	-	26.3g	0.0602	7.36b	0.0185	4.42e	0.0341	71.07c	0.0309	11.82b	0.0128	19.22d	0.0219
A-Rcc 5	3	-	-	36.9f	0.0730	4.13d	0.0253	2.16	0.0129	70.13c	0.0402	11.22b	0.0138	20.16d	0.0108
A-Rcc 6	3	-	-	43.2e	0.0415	6.33c	0.0413	3.61f	0.0902	44.49e	0.0103	14.36a	0.0340	15.32e	0.0111
A-Rcc 7	3	-	-	130.6a	0.0532	4.09d	0.0901	-	-	45.01e	0.0109	9.08c	0.0324	9.83f	0.0805
A-Rcc 8	3	1.16b	0.0234	68.3c	0.0632	3.12f	0.0403	2.21e	0.0342	112.34a	0.0105	5.82e	0.0178	9.71f	0.0502
B-Rnc 9	3	-	-	8.90h	0.0830	8.20a	0.0831	-	-	88.11b	0.0107	-	-	32.94c	0.0731
B-Rnc 10	3	-	-	54.4d	0.0911	3.10f	0.0281	-	-	53.16d	0.0109	4.52e	0.0345	16.04e	0.0739
B-Rcc 11	3	2.34a	0.0432	92.7b	0.0203	4.41d	0.0176	9.03b	0.0216	53.45d	0.0206	14.76a	0.0309	-	-
B-Rcc 12	3	2.41a	0.0345	52.9d	0.0801	6.19c	0.0718	6.8d	0.0229	69.05c	0.0304	15.22a	0.0234	19.01d	0.0714
B-Rcc 13	3	-	-	7.96h	0.0567	5.11d	0.0514	-	-	44.23e	0.0120	8.87c	0.0345	19.17d	0.0936
C-Rcc 14	3	-	-	10.1g	0.0319	2.96f	0.0132	1.16g	0.0123	44.21e	0.0323	6.17d	0.0567	10.19	0.0103
C-Rac 15	3	1.08b	0.0234	8.11h	0.0109	1.12g	0.0332	-	-	87.11b	0.0932	-	-	56.12b	0.0203
C-Rac 16	3	-	-	36.4f	0.0105	2.61g	0.0234	11.01a	0.0921	55.13d	0.0734	-	-	20.45d	0.0216
C-Rac 17	3	-	-	41.2e	0.0253	4.15d	0.0812	10.91a	0.0134	33.11h	0.0108	-	-	31.03c	0.0123
C-Rac 18	3	-	-	37.2f	0.0203	6.21c	0.0916	4.62	0.0409	38.09g	0.0401	3.24f	0.0567	-	-
D-Rnc 19	3	-	-	67.1c	0.0313	3.09f	0.0760	7.91c	0.0271	71.18c	0.0731	3.56f	0.0234	15.62e	0.0109
D-Rnc 20	3	2.16a	0.0127	37.0f	0.0419	6.40c	0.0411	-	-	70.90c	0.0339	-	-	55.19b	0.0126
D-Rnc 21	3	-	-	10.1g	0.004	3.71f	0.0810	1.27g	0.0134	55.16d	0.0228	-	-	66.03a	0.0123
D-Rnc 22	3	0.91c	-	5.4j	0.0108	2.60g	0.0167	-	-	45.13e	0.0534	2.18g	0.0124	9.83f	0.0342
D-Rac 23	3	-	-	8.1h	0.0203	2.64g	0.0186	6.92d	0.0309	33.11h	0.0329	-	-	15.02e	0.0437
E-Rac 24	3	-	-	5.6j	0.0133	5.12d	0.0130	4.72e	0.0631	71.12c	0.0287	12.11b	0.0304	20.09d	0.0341
E-Rac 25	3	-	-	38.2f	0.011	6.23c	0.0201	-	-	87.12b	0.0560	-	-	-	-
F-Rac 26	3	-	-	55.6d	0.0542	8.12a	0.0173	3.82f	0.0260	41.11b	0.0317	-	-	15.43c	0.0203

^a The differences between the two means shown with different letters in the same column are statistically significant (p < 0.01).^b A-F: The wineries; nc: natural cork; cc: colmate cork; ac: agglomerated cork; R: red wines.**Table IV.** Sensorial analysis results of the red wines.^a

Sample ^b	T1	T2	T3	T4	T5	T6	T7	Final taint
								score
A-Rnc 1	nd	nd	nd	nd	nd	nd	nd	nd
A-Rnc 2	nd	nd	nd	nd	nd	nd	nd	nd
A-Rnc 3	+++	+++	++	++	++	+++	+++	+++
A-Rnc 4	nd	nd	nd	nd	nd	nd	nd	nd
A-Rnc 5	nd	nd	nd	nd	nd	nd	nd	nd
A-Rcc 6	nd	nd	nd	nd	nd	nd	nd	nd
A-Rcc 7	+++	+++	+++	+++	+++	+++	+++	+++
A-Rcc 8	++	++	+++	++	+++	++	++	++
B-Rnc 9	nd	nd	nd	nd	nd	nd	nd	nd
B-Rnc 10	nd	nd	nd	nd	nd	nd	nd	nd
B-Rcc 11	nd	nd	nd	nd	nd	nd	nd	nd
B-Rcc 12	++	++	++	++	++	+	+	++
B-Rcc 13	nd	nd	nd	nd	nd	nd	nd	nd
C-Rcc 14	nd	nd	nd	nd	nd	nd	nd	nd
C-Rac 15	nd	nd	nd	nd	nd	nd	nd	nd
C-Rac 16	nd	nd	nd	nd	nd	nd	nd	nd
C-Rac 17	+	++	++	+	++	++	+	++
C-Rac 18	+	+	nd	+	+	+	+	+
D-Rnc 19	+++	++	+++	++	+++	+++	++	+++
D-Rnc 20	nd	nd	nd	nd	nd	nd	nd	nd
D-Rnc 21	nd	nd	nd	nd	nd	nd	nd	nd
D-Rnc 22	nd	nd	nd	nd	nd	nd	nd	nd
D-Rac 23	nd	nd	nd	nd	nd	nd	nd	nd
E-Rac 24	nd	nd	nd	nd	nd	nd	nd	nd
E-Rac 25	+	+	+	+	+	+	+	+
F-Rac 26	nd	nd	nd	nd	nd	nd	nd	nd

^a nd: Not detected, +: slightly detected, ++: moderately detected, +++: distinctly detected.^b A-F: The wineries; nc: natural cork; cc: colmate cork; ac: agglomerated cork; T1-T7: tasters.

On the other hand, the high concentrations of PCP that occurred in some wine samples, such as AR-5, AR-6 and DR-20 and DR-21, was probably not due to the cork stoppers but was due to the contamination at the wineries.

CONCLUSIONS

The method used for the quantitative determination of the chloroanisole and chlorophenol compounds in cork stoppers and wines gave excellent calibration lines, recoveries, LOD, LOQ repeatability and intermediate precision. Moreover, the method utilized a short extraction time.

According to the research results presented, there was a correlation between the TCA in wines and the TCP in cork stoppers. TCA is the primary compound responsible for the cork taint in wines, especially when its concentration is higher than 5 ng/L, and levels above this can also be detected by sensorial analysis. At lower levels, other chlorinated compounds, especially PCP, can also play a role in the cork taint problem and their presence can be due to contamination of the wine cellar.

ACKNOWLEDGEMENTS

We thank the following Turkish wineries (Kavaklidere S. A, Sevilen S. A, Doluca S. A, Mey S. A, Pamukkale S. A, Melen S. A, K p, S. A, Diren, S. A, Ganos S. A, Umurbey S. A) for providing wine samples.

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(Manuscript accepted for publication March 2009)