CHIMIA **2004**, *58*, No. 7/8

FH – HES Fachhochschule – Hautes Ecoles Spécialisées

Chimia 58 (2004) 560–562

ISSN 0009–4293

Fast Determination of Chlorophenols at the ppt Level. A New Analytical Tool for Quality Control of Cork Stoppers?

Raphaël Berthod, Olivier Flaction[a], and Urban Frey*

Abstract: Chlorophenols (CPs) are the precursors of the main source for the so-called musty taint, especially in wine. Currently, different fast methods for the determination of the chloroanisoles (CAs), mainly 2,4,6-trichloroanisole (TCA), the principal causative agent, are used for the quality control of cork stoppers. The methods available for CPs present either a long sample preparation step or are less representative for a batch, because of the very small sample size. We present a new fast method for the determination of chlorophenols and chloroanisoles at the ppt level by GC-MSMS using *in situ* derivatization and solid-phase microextraction (SPME) preconcentration. We present first results of the development of a new tool for efficient quality control in the cork stopper production.

Keywords: Chloroanisoles · Chlorophenols · Cork · Derivatization · GC-MSMS · Musty taint · Solid-phase microextraction · Wine

Introduction

Cork has been the most popular material for wine stoppers for many years and is regarded as the standard in the case of quality wines. However, it is not entirely without problems. The most important of these problems is a musty/mouldy taint known as 'cork taint', which is often attributed to chemical compounds present in the cork stoppers, but may also be caused by the barrels or the atmosphere in the cellar. The economical loss as a result of cork taint has been estimated to be approximately 10 billion US dollars worldwide [1]. Reasonable estimates of the incidence of corked bottles range from 2.5 to 5% [1–3]. Among these, chloroanisoles (CAs), especially 2,4,6-trichloroanisole (TCA) and 2,3,4,6-tetrachloroanisole (TeCA), are the most frequently identifiable compounds in wine criticized on tasting as 'musty' or 'corked'. Recently, 2,4,6-tribromoanisole (TBA) was shown to give similar musty/mouldy taint problems, either with screw caps [4], cellar atmosphere or plastic stoppers [5].

TCA, while not the only causal agent, is certainly most commonly encountered. Amon *et al.* [6] observed that TCA was present in 62% of the tainted wines they analysed. An additional report suggests that TCA is present in 70–80% of all tainted wines [1].

It has been found [1][7] that fungi may biosynthesise TCA along with other chloroanisoles as a detoxification mechanism in order to remove chlorophenols from their environment and these may migrate to wines from contaminated cork stoppers.

Few currently available analytical techniques allow the direct determination of chlorophenols at ppt level [8][9]. This has promoted the development of various procedures for the extraction from their matrices using organic solvents or solid-phase extraction with different sorbents. In every case, large volumes of sample have to be processed and the final extracts must be concentrated. Chlorophenols are then usually determined by chromatographic techniques such as HPLC or GC. However, because of their high polarity, they give broad, tailed peaks if separated directly (without prior derivatization) by GC. It is therefore advisable to convert chlorophenols into less polar forms in order to improve peak shape, resolution and sensitivity. Acetylation is the most frequently used reaction for this purpose [8][9].

For these reasons, we wanted to set up a reliable, sensitive, and fast analytical

*Correspondence: Dr. U. Frey
University of Applied Sciences Valais
Institute of Life Technologies
Route du Rawyl 64
CH–1950 Sion 2
Tel.: +41 27 606 86 57
Fax: +41 27 606 86 11
E-Mail: fru@hevs.ch
[a]Chaillot SA
Z.I. au Glapin
CH–1162 St. Prex

method for wine and corks, allowing the evaluation of possible transformation of CPs to CAs and the implementation of a specific quality control procedure during the different production stages of cork. Solid-phase microextraction (SPME), a fast, simple, sensitive, and inexpensive technique, is used for the concentration step. Tandem mass spectrometry allows further selectivity, since two mass separations take place. In fact, this technique is particularly useful for the analysis of very complex mixtures, as it allows the separation and identification of components with different structures that are eluted at similar retention times and at widely different concentration levels. Also, increased signal-to-noise ratios afford the sensitivity needed for low trace level analysis without having to process large volumes of sample.

Experimental

For the determination of 2,4,6-trichlorophenol (TCP), 2,3,4,6-tetrachlorophenol (TeCP), pentachlorophenol (PCP), TCA, TeCA, and pentachloroanisole (PCA) in wine, 10 ml of wine were placed in a 20 ml vial, made slightly basic with K_2CO_3 aqueous solution, and then acetic anhydride was added for the *in situ* derivatisation of the CPs. After adding NaCl, the headspace was sampled using SPME (Supelco, polydimethylsiloxane fibre, 100 µm) and a Combi-Pal System (CTC analytics). The fibre was exposed to the headspace over the wine at 333 K for 20 min and then directly analysed by GC-MSMS (Varian, 3800 GC system, Saturn 2000 ion trap MS system). Chromatographic separation was performed using a CP-Sil8 CB lowbleed MS (Varian) capillary column. MS detection was performed under electron impact ionisation (EI) and, in order to increase the selectivity and sensitivity, MSMS experiments were carried out. For the determination of CPs and CAs in cork material, the same sample preparation method was applied as for TCA analysis [10], which means that only the compounds extractable with wine are quantified. The corks are soaked in white wine (chasselas type) for 24 h at room temperature and this wine soak is analysed using the method described above.

Results and Discussion

EI-MS mass spectra of the CP-acetates show a predominant $[M\text{-}C_2H_2O]^+$ fragment and the corresponding isotopic pattern due to the chlorines. To enhance sensitivity and selectivity MSMS spectra were recorded using the most intense peak in this pattern of the fragment as parent ion. The obtained MSMS spectra are shown in Fig. 1. In the case of the CAs, the most intense isotopic peak of the $[M]^{+\cdot}$ ion was chosen as parent ion in the MSMS detection mode.

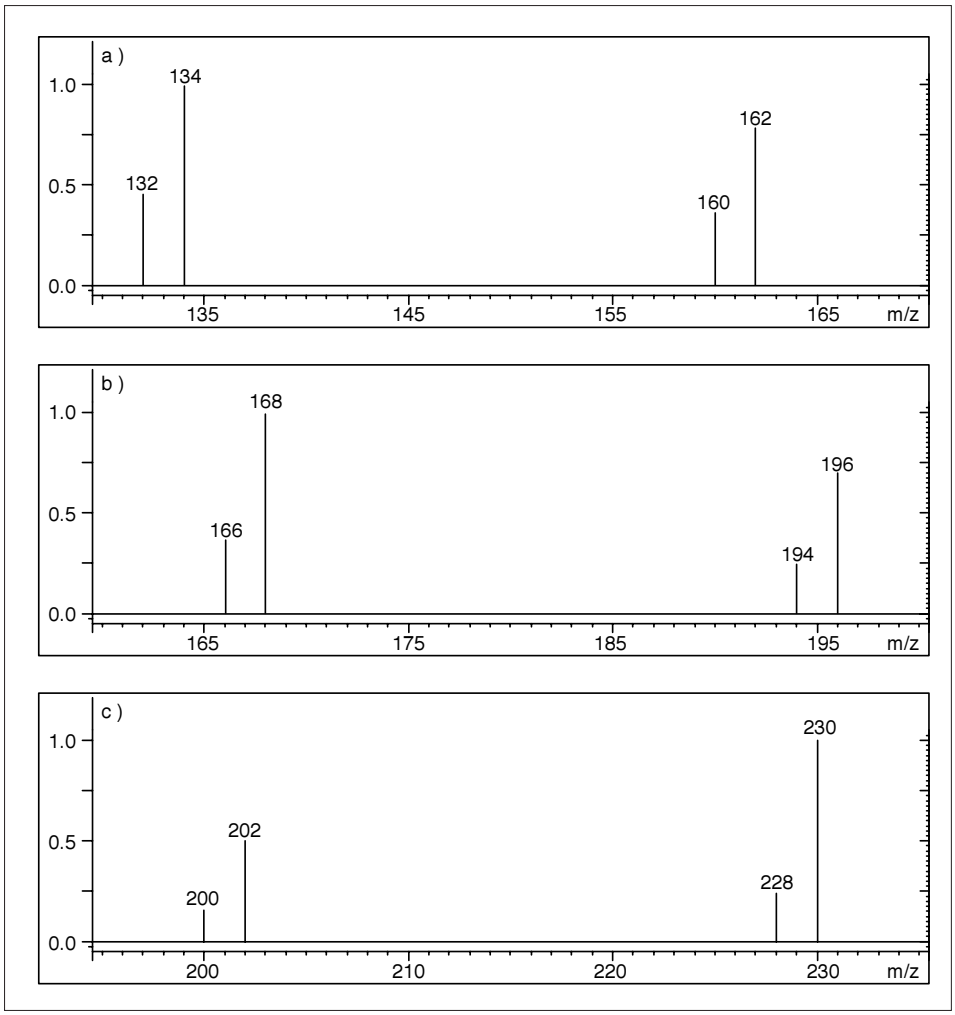

Fig. 1. Fragment ion MSMS spectra of a) 2,4,6-trichlorophenol acetate, b) 2,3,4,6-tetrachlorophenol acetate, and c) pentachlorophenol acetate.

The quantification is undertaken with the fragment ions 134+162, 168+196 and 202+230 for TCP, TeCP and PCP, respectively. In a total analysis time of 24.5 min a good separation of all six compounds (TCP, TeCP, PCP, TCA, TeCA, PCA) is obtained. Fig 2. shows a chromatogram of a wine spiked with 50 ng/l of each compound. The limit of detection (LOD) ranges from 1 to 4 ng/l in a wine or wine soak matrix. A very important parameter for an analytical method is the time required for the analysis, and especially the man-time necessary for sample preparation and machine-time necessary for the determination. This method requires only a few minutes to prepare the sample (soaking and adding the reactants), 20 min for the headspace SPME concentration step and <30 min for the GC-MSMS analysis. Therefore, using the autoanalyser in routine work, a sample can be analysed every 30 min.

To show the possibility of using this method in the quality control of cork stoppers, we first analysed two batches of cork (batch C and batch N). Both were previously analysed for TCA using the standard quality control method. Batch C showed an acceptable TCA concentration (<3 ng/l) determined in the wine soak and batch N a concentration near the limit of acceptance (5 ng/l). The individual determination of CPs and CAs detected only TCP and TCA.

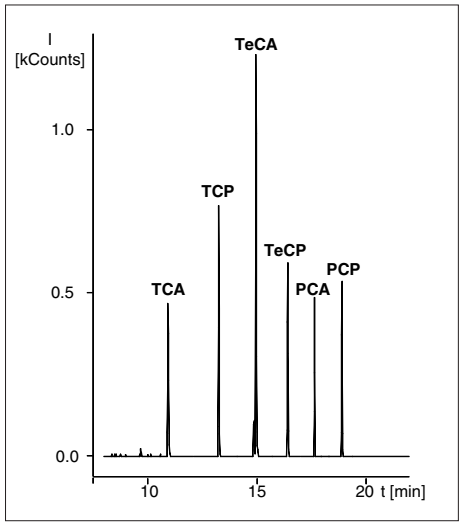

Fig. 2. GC-MSMS chromatogram of CPs and CAs obtained for a spiked wine containing 50 ng/l of each compound.

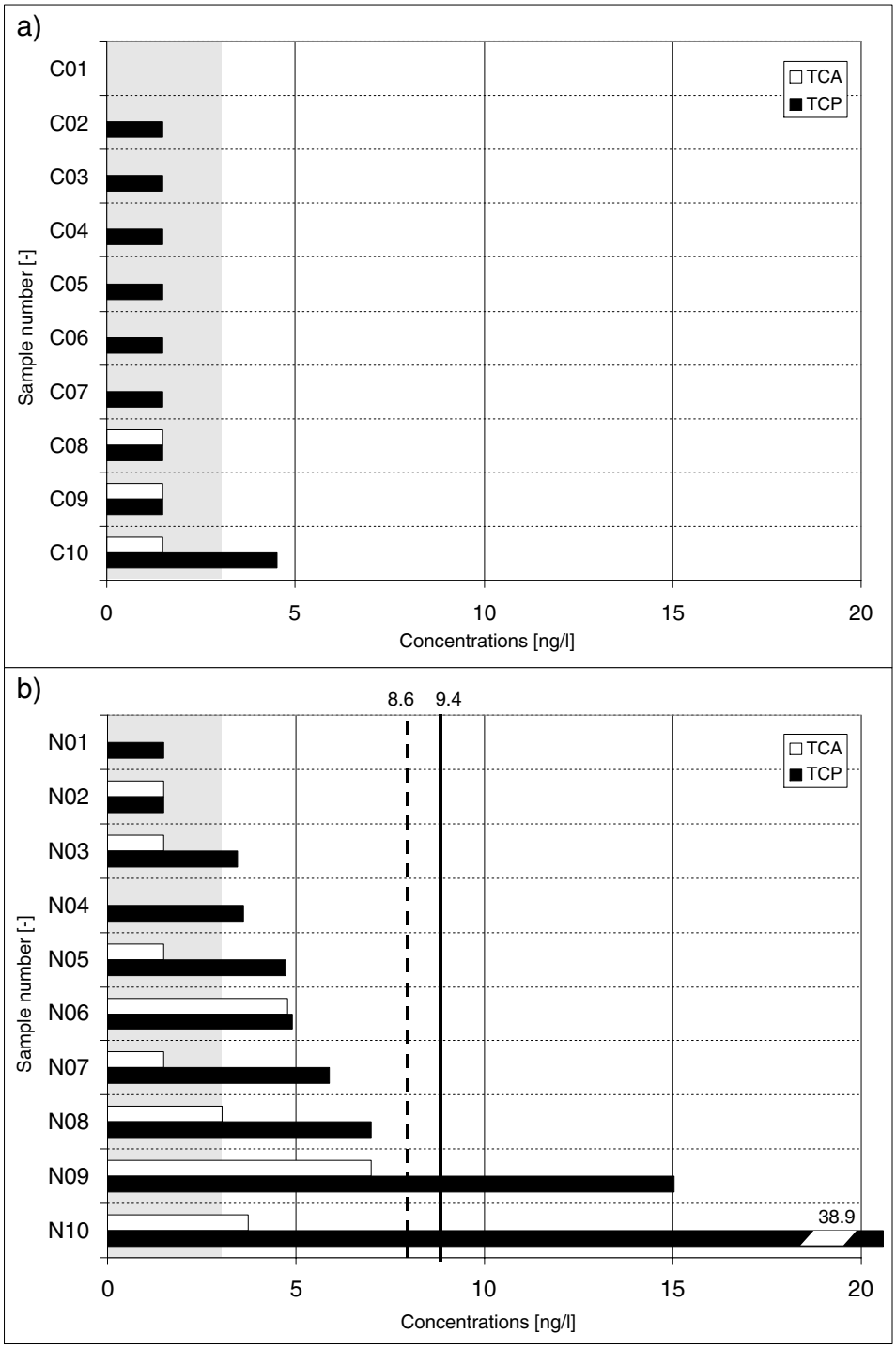

Fig. 3. TCA and TCP concentration distribution in individual wine soak of a) a cork batch with very low contamination and b) a cork batch with a medium contamination. The dotted line represents the mean TCP concentration of the individual soaks and the straight line shows the TCP concentration of the global soak of 10 corks. Limit of detection (LOD) = 1 ng/l. Limit of quantification (LOQ) = 3 ng/l, indicated by the grey zone.

A clear difference in concentration in both batches is visible (Fig. 3). In general, the determined concentration of TCP, the precursor of the anisoles, is about twice that of TCA. This preliminary investigation shows that the determination of TCP may be used for quality control, knowing that a high concentration of TCP can lead to the appearance of critical TCA levels. However, further investigations must be undertaken to determine more precisely the relevance of this method for quality control and to define the most appropriate moment, during natural cork stopper production, for implementation of this control. Both aspects are the main goal of our further studies.

Acknowledgments

The authors wish to thank the Swiss Innovation Promotion Agency (CTI/KTI) for financial funding on this research in the framework of project 6722.2.

Received: July 1, 2004

[1] P. Fuller, *Aust. N. Z. Wine Ind. J.* **1995**, *10*, 58.
[2] C. Smith, *Practical vineyard and winery* **1992**, *Nov/Dec*, 49.
[3] T.H. Lee, R.F. Simson, *Wine microbiology and biotechnology* **1993**, 353.
[4] U. Frey, P. Jacquemettaz, *Chimia* **2003**, *57*, 389.
[5] P. Chatonnet, S. Bonnet, S. Bouton, M.-D. Labadie, *J. Agr. Food Chem.* **2004**, *52*, 1255.
[6] J.M. Amon, A.P. Pollnitz, K.H. Pardon, D. Liacopoulos, G.K. Skouroumounis, *Austr. J. Grape Wine Res.* **1996**, *2*, 184.
[7] QUERCUS – Qualitative Experiments to Determine the Components Responsible and Eliminate the Causes of Undesirable Sensory Characteristics in Drinks Stoppered With Cork, European Union and C.E. Liège contract N° AIR1-CT92-0372, **1996.**
[8] I. Turnes, I. Rodríguez, C.M. García, R. Cela, *J. Chromatogr. A.* **1996**, *743*, 283.
[9] I. Rodríguez, I. Turnes, M.C. Mejuto, R. Cela, *J. Chromatogr. A.* **1996**, *721,* 297.
[10] U. Frey, J.-L. Luisier, *Chimia* **2000**, *54*, 771.