MICROEXTRACTION BY PACKED SORBENT: A NEW TECHNIQUE FOR THE ANALYSIS OF SMALL BROMINATED AND CHLORINATED AROMATIC COMPOUNDS

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Introduction

The wine industry has long been a sufferer of TCA and TBA contamination or "cork" taint. The compound 2,4,6-trichloroanisole (a musty smell synonymous with a "musty basement" otherwise known as TCA) and more recently 2,4,6 tribromoanisole has been found to be present in corks, packaging material, cardboard boxes, wooden products etc etc. The list is endless. The use of chlorine or bromine compounds in everyday life from the sterilization of water to the bleaching of wood and paper products continues unabated. Chlorine will continue to be used in years to come, since it is the cheapest sterilization agent available to date. In water, the levels of THM's (or trihalomethanes) has increased considerably since chlorine was first used to sterilize water supplies and is a common compound on a water treatment analysis these days. Only 1-2 ng L^{-1} of TCA or TBA is required to give wine that "musty" aroma. It has been proposed that chlorophenols are the precursor for TCA, also dioxins might be present as contaminants in the chlorophenols[1] and might therefore also be present in the corks, this needs to be investigated further. Several studies have been published in the literature for determination of haloanisoles using sample preparation methods based on LLE[2,3], SBSE[4,5], SPME[6-11] and SPE[12-14]. In this work, the analysis of TCA and TBA is performed by a new technique for sample preparation called Microextraction by packed sorbent (MEPS). This technique in combination with different GCMS techniques is described below and compared with other methods.

Material and methods

All stock solutions were prepared in methanol and all working solutions were prepared by dilution of the stock solution with methanol. The internal standard was added to the sample just before extraction. Wine was spiked with the working solution to a final concentration of 0.001-100 μg L^{-1}. The wine used in the study (Sangiovese and Sauvignon Blanc) was purchased from the Swedish Alcohol Retailing Monopoly

MEPS was performed using a 100 μL gas-tight syringe filled with 4 mg of C18 (SGE, Ringwood, Australia) and it was conditioned using 30 μL MeOH and 30 μL water. Extraction was performed by drawing 100 μL or 10×100 μL of the sample through the syringe and the C18 solid phase. The C18 sorbent bed was dried by 3×80 μL of dry air. The analytes were then eluted with 10 μL solvent into a GC-vial with conical insert and injected using a standard GC autosampler. All samples have been manually extracted using MEPS and transferred to GC-vials for analysis in the GC-MS. Between sample extractions, the C18 adsorbent in the barrel insert and needle assembly was washed with methanol (5×80 μL) and water (4× 80 μL).

The GC-MS system consisted of a Hewlett Packard 6890 gas chromatograph coupled to a HP 5973 low-resolution-mass spectrometer using electron ionization (EI) at 70 eV or negative chemical ionization (NCI). The analytical column was a 30 m BPX5 (5% Phenyl Polysilphenylene) (0.25 mm 0.25 μm film thickness) with an integrated guard column (SilGuard) from SGE (Ringwood, Australia). The GC temperature program started with an initial oven temperature of 70 °C which was held for 3 min, then heating to 180 °C at 5 °C/min and then to 320 °C at 20 °C/min and then held at 320 °C for 5 min. Splitless injection was used to inject 1 μL at 250°C. Helium was used as carrier gas, when using NCI, methane was used as reagent gas. The mass spectrometer was run using single ion monitoring (SIM) mode, after a solvent delay of 9 minutes. Quantification was performed using m/z 195 for TCA, m/z 210 for the internal standard (2,3,6-TCA) and m/z 346 for TBA. When using NCI, quantification was performed using m/z 174 for IS and TCA and m/z 79 for TBA.

GC-high resolution (HR) MS was performed on a Micromass Autospec Ultima operating at 10,000 resolution using electron ionization at 35 eV. A 15 m SGE BPX5 (0.25 mm, 25 μm) GC column with SilGuard was used. The temperature program was started at 70 °C held for 3 min, then heating to 160 °C at 5 °C/min and then to 300 °C at 32 °C/min and then held at 300 °C for 5 min and at last heated to 320 °C at 20 °C/min and held for 2 min. The injector temperature was at 280°C. Solvent delay time was 7 min. In the SIM mode m/z 209.9406 and m/z 211.9377 were used for TCA and the internal standard. Mass 343.8780 and 345.7850 were used for TBA. For quantification, m/z 209.9406 was used for TCA and IS and mass 343.7870 for TBA.

Results and discussion

Optimization

The MEPS optimization experiments were conducted on spiked red wine samples (10 μg L^{-1} TCA and TBA and 100 μg L^{-1} IS) analyzed by GC-EI-MS. Dichloromethane and toluene, were tested for the elution of the target compounds from a standard MEPS containing 4 mg C18 adsorbent. The recoveries were similar, 55% TCA and 77% TBA using 10 μl of both solvents, compared to standard with analytes dissolved in toluene and directly injected into the GC. Toluene was used as eluant for the subsequent experiments, mainly due to the lower volatility of toluene, which makes it more suitable when considering storage of samples. The elution efficiency was further tested by a second and third portion of 10 μL toluene. The amount of analytes eluted with the second portion toluene was 7% TCA and 11% TBA. The third elution volume contained 2% TCA and 3% TBA. To be able to use the method on line, the elution volume has to be reduced as much as possible and the amount of elution solvent was limited to 10 μL of toluene. To test the extraction efficiency the same sample of 100 μL was pumped ten times through the C18 bed volume and the extraction efficiency was not increased by multiple extractions of the same sample. The influence of different sample volumes on the extraction efficiency was also studied (1×100 μL, 3×100 μL, 5×100 μL and 10×100 μL). The amount of analytes increased with the larger number of extraction volumes, but the extraction efficiency decreased after each extraction volume. By sampling several volumes of the wine, the competition for the active adsorption sites of the C18 sorbent seemed to increase. The amount of sorbent is very small, only 4 mg and the capacity might be limited for sample volumes of 1 mL, due to the results of different extraction volumes. Ten extraction volumes resulted in 5.5 times the amount of TCA extracted and 6.8 times the amount TBA extracted, compared to one extraction volume. The recovery from elution with a fresh second and third portion of toluene was similar to the values reported above for only one extraction volume of the sample.

To clean the MEPS system between the extractions it was washed with methanol and water. After the washing procedure between the extractions, the MEPS were clean for re-use. No memory effects or loss of performance was observed after more than 30 wine extractions. None of the blank samples contained any TCA or TBA. MEPS proved to be very specific and the eluted extracts were free of interference and no further clean up washing step of the MEPS solid phase was found to be necessary.

Performance of the method

The linearity of the method was studied using relative responses between the area of the internal standard and the area of the analytes, for samples of spiked red wine. For GC-EI-MS a five point linearity test resulted in correlation coefficients > 0.999 for both TCA and TBA in the range 1-100 μg L^{-1}. For GC-HRMS the linearity was tested in the range 1-100 ng L^{-1} (1, 10, 50, 100 ng L^{-1}) with correlation coefficients > 0.962 for both TCA and TBA. Repeatability was studied using triplicate analysis of spiked wine at the low end of the calibration curve (Table 1). The RSD for TCA and TBA in spiked red and white wine was between 2-5%. RSD for triplicates in red wine extracted and analyzed on two consecutive different days were 11% for TCA and 3% for TBA based on the internal standard method. RSD-values for the GC-HRMS method at 1 ng L^{-1} were 10% for TCA and 4% for TBA, respectively. TCA is more volatile than TBA and this might be the reason for the slightly increased RSD values for TCA. Repeatability was also studied for the GC-HRMS method analyzing the same extract (spiked red wine, 1 ng L^{-1}) three times, RSD was then 5% for TCA and 7% for TBA, respectively. For

extraction and preconcentration of TCA and TBA in these complex wine matrices, MEPS showed to be a robust technique with good linearity over a large concentration range and good reproducibility.

Limits of detection (LOD) were calculated based on signal-to noise (S/N) 3/1 from spiked wine samples at low concentrations. LODs for TCA and TBA in the present study for red wine using GC-EI-MS was 490 and 450 ng L^{-1} for TCA and TBA, respectively. For white wine the LOD was 270 ng L^{-1} for TCA and 170 ng L^{-1} for TBA. The LODs for the MEPS method for red wine using GC-NCI MS was improved to 20 ng L^{-1} for TCA and 5 ng L^{-1} for TBA (the sample volume was increased to 10 × 100 µL). Even further improvement was achieved by running the MEPS extracts on the high resolution GC-MS system. LODs for TCA and TBA in red and white wine were 0.67-0.75 and 0.22-0.23 ng L^{-1} for TCA and TBA, respectively. Chromatogram of a spiked red wine samples run using GC-HRMS is presented in Figure 1. The LOD values for MEPS-NCI-MS and MEPS-GC-HRMS are similar to those found in the literature[9,14,15]. The advantage of MEPS compared to SPME is the extraction time of only about 5 minutes per sample while SPME needs about 30 minutes[16].

A selective and fast sample preparation method using MEPS in combination with different GC-MS techniques has been developed and validated for the determination TCA and TBA in small volumes (0.1-1mL) of wine. TCA and TBA were selectively extracted from complex wine matrices resulting in interference free and reproducible GC-MS chromatograms even without clean up washing steps. The reproducibility of the method was increased by the usage of an internal standard (2,3,6-TCA) for quantification. LODs were extremely low for GC-NCI-MS and GC-HRMS and TCA and TBA can be detected in the wine before it is sensorically noticed as cork tainted.

It is speculated that the usage of chlorophenol as a disinfectant might lead to TCA formation. As it is known that several chlorophenol formulations contain dioxins, further research will be focused on the source of TCA and levels of PCDD/DFs in natural cork.

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Table 1. Validation for TCA and TBA in spiked samples of red and white wine analyzed by MEPS-GC-EI-MS and MEPS-GC-HRMS.

Method	Wine	Sample (n=3)	Concentration	TCA RSD (%)	TBA RSD (%)
GC-EI-MS	White	Different extractions	10 µg L^{-1}	2%	3%
GC-EI-MS	White	Different extractions	1 µg L^{-1}	5%	2%
GC-EI-MS	Red	Different extractions	10 µg L^{-1}	4%	4%
GC-EI-MS	Red	Different extractions, different days	10 µg L^{-1}	11%	3%
GC-HRMS	Red	Different extractions	1 ng L^{-1}	10%	4%
GC-HRMS	Red	Same extract	1 ng L^{-1}	5%	7%

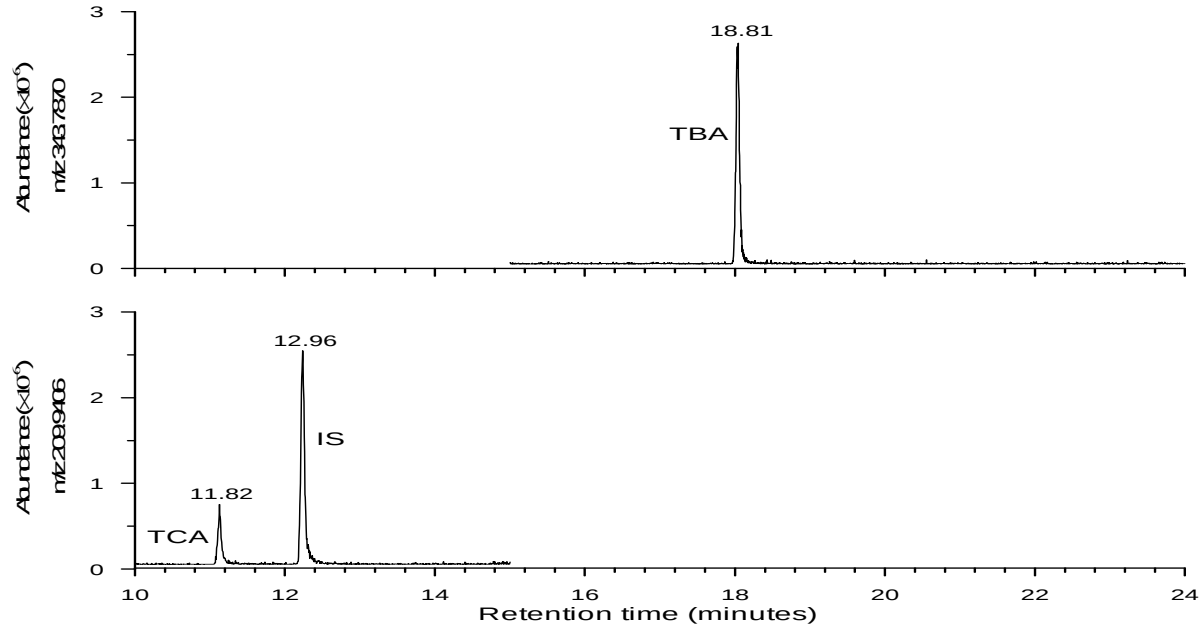

Figure 1. Chromatogram obtained from spiked red wine containing 1 ng L-1 of TCA and TBA and 10 ng L-1 internal standard (2,3,6-TCA) using MEPS-GC-HRMS-SIM.