Detection of corkiness in wine by analysis of 2,4,6-Trichloroanisole with Stir Bar Sorptive Extraction (SBSE) and Thermal Desorption GC/MS

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2,4,6-Trichloroanisole (TCA) is well known as being the main cause for corkiness in wine. Its extremely low odor threshold usually mandidates enrichment and concentration steps before it is available for precise quantitation. This paper describes the applicability of a recently developed, novel and simple approach for sorptive extraction of organic compounds from aqueous samples for the determination of 2,4,6-Trichloroanisole in wine without the necessity of any classical sample preparation. Calibration curves will show linear responses over more than three magnitudes down to 10 ng/l in full scan and below 1 ng/l selected ion monitoring. The method shows good ruggedness, high precision, speed, high sensitivity and due to the absence of any organic solvents involved in the analysis it is environmentally friendly.

Nachweis von Korkgeschmack in Wein durch die Analyse von 2,4,6-Trichloranisol mittels adsorptiver Extraktion mit Rührstäbchen (Stir Bar Sorptive Extraction - SBSE) und thermischer Desorptions-GC/MS. *2,4,6-Trichloranisol (TCA) wird als Hauptursache für Korkgeschmack in Wein verantwortlich gemacht. Seine extrem niedrige geruchliche Wahrnehmungsschwelle verlangt normalerweise Konzentrierungsschritte, bevor es für eine präzise Quantifizierung verfügbar ist. Diese Arbeit beschreibt die Anwendung eines neu entwickelten und einfachen Verfahrens für die adsorptive Extraktion organischer Substanzen aus wässrigen Lösungen für den Nachweis von 2,4,6-Trichloranisol in Wein ohne Notwendigkeit einer klassischen Probenaufbereitung. Die Kalibrationskurven verlaufen über einen Bereich von drei Zehnerpotenzen linear bis zu den unteren Nachweisgrenzen von 10 ng/l beim Gesamt-Ionen- bzw. 1 ng/l im Ausgewählten-Ionen-Monitoring. Die Methode ist schnell, zeigt gute Robustheit, hohe Präzision und Sensitivität und ist wegen der Nichtverwendung organischer Lösungsmittel umweltfreundlich.*

La détection du goût de bouchon dans les vins par analyse du 2,4,6-Trichloroanisole au moyen de l'extraction par adsorption à l'aide d'un agitateur (Stir Bar Sorptive Extraction - SBSE) et par désorption thermique GC/MS (chromatographie gazeuse/spectrométrie de masse). *Le 2,4,6-Trichloroanisole (TCA) est réputé être la cause principale du goût de bouchon des vins. Son seuil extrêmement faible de perception par l'odorat nécessite d'habitude des mesures de concentration avant qu'il ne soit possible de procéder à une quantification précise. Le présent article décrit l'application d'une nouvelle procédure simple d'extraction par adsorption de substances organiques de solutions aqueuses, permettant la détection du 2,4,6-Trichloroanisole dans le vin, un traitement classique des échantillons*

n'étant plus nécessaire. Les courbes de calibrage passent de façon linéaire à travers une gamme de trois puissances de dix jusqu' aux limites de détection inférieures de 10 ng/l pour le monitoring de la totalité des ions et/ou de 1 ng/l pour le monitoring d'ions sélectionnés. La méthode est rapide, elle présente une bonne robustesse, une précision et une sensibilité élevées et elle est favorable à l'environnement en raison de la non-utilisation de solvants organiques.

2,4,6-Trichloroanisole is an off-flavor substance which is believed to be produced by fungal degradation and methylation of pentachlorophenol fungicides (NEIDLEMAN and GEIGERT, 1986; NICHOLSON et al., 1992). Its origin in wine can be traced back to contaminated cork stoppers and is either already present in the corkwood or produced during corkwood-processing. Above concentrations as low as 15 to 20 ng/l TCA contribute to a corky off-flavor in wine, and can result in complete spoilage (BELITZ and GROSCH, 1990). A common procedure to analyze cork for TCA is to leach it out of the cork into a reference wine or an ethanol-water mixture at consistent parameters. After extraction with an organic solvent TCA can be quantified by reconcentration of the extract and headspace sampling or direct injection into a standard GC-system (KLEE and MENG, 1999). Another technique applicable is direct thermal extraction: a small piece of the cork stopper is placed into an empty glass tube of a thermal desorption system and heated to a temperature suited to release the TCA from the corkwood matrix followed by GC analysis. Here sample amounts lower than 10 mg are sufficient for extremely low detection limits (HOFFMANN and SPONHOLZ, 1997). All these methodologies have in common that they solve the analytical problem at its source (which can be desired as a preventive task). Regarding especially legal aspects, it is desireable to determine TCA in wine directly, not indirectly through corkwood analysis. The problem here is the extremely low odor threshold of TCA, which requires cumbersome extraction and enrichment steps in order to achieve the necessary sensitivity. On the other hand liquid-liquid extractions require the use of organic solvents and often produce more toxic waste than the trace level pollutants to be determined. State-of-the-art procedures should be designed to minimize or even completely avoid organic solvent consumption and therefore provide an alternative 'environmentally friendly' approach. About ten years ago ARTHUR and PAWLISZYN (1990) developed such a technique called solid phase micro extraction (SPME). Extraction of organics from water using polydimethyl-siloxane (PDMS) sorbents (PDMS coated open tubular traps) has been described by various groups (MOL et al., 1993) in the mid 80's, but practical limitations like low sample capacity and low sample breakthrough volumes, have prevented its acceptance. It was the practical approach of SPME with its PDMS-coated fiber that led to its success. SPME is an equilibrium technique based on partitioning between the coating and the aqueous matrix. This equilibrium can be correlated with octanol/water distribution coefficients (K(o/w)) recently published in several studies (DUGAY, et al., 1998; DE BRUIN et al., 1986). For low coefficients (< 10000) low recoveries are obtained, an effect of the phase ratio between PDMS and aqueous phase. As a consequence enlarging the amount of PDMS relative to the aqueous matrix would dramatically increase recovery of analytes and therefore sensitivity. The amount of phase usually used for SPME is typically in the order of 0.5 µl. Using a sample size of 10 ml this corresponds to a phase ratio of 20000. That implies that for 50 % recovery of a given compound (50 % in the PDMS, 50 % left in the aqueous matrix) a K(o/w) larger than 20000 is necessary. The studies showed clearly that for compounds with a K(o/w) lower than 10000 only low or no recoveries are obtained.

Figure 1. Gerstel Twister.

This paper describes the applicability of a recently developed technique (Stir Bar Sorptive Extraction, SBSE) based on this sorption principle for the determination of 2,4,6-Trichloroanisole in wine. Here a stir bar (figure 1) instead of a fiber is coated with PDMS and used for extraction. The coating volume is in the range of 55 µl,

which corresponds to a phase ratio 200 and therefore leads to an increase in sensitivity by a factor of more than 100 compared to SPME. 50 % recovery can now be reached for compounds with a K(o/w) as low as 200. Figure 2 compares the different recoveries for SPME and SBSE.
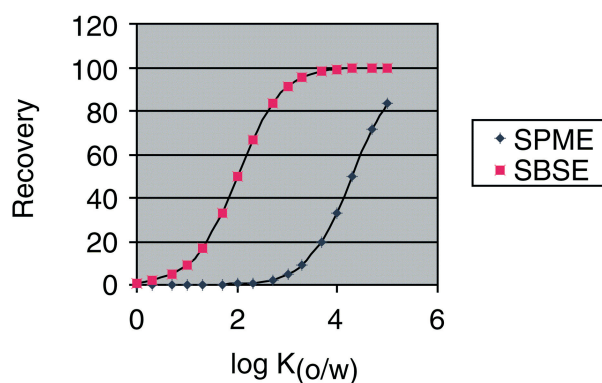

Figure 2. Recovery as function of log $K_{(o/w)}$.

Materials and Methods

Instrumentation

The stir bars (Twister, Fa. Gerstel GmbH & Co.KG, Mülheim an der Ruhr, Germany) consist of a 10 mm magnetic stirring rod incorporated in a glass jacket and coated with a 0.5 mm layer of PDMS.
The analytical system consists of a thermodesorption system (TDS 2, Fa. Gerstel), a temperature programmable vaporization inlet (CIS 4, Fa. Gerstel), a gas chromatograph (6890, Fa. Agilent Technologies, Little Falls, USA) and a mass selective detector (5973, Fa. Agilent Technologies).

Operation

Sample extraction is performed by placing 10 ml of wine in a 10 ml headspace vial, adding a stir bar, crimping the vial to avoid evaporation of volatiles into the air and stirring for 30 to 120 minutes. After extraction the stir bar is removed, rinsed with water, dried with a lint free tissue and placed in a glass thermal desorption tube. No further sample preparation is necessary. The thermal desorption tube is then introduced into the thermal desorption unit, where the stir bar is thermally desorbed at a temperature of 200 °C to release the ex-

tracted compounds into the cryogenically precooled PTV for subsequent GC/MS analysis.

Table I:
Analysis conditions.

Twister	10 mm, 55 μl PDMS
Column	30 m HP 5 (Fa. Agilent), di = 0.25 mm, df = 0.25 μm
Pneumatics	He, Pi = 56.6 kPa, constant flow = 1 ml/min
	TDS-desorption flow = 50 ml/min (splitless)
	PTV-splitless time = 1 min
TDS Temperatures	20 °C, 60 °C/min, 180 °C (5 min)
PTV Temperatures	-150 °C, 12 °C/s, 280 °C (5 min)
Oven Temperatures	60 °C (1 min), 10 °C/min, 150 °C, 25 °C/min, 300 °C (30 min)
Detector	Mass selective detector (= MSD), 230 °C / 150 °C, Scan 35-350 amu, SIM m/z 212/197/169

Two different series of wine samples were analyzed: The first series contained wines which showed a clear and distinctive cork off-flavor together with some non-tainted wines for comparison. The second series contained wines from the grape cultivar 'Welschriesling' which were found to have an off-flavor, but could not be definitely described as "corky". 4-Bromoanisole (10ng/l, Reinheit 99%, Fa. Aldrich-Ega) was added as internal standard.

Results and Discussion

Calibration

Two calibration curves were prepared: One for TIC-mode (TIC = total ion chromatogram) with calibration levels at 100.000, 50.00, 10.000, 5.000, 1.000, 500, 100, 50 and 10 ng/l (Fig. 3), another one for SIM-mode (SIM = selected ion monitoring with calibration levels at 10.000, 5.000, 1.000, 500, 100, 50, 10, 5 and 1 ng/l (Fig. 4). The TIC-standard was prepared by adding TCA to a non-tainted wine, the SIM-standard by adding TCA to a 12 % ethanol-water mix.

Figure 3:
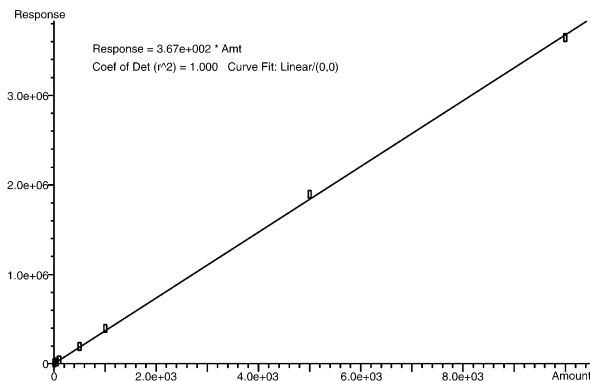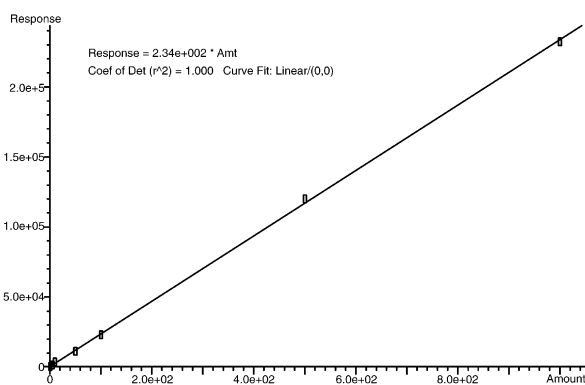Calibration curve for total ion chromatogram mode.

Figure 4:
Calibration curve for selected ion chromatogram mode.

Figure 5:
Extracted ion chromatogram of 160 ng TCA per litre wine (cultivar: 'Silvaner') (160 ppt).
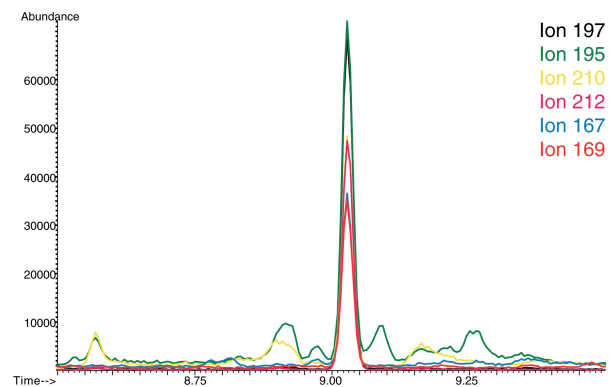
Figure 6:
Spectrum of 160 ng TCA per litre wine (cultivar: 'Silvaner')

For each standard and for each sample a new stir bar was used. It is not necessary to use only one and the same stir bar for quantitation.

Both calibration curves showed excellent linearity over three orders of magnitude analyte concentration. Several wines from the first series were analyzed and two of them were chosen as examples: Figure 5 shows the extracted ion chromatogram of a wine sample of the cultivar 'Silvaner', with a TCA-content of 160 ng/l, which was the highest level found in all wine samples investigated.

Even at this low level (ppt) very clean mass spectra are obtained (Fig. 6).

The other extracted ion chromatogram shows a wine sample (cultivar: 'Rheinriesling') where the TCA-content was determined to be at 9.5 ng/l (Fig. 7). Although

here no clean mass spectrum could be obtained due to overlapping of other compounds the ion traces still allow very accurate and reliable qualification and quantification.

Operating the MSD in selected ion monitoring mode (SIM) further increases the sensitivity of the method. Figures 8 and 9 show the same samples as in Figures 5 and 7, but this time in SIM. The enhanced signal to noise ratio further improves quantitation quality.

The second series of wine samples consisted of eight bottles of the grape cultivar 'Welschriesling'. Here Bromoanisole was added as internal standard, the TCA-content found ranged between 0.3 and 1.3 ng/l. Figure

Figure 7:
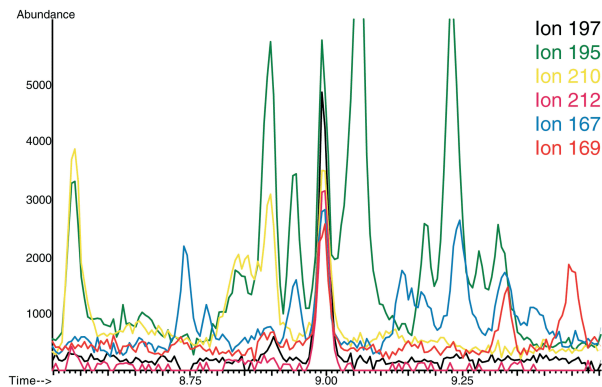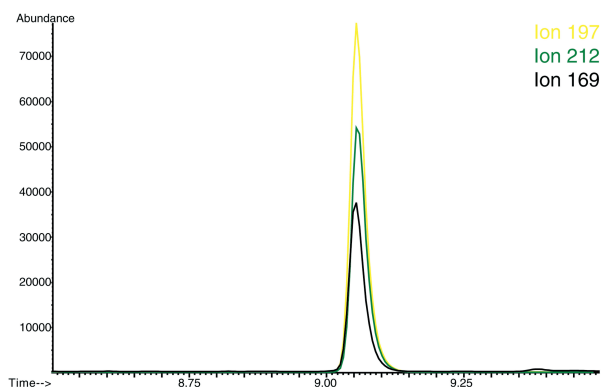Extracted ion chromatogram of 9.5 ng TCA per litre wine (cultivar: 'Rheinriesling').

Figure 8:
Selected ion monitoring chromatogram of 160 ng per litre wine (cultivar: 'Silvaner')

Figure 9:
Selected ion monitoring chromatogram of 9.5 ng TCA per litre wine (cultivar: 'Riesling')
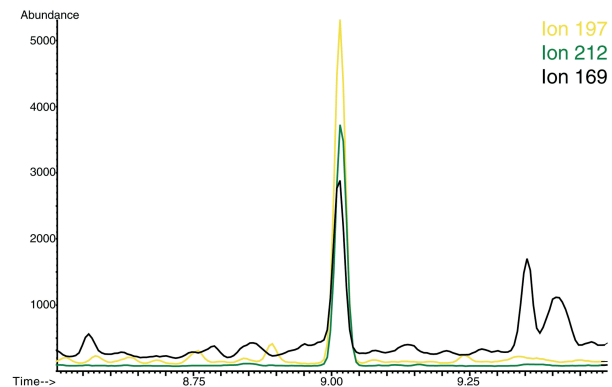
Figure 10:
Selected ion monitoring chromatogram of 0.3 ng TCA per litre wine (cultivar: 'Welschriesling')

10 shows a SIM-chromatogram of a wine sample with the lowest TCA concentration found (0,3 ng/l).

The wines of the second series cannot really be regarded as being "corky", since the sensory detection limit of TCA is somewhere between 3 and 15 ng/l (AMON et al., 1989). All of the wines in this study, even those not determined to be "corky", were found to contain some TCA, they only differed in amount.

Stir Bar Sorptive Extraction (SBSE) turned out to be an extremely powerful tool for the determination of 2,4,6-Trichloroanisole in wine. The combination of ease of use, ruggedness, precision, speed and sensitivity is a big step forward in trace analysis of aqueous samples. No sample preparation is necessary, other than simply stirring, yet detection limits in the sub-ppt level can be reached with a standard benchtop mass selective detector. In addition the entire methodology is "environmentally friendly" due to the absence of any organic solvents involved in the analysis.

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