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Headspace GC-SCD monitoring of low volatile sulfur compounds during fermentation and in wine*

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Abstract. A rapid and reliable method for the determination and quantification of volatile sulfur compounds in wine is reported. The analysis was carried out with an automatic headspace injection sampler combined with a cooled injection system and coupled with a gas chromatograph and a sulfur chemiluminescence (GC-SCD) detector. The calibration curves were calculated in a wine with a very low amount of sulfur compounds. As internal standard ethyl methyl sulfide was used. In dependence on the S-compound the detection limit was in the range of 0.1 to 3 µg/L. A linear response was obtained up to a concentration of about 100 µg/L with an average correlation coefficient of 0.998. Major advantages are the small sample volume (3 mL), the high repeatability or reproducibility and a short total analysis time.

Key words. Low volatile sulfur compounds in wine – automatic headspace technique – sulfur chemiluminescence detector.

Introduction

Very low levels of sulfur (S)-compounds give wines their distinctive aroma, but in higher concentrations they can create unappealing flavours. A rapid and reproducible detection of S-compounds in wine becomes more and more important for quality control and research analysis. Therefore a multi-purpose-sampler, designed for liquid large volume, gaseous and headspace samples was tested for its suitability in the headspace analysis of low volatile S-compounds in wine and during fermentation.

Experimental

An automatic headspace sampling system (Multi Purpose Sampler used as Headspace-Sampler, Gerstel GmbH, Mülheim a. R., Germany) with a volume, temperature and speed controlled 1000 µL gas tight syringe was combined with a temperature controlled cooled injection system (CIS-3, Gerstel GmbH). Regular 5 mL GC-vials filled with only 3 mL sample were used as headspace sampling vials. The wine samples are stored at 4 °C before sample preparation. A high advantage of this method is the low sample volume. Each sample is heated for the same period of time at the same temperature in the preheating station (45 min). After preheating the sample is injected into the cooled CIS-3 (Gerstel) for cold trapping, focussing and enrichment and after heating up to the desired temperature transferred to the capillary column in either split or splitless mode. The most suitable column for the detection of low volatile S-compounds in wine seems to be a SPB-1-Sulfur (Supelco INC., Bellefonte PA, USA) which was also used in this study. The samples were investigated on a Siemens Sichromat 2 (Siemens, Karlsruhe, Germany) fitted with a SCD 350 B (Sievers Instrument, Boulder, Colorado,USA) sulfur chemiluminescence detector. The SCD 350 is coupled to a flame ionization detector (FID). The SCD is a very sensitive and selective detector for sulfur compounds in different kinds of beverages. It gives a linear response over a wide concentration range and there is no problem of hydrocarbon quenching [1,3,4,6].

The liner of the CIS-3 was filled with a porous polymer to increase the enrichment capacity. Sensitivity of the system was enhanced by the addition of sodium chloride to the sample. A concentration of 0.8 g NaCl/3 mL sample (0.27 g/mL) enhanced the response of the sulfur compounds about 80 to 100 per cent.

The first studies were carried out with hydrogen as carrier gas. Afterwards helium (He) was used, because in future the GC Sichromat 2 will be coupled with a mass spectrometer system.

Ethyl methyl sulfide (EMS) was used as internal standard (30 µg/L EMS).

Analysis conditions

• *Headspace Single Injection (HSI)*

Injection: total volume 5000 µL (5 times 1000 µL of each sample).
Temperature: syringe 60 °C and preheating station 60 °C.
Preheating time: 45 min.

• *Cooled injection System CIS-3*

Liner: filled with 25 mg Porapak Q 80/100 mesh (WGA, Düsseldorf, Germany.
Temperature program: − 60 °C, 12 °C/s ↗ 180 °C for 8 min.
Split flow through the liner: 50 mL/min.
Splitless time: 8 min.

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- *Gas chromatograph*

Column: SPB-1 Sulfur.
(30 m × 0.32 mm I. D., 4 µm film thickness, Supelco).
Temperatures: 35 °C for 5 min, 10 °C/min ↗ 180 °C for 8 min.
Linear gas velocity for hydrogen was 40 cm/s at 60 °C.
Linear gas velocity for helium was 22 cm/s at 60 °C.
Split: 1:20.

- *Sulfur chemiluminescence detector:*

Detector temperature of the FID: 240 °C.
Gas flow conditions: 250 mL/min H_2. 375 mL/min air. 27 mL/min air for make-up gas.

Results and discussion

The analytical method can be practised for detection and quantification of volatile sulfur compounds in wine like hydrogen sulfide (H_2S), methanethiol (MeSH), carbon disulfide (CS_2), ethanethiol (EtSH), dimethyl sulfide (DMS), thioacetic acid methyl ester (MeSAc), thioacetic acid ethyl ester (EtSAc), dimethyl disulfide (DMDS), ethyl methyl disulfide (MeSSEt) and diethyl disulfide (DEDS). An oxidation of the mercaptanes to the disulfides during sample preparation could be eliminated by the addition of an antioxidant like 4 mg/L 2,6-di-tert-butyl-4-methyl-phenol. EDTA (ethylenediamine tetraacetic acid) can be used to avoid the interference with metals.

The calibration curves were calculated in wine and in synthetic must- and wine-like media. In dependence on the S-compound the detection limit was in the range of 0.1 to 3 µg/L. A linear response was obtained up to a concentration of about 100 µg/L with an average correlation coefficient of 0.998. The calibration graphs for MeSH (r = 0.991) and for EtSAc (r = 0.999) are shown in figures 1 and 2 (A. is used as abbreviation for area).

The repeatability and reproducibility error is between 5 − 10%. The calibration in wine was carried out by the addition of the S-compounds in different quantities to a wine with a low content of the S-substances and without addition of sulfur dioxide (SO_2).

Sulfur dioxide can be added to wine as an antioxidant and antimicrobial agent. Commercial wines contain up to 50 mg/L free SO_2 or even more. High concentrations of SO_2 interfere with the detection of the other volatile S-compounds, which occur in wine in significant lower concentrations. SO_2 reacts with carbonyl compounds. The reaction depends upon pH, temperature and concentration of reactants [2]. Therefore ethanal, propanal, pyruvic acid and 2-ketoglutaric acid (also in combination) were given to the wines to eliminate free SO_2.

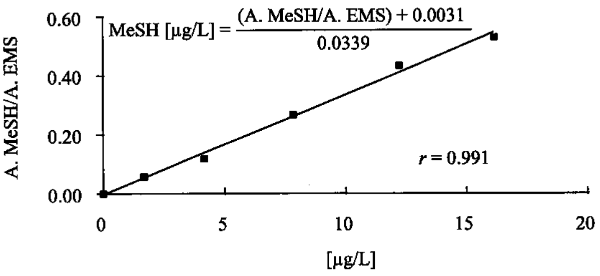

Fig. 1. Calibration graph for dimethyl sulfide (MeSH) from wine. Each point represents the average value of five independent measurements.

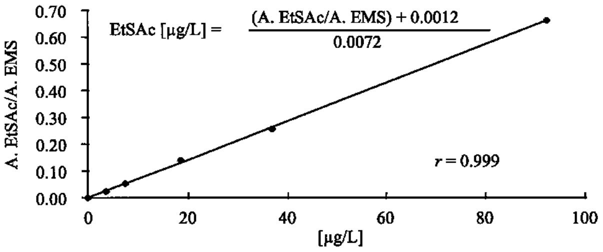

Fig. 2. Calibration graph for thioacetic acid ethyl ester (EtSAc) from wine. Each point represents the average value of five independent measurements.

The best results were obtained with the addition of propanal. In most cases 500 mg/L of propanal seem to be enough to bind free SO_2 in wines (see Fig. 3). The influence of propanal on the quantitative measurement of the S-compounds was tested in wines with and without free SO_2, especially with different amounts of H_2S and EtSH. No artefact production could be observed during the normal analysis time even if 1000 mg/L propanal were added to the samples. There was also no change in the repeatability.

Known amounts of the S-compounds (n = 5 for each concentration) under investigation are added to different wines to determine the recoveries. For all S-substances the recovery was in the range of 85 − 110%.The standard deviations of the various amounts of the investigated S-substances were very low (s = ± 1.3 − ± 2.1 µg/L S-substance). This is a difference in the measurements about ± 5 − 10%.

Figure 4 shows a chromatogram of a wine with a sulfurous off-flavour. In this case elemental sulfur residues in the must were the cause for the production of increased amounts of thioacetic acid esters and mercaptanes during fermentation [5].

Table I gives an overview about the different concentrations of low volatile S-substances in wines with and without sulfurous off-flavour from different wine growing countries.

Methanethiol was detected in every wine. Concentrations of more than 1.5 µg/L of methanethiol seem to be responsible for the occurring of off-flavours in wine in connection with a significant increase of other S-components with higher boiling points [5]. The detected levels of H_2S are far away from the threshold value which normally is in the range of 50 to 80 µg/L. Therefore H_2S cannot be the reason for the off-flavour in these wines.

Conclusion

Headspace sample injection combined with a cooled injection system and coupled with a GC-SCD is an attractive alternative to classical static or dynamic headspace methods for the detection and quantification of low molecular sulfur compounds in different beverages. Furthermore the injection

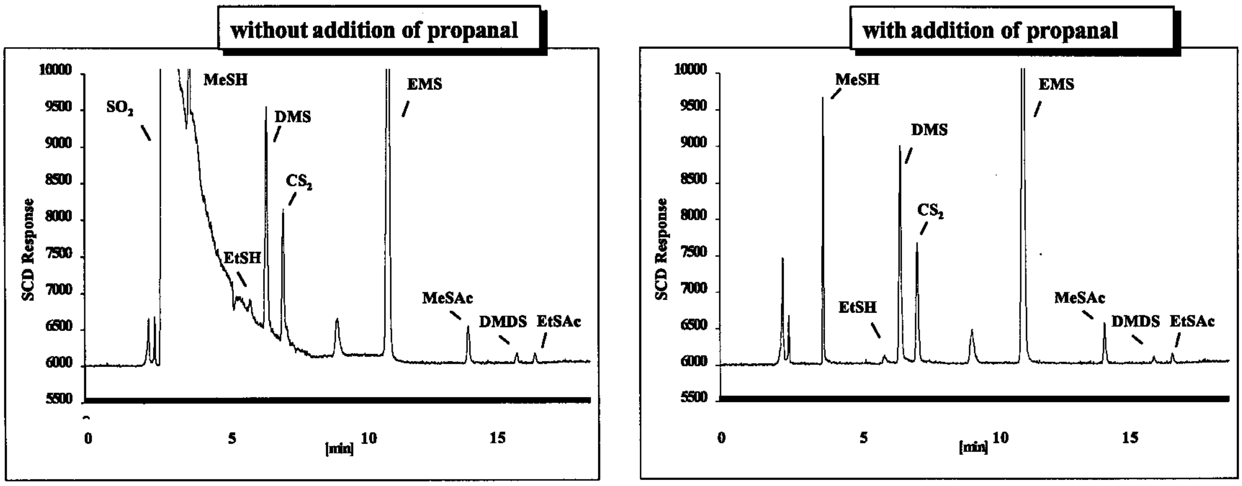

Fig. 3. Chromatograms of a wine with off-flavour before (A) and after (B) addition of 500 mg/L propanal by HSI-GC-SCD-Analysis(GC-column: SPB-1-sulfur, carrier gas: He).

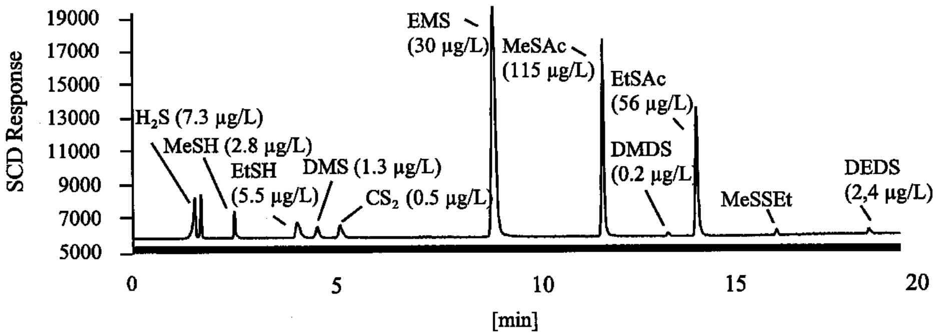

Fig. 4. Chromatogram of a young wine with a sulfur off-flavour by Headspace GC-SCD Analysis (GC-column: SPB-1-sulfur, carrier gas: H_2).

Table I. Concentrations of low volatile sulfur compounds in different wines.

wine sample	*H_2S µg/L*	*MeSH µg/L*	*EtSH µg/L*	*DMS µg/L*	*CS_2 µg/L*	*MeSAc µg/L*	*DMDS µg/L*	*EtSAc µg/L*	*MeSSEt µg/L 1)*	*DEDS µg/L*	*Off-Flavour*
1985 Riesling	n. d. 2)	2.8	n. d.	41.7	1.6	n. d.	2.4	n. d.	n. d.	n. d.	yes
1985 Riesling	traces	4.8	n. d.	52.7	1.9	n. d.	2.3	n. d.	n. d.	n. d.	yes
1989 Müller-Thurgau	3.9	0.5	traces	28.5	2.2	traces	Traces	n. d.	n. d.	n. d.	no
1991 Riesling	1.6	1.2	n. d.	18.2	3.0	n. d.	Traces	n. d.	n. d.	n. d.	no
1992 Pinot noir	3.2	7.1	n. d.	45.5	1.2	traces	Traces	n. d.	n. d.	n. d.	yes
1992 Riesling	8.2	4.5	traces	22.9	1.3	n. d.	n. d.	n. d.	n. d.	traces	yes
1993 Scheurebe	n. d.	1.0	traces	9.2	4.9	3.1	Traces	traces	n. d.	n. d.	no
1994 Riesling	traces	1.6	n. d.	11.7	2.4	10.2	Traces	traces	n. d.	traces	yes
1995 Riesling	2.9	0.6	n. d.	10.1	1.1	traces	n. d.	n. d.	n. d.	n. d.	no
1996 Chardonnay	6.5	0.5	traces	25.0	1.1	n. d.	n. d.	n. d.	n. d.	n. d.	no
1996 red wine	2.6	0.5	traces	9.1	1.8	traces	n. d.	n. d.	n. d.	n. d.	no
1996 Müller-Thurgau	4.1	4.4	n. d.	1.6	1.4	traces	1.7	n. d.	n. d.	n. d.	yes
1996 Muscat	4.1	3.1	traces	2.9	2.7	12.3	Traces	traces	n. d.	n. d.	yes
Pinot noir	7.3	2.8	5.5	1.3	0.5	115.0	0.2	56.0	3.0	2.4	yes
1996 Riesling	2.2	1.0	n. d.	9.5	3.6	n. d.	n. d.	n. d.	n. d.	n. d.	no

1) calculated with the calibration graph of diethyl disulfide (DEDS), 2) not detected.

compounds in different beverages. Furthermore the injection operation can be automated.

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