The Analysis of Resveratrol in Red Wine by On-Fiber Derivatization/SPME

Katherine Stenerson
katherine.stenerson@sial.com

Introduction

Resveratrol is a phytoallexin produced by grapes and other plants to increase resistance to fungal infection. Recent research suggests that consumption of resveratrol may reduce the risk of certain cancers, heart disease, and other age-related disorders (1). Red wine, which is produced by fermentation of juice on the crushed grapes, has been found to contain a greater amount of resveratrol than white wine, which is produced by fermentation of the juice alone.

In this study, the extraction and analysis of resveratrol from red wine is demonstrated using solid phase microextraction (SPME) and GC-MS. The presence of 3-hydroxyl (OH) groups make it necessary to derivatize this compound prior to GC analysis. Derivatization was conducted, after extraction, directly on the SPME fiber by exposing it to the vapors of a silyating reagent. The derivative of resveratrol was then analyzed directly from the fiber using GC-MS.

Experimental

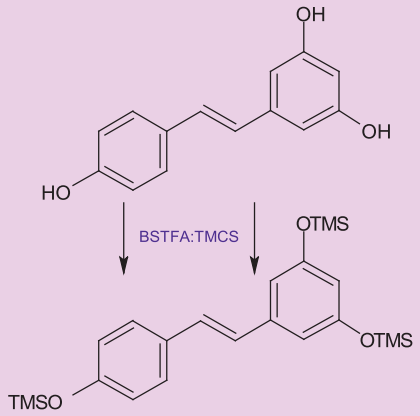
The extraction and derivatization conditions used are summarized in Table 1. The polyacrylate fiber, which is suitable for the extraction of polar semivolatiles and is more resistant to swelling than other types of fibers, was chosen based on recently published findings (2,3). The extraction conditions were based on these works as well, with some modifications made to extraction and desorption times to decrease matrix interference from the wine sample.

Table 1. SPME – On-Fiber Derivatization Conditions

sample/matrix:	3 mL of red wine (California merlot) diluted 3:1 in 12% ethanol:water
SPME fiber:	85 µm polyacrylate
extraction:	immersion at room temperature, 15 min., with stirring at 400 rpm
derivatization:	20 min. in 4 mL vial containing 5 µL of Sylon-BFT
desorption:	280 °C, 2 minutes

After sample extraction, the SPME fiber was gently blotted with a Kimwipe® to remove excess water. The fiber was then inserted into a 4 mL vial containing 5 µL of Sylon™ -BFT (BSTFA + 1% TMCS) reagent. For consistency, the vial containing the Sylon-BFT was allowed to equilibrate for 60 – 90 minutes prior to use, and a new reagent vial was used for each extraction. The fiber was desorbed at 280 °C for 2 minutes directly in the injection port of the GC-MS. The derivatization procedure resulted in silylation of all three –OH groups present in resveratrol (Figure 1). The resulting derivative has a molecular weight of 444, and subsequent GC-MS analysis showed a predominance of the molecular ion, which was used for quantification.

Figure 1. Derivatization of Resveratrol



Calibration standards ranging from 10 – 300 µg/L were prepared by spiking a solution of 12% ethanol in water with trans resveratrol. Red wine (California merlot), diluted 3:1 in 12% ethanol in water, was analyzed both "as is" and spiked with a known level of trans-resveratrol.

Results/Discussion

A plot of the calibration standards as absolute response vs. concentration is presented in Figure 2. Linearity was very good, with a correlation coefficient of 0.9987, indicating that the extraction and derivatization procedure is quantitative. Peak responses obtained using the technique were adequate to allow for detection of the lowest calibration standard while using the MSD in full scan mode.

Chromatograms of the unspiked and spiked red wine samples are presented in Figures 3 and 4. Using the average response factor from the calibration, the levels of trans-resveratrol in each were calculated, along with a percent accuracy of this experimental value relative to the known spiking level of the wine sample (Table 2).

Table 2. Wine Spike Levels

	Unspiked Red Wine	Spiked Red Wine (100 µg/L)
Conc. of trans-resveratrol (µg/L)	22.6	134.7
% Accuracy	---	110%

Figure 2. SPME Calibration Standards; 10-300 µg/L

SPME - on-fiber derivatization analysis of trans-resveratrol

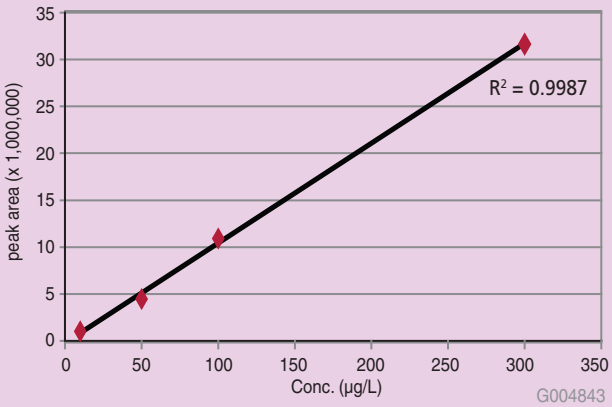


Figure 3. SPME On-Fiber Derivatization Analysis of Resveratrol in Unspiked Red Wine Sample

column: SLB-5ms, 30 m x 0.25 mm I.D., 0.25 µm (28471-U)
oven: 100 °C (1 min.), 10 °C/min. to 325 °C (3 min.)
MSD interface: 325 °C
scan range: m/z 40-450
carrier gas: helium, 1 mL/min, constant
liner: 0.75 mm I.D. SPME

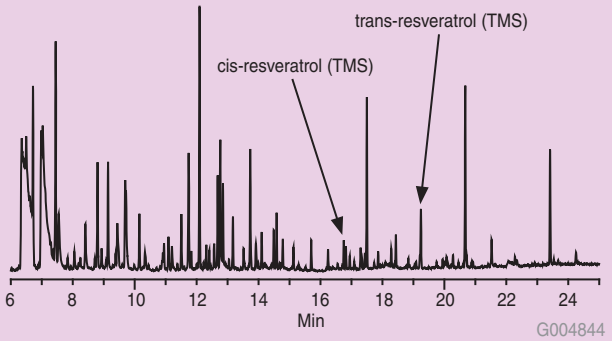
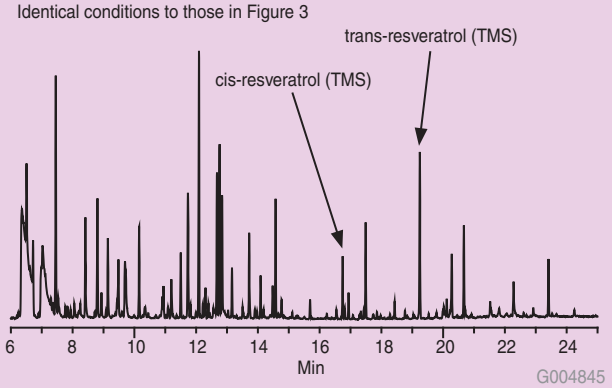


Figure 4. SPME On-Fiber Derivatization Analysis of Resveratrol in Spiked Red Wine Sample

Identical conditions to those in Figure 3



In addition to the trans form, a sizable cis-resveratrol peak was detected in the wine samples. The cis form is not naturally found in grapes, however it has been theorized that it can be formed from the trans during analysis, or the production and/or aging of wine (2).

Conclusions

SPME when used in combination with on-fiber derivatization was found to be applicable to the extraction of resveratrol from red wine. The technique was found to be highly sensitive, simple, and quantitative. The polyacrylate fiber was also found to withstand exposure to the vapors of the silylating reagent without damage resulting from swelling.

References

1. Red Wine Compound Resveratrol Demonstrates Significant Health Benefits. ScienceDaily. Retrieved June 18, 2009, from http://www.sciencedaily.com / releases/2009/06/090611174052.htm
2. Cai, Lingshuang, Koziel, Jacek A., Dharmadhikari, Murli, van Leeuwen, J. (Hans), Rapid Determination of trans-resveratrol in red wine by solid-phase microextraction with on-fiber derivatization and multidimensional gas chromatography-mass spectrometry. J. Chrom. A (2009), 1216, 281-287.
3. Vinas, Pilar, Campillo, Natalia, Martinez-Castillo, Nelson, Hernandez-Cordoba, Manuel, Solid-phase microextraction on-fiber derivatization for the analysis of some polyphenols in wine and grapes using gas chromatography-mass spectrometry. J. Chrom A (2009), 1216, 1279-1284.

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Description	Qty.	Cat. No.
SPME Fibers		
Manual, w/ 85 µm polyacrylate coating	3	**57304**
Auto, w/ 85 µm polyacrylate coating	3	**57305**
Auto, 23 GA w/ 85 µm polyacrylate coating	3	**57294-U**
GC Column		
Fused silica capillary column, SLB-5ms, 30 m x 0.25 mm I.D., 0.25 µm		**28471-U**
Chemical Standards and Reagents		
Resveratrol	100 mg	**R5010**
BSTFA + TMCS, 99:1 (Sylon BFT)	20 x 1 mL 25 mL 50 mL	**33148** **33155-U** **33149-U**

Related Information

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